8-2004

Systematics and species concepts in the genera Lentinus Fr. and Panus Fr., with emphasis on the Lentinus tigrinus, L. crinitus and Panus lecomtei complexes

Edward A. Grand
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Edward A. Grand entitled "Systematics and species concepts in the genera Lentinus Fr. and Panus Fr., with emphasis on the Lentinus tigrinus, L. crinitus and Panus lecomtei complexes." 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.

Ronald H. Petersen, Major Professor

We have read this dissertation and recommend its acceptance:

Karen W. Hughes, Randall L. Small, Robert N. Trigiano

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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A Dissertation
Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Edward A. Grand
August 2004

Acknowledgements

I would like to acknowledge my advisor Ron Petersen and committee for guidance during the preparation of this work. Funding was provided by NSF-PEET Grants (DEB 9521526 and DEB 9978011) and the University of Tennessee Botany Department. Collection and observation of fresh material would not have been possible without this funding. Collaborators (Dirk Krüger, Tage Rolen) and travel partners (Tim Baroni, Joaquin Cifuentes, Dennis Desjardin, Jean Lodge and many others) were also essential for this study. Several culture collections graciously provided material [mainly Forest Products Laboratory, Madison, WI and Leningrad culture collection (Nadia Psurtseva, St. Petersburg, Russia)]. I would also like to thank David N. Pegler for morphological identifications and Tage Rolen (2001) for providing access to his thesis information and the sequence alignments used in it. Thank you to the many students from the University of Tennessee Botany Department that provided moral support and friendly advice.

Abstract

The monographic work of Pegler (1983) on *Lentinus* has established the taxonomic guidelines for most recent studies involving members of the genus (Moncalvo *et al.* 2002, Rolen 2001, Krüger 2002). Pegler's taxonomic hierarchy combined both *Lentinus* Fr. and *Panus* Fr. into one large genus, *Lentinus* Fr. The combination of these genera and its validity was one of the reasons for beginning this study. For generic level comparisons, ribosomal DNA sequence data can be helpful for determining relationships among taxa (Binder and Hibbett 2002, Hibbett and Vilgalys 1991, 1993, Hibbett and Donoghue 2001, Moncalvo *et al.* 2002, Thorn *et al.* 2000). In this study, ribosomal large subunit (LSU) sequences were used to determine if *Lentinus sensu* Pegler should contain both *Lentinus* Fr. and *Panus* Fr. LSU data were also used to explore the relationships of *Lentinus sensu* Pegler (1983) to genera affiliated with it in other works (Krüger 2002, Hibbett and Donoghue 2001, Moncalvo *et al.* 2002). I sought to assess various taxonomic schemes and the delineation of taxa using techniques such as morphology, sexual intercompatibility, and DNA sequence data. In order to determine if ITS data could be useful in elucidating biogeographical patterns, this study concentrated on three morphological species complexes: *Lentinus crinitus* (Linn.: Fr.) Fr., *L. tigrinus* (Bull.: Fr.) Fr., and *L. strigosus* (Schwein.) Fr. [*Panus lecomtei* (Fr.) Corner in this study].

The ribosomal ITS1 – 5.8S – ITS2 (ITS) region evolves faster and mutates more frequently than LSU (Hibbett 1992). ITS sequence data was used to study species circumscriptions and delineations among *Lentinus sensu* Pegler (1983) and its segregates. In some cases ITS sequence patterns can also be used to determine biogeographical patterns (Hughes *et al.* 1999, Petersen and Bermudes 1992, Petersen 1995a, 1995b).

Lentinus and *Panus* were found to be separable at the generic level based on LSU sequence data. Several morphological sections (Pegler 1983) of the genera were polyphyletic in maximum parsimony and neighbor-joining analyses (sects. *Rigidi*, *Velutini*, and *Panus*). Group *Polyporellus* (Nuñez and Ryvarden 1995) was closely related to *Lentinus tigrinus* and sect. *Tigrini* (Pegler 1983). Synonymization of *L. lindquistii* Lechner and Albertó (2000) and *L. glabratus* under *L. tigrinus* is suggested.

Data also suggests that *Panus fragilis* O. K. Miller (1965) should be synonymized under *P. lecomtei* Fr. *Lentinus suavissimus* Fr. is not part of either generic clade containing *Lentinus* or *Panus* spp. The transfer of *Lentinus suavissimus* Fr. to another genus is necessary.

A bioegeographical pattern observed in sect. *Tigrini* showed a correlation between geography and clades based on ITS data. Synonymization of *L. lindquistii* Lechner and Albertó and *L. glabratus* Mont. under *L. tigrinus* (Bull.: Fr.) Fr. is suggested based on sexual intercompatibility studies and molecular data. *Polyporus* group *Polyporellus sensu* Nuñez and Ryvarden appears to be a monophyletic group related to *Lentinus* sect. *Tigrini.*

This study concentrated on the circumglobal species complex *Panus lecomtei* Fr. to access biogeographical relationships in that group. Sexual intercompatibility studies indicated that seven collections of this complex formed a cohesive intersterility group. Ribosomal ITS sequence data for all collections of *P. lecomtei* Fr. sampled were nearly 100 % identical. Two collections of *Panus fragilis* O. K. Miller (1965) were also included and found to be conspecific with *P. lecomtei* Fr. based on ITS and LSU sequence data. Because of the macromorphological similarity of *Panus conchatus* and *P. lecomtei*, data from both species were collected. Eight collections of *P. conchatus* were shown to form an intersterility group. Other species of subg. *Panus sensu* Pegler (1983) were sequenced for ITS data, but not used for intercompatibility studies. These species include the following: *Panus ciliatus* Lév. (= *Lentinus ciliatus* Lév. *sensu* Pegler 1983), *Panus strigellus* Berk. (= *Lentinus strigellus* Berk. *sensu* Pegler 1983), *Panus fulvus* (Berk.) Pegler and Rayner (= *Lentinus velutinus sensu* Pegler 1983), and *Panus similis* Berk. and Br. (= *Lentinus similis sensu* Pegler 1983). *Lentinus suavissimus* Fr., group *Polyporellus* (Nuñez and Ryvarden 1995), *Ganoderma* and *Neolentinus* Redhead and Ginns (1985) were included to explore possible supra-generic relationships.

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Part 1 – Overview.

General introduction

The monographic work of Pegler (1983) on *Lentinus* has established the taxonomic guidelines for most recent studies involving members of the genus (Moncalvo *et al.* 2002, Rolen 2001, Krüger 2002). Pegler's taxonomic hierarchy combined both *Lentinus* Fr. and *Panus* Fr. into one large genus, *Lentinus* Fr. The combination of these genera and its validity was one of the reasons for beginning this study. Most authors have separated *Lentinus sensu* Pegler (1983) into at least two (*Lentinus* and *Panus*), and as many as five genera (*Lentinus*, *Panus*, *Neolentinus*, *Pleurotus*, and *Heliocybe*), in various ways (Corner 1981, Rune 1994, Redhead and Ginns 1985, Singer 1986). I sought to assess these various taxonomic schemes and the separation of taxa using techniques such as sexual intercompatibility studies and DNA sequence data.

For generic level comparisons, ribosomal DNA sequence data can be helpful for determining relationships among taxa (Binder and Hibbett 2002, Hibbett and Vilgalys 1991, 1993, Hibbett and Donoghue 2001, Moncalvo *et al.* 2002, Thorn *et al.* 2000). In this study, ribosomal large subunit (LSU) sequences were used to determine if *Lentinus sensu* Pegler should contain both *Lentinus* Fr. and *Panus* Fr. LSU data were also used to explore the relationships of *Lentinus sensu* Pegler (1983) to genera affiliated with it in other works (Krüger 2002, Hibbett and Donoghue 2001, Moncalvo *et al.* 2002). These included some *Polyporus s.l.* (Nuñez and Ryvarden 1995), group *Polyporellus* (Nuñez and Ryvarden 1995) and *Neolentinus* Redhead and Ginns (1985). LSU data was also used to determine the monophyly of Pegler's (1983) sections and subgenera.

The ribosomal ITS1 – 5.8S – ITS2 (ITS) region evolves faster and mutates more frequently than LSU (Hibbett 1992). ITS sequence data was used to study species circumscriptions and delineations among *Lentinus sensu* Pegler (1983) and its segregates. In some cases ITS sequence patterns can also be used to determine biogeographical patterns (Hughes *et al.* 1999, Petersen and Bermudes 1992, Petersen 1995a, 1995b). In order to determine if ITS data could be useful in elucidating biogeographical patterns, this study concentrated on three morphological species complexes: *Lentinus crinitus* (Linn.: Fr.) Fr., *L. tigrinus* (Bull.: Fr.) Fr., and *L. strigosus* (Schwein.) Fr. [*Panus lecomtei* (Fr.) Corner in this study].

Synopsis of dissertation parts

Phylogenetic reconstruction of selected *Lentinus*, *Panus* and *Polyporus* taxa based on nrLSU sequences. (Part 2)

Ribosomal large subunit (LSU) data was used to infer relationships among the aphyllophoralean genera *Lentinus* Fr., *Panus* Fr. and some members of *Polyporus* Adans.: Fr.. Correlation of morphological, biological and phylogenetic species concepts was used to investigate the sections and species of the genera. *Polyporus* and related taxa [*Neolentinus* Redhead and Ginns (1985) and group *Polyporellus* Nuñez and Ryvarden (1995)] were included in the analysis. *Lentinus* and *Panus* were found to be separable at the generic level based on LSU sequence data. Several morphological sections (Pegler 1983) of the genera were polyphyletic in maximum parsimony and neighbor-joining analyses (sects. *Rigidi*, *Velutini*, and *Panus*). Group *Polyporellus* (Nuñez and Ryvarden 1995) was closely related to *Lentinus tigrinus* and sect. *Tigrini* (Pegler 1983). Synonymization of *L. lindquistii* Lechner and Albertó (2000) and *L. glabratus* under *L. tigrinus* is suggested. Data also suggests that *Panus fragilis* O. K. Miller (1965) should be synonymized under *P. lecomtei* Fr. *Lentinus suavissimus* Fr. is not part of either generic clade containing *Lentinus* or *Panus* spp. The transfer of *Lentinus suavissimus* Fr. to another genus is necessary.

Biogeography and species concepts in the genus *Lentinus*, with emphasis on sects. *Lentinus* and *Tigrini*. (Part 3)

Two sections of the genus *Lentinus* subg. *Lentinus sensu* Pegler were examined in detail for this paper. Sects. *Lentinus* and *Tigrini* were evaluated using ribosomal ITS sequence data, sexual intercompatibility studies and morphological data. Both sections were monophyletic with respect to the taxa sampled. A bioegeographical pattern was not observed in sect. *Lentinus*, but sect. *Tigrini* members showed a correlation between geography and clades based on ITS data. Synonymization of *L. lindquistii* Lechner and Albertó and *L. glabratus* Mont. under *L. tigrinus* (Bull.: Fr.) Fr. is suggested based on

sexual intercompatibility studies and molecular data. Several members of sects. *Rigidi* and *Lentodiellum sensu* Pegler were also sequenced for the ITS analysis. No intercompatibility data was collected for these sections. Group *Polyporellus sensu* Nuñez and Ryvarden was included to explore possible supra-generic relationships. *Polyporus* group *Polyporellus* appears to be a monophyletic group related to *Lentinus* sect. *Tigrini*. Related genera and the other sections within *Lentinus sensu* Pegler were analyzed and discussed.

Biogeography and species concepts in the genus *Panus* Fr., with emphasis on *Panus lecomtei* and *Panus conchatus*. (Part 4)

Members of *Lentinus* subg. *Panus sensu* Pegler (1983) were analyzed in this study. Taxa from two sections were included (sects. *Panus* and *Velutini*). This study concentrated on the circumglobal species complex *Panus lecomtei* Fr. to access biogeographical relationships in that group. Sexual intercompatibility studies indicated that seven collections of this complex formed a cohesive intersterility group. Ribosomal ITS sequence data for all collections of *P. lecomtei* Fr. sampled were nearly 100 % identical. Two collections of *Panus fragilis* O. K. Miller (1965) were also included and found to be conspecific with *P. lecomtei* Fr. based on ITS and LSU sequence data. Because of the macromorphological similarity of *Panus conchatus* and *P. lecomtei*, data from both species were collected. Eight collections of *P. conchatus* were shown to form an intersterility group. Other species of subg. *Panus sensu* Pegler (1983) were sequenced for ITS data, but not used for intercompatibility studies. These species include the following: *Panus ciliatus* Lév. (= *Lentinus ciliatus* Lév. *sensu* Pegler 1983), *Panus strigellus* Berk. (= *Lentinus strigellus* Berk. *sensu* Pegler 1983), *Panus fulvus* (Berk.) Pegler and Rayner (= *Lentinus velutinus sensu* Pegler 1983), and *Panus similis* Berk. and Br. (= *Lentinus similis sensu* Pegler 1983). *Lentinus suavissimus* Fr., group *Polyporellus* (Nuñez and Ryvarden 1995), *Ganoderma* and *Neolentinus* Redhead and Ginns (1985) were included to explore possible supra-generic relationships.

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Part 2 – Phylogenetic reconstruction of selected *Lentinus*, *Panus* and *Polyporus* taxa based on nrLSU sequences.

Abstract

Ribosomal large subunit (LSU) data was used to infer relationships among the aphyllophoralean genera *Lentinus* Fr., *Panus* Fr. and some members of *Polyporus* Adans.: Fr.. Correlation of morphological, biological and phylogenetic species concepts was used to investigate the sections and species of the genera. *Polyporus* and related taxa [*Neolentinus* Redhead and Ginns (1985) and group *Polyporellus* Nuñez and Ryvarden (1995)] were included in the analysis. *Lentinus* and *Panus* were found to be separable at the generic level based on LSU sequence data. Several morphological sections (Pegler 1983) of the genera were polyphyletic in maximum parsimony and neighbor-joining analyses (sects. *Rigidi*, *Velutini*, and *Panus*). Group *Polyporellus* (Nuñez and Ryvarden 1995) was found to be closely related to *Lentinus tigrinus* and sect. *Tigrini* (Pegler 1983). Synonymization of *L. lindquistii* Lechner and Albertó (2000) and *L. glabratus* under *L. tigrinus* is suggested. Data also suggests that *Panus fragilis* O. K. Miller (1965) should be synonymized under *P. lecomtei* Fr. *Lentinus suavissimus* Fr. is not part of either generic clade containing *Lentinus* or *Panus* spp. The transfer of *Lentinus suavissimus* Fr. to another genus will be necessary.

Introduction

Taxa of *Lentinus sensu* Pegler (1983) have been treated in as many as five genera by recent authors (Corner 1981, Singer 1986, Redhead and Ginns 1985, Rune 1994). These segregations have been based mainly on morphological characters such as hyphal construction of the basidiome, gill ontogeny, rot-type and presence of sterile elements in the hymenium (Corner 1981, Hibbett *et al.* 1993, Pegler 1983, Redhead and Ginns 1985, Singer 1986). The genus and its segregates have usually been placed in the family Agaricaceae because of their lamellate hymenophores (Donk 1962).

It has previously been suggested that taxa included in *Lentinus* Fr. and *Panus* Fr. actually belonged in the Aphyllophorales based on dimitic hyphal construction (Kühner 1980, Moser 1978, Pegler 1983, Singer 1986), a common characteristic of poroid, resupinate, clavarioid and hydnoid fungi (Donk 1960). The sporocarp is composed of

thin-walled generative hyphae along with a second hyphal type, either thick-walled skeletal or skeleto-ligative hyphae (Corner 1981, Pegler 1983). The dimitic hyphal construction of many *Lentinus sensu* Pegler species is shared by members of *Polyporus sensu lato* and its allies (Corner 1953). In contrast, basidiomata of members of the Agaricales are typically monomitic, composed of only generative hyphae (Pegler 1983).

Some species of *Lentinus sensu stricto* produce hymenial fascicles of sterile hyphae (termed hyphal pegs), which may indicate a common ancester of polyporoid origin (Hibbett and Vilgalys 1993). Ontogenetic studies have also shown early poroid hymenophoral stages in young basidiomata that develop into mature lamellae (Hibbett *et al.* 1993, pers. obs.). This early hymenial state is very similar to that in the stipitate polypores.

Circumscriptions of *Lentinus* Fr. and *Panus* Fr. have been problematic since their proposals (Fries 1825, 1838). Fries (1825) initially circumscribed the genus *Lentinus* based on the tough, coriaceous basidiome consistency. He included 20 species in 1825, but later expanded it to include 43 (Fries 1836) and then 50 (Fries 1838). *Panus* was proposed by Fries in 1838 to accommodate 16 species with coriaceous basidiomata and radiately constructed lamellae. This brought the total number of *Lentinus* and *Panus* to 66 (Fries 1838). Disagreement about generic types, nomenclature, morphological plasticity, species concepts, and geographical morphotypes has continued to cause turmoil (Corner 1981, Kühner 1980, Pegler 1983, Singer 1975, 1986).

Although many members of *Pleurotus* (Fr.) Kummer have been placed in *Lentinus* and *Panus*, it is now clear that the monophyletic family Pleurotaceae, containing *Pleurotus* and *Hohenbuehelia*, belongs with the Tricholomataceae, among the Agaricales (Moncalvo *et al.* 2002, Thorn *et. al* 2000). *Pleurotus* is distinguished by the production of nematotoxic droplets (Hibbett and Thorn 1994, Hilber 1997, Petersen and Greilhuber 1996, Thorn and Barron 1984, Thorn *et al.* 2000, Zervakis and Balis 1996). Because of this, *Pleurotus* species have not been not included in this analysis.

Several monographic works form the basis for what are generally accepted as members of *Lentinus*, *Panus*, and *Pleurotus* (Corner 1981, Hilber 1982, Pegler 1983, Pilát 1946). These works have since been supplemented by many authors (Binder and

Hibbett 2002, Hibbett and Vilgalys 1993, Hibbett and Donoghue 2001, Hibbett *et al.* 1994, Hilber 1997, Moncalvo *et al.* 2002, Redhead and Ginns 1985, Rune 1994, Thorn *et al.* 2000). Recent large phylogenies based on rDNA sequences have shown members of *Lentinus*, *Panus*, *Neolentinus* and *Polyporus* to be related (Binder and Hibbett 2002, Krüger 2002, Moncalvo *et al.* 2002), but separable into clades which correspond to morphological genera. Most recent studies rely heavily on DNA sequence data, as does this paper.

In a study including five species of *Lentinus sensu* Pegler, three species in the Polyporaceae, and two in the Tricholomataceae, Hibbett and Vilgalys (1991) found support for the segregate genera *Neolentinus* Redhead and Ginns, *Panus* Fr., and *Lentinus* Fr. using physiological, morphological and nucleic acid data (PCR-RFLPs of rDNA). This provided support for the earlier segregation of *Neolentinus* Redhead and Ginns, based on binucleate spores, a bipolar mating system and the ability to cause brown rots (Redhead and Ginns 1985). Several *Neolentinus* species were included in this analysis to explore possible relationships to *Lentinus* and *Panus*.

Ribosomal DNA sequences [large subunit (LSU), small subunit (SSU), internally transcribed spacer (ITS)] have been used to elucidate relationships at specific and generic ranks in many groups of fungi [Binder and Hibbett 2002, Hibbett and Vilgalys 1991 (PCR-RFLP), Hibbett and Donoghue 2001, Hibbett and Thorn 2001, Moncalvo *et al.* 2002, Thorn *et al.* 2000]. Separation of species based on sequence divergence is an integral part of many recent taxonomic schemes (Binder and Hibbett 2002, Moncalvo *et al.* 2002). Primers for other nuclear and mitochondrial DNA regions are currently being developed to provide greater support and resolution for current systematic studies [Kretzer and Bruns 1999 (mt *atp*6 and mt *cox*3), Matheny *et al.* 2002 (RPB1), O'Donnell *et al.* 2001 (EF1-α)].

Morphological species concept

The first step in these analyses was the assignment of the available collections to morphological species. Many taxa of *Lentinus sensu* Pegler fall into "species complexes" (Pegler 1983). Traditionally, these complexes have been separated based on micro- and

macromorphological phenotypes. The morphological species is based on the following concept – "characters (phenotypes) of individual organisms are compared, and similar individuals are designated as a species." (Petersen and Hughes 1999). This strictly morphological approach may be inadequate for delimiting species when it is difficult to delineate character states (Anderson 1986, Anderson and Ullrich 1979, Clémençon 1977, Petersen and Hughes 1999, Smith 1968, Ullrich and Anderson 1978, Vilgalys 1991, Vilgalys *et al.* 1993).

Three of the species complexes in this study, *L. crinitus* (Linn.: Fr.) Fr., *L. tigrinus* (Bull.: Fr.) Fr., and *L. strigosus* (Schwein.) Fr. [*Panus lecomtei* (Fr.) Corner in this study], could include taxa that have been separated based strictly on morphological characters (Pegler 1983, Corner 1981). The convergence of characters in these three complexes makes it difficult to separate species based on morphology alone. A goal of this study was to determine if a strictly morphological approach could be used to separate species. The correlation of morphology with biological and phylogenetic data was also investigated.

Biological species concept and intercollection pairings

The biological species concept is based on the idea that genetic isolation leads to speciation (Mayr 1942, Dobzhansky 1951, Petersen and Hughes 1999). If two populations have the ability to interbreed successfully, they belong to the same gene pool, and therefore are considered the same biological species.

Although the techniques are relatively straightforward, the conclusions drawn from mating studies have been questioned (Boidin 1986, Mishler and Donoghue 1982, Mishler and Brandon 1987, Vilgalys 1991, Worrall 1997). Successful intercollection pairings are usually not tested for the production of fertile basidiomata (Gordon and Petersen 1991, 1992, Hallenberg 1983, Johnson and Methven 1994, Petersen 1995a). In most organisms the production of fertile offspring is necessary for two taxa to be considered biologically conspecific (Mayr 1942). In this study, clamp connection formation after contact between two monokaryotic mycelia indicated biological

conspecificity. If clamp formation was not observed, some barrier to gene flow was assumed to exist (*i.e.* speciation, see Brasier 1987).

Pairings among monokaryotic mycelia have been used for some years to determine relationships among closely related taxa (Gordon and Petersen 1991, 1992, 1998 (*Marasmius*), Hallenberg 1983 (*Hericium*), Johnson and Methven 1994 (*Panus*), McCleneghan 1996 (*Pholiota*), Petersen and Bermudes 1992 (*Panellus*), Petersen 1995a, 1995b (*Flammulina*, *Omphalotus*). This study attempts to use biological compatibility in concert with morphological and phylogenetic data, in order to determine any correlations.

Phylogenetic species concept

A phylogenetic species is composed of a group of organisms (Hennig 1966) that contains all descendents of an evolutionary lineage (Petersen and Hughes 1999). Taxa that do not share synapomorphic (= derived) character states are inferred to be reproductively isolated and genetically diverged (Davis 1996), *i.e.* they are not monophyletic. In a monophyletic group, some of the characters states must be present in all taxa of that group. The commonality of these character states among the taxa indicates relatedness. States can be based on many things, such as morphology, DNA sequences and isozyme patterns, as long as that character can be delineated into states. Polyphyletic groups do not share an immediate common ancestor and therefore are not considered a species. This study used LSU rDNA data to infer phylogenetic relationships among taxa.

In order to understand relationships among these genera and sections, this study utilized greater sampling in the genus *Lentinus s.s.* (this paper, Grand 2004: pt. 3) and *Panus s.s.* (this paper, Grand 2004: pt. 4) than previously published papers. Using additional sequences acquired from Krüger's (2002) dissertation and GenBank, robust phylogenetic reconstructions were created.

Nomenclature

In an effort to establish stability at the generic rank, *Panus* Fr. has been conserved with the type species *Panus conchatus* (Bull.: Fr.) Fr. (International Code of Botanical Nomenclature 2000).

Pegler (1983) considered the lectotype species of *Lentinus* subg. *Lentinus* to be *L. crinitus* (Linn.:Fr.) Fr. Donk (1962) also supported *L. crinitus* as the type, but others, including Clements and Shear (1931), have considered it to be *L. tigrinus* Fr. Singer (1975) believed the type to be *L. lepideus* Fr., but found little support for this idea. Corner (1981) discussed the advantages of using *L. crinitus* as the type of the *Lentinus*, but then stated two possibilities for the type in his circumscription of the genus (*L. crinitus* or *L. tigrinus*). A type has not been officially designated for *Lentinus* in the latest edition of the International Code of Botanical Nomenclature (2000) or in recent literature.

Redhead and Ginns (1985) discussed the classification systems proposed by several different authors and the validity of them. The earliest lectotypification for *Lentinus* was by Clements and Shear (1931). According to Art. 9.17 of the International Code of Botanical Nomenclature (St. Louis Code 2000), the first lectotypification of the genus is the one that will be followed. In this study, *L. tigrinus* is considered the type for *Lentinus*, following the discussion of Redhead and Ginns (1985).

Mating systems

Mating systems have been emphasized as a key character when considering the taxonomy of lentinoid species (Johnson and Methven 1994, Petersen *et al.* 1997, Hibbett and Donoghue 2001), as well as other groups of fungi (Petersen 1995a, 1995b).

Petersen *et al.* (1997) reported *L. crinitus* (Linn.: Fr.) Fr., *L. bertieri* (Fr.) Fr., *Panus lecomtei* (as *L. strigosus*), *L. cf. puntaticeps* Berk. & Br. (actually *L. copulatus*), *L. strigellus* Berk. and *L. suavissimus* Fr. as having tetrapolar mating systems. *L. torulosus* (Pers.: Fr.) Lloyd [= *Panus conchatus* (Bull: Fr.) Fr.] has been reported as tetrapolar (Johnson and Methven 1994). Petersen *et al.* (1997) also reported two stipitate polypores, *Polyporus ciliatus* Fr. and *P. varius* Fr., as exhibiting tetrapolar mating

systems. Shared tetrapolarity of polyporoid and lentinoid species would be expected if these groups were closely related.

This study used LSU sequence data to explore the validity of subg. *Lentinus* and *Panus sensu* Pegler (1983) and the sections contained within them. Several morphospecies included in these analyses where investigated using various techniques, including intercompatibility studies, to ascertain their rank of species. Supra-generic relationships to *Neolentinus* and some polyporoid groups were also studied.

Materials and methods

Abbreviations and acronyms

Collection data for the specimens and cultures used in this study are shown in Tab. 1. FB indicates the Tennessee Field Book number and accession in the Tennessee culture collection (CULTENN). TENN indicates the dried voucher specimen's location in the University of Tennessee fungal herbarium. If no TENN number is present, only a culture and the received identification were available. FPLM indicates that the culture was obtained from the Forest Products Laboratory culture collection in Madison, Wisconsin.

Morphology and microscopy

Macro- and micromorphological observations and putative species determinations followed those of Pegler (1983) and Corner (1981). More current literature was used to supplement and amend taxonomic conclusions (Redhead and Ginns 1985, Lechner and Albertó 2000, 2002, Rune 1994). Dried collections were hand-sectioned with a double-edged razor blade, rehydrated in 3 % KOH, and stained with various stains (i.e. phloxine, Congo red) for micromorphological examination using bright field and phase contrast optics.

Tab. 1 - Fungal specimens and cultures examined for LSU phylogeny.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
---	Datronia mollis	AF261541	---	Datronia mollis	NA
---	Ganoderma applanatum	AJ406526	---	Ganoderma applanatum	NA
---	Ganoderma australe	X78780	---	Ganoderma australe	NA
---	Ganoderma lucidum	X78776	---	Ganoderma lucidum	
---	Ganoderma tsugae	X78778	---	Ganoderma tsugae	NA
---	Gloeophyllum abietinum	AJ583431	---	Gloeophyllum abietinum	NA
---	Gloeophyllum sepiarium	AF393059	---	Gloeophyllum sepiarium	NA
---	Gloeophyllum trabeum	AF139948	---	Gloeophyllum trabeum	NA
---	Heliocybe sulcatus	AF518619	---	Heliocybe sulcatus	subg. *Panus* sect. *pulverulenti*
FB11754 (DEH2430)	Lentinus bertieri	(this study)	USA, Hawaii	Lentinus bertieri	subg. *Lentinus* sect. *lentinus*
FB11756 (DEH2432)	Lentinus bertieri	(this study)	USA, Hawaii	Lentinus bertieri	subg. *Lentinus* sect. *lentinus*
FB11708 (TENN59773)	Lentinus bertieri	(this study)	Dominican Republic	Lentinus bertieri	subg. *Lentinus* sect. *lentinus*
FB11118 (TENN59659)	Lentinus crinitus	(this study)	USA, Florida	Lentinus crinitus	subg. *Lentinus* sect. *lentinus*
FB10688 (TENN58775)	Lentinus crinitus	(this study)	USA, Texas	Lentinus sp.	subg. *Lentinus* sect. *rigidi*
FB11196 (TENN59732)	Lentinus crinitus	(this study)	Dominican Republic	Lentinus crinitus	subg. *Lentinus* sect. *lentinus*
FB9145 (TENN54876)	Lentinus crinitus	(this study)	USA, Florida	Lentinus crinitus[1]	subg. *Lentinus* sect. *lentinus*
FB10235 (TENN51836)	Lentinus polychrous	(this study)	Thailand	Lentinus polychrous[1]	subg. *Lentinus* sect. *rigidi*
FB11731 (TENN59788)	Lentinus sajor-caju	(this study)	Thailand, Chiang Mai Province	Lentinus sajor-caju	subg. *Lentinus* sect. *rigidi*
FB11736 (TENN59793)	Lentinus sajor-caju	(this study)	Thailand, Chiang Mai Province	Lentinus sajor-caju	subg. *Lentinus* sect. *rigidi*
FB11739 (TENN59796)	Lentinus sajor-caju	(this study)	Thailand, Chiang Mai Province	Lentinus sajor-caju	subg. *Lentinus* sect. *rigidi*
FB11164 (TENN59704)	Lentinus scleropus	(this study)	Mexico	Panus hirtus	subg. *Lentinus* sect. *lentodiellum*
---	Lentinus squarrosulus	AF261563[4]	---	Lentinus squarrosulus	subg. *Lentinus* sect. *rigidi*
FB11130 (TENN59671)	Lentinus suavissimus	(this study)	France	Lentinus suavissimus	subg. *Lentinus* sect. *pleuroti*

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB11096 (TENN59836)	Lentinus suavissimus	(this study)	USA, Tennessee	Lentinus suavissimus	subg. *Lentinus* sect. *pleuroti*
FB11656 (TENN59822)	Lentinus suavissimus	(this study)	Russia	Lentinus suavissimus	subg. *Lentinus* sect. *pleuroti*
FB10259 (TENN57582)	Lentinus swartzii	(this study)	Costa Rica	Lentinus swartzii	subg. *Lentinus* sect. *lentinus*
FB5206 (TENN51531)	Lentinus swartzii[1]		Costa Rica	Lentinus bertieri	subg. *Lentinus* sect. *lentinus*
FB5206 (TENN51531)	Lentinus swartzii	(this study)	Costa Rica	Lentinus swartzii[1]	subg. *Lentinus* sect. *lentinus*
FB10672 (TENN59833)	Lentinus tigrinus	(this study)	Austria	Lentinus tigrinus	subg. *Lentinus* sect. *tigrini*
FB10236 (Type Culture)	Lentinus tigrinus	BAFC 2117, 2132, 2266, 2274	Argentina	Lentinus lindquistii (Type cultures)	subg. *Lentinus* sect. *tigrini*
FB11746 (IRAN279C Culture)	Lentinus tigrinus	(this study)	Iran	Lentinus tigrinus	subg. *Lentinus* sect. *tigrini*
FB9093 (TENN54918)	Lentinus tigrinus	(this study)	USA, Louisiana	Lentinus tigrinus[1]	subg. *Lentinus* sect. *tigrini*
FB6954 (TENN56194)	Lentinus tigrinus	(this study)	USA, North Carolina	Lentinus glabratus[1]	subg. *Lentinus* sect. *tigrini*
FB11101 (TENN59644)	Lentinus tigrinus	AJ487929[3]	USA, Louisiana	Lentinus tigrinus	subg. *Lentinus* sect. *tigrini*
---	Lentinus tigrinus	AF135173[4]		Lentinus tigrinus	subg. *Lentinus* sect. *tigrini*
FB11279 (TENN59088)	Mycobonia flava	AJ487933[3]	Argentina	Mycobonia flava	NA
FB11104 (TENN59647)	Neolentinus adhaerens	this study	USA, Tennessee	Neolentinus adhaerens	subg. *Panus* sect. *pulverulenti*
---	Neolentinus dactyloides	AF135174	---	Neolentinus dactyloides	subg. *Panus* sect. *squamosi*
FB10293 (TENN59824)	Neolentinus schaefferi	(this study)	Austria	Lentinus cyathiformis	subg. *Panus* sect. *squamosi*
FB11728 (TENN59785)	Panus ciliatus	(this study)	Thailand	Panus ciliatus	subg. *Panus* sect. *velutini*
FB11729 (TENN59786)	Panus ciliatus	(this study)	Thailand	Panus ciliatus	subg. *Panus* sect. *velutini*
FB11737 (TENN59808)	Panus ciliatus	(this study)	Thailand	Panus ciliatus	subg. *Panus* sect. *velutini*
FB11755 (DEH2430A)	Panus ciliatus	(this study)	Hawaii	Panus ciliatus	subg. *Panus* sect. *velutini*
FB4314 (TENN50394)	Panus conchatus	(this study)	Switzerland	Panus conchatus	subg. *Panus* sect. *panus*
FB6254 (TENN52912)	Panus conchatus	(this study)	Mexico	Lentinus strigellis[1]	subg. *Panus* sect. *panus*

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
LCF573[2] (Culture)	Panus fulvus	(this study)	Argentina	Panus fulvus	subg. *Panus* sect. *velutini*
FB10689 (TENN58776)	Panus fulvus	(this study)	USA, Texas	Panus fulvus	subg. *Panus* sect. *velutini*
HHB6616[2] (Culture)	Panus lecomtei	(this study)	USA, Florida	Panus fragilis	subg. *Panus* sect. *panus*
PR1116[2] (Culture)	Panus lecomtei	(this study)	USA, Puerto Rico	Panus rudis	subg. *Panus* sect. *panus*
FB11125 (TENN59666)	Panus lecomtei	(this study)	USA, Florida	Panus rudis	subg. *Panus* sect. *panus*
FB5525 (TENN51805)	Panus lecomtei	(this study)	USA, North Carolina	Lentinus strigosus[1]	subg. *Panus* sect. *panus*
---	Panus lecomtei	AF287878	---	Panus lecomtei	subg. *Panus* sect. *panus*
FB10747 (TENN58955)	Panus similis	(this study)	Argentina	Panus similis	subg. *Panus* sect. *velutini*
FB11302 (TENN59008)	Panus similis	(this study)	Argentina	Panus similis	subg. *Panus* sect. *velutini*
FB9854 (TENN59829)	Panus similis	(this study)	Argentina	Panus similis	subg. *Panus* sect. *velutini*
---	Panus sp.	AF261564[4]	---	Panus sp.	---
---	Panus sp.	AF261565[4]	---	Panus sp.	---
FB9215 (TENN55993)	Panus strigellis	(this study)	USA, Louisiana	Lentinus strigellis[1]	subg. *Panus* sect. *panus*
FB9114 (TENN56192)	Panus strigellis	(this study)	USA, Louisiana	Lentinus strigellis[1]	subg. *Panus* sect. *panus*
DSH90.36	Polyporus alveolaris	AJ487937[3]	---	Polyporus alveolaris	NA
FB10299 (TENN58370)	Polyporus arcularius	AJ487938[3]	Austria	Polyporus arcularius	NA
FB5085 (TENN59136)	Polyporus badius	AJ487941[3]	USA	Polyporus badius	NA
FB10908 (TENN58391)	Polyporus brumalis	AJ487942[3]	Germany	Polyporus brumalis	NA
FB10167 (TENN57698)	Polyporus ciliatus	AJ487943[3]	Denmark	Polyporus ciliatus	NA
FB11254 (TENN58943)	Polyporus grammocephalus	AJ487946[3]	Paraguay	Polyporus grammocephalus	NA
FB10921 (TENN58404)	Polyporus guianensis	AJ487948[3]	Venezuela	Polyporus guianensis	NA
FB10489 (TENN58597)	Polyporus leprieurii	AJ487949[3]	Costa Rica	Polyporus leprieurii	NA
---	Polyporus melanopus	AF261545	---	Polyporus melanopus	NA
FB11465 (TENN59326)	Polyporus melanopus	AJ487951[3]	Austria	Polyporus melanopus	NA

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB10298 (Thorn567 Culture)	Polyporus pseudobetulinus	AJ487954[3]	Finland	Polyporus pseudobetulinus	NA
---	Polyporus squamosus	AF135181	---	Polyporus squamosus	NA
FB10831 (TENN58380)	Polyporus squamosus	AJ488106[3]	USA	Polyporus squamosus	NA
---	Polyporus tuberaster	AF261544	---	Polyporus tuberaster	NA
FB10197 (TENN57727)	Polyporus tuberaster	AJ488116[3]	Germany	Polyporus tuberaster	NA
FB9579 (TENN56491)	Polyporus tricholoma	AJ488115[3]	Puerto Rico	Polyporus tricholoma	NA
FB10962 (TENN58587)	Polyporus varius	AJ488121[3]	USA	Polyporus varius	NA
---	Polyporus varius	AF261540	---	Polyporus varius	NA
FB11219 (TENN58908)	Polyporus virgatus	AJ488122[3]	Argentina	Polyporus virgatus	NA
FB8744 (TENN55173)	Pseudofavolus cucullatus	AJ488125[3]	Mexico	Pseudofavolus cucullatus	NA
---	Pseudotomentella ochracea	AF092847[4]	---	Pseudotomentella ochracea	NA

[1]Annotated by D. N. Pegler

[2]Culture obtained from Forest Products Laboratory, Madison

[3]Sequence obtained from Krüger (2002)

[4]Sequence used in Moncalvo *et al.* (2002)

Field collection and specimen processing

Field collection and processing followed standard procedure as outlined by Largent *et al.* (1977), Largent (1986), and Largent and Baroni (1988). Field notes were recorded in numbered, bound fieldbooks. Putative species identification, date, location (including latitude and longitude), collector, and other pertinent and transient information such as color, taste, and smell were recorded. Photographs were taken using Kodachrome ASA 200 slide film. Collections were then exposed to low heat until thoroughly dry. Dried collections were accessioned into the TENNFU (TENN) database at The University of Tennessee as indicated in Tab. 1. Field book numbers (FB) in Tab. 1 indicates that cultures were established and preserved in The University of Tennessee culture collection (CULTENN). If only a culture was available (*i.e.* no basidiomata), this is indicated by the absence of a TENN number in Tab. 1. The origins of these cultures are listed as footnotes in Tab. 1.

Culture techniques

Single-basidiospore isolates (SBIs) were obtained following the method of Gordon and Petersen (1991). Another method utilized for obtaining SBIs was to collect a spore print on autoclaved aluminum foil, after which the spores were diluted in distilled water and spread on 1.5 % malt extract agar plates to germinate (Petersen and Greilhuber 1996). After germination, single germinating spores were harvested using a sharpened dental pick and placed on individual agar plates.

In vitro fruiting of dikaryon cultures

In order to obtain monokaryotic SBIs for intercompatibility studies and DNA extractions, collections not available either through CULTENN or collaborators (*i.e.* from spore prints) were fruited *in vitro*. In cases where only a dikaryon culture was available, cultures were fruited on a mixture of sawdust (95 % by weight) and bran (5% by weight) (Stamets and Chilton 1983, Stamets 1993). After confirmation of a clamped dikaryotic culture, the isolate was grown on sterilized rye grain. 100 grams of dry rye grain was

mixed with 100 ml of water, then autoclaved for 1 hour at 15 psi. The cooled rye grain was then inoculated with several small chunks (*ca.* 0.5 cm^2) of agar and allowed to colonize for several weeks. Fully colonized rye grains were broken into individual grains by manual shacking. The individual grains were then used to inoculate a polyethylene autoclavable bag (Unicorn Bag Company) containing water saturated, sterilized *Liriodendron* sawdust. The colonized block was then subjected to fruiting conditions (~95 % humidity, ambient light) for several weeks, after which mature, sporulating basidiomata formed. Spore prints were obtained from *in vitro* fruited basidiomata and handled in the same way as those from naturally grown basidiomata.

Molecular techniques

Molecular techniques and data analysis followed those described by Hughes *et al.* (2001). Monokaryon cultures were chosen for DNA extraction after verification that the cultures were clampless. Monokaryon isolates were used to help prevent sequencing problems due to heterozygosity for insertions or deletions (indels) that are sometimes observed when using dikaryons. To obtain fungal tissue suitable for DNA extraction, a *ca.* 0.5 cm^2 piece of colonized agar was placed in a jar containing PD broth (24 g/L Difco Potato Dextrose Broth) and allowed to grow at room temperature for several weeks. When the culture reached a diameter of *ca.* 3-4 centimeters, DNA was extracted from the culture using the procedure of Cifuentes *et al.* (2003). When cultures were not available, dried herbarium material was extracted in a similar fashion after a small piece of dried tissue (*ca.* 0.5 cm^2) was ground with the aid of sterile grinding sand.

Large subunit (25S) ribosomal DNA was amplified using primers LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) and ITS3 (White *et al.* 1990). Generally, 1µl of DNA extract was used for amplification. When standard amplification procedure failed, RedMix Plus mixture (Gene Choice, PGC) was usually successful using the same primers and amplification protocol. The amplification protocol was: 3 mins at 94 °C, 1 cycle; 0:30 min at 94 °C, 1:00 min at 50 °C 1:30 min at 72 °C, 35 cycles; 3:00 mins at 72 °C, 1 cycle; hold at 4 °C. Five µl of the PCR product was then examined by gel electrophoresis (in a 1.5 % TBE agarose gel) to confirm amplification.

Primers and unincorporated nucleotides were removed from the PCR product by digestion with ExoSAP-IT (Amersham Biosciences) following manufacturer's directions. Sequencing was performed using ABI's Big Dye Terminator Cycle Sequencing Kit Version 3.1. Primers were LR7, ITS3 or LR5. The sequencing protocol was: 0:30 min at 96 °C, 0:15 min at 50 °C, 4 min at 60 °C, 25 cycles; hold at 4 °C. Depending on the quality of the sequence, both ITS 3 and either LR5 or LR7 primers were used to form an overlapping contig sequence. LR5, a primer internal to the LR7/ITS3 was occasionally necessary to selectively amplify the proper PCR product when multiple bands appeared on the agarose gel. The sequencing reaction was cleaned with a Sephadex G-50 column to remove dyes, dried in a spinvac, and sequenced using an automated ABI 3100 DNA sequence (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc.).

DNA extraction and amplification of representative collections from all available sections of *Lentinus*, *Panus*, *Neolentinus* and *Polyporus* was attempted, but not always with success.

Choosing an outgroup

A preliminary tree was constructed using sequences retrieved from BLAST (Altschul *et al.* 1997) searches of published Genbank accessions and data from this study. *Pseudotomentella ochracea* was chosen as the outgroup for the initial analysis involving the LSU data set. The choice of outgroup was based on recent phylogenetic trees produced by Moncalvo *et al.* (2002), Binder and Hibbett (2002), and Hibbett and Donoghue (2001). These trees incorporated LSU data to some degree, but surveyed a greater breadth of taxa than this study. All members of interest in this study were contained in the /polyporaceae and /corticioid clades of Moncalvo *et al.* (2002). The next closely related clade on the Moncalvo *et al.* (2002) phylogeny was the Thelephoroid clade, for which *Pseudotomentella ochracea* is the only representative.

The inclusion of *Polyporus* taxa was based on work by Hibbett and Donoghue (2001), Krüger (2002), and Moncalvo *et al.* (2002). In an analysis using SSU sequence data (mt-rDNA and nu-rDNA), Hibbett and Donoghue (2001) found that *L. tigrinus, P. rudis (= P. lecomtei* here), and several polypores fell into one monophyletic polyporoid

clade. This grouping was also hypothesized by Pegler (1983) and Corner (1981) while discussing the generic circumscriptions of these taxa. Hibbett and Donoghue's (2001) polyporoid clade also included members not traditionally thought to be closely related to *Lentinus s.s.*, but some of them appeared related to *Panus rudis* (= *Panus lecomtei* here) in their phylogeny. Some of these were explored for use as outgroups before settling on *Pseudotomentella ochracea.* According to Hibbett and Donoghue's (2001) phylogeny, *Pleurotus* was not placed in the same clade as the bulk of the polypores, *Lentinus* or *Panus*. *Pleurotus* was eventually excluded from the final analysis because of this and alignment difficulties.

Sequence analysis

Sequences were aligned and edited manually using SEQLAB in the Genetics Computer Group package (GCG 2000), followed by analysis using maximum parsimony (MP) and neighbor-joining (NJ) in PAUP* 4.0 (Swofford 2001). Gaps were treated as missing data or new states, but tree topology remained the same. 1000 bootstrap replicates were performed for a 50 % majority rule consensus trees. The trees were estimated using an heuristic search and retaining branches consistent with the 50 % majority rule. Sequence addition = furthest. The NJ tree was estimated using Jukes-Cantor as the nucleotide substitution model. Trees were visualized in TreeView (Page 1996) and edited using Powerpoint (Microsoft Corp.) and Illustrator 10.0 (Adobe). Bootstrap values and support indexes are displayed in the legend of figures.

Because of a deletion in the *Neolentinus* taxa used, analysis was done with and without that region (~14 bases). Trees were visualized in TreeView (Page 1996) and modified using Powerpoint (Microsoft Corp.) and Illustrator (Adobe).

Some problems were encountered when trying to incorporate Genbank accessions into the data set. This was due to insertions and deletions in the Genbank sequences. Many of the sequences also had considerable ambiguity (*e.g.* "n", r, y, m, k, etc.). Too many "n"s in several sequences resulted in poor resolution and uncertainty as to whether the sequence was accessioned with the correct morphological identification.

Phlebia, *Ceraceomyces* and several potentially related species were included in the initial LSU tree because of their placement in the Moncalvo *et al.* (2002) phylogeny. *Phlebia* has a bipolar mating system, relatively uncommon in the homobasidiomycetes (Hibbett and Donoghue 2001, Gilbertson and Ryvarden 1986, Nakasone 1990), but appears to be sister to all *Panus* spp. used in this study. It was therefore used as an outgroup for the *Panus s.s* ITS phylogeny presented in this dissertation (Grand 2004: pt. 4).

Results

Maximum parsimony and neighbor-joining analyses were performed using the LSU data set. The topologies of both analyses were nearly identical, with only a few taxa changing positions. The same conclusions can be inferred from either maximum parsimony or neighbor-joining methods. Comments below pertaining to the clades represented in the phylogenetic reconstructions apply to both trees (Figs. 1, 2).

LSU phylogenetic reconstructions show all members of *Lentinus s.s.* (except *L. suavissimus*) residing in a monophyletic clade (/eulentinus) with a bootstrap value of 90%. The three sequences of *L. suavissimus* all cluster separately in a highly supported /suavissimus (bootstrap value = 100%). The position of *L. suavissimus* in the phylogenies will be discussed below.

All *Panus s.s.* LSU sequences used in this study are in /eupanus. This clade is highly supported (bootstrap = 100 %) in both maximum parsimony (Fig. 1) and neighbor-joining phylogenies (Fig. 2). /eupanus is sister to a well supported clade of brown rot fungi (/brownrot), although this is weakly supported (bootstrap value = 31 %).

The *Panus s.s.* portion of the trees (/eupanus) contain two morphological sections (Pegler 1983) that are polyphyletic. /eupanus is well-supported (bootstrap = 100 %), but the sections designated by Pegler (1983) are not. Pegler's (1983) separation of sections and taxa was different than that of Corner (1981). These differences are discussed below.

Taxa of *Polyporus s.l.* sampled in this LSU study are clearly polyphyletic and separable into at least four clades. Lack of robust sampling precludes detailed comment on these taxa. For discussion of *Polyporus s.l.*, see Krüger (2002).

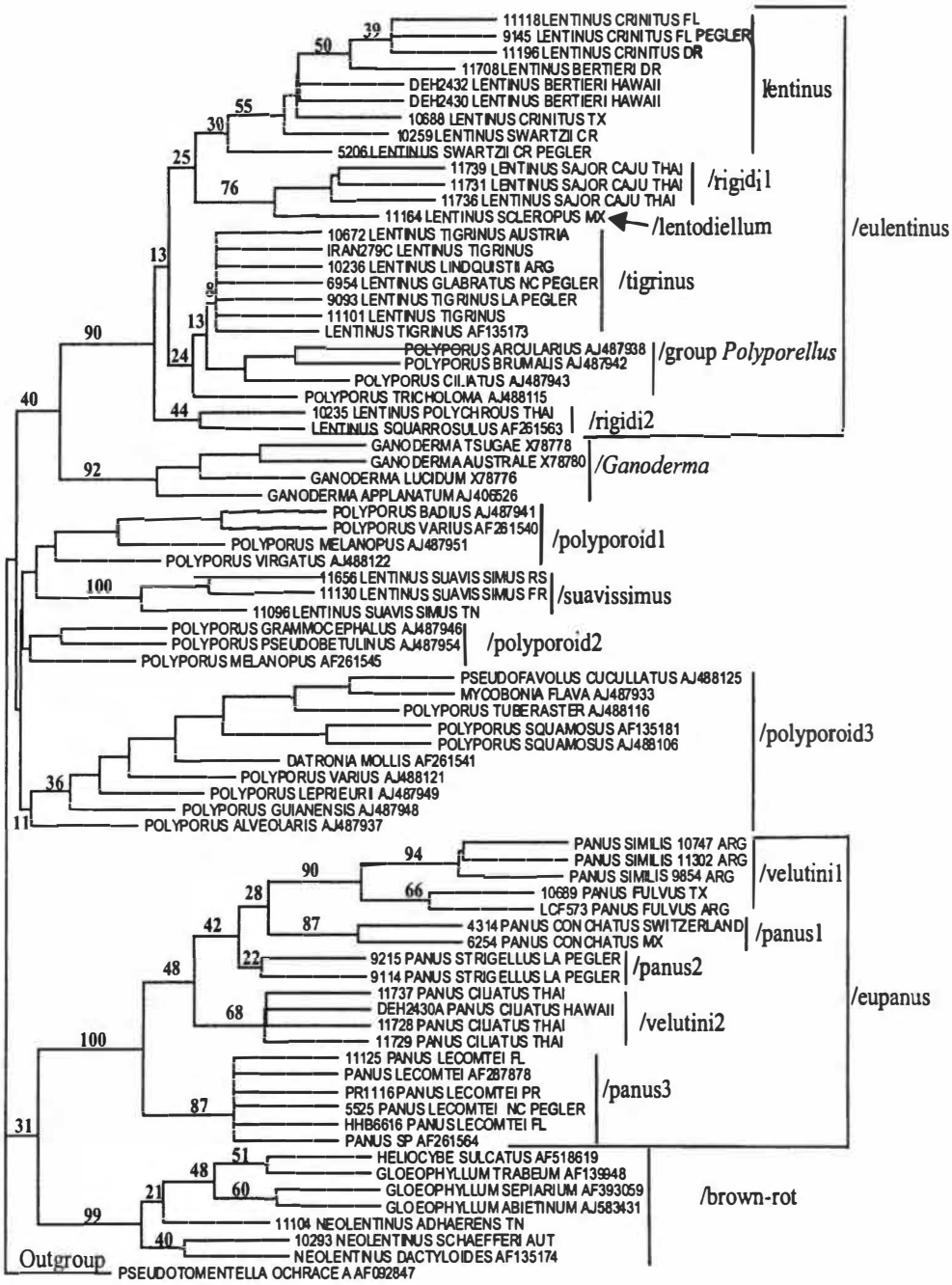

Fig. 1 - Maximum parsimony 50 % majority rule consensus LSU phylogeny. Bootstrap values are proportional to branch lengthes and reported on branches preceding clades. 1000 bootstrap replicates were performed. Tree length = 537, consistency index (CI) = 0.4525, homoplasy index (HI) = 0.5475, retention index (RI) = 0.8344.

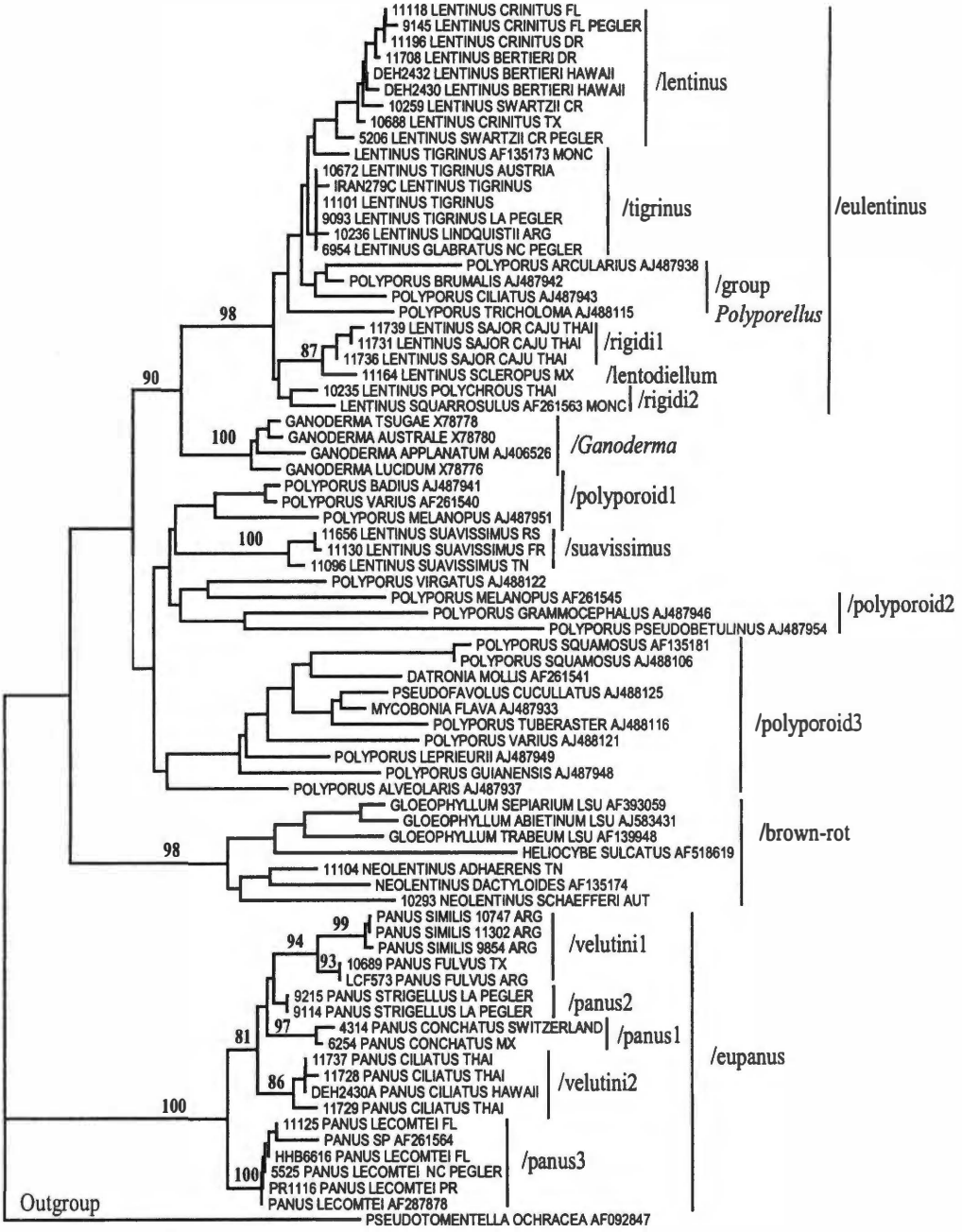

Fig. 2 - Neighbor-joining LSU phylogeny. Bootstrap values are proportionate to distances and reported on branches preceding clades. 1000 bootstrap replicates were performed using the Jukes-Cantor nucleotide substitution model. Tree length = 542.

The four species included in */Ganoderma* are sister to /eulentinus. The clade has high bootstrap support (92 %) and is monophyletic. For discussion of *Ganoderma* and its possible relationship to *Lentinus* see Grand (2004: pt. 3).

A close relationship among members of *Polyporellus* Karst. and *Lentinus* sect. *Tigrini* (Pegler 1983) has long been suspected (Pegler 1983: 10). LSU data indicate they are both within the same monophyletic /eulentinus (bootstrap value = 90 %).

Discussion

During preliminary analyses, it was found that both *Neolentinus* spp. and *Pleurotus* spp. sequences shared a deletion not found in any other included taxa. To investigate whether this deletion was of potential phylogenetic importance, maximum parsimony analyses were done with and without those sequence characters. Bootstrap support for the major clades dropped slightly (~ 1%) when this region was included, but tree topology was similar, regardless of these characters being included or excluded. In all analyses, *Pleurotus s.s.* formed a monophyletic clade not related to either *Neolentinus* or other aphyllophoralean taxa. Although *Neolentinus* and *Pleurotus* share this deletion, it appears not to be a synapomorphy and is therefore phylogenetically homoplasious. Although different taxa were sampled, the topology of the major clades in the LSU phylogenies was consistent with the trees of Moncalvo *et al.* (2002) and Hibbett and Donoghue (2001).

In Krüger's (2002) analysis of *Polyporus s.l.*, he reported the genus as polyphyletic. He included taxa more distantly related to *Polyporus* in that study, along with several *Lentinus s.s.* sequences. Representative samples from his work were included here. LSU analyses in this study (Figs. 1, 2) also showed *Polyporus sensu* Krüger (2002) to be polyphyletic, with sequences from Krüger's (2002) work appearing in four clades (/polyporoid1, /polyporoid2, /polyporoid3, /group *Polyporellus*). Detailed discussion on *Polyporus s.l.* and its pertinence to *Lentinus s.s.* was covered by Krüger (2002). For the sake of clarity and focus, no conclusions were drawn from the members contained in /polyporoid1, /polyporoid2 and /polyporoid3 except in reference to other taxa included in this study. /group *Polyporellus* will be discussed below.

Genus *Lentinus* sect. *Lentinus* (= *Lentinus* subg. *Lentinus* sect. *Lentinus* of Pegler 1983)

The *Lentinus crinitus* complex forms the core of Pegler's (1983) subg. *Lentinus*, sect. *Lentinus*. Pegler separated eight morphotaxa in this section by the length and density of pileal hairs, pileus margin (*i.e.* presence of hairs), geography, and color of basidiome. Three of these morphotaxa were sampled here: *L. crinitus, L. bertieri*, and *L. swartzii*. Morphospecies in this section not available for this study were: *L. villosus* Klotzsch, *L. stupeus* Klotzsch, *L. atrobrunneus* Pegler, *L. nigroosseus* Pilát, *L. zeyheri* Berk.

Sampling included two specimens identified and annotated by Pegler (FB9145 = *L. crinitus* and FB5206 = *L. swartzii*). The *L. crinitus* LSU sequence (FB9145) clusters with two other *L. crinitus* sequences, but there is a lot of intercollation among morphospecies in this section. Although /lentinus (bootstrap value = 30 %) is inclusive of all morphospecies of sect. *Lentinus* (Pegler 1983) sampled in this study, clade swapping of the terminal branches and sequence homoplasy prevents the distinction of species based on LSU sequence data.

All three species of sect. *Lentinus* (Pegler 1983) sequenced for these LSU analyses fall into the monophyletic /lentinus clade supported by 30 % bootstrap value in the maximum parsimony phylogeny (Fig. 1). Such weak support for this clade and the intermingling of morphospecies is the result of an ambiguous region (base position 165-176 in sequence alignment) in the LSU alignment, and little variation among sequences. This ambiguous region accounts for more sequence divergence among the species than any other region of the LSU sequence. The electropherograms indicate double peaks in this region which could be the result of heterologous copies of the rDNA tandem repeats. There are also several sequences that contain autapomorphic base substitutions that do not follow any pattern. These apparently random substitutions do not add information to the phylogeny, but do affect the order of the terminal taxa and support values for the tree.

The electropherograms for all of these aberrant sequences were examined and found to be real substitutions and not sequencing artifacts.

Lentinus swartzii is represented by two sequences in the LSU phylogenies (Figs. 1, 2). One of these collections was identified by Pegler as *L. swartzii* (FB5206). Both of these sequences are nested inside the /lentinus clade, but are not resolved together due to ambiguous sequence regions discussed above. Deletion of this ambiguous region from the analysis was explored, but resulted in a less resolved tree. Because this region was common to both sequences of the *L. swartzii* morphospecies, it was included in the analysis.

Genus *Lentinus* sect. *Tigrini* (= *Lentinus* subg. *Lentinus* sect. *Tigrini* of Pegler 1983)

Lentinus sect. *Tigrini* includes six morphospecies according to Pegler's taxonomic scheme (1983). The section is united by the presence of inflated generative hyphae in the stipe and pileus trama. Sect. *Tigrini* was split into two major groups by Pegler (1983). The first group consisted of three species with true lamellate gill construction; *L. tigrinus* (Bull.: Fr.) Fr., *L. concinnus* Pat., and *L. sclerogenus* Sacc. A fourth species, *L. lindquistii* (Singer) Lechner and Albertó, was transferred to sect. *Tigrini sensu* Pegler (1983) by Lechner and Albertó (2000). The second group contained three morphospecies, *L. lamelliporus* Har. & Pat., *L. glabratus* Mont., and *L. retinervis* Pegler. These taxa exhibited interveined to reticulate lamellae, with some specimens approaching sub-poroid configuration.

Lentinus tigrinus s.s. has many macroscopic similarities to other taxa such as *L. crinitus*, *L. concinnus* Pat., and *L. squarrosulus* Mont. and is often confused with them. Microscopically, however, the inflated generative hyphae in the basidiome flesh of *L. tigrinus* are very distinctive, and separate it from these species. The inflated generative hyphae resemble those of typical members of the Agaricales (Pegler 1983). Also, the lamellae of *L. tigrinus* are formed in a descending manner ("regular" of Hibbett *et al.* 1993, Pegler 1983), similar to many agarics (Hibbett and Thorn 2001). Contrasting with

this similarity to the gilled mushrooms is the presence of a dimitic hyphal system such as that of many members of the Aphyllophorales (Hibbett and Thorn 2001).

In *Lentinus tigrinus*, an evanescent partial veil forms a cortinoid annulus in young specimens, which is soon eroded in age (Pegler 1983, Singer 1975). Hibbett *et al.* (1993) observed no veil formation and reported basidiome development as strictly gymnocarpic. Gymnocarpic development is the formation of the hymenophore on the outside of the developing primordium at very early stages (Singer 1975: 26). Personal observation by the author of *in vitro* basidiomata indicates an evanescent veil, along with poroid lamellar precursors in very young basidiomata.

Lentinus tigrinus is a common species that is often collected and easily identified as belonging to the complex. Because of this North Temperate ubiquity, many herbarium and culture collection accessions exist. Cultures from varied collecting locales are available, and respond well to induced fruiting under controlled conditions (Grand 2004: pt. 3, Hibbett *et al.* 1993, 1994, Rosinsky and Robinson 1968.).

Lentinus tigrinus complex

Lentinus lindquistii is a morphospecies belonging to the "*L. tigrinus*" complex. It was originally described by Singer (1960) as a *Pleurotus* from Argentina. Subsequent studies of basidiomata fruited *in vitro* resulted in a transfer to *Lentinus* (Lechner and Albertó 2000). This study has shown "*L. lindquistii*" (FB10236) to be sexually intercompatible with ten *L. tigrinus* isolates of worldwide distribution (Grand 2004: pt. 3, Tab. 4).

Lentinus glabratus is another morphospecies separated by Pegler on the basis of a more glabrous pileus and stronger intervenation between the lamellae than that of its close relative, *L. tigrinus*. This species is represented by one collection identified by Pegler (FB6954).

The data presented here show the LSU sequences of *L. lindquistii* and *L. glabratus* to be nearly identical to two geographically disjunct representatives of *L. tigrinus* (FB10672, Austria; FB9093, USA). Among the seven sequences represented by this morphospecies complex, only four polymorphic sites in the LSU region were

observed. All sequences from sect. *Tigrini* form a weakly supported clade (MP bootstrap value = 8 %) that is sister to the three species in /group *Polyporellus.*

Separation of the /tigrinus clade and the /group *Polyporellus* clade is weakly supported (13 % MP bootstrap value), and the /group *Polyporellus* clade is nested among the other clades containing species of *Lentinus* (except *L. suavissimus*). This might indicate that both clades should be combined into one section of either *Lentinus* or *Polyporus*. This combination would cause great nomenclatural instability in the two morphogenera and is not suggested. Nomenclatural changes would be a topic of further research, but would require a greater sampling of taxa in the /rigidi1, /rigidi2, and /lentodiellum clades that flank /tigrinus and /group *Polyporellus*.

Other data sets [(rDNA ITS, Grand 2004: pt. 3) and (mtCOX3, data not shown)] have also shown *L. lindquistii* and *L. glabratus* sequences to be nearly identical to other clearly defined clades containing *L. tigrinus.* Morphological similarity, mating studies, and sequence data all indicate that these two taxa, and possibly other members of the sect. *Tigrini* (Pegler 1983) should be synonymized under *L. tigrinus* (Bull.: Fr.) Fr.

The work in this paper tested F1 compatible matings for potential production of viable basidiomata (*i.e.* fertility) in one case (FB8937-3 X FB10236-1). One of the SBIs used in this pairing was from a "*L. tigrinus*" morphospecies annotated by Pegler (FB8937), and the other was an *ex typus* culture obtained from *in vitro* fruited basidiomata of "*L. lindquistii*" (FB10236). After isolating and confirming a compatible pairing and formation of clamped dikaryon hyphae, the culture was submitted to fruiting conditions with the successful production of fertile basidiomata bearing viable spores (Grand 2004: pt. 3). The spores produced from this *in vitro* fruited F1 compatible pairing grew similarly to the parental spore isolates. This would indicate that "*L. lindquistii*" and "*L. tigrinus*" are not only capable of together forming a dikaryotic mycelium, but also producing fertile basidiomata.

Genus *Lentinus* sect. *Rigidi* (= *Lentinus* subg. *Lentinus* sect. *Rigidi* of Pegler 1983)

Three species representing this section in LSU phylogenies are *Lentinus sajor-caju* (Fr.) Fr., *L. polychrous* Lév. and *L. squarrosulus* Mont. The three sequenced collections of *L. sajor-caju*, all from Thailand, form a monophyletic group (/rigidi1, MP bootstrap value = 76 %). This would be expected due to their collection proximity. *L. polychrous* and *L. squarrosulus* also cluster together (/rigidi2, MP bootstrap value = 44 %), but not within the same clade as the three sequences of *L. sajor-caju.* Pegler's sect. *Rigidi* appears polyphyletic in the analyses.

The sequence for *L. squarrosulus* (Genbank AF261563, derived from Strain NEDA 500) deposited by Moncalvo *et al.* (2002) appears to be aberrant when compared to other *Lentinus s.s.* LSU sequences. The sequence has many bases that are autapomorphic with respect to the other *Lentinus* sequences included here. Perhaps there were some sequencing errors that were not detected in other studies more limited in *Lentinus* taxa (*i.e.* Moncalvo *et al.* 2002).

Genus *Lentinus* sect. *Lentodiellum* (= *Lentinus* subg. *Lentinus* sect. *Lentodiellum* of Pegler 1983)

Sect. *Lentodiellum sensu* Pegler (1983) contains *L. scleropus* (Pers.) Fr. and three other species not included in the LSU analyses. The single representative (*L. scleropus*) appears sister to the /rigidi1 clade that contains three sequences of *L. sajor-caju* from sect. *Rigidi sensu* Pegler (1983).

Pegler separated sects. *Rigidi* and *Lentodiellum* based on geographic distribution and the presence (sect. *Rigidi*) or absence (sect. *Lentodiellum*) of hyphal pegs. The validity of this separation is difficult to ascertain based on the limited LSU data set of six sequences.

Genus *Polyporus* group *Polyporellus sensu* Nuñez and Ryvarden (1995) (= *Polyporellus* Karst. 1880)

Members of the polyphyletic genus *Polyporus s.l.* (Krüger 2002) were included to determine relationships between some of the gilled and poroid taxa included in this study. Some of these appear to be intermediates between the development of a gilled or poroid hymenophore.

Group *Polyporellus* of the genus *Polyporus* (Nuñez and Ryvarden 1995) contains seven species: *P. arcularius* Batsch: Fr., *P. brumalis* Pers.: Fr., *P. ciliatus* Fr., *P. corylinus* Mauri, *P. meridionalis* (A. David) Jahn, *P. rhizophilus* Pat., and *P. tricholoma* Mont. According to analyses by Hibbett and Vilgalys (1993) and Hibbett and Donoghue (2001), several of these species are in the same phylogenetic clades as members of the genus *Lentinus s.s.* Krüger (2002) sampled more extensively members of group *Polyporellus* (Nuñez and Ryvarden 1995) and other *Polyporus s.l.* He found that three LSU sequences of *L. tigrinus* clustered within a monophyletic clade containing *P. arcularius*, *P. brumalis*, *P. ciliatus*, and *P. tricholoma* (Krüger 2002). He named this the "*Polyporellus*" clade.

/eulentinus presented in this paper includes all species of *Lentinus s.s.*(except *L. suavissimus*) and the related *Polyporus* group *Polyporellus* (Nuñez and Ryvarden 1995). A close relationship has long been suspected (Nuñez and Ryvarden 1995, Pegler 1983, Singer 1986) and is shown here using LSU sequence data.

Members of *Polyporus* group *Polyporellus sensu* Nuñez and Ryvarden (1995) typically have inflated generative hyphae, similar to all members of *Lentinus* sect. *Tigrini* (Pegler 1983). *Polyporus arcularius*, *P. brumalis*, *P. ciliatus* and *P. tricholoma*, all members of *Polyporus* group *Polyporellus* (Nuñez and Ryvarden 1995) are embedded in /group *Polyporellus* clade which is sister to /tigrinus. The /tigrinus clade includes all members of the "*Lentinus tigrinus* complex."

Krüger (2002) expressed confidence in the relationship between the /tigrinus and /group *Polyporellus* clades and proposed nomenclatural changes. I do not believe there are enough data available to warrant nomenclatural changes. Members of /eulentinus

contained in sect. *Lentinus* (*e.g. L. crinitus*), do not have the inflated generative hyphae typical of sect. *Tigrini*. LSU data presented here do not indicate a strong relationship between /lentinus and /group *Polyporellus*.

Lentinus sect. *Pleuroti* (= *Lentinus* subg. *Lentinus* sect. *Pleuroti* of Pegler 1983)

One species of *Lentinus* subg. *Lentinus sensu* Pegler that was not part of the monophyletic /eulentinus (90 % MP bootstrap value) is *Lentinus suavissimus* Fr. Three collections of this species were sequenced here. All three collections cluster in a monophyletic clade (100% MP bootstrap value) interdigitated between /polyporoid1 and /polyporoid2. These two flanking clades contain members of seven species of *Polyporus s.l.*, belonging to three groups (Nuñez and Ryvarden 1995). Separation of the *L. suavissimus* clade from other members of *Lentinus* subg. *Lentinus sensu* Pegler was also observed with ITS sequence data (Grand 2004: pt. 3). It appears that the monotypic section (Pegler 1983) that contains *L. suavissimus* does not fit genetically among *Lentinus*, *Panus* or *Neolentinus* (Figs. 1, 2).

Pegler (1983: 105) noted that the hyphal structure of *L. suavissimus* differed considerably from other members of his *Lentinus* subg. *Lentinus*. The skeletal-ligative hyphae do not taper as in most of subg. *Lentinus*, but more closely resemble the skeletal hyphae of Pegler's (1983) *Lentinus* subg. *Panus*, which contains *Lentinus sulcatus* Berk. *Lentinus sulcatus* was subsequently transferred to *Heliocybe* by Redhead and Ginns (1985) and then to *Neolentinus* by Rune (1994).

Material of *Heliocybe sulcatus* (Berk.) Redhead and Ginns (1985) was not available for this study. Instead, a very poor Genbank accessioned sequence was used (AF518619). Because the sequence contained many indels and ambiguities, it was difficult to make any specific taxonomic judgment. It appears at least to represent a brown-rot species and is in the /brown-rot clade (bootstrap value = 99 %) with *Neolentinus*.

Lentinus suavissimus is thought to be a white-rot species, with obvious clamps and has been reported as tetrapolar (Petersen *et al.* 1997). Because of these inconsistencies, *Heliocybe* as proposed by Redhead and Ginns (1985) would be an inappropriate genus for *L. suavissimus*. *Neolentinus* Redhead and Ginns (1985), which causes a brown-rot, has binucleate spores, and is bipolar would also not be a good genus for the transfer of *L. suavissimus*. It is difficult, therefore, to comment on the proper taxonomic position of *L. suavissimus* and/or its placement in *Lentinus* subg. *Lentinus sensu* Pegler. It is clear from the LSU data that *L. suavissimus* is not part of the monophyletic /eulentinus. A BLAST search (Altschul 1997) using the entire LSU sequence indicates homology with a range of genera, some of which are not represented in the LSU phylogenies. The transfer of *L. suavissimus* or proposal of a new genus to accommodate *L. suavissimus* may be necessary in future work.

/brown-rot

Three species of the brown-rot causing, bipolar genus *Gloeophyllum* (Ryvarden and Gilbertson 1993, Nobles *et al.* 1957) were included this analysis to ascertain congruence of rot-type and mating systems. *Gloeophyllum*, *Neolentinus*, and *Heliocybe* all contribute to the monophyletic /brown-rot clade (99 % MP bootstrap value).

Genus *Panus* (= *Lentinus* subg. *Panus* of Pegler 1983)

/eupanus is highly supported (MP bootstrap value = 100 %) and contains all members of *Panus s.s.* In both analyses, sections of subg. *Panus* (Pegler 1983) are not separable based on LSU data. The two represented sections in this study are polyphyletic.

The genus *Panus s.s.* appears in LSU phylogenetic reconstructions to be a monophyletic group (/eupanus) with very high bootstrap support (100%). Several species that have been transferred to other genera (*Neolentinus* and *Heliocybe*) were included in LSU analyses. All *Neolentinus* and *Heliocybe* fell into a monophyletic clade (/brown-rot clade, MP bootstrap value = 99%) sister to /eupanus. The /brown-rot clade contains

Neolentinus, *Heliocybe* and another brown rot-causing genus *Gloeophyllum*. *Gloeophyllum* was included because of its high homology to *Neolentinus* species according to a BLAST search (Altschul *et al.* 1997). The relationship of the /brown-rot clade to /eupanus is weakly supported (bootstrap value = 31 %).

Genus *Panus* sect. *Panus* (= *Lentinus* subg. *Panus* sect. *Panus* of Pegler 1983)

Panus conchatus Fr. (= *Lentinus torulosus* of Pegler 1983)

Panus conchatus is represented by two collections in LSU phylogenies. The two sequences form a monophyletic clade (/panus1) supported by an 87 % bootstrap value in the maximum parsimony analysis (Fig. 1). This clade appears sister to /velutini1, but is weakly supported (28 % bootstrap value).

Two other clades in LSU phylogenies contain species in sect. *Panus sensu* Pegler (1983). These species are in the /panus2 and /panus3 clades discussed below. Sect. *Panus sensu* Pegler (1983) is polyphyletic in the LSU analyses (Figs. 1, 2).

Panus strigellus Berk. (= *Lentinus strigellus* Berk. of Pegler 1983)

LSU phylogenies contain two sequences of *Panus strigellus*, both from the USA (Louisiana) and annotated by Pegler as *Lentinus strigellus* (= *P. strigellus* here). The two form the weakly supported /panus2 (22 % bootstrap value) which is part of /eupanus that contains all members of the genus *Panus*.

The morphological sections *Panus* and *Velutini* (Pegler 1983) are both polyphyletic in LSU phylogenies. Section *Panus* (and sect. *Pulverulenti*) *sensu* Pegler (1983) are distinguished by the presence of conspicuous hymenial cystidia. Section *Velutini* may have skeletocystidia, but they are never thick-walled or metuloidal as in sect. *Panus* (Pegler 1983). Sections *Panus* and *Velutini* both appear polyphyletic in /eupanus, suggesting that the presence and type of cystidia may not be important for separating these two sections.

Panus lecomtei Fr. (= *Lentinus strigosus* (Schwein.) Fr. of Pegler 1983)

Several species of Pegler's subg. *Lentinus* and subg. *Panus* (1983) may be confused macroscopically with *P. lecomtei* Fr. (1825: 77, Syst. Orb. Veg.) [= *Panus rudis* Fr. (1838: 328, Epicr.)], including *L. velutinus* [= *P. fulvus* (Berk.) Pegler and Rayner here], *L. strigellus* Berk. (= *P. strigellus* Berk. here), *L. hirtiformis* Murr., and *Lentinus torulosus* (Pers.: Fr.) Lloyd (= *Panus conchatus* Fr. here). These species are distinguished from *P. lecomtei* by pileus surface texture, color, and thinner-walled gloeocystidia (if any), rather than thick-walled metuloids (Johnson 1992, Johnson and Methven 1994, Pegler 1983).

The *Panus lecomtei* complex includes several morphospecies treated by Pegler as *L. strigosus* (Schw.) Fr. In this study, several collections annotated under a variety of names, including *Panus strigosus*, *Lentinus strigosus*, *Panus rudis*, *Panus lecomtei*, and *Panus fragilis* O. K. Miller (1965) were utilized. For clarity, the sequences received under these names are all annotated as *P. lecomtei* in Fig. 1. For species annotation as received, see Tab. 1. *Panus lecomtei* Fr. and *P. fragilis* O. K. Miller were regarded by Pegler as taxonomic synonyms under *L. strigosus*. In Grand (2004: pt. 4), I review some of the nomenclatural issues among these taxa.

Pairing experiments using five collections of the "*Panus lecomtei*" from Minnesota, Florida, Tennessee, and New Mexico were shown to be 100% intercompatible (Grand 2004: pt. 4). Two collections of *P. conchatus* were used as negative controls. All of the negative control pairings lacked clamps at the interface of the monokaryotic mycelia (Grand 2004: pt. 4, Tab. 2).

The /panus3 clade includes six sequences belonging to the *P. lecomtei* species complex. This clade is well-supported (87 % bootstrap value) and contains five LSU sequences from collections and cultures received as *P. lecomtei*, *P. rudis*, *P. fragilis* and *L. strigosus*. The sixth member in /panus3 is a Genbank sequence (AF261564) accessioned as *Panus sp.*, and used by Moncalvo *et al.* (2002).

Panus fragilis was described by O. K. Miller as a new species in 1965. The holotype is based on basidiomata fruited *in vitro* on malt extract agar medium. Miller

noted that basidiomata had a lighter coloration and were slightly smaller than those of the related *P. rudis* (= *P. lecomtei* here).

P. fragilis appears to be conspecific with *P. lecomtei* based on LSU data. The dikaryon culture of *P. fragilis* sequenced in this study (HHB6616) was obtained from the Forest Products Laboratory culture collection in Madison, Wisconsin (FPLM). Several *P. fragilis* cultures annotated by O. K. Miller and H. B. Burdsall were obtained from FPLM for use in ITS studies (Grand 2004: pt. 4). ITS sequence data also support the conspecificity of *P. fragilis* and *P. lecomtei* (Grand 2004: pt. 4, Figs. 1, 2).

Genus *Panus* sect. *Velutini* (= *Lentinus* subg. *Panus* sect. *Velutini* of Pegler 1983)

Panus similis Berk. and Br. (= *Lentinus similis* of Pegler 1983)

Corner (1981: 85) considered *Panus similis* to be a variety of *Panus fulvus*. Following the opinion of Pegler (1983), I recognize this taxon as a species distinct from *P. fulvus* based on LSU sequence, ITS sequence (Grand 2004: pt. 4), basidiome coloration, and other morphological differences (*i.e.* pileus surface and basidiome stature). Pegler's (1983) collections were reported from Africa, Asia and Australia. Although not previously reported from the Americas, this study includes three collections from Argentina that are morphologically *P. similis*. In addition, sequences from an independently identified specimen of *P. similis* from Argentina were homologous to those in /velutini1 (Bernardo Lechner, pers. comm.).

Sequences of *P. similis* and *P. fulvus* are grouped in /velutini1 (MP bootstrap value = 90 %) which is a subclade of the larger /eupanus. LSU sequences of *P. similis* are distinct from the closely related and often confused *P. fulvus* (Pegler 1983). There is bootstrap support for each infraspecific clade containing *P. similis* (bootstrap value = 94 %) and *P. fulvus* (bootstrap support = 66 %). This separation is distinct in both LSU and ITS phylogenies (Grand 2004: pt. 4). *P. similis*, *P. fulvus* and several other species in sect. *Velutini sensu* Pegler (1983) are frequently misidentified. I believe the exclusion of *P. similis* from the Americas to be in error.

Panus fulvus (Berk.) Pegler and Rayner (= *Lentinus velutinus* of Pegler 1983)

One collection (FB10689) and one dikaryon culture (LCF573) were available for sequencing in this study. These sequences cluster in a subclade of /velutini1 that also contains *Panus similis*. *P. similis* was treated as a variety of *P. fulvus* by Corner (1981), but is separable from it based on LSU sequence.

Sect. *Velutini* of Pegler (1983) is polyphyletic in LSU analyses. The /velutini1 and /velutini2 are separated by two species of sect. *Panus sensu* Pegler (*P. conchatus* and *P. strigellus*).

Panus ciliatus Lév. (= *Lentinus ciliatus* Lév. of Pegler 1983)

The monophyletic clade /velutini2 contains four sequences of *P. ciliatus* from the USA (Hawaii) and Thailand. The clade is supported by 68 % bootstrap value. This species is macromorphologically similar to *P. lecomtei*, but the lack of metuloidal pleurocystidia separates it microscopically. The four sequences are distinct from other members of Pegler's (1983) sect. *Velutini*, making Pegler's section polyphyletic based on LSU data.

Genus *Panus* sects. *Pulverenti* and *Squamosi* (= *Lentinus* subg. *Panus* sects. *Pulverenti* and *Squamosi* of Pegler 1983)

Some members of Pegler's (1983) subg. *Panus* sects. *Pulverulenti* and *Squamosi* have been transferred to *Neolentinus* Redhead and Ginns (1985). Collections of *Neolentinus adhaerens* (Alb. and Schw.: Fr.) Redhead and Ginns, *N. schaefferi* (Weinm.) Redhead and Ginns [= *L. cyathiformis* (Schaeff.) Bres.], and *N. dactyloides* (Clel.) Redhead and Ginns were included in the analyses. Although these collections (and *Heliocybe*) clearly form a monophyletic group (99% MP bootstrap value) with *Gloeophyllum*, their relationship to other taxa sampled in this study was not explored.

While searching for potential outgroups for LSU analyses, three *Gloeophyllum* species were included. *Neolentinus* appears to be closely related to *Gloeophyllum*, and

consistently clustered with it in initial analyses. Both genera cause brown-rots and have bipolar mating systems. *Gloeophyllum* spp. and related taxa would be important to include in any further analysis of *Neolentinus*.

Lentinus subg. *Panus* sect. *Squamosi sensu* Pegler (1983) formerly contained *Lentinus levis* (Berk. and Curt.) Murr., *L. lepideus* (Fr.: Fr.) Fr., *L. ponderosus* O. K. Miller, *L. dactyloides* Clel., *L. cyathiformis* (Schaeff.) Bres., and *L. pallidus* Berk. and Curt. All of these species have been transferred to *Neolentinus* Redhead and Ginns (1985) except for *L. levis*, which was transferred to *Pleurotus levis* (Berk. and Curt.) Singer.

Pleurotus (Fr.) Kummer

Several taxa of what is now considered the monophyletic genus *Pleurotus* (Moncalvo *et al.* 2000, 2002, Thorn and Hibbett 2001) have traditionally been included in *Lentinus* and *Panus* (Fries 1825, Singer 1960, 1986, Corner 1981, Pegler 1983). Material received as *Panus* (*P. levis* and *P. giganteus*) was sequenced and initially included in the analyses. LSU sequence analysis, using a total of ten species of *Pleurotus* (Fr.) Kummer (*P. ostreatus* (Jacq.: Fr.) Kummer, *P. pulmonarius* (Fr.) Quel., *P. geesterani* Singer, *P. abieticola* Petersen and Hughes, *P. cornucopiae* (Pau.: Pers.) Rolland, *P. opuntiae* (Dur. and Lév.) Sacc., *P. calyptratus* (Lindb. *apud* Fr.) Sacc., *P. dryinus* (Pers.: Fr.) Kummer, *P. levis* (Berk. and Curt.) Singer, "*Panus*" *giganteus* Berk.), has shown that including these sequences in the analyses was inappropriate. *Panus levis* and *P. giganteus* are more closely affiliated with *Pleurotus* and other "eu-agarics". This conclusion agrees with data presented by Moncalvo *et al.* (2000) and Thorn and Hibbett (2001), who showed *Pleurotus* to be much more closely related to the "eu-agarics", including *Hohenbuehelia.*

Panus giganteus Berk. is a southeast Asian taxon placed in *Panus* by Corner (1981) and in *Lentinus* by Pegler (1983). Both authors suggested that these were probably inaccurate placements but did not transfer the name. LSU and ITS data show that this taxon is a member of the genus *Pleurotus* (Fr.) Kummer (data not shown).

Other putative species of *Panus* (*P. levis*, *P. tuberregium*) have also been transferred to *Pleurotus* based on several characters, including the ability to form white-

rots, tetrapolar mating systems, and the production of nematotoxic droplets (Hibbett and Thorn 1994, Isikhuemhen *et al.* 2000, Thorn and Barron 1984, Thorn *et al.* 2000).

The possibility of transferring *Panus giganteus* to *Pleurotus* was discussed briefly by Hibbett and Thorn (1994). Further analysis is needed before those results can be accessed. However, in initial analyses, members currently included in *Pleurotus s.s.* form a distinct monophyletic clade that includes a sequence of *Panus giganteus* Berk.

Acknowledgements

I would like to acknowledge my advisor Ron Petersen and my committee for guidance during the preparation of this work. An NSF-PEET grant (DEB9978011) and University of Tennessee Botany Department provided funding for my research and travel during my study. Collection and observation of freshly collected material would not have been possible without this funding. Collaborators (Dirk Krüger, Tage Rolen) and travel partners (Tim Baroni, Joaquin Cifuentes, Dennis Desjardin, Jean Lodge) were also essential for this study. Several culture collections graciously provided material (mainly Forest Products Laboratory, Madison, WI and Leningrad culture collection (Nadia Psurtseva, St. Petersburg, Russia)). I would also like to thank David N. Pegler for morphological identifications.

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Part 3 – Biogeography and species concepts in the genus *Lentinus* Fr., with emphasis on sects. *Lentinus* and *Tigrini sensu* Pegler (1983).

Abstract

Two sections of the genus *Lentinus* subg. *Lentinus sensu* Pegler were examined in detail for this paper. Sects. *Lentinus* and *Tigrini* were evaluated using ribosomal ITS sequence data, sexual intercompatibility studies and morphological data. Both sections were shown to be monophyletic with respect to the taxa sampled. No bioegeographical pattern was observed in sect. *Lentinus*, but sect. *Tigrini* members showed a correlation between geography and clades based on ITS data. Synonymization of *Lentinus lindquistii* Lechner and Albertó and *Lentinus glabratus* Mont. under *L. tigrinus* (Bull.: Fr.) Fr. is suggested based on sexual intercompatibility studies and molecular data. Several members of sects. *Rigidi* and *Lentodiellum sensu* Pegler were also sequenced for the ITS analysis. No intercompatibility data was collected for these sections. Group *Polyporellus sensu* Nuñez and Ryvarden was included to explore possible supra-generic relationships. *Polyporus* group *Polyporellus* appears to be a monophyletic group related to *Lentinus* sect. *Tigrini.* Related genera and the other sections within *Lentinus sensu* Pegler were analyzed and discussed.

Introduction

Lentinus sensu Pegler (1983) has been split into segregate genera (*Lentinus* and *Panus*) by most authors (Corner 1981, Moser 1978, Singer 1975, 1986). The genera *Lentinus* Fr., *Panus* Fr. and their segregates have been the focus of several phylogenetic studies (Grand 2004, Hibbett and Vilgalys 1991, 1993, Moncalvo *et al.* 2002, Redhead and Ginns 1985, Thorn *et al.* 2000). These studies have elucidated the relationships among these genera and identified possible problems in a strictly morphological approach to circumscribing the genera. Other characters such as DNA sequence data, sexual intercompatibility, nuclear behavior during meiosis, culture characteristics and physiology (rot-type) have also been used to explore the taxonomy and phylogenetic relationships of *Lentinus* and *Panus* to other genera such as *Polyporus*, *Pleurotus* and *Neolentinus* (Hibbett and Thorn 1994, Hibbett *et al.* 1994, Hibbett and Donoghue 2001, Krüger 2002, Redhead and Ginns 1985).

Neolentinus spp. were separated from *Panus* sects. *Squamosi* and *Pulverulenti sensu* Pegler (1983) by Redhead and Ginns (1985) based on bipolar mating systems and the ability to cause brown-rots. All currently accepted members of *Lentinus*, *Panus*, and *Polyporus* (*sensu* Nuñez and Ryvarden 1995) form white rots (Nuñez and Ryvarden 1995).

Pleurotus spp. were initially included, but after aligning and analyzing preliminary data, it was clear that these species were inappropriate for a *Lentinus* study. Based on preliminary work and the conclusions of three recent phylogenetic papers (Hibbett and Donoghue 2001, Moncalvo *et al.* 2000, 2002), *Pleurotus* was excluded from the analyses.

The morphological similarity of the polyporoid group *Polyporellus* (*sensu* Nuñez and Ryvarden 1995) and *Lentinus* sect. *Tigrini sensu* Pegler (1983) has been discussed by several authors (Krüger 2002, Nuñez and Ryvarden 1995, Pegler 1983, Singer 1986). Both groups exhibit inflated generative hyphae and dimitic hyphal systems with skeleto-ligative hyphae (Pegler 1983). In order to explore this relationship, four species of group *Polyporellus* (*sensu* Nuñez and Ryvarden 1995) were included in the analyses.

Several species of *Lentinus sensu* Pegler (1983) are part of "species complexes" (*e.g. L. crinitus, L. tigrinus*). These complexes consist of morphotaxa that are sometimes difficult to distinguish from each other and identify to species. Pegler (1983) postulated that some members of these complexes were capable of interbreeding. Interbreeding syngameons may express slightly different morphotypes, but are capable of successful genetic exchange if they contact each other (Lotsy 1925).

Sexual intercompatibility studies in basidiomycetous fungi have been outlined and used elsewhere [Gordon and Petersen 1991, 1992, 1998 (*Marasmius*), Grand 2004 (*Panus* and *Lentinus*), Hallenberg 1983 (*Hericium*), Johnson and Methven 1994 (*Panus*), McCleneghan 1996 (*Pholiota*), Petersen and Bermudes 1992 (*Panellus*), Petersen 1995a, 1995b (*Flammulina*, *Omphalotus*)]. The premise of these studies is that genetic isolation leads to speciation (Mayr 1942, Dobzhansky 1951, Petersen and Hughes 1999). If two individuals cannot exhange genetic material and interbreed, they are separate biological species. These separate biological species will eventually accumulate mutations that will

be expressed in morphology as well as DNA sequence. Some authors have questioned the validity of such techniques because they typically do not involve the production of progeny (Boidin 1986, Mishler and Donoghue 1982, Mishler and Brandon 1987, Vilgalys 1991, Worrall 1997). Intercollection pairings were used in this study to determine intersterility groups and conducted in the same manner outlined by Grand (2004: pt. 2).

To determine phylogenetic relationships among members of *Lentinus s.s.*, ribosomal ITS sequence data, morphology and intercollection pairings were used. *Panus s.s.* was excluded from these analyses due to the distant relationship shown in LSU phylogenies (Grand 2004: pt. 2) and alignment difficulties. The results of a study similar to this one, but utilizing *Panus s.s.* are in Grand (2004: pt. 4).

Sequence data from the ITS region were also used to explore biogeographical trends, species distributions and population differences within infraspecific groups. *Lentinus crinitus* (Linn.: Fr.) Fr. and *L. tigrinus* (Bull.: Fr.) Fr. are two species that form complexes within which it is difficult to distinguish separate morphospecies (Pegler 1983). DNA sequences of the ITS region were used in an attempt to delineate species within the *L. crinitus* and *L. tigrinus* complexes. An adequate number of collections of *L. crinitus* and *L. tigrinus* were available for this purpose. Collections and cultures of other species in *Lentinus s.s.* were not as readily available, making sampling in those species more limited than in the *L. crinitus* and *L. tigrinus* complexes. One of the goals of this study was to further understand the distribution and biogeography of these complexes.

Taxonomic background

Genus *Lentinus* subg. *Lentinus* sect. *Lentinus sensu* Pegler (1983)

There are four species in sect. *Lentinus* Pegler, which along with *L. crinitus*, are restricted to the Americas. *Lentinus bertieri* (Fr.) Fr., *L. swartzii* Berk., and *L. nigroosseus* Pilát are distinguished from *L. crinitus* by their basidiome stature, pileus surface, microstructure, and pileus margin. Three were sampled for this ITS analysis: *L. crinitus, L. bertieri*, and *L. swartzii*. The four African representatives in sect. *Lentinus* Pegler are *L. zeyheri* Berk., *L. villosus* Klotzsch, *L. stupeus* Klotzsch, and *L.*

atrobrunneus Pegler. Material of these taxa was not available for this study. These four African representatives can be separated from other members of sect. *Lentinus* based on spore morphology, persistently involute pileus margin and their distribution in Africa.

The *Lentinus crinitus* (Linn.: Fr.) Fr. species complex forms the core of Pegler's (1983) *Lentinus* subg. *Lentinus* sect. *Lentinus*. It is one of the most commonly collected species, but it and the other members of this section often have similar morphological characters. The difficulty of correctly identifying members of this section by morphology alone was a primary reason for concentrating on this section.

Basidiomata of *L. crinitus s.s.* exhibit a pale stipe with white scurfy squamules and a glabrescent pileus surface. A non-involute pileus margin, crowded lamellae, abundant hyphal pegs and squat basidiome stature distinguish this species. Two other species that occur in tropical America are *Lentinus bertieri* (Fr.) Fr. and *L. swartzii* Berk. *Lentinus bertieri* is distinguished morphologically by a densely pilose pileus and initially involute margin. *L. swartzii* has a darker colored fruitbody and well-developed, erect squamules on a more robust pileus.

Pegler (1983) considered *Lentinus crinitus* (Linn: Fr.) Fr. the type species of *Lentinus* Fr. This typification is not universally accepted. For discussion regarding the differing opinions about the type species for *Lentinus* Fr., see Grand (2004: pt. 2 under "Nomenclature") and Redhead and Ginns (1985). The earliest lectotype of *Lentinus* was designated by Clements and Shear (1931) as *Lentinus tigrinus* (Bull.: Fr.) Fr. Art. 9.17 of the International Code of Botanical Nomenclature (St. Louis Code 2000) states that "the author that first designates a lectotype or a neotype must be followed...". Therefore, in this study, the type species of *Lentinus* is accepted as *L. tigrinus*.

Genus *Lentinus* subg. *Lentinus* sect. *Tigrini sensu* Pegler (1983)

Lentinus subg. *Lentinus* sect. *Tigrini* includes at least seven morphospecies. The section is united by the presence of inflated generative hyphae. Inflated hyphae are also conspicuous in basidiomata of taxa of *Polyporellus* Karsten (1880). The presence of skeleto-binding hyphae, dimitic hyphal construction, inflated generative hyphae and the

ability to cause white-rots are shared by sect. *Tigrini sensu* Pegler (1983) and *Polyporellus* Karsten (1880) (Krüger 2002, Nuñez and Ryvarden 1995).

The inflated generative hyphae of the *L. tigrinus* complex are very distinctive, and separate the complex from other species. The generative hyphae are inflated up to 20 µm in diameter. This degree of inflation in the generative hyphae is typical for members of the Agaricales (Pegler 1983). The lamellae of *L. tigrinus* are also formed in a descending (= regular) manner (Hibbett *et al.* 1993, Pegler 1983), similar to many agarics (Hibbett and Thorn 2001, Reijnders and Stalpers 1992, Reijnders 1993). Contrasting with this similarity to the gilled mushrooms is the presence of a dimitic hyphal system such as those of many members of the Aphyllophorales (Corner 1953, Donk 1960, Hibbett and Thorn 2001).

Sect. *Tigrini* has been split into two major groups by Pegler (1983). The first group consists of three species with true lamellate gill construction; *L. tigrinus* (Bull.: Fr.) Fr., *L. concinnus* Pat., and *L. sclerogenus* Sacc. The second group contains three morphospecies with lamellae that are interveined or reticulated; *L. lamelliporus* Har. & Pat., *L. glabratus* Mont., and *L. retinervis* Pegler. The ontogenetic precursors to these reticulated lamellae sometimes are sub-poroid, especially near the stipe. *L. lindquistii* (Singer) Lechner and Albertó (2000) is a species that has similar morphology to *L. tirgrinus*, and was transferred to *Lentinus* Fr. subg. *Lentinus* Fr. sect. *Tigrini sensu* Pegler (1983) by Lechner and Albertó (2000).

Lentinus tigrinus is a morphologically variable but common species that is often collected and easily identified as belonging to the complex. Because of its North Temperate ubiquity, many herbarium and culture collection accessions exist. Some authors have considered its range as extending into the tropics (Corner 1981, see *L. lindquistii* discussion below). Cultures of *L. tigrinus* from widely distributed geographic locations respond well to induced fruiting under controlled conditions (Hibbett *et al.* 1993, 1994, Rosinsky and Robinson 1968). *L. tigrinus* can be confused with other species such as *L. crinitus* (Linn.:Fr.) Fr., *L. concinnus* Pat., and *L. squarrosulus* Mont., especially in tropical locales.

Morgan (1895) proposed the genus *Lentodium* for a single gastroid species, *L. squamulosum*. A close relationship to *L. tigrinus* was long suspected, and both *L. tigrinus* and *Lentodium squamulosum* have been shown to be biologically conspecific using monokaryon crossing experiments (Rosinsky and Robinson 1968). Fruiting of the resultant "hybrids" also produced normal *L. tigrinus* basidiomata.

Lentinus glabratus Mont. is a species known originally from Cuba and described as having a more glabrous pileus than *L. tigrinus*. Pegler (1983) noted that although *L. glabratus* was described as having a glabrous pileus, there were indeed small squamules on the type basidiomata. These squamules and similar micromorphology indicate a close affiliation with *L. tigrinus*. Material from North Carolina, USA, annotated by Pegler as *L. glabratus*, was available for this study (FB6954). Using ITS (this study) and LSU (Grand 2004: pt. 2) sequence data, the possible conspecificity of these species was investigated.

Lentinus lindquistii is another morphospecies closely related to *L. tigrinus*. The species was named from Argentinian material and originally described by Singer (1960) as a *Pleurotus*. Subsequent studies of basidiomes fruited *in vitro* resulted in a transfer to *Lentinus* (Lechner and Albertó 2000). SBIs derived from the type collection of this species were available for this study. Intercompatibity experiments, as well as ITS and LSU data were used to determine if the separation of this species from *L. tigrinus* based on non-inflated generative hyphae and spore size differences was valid.

Genus *Lentinus* subg. *Lentinus* sects. *Dicholamellatae*, *Rigidi,* and *Lentodiellum sensu* Pegler (1983)

The three species in Pegler's (1983) *Lentinus* sect. *Dicholamellatae* are: *Lentinus badius* (Berk.) Berk., *L. araucariae* Har. and Pat., and *L. brunneofloccosus* Pegler. These taxa have paleotropical and Australasian distributions that are not well represented in the material available for this study, thus no representatives of sect. *Dicholamellatae* were included in this study.

Sect. *Rigidi sensu* Pegler (1983) contains five morphospecies. It is represented in these ITS phylogenies by one species, *Lentinus sajor-caju* (Fr.) Fr. There are two

representative sequences of *L. sajor-caju* included. Another species available for study in this section was *L. polychrous* Lév. PCR amplification was attempted from dried herbarium material with no success.

Two species were available from sect. *Lentodiellum sensu* Pegler (1983); one collection of *L. scleropus* (Pers.) Fr. and a sequence of *L. striatulus* Lév. (MO135; Rolen 2001). This is the only section of the genus *Lentinus* that does not have hyphal pegs in the hymenium.

The purpose of this study was to investigate relationships in subg. *Lentinus sensu* Pegler (1983). The *L. crinitus* and *L. tigrinus* species complexes were the most accessible material for research involving ITS sequence data and intercompatibility studies. ITS data was used in an attempt to elucidate biogeographical patterns in two morphologically separated sections. Two morphotaxa were found to be candidates for synonymization.

Materials and methods

Abbreviations and acronyms

Tab. 1 shows collection data for the specimens and cultures used in this study. FB indicates the Tennessee Field Book number and location in the Tennessee culture collection (CULTENN). The TENN indicates the dried voucher specimen's accession in the University of Tennessee fungal herbarium. If no TENN is present, only a culture and the received identification were available. FPLM indicates that the culture was obtained from the Forest Products Laboratory culture collection in Madison, Wisconsin.

Morphology

Macro- and micromorphological observations and putative species designations followed keys and descriptions by Pegler (1983) and Corner (1981). Field collection and processing techniques followed those of Largent *et al.* (1977), Largent (1986), and Largent and Baroni (1988).

Tab. 1 - Fungal specimens and cultures examined for ITS sequences.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
--	Ganoderma sp.	AF455510	--	Ganoderma sp.	NA
--	Ganoderma sp.	AY508882	--	Ganoderma sp.	NA
--	Ganoderma lucidum	AY456341	--	Ganoderma lucidum	NA
FB10791 (TENN58997)	Lentinus bertieri		Argentina	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB11702 (TENN59770)	Lentinus bertieri		Dominican Republic, La Placeta	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB11708 (TENN59773)	Lentinus bertieri		Dominican Republic, La Celestina	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB11723 (TENN59781)	Lentinus bertieri		Dominican Republic, Carrizal	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB11754 (DEH2430)	Lentinus bertieri		USA, Hawaii	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB11756 (DEH2432)	Lentinus bertieri		USA, Hawaii	Lentinus bertieri	subg. Lentinus sect. Lentinus
EC79_5[2]	Lentinus crinitus		Ecuador, Napo	Lentinus crinitus	subg. Lentinus sect. Lentinus
HHB6558[2]	Lentinus crinitus		USA, Florida	Lentinus crinitus	subg. Lentinus sect. Lentinus
PR917[2]	Lentinus crinitus		Puerto Rico, Mount Britton	Lentinus swartzii	subg. Lentinus sect. Lentinus
PR2058[2]	Lentinus crinitus		Puerto Rico, Luquillo Mountains	Lentinus crinitus	subg. Lentinus sect. Lentinus
Tage35436	Lentinus crinitus		Venezuela	Lentinus crinitus	subg. Lentinus sect. Lentinus
TageLR24129	Lentinus crinitus		Brazil	Lentinus crinitus	subg. Lentinus sect. Lentinus
TageTR10	Lentinus crinitus		Costa Rica	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB8680 (TENN55401)	Lentinus crinitus		USA, Louisiana	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB9145 (TENN54876)	Lentinus crinitus		USA, Florida, Leon County	Lentinus crinitus[1]	subg. Lentinus sect. Lentinus
FB9146 (TENN54877)	Lentinus crinitus		USA, Florida, Leon County	Lentinus crinitus[1]	subg. Lentinus sect. Lentinus
FB9218 (TENN55994)	Lentinus crinitus		USA, Florida, Liberty County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB9263 (56452)	Lentinus crinitus		USA, Florida, Putnam County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB9505 (TENN56265)	Lentinus crinitus		USA, Louisiana, West Feliciana Parrish	Lentinus crinitus	subg. Lentinus sect. Lentinus

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB10431 (TENN59832)	Lentinus crinitus		Costa Rica	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11117 (TENN59658)	Lentinus crinitus		USA, Florida, Calhoun County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11118 (TENN59659)	Lentinus crinitus		USA, Florida, Calhoun County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11121 (TENN59662)	Lentinus crinitus		USA, Florida, St. Johns County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11122 (TENN59663)	Lentinus crinitus		USA, Florida, St. Johns County	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11196 (TENN59732)	Lentinus crinitus		Dominican Republic, Jarabacoa	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11534 (TENN59415)	Lentinus crinitus		Belize, Eligio Panti Nat'l Park	Lentinus crinitus	subg. Lentinus sect. Lentinus
FB11736 (TENN59793)	Lentinus sajor-caju		Thailand, Chiang Mai Province	Lentinus sajor-caju	subg. Lentinus sect. Rigidi
FB11739 (TENN59796)	Lentinus sajor-caju		Thailand, Chiang Mai Province	Lentinus sajor-caju	subg. Lentinus sect. Rigidi
FB11164 (TENN59704)	Lentinus scleropus		Mexico, Villahermosa	Panus hirtus	subg. Lentinus sect. Lentodiellum
TageMO135	Lentinus striatulus		Costa Rica	Lentinus striatulus	subg. Lentinus sect. Lentodiellum
TageMO166	Lentinus swartzii		Costa Rica	Lentinus bertieri	subg. Lentinus sect. Lentinus
FB5206 (TENN51531)	Lentinus swartzii[1]		Costa Rica	Lentinus bertieri	subg. Lentinus sect. Lentinus
WC286 (ATCC9406)	Lentinus tigrinus		USA, Louisiana	Lentinus tigrinus	subg. Lentinus sect. Tigrini
WC288 (ATCC28757)	Lentinus tigrinus		USA, Iowa	Lentinus tigrinus	subg. Lentinus sect. Tigrini
FP100141 (culture_only)	Lentinus tigrinus		USA, Tennessee	Lentinus tigrinus	subg. Lentinus sect. Tigrini
FP102501 (culture_only)	Lentinus tigrinus		USA, Illinois	Lentinus tigrinus	subg. Lentinus sect. Tigrini
LE0789[3]	Lentinus tigrinus		Armenia	Lentinus tigrinus	subg. Lentinus sect. Tigrini
FB9770 (LE0861)[3]	Lentinus tigrinus		Mongolia	Lentinus tigrinus	subg. Lentinus sect. Tigrini
LE0845[3]	Lentinus tigrinus		Azerbaijan	Lentinus tigrinus	subg. Lentinus sect. Tigrini
LE1305[3]	Lentinus tigrinus		Ukraine, Kiev Region	Lentinus tigrinus	subg. Lentinus sect. Tigrini
RLG9953[2] (culture_only)	Lentinus tigrinus		USA, Arizona	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB6954 (TENN56194)	Lentinus glabratus[1]		USA, North Carolina	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB8937 (TENN55557)	Lentinus tigrinus[1]		Russia, Caucasia	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB9066 (TENN55773)	Lentinus tigrinus[1]		USA, Mississippi	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB9093 (TENN54918)	Lentinus tigrinus[1]		USA, Louisiana	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB9209 (culture_only)	Lentinus tigrinus		USA, Louisiana	Lentinula sp.	subg. *Lentinus* sect. *Tigrini*
FB10236 (Type_cultures)	Lentinus tigrinus ("lindquistii" in tree)	BAFC 2117, 2132, 2266, 2274	Argentina, Buenos Aires	Lentinus lindquistii	subg. *Lentinus* sect. *Tigrini*
FB10672 (TENN59833)	Lentinus tigrinus		Austria	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB10674 (TENN59834)	Lentinus tigrinus		Austria	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB10832 (TENN59835)	Lentinus tigrinus		USA, Texas	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11101 (TENN59644)	Lentinus tigrinus		USA, Louisiana	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11102 (TENN59645)	Lentinus tigrinus		USA, Louisiana	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11170 (TENN59709)	Lentinus tigrinus		USA, Florida	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11171 (TENN59710)	Lentinus tigrinus		USA, Florida	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11746 (IRAN279C)	Lentinus tigrinus		Iran, Gilan Province	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11747 (IRAN300C)	Lentinus tigrinus		Iran, Tehran Province	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11748 (IRAN309C)	Lentinus tigrinus		Iran, Gilan Province	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11750 (IRAN315C)	Lentinus tigrinus		Iran, Lorestan Province	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11751 (CCBAS391)	Lentinus tigrinus		Czech Republic, Moravia	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11752 (CCBAS392)	Lentinus tigrinus		Czech Republic, Moravia	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB11753 (CCBAS826)	Lentinus tigrinus		Uzbekistan	Lentinus tigrinus	subg. *Lentinus* sect. *Tigrini*
FB7883 (TENN53747)	Polyporus arcularius	AF516524	Costa Rica	Polyporus arcularius	NA
DSMZ-H17 (DAOM 31983)	Polyporus brumalis	AB070870	Canada	Polyporus brumalis	NA
FB7480 (TENN53639)	Polyporus ciliatus	AB070880	Finland	Polyporus ciliatus	NA

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB9591 (TENN56503)	Polyporus tricholoma	AJ132941	Puerto Rico	Polyporus tricholoma	NA
FB10241 (TENN57564)	Polyporus tricholoma	AF516541	Costa Rica	Polyporus tricholoma	NA
FB11722 (TENN59780)	Polyporus tricholoma	AFXXXXX	Dominican Republic	Polyporus tricholoma	NA

[1]Annotated by D. N. Pegler

[2]Culture obtained from FPLM

[3]Culture obtained from Leningrad Culture Collection

Culture techniques

Single-basidiospore isolates (SBIs) for sexual intercompatibility studies were obtained following the method of Gordon and Petersen (1991). Another method of obtaining SBIs was to collect a spore print on autoclaved aluminum foil (Petersen and Greilhuber 1996) followed by dilution in sterile water and dispersal on malt extract agar plates. Spores were allowed to settle on the agar surface before the excess water was decanted from the surface. Germinating spores were harvested and transferred to fresh plates. Before use in intercompatibility studies, each SBI was checked for the absence of clamp connections to verify monokaryon status.

In vitro fruiting of dikaryon cultures

In order to obtain monokaryotic SBIs for intercompatibility studies and DNA extractions, collections not available either through CULTENN or collaborators (*i.e.* from spore prints) were fruited *in vitro*. In cases where only a dikaryon culture was available, cultures were fruited on a mixture of sawdust (95 % by weight) and bran (5% by weight) (Stamets and Chilton 1983, Stamets 1993). After confirmation of a clamped dikaryotic culture, the isolate was grown on sterilized rye grain. 100 grams of dry rye grain was mixed with 100 ml of water, then autoclaved for 1 hour at 15 psi. The cooled rye grain

was then inoculated with several small chunks (*ca.* 0.5 cm^2) of agar and allowed to colonize for several weeks. Fully colonized rye grains were broken into individual grains by manual shacking. The individual grains were then used to inoculate a polyethylene autoclavable bag (Unicorn Bag Company) containing water saturated, sterilized *Liriodendron* sawdust. The colonized block was then subjected to fruiting conditions (~95 % humidity, ambient light) for several weeks, after which mature, sporulating basidiomata formed. Spore prints were obtained from *in vitro* fruited basidiomata and handled in the same way as those from naturally grown basidiomata.

Intercollection pairings

To ascertain biological conspecificity among collections, pairs of SBIs (n = 4 or 8) were placed in close proximity on a fresh 1.5 % malt extract agar plate (Difco). After growing for several weeks, the two monokaryotic isolates formed a contact zone. From this contact zone, a small portion was excised for microscopic examination. The observation of clamped hyphae (*i.e.* dikaryon formation) in this contact zone meant that the two collections used in the pairing belonged to the same intersterility group. Pairings were performed among members of putative morphospecies complexes and other problematic taxa.

Molecular Techniques

Molecular techniques and data analysis followed those described by Hughes *et al.* (2001) and Cifuentes *et al.* (2003). Monokaryon cultures were chosen for DNA extraction after verification that the culture was clampless. Monokaryons were used to help prevent sequencing problems due to heterozygosity for insertions or deletions (indels) that were sometimes observed when using dikaryons. To obtain fungal tissue suitable for DNA extraction, a *ca.* 0.5 cm^2 piece of inoculant agar was placed in a jar containing PD broth (24 g/L Difco Potato Dextrose Broth) and allowed to grow for several weeks. When the culture reached a diameter of *ca.* 3-4 centimeters, the culture was sacrificed and DNA extracted using the procedure of Cifuentes *et al.* (2003). Dried

herbarium tissue was extracted in a similar fashion after a small piece of dried tissue (*ca.* 0.5 cm^2) was ground with the aid of sterile grinding sand.

ITS1-5.8S-ITS2 ribosomal DNA (ITS) was amplified using primers ITS1F, ITS4B, and ITS4 (Gardes and Bruns 1993, White *et al.* 1990) in various combinations (ITS4, ITS5; ITS1F, ITS4B; ITS1F, ITS4). These primer pairs did not work equally well for all taxa and needed to be changed several times during this study. 1 µl of DNA extract was used for amplification. The amount of extract was sometimes adjusted, depending on the efficiency of the extraction procedure. When standard amplifications failed, RedMix Plus mixture (Gene Choice, PGC) was usually successful during subsequent attempts using the same primers and amplification protocol. The amplification protocol was: 4 mins at 94 °C, 1 cycle; 1 min at 94 °C, 1 min at 52 °C 1 min at 72 °C, 35 cycles; 3 mins at 72 °C, 1 cycle; hold at 4 °C. Five µl of the PCR product was then examined by gel electrophoresis (in 1.5 % TBE agarose gel) to confirm amplification.

Primers and unincorporated nucleotides were removed from the PCR product by digestion with ExoSAP-IT (Amersham Biosciences) following manufacturer's directions. Sequencing was performed using the ABI Big Dye Terminator Cycle Sequencing Kit Version 3.1. Sequencing primers were ITS4 and ITS5. Depending on the quality of the sequence, both forward and reverse primers may have been used to form an overlapping contig sequence.

The sequencing reaction was cleaned with a Sephadex G-50 column to remove dyes, dried in a spinvac, and sequenced using an automated ABI 3100 DNA sequence (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc.).

Data Analysis

Sequences were aligned manually using SEQLAB in the Genetics Computer Group package (GCG 2000), followed by analysis using maximum parsimony (MP) and neighbor-joining (NJ) in PAUP* 4.0 (Swofford 2001). Gaps were treated as missing data because of uncertain alignment of some sequences near gap regions. 1000 bootstrap

replicates were performed for a 50 % majority rule consensus trees. The trees were estimated using an heuristic search and retaining branches consistent with the 50 % majority rule. Sequence addition = furthest. The NJ tree was estimated using Jukes-Cantor as the nucleotide substitution model. Trees were visualized in TreeView (Page 1996) and edited using Powerpoint (Microsoft Corp.) and Illustrator 10.0 (Adobe). Bootstrap values and support indexes are displayed in the legend of figures.

Choosing an outgroup for *Lentinus* ITS analysis

Examination of preliminary data (Grand 2004: pt. 2), recent large-scale phylogenies (Moncalvo *et al.* 2000, 2002, Thorn *et al.* 2000) and BLAST (Altschul *et al.* 1997) searches were used to decide on *Ganoderma* as the outgroup for the *Lentinus* analysis.

Related taxa included in analysis

Polyporus group *Polyporellus* (Nuñez and Ryvarden 1995) taxa were included in the ITS analysis because of earlier work using rDNA LSU by Binder and Hibbett (2002), Hibbett and Donoghue (2001), Grand (2004: pt. 2), and Krüger (2002) that showed a close relationship to some species of *Lentinus s.s.* Ko and Jung (2002) also found *Polyporus arcularius* (= *Polyporellus arcularius*) to be closely related to *Lentinus* spp. based on a mitochondrial SSU rDNA analysis.

Results

Phylogenetic reconstructions of subg. *Lentinus sensu* Pegler (1983) and related taxa based on ITS rDNA sequences are shown in Figs. 1 and 2. Topology of major clades is similar in both maximum parsimony (Fig. 1) and neighbor-joining (Fig. 2) analyses. There are several large clades that correspond to morphological sections circumscribed by Pegler (1983). Because sects. *Lentinus* and *Tigrini sensu* Pegler (1983) were part of the concentration in this study, more detailed explanation follows below in results and discussion.

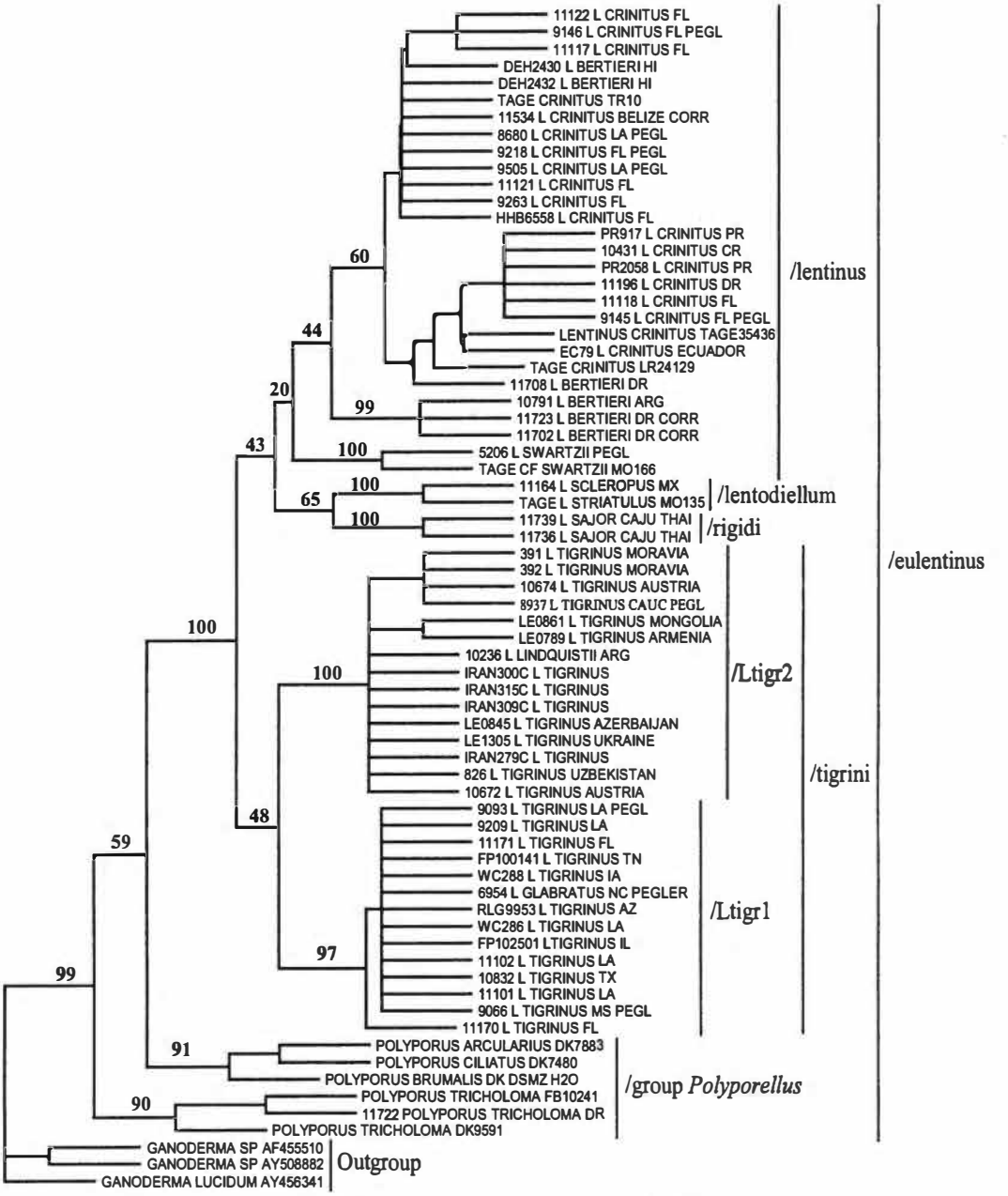

Fig. 1 - Maximum parsimony 50 % majority rule consensus ITS *Lentinus* phylogeny. Bootstrap values are on branches preceding clades. 1000 bootstrap replicates were performed. Tree length = 377, Consistency index (CI) = 0.7507, Homoplasy index (HI) = 0.2493, Retention index (RI) = 0.9170

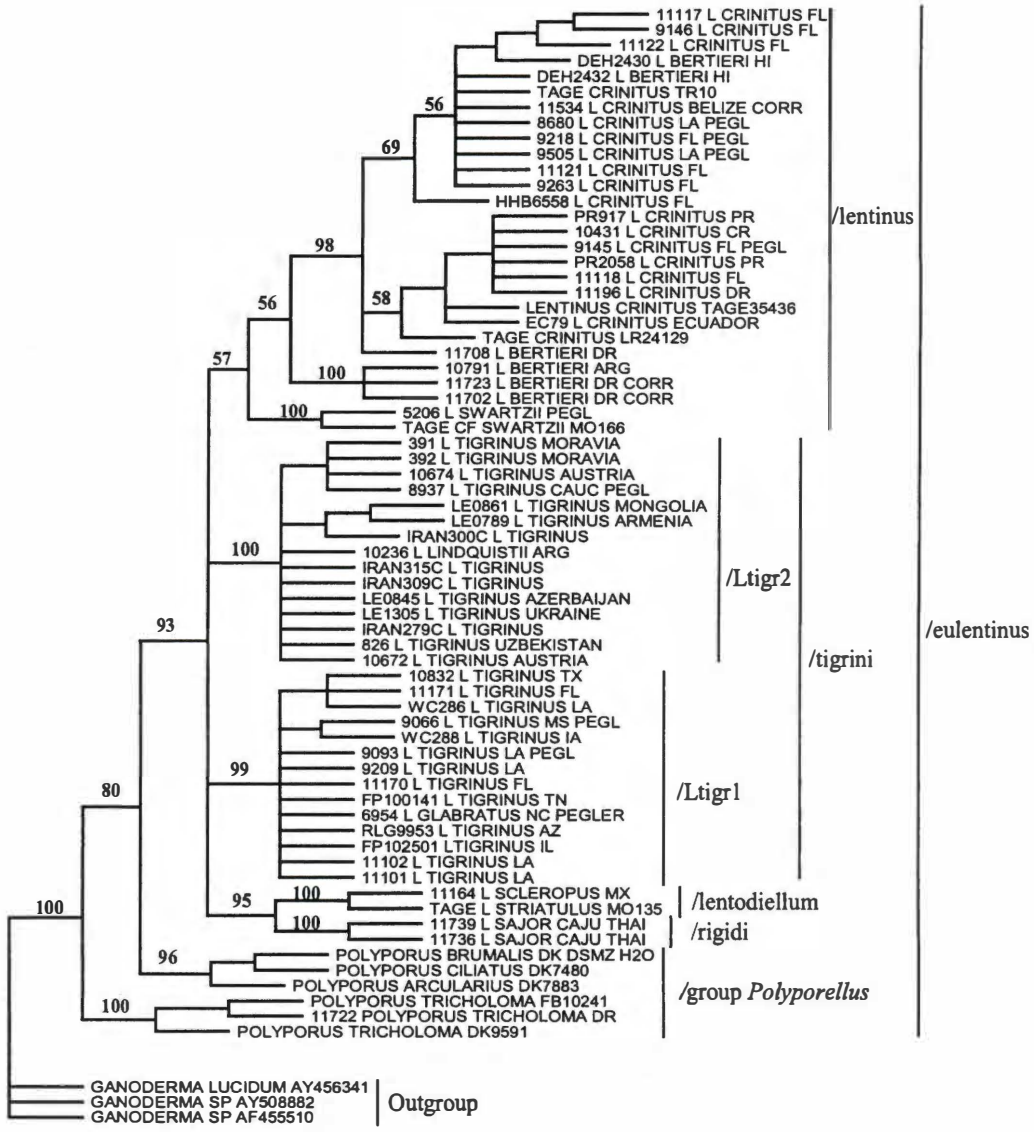

Fig. 2 - Neighbor-joining ITS *Lentinus* phylogeny. Bootstrap values are on branches preceding clades. 1000 bootstrap replicates were performed using the Jukes-Cantor nucleotide substitution model. Tree length = 346.

/eulentinus includes all sampled members of subg. *Lentinus sensu* Pegler (1983) and group *Polyporellus sensu* Nuñez and Ryvarden (1995). This clade is supported a MP bootstrap value of 99 %.

The /lentinus clade corresponds to sect. *Lentinus sensu* Pegler (1983), which includes three species sequenced for this study (*L. bertieri*, *L. crinitus*, *L. swartzii*). All of these sequences reside in /lentinus. The positions of *L. crinitus* and *L. bertieri* change rather easily depending on the sequences included or excluded from the analyses. The sources of this shifting topology are several regions of highly polymorphic DNA located in the non-coding ITS1 and ITS2 regions (see Fig. 3). These polymorphisms do not correspond to any pattern that relates to the morphological species. No biogeographical pattern can be distinguished involving *L. bertieri*, *L. crinitus* or *L. swartzii* sequences used in the ITS analyses.

Two sequences of *L. swartzii* form a highly supported clade (100% bootstrap value) within the larger /lentinus clade. One of these (FB5206) was annotated by David N. Pegler as *L. swartzii*, and the other was contributed by Rolen (2001). The identity of these collections and the nearly complete identity between the sequences indicate that they were correctly identified.

The /tigrini clade contains three morphological species and has bootstrap support of 48 % in the MP analysis (Fig. 1). Two sub-clades (/Ltigr1 and /Ltigr2), each with very high bootstrap support (98 % and 100 %), are embedded within the /tigrini clade. Most of these collections were identified and annotated as *L. tigrinus* (some by D. N. Pegler, see footnotes of Tab. 1). /tigrini also includes two other members of sect. *Tigrini sensu* Pegler (1983); *L. glabratus* Mont. (FB6954) and *L. lindquistii* Lechner and Albertó (2000) (FB10236). The position of these taxa in the /tigrini clade is discussed below.

The /lentodiellum clade contains sequences of one collection of *L. scleropus* (FB11164) from Mexico and one sequence of *L. striatulus* (MO135) contributed by Rolen (2001). Both species are in a clade that is highly supported (100% bootstrap value) and corresponds to sect. *Lentodiellum* of Pegler (1983). Two species in this section were not represented (*L. patulus* Lév. and *L. concavus* (Berk.) P. Henn.) in the data set.

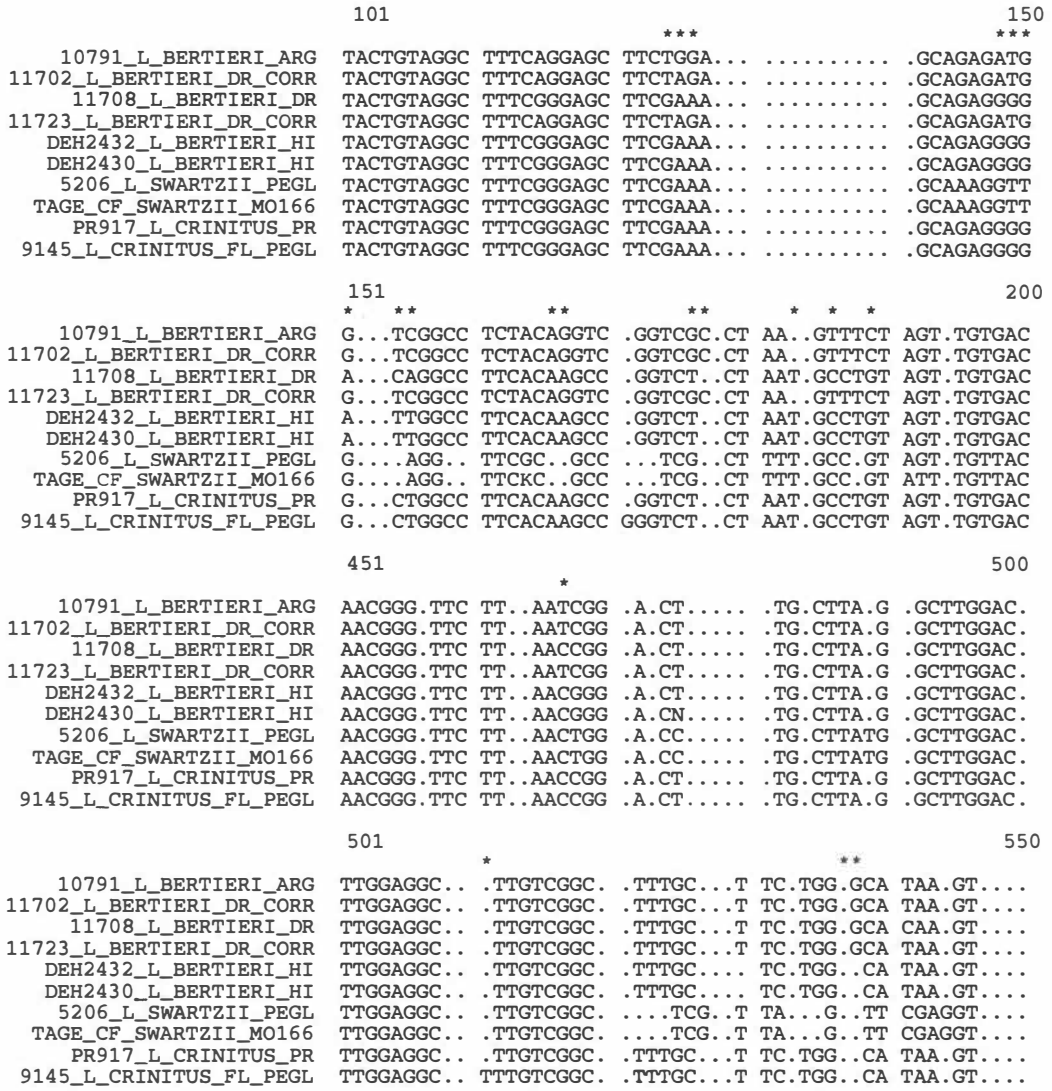

Fig. 3 - ITS hyper-variable region in /lentinus. "*" indicates alignment positions where random polymorphism occurs. Numbers above alignment indicate position in data file.

Two sequences of *L. sajor-caju* are in a clade with 100 % bootstrap support. This is the /rigidi clade in Figs. 1 and 2. No other species or representative sequences for this section were available for this study.

The four species of *Polyporus* group *Polyporellus* (Nuñez and Ryvarden 1995) are all in /group *Polyporellus*. The same sequences are paraphyletic in the MP and NJ analyses (Fig. 1, 2).

Lentinus subg. *Lentinus* sect. *Lentinus sensu* Pegler (1983)

Lentinus crinitus (Linn.: Fr.) Fr.

Pairing experiments of *L. crinitus* completed in this study are shown in Tab. 2. Using collections identified morphologically as *L. crinitus* and two frequently confused taxa (*P. strigellus* Berk. and *L. bertieri*), pairing experiments were done to ascertain biological compatibility. The results of these experiments (n = 4) indicate that among three collections of *L. crinitus* from Florida, and one from Belize (FB11534), there is a single intersterility group. Pairings among collections of the *L. crinitus* intersterility group and negative controls (*P. strigellus* and *L. bertieri*) were all incompatible as judged by the absence of clamp connections (n = 4). The pairing labeled "Self" was between single basidiospore cultures obtained from basidiomata collected in very close proximity. The incomplete compatibility of the cross suggests that the two collections shared one parent mycelium. If this was the case, some spores (and the cultures grown from them) likely contained the same mating alleles and therefore would not form clamped, dikaryotic mycelium.

L. crinitus was found to be conducive to fruiting *in vitro* by Grand (2004) and others (Hibbett *et al.* 1993, 1994). Production of fertile basidiomata was accomplished for two dikaryon cultures; FB11122 and MUCL41626. The ontogeny of these basidiomes was gymnocarpic as in most members of the Aphyllophorales (Singer 1975).

Tab. 2 - *L. crinitus, L. bertieri* and *P. strigellus* intercollection pairings.

		Lentinus crinitus USA, Florida FB11117	*Lentinus crinitus* USA, Florida FB11121	*Lentinus crinitus* USA, Florida FB11122	*Lentinus crinitus* Belize FB11534
Lentinus crinitus USA, Florida	FB11117	NA	4/4	4/4	
Lentinus crinitus USA, Florida	FB11121		NA	2/4 Self	4/4
Lentinus crinitus USA, Florida	FB11122			NA	4/4
Lentinus crinitus Belize	FB11534				NA
Lentinus bertieri Dominican Republic Negative control	FB11708		0/4	0/4	
Panus strigellus Mexico, Calakmul Negative control	FB11161	0/4			0/4

Lentinus bertieri

Lentinus bertieri and *L. swartzii* are two morphospecies in *Lentinus* sect. *Lentinus* that were available for this study. Three putative collections of *L. bertieri* from the Dominican Republic were paired amongst themselves (Tab. 3). Almost 100% compatibility was exhibited (n = 4). The two incompatible pairings involved an isolate that was most likely a contaminant (*Penicillium* sp.). One collection of *L. bertieri* (FB11708) was also paired with known *L. crinitus* isolates (n = 4, see Tab. 2). All of these pairings were incompatible, indicating a barrier to gene flow between these collections of *L. crinitus* and *L. bertieri*.

Lentinus swartzii

Although herbarium collections and dikaryon cultures of *L. swartzii* were available, haploid SBIs were not. Morphological and sequence analysis was done, but no pairings were attempted.

Tab. 3 - *Lentinus bertieri* intercollection crosses.

		Lentinus bertieri Dominican Republic FB 11702	*Lentinus bertieri* Dominican Republic FB 11708	*Lentinus bertieri* Dominican Republic FB 11723
Lentinus bertieri Dominican Republic	FB 11702	NA		
Lentinus bertieri Dominican Republic	FB 11708	3/4	NA	
Lentinus bertieri Dominican Republic	FB 11723	4/4	3/4	NA

Lentinus subg. *Lentinus* sect. *Tigrini sensu* Pegler (1983)

Lentinus tigrinus

Collections used for intercompatibility studies and the ITS data set are listed in Tab. 1. The collections were from widely dispersed geographic origins. ITS sequence data indicated that there were two strongly supported clades within the larger /tigrini clade which is supported by a bootstrap value of 48 % (Fig. 1). These two clades correspond geographically to North American (/Ltigr1 clade) and Eurasian (/Ltigr2 clade) distributions.

Twelve collections of the *L. tigrinus* morphospecies were paired in various combinations (n = 4 or 8, see Tab. 4). This indicates a widespread potential for genetic exchange among these collections. Two collections, *Panus lecomtei* (FB7980) and *Panus conchatus* (FB10642), were used as negative controls. Both of these pairings were incompatible.

In one case, the dikaryon from a successful pairing was isolated. The "hybrid" dikaryon was formed between *L. tigrinus* (FB8937-3) and *L. lindquistii* (FB10236-1). After growing the dikaryon and fruiting *in vitro*, basidiomata were obtained. These basidiomata released viable spores that germinated and grew in a manner like *L. tigrinus*. The basidiomata formed were also macromorphologically *L. tigrinus* (FB11188).

Tab. 4 - *Lentinus tigrinus, L. lindquistii,* and *Panus* spp. intercollection crosses.

Geographic origin		Mississippi FB9066	Louisiana FB9093	Louisiana FB11101	Louisiana FB11102	Texas FB10832	Austria FB10672	Austria FB10674	Caucasus FB8937	Armenia LE0789	Azerbaijan LE0845	Mongolia LE0861	Ukraine LE1305	Argentina "Lentinus lindquistii" FB10236
Mississippi	FB9066	NA	4/4	-	-	4/4	4/4	-	8/8	7/8	8/8	6/8	-	-
Louisiana	FB9093		NA	4/4	4/4	4/4	4/4	-	8/8	8/8	4/4	8/8	4/4	-
Louisiana	FB11101			NA	4/4	4/4	-	-	3/4	-	-	-	4/4	4/4
Louisiana	FB11102				NA	4/4	-	-	1/4	-	-	-	4/4	4/4
Texas	FB10832					NA	4/4	-	8/8	8/8	3/4	8/8	4/4	4/4
Austria	FB10672						NA	4/4	4/4	4/4	4/4	4/4	-	-
Austria	FB10674							NA	-	4/4	3/4	4/4	-	4/4
Caucasus	FB8937								NA	4/4	4/4	4/4	3/4	-
Armenia	LE0789									NA	3/4	-	-	4/4
Azerbaijan	LE0845										NA	3/4	-	-
Mongolia	LE0861											NA	-	-
Ukraine, Kiev Region	LE1305												NA	4/4
Argentina "Lentinus lindquistii"	FB10236													NA
Panus lecomtei	FB7980												Negative control	0/4
Panus conchatus	FB10642												Negative control	0/4

Lentinus lindquistii (Sing.) Lechner & Albertó (= *Pleurotus lindquistii* Singer)

The SBIs available for this study (FB10236) were obtained from an *in vitro* fruited dikaryon culture that was derived from the type collection of *Lentinus lindquistii* (Sing.) Lechner & Albertó. Research has shown that *Lentinus lindquistii* (Sing.) Lechner & Albertó is sexually intercompatible with *L. tigrinus* isolates of world-wide distribution (Tab. 4). Four SBIs from *L. lindquistii* (FB10236) were paired with those of six other collections of *L. tigrinus* and shown to be 100 % intercompatible.

Lentinus glabratus Mont.

One collection (FB6954) and associated cultures of *L. glabratus* were available for this study. The ITS sequence of this collection is identical to several other collections of the *L. tigrinus* morphospecies. This collection of *L. glabratus* is from North Carolina, USA and is part of /Ltigr1. /Ltigr1 contains all of the North American collections of *L. tigrinus* and *L. glabratus*.

Genus *Polyporus* group *Polyporellus sensu* Nuñez and Ryvarden (1995) (= *Polyporellus* Karst. 1880)

ITS analyses (Figs. 1, 2) showed four species of group *Polyporellus sensu* Nuñez and Ryvarden (1995) sister to other clades containing members of subg. *Lentinus sensu* Pegler (1983). In maximum parsimony and neighbor-joining phylogenetic reconstructions, /group *Polyporellus* is paraphyletic (Figs. 1, 2). In the analyses, *P. arcularius*, *P. brumalis* and *P. ciliatus* are more closely related to subg. *Lentinus sensu* Pegler (1983) than the three sequences of *P. tricholoma.*

Discussion

Lentinus subg. *Lentinus* sect. *Lentinus sensu* Pegler (1983)

The /lentinus clades shown in Figs. 1 and 2 contain all sequences of sect. *Lentinus sensu* Pegler (1983). Ribosomal ITS data indicate a high level of sequence polymorphism among the *L. swartzii*, *L. bertieri*, and *L. crinitus* collections used in this study. The weak support of /lentinus (MP bootstrap value = 20 %, NJ bootstrap value = 57 %) is caused by the highly polymorphic and chaotic base substitutions in certain regions of the ITS sequences (see Fig. 3). Because of the weak support for /lentinus, it is impractical to draw biogeographical conclusions based on this data.

Fig. 3 shows several of the ITS sequence regions that make correlating morphological species with phylogenetic clades difficult. Pegler (1983) reported *L. swartzii*, *L. bertieri*, and *L. crinitus* morphospecies only from tropical America. The geographical locations of material available for this study are centered around the Caribbean and the Gulf of Mexico (except DEH2430 and DEH2432 from Hawaii).

One particular case in the phylogenetic reconstructions where this disarray is particularly evident is with the morphospecies *L. bertieri.* The six sequences of *L. bertieri* appear in several different positions in maximum parsimony and neighbor-joining phylogenetic reconstructions (Figs. 1 and 2). This is caused by the same polymorphism (*e.g.* Fig. 3) that does not allow morphospecies to be correlated with phylogenetic clades in sect. *Lentinus sensu* Pegler (1983).

Three collections of *L. bertieri* (FB11702, FB11708, FB11723) from the Dominican Republic were shown to be biologically conspecific (Tab. 3). Although these three collections were sexually intercompatible (*i.e.* form clamps), one of the three sequences (FB11708) is contained in another phylogenetic clade consisting of all *L. crinitus* collections. This disjunction between morphological species and phylogenetic clades is characteristic in both maximum parsimony and neighbor-joining ITS analyses of sect. *Lentinus sensu* Pegler (1983).

Two representative of *Lentinus swartzii* were available for this study. One collection was annotated by Pegler (FB5206). The sequence for this collection, along

with another obtained from Rolen (2001), together form a clade that is strongly supported (100 % bootstrap value, Figs. 1, 2). More sequences would be helpful in elucidating the relationship of *L. swartzii* to other taxa in sect. *Lentinus*, and in determining if this morphospecies also exhibits the same level of ITS polymorphism as *L. crinitus* and *L. bertieri*.

An explanation for the confusion in /lentinus could be that these morphospecies have recently diverged from a common gene pool that was highly variable. Although distinguishable based on morphology, these taxa still share the variability in ITS sequences that was present in their common ancestor.

The three morphospecies in /lentinus typically fruit on small pieces of wood. This ecotype would allow for convenient dispersal by water (*e.g.* floating wood). Considering the geographic proximity of most collections and their accessibility to water, the random ITS sequence patterns may be a result of hybridization among infraspecific groups. Hybridization between morphospecies was not shown in this study using *L. crinitus* and *L. bertieri*, but was only attempted twice (Tab. 2).

Lentinus subg. *Lentinus* sect. *Tigrini sensu* Pegler (1983)

Lentinus sect. *Tigrini sensu* Pegler (1983) contains six morphospecies according to Pegler's taxonomic scheme (1983). In ITS analyses, I also included *L. lindquistii* which was not included in sect. *Tigrini* by Pegler, but was noted to be closely related to the type for his section, *L. tigrinus* (Bull.: Fr.) Fr., by Lechner and Albertó (2000).

Both maximum parsimony and neighbor-joining analyses show two highly supported clades of *L. tigrinus* sequences (/Ltigr1 and /Ltigr2). These two subclades are part of the larger /tigrini clade that corresponds to sect. *Tigrini sensu* Pegler (1983). All morphological species of Pegler's sect. *Tigrini* sequenced in this study are contained in /tigrini.

Within the /tigrini clade there is clear phylogeographic signal among ITS sequences. There are many polymorphic sites among these sequences. At nearly every polymorphic site the North American collections all have the same base, while the

	*191
9093 Lentinus tigrinus, Louisiana, USA	GTT CTT ACG TCGTG GTTG
WC288 Lentinus tigrinus, Iowa, USA	GTT CTT ACG TCGTG GTTG
10832 Lentinus tigrinus, Texas, USA	GTT CTT ACG TCGTG GTTG
11171 Lentinus tigrinus, Florida, USA	GTT CTT ACG TCGTG GTTG
LE0845 Lentinus tigrinus, Azerbaijan	GTTT CTT ACG CCGGA GTTG
LE1305 Lentinus tigrinus, Ukraine	GTTT CTT ACG CCGGA GTTG
IRAN279C Lentinus tigrinus, Iran	GTTT CTT ACG CCGGA GTTG
10672 Lentinus tigrinus, Austria	GTTT CTT ACG CCGGA GTTG

Fig. 4 – Portion of *Lentinus tigrinus* ITS sequence.

European and Asian isolates consistently contain a different base (*i.e.* G for North Americans and A for the Europeans and Asians at character *191, see Fig. 4). These consistent base change sites represent 84% of the polymorphic sites (26 total) between the two geographically correlated clades. This polymorphism provides very strong support for /Ltigr1 (North American collections; MP bootstrap value = 97 %) and /Ltigr2 (European and Asian collections; MP bootstrap value = 100 %) in both maximum parsimony (Fig. 1) and neighbor-joining (Fig. 2) phylogenies.

Sequence analysis of the ITS1-5.8S-ITS2 nuclear rDNA suggests that widely scattered geographical populations of the *L. tigrinus* complex are genetically divergent, but are still capable of exchanging genetic material when they contact each other *in vitro* (Tab. 4). Although sequence differences imply some type of barrier to gene flow, that separation is not old enough to prevent recognition within the *L. tigrinus* intersterility group (Tab. 4). The disappearance of the Bering Straight land bridge (10,000 - 20,000 years ago) or the north Atlantic land bridge (3-4 million years ago) may have provided a geographical barrier to gene flow between North America and Eurasia.

Lentinus lindquistii Lechner and Albertó (2000)

Lentinus lindquistii is a morphospecies belonging to the "*L. tigrinus*" complex (Lechner and Albertó 2000). It was originally described from Argentina by Singer (1960) as *Pleurotus lindquistii*. After examining basidiomes fruited *in vitro* from the *ex typus* dikaryon culture, Lechner and Albertó (2000) transferred it to *Lentinus*. Data shows this taxon to be intercompatible with six *L. tigrinus* collections of worldwide distribution (Tab. 4). The collections paired with *L. lindquistii* include three from the North American clade (/Ltigr1) and three from the Eurasian clade (/Ltigr2). The SBIs of *L. lindquistii* did not form clamped dikaryotic hyphae after contact with two negative controls.

Although this collection of *L. lindquistii* is from Argentina, the ITS sequence data suggests that it is closely related to the Eurasian members (/Ltigr2) of the complex. This biogeographical pattern was also noted by Hughes *et al.* (1999) when examining the *Flammulina velutipes* complex. Vilgalys (1991), using the *Collybia dryophila* complex, noted high DNA polymorphism within the same intersterility groups from different continents, while different intercompatibility groups from the same continent were more similar. These studies and data involving the *L. tigrinus* complex indicate that intersterility may not be directly correlated to sequence divergence in the ITS region.

Testing of F_1 compatible pairings for production of viable basidiomata (*i.e.* fertility) was completed in one case. The dikaryon product of one pairing using *L. lindquistii* (FB 10236) and *L. tigrinus* (FB8937) was isolated and grown on a fresh agar plate. After following the procedure for *in vitro* fruiting outlined in materials and methods, basidiomata were obtained from this pairing. The basidiomata produced were macromorpholocially *L. tigrinus* and produced viable spores that germinated and grew similarly to those of other collections of *L. tigrinus*.

LSU sequence of *L. lindquistii* is almost completely identical to well-defined members of the *L. tigrinus* morphospecies (Grand 2004: pt. 2). The results of the ITS and LSU sequence analyses, intercompatibility experiments, and production of viable F_1 basidiomata indicates that *L. lindquistii* should be synonymized under *L. tigrinus*.

Lentinus tigrinus is thought to be a North Temperate species (Pegler 1983). Based on the results of this study, this geographical restriction is inaccurate. *L. lindquistii* from Argentina was shown to be biologically (Tab. 4) and molecularly conspecific with *L. tigrinus* (Figs. 1, 2). Morphology of *in vitro* fruited material of *L. lindquistii* is similar to that of *L. tigrinus*, but the generative hyphae are not as inflated and there are slight differences in spore size and cheilocystidia (Lechner and Albertó 2000). These differences lead Lechner and Albertó (2000) to transfer the species to subg. *Lentinus* sect. *Tigrini sensu* Pegler (1983), but were not enough to warrant synonymization under *L. tigrinus*. Synonymization of *L. lindquistii* would extend the range of *L. tigrinus* into South America.

Lentinus glabratus Mont.

Lentinus glabratus is a member of sect. *Tigrini* separated by Pegler (1983) based on a more glabrous pileus and stronger intervenation of the lamellae than its close relative, *L. tigrinus*. A collection confirmed morphologically by Pegler (FB9854) as *L. glabratus* was shown to be completely identical to other collections of the *L. tigrinus* morphospecies in ITS sequence. The sequence is in the /Ltigr1 clade (Figs. 1 and 2) which contains other collections from North America. LSU sequence also places *L. glabratus* in the same clade that contains five sequences of the *L. tigrinus* morphospecies (and *L. lindquistii*). These data are shown in Grand (2004: pt. 2, Figs. 1, 2).

This collection of *L. glabratus* (FB9854) was made in North Carolina, USA, where *L. tigrinus* is also known to occur. Because of the morphological differences, homology in ITS, LSU, and mtCOX3 sequences, and geographic overlap, *L. glabratus* should be synonymized under *L. tigrinus* (Bull.: Fr.) Fr.

Genus *Lentinus* subg. *Lentinus* sect. *Rigidi sensu* Pegler (1983)

The representative sequences for this section are two collections of *Lentinus sajor-caju* (Fr.) Fr. The sequences, both from basidiomata collected in Thailand, form a clade (MP and NJ bootstrap values = 100 %) that is sister to members of sect.

Lentodiellum sensu Pegler (1983). The clade that contains both /rigidi and /lentodiellum is supported by a 65 % bootstrap value in the MP phylogeny (Fig. 1) and a 95 % bootstrap value in the NJ phylogeny (Fig. 2). Sects. *Rigidi* and *Lentodiellum* sensu Pegler (1983) are united by a radiately constructed hymenophoral trama, non-inflated generative hyphae and the presence of lamellae and lamellulae. Sect. *Rigidi* is separated by the presence of hyphal pegs in the hymenium.

Genus *Lentinus* subg. *Lentinus* sect. *Lentodiellum sensu* Pegler (1983)

This section contains *Lentinus scleropus* (Pers.) Fr. and *L. striatulus* Lév. along with two other species not available for this study (*L. patulus* Lév and *L. concavus* (Berk.) P. Henn.). The two members of this section form a clade with a bootstrap value of 100 % in phylogenetic reconstructions using ITS sequence data (Figs. 1, 2). Members of this section are separated morphologically by the absence of hyphal pegs in the hymenium. All other species in subg. *Lentinus sensu* Pegler (except *Lentinus brunneofloccosus* Pegler) have hyphal pegs.

Genus *Polyporus* group *Polyporellus sensu* Nuñez and Ryvarden (1995) (= *Polyporellus* Karst. 1880)

The topology of phylogenetic reconstructions created using species of group *Polyporellus* and other *Polyporus s.l.* changes dramatically depending on the region of DNA sequenced (Binder and Hibbett 2002, Hibbett and Donoghue 2001, Ko and Jung 2002). Correct taxonomic placement for some of these taxa is uncertain. In ITS analyses, four species (six sequences) were representative of the *Polyporellus* group (Nuñez and Ryvarden 1995). The four species form clades that are sister to the /eulentinus, which contains all other members of subg. *Lentinus sensu* Pegler (1983) sampled in this study.

/eulentinus includes all members of Pegler's (1983) subg. *Lentinus*, along with /group *Polyporellus*, and is well-supported (MP bootstrap value = 99 %). Krüger (2002)

sampled group *Polyporellus* (Nuñez and Ryvarden 1995) and *Polyporus s.l.* more extensively and found that three LSU sequences of *L. tigrinus* clustered among his "*Polyporellus*" LSU sequences. LSU sequence data (Grand 2004: pt. 2) also indicate that group *Polyporellus* (Nuñez and Ryvarden 1995) is within the same clade that (/eulentinus of Grand 2004: pt. 2) includes all members of subg. *Lentinus sensu* Pegler (1983).

Group *Polyporellus* (Nuñez and Ryvarden 1995) is paraphyletic in MP and NJ analyses (Figs. 1, 2). Within /eulentinus, three sequences of *P. tricholoma* are separated into a different subclade that is sister to the rest of /eulentinus.

A relationship between members of group *Polyporellus* (Nuñez and Ryvarden 1995) and subg. *Lentinus sensu* Pegler (1983) has been suspected (Nuñez and Ryvarden 1995, Pegler 1983), especially between group *Polyporellus* (Nuñez and Ryvarden 1995) and sect. *Tigrini sensu* Pegler (1983). Members of group *Polyporellus* (Nuñez and Ryvarden 1995) typically have inflated generative hyphae, similar to members of subg. *Lentinus* sect. *Tigrini* (Pegler 1983). Krüger (2002) suggested nomenclatural changes and emendation of *Lentinus* to allow for pored species. Sequence data and other common characteristics such as tetrapolar mating systems (Petersen *et al.* 1997) and dimitic hyphal construction (with skeletal-ligative hyphae) provide corroborating evidence that these taxa are related, but taxonomic and nomenclatural changes are not suggested in this work.

Acknowledgements

I would like to thank Dr. Nadya Psurtseva, Komarov Institute, St. Petersburg, Russia for spore prints from LE0789, LE0845, LE0861, and LE1305. Funding was provided by NSF-PEET Grants DEB 9521526 and DEB 9978011.

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Part 4 – Biogeography and species concepts in the genus *Panus* Fr., with emphasis on *Panus lecomtei* and *Panus conchatus*.

Abstract

Members of *Lentinus* subg. *Panus sensu* Pegler (1983) were analyzed in this study. Taxa from two sections were included (sects. *Panus* and *Velutini*). This study concentrated on the circumglobal species complex *Panus lecomtei* Fr. to access biogeographical relationships in that group. Sexual intercompatibility studies indicated that seven collections of this complex formed a cohesive intersterility group. Ribosomal ITS sequence data for all collections of *P. lecomtei* Fr. sampled were nearly 100 % identical. Two collections of *Panus fragilis* O. K. Miller (1965) were also included and found to be conspecific with *P. lecomtei* Fr. based on ITS and LSU sequence data. Because of the macromorphological similarity of *Panus conchatus* and *P. lecomtei*, data from both species were collected. Eight collections of *P. conchatus* were shown to form an intersterility group. Other species of subg. *Panus sensu* Pegler (1983) were sequenced for ITS data, but not used for intercompatibility studies. These species include: *Panus ciliatus* Lév. (= *Lentinus ciliatus* Lév. *sensu* Pegler 1983), *Panus strigellus* Berk. (= *Lentinus strigellus* Berk. *sensu* Pegler 1983), *Panus fulvus* (Berk.) Pegler and Rayner (= *Lentinus velutinus sensu* Pegler 1983), and *Panus similis* Berk. and Br. (= *Lentinus similis sensu* Pegler 1983). *Lentinus suavissimus* Fr., group *Polyporellus* (Nuñez and Ryvarden 1995), *Ganoderma* and *Neolentinus* Redhead and Ginns (1985) were included to explore possible supra-generic relationships.

Introduction

Pegler (1983) recognized *Panus* Fr. as a subgenus of *Lentinus* Fr., but other authors (Corner 1981, Donk 1960, Singer 1975, 1986, Redhead and Ginns 1985) have recognized *Panus* and *Lentinus* at the generic level. Pegler combined *Lentinus* and *Panus* based on dimitic hyphal construction. *Lentinus* subg. *Panus* was distinguished by the presence of skeletal hyphae, as opposed to the skeletal-ligative hyphae of subg. *Lentinus sensu* Pegler (1983). Subg. *Panus* Fr. *sensu* Pegler (1983) contained 36 morphospecies, separated into nine sections.

The systematics of *Panus* and the closely allied *Lentinus* are often misunderstood. A variety of taxonomic schemes and nomenclatural changes have been proposed. *Panus* has been typified with *Panus conchatus* (Bull.: Fr.) Fr. (International Code of Botanical Nomenclature 2000). Different generic circumscriptions and ranking of taxa provide difficulties in finding the correct name for a taxon.

Lentinus sensu Pegler (1983) was investigated using ITS (here) and LSU (Grand 2004: pt. 2) sequence data. This paper deals with subg. *Panus sensu* Pegler (1983), without members of subg. *Lentinus* (except *L. suavissimus* Fr. and transferred *Neolentinus* spp.).

Lentinus Fr. and *Panus* Fr. were separable based on LSU data analysis (Grand 2004: pt. 2). Using ITS data, some of the sections within *Panus* were explored. Putatively related taxa, and species that have been transferred from *Panus* were also included to examine their possible relationships to *Panus*.

Nine species of subg. *Panus sensu* Pegler (1983) were transferred to *Neolentinus* (Redhead and Ginns 1985). The separation of these species was based on binucleate spores and the ability to form brown rots (Redhead and Ginns 1985). One species (*Lentinus sulcatus* Berk.) was transferred to *Heliocybe* by Redhead and Ginns (1985), but subsequently transferred to *Neolentinus* (Rune 1994).

Some species of *Panus* have also been transferred to *Pleurotus* (Fr.) Kummer. *Pleurotus* is now confined to species that produce nematotoxic droplets (Hibbett and Thorn 1994, Isikhuemhen *et al.* 2000, Thorn and Barron 1984, Thorn *et al.* 2000). The segregation of these species [*Pleurotus levis* (Berk. and Curt.) Singer (= *Lentinus levis* (Berk and Curt.) Murr., *Pleurotus tuber-regium* (Fr.) Singer (= *Lentinus tuber-regium* (Fr.) Fr.] is well-supported by recent phylogenetic studies (Hibbett and Donoghue 2001, Moncalvo *et al.* 2000, 2002, Thorn *et al.* 2000).

Transfer of species to well-established and/or newly proposed genera has led to a complex nomenclatural history. *Panus lecomtei* Fr. (1825: 77, Syst. Orb. Veg.) is a good example of this difficulty. The list of published names includes *Lentinus lecomtei* Fr. (1825), *L. strigosus* (Schw.) Fr. (1825), and *Panus rudis* Fr. (1838). Annotations for this species are usually a combination of *Panus* or *Lentinus*, and one of these three species

epithets. Annotation lacking authority has lead to confusion in herbarium collections and the identity of cultures established from them. The acceptance of *Panus* at generic rank in this study and the discussion of Hrouda (2001) led to *P. lecomtei* Fr. as the correct name for this species. *Panus rudis* (Fries 1838) is a commonly used name for this species, but because the epithet *lecomtei* is older (Schweinitz 1822) than *rudis*, it has priority according to Art. 11.4 (International Code of Botanical Nomenclature 2000).

Panus lecomtei and *Panus conchatus* share macromorphological characters such as stature and purplish coloration in young fruitbodies. Because of these overall similarities and geographical overlap of both species, some material of *P. conchatus* was included in this study.

A phylogenetic reconstruction of *Panus* was used to explore the infraspecific groups and the sections of the genus. Because of the circumglobal distribution of *P. lecomtei*, and abundant material available in culture collections, I concentrated on this species to determine if any biogeographical relationships could be inferred.

Pairing studies were also used to determine intersterility groups in *P. lecomtei* and *P. conchatus*. It was a goal of this study to correlate confirmed members of biological intersterility groups and ITS sequence data.

Other species in *Panus* were also sampled to investigate the relationships among sections (*sensu* Pegler 1983). No sexual intercompatibility studies were done with these species.

Materials and methods

Abbreviations and acronyms

Tab. 1 shows collection data for the specimens and cultures used in this study. FB indicates the Tennessee Field Book number and location in the Tennessee culture collection (CULTENN). TENN indicates the dried voucher specimen's location in the University of Tennessee fungal herbarium. If no TENN number is present, only a culture and the received identification were available. FPLM indicates that the culture was obtained from the Forest Products Laboratory culture collection in Madison, Wisconsin.

Tab. 1 - Fungal specimens and cultures examined for ITS data set.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
--	Ganoderma lucidum	AY456341	--	Ganoderma lucidum	NA
--	Ganoderma sp.	AF455510	--	Ganoderma sp.	NA
--	Ganoderma sp.	AY508882	--	Ganoderma sp.	NA
LE127[1]	Lentinus suavissimus	--	Russia, Chelyabinsk Region	Lentinus suavissimus	subg. *Lentinus* sect. *Pleuroti*
FB11129 (TENN59670)	Lentinus suavissimus	--	France, Chambery Municipality	Lentinus suavissimus	subg. *Lentinus* sect. *Pleuroti*
FB11130 (TENN59671)	Lentinus suavissimus	--	France, Chambery Municipality	Lentinus suavissimus	subg. *Lentinus* sect. *Pleuroti*
FB11656 (TENN59822)	Lentinus suavissimus	--	Russia	Lentinus suavissimus	subg. *Lentinus* sect. *Pleuroti*
FB11315 (TENN59823)	Neolentinus lepideus	AFXXXX	USA, New Mexico	Neolentinus lepideus	subg. *Panus* sect. *Squamosi*
FB11717 (TENN59777)	Neolentinus lepideus	--	Dominican Republic	Neolentinus lepideus	subg. *Panus* sect. *Squamosi*
FB11104 (TENN59647)	Neolentinus adhaerens	--	USA, Tennessee	Neolentinus adhaerens	subg. *Panus* sect. *Pulverulenti*
FB11726 (TENN59783)	Neolentinus adhaerens	--	USA, Tennessee	Neolentinus adhaerens	subg. *Panus* sect. *Pulverulenti*
FB10293 (TENN59824)	Neolentinus schaefferi	--	Austria	Lentinus cyathiformis	subg. *Panus* sect. *Squamosi*
FB11728 (TENN59785)	Panus ciliatus	--	Thailand, Chiang Mai Province	Lentinus ciliatus	subg. *Panus* sect. *velutini*
FB11729 (TENN59786)	Panus ciliatus	--	Thailand, Chiang Mai Province	Lentinus ciliatus	subg. *Panus* sect. *velutini*
FB11737 (TENN59808)	Panus ciliatus	--	Thailand, Chiang Mai Province	Lentinus ciliatus	subg. *Panus* sect. *velutini*
FB11755 (DEH2430A)	Panus ciliatus	--	USA, Hawaii	Lentinus ciliatus	subg. *Panus* sect. *velutini*
FB11105 (TENN59648)	Panus conchatus	--	USA, Tennessee	Lentinus torulosus	subg. *Panus* sect. *panus*
FB10642 (TENN58250)	Panus conchatus	--	Russia, Leningrad Region	Lentinus torulosus	subg. *Panus* sect. *panus*
FB6254 (TENN52912)	Panus conchatus	--	USA, Louisiana	Lentinus strigellus[2]	subg. *Panus* sect. *panus*
OKM2412[3]	Panus conchatus	--	USA, Idaho	Panus conchatus	subg. *Panus* sect. *panus*
FB4314 (TENN50394)	Panus conchatus	--	Switzerland	P. conchatus	subg. *Panus* sect. *panus*
FB9886 (TENN59519)	Panus conchatus	--	Austria	Panus conchatus	subg. *Panus* sect. *panus*

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB9353 (TENN59825)	Panus conchatus	--	USA, Tennessee	Panus sp.	subg. *Panus* sect. *panus*
LCF573[3]	Panus fulvus	--	Argentina, Puerto Londero Missiones	Lentinus velutinus	subg. *Panus* sect. *velutini*
FB10689 (TENN58776)	Panus fulvus	--	USA, Texas	Lentinus velutinus	subg. *Panus* sect. *velutini*
TageIL295	Panus fulvus	--	Costa Rica	Panus fulvus	subg. *Panus* sect. *velutini*
TageMU152	Panus fulvus	--	Costa Rica	Panus fulvus aff. fulvus	subg. *Panus* sect. *velutini*
FB9888 (LE59)[1]	Panus lecomtei	--	Russia, Chelyabinsk Region	Panus rudis	subg. *Panus* sect. *panus*
OKMCHD30684[3]	Panus lecomtei	--	USA, Georgia	Panus fragilis	subg. *Panus* sect. *panus*
OKM6666[3]	Panus lecomtei	--	USA, Montana	Panus lecomtei	subg. *Panus* sect. *panus*
FB10676 (TENN59826)	Panus lecomtei	--	USA, Virginia	Lentinus strigosus	subg. *Panus* sect. *panus*
FB10677 (TENN59827)	Panus lecomtei	--	USA, Tennessee	Lentinus strigosus	subg. *Panus* sect. *panus*
HHB6616[3]	Panus lecomtei	--	USA, Florida	Panus fragilis	subg. *Panus* sect. *panus*
PR1116[3]	Panus lecomtei	--	Puerto Rico	Panus rudis	subg. *Panus* sect. *panus*
FB11125 (TENN59666)	Panus lecomtei	--	USA, Florida	Panus rudis	subg. *Panus* sect. *panus*
FB11126 (TENN59667)	Panus lecomtei	--	USA, Tennessee	Panus rudis	subg. *Panus* sect. *panus*
FB11120 (TENN59661)	Panus lecomtei	--	USA, Tennessee	Panus rudis	subg. *Panus* sect. *panus*
HHB18705[3]	Panus lecomtei	--	USA, Alaska	Panus crinitus	subg. *Panus* sect. *panus*
FB11744 (NN050189)	Panus lecomtei	--	Russia, Kamchatka Region	Panus rudis	subg. *Panus* sect. *panus*
FB11165 (TENN59705)	Panus lecomtei	--	Mexico, Villahermosa	Panus rudis	subg. *Panus* sect. *panus*
FB7980 (TENN54369)	Panus lecomtei	--	USA, Minnesota	Panus rudis	subg. *Panus* sect. *panus*
FB11319 (TENN59828)	Panus lecomtei	--	USA, New Mexico	Panus rudis	subg. *Panus* sect. *panus*
FB5525 (TENN51805)	Panus lecomtei	--	USA, North Carolina	Lentinus strigosus[1]	subg. *Panus* sect. *panus*
TageTR25	Panus lecomtei	--	Costa Rica	Panus lecomtei	subg. *Panus* sect. *panus*
TageAR3082	Panus lecomtei	--	French Guyana	Panus lecomtei	subg. *Panus* sect. *panus*

Tab. 1 - Continued.

Strain numbers and/or herbarium voucher numbers if known	Species in tree	GenBank accession number and study	Geographic origin	Received as	Pegler (1983) subgenus and section
FB10747 (TENN58955)	Panus similis	--	Argentina, Province Missiones	Lentinus similis	subg. *Panus* sect. *velutini*
FB11302 (TENN59008)	Panus similis	--	Argentina	Lentinus similis	subg. *Panus* sect. *velutini*
LR41455	Panus similis	--	Venezuela	Panus similis	
FB9854 (TENN59829)	Panus similis	--	Argentina, Province Missiones	Lentinus similis	subg. *Panus* sect. *velutini*
FB9215 (TENN55993)	Panus strigellus	--	USA, Louisiana	Lentinus strigellus[1]	subg. *Panus* sect. *panus*
FB9114 (TENN59830)	Panus strigellus	--	USA, Louisiana	Lentinus strigellus[1]	subg. *Panus* sect. *panus*
FB11161 (TENN59701)	Panus strigellus	--	Mexico	Lentinus strigellus	subg. *Panus* sect. *panus*
FB9118 (TENN56189)	Panus strigellus	--	USA, Louisiana	Lentinus strigellus[1]	subg. *Panus* sect. *panus*
FB8762 (TENN55190)	Panus strigellus	--	Mexico, San Blao	Lentinus strigellus[1]	subg. *Panus* sect. *panus*
TageTR31	Panus strigellus	--	Costa Rica	Panus strigellus	subg. *Panus* sect. *panus*
TageTR32	Panus cf. strigellus	--	Costa Rica	Panus strigellus	subg. *Panus* sect. *panus*
TageTR39	Panus strigellus	--	Costa Rica	Panus strigellus	subg. *Panus* sect. *panus*
--	Phlebia albida	AY219368	--	Phlebia albida	NA
--	Phlebia albomellea	AY219369	--	Phlebia albomellea	NA
--	Phlebia chrysocrea	AY219367	--	Phlebia chrysocrea	NA
--	Phlebia radiata	AY219366	--	Phlebia radiata	NA
FB7883 (TENN53747)	Polyporus arcularius	AF516524	Costa Rica	Polyporus arcularius	NA
DSMZ-H17 (DAOM 31983)	Polyporus brumalis	AB070870	Canada	Polyporus brumalis	NA
FB7480 (TENN53639)	Polyporus ciliatus	AB070880	Finland	Polyporus ciliatus	NA
FB9591 (TENN56503)	Polyporus tricholoma	AJ132941	Puerto Rico	Polyporus tricholoma	NA
FB10241 (TENN57564)	Polyporus tricholoma	AF516541	Costa Rica	Polyporus tricholoma	NA
FB11722 (TENN59780)	Polyporus tricholoma	--	Dominican Republic	Polyporus tricholoma	NA

[1]Culture obtained from Leningrad Culture Collection

[2]Annotated by D. N. Pegler

[3]Culture obtained from FPLM

Morphology

Micromorphological observations and putative species designations followed those of Pegler (1983), and Corner (1981). Field collection and processing techniques followed those of Largent *et al.* (1977), Largent (1986), and Largent and Baroni (1988).

Culture techniques

Single-basidiospore isolates (SBIs) for sexual intercompatibility studies were obtained following the method of Gordon and Petersen (1991). Another method of obtaining SBIs was to collect a spore print on autoclaved aluminum foil (Petersen and Greilhuber 1996). Before use in intercompatibility studies, each SBI was checked for the absence of clamp connections to verify monokaryon status.

Molecular Techniques

Molecular techniques and data analysis followed those described by Hughes *et al.* (2001) and Cifuentes *et al.* (2003). Monokaryon cultures were chosen for DNA extraction after verification that the culture was clampless. Monokaryons were used to help prevent sequencing problems due to heterozygosity for insertions or deletions (indels) that were sometimes observed when using dikaryons. To obtain fungal tissue suitable for DNA extraction, a *ca.* 0.5 cm^2 piece of inoculant agar was placed in a jar containing PD broth (24 g/L Difco Potato Dextrose Broth) and allowed to grow for several weeks. When the culture reached a diameter of *ca.* 3-4 centimeters, the culture was sacrificed and DNA extracted using the procedure of Cifuentes *et al.* (2003). Dried herbarium tissue was extracted in a similar fashion after a small piece of dried tissue (*ca.* 0.5 cm^2) was ground with the aid of sterile grinding sand.

ITS1-5.8S-ITS2 ribosomal DNA (ITS) was amplified using primers ITS1F, ITS4B, and ITS4 (Gardes and Bruns 1993, White *et al.* 1990) in various combinations (ITS4, ITS5; ITS1F, ITS4B; ITS1F, ITS4). These primer pairs did not work equally well for all taxa and needed to be changed several times during this study. 1 µl of DNA extract was used for amplification. The amount of extract was sometimes adjusted,

depending on the efficiency of the extraction procedure. When standard amplifications failed, RedMix Plus mixture (Gene Choice, PGC) was usually successful during subsequent attempts using the same primers and amplification protocol. The amplification protocol was: 4 mins at 94 °C, 1 cycle; 1 min at 94 °C, 1 min at 52 °C 1 min at 72 °C, 35 cycles; 3 mins at 72 °C, 1 cycle; hold at 4 °C. Five µl of the PCR product was then analyzed by gel electrophoresis (1.5 % TBE agarose gel) to confirm amplification.

Primers and unincorporated nucleotides were removed from the PCR product by digestion with ExoSAP-IT (Amersham Biosciences) following manufacturer's directions. Sequencing was performed using the ABI Big Dye Terminator Cycle Sequencing Kit Version 3.1. Sequencing primers were ITS4 and ITS5. The sequencing protocol was: 0:30 min at 96 °C, 0:15 min at 50 °C, 4 min at 60 °C, 25 cycles; hold at 4 °C. Depending on the quality of the sequence, both forward and reverse primers may have been used to form an overlapping contig sequence.

The sequencing reaction was cleaned with a Sephadex G-50 column to remove dyes, dried in a spinvac, and sequenced using an automated ABI 3100 DNA sequence (ABI Prism Dye Terminator cycle sequencing, Perkin-Elmer, Inc.).

Data Analysis

Sequences were edited and aligned manually using SEQLAB in the Genetics Computer Group package (GCG 2000). Phylogenetic reconstructions using maximum parsimony (MP) and neighbor-joining (NJ) were done in PAUP* 4.0 (Swofford 2001). Gaps were treated as missing data because the length and phylogenetic importance of such regions was uncertain. 1000 bootstrap replicates were performed for a 50 % majority rule consensus trees. The trees were estimated using an heuristic search and retaining branches consistent with the 50 % majority rule. Sequence addition = furthest. The NJ tree was estimated using Jukes-Cantor as the nucleotide substitution model. Trees were visualized in TreeView (Page 1996) and edited using Powerpoint (Microsoft Corp.) and Illustrator 10.0 (Adobe). Bootstrap values and support indexes are displayed in the legend of figures.

Choosing an outgroup for *Panus* ITS analysis

Based on recent phylogenies (Moncalvo *et al.* 2000, 2002, Thorn *et al.* 2000) that used sequences labeled as *Panus* spp., potential outgroups were investigated. Sequences from preliminary data, and taxa shown to be related in these phylogenies, were used to do BLAST (Altschul *et al.* 1997) searches. Four species of *Phlebia* were used as the outgroup for maximum parsimony and neighbor-joining analyses (Figs. 1, 2).

Results

Phylogenetic reconstructions were created using ITS sequence data for subg. *Panus sensu* Pegler (1983), *Neolentinus* spp. previously included in *Panus*, and other species whose taxonomic positions were uncertain (*e.g. L. suavissimus* Fr.). The phylogenetic reconstruction using maximum parsimony as the optimality criterion is shown in Fig. 1. Neighbor-joining algorithms were used to create the phylogeny shown in Fig. 2. The topology of the major clades, and the taxa included in those clades, was identical in both maximum parsimony (MP) and neighbor-joining (NJ) analyses. Bootstrap values and statistical measure are shown in the legend of Figs. 1 and 2. Comments that follow regarding bootstrap values refer to the maximum parsimony analyses unless otherwise noted.

All sampled morphospecies in subg. *Panus sensu* Pegler reside in clade /eupanus. This is highly supported by a 100 % bootstrap value (MP phylogeny). Separation of /eupanus from the outgroup and possible relatives (/nonpanus, *e.g. Neolentinus*) is distinct (MP bootstrap value = 93 %).

Group *Polyporellus* spp. were included to contrast their suspected relationship to subg. *Lentinus* sect. *Tigrini sensu* Pegler (1983). /group *Polyporellus* is sister to */Ganoderma*. *Ganoderma* was included because BLAST searches revealed homology with members of subg. *Lentinus sensu* Pegler (1983). Three sequences form a well-supported monophyletic clade (MP bootstrap value = 92 %).

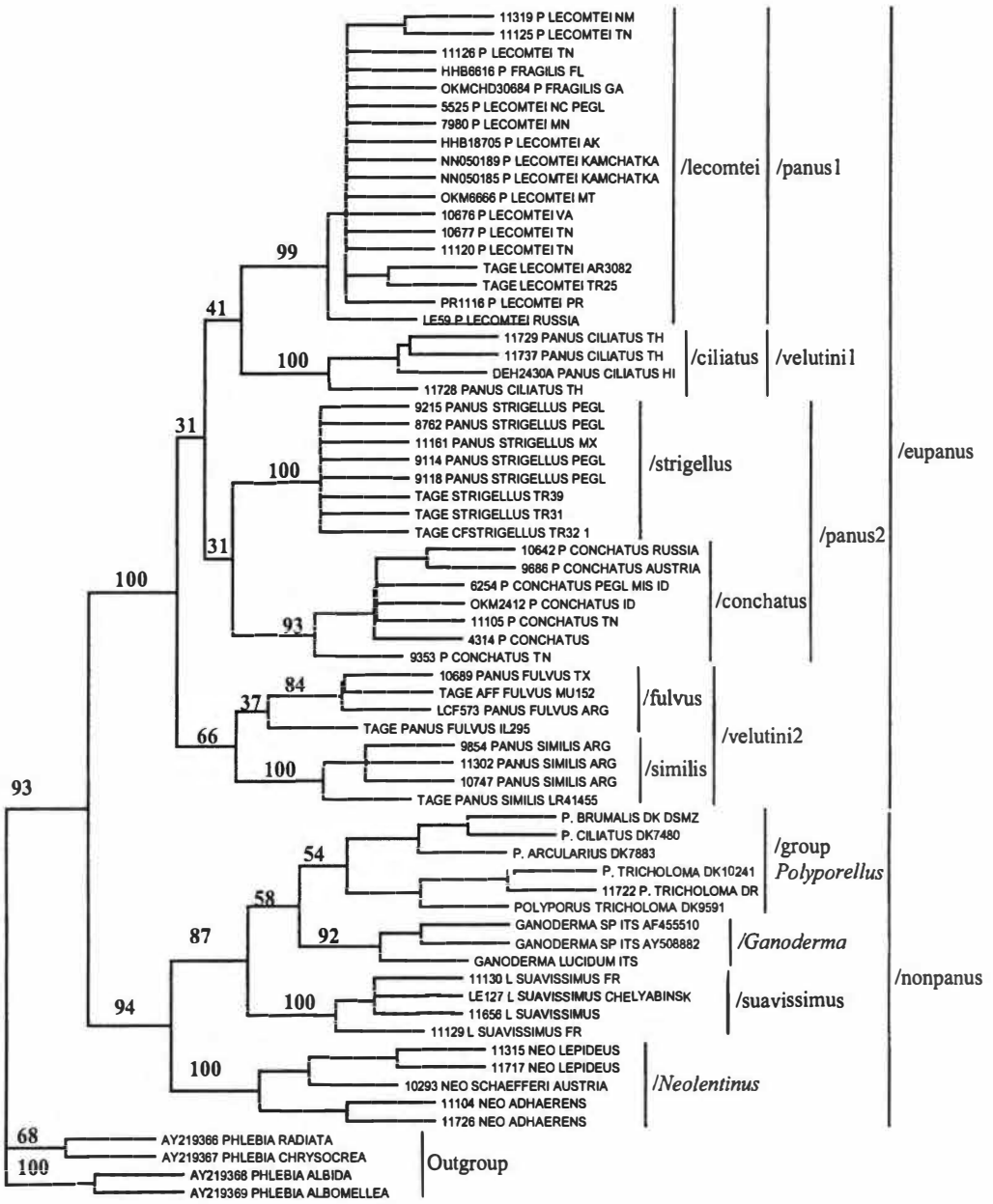

Fig. 1 - Maximum parsimony 50 % majority rule consensus ITS *Panus* phylogeny. Bootstrap values are on branches preceding clades. 1000 bootstrap replicates were performed. Tree length = 1027, consistency index (CI) = 0.6748, homoplasy index (HI) = 0.3252, retention index (RI) = 0.8900

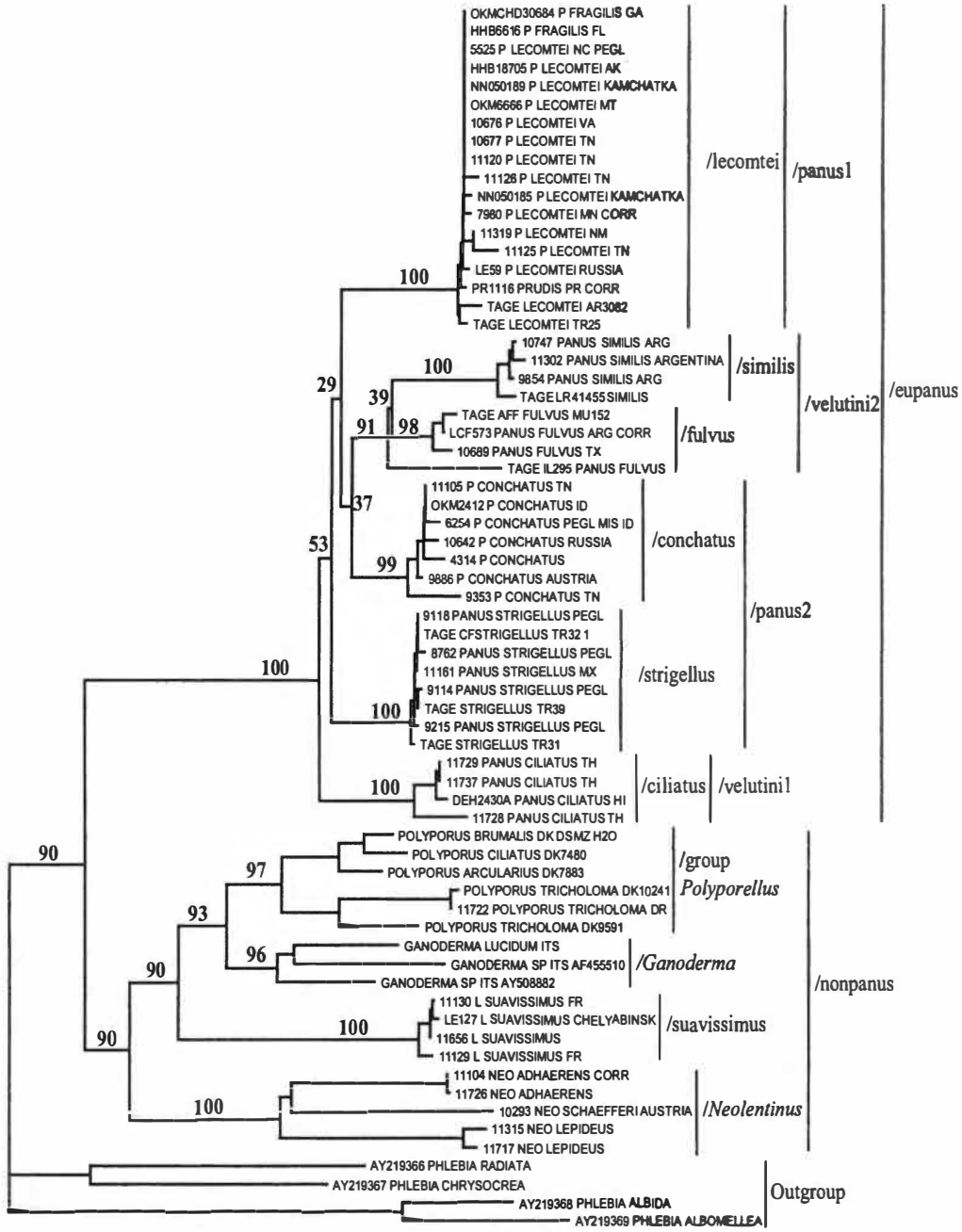

Fig. 2 - Neighbor-joining ITS *Panus* phylogeny. Bootstrap values are on branches preceding clades. 1000 bootstrap replicates were performed using the Jukes-Cantor nucleotide substitution model. Tree length = 1031.

The four clades, /group *Polyporellus*, /*Ganoderma*, /suavissimus, /*Neolentinus* and the outgroup are not part of clade /eupanus. All belong to a larger clade (/nonpanus) that is supported by 94 % bootstrap value. The relationships in /nonpanus will only be commented on briefly because of inadequate sampling in those taxa. /group *Polyporellus*, /*Ganoderma* and /suavissimus have been included and discussed in Grand (2004: pt. 3). /*Neolentinus* will be commented on with regard to its separation from subg. *Panus sensu* Pegler (1983).

Subg. *Panus* sect. *Panus* is represented by four morphospecies in the ITS phylogenies (Figs. 1, 2). The section is polyphyletic and appears in two areas in the phylogenetic reconstructions (/panus1 and /panus2). All *P. lecomtei* and *P. fragilis* sequences are nested in /panus1. The clade is supported by a MP bootstrap value of 99%, and contains no other morphospecies. /panus2 contains all sequences of the morphospecies *P. strigellus* and *P. conchatus*. /panus2 is supported by 31 % bootstrap value.

Within /panus2, two subclades separate the constituent morphospecies into two well-supported clades. /strigellus is supported by a 100 % bootstrap value and contains all sequences of the morphospecies *P. strigellus*. /conchatus is also highly supported (bootstrap value = 93 %) and is inclusive of all *P. conchatus* sequences.

Subg. *Panus* sect. *Velutini sensu* Pegler (1983) appears polyphyletic in the MP and NJ analyses. The section is represented by three morphospecies in the phylogenies, *Panus ciliatus* Lév, *Panus fulvus* (Berk.) Pegler and Rayner and *Panus similis* Berk. and Br. /velutini1 is interdigitated between /panus1 and /panus2. /velutini1 contains all sequences of the morphospecies *P. ciliatus* and is supported by 100 % bootstrap value. /velutini2 contains sequences from two morphospecies (*P. fulvus* and *P. similis*) and is supported by a 66 % bootstrap value.

/velutini2, which contains *P. fulvus* and *P. similis* sequences, is broken into two subclades (/fulvus and /similis) that correspond to the clusters representing each morphospecies. /velutini2 is supported by a 66 % bootstrap value. The two subclades of /fulvus and /similis are supported by 37 % and 100 % bootstrap values, respectively.

Panus lecomtei Fr. intercompatibility experiments

Five collections of *Panus lecomtei* were paired in various combinations (Tab. 2). These five North American collections were shown to all be part of a single intersterility group. Two collections of *Panus conchatus* (FB10642, FB11105) were used as negative controls. Nearly all pairings of *P. lecomtei* with the negative controls were incompatible. The pairings of the *P. lecomtei* group with the *P. conchatus* controls that were positive involved an SBI that was already a dikaryon.

The pairing labeled "Self" was between two collections obtained from the same stump at different times. These two collections, and the SBIs derived from them, most likely shared one parent mycelium. Because of the identical mating alleles contained in both isolates and the outcrossing nature of this fungus, only 2 of 4 pairings were positive.

Tab. 2 – *P. lecomtei* intercollection pairings.

	FB10642	FB7980	FB11105	FB11120	FB11125	FB11126	FB11319
Geographic origin	Russia	Minnesota	North Carolina	Tennessee	Florida	Tennessee	New Mexico
FB10642[1] Russia	NA						
FB7980 Minnesota	0/4	NA					
FB11105[1] North Carolina	3/4	0/4	NA				
FB11120 Tennessee	0/4	3/4	0/4	NA			
FB11125 Florida	1/4	4/4	0/4	4/4	NA		
FB11126 Tennessee	1/4	4/4	0/4	2/4 Self	4/4	NA	
FB11319 New Mexico	0/4	4/4	0/4	4/4	4/4	4/4	NA

[1]FB10642 and FB11105 are *Panus conchatus* negative controls.

Tab. 3 - *P. conchatus* intercollection pairings.

Geographic origin	Maggia Switzerland	Austria	Leningrad Russia	Chelyabinsk Russia	Argentina	Argentina	North Carolina	Sugarlands Tennessee
	FB4314	FB9886	FB10642	FB9885	FB9854	FB11302	FB11105	FB9353
FB4314	NA							
FB9886		NA						
FB10642			NA					
FB9885	4/4	4/4		NA				
FB9854			1/4*		NA			
FB11302	2/2		2/4		3/4	NA		
FB11105		4/4	4/4		1/4*		NA	
FB9353	4/4					4/4	4/4	NA

* = These pairings had contamination problems.

Panus conchatus (Bull.: Fr.) Fr. intercompatibility experiments

The results of intercompatibility studies using *P. conchatus* are shown in Tab. 3. Eight collections from Europe, North America, South America and Russia were shown to belong to the same intercompatibility group.

Discussion

Lentinus subg. *Panus sensu* Pegler (1983) status and nomenclature

I have used the taxonomy and nomenclature of Pegler (1983) throughout this paper for clarity and ease of reading. According to the LSU (Grand 2004: pt.2), ITS (here), and mtCOX3 (data not shown) sequence data, subg. *Panus* of Pegler (1983) is genetically divergent from subg. *Lentinus* (Pegler 1983) and putatively related genera.

This level of divergence is typical among different genera and suggests that *Panus* Fr. should be accepted at the rank of genus.

Lentinus Fr. and *Panus* Fr. are genetically divergent enough at the ITS level to make sequence alignment ambiguous. Because of this, ITS analyses for *Panus* and *Lentinus* (Grand 2004: pt. 3) were completed separately.

The ascension of *Panus* to genus rank raises numerous nomenclatural issues with several of the taxa involved in this study (*e.g.* "*P. lecomtei*", "*P. ciliatus*", "*P. similis*"). Pegler's (1983) nomenclature and lists of synonymy are detailed, but are based on the combination of *Panus* Fr. and *Lentinus* Fr. into one large *Lentinus* Fr. *sensu* Pegler (1983). When taxa are transferred among segregate genera, species epithets may require changes to reflect correct nomenclature (International Code of Botanical Nomeclature 2000). In this study, significant data were accumulated for the morphospecies *P. lecomtei* and *P. conchatus*. *P. lecomtei* and *P. conchatus* are the correct names when these taxa are placed in *Panus*, according to the International Code of Botanical Nomenclature (2000). For some morphospecies, data were limited to sequences (*e.g. P. fulvus*). Because these taxa involved limited sampling, searches for the correct name were not exhaustive. This will be part of future work with greater sampling and more detailed analysis.

Lentinus subg. *Panus* sect. *Panus sensu* Pegler (1983)

Panus lecomtei Fr. (= *Lentinus strigosus* (Schwein.) Fr. of Pegler 1983), *Panus fragilis* O. K. Miller (1965), *Panus strigellus* Berk. (= *Lentinus strigellus* Berk. of Pegler 1983), *Panus conchatus* (Bull.: Fr.) Fr. (= *Lentinus torulosus* (Pers.: Fr.) Lloyd of Pegler 1983)

Sect. *Panus sensu* Pegler (1983) appears polyphyletic in these analyses. The weak support along the entire backbone of *Leupanus*, and limited sampling from other sections of subg. *Panus sensu* Pegler (1983) prevents conclusions about the relationship of sect. *Panus* to other sections of subg. *Panus sensu* Pegler (1983).

Three of the four morphospecies included in the analyses are in sect. *Panus sensu* Pegler (1983); *P. lecomtei*, *P. strigellus* and *P. conchatus*. The fourth species, *P. fragilis* O. K. Miller (1965) was regarded by Pegler (1983) as a synonym of *P. lecomtei* (as *Lentinus strigosus*).

Sequences of Pegler's (1983) sect. *Panus* appear in two places in phylogenetic reconstructions (/panus1 and /panus2). /panus1 contains all sequences of *P. lecomtei* and *P. fragilis*, and is highly supported (MP bootstrap value = 99 %).

In phylogenetic reconstructions (Figs. 1, 2), I have labeled everything in the /lecomtei clade as *P. lecomtei* (except *P. fragilis*) for clarity in discussion. Many of these cultures were actually received and annotated under other names. The initial annotations of these cultures are listed in Tab. 1. Discussion of the nomenclature in this group is discussed above and also in Hrouda (2001).

The two sequences of the *P. fragilis* morphospecies are surrounded by *P. lecomtei* sequences. Examination of the actual sequences indicates that these two *P. fragilis* are 100 % identical to several other collections of *P. lecomtei*. Given the quickly evolving nature of the ITS region, this homology indicates a very close relationship. Nuclear ribosomal LSU (Grand 2004: pt. 2) and mitochondrial COX3 (data not shown) sequences also show nearly complete homology between *P. fragilis* and *P. lecomtei*.

Miller (1965) based his description of *P. fragilis* on basidiomata fruited *in vitro*. He described the coloration of *P. fragilis* as being lighter than that of the closely related *P. lecomtei* (as *P. rudis* Fr.). *P. fragilis* basidiomata (grown on malt extract agar) were also smaller than those of *P. lecomtei*. Sequence data, and the morphological similarities of these taxa indicate that the synonymization of *P. fragilis* O. K. Miller (1965) under *P. lecomtei* Fr. is appropriate.

Biological compatibility was explored in *P. lecomtei*. The results of these pairing experiments are shown in Tab. 2 in results. Five collections of *P. lecomtei* were paired in all combinations with each other, and with two negative controls (*P. conchatus*). The results show that the five collections identified morphologically as *P. lecomtei* were all part of the same intersterility group. The pairings of the *P. lecomtei* group with the *P. conchatus* controls were all negative except for two cases where one of the SBIs used in

the pairing was already a dikaryon. Sequences from all five members of the intersterility group clustered in /lecomtei in phylogenetic reconstructions (Figs. 1, 2). Biological conspecificity and phylogenetic clades are very well-correlated in *P. lecomtei*.

/panus2 includes all sequences from two morphospecies, *P. strigellus* and *P. conchatus*. The two morphospecies are separated into two highly supported subclades, /strigellus (100 % bootstrap value) and /conchatus (93 % bootstrap value). The separation of the two subclades is not well-supported (MP bootstrap value = 31 %), as is the case for many of the deeper nodes in /eupanus.

Panus strigellus is separated from other members of sect. *Panus sensu* Pegler (1983) by a glabrescent pileus with scattered squamules and refractive gloeocystidia in the hymenium. Its range is also limited to the tropical Americas. All sequences of *P. strigellus* used in this study form a highly supported clade (/strigellus) exclusive of any other morphospecies.

Although /strigellus appears sister to /conchatus in MP and NJ phylogenies, it is weakly supported (bootstrap value = 31 %). This relationship is probably not more significant than the relationship among the species in /panus1 and /panus2 because of such weak support.

All sequences of *Panus conchatus* used for this study are included in /conchatus. This clade is supported by a bootstrap value of 93 %. /conchatus appears sister to /strigellus, but this relationship is weakly supported. *P. conchatus* is separated from *P. strigellus* by glabrous pileus and North temperate distribution: *P. strigellus* has scattered squamules on the pileus and is restricted to tropical America (Pegler 1983).

Basidiomata of *Panus conchatus* are often confused with *P. lecomtei* due to their similar stature, geographical confluence, purplish tint in young basidiomata and velutinate stipes. In order to establish a group of collections belonging to the same intersterility group, I performed pairing studies with eight collections of *P. conchatus*. The results of these pairings are shown in Tab. 3. ITS sequences from four of the successfully paired collections are in /conchatus (Figs. 1, 2). The correlation of morphological, biological and phylogenetic data indicate that the *P. conchatus* collections utilized in this study are a conspecific, monophyletic group.

Lentinus subg. *Panus* sect. *Velutini sensu* Pegler 1983

Panus ciliatus Lév. (= *Lentinus ciliatus* Lév. of Pegler 1983), *Panus similis* Berk. and Br. (= *Lentinus similis* Berk. and Br. of Pegler 1983), *Panus fulvus* (Berk.) Pegler and Rayner (= *Lentinus velutinus* Fr. of Pegler 1983)

Panus ciliatus, *P. fulvus*, and *P. similis* (as *Lentinus* spp.) are all members of Pegler's (1983) sect. *Velutini*. Sect. *Velutini sensu* Pegler (1983) is distinguished from other sections of subg. *Panus sensu* Pegler (1983) by a velutinate stipe and pileus, small spores and the absence of thick-walled metuloids or gloeocystidia. Skeletocystidia (Pegler 1983) may be present, but are not thick-walled or refractive.

Maximum parsimony (Fig. 1) and neighbor-joining (Fig. 2) analyses of ITS sequence data both indicate that sect. *Velutini sensu* Pegler (1983) is polyphyletic. /ciliatus (= /velutini1) (MP bootstrap value = 100%), which contains all sequences of the *P. ciliatus* morphospecies, is sister to /lecomtei. This sister relationship is weakly supported (bootstrap value = 41%), as are many of the deeper nodes that separate the clades of the phylogenies.

Both sects. *Panus* and *Velutini sensu* Pegler (1983) have members with velutinate to strigose stipes and pilei. Sect. *Velutini sensu* Pegler is his only section of subg. *Panus* that contains skeletocystidia. This character distinguishes basidiomata microscopically from those in other sections. According to the analyses, this may not be an adequate character for separating sect. *Velutini sensu* Pegler from the other sections in subg. *Panus sensu* Pegler.

Panus fulvus and *P. similis* are two morphological species that occur in /velutini2 in these analyses. The separation of the sequences is not well-supported (bootstrap = 66 %). Some of the sequences used here (Tage MU152, IL295, LR41455) contained ambiguous regions that might have helped separate *P. fulvus* and *P. similis*, but were difficult to align. These collections (Tage MU152, IL295, LR41455) could not be examined morphologically and should be omitted from future work until the original material can be examined and resequenced.

P. similis sensu Pegler (1983) is geographically limited to southeast Asia, Australasia and equatorial Africa. *P. fulvus* is a pantropical species that produces a larger, more robust basidiomata. The material of *P. similis* used in this study is from Argentina and Venezuela. These collections (except LR41455) were examined by the author and found to be morphologically distinct from the pantropical *P. fulvus*. These results expand the geographical range of *P. similis* into tropical America where *P. fulvus* is commonly collected. Because *P. fulvus* and *P. similis* are macroscopically similar, misidentification in the past has led to confusion regarding the range of both species.

/Neolentinus

Three former members of *Lentinus* subg. *Panus sensu* Pegler (1983) that have been transferred to *Neolentinus* Redhead and Ginns (1985) were included in analyses. One species from sect. *Pulverenti sensu* Pegler (1983) [*N. adhaerens* (Alb. and Schw.: Fr.) Redhead and Ginns] and two from *Lentinus* subg. *Panus* sect. *Squamosi sensu* Pegler (1983) [*N. lepideus* (Fr.: Fr.) Redhead and Ginns and *N. schaefferi* (Weinm.) Redhead and Ginns] were represented in phylogenetic reconstructions (Figs. 1, 2).

All five sequences of *Neolentinus* cluster in */Neolentinus* (MP and NJ bootstrap values = 100 %). According to ITS (here) and LSU sequences (Grand 2004: pt. 2), it appears that the segregation of *Neolentinus* by Redhead and Ginns (1985) based on bipolar mating systems, binucleate spores and ability to form brown-rots is justifiable.

Lentinus suavissimus Fr.

Lentinus suavissimus Fr. is the only member of *Lentinus* sect. *Pleuroti sensu* Pegler (1983). The placement of this taxa in LSU (Grand 2004: pt. 2) and ITS phylogenies (Grand 2004: pt. 3) was not within the large /eulentinus clades that contained all other members of subg. *Lentinus sensu* Pegler (1983). To explore the placement of this taxa, I included four sequences in the analysis involving subg. *Panus sensu* Pegler (1983). These four sequences form /suavissimus, which is part of /nonpanus. The placement of /suavissimus inside /nonpanus is well-supported (bootstrap

value = 100 %), as is the separation of /nonpanus from /eupanus (bootstrap value = 93 %). According to this analysis, *L. suavissimus* is not closely related to subg. *Panus sensu* Pegler (1983).

/nonpanus

Some species included in the analyses that were segregated from *Lentinus sensu* Pegler (1983), (*e.g. Neolentinus*), and others whose relationships to *Lentinus sensu* Pegler were intriguing (*e.g. Ganoderma*, *L. suavissimus* Fr., group *Polyporellus*) based on other data (Grand 2004: pts. 2, 3)

All of these taxa clustered in the highly supported (MP bootstrap value = 94 %) /nonpanus clade. The separation of /nonpanus from /eupanus, which includes all members of subg. *Panus sensu* Pegler (1983) used in this study, is also well-supported (MP bootstrap value = 93 %). Because of this separation, a close relationship between /eupanus taxa, and any member contained in /nonpanus is doubtful.

Acknowledgements

I would like to acknowledge my advisor Ron Petersen and my committee for guidance during the preparation of this work. An NSF-PEET grant (DEB9978011) and the University of Tennessee Botany Department provided funding for research and travel during this study. Collection and observation of freshly collected material would not have been possible without this funding. Collaborators and travel partners were also essential. Several culture collections graciously provided material (mainly Forest Products Laboratory, Madison, WI and Leningrad culture collection (Nadya Psurtseva, St. Petersburg, Russia). I would like to thank Tage Rolen (2001) for providing access to his thesis and the sequence alignments used in it. David N. Pegler provided morphological identifications for some collections.

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Vita

Edward Grand was born June 6th, 1974 in Jackson, Michigan. He spent a large amount of time in his youth wandering the woods and observing nature's wonder. He graduated from Jackson High School in June, 1992. Fall semester of that year he began attending classes at Jackson Community College. Unsure of the possibility of turning his passion for the outdoors into a career, he applied for entry into the Chemical Engineering program at the University of Michigan and was accepted. After completing a Bachelor's degree in Chemical Engineering, he began working in a medical lab doing research with a Gastroenterologist at the University of Michigan Medical Center. Although that job provided sustenance and exposure to some interesting tools for research, it was not where his passion lied. After three years, it was time for a change.

After several years of assisting Dr. Robert Shaffer during his annual Fall Mycology class, Edward began to asking Dr. Shaffer questions about academics. He started a search for academic institutions that offered graduate studies in Mycology during the Fall of 1999. That is when he found the University of Tennessee Mycology Group website. After a series of letters between Edward and Dr. Ronald H. Petersen, plans were made for a visit to Tennessee and potential acceptance as a graduate student. He was accepted for admission into the doctoral program of the Botany Department in May, 2000.

Edward is graduating in 2004 and moving to Thailand for a position at the Mushroom Research Centre in Chiang Mai, Thailand. He will be conducting research, providing assistance for several students residing at the Centre and acting as intermediary among the public, students and academians associated with the Centre.