## **MISCELLANEOUS NOTES ON PLEUROTUS**

# EDGARDO O. ALBERTÓ<sup>1</sup>, RONALD H. PETERSEN<sup>2</sup>, KAREN W. HUGHES<sup>2</sup> & BERNARDO LECHNER<sup>3</sup>

The paper takes up four points: 1) A previously unnamed intersterility group in *Pleurotus* (ISG XIII) has been identified as *P. albidus*, which fruits from Central America to central Argentina. The species is genetically isolated and phylogenetically it is placed in the '*P. ostreatus*' group of monomitic *Pleurotus* basidiomata. 2) The distributional range of *P. abieticola* is extended to far northwestern Russia and northern China. 3) A partial nomenclator is furnished for *P. djamor* 4) The use of the term 'dimitic' is discussed as it pertains to *Pleurotus*.

The past decade or more has seen an increase in systematic research on *Pleurotus* ('oyster mushrooms'). In taxonomy, there has been an effort to circumscribe the infrageneric taxa morphologically (Hilber, 1982, 1997; Zervakis & Balis, 1991; Vilgalys et al., 1993; Petersen & Krisai-Greilhuber, 1996, 1999; Petersen & Hughes, 1997), to extend informative taxonomic characters to mating patterns (Vilgalys et al., 1993; Petersen & Hughes, 1993; Petersen, 1995a, b; Zervakis, 1998), physiology (Zervakis & Balis, 1991, 1992, 1996) and enzyme patterns (Zervakis & Labarère, 1992), and to reconstruct the phylogeny of the genus (Vilgalys & Sun, 1994; Zervakis et al., 1994; Vilgalys et al., 1996; Gonzalez & Labarère, 2000; Thorn et al., 2000; Montcalvo et al., 2000). As this research has emerged, the stringency of proposing new taxa has also increased, so the standard now assumed is that proposal of a new species name should be accompanied by data on its genetic isolation and phylogenetic placement.

As pointed out elsewhere (Petersen & Hughes, 1998), there are at least three means through which collections (= basidiomata and/or cultures) can be judged contaxic or segregated as separate taxonomic entities: 1) morphological similarity/dissimilarity, usually as judged through basidiomata; 2) ability or potential ability to interbreed, requiring gametic (= haploid, monokaryon) isolates with which to conduct crossing experiments; and 3) placement on a phylogenetic reconstruction, requiring data (phenetic or molecular) from other congeneric taxa for comparative purposes.

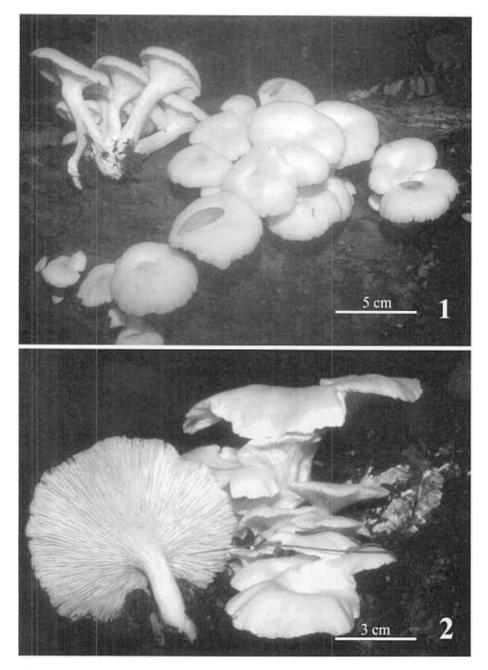
## INTERSTERILITY GROUP XIII IS PLEUROTUS ALBIDUS

Vilgalys & Sun (1994) initiated a system of numbering intersterility groups (ISGs) in *Pleurotus* in the same way that *Armillaria* ISGs had been numbered previously (Anderson & Ullrich, 1979). In the first such enumeration, eight ISGs were identified in *Pleurotus*, but later (Vilgalys et al., 1996), 15 were listed in a phylogenetic recon-

<sup>1)</sup> Instituto de Investigaciones Biotecnológicas, IIB-INTECH (UNSAM-CONICET), Camino de Circumv. Km 6, CC 164, 7130 Chascomús, Buenos Aires, Argentina.

<sup>2)</sup> Department of Botany, University of Tennessee, Knoxville, Tennessee 37996-1100, USA.

<sup>3)</sup> PRHIDEB-CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina.



Figs. 1 & 2. Basidiomata of Pleurotus albidus in nature. BAFC 50.047 (also known as 'PSTAC').

struction. One of these, ISG XIII, labelled as 'Brazil' furnished no supporting morphological or herbarium data. Until this paper, ISG XIII has remained unsecured to a morphological or biological species.

In South and Central America, white, cornucopioid basidiomata of a *Pleurotus* taxon fruit on dead trunks (Figs. 1 & 2). Two collections were gathered and cultured in Costa Rica, while two others were isolated in central Argentina. Gametic cultures of the Argentine collections were brought to the Tennessee laboratory by EA. All four collections were found to be intercompatible, and for some time it was thought that they represented a new species of *Pleurotus*.

A visit to the Royal Botanic Gardens Herbarium (Kew, by RHP) allowed examination of type material of several *Pleurotus* species, to which was added that of *Panus laciniato-crenatus* Speg. Four species epithets were found to represent this organism, and a nomenclator is offered below.

#### MATERIALS AND METHODS

#### Morphological analyses

Basidiomata of Argentine collections were examined by RHP and EA separately, while those of 9498 and 10056 were examined by RHP. Macromorphological notes were taken on relevant type specimens by RHP. Micromorphological analyses and measurements of various structures were conducted using standard procedures (i.e. thin-sections of pileipellis, pileus trama and lamellae; squash mounts of lamellae using either bright field microscopy with or without aqueous phloxine or phase contrast microscopy without stain).

In descriptions below, colours appearing within quotation marks are from Ridgway (1912); those listed alphanumerically are from Kornerup & Wanscher (1978). BAFC = Herbarium, Universidad de Buenos Aires, Facultad de Ciencias; TENN = Herbarium, University of Tennessee.

## Mating studies

Single-basidiospore isolates (SBIs) were obtained from two sources: 1) basidiomata collected in nature (i.e. BAFC 50.047, BAFC 50.261, TENN 57623, TENN 56526); or 2) from basidiomata of the BAFC collections fruited on *Liriodendron tulipifera* sawdust in the laboratory at TENN. SBIs from fruited basidiomata did not differ from those isolated from nature.

Two types of pairing experiments were performed: 1) self-crosses of BAFC 50.047 and 10056 (= TENN 57623) using 12 SBIs; and 2) intercollection pairings. Intercollection pairings comprised two methodologies: 1) pairing of four SBIs of four putative collections of *P. albidus* (i.e. those mentioned above) in which n = 4; and 2) pairings of SBIs of BAFC 50.047 and 10056 with a battery of standard SBIs (see Petersen, 1995a for use of standard battery) of the following: *P. abieticola* (see Petersen & Hughes, 1997), *P. eryngii, P. tuber-regium* (see Petersen & Nicholl, 1997), *P. djamor, P. populinus, P. cystidiosus, P. cornucopiae, P. opuntiae* (for use of the latter two names, see Petersen & Krisai-Greilhuber, 1999) *P. ostreatus* (see Petersen & Krisai-Greilhuber, 1996), and *P. pulmonarius* (see Petersen & Hughes, 1993). In all intercollection pairings, n = 4.

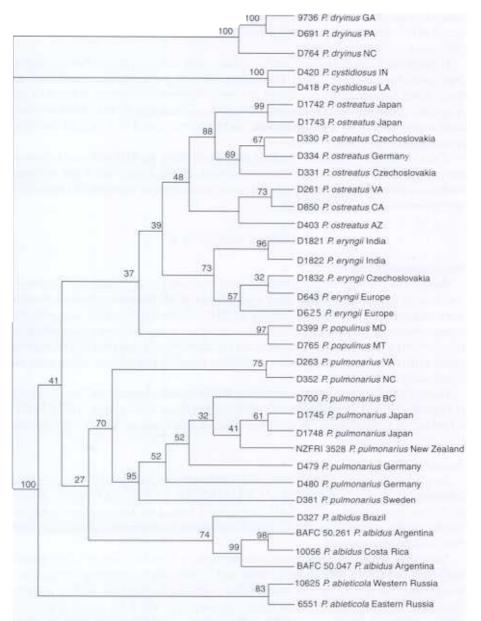


Fig. 3. Phylogenetic reconstruction of the P. 'ostreatus' clade based on ITS DNA sequences. Note monophyly of P. albidus and P. abieticola collections. GenBank numbers for remaining collections are: 6551 = U 59326, 9763 = AF 345662, 10056 = AF 345661, 10625 = AF 345656, BAFC 50.047 = AF 345660, BAFC 50.261 = AF 345659, NZFRI = U 60648. Collections beginning with 'D' are from Vilgalys & Sun (1994). Numbers are bootstrap values for the nodes to the right of the number. Parsimony-informative characters = 151. Consistency index = 0.83. Homoplasy index = 0.17.

## 59

#### Molecular studies

Material used: for a summary of specimens used for molecular sequence data see Fig. 3.

DNA was extracted from monokaryon cultures as described by Hughes et al. (1999). The ribosomal ITS1-5.8S-ITS2 was amplified with primers ITS5 and ITS4 using 1 µl extracted DNA in a 50 µl reaction mixture. PCR parameters were 3 mins at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 52 °C and 1 min at 72 °C. The final extension was 3 mins at 72 °C. PCR products were purified with a Wizard PCR purification system (Promega), following manufacturer's directions. Both strands of the PCR product were reamplified for cycle sequencing using ITS2, ITS3, ITS4 and ITS5 primers (White et al., 1990). Products were sequenced using ABI automated sequencing systems at the University of Tennessee Sequencing Facility. Sequences were edited using the Genetics Computer Group Sequence Analysis Software Package gap and line-up programs (GCG 2000) and deposited with GenBank (see Fig. 3). Sequences were aligned using the GCG Seqlab program and adjusted manually. Regions of homology to the yeast (Saccharomyces cerevisiae) 18S gene to the Heterobasidion annosum 5.8S gene and to the yeast 25S gene were determined by sequence comparison. Pleurotus ITS1 and ITS2 sequences (Vilgalys & Sun, 1994) were retrieved from GenBank. For each collection, ITS1 sequences were reversed and complemented and appended to ITS2 sequences for the same collection with a gap representing a section of the 5.8S gene that was not sequenced in those studies. Vilgalys furnished an ITS sequence for strain D 327, the basis for ISG XIII (Vilgalys et al., 1996).

#### Gap coding and phylogeny estimations

Phylogenetic relationships were computed using PAUP 4.0 using a branch and bound search option. Node support was estimated from 100 bootstrapped replicates. There were few gaps and they were treated as a fifth base. *Pleurotus cystidiosus* and *P. dryinus* were used as outgroups based on studies showing that these species were basal to the monomitic *Pleurotus* clade (Vilgalys & Sun, 1994).

#### NOMENCLATOR AND TAXONOMIC DESCRIPTION

#### Pleurotus albidus (Berk.) Pegler, Kew Bull., Addit. ser. 10 (1983) 219.

 $\equiv$  Lentinus albidus Berk., Hooker's J. Bot. 2 (1843) 633.

Holotype: K, Brazil, Prov. Minas-Geraes, Inficionade, 'ad citrum', X.1840, coll. Gardner [!].

- = Lentinus calvescens Berk. & Curtis, Hooker's J. Bot. 8 (1856) 143.
- Holotype: K, Brazil, Panuré, II.1853, 'on decaying trunks of trees', Spruce no. 136 [!].

= Pleurotus jacksonii Berk. & Cooke, J. Linn. Soc. 15 (1877) 363.

- Holotype: K, Brazil, Mahues, no date, 'in lignis marsescentibus', Traill, s.n. [!].
- = Panus laciniato-crenatus Speg., An. Soc. Cient. Arg. 9 (1880) 164.
- = Pleurotus laciniato-crenatus (Speg.) Speg., Bol. Acad. Nac. Cienc. Córdoba 23 (1919 381-382.

Holotype: LPS, Argentina, Buenos Aires, 25.II.1880, leg. O. Schnyder, LPS no. 17095 [!].

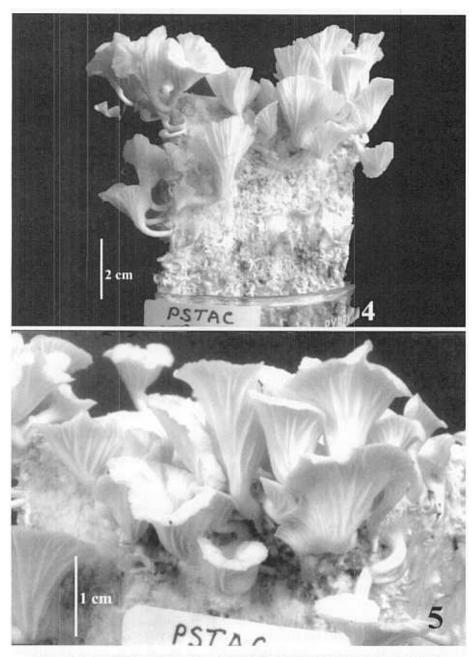
Basidiomata cornucopioid, clitocyboid, omphalioid to lentinoid (i.e. *L. crinitis*), usually gregarious in troops of 2-30 basidiomata. Pileus circular, infundibuliform, up to 135 mm broad, smooth to innately radially fibrillose, hygrophanous, white to

cream to dull greyish tan ['sayal brown' (6C5) inward, outward 'cartridge buff' (30A2) to 'pale olive buff' (3B2)]; margin entire to lacerate-crenate, thin, usually somewhat inturned (especially in drying); pileus often becoming pallid lemon yellow through drying. Lamellae deeply decurrent, extending as ridges through much of stipe length, tough (resisting fragmentation and squashing in KOH), subdistant, up to 6 mm deep, in at least three ranks, without interveining, white to dull off-white (to 'cartridge buff'); dried portions of lamellae turning dull golden yellow in 3% KOH. Stipe  $35-80 \times 5-13$  mm, terete, upward fluted through lamellar bases, tapering downward, usually curved-ascendant, hollow to lightly stuffed, white, sometimes streaked with 'tilleul buff' (7B2); surface matt to minutely loosely felty. Taste mild; odor mild to resembling sweet lemon. Usually on dead wood including (but probably not limited to) *Salix* and *Ulmus*, rarely found on living trees.

Pileus surface a generally repent layer of hyphae; hyphae 2.5-4.5 µm diam., hyaline, thin- to thick-walled (wall up to 0.5 µm thick), often adherent (? through drying); occasional coralloid hyphal tips emergent; mucronate pileicystidia not observed. Pileus trama monomitic; hyphae 4.0-12.5 µm diam., hyaline, frequently branched, conspicuously clamped, interwoven. Lamellar trama tightly interwoven, with distinct mediostratum up to 320 µm broad, and lateral strata up to 32 µm thick. Hyphae of mediostratum as in pileus trama; gloeoplerous hyphae occasional to rare (or absent), 4.5-6.5 um diam., yellow-refringent under phase contrast microscopy, glassy (not coscinoidal). Hyphae of lateral stratum pseudoparenchymatous, isodiametrical, hyaline. Subhymenium rudimentary, rarely over 15 µm thick. Hymenium composed of basidia and non-basidial elements; basidia 18.5-24 × 4.5-5.2 µm, narrowly clavate to subcylindrical, hyaline, clamped; sterigmata 4, slender, curved; non-basidial elements fusiformmucronate, non-emergent, of similar length but somewhat stouter than basidia (these elements could be interpreted as pleurocystidia, but in all cases hymenial elements have proliferated somewhat, perhaps as a function of slow drying or confinement of the basidiomata for some time before drying). Cheilocystidia absent. Stipe trama monomitic; hyphae 4.5-18.5 µm diam., hyaline, consistently thick-walled (wall up to 4.0 µm thick), branched, conspicuously clamped, loosely interwoven. Stipe surface mattplushy, white, forming a thatch; hyphae 2.5-5.5 µm diam., frequently branched and entangled, conspicuously clamped, thick-walled (wall 0.3-0.7 µm thick), hyaline.

Basidiospores (n = 45)  $(5.2-)8-9.5(-10.5) \times (3.1-)3.5-4.5 \mu m$ ; E = 2.00–2.38; Em = 2.15; Lm 8.53 µm, very variable in length, narrowly ellipsoid to subcylindrical and slightly adaxially flattened, smooth, hyaline, thin-walled, inamyloid, with one or two refringent guttules and some small granular inclusions; hilar appendix asymmetrical, small. Spore-print white to cream.

Specimens examined. ARGENTINA: Buenos Aires, Partido de la Costa, San Clemente del Tuyu, Parque Municipal, 13.VI.99, leg. & det. E. Alberto ('Ed 772'), on dead Salix species, BAFC no. 50.261; Buenos Aires, Llavallol, Sta. Catalina, 26.III.98, coll. E. Alberto, no. 'Ed 519', (also known as 'PSTAC'; see Figs. 4 & 5), det. Lechner & Albertó. Sobre tronco caido; muy abundante (as *P. cornucopiae*), BAFC no. 50.047; Buenos Aires, 25.II.1880, leg. O. Schnyder (holotype specimen of *Panus laciniato-crenatus*, LPS no. 17095); Buenos Aires, Santa Catalina Forest, 6.V.99, leg. E. Albertó & G. Pire, det. Albertó & Lechner ('Ed 751'), growing on dead wood, BAFC no. 50.413; Buenos Aires, La Lucila, 5.V.96 ('Ed 808'), basidiomata produced from dikaryon culture BAFC 2787.



Figs. 4 & 5. Basidiomata of P. albidus (BAFC 50.047) fruited on Liriodendron sawdust.

BRAZIL: Prov. Minas Geraes, Inficionade, ad citrum, X.1840, coll. Gardner (holotype specimen of Lentinus albidus, K).

COSTA RICA: Prov. Puntarenas, Co. Coto Brus, Hacienda la Amistad, trail from Ave. Pizoté, N 08°54.218', W 82°47.401', ~1328 m, 4.VII.98, coll. RHP, fieldbook no. 9498 (TENN 56526); Prov. Puntarenas, vic. Vulcan Arenal, Heliconia Hotel Sanctuary Reserve, 400 m before entrance to Sta Elena Reserve, N 10°20.32', W 84°47.55', 17.III.99, coll. J.L. Mata, fieldbook no. 10056 (TENN 57633).

TRINIDAD: St. Augustine, 20. VIII.47, R.E.D. Bahn no. 1545, annot. R.W.G. Dennis, K.

#### Cultural characters

Colony growth rate up to one cm per week on malt extract (15 mg/L) agar (20 g/L). Difco-bacto), white, substantially aerial, appearing combed to plumed; small orange exudate droplets produced in some aged cultures; hyphae all generative,  $3.5-6.5 \mu m$ diam., clamped. Colony odour moderate, perfumed or floral, similar to that of cultures of *P. pulmonarius*. Microdroplets produced on aerial hyphae, up to  $4.5 \mu m$  diam.

#### Mating experiments

Self-crosses using 12 SBIs of collections BAFC 50.047, BAFC 50.261, and 10056 independently revealed a tetrapolar mating system. SBIs of these three collections, as well as those from 9489, were all intercompatible (n = 4 throughout).

Once it was clear that hyphal construction of basidiomata of *P. albidus* was monomitic, SBIs of 10056 and 9498 were paired with SBIs of *P. ostreatus*, *P. pulmonarius*, *P. populinus*, and *P. abieticola* (see Petersen, 1995b for strains and SBI numbers). All pairings lacked clamp-connections. Because Spegazzini mentioned pinkish colours of lamellae, and because the only known *Pleurotus* species with such colours is *P. djamor* (Nicholl & Petersen, 2000), SBIs of BAFC 50.261, BAFC 50.047 and 9498 were paired with SBIs of *P. djamor* (strain 6346, see Petersen, 1995b). All such pairings lacked clamp-connections.

#### DISCUSSION

Three subtle morphological taxonomic characters seem to segregate one taxonomic complex within *Pleurotus*: 1) monomitic hyphal construction of basidiomata; 2) small size of microdroplets in culture; and 3) production of microdroplets on aerial hyphae (rather than from hyphae on the agar surface) in culture. This complex appears to be congruent with what Vilgalys has called the '*P. ostreatus* clade' (Vilgalys et al., 1993, 1996; Vilgalys & Sun, 1994; Vilgalys, 1997; Thorn et al., 2000). Within this complex, basidiomata of *P. ostreatus*, *P. pulmonarius*, *P. abieticola*, and *P. populinus* share a 'pleurotoid' stature (i.e. more or less shelf-like; radically eccentrically stipitate). Basidiomata of *P. eryngii* are centrally to eccentrically stipitate, but the species appears unique also as a root parasite of plants in the Umbelliferae. Thus, while it is not unprecedented to find omphalioid or clitocyboid stature within the complex, basidiomata of *P. albidus* are deeply infundibuliform and occur on wood, while those of *P. eryngii* are planar to slightly depressed, and appear to be root parasites with basidiomata produced through soil. Thus, *P. albidus* basidiomata are unique to the 'ostreatus clade' (Fig. 3).

We have found additional records of Spegazzini specimens listed under *P. laciniato-crenatus*, and have examined the pertinent specimens. Data are as follows: 1) LPS

17060 (Uruguay, Montevideo, V.1914), a monomitic stipitate *Pleurotus*, probably correctly identified by Spegazzini; 2) LPS 17061 (Argentina, Tucuman, VII.1918), almost destroyed by insects and now unidentifiable; 3) LPS 17062 (Argentina, La Plata, IV.1919), a monomitic *Pleurotus* with several spore types, one of which gives dimensions as  $8-12 \times 3-4 \mu$ m, probably correctly identified by Spegazzini; 4) LPS 17074 (Paraguay, Villa Moira, 1893), a marasmielloid, laterally stipitate fungus, similar to *Neonothopanus nambi*; and 5) LPS 17099 (Paraguay, Garapeguá, 28.VI.1883), dimidiate, imbricate pleurotoid basidiomata with lobed, fimbriate margin, perhaps *Pleurotus djamor*.

No more recent reports (i.e. notes with herbarium specimens used in this study) of pinkish colouration of lamellae have been seen since Spegazzini's initial proposal of the species epithet. Such colouration is common in *P. djamor* (see Corner, 1981; Petersen, 1995a; Nicholl & Petersen, 2000), but Spegazzini's mention represents the only such report for the 'ostreatus clade'.

In the process of separating basidiomata of *Pleurotus* from those of other superficially similar taxa (i.e. *Panus*), it is partially diagnostic to observe mucronate cheilocystidia (with a capitula of resinous liquid when fresh) or occasionally such pileicystidia. These have not been seen on these cornucopioid basidiomata. The condition of the relevant type specimens was relatively poor except for that of *Pleurotus laciniatocrenatus*. In all cases (including type specimens), pilei were thin, infundibuliform, and stipes were central to somewhat eccentric. Argentine strains Ed 519 (BAFC 50.261) and Ed 772 (BAFC 50.047) were fruited at Tennessee on *Liriodendron* sawdust blocks and Ed 808 (BAFC 2787) was fruited in Argentina on sawdust, and all produced similar basidiomata (Figs. 4 & 5). Although all such basidiomata were smaller than those from nature, all other morphological characters matched those of natural basidiomata, including both lacerate-crenate and entire pileus margin.

Singer (1950), although in a paper purporting to report on type specimens, apparently reported on his own fresh collections identified as *Pleurotus laciniato-crenatus*, and redescribed the taxon. He judged that "Although this species belongs in the *Pleurotus*-ostreatus-complex, I believe it to be an outstanding form of the latter." Singer's conclusion was correct, for although *Pleurotus ostreatus* exhibits a rather different habit (i.e. shelf-like, imbricate – in short, 'pleurotoid'), it is also monomitic. This conclusion is even more surprising, for Singer's experience with *Pleurotus* was marginal, especially in South America.

Horak (1968: 681) compared the type specimen of *Pleurotus laciniato-crenatus* to *P. eugrammus*. Although basidiome habit was decidedly different, he found micromorphology to be 'identisch'. Petersen & Krisai-Greilhuber (1999) redescribed *P. eugrammus* from type material, noting that hyphal construction was dimitic, this contrary to Horak's comparison of micromorphology. Petersen (below) has questioned the use of the term dimitic in *Pleurotus*, but the meaning of the term as applied to *Pleurotus* remains clear.

## RANGE EXTENSIONS FOR PLEUROTUS ABIETICOLA

Pleurotus abieticola Petersen & Hughes (1997) was proposed based on two specimens on conifer logs in far-eastern Russia (Sichote Alin Biosphere Preserve). Those collections were unique morphologically, genetically isolated (i.e. interINcompatible with a battery of other *Pleurotus* taxa) and ITS sequences formed an independent clade within the genus.

Now, three more collections can be reported which greatly extend the known distribution of the species. Two collections were made some years prior to description of *P. abieticola*, and were 'discovered' during an attempt to identify all collections of *Pleurotus* at TENN. From northern Jilin Province, China, basidiomata formed on conifer logs. The other collection was made in far northwestern Russia, north of St. Petersburg. Substratum was thought to be *Salix* or *Alnus*.

All three collections were intercompatible with the original two collections, but were interINcompatible with all other *Pleurotus* taxa. ITS sequences of collection 10625 also places this collection within the same clade as the original material (Fig. 3).

With these range extensions, the species should be sought in northern Scandinavia, in northern Japan, and on Kamchatka Peninsula. Basidiomata are easily confused with those of *P. ostreatus*, the pileus of which is also often brown. Specimens and field notes follow.

Specimens examined. CHINA: Jilin Prov., Songjianghe, Chang Bai Shan Preserve, 9.VIII.88, coll. R.H. Petersen, on *?Picea*, field no. 1425 (TENN 48301). Pileus 'Verona brown' (6E5) over disc, 'wood brown' (7C4) outward. Lamellae close, 'tilleul buff' (7B2) outward, 'pale pinkish buff' (6A2) inward. Stipe eccentric, off-white, discoloured at base to 'avellaneous' (7B3). Odor negligible.

CHINA: Jilin Prov., Antu Co., Beihe, forest behind fire tower, 14.VIII.88, coll. R.H. Petersen, field no. 1456 (TENN 48298). Pileus 'pale pinkish cinnamon' (6A2) at margin, inward 'tilleul buff' (7B2), 'avellaneous' (7B3), to 'wood brown' (7C4) near stipe attachment. Lamellae 'pale pinkish buff' (6A2). Stipe nearly lateral, 'tilleul buff' (7B2).

RUSSIA: Leningrad Reg., Nyzhnesvirsky Reserve, trail to Svir River, N 60°36.775', E 33°07.628', 28.VIII.99, coll. S.A. Redhead, on *Salix* or *Alnus*, field no. 10625 (TENN 58284). Pileus hygrophanous, 'vinaceous buff' (9B2) where moist, 'pale pinkish cinnamon' (6A2) where dry. Lamellae deep, mostly 'pale pinkish cinnamon' (6A2), near margin 'tilleul buff' (7B2). Stipe distinct, matt, 'tilleul buff' (7B2).

## A NOMENCLATOR FOR PLEUROTUS DJAMOR

Based on the parameters discussed above and previous experiments (Nicholl & Petersen, 2000), the following nomenclator can be offered for *Pleurotus djamor*. In most cases, links among these names are based solely on morphological analysis of type specimens ('[!]') often in less than pristine condition, but in all cases habit is pleurotoid and hyphal construction of stipe medullary tissue is 'dimitic'.

Pleurotus djamor (Rumph. apud Fr.) Boedijn, in: H.C.D. de Wit (ed.), Rumphius Memorial Vol. (1959) 292.

≡ Agaricus djamor Rumph. apud Fr., Syst. Mycol. 1 (1821) 185.

= Agaricus arboreus secundus Rumph., Fl. Amboin. 11 (1750) 125.

= Agaricus caryophyllus Berk., J. Linn. Soc. 13 (1872) 157 [!].

= Agaricus emerici Berk., Gard. Chron. 21 (2) (1880) 240 [!].

= Agaricus eous Berk., Hooker's J. Bot. 2 (1850) 83 [!]. [Type specimen unclear; description fits.]

= Pleurotus eous (Berk.) Sacc., Syll. Fung. 5 (1887) 361.

- = Agaricus flabellatus Berk. & Curtis, J. Linn. Soc. 11 (1871) 528 [!].
- = Agaricus griseo-roseus Mont., Syll. Gen. Spec. Cryptog. (1851) 114 [!].
- = Pleurotus griseo-roseus (Mont.) Sacc., Syll. Fung. 5 (1887) 386.
- = Agaricus luteoalbus Beeli, Bull. Soc. Roy. Bot. Belge 60 (1928) 163 [!].
- = Agaricus leptogramme Berk. & Broome, J. Linn. Soc. 11 (1871) 529 [!].
- = Agaricus moselei Berk., Challenger 37 (1878) [!].
- = Agaricus ninguidus Berk., Hooker's J. Bot. 2 (1850) 84 [!].
- ≡ Pleurotus ninguidus (Berk.) Sacc., Syll. Fung. 5 (1887) 361.
- = Pleurotus ostreatoroseus Singer, Publ. Inst. Mic., Univ. Recife 304 (1961) 10.
- = Agaricus pacificus Berk., London J. Bot. 1 (1842) 451 [!].
- = Agaricus placentodes Berk., Hooker's J. Bot. 4 (1852) 104 [!].
- = Agaricus prometheus Berk. & Curtis, Amer. Acad. Arts & Sci. 4 (30) (1858) [!].

= Pleurotus salmoneostramineus Vasilyeva, [Russian title] Agar. & Bol. Primorsk Reg. (1973) 85.

= Agaricus scabriusculus Berk., J. Linn. Soc. Bot. 13 (1873) 157 [!].

= Pleurotus scabriusculus (Berk.) Sacc., Syll. Fung. 5 (1887) 374.

≡ Pleurotus scabellus Sacc., Syll. Fung. 5 (1887) 374 [nom. nov., non P. scabriusculus Berk. Australian Fungi no. 18.]

In addition, another name represents pleurotoid basidiomata with partial veil covering lamellae in juvenile state. Vilgalys et al. (1996) has shown that *P. calyptratus* is partially sexually compatible with *P. djamor*, DNA sequences are contaxic, basidiomata are dimitic, and both names were included in ISG V.

## Pleurotus djamor forma calyptratus (Lindblad apud Fr.) R.H. Petersen, comb. & stat. nov.

siai. nov.

Basionym: Agaricus calyptratus Lindblad apud Fr., Monog. Hymen. Suecici (1857) 238.

- = Pleurotus calyptratus (Lindblad apud Fr.) Sacc., Syll. Fung. 5 (1887) 341.
- = Tectella calyptratus (Lindblad apud Fr.) Singer, Agar. Mod. Tax. (1951) 263.

Typification: Fries (ibid.) saw an illustration by Lindblad, now in the Stockholm Museum, and that illustration can serve as lectotype. An epitype should be sought among material from vic. Högholm in Sudermannia, referred to by Fries.

## NOTES ON MITICITY IN PLEUROTUS

Corner (1932a, b) developed terms and definitions dealing with hyphal construction in polyporoid fungi and elaborated on these concepts in the clavarioid fungi (Corner, 1950). In 1953, Corner recapitulated definitions formulated in 1932, and defined a skeletal hypha as follows: "... unbranched, thick-walled, commonly aseptate, longitudinal, constructional hyphae of the first order in the growing region." Likewise, Corner (1953) circumscribed generative hyphae as follows: "The thin-walled hyphae usually remain thin-walled and are very inconspicuous for this reason ... or parts of them become thick-walled and mistakable for skeletal or binding hyphae ... Because they *produce* the system of skeletal hyphae ... and the system of binding hyphae ... I called them generative hyphae." [Italics ours]. Specifically, when only one hyphal type was present in basidiome tissues, those tissues were termed 'monomitic', those with two hyphal types were 'dimitic', and those with three were 'trimitic'. Corner's personal experience allowed 'dimitic' to include a combination of generative plus skeletal hyphae or generative plus binding hyphae, but other variations of miticity (i.e. inclusion of gloeoplerous or laticiferous hyphae in combination with generative hyphae, etc.; see Pegler, 1983, 1996, for a more complete exposition) were largely overlooked for they did not seem important to the classification of the polypores.

These definitions were carried to the clavarioid fungi (Corner, 1950), where skeletal hyphae were found in certain basidiomata, and also in the rhizomorphs of some *Ramaria* taxa (Petersen, 1975). In *Lachnocladium*, skeletal hyphae were modified into highly branched structures, while in *Ramaria* they were largely unbranched. Later (Corner, 1970) further modifications to the original definition were made, chiefly that skeletal hyphae could appear as *intercalary* segments, arising from and reverting to the generative morphology (i.e. as found in *Ramaria gracilis*, *R. rubella* and others).

As time elapsed, these terms of miticity and hyphal types were augmented so that now they have become somewhat blurred. Nonetheless, these concepts were applied to other fungi, and in fact, di- or trimitic hyphal construction (sensu Corner) came to exclude members of the Agaricales, and became semi-diagnostic for members of the Aphyllophorales. If a basidioma was monomitic it could be placed in the Agaricales or the Aphyllophorales, but if it was dimitic or trimitic (sensu Corner), it was excluded from the Agaricales. While it was recognized that other hyphal types might be found in basidiomatal tissues (i.e. gloeoplerous hyphae, subdivided by staining affinities of contents; viz. the Gomphaceae, where gloeoplerous hyphae are cyanophilous, versus the Auriscalpiaceae where they are blackish in sulfobenzaldehyde), and while presence of such hyphal types alter Corner's terms (i.e. *Lentinellus*, in which basidiomata of several species exhibit generative, skeletal and gloeoplerous hyphal types, which Corner would term dimitic, gloeoplerous hyphae having never been included in his summary of hyphal construction), this discussion pertains to *Pleurotus* and definition of its basidiomatal tissue.

Corner (1983) became discontent with attempts to refine his basic mitic plan and put forward subcategories of dimiticity. Later (Corner, 1991) the expanded system was outlined again. Pegler (1996) furnished a history of research on miticity in Basidiomycotina, including *Pleurotus* under dimitic construction with "limited skeletal hyphae ... 'type d4' of Corner" (1983, 1991). The word 'limited', however, indicated the termination of individual skeletal hyphae within the tissue (I cannot conceptualize an alternative), not limitation as intercalary lengths limited by clamp-connections at origin from and reversion to generative hyphae.

Micromorphological examination of some *Pleurotus* basidiomata, especially of stipe medullary tissue, reveals the presence of two hyphal types (and occasionally, rare gloeoplerous hyphae). One hyphal type seems to form the flesh substance and, while often thick-walled, hyphae are frequently septate/clamped so in traditional Cornerian terminology are generative hyphae. A second hyphal type (again in basidiomata of only some species) appears long-celled, thick-walled, and refringent under phase contrast microscopy. Superficially, these resemble skeletal hyphae, and based on the presence of such hyphae, such tissues have been called dimitic (Stankovicova, 1974; Pegler, 1977). Although occasionally these skeletal hyphae are found in pileus and lamellar tramae, the most reliable tissue in which they are to be seen is the stipe medulla. When carefully analysed, however, these skeletal hyphal segments are seen often to be inter-

calary, and/or to bear occasional internal clamp-connections. They are not skeletal hyphae sensu Corner, therefore, and tissues which include them are not strictly dimitic sensu Corner. Pilát (1965) recognized this discrepancy in a discussion of *P. calyptratus*.

Stankovicova (1974) reported that certain species of *Pleurotus* were monomitic (i.e. *P. ostreatus*) while others (i.e. *P. calyptratus*, *P. 'sajor-caju* var. *dactyliophora'*) were considered dimitic or perhaps trimitic. The accurate identity of the latter taxon cannot be judged, for Pegler (1983) ascertained that true *P. sajor-caju* was a *Lentinus*, not *Pleurotus*. Stankovicova (1974) made no distinction between various types of skeletal hyphae.

Perhaps based on this hyphal construction (i.e. 'dimitic'), *Pleurotus* has been linked with the genera *Polyporus* (Pegler, 1983; Hibbett & Vilgalys, 1993) and *Lentinus* (Corner, 1981; Petersen & Nicholl, 1997). Taxa of all three genera produce large, non-amyloid basidiospores of comparable size and shape and basidiomata of 'pleurotoid' habit (i.e. imbricate, stipe-less basidiomata to stoutly, usually eccentrically or laterally stipitate habit). Early molecular evidence included *Pleurotus* in phylogenetic reconstructions concerned chiefly with *Lentinus* (Hibbett & Vilgalys, 1993), but later refinements showed that *Pleurotus* was actually within the Agaricales (Montcalvo et al., 2000; Thorn et al., 2000). With this phylogenetic alignment, *Pleurotus* appears to be one of very few agaric genera (or perhaps the only genus) with 'dimitic' hyphal construction.

Should a new term be coined for the 'dimitic' hyphal construction in some *Pleurotus* species? We think not, but awareness of this anomaly in *Pleurotus* is urged, with the suggestion that future descriptions be worded carefully to take such hyphal construction into account.

We tentatively conclude that *Pleurotus* includes three groups based on a combination of hyphal construction and general habit. In all cases occasional to rare gloeoplerous hyphae are also found.

Type I — Stipe trama: hyphae frequently clamped, often somewhat inflated, usually thick-walled, relatively uniform in appearance (i.e. skeletalized generative hyphae; monomitic). Lamella trama: hyphae as in stipe tissue but less thick-walled. Basidiomata strongly eccentrically stipitate with inconspicuous pileus extension over stipe [i.e. *P. ostreatus, P. populinus, P. pulmonarius, P. abieticola*) to centrally or subcentrally stipitate (i.e. *P. albidus, P. eryngii*), soft-fleshy; pileus trama usually over 5 mm thick near stipe, etc.].

Type II — Stipe medullary tissues comprising two elements: 1) generative hyphae as in type I; 2) imperfect skeletal hyphae long-celled, skeletalized (i.e. wall usually thicker than 0.7  $\mu$ m thick), refringent under phase contrast microscopy; clamp-connections initiative to occasionally intercalary, especially near bases of hyphal branches. Lamella trama: usually as in Type I, occasionally with 'skeletal' hyphae as in stipe tissues of Type II. Basidiomata stoutly stipitate, usually large; stipe central (*P. dryinus*, *P. levis*) eccentric to almost lateral (*P. opuntiae*, *P.* cf. gemmellarii, *P. fossulatus*); pileus trama usually over 5 mm thick near stipe.

Type III — Stipe medullary tissues comprising two elements: 1) generative hyphae as in Type I; 2) imperfect skeletal hyphae similar to those of Type II; clamp-connections

rare, initiative and intercalary. Lamellar trama: juvenile basidiomata as in Type I or exhibiting 'skeletal' hyphae (usually late in age). Immature basidiomata laterally stipitate and then the stipe reduced to a knot; pileus trama usually less than 5 mm thick near stipe (*P. djamor* and its forms).

#### **ACKNOWLEDGEMENTS**

Dr. Rytas Vilgalys generously furnished an ITS sequence for D327 (ISG XIII): it appears in the phylogenetic reconstruction. This work was supported, in part, by National Science Foundation grant DEB-9521526 and DEB-9878011 (PEET grants). Dr. Alberto's time at Tennessee was supported by the Hesler Endowment Fund of the Botany Department, University of Tennessee.

#### REFERENCES

- Anderson, J.B. & R.C. Ullrich. 1979. Biological species of Armillaria mellea in North America. Mycologia 71: 402-414.
- Corner, E.J.H. 1932a. The fruit-body of Polystictus xanthopus. Ann. Bot. 46: 71-111.
- Corner, E.J.H. 1932b. A Fomes with two systems of hyphae. Trans. Brit. Mycol. Soc. 17: 51-81.
- Corner, E.J.H. 1950. A monograph of Clavaria and allied genera. Ann. Bot. Mem. 1: 1-740.
- Corner, E.J.H. 1953. The construction of polypores 1. Introduction: Polyporus sulphureus, P. squamosus, P. betulinus and Polystictus microcyclus. Phytomorph. 3: 152–167.
- Corner, E.J.H. 1970. Supplement to 'A monograph of Clavaria and allied genera'. Beih. Nova Hedwigia 33: 1-299.
- Corner, E. J. H. 1981. The agaric genera Lentinus, Panus, and Pleurotus with particular reference to Malaysian species. Beih. Nova Hedwigia 69: 1–169.
- Corner, E.J.H. 1983. Ad Polyporaceas I. Amauroderma and Ganoderma. Beih. Nova Hedwigia 75: 1–182.
- Corner, E.J.H. 1991. Ad Polyporaceas VII. The Xanthochroic polypores. Beih. Nova Hedwigia 101: 1–175.
- GCG. 2000. Wisconsin package 10.1, Genetics Computer Group (GCG). Madison, WI.
- Gonzalez, P. & J. Labarère. 2000. Phylogenetic relationships of Pleurotus species according to the sequence and secondary structure of the mitochondrial small-subunit rRNA V4, V6 and V9 domains. Microbiology 146: 209-221.
- Hibbett, D.S. & R. Vilgalys. 1993. Phylogenetic relationships of Lentinus (Basidiomycotina) inferred from molecular and morphological characters. Syst. Bot. 18: 409-433.
- Hilber, O. 1982. Die Gattung Pleurotus. Biblioth. Mycol. 87: 1-448.
- Hilber, O. 1997. The genus Pleurotus (Fr.) Kummer (2). Publ. priv.
- Horak, E. 1968. Synopsis generum Agaricalium (Die Gattungstypen der Agaricales). Beitr. Kryptogamenfl. Schweiz 13: 1–741.
- Hughes, K.W., L.L. McGhee, A.S. Methven, J. Johnson & R.H. Petersen. 1999. Patterns of geographic speciation in the genus Flammulina based on sequences of the ribosomal ITS1-5.8S-ITS2 area. Mycologia 91: 978–986.
- Kornerup, A. & J.F. Wanscher. 1978. Methuen Handbook of Colour. 3rd edition. London.
- Montcalvo, J-M., F.M. Lutzoni, S.A. Rehner, J. Johnson & R. Vilgalys. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Biol. 49: 278-305.
- Nicholl, D.B.G. & R.H. Petersen. 2000. Phenetic plasticity in Pleurotus djamor. Mycotaxon 76: 17-37.
- Pegler, D.N. 1977. A preliminary agaric flora of east Africa. Kew Bull, Addit. ser. VI.
- Pegler, D.N. 1983. The genus Lentinus. Kew Bull., Addit. ser. X: 28.
- Pegler, D.N. 1996. Hyphal analysis of basidiomata. Mycol. Res. 100: 129-142.
- Petersen, R.H. 1975. Ramaria subgenus Lentoramaria, with emphasis on North American taxa. Biblioth. Mycol. 43: 1–161.

- Petersen, R.H. 1995a. There's more to a mushroom than meets the eye: Mating studies in the Agaricales. Mycologia 87: 1–17.
- Petersen, R.H. 1995b. Contributions of mating studies to mushroom systematics. Canad. J. Bot. 73 (Suppl. 1): S831-S842.
- Petersen, R.H. & K.W. Hughes. 1993. Intercontinental interbreeding collections of Pleurotus pulmonarius, with notes on other species. Sydowia 25: 139–152.
- Petersen, R.H. & K.W. Hughes. 1997. A new species of Pleurotus. Mycologia 89: 173-180.
- Petersen, R.H. & K.W. Hughes. 1998. Mating systems in Omphalotus (Paxillaceae, Agaricales). Plant Syst. Evol. 211: 217-229.
- Petersen, R.H. & I. Krisai-Greilhuber. 1996. An epitype specimen for Pleurotus ostreatus. Mycol. Res. 100: 229-238.
- Petersen, R.H. & I. Krisai-Greilhuber. 1999. Type specimen studies in Pleurotus. Persoonia 17: 201-220.
- Petersen, R.H. & D.B.G. Nicholl. 1997. Mating systems of some putative polypore-agaric relatives. Pl. Syst. Evol. 207: 135–158.
- Pilát, A. 1965. Further finds of Pleurotus calyptratus (Lindbl. in Fr.) Sacc. in Czechoslovakia. Česka Mykol. 19: 53-56.
- Ridgway, R. 1912. Color standards and color nomenclature. Washington, DC. Publ. priv.
- Singer, R. 1952 ('1950'). Type studies on Basidiomycetes. Lilloa 23: 147-246.
- Stankovicova, L. 1974 ('1973'). Hyphal structures in some pleurotoid species of Agaricales. Nova Hedwigia 24: 61–120.
- Thorn, R.G., J-M. Montcalvo, C.A. Reddy & R. Vilgalys. 2000. Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the polyphyletic pleurotoid-lentinoid fungi. Mycologia 92: 241–252.
- Vilgalys, R. 1997. Biodiversity of the oyster mushroom Pleurotus. Mushroom News 1997: 32-35.
- Vilgalys, R., J-M. Montcalvo, S-R. Liou & M. Volovsek. 1996. Recent advances in molecular systematics of the genus Pleurotus. In: D. Royse (ed.), Mushroom biology and mushroom products, Penn. State Press: 91–101.
- Vilgalys, R., A. Smith & B.L. Sun. 1993. Intersterility groups in the Pleurotus ostreatus complex from the continental United States and adjacent Canada. Canad. J. Bot. 71: 113–128.
- Vilgalys, R. & B.L. Sun. 1994. Ancient and recent patterns of geographical speciation in the oyster mushroom Pleurotus revealed by phylogenetic analysis of ribosomal DNA sequences. Proc. Nat. Acad. Sci. USA 91: 4599–4603.
- White, T.J., T. Bruns, S. Lee & J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (eds.), PCR Protocols, A Guide to Methods and Applications: 315–322. Academic Press, San Diego.
- Zervakis, G.I. 1998. Mating competence and biological species within the subgenus Coremiopleurotus. Mycologia 90: 1063-1074.
- Zervakis, G.[I.] & C. Balis. 1991. Pleurotus species in Greece: and evaluation of their morphological and physiological characteristics. In: M.J. Maher (ed.), Science and cultivation of edible fungi: 537-544. Rotterdam.
- Zervakis, G. [I.] & C. Balis. 1992. Comparative study on the cultural characters of Pleurotus species under the influence of different substrates and fruiting temperatures. Micol. Neotrop. Appl. 5: 39-47.
- Zervakis, G.[I.] & C. Balis. 1996. A pluralistic approach in the study of Pleurotus species with emphasis on compatibility and physiology of the European morphotaxa. Mycol. Res. 100: 717–731.
- Zervakis, G.[I.] & J. Labarère. 1992. Taxonomic relationships within the fungal genus Pleurotus as determined by isoelectric focusing analysis of enzyme patterns. J. Gen. Microbiol. 138: 635–645.
- Zervakis, G.[I.], J. Sourdis & C. Balis. 1994. Genetic variability and systematics of eleven Pleurotus species based on isozyme analysis. Mycol. Res. 98: 329-341.