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Biological species concepts in eastern North American populations of *Lentinellus*

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Abstract: As part of a biosystematic study of the *Lentinellus ursinus* species complex in eastern North America, field and herbarium specimens from North America, Central America, and Europe were examined. Crossing single basidiospore isolates demonstrated tetrapolar (bifactorial) mating systems. Three biological species are recognized as *L. ursinus, L. angustifolius,* and *L. vulpinus*. The production of gloeocystidia and/or chlamydospores is considered taxonomically significant for identifying somatic cultures of these species. All three taxa produced laccase, tyrosinase, and peroxidase in culture and are white-rot fungi. This is the first confirmed report of *L. angustifolius* from North America.

Key Words: Auriscalpiaceae, Basidiomycetes, culture mat analyses, Hericiales, mating systems, phenoloxidase enzymes, systematics

INTRODUCTION

The genus *Lentinellus* Karsten (Hericiales, Auriscalpiaceae) includes a group of clitocyboid to pleurotoid fungi most commonly collected in deciduous and coniferous forests throughout North temperate regions. Although the genus *Lentinellus* is easily recognized by serrate lamellae and amyloid, ornamented basidiospores, many taxa are extremely variable and often difficult to distinguish macroscopically and/or microscopically. The difficulty in segregating taxa is due primarily to the relatively long life of the basidiomata, which results in considerable morphological variation especially in pileus tomentum and degree of tramal amyloidity (Miller and Stewart 1971). The use of morphological characters alone to segregate taxa has led to considerable taxonomic confusion as numerous taxa have been proposed only to later be reduced to synonymy (Miller and Stewart 1971, Pegler 1983, Segedin 1996). Among the most confusing species complex is the one which includes *L. ursinus* (Fr.) Kühner, *L. castoreus* (Fr.) Romag., a European taxon, *L. vulpinus* (Sow.: Fr.) Kühner & Maire, and several other morphospecies (Stalpers 1996) which have not been adequately differentiated biologically or phylogenetically from *L. ursinus* s.s. Although morphotaxonomy has been used to distinguish *L. ursinus* from *L. vulpinus* (Miller and Stewart 1971), it has not accounted for additional cryptic taxa within the *L. ursinus* species complex and reveals nothing about reproductive compatibility between morphospecies. Since preliminary data suggested that more than one biological species was represented in the *L. ursinus* species complex in eastern North America, intercollection matings were proposed to identify sexually compatible populations (i.e., biological species) in the *L. ursinus* species complex.

The objectives of this study were: (i) to determine the mating systems operating in the *L. ursinus* complex; (ii) to identify sexually compatible and incompatible populations; (iii) to determine the taxonomic value of culture mat analyses and phenoloxidase reactions, and (iv) to identify additional morphological characters which might be used to more readily segregate taxa.

MATERIALS AND METHODS

Basidiomata were collected in six states in eastern North America from Aug through Nov, 1995 and 1996. Macromorphological features were recorded using standard techniques (Largent 1977) with colors terms taken from Kornerup and Wanscher (1967). Basidiomata were dried and deposited in the herbarium at Eastern Illinois University (EIU). Additional specimens were obtained from herbaria at the Royal Botanical Gardens, Edinburgh (E), the University of Tennessee (TENN), the University of Michigan (MICH), and Uppsala (UPS). Supplementary specimens with spore prints were also provided by Thomas J. Volk (TJV). The names of the authors are abbreviated ANM for Andrew N. Miller and ASM for Andrew S. Methven.

Micromorphological analyses of specimens followed standard practices and terminology (Largent et al 1977). Sections were mounted in 10% NH_4OH, Melzer's reagent, or

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sulfobenzaldehyde and observed under bright field optics with a Nikon research microscope. A minimum of 10 basidia, 10 pseudocystidia, and 30 basidiospores were measured for each collection. For basidiospore data, $\bar{x} \pm$ SD and Q (spore length/width) were calculated for each species.

Monospore and polyspore isolates.—Single basidiospore isolates (SBIs) were collected utilizing one of the following methods: (i) small pileus sections from fresh basidiomata were suspended from the lid of a 90-mm Pyrex Petri plate with petroleum jelly and basidiospores allowed to fall onto malt extract (1.25% Difco) agar (2% Difco Bacto) (MEA) for 15–60 min; or (ii) basidiospores from a spore print were affixed to a moistened, sterilized loop and streaked onto MEA in a 90-mm Pyrex Petri plate. Basidiospore germination occurred after 3–6 d of incubation in the dark at 23 C. Individual germlings were initially transferred to MEA in 90-mm Pyrex Petri plates and then subsequently transferred to individual screw cap test tubes containing MEA after the colonies reached ca 1 cm diam. Polyspore isolates were obtained by transferring a 5-mm^2 block of MEA containing numerous germlings to MEA in screw cap test tubes which were then stored in the dark at 23 C. In the case of ANM 321 and RHP 8768, polyspore isolates were created by self-crossing the mating types identified in each collection. Additional sets of SBIs, along with accompanying polyspore isolates and voucher specimens, were obtained from R. H. Petersen (RHP) (TENN). Ancillary sets of SBIs and voucher specimens from Austria and Costa Rica were obtained from I. Krisai-Greilhuber and R. H. Petersen, respectively. All SBIs (including tester strains) and polyspore isolates utilized in this study were deposited in the University of Tennessee Fungus Culture Collection (TENN).

Intra- and intercollection matings.—Intra- and intercollection matings followed Petersen and Cifuentes (1994) with minor modifications. Clamp connections were observed in a 1:1 mixture of 2% phloxine and 2% congo red at ×400 under bright field illumination using a Nikon research microscope. Intra- and intercollection matings were scored on a grid for the presence or absence of clamp connections and notes on contact zone morphology recorded. Macromorphological features were recorded using the same procedure outlined under culture mat analyses (see below). After intracollection matings were scored, the grid was rearranged to produce the most discernable pattern (Ginns 1974), and mating types were arbitrarily assigned for each collection. Intracollection matings were conducted on 13 of 17 collections (ANM 473 and ASM 8109 consisted of only 3 SBIs each and ANM 511 and RHP 7880 consisted of only 4 SBIs each). Tester strains were then arbitrarily selected for intercollection matings. All tester strains (or designated SBIs if mating types were not identified) were crossed as above in all possible combinations except in ANM 511 and RHP 7880. After ample time for development of a contact zone, matings were examined for clamp connections and scored as above.

Culture mat analysis and phenoloxidase activity.—Polyspore isolates were transferred to the center of MEA in 90 mm Pyrex Petri plates and incubated in the dark at 23 C. After 11–14 d (28 d for RHP 7966), 5-mm-diam plugs were removed from the margin of the hyphal mat and transferred to 12 replicate plates each of MEA and Difco potato dextrose agar (PDA). Growth rates and macromorphological and micromorphological features were recorded at 2-wk intervals according to Nobles (1948, 1965), Stalpers (1978), and Desjardin (1990). Color terms were taken from Kornerup and Wanscher (1967). Nobles Species Codes (1965) and Staplers Species Codes (1978) were determined for each species.

Spot tests for the phenoloxidase enzymes laccase, tyrosinase, and peroxidase were conducted on culture mats at weeks 2 and 6 (Desjardin 1990). Syringaldazine (Harkin and Obst 1973, Harkin et al 1974) and α-naphthol (Käärik 1965) were used to test for laccase activity, L-tyrosine and *p*-cresol for tyrosinase (Marr 1984, Marr et al 1986), and pyrogallol plus H_2O_2 for peroxidase (Taylor 1974, Staplers 1978). Distilled water and 95% ethanol were used as negative controls.

RESULTS

Lentinellus ursinus (Fr.) Kühner, Le Botaniste 17:99, 1926.

Basidiomata morphology. Miller and Stewart (1971) adequately described this taxon and provided photographs and line drawings of salient microscopic features. Although minor variations in some characters were found in this study, none were significantly different from those reported by Miller and Stewart (1971) except for the presence of oleiferous hyphae which were: (i) abundant throughout pileipellis, at times terminating as solitary, cylindric to narrowly clavate, thin-walled pileocystidia; (ii) infrequent to common in trama; (iii) abundant in subhymenium, terminating as clavate pseudocystidia, and (iv) thin-walled, unclamped, unbranched, inamyloid, yellowish in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Specimens examined. RUSSIA. PRIMORSKI REGION: District Ternei, Sichote Alin Biosphere Reserve, Kabanya, 18 Sep 1990, *R.H. Petersen 3307* (TENN 53150); Meise, 8 Sep 1993, *R.H. Petersen 6556* (TENN 52995). SCOTLAND. Hollands Wood, New Forest, Hants., 23 Oct 1969, *Orton 3663* (E00028785) (E). SWEDEN. SKÅNE: Osby, Kjelsved, 28 Sep 1980, *S. Ryman s.n.* (UPS). UPPLAND: Bondkyrka Par., Vardsatra Nature Reserve, 16 Oct 1980, *S. Ryman 6132* (as *Lentinellus castoreus*) (UPS); Sollentuna Par., 400 m N. of Hagerstalund, 25 Sep 1984, *Nils Lundqvist 15259* (F-01752) 18676 (UPS). USA. ILLINOIS: Jackson Co., Touch of Nature Preserve, 20 Oct 1989, *R.H. Petersen 2414* (TENN 48582); Gallatin Co., Rim Rock Nature Preserve, 15 Oct 1994, *A.N. Miller 210* (EIU); Douglas Co., Walnut Point State Park, 7 Oct 1995, *A.N. Miller 321* (EIU); Ogle Co., White Pines Forest State Park, 28 Sep 1996, *A.N. Miller 497* (EIU); Douglas Co., Walnut Point State Park, 4 Nov 1996, *A.N. Miller 512* (EIU). INDIANA: Brown Co., Yellowwood State Park, 20 Oct 1996, *A.N. Miller 508* (EIU); Monroe

Co., Hoosier National Forest, Col. Joshua Dettmer, 21 Oct 1996, *A.N. Miller 510* (EIU). IOWA: Linn Co., Palisades-Kepler State Park, 31 Aug 1996, *A.N. Miller 480* (EIU); Muscatine Co., Wildcat Den State Park, Griss Mill Trail, 1 Sep 1996, *A.N. Miller 482* (EIU). MICHIGAN: Leelanau Co., North Bar Lake, 25 Jul 1995, *A.S. Methven 8027* (EIU); Benzie Co., Esch Road, 7 Aug 1996, *A.S. Methven 8109* (EIU); Leelanau Co., Shell Lake, 24 Jul 1997, *A.S. Methven 8155* (EIU). MISSOURI: Stoddard Co., Mingo Wildlife Refuge, 17 Sep 1994, *A.N. Miller 169* (EIU); Wayne Co., Sam A. Baker State Park, Shut-in Trail, 20 Sep 1996, *A.N. Miller 491* (EIU); Mingo Wildlife Refuge, Ditch 6, 21 Sep 1996, *A.N. Miller 493* (EIU). NORTH CAROLINA: Transylvania Co., Pisgah National Forest, Coon Tree Trail, 29 Aug 1989, *R.H. Petersen 2210* (TENN 48125). WISCONSIN: La Crosse Co., Hixon Forest, 21 Aug 1996, *T.J. Volk 96–105* (*A.N. Miller 473,* EIU).

Intracollection matings. Two collections (ANM 473 and ASM 8109) of *L. ursinus* consisted of only 3 SBIs each. Therefore intracollection matings were conducted on only eight of the ten collections of *L. ursinus* from which SBIs were obtained. All intracollection matings revealed tetrapolar mating systems.

Specimens and tester strains (in the order A_1B_1, A_2B_2, A_1B_2, A_2B_1) used in mating studies were as follows: *ANM 321-1,2,3,5, ANM 473-1,2,3, ANM 482-1,3,6,8, ANM 491-1,7,4, ANM 497-1,2,10,14, ANM 508-1,3,2,4, ASM 8027-1,2,13,7, ASM 8109-1,3,2, RHP 2210-1,3,2,5, RHP 2414-1,5,13* (all TENN).

Macromorphology. MEA: Radius (and means): Wk II 37–65 mm (52.9 mm), plates covered by wk IV. Mat initially silky, translucent, becoming farinaceous, white (1A1) to light yellow (4A4-5) by wk VI; advancing zone appressed, silky, margin occasionally plumose, not distinct, translucent; plug initially undifferentiated, translucent, becoming farinaceous, white (1A1) to light yellow (3A4-5) by wk VI; reverse unchanged; odor none to faintly fruity. PDA: Radius (and means): Wk II 27–66 mm (44 mm), wk IV 64–84 mm (71.5 mm), plates covered by wk VI; mat subfelty, white (1A1) to light yellow (3A4), becoming farinaceous by wk VI; advancing zone appressed, plumose, narrow (2–5 mm), not distinct, translucent to yellowish white (1A2); plug farinaceous, white (1A1) to light yellow (3A4-4A5) by wk VI; reverse variable, unchanged to yellow-brown (5E4-7) or brown (6D7-6E6) under plug by wk VI; odor none to faintly fruity.

Micromorphology. MEA: Advancing zone hyphae 1.3–6.3 μm diam, thin-walled, clamped, infrequently branched, rarely intertwined into mycelial cords, hyaline, contents light yellow in KOH, inamyloid; aggregated crystals surrounding hyphae, crystals 5–9 × 5.1–8 μm, globose to subglobose, hyaline to pale yellow in KOH; diffuse crystals absent in agar. Aerial hyphae 1–6 μm diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; gloeocystidia common to abundant, cylindric [25–101 (-166) × 2–5 μm], clavate (13–60 × 3–8 μm) or ventricose-rostrate [15–68 × 3–8 (-10.5) μm], terminal, basally clamped, thin-walled, rarely forked, yellow in KOH, contents resinous, partially refractive, staining dark purple in sulfobenzaldehyde; chlamydospores abundant, 4–9.5 (-15) × (3-) 4–6.3 (-7.7) μm (7.4 ± 1.7 × 5.1 ± 1.5), subglobose to pyriform or broadly ellipsoid, mostly terminal, rarely intercalary, thick-walled (wall ≤ 1 μm thick), hyaline to yellow in KOH, contents amorphous to resinous; diffuse crystals in agar few to abundant, grain-like to globular, rarely needle-like, refractive. Submerged hyphae 1–6 (-6.9) μm diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; diffuse crystals in agar few to abundant, prismatic and slightly refractive or grain-like to globular and refractive. PDA: Advancing zone hyphae same as MEA except aggregated crystals absent. Aerial hyphae same as MEA. Submerged hyphae same as MEA except diffuse crystals in agar cuboidial to quartz-like, infrequently globular or rarely needle-like, refractive. Nobles species code: 2, 3, 15, 34, 36, 38, 39, 44, 50, 54, 60. Stalpers species code: 2, 3, 6, 13, 14, 15, 18, 20, 24, 30, 31, 36, 38, 39, 45, 52, 53, 73, 81, 82, 83, 85, 89, 94.

Phenoloxidase reactions tested at the margin and 1 cm from the inoculation plug for *L. ursinus* were as follows: Wk II: On MEA, L-tyrosine (−), *p*-cresol (−), pyrogallol plus hydrogen peroxide (+), α-naphthol (+) at margin and (weakly + to +) 1 cm from inoculation plug, syringaldazine (+); On PDA, L-tyrosine (− to weakly +) at margin and (− to +) 1 cm from inoculation plug, *p*-cresol (− to +), pyrogallol plus hydrogen peroxide (+), α-naphthol (weakly + to +) at margin and (+) 1 cm from inoculation plug, syringaldazine (+); Wk VI: On MEA, similar results except α-naphthol more variable (− to +); On PDA, similar results except L-tyrosine more variable (− to +) at margin, α-naphthol more variable (− to +).

Specimens used in culture studies were *ANM 321, ANM 473, ANM 482, ANM 491, ANM 497, ANM 508, ANM 512, ASM 8027, ASM 8109, RHP 2210, RHP 2414* (all TENN).

Commentary. Lentinellus ursinus is characterized macroscopically by the large (2–14 cm broad), brownish orange to yellow-brown pileus, subdistant to close, broad lamellae, and sessile habit. *Lentinellus angustifolius* has smaller basidiomata and close to crowded, narrow lamellae, while *L. vulpinus* has a white to yellowish pileus, close to crowded lamellae, and short, fused stipes. Cultures of *Lentinellus ursinus* covered the surface of both MEA and PDA plates in four wk, produced silky to subfelty, white to light yel-

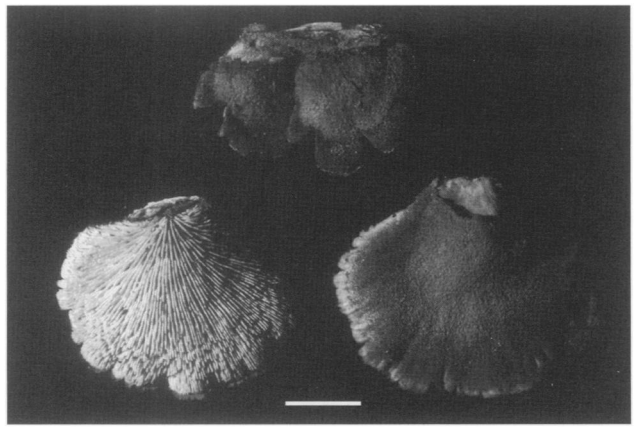

FIG. 1. *Lentinellus angustifolius* (ANM 495). Bar = 1 cm.

low, farinaceous colonies, and developed abundant chlamydospores and cylindric, ventricose-rostrate, or clavate gloeocystidia. *Lentinellus angustifolius* formed translucent to orange-yellow colonies which were never farinaceous and produced few chlamydospores. *Lentinellus vulpinus* grew much slower, formed colonies which were never farinaceous, produced no chlamydospores, and developed only cylindric gloeocystidia.

Lentinellus angustifolius (Romell) Singer, Lilloa 25: 91, 1952. FIG. 1

≡ *Lentinus angustifolius* Romell, Bih K Svenska Vetensk Akad Handl 26, Afd 3, no 16:7–8, pl. 1/11, 1901.

Basidiomata morphology. Pileus 1–2.5 (-5) × 1–3.5 cm, conchate to plane in profile, dimidiate to spathulate from above; margin decurved to plane, even to lobed, at times rimose, glabrous, yellowish white (4A2), light yellow (4A4), greyish yellow (4B4), greyish orange (5B6) or brownish orange (5C6); surface moist to dry, hygrophanous, minutely tomentose to matted fibrillose towards point of attachment, hispid hairs sometimes present, yellowish brown (5D6-5F6), brown (6E8) or dark brown (6F7) at point of attachment. Pileus context 1–1.5 mm thick, yellowish white (2A2) to pale yellow (3A3), not staining on exposure. Lamellae radiating outward from point of attachment, close to crowded, thin, 2–3 mm broad, not forked, margins serrulate to serrate, yellowish white (3A2) to pale yellow (3A3-4A3); lamellulae in 3–4 tiers. Stipe absent. Odor pleasant, faintly fruity. Taste absent to slightly acrid, numbing tip of tongue for 5 min or more.

Basidiospores white in mass, (3.1-) 3.9–5 × (2.5-) 2.9–3.9 μm (4.1 ± 0.3 × 3.1 ± 0.2, Q = 1.3 ± 0.1), subglobose to broadly ellipsoid, thin-walled, minutely echinulate, amyloid; contents hyaline, light yellow in NH_4OH, often containing a globose, centric to subcentric, light yellow, refractive oil droplet. Basidia (9-) 12–21 × 3–5.5 μm, narrowly clavate to clavate, thin-walled, four-sterigmate, contents hyaline to light yellow in NH_4OH. Pseudocystidia common on lamellar faces and edges, of two types: type 1) 13–29 (-39) × 3–6.5 μm, subulate (fusiform) to obclavate, apices occasionally acuminate to mucronate, thin-walled, contents hyaline to light yellow in NH_4OH, occasionally resinous, staining dark purple in sulfobenzaldehyde; type 2) 13–33 × 2–6 (-9) μm, cylindric to clavate, apices obtuse, otherwise similar to type 1 except contents frequently resinous. Pileipellis a cutis of radial hyphae and erect pileocystidia; radial hyphae 1.7–8.5 μm diam, thin- to slightly thick-walled (wall <1 μm thick), clamped, inamyloid; contents light yellow to yellow-brown in NH_4OH; pileocystidia 30–900 (-1850) × 1.7–5 (-8) μm, solitary, tangled or frequently in fascicles, thin-walled to slightly thick-walled (wall <1 μm thick), clamped, inamyloid; contents light yellow to yellow-brown in NH_4OH. Pileus trama interwoven; hyphae 1.9–7 μm diam, thin- to slightly thick-walled (wall <1 μm thick), clamped, inamyloid; contents hyaline in NH_4OH; thick-walled (wall >1 μm thick) hyphae rarely clamped, walls weakly amyloid, amyloid reaction occasionally occurring in spiral bands; contents light yellow in NH_4OH. Lamellar trama similar to pileus trama. Subhymenium composed of parallel hyphae 1.7–6.5 μm diam, thin-walled, frequently clamped, inamyloid; contents light yellow to light caramel brown in NH_4OH. Oleiferous hyphae (i) abundant throughout pileipellis, at times terminating as solitary, cylindrical, thin-walled pileocystidia; (ii) common in trama; (iii) abundant in subhymenium, terminating as pseudocystidia; and (iv) thin-walled, unclamped, unbranched, inamyloid, yellowish in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Habitat. Solitary to gregarious or in imbricate clusters on dead, decorticated, deciduous logs.

Distribution. Throughout temperate regions in the USA, Illinois south to Mississippi and North Carolina, west to Missouri, infrequent. Reported from Florida by Singer (Singer and Digilio 1952). Also known from Costa Rica and Austria.

Specimens examined. AUSTRIA. FEDERAL STATE: Burgenland, Jennersdorf District, Minihof-Liebau Community, forest along Mlhlgraben Street, grid square 9162/1, 21 Sep 1996, *I. Krisai-Greilhuber s.n.* (*A.N. Miller 511*, EIU). BRAZIL. RIO GRANDE DO SUL: Cachoeira, 20 Feb 1893, *G. Malme 256* (LECTOTYPE, S); Porto Alegro, 29 May 1893, *G. Malme 364* (PARATYPE, S). COSTA RICA. SAN JOSE: Jardin de Dota, N 9° 42′52″ W 83° 58′28″, 15 Jun 1995, *R.H. Petersen 7808* (TENN 53758); Valle Rio Savegre, San Gerardo, N 9° 33′2″ W 83° 48′27″, 21 Jun 1995, *R.H. Petersen 7880* (TENN 53831); 21 Jun 1995, *R.H. Petersen 7876* (TENN 53748). USA. ILLINOIS: Ogle Co., Castle Rock

State Park, Wildlife Viewing Trial, 27 Sep 1996, *A.N. Miller 495* (EIU). MISSISSIPPI: Pearl River Co., Henleyfield, N 30° 40′10″ W 89° 47′74″, 1 Dec 1995, *T.J. Volk 95-96* (EIU). MISSOURI: Wayne Co., Mingo National Wildlife Refuge, Flat Banks, 21 Sep 1996, *A.N. Miller 492* (EIU). NORTH CAROLINA: Macon Co., Blue Valley, Pickelsimer's Falls, Forest Service Road 79, 4 Aug 1996, *R.H. Petersen 8768* (TENN 55196, *A.N. Miller 377,* EIU). TENNESSEE: Sevier Co., Great Smoky Mountains National Park, Rainbow Falls Parking Lot, 28 Jul 1989, *R.H. Petersen 2036* (TENN 48636).

Intracollection matings. Two collections (ANM 511 and RHP 7880) of *L. angustifolius* consisted of only 4 SBIs each. Therefore intracollection matings were conducted on four of the six collections of *L. angustifolius* from which SBIs were obtained. Two collections (ANM 492 and ANM 495) initially appeared to have bipolar mating systems as only two mating types were identified from each collection. After additional SBIs were crossed with previously assigned mating types, three mating types were identified for each collection indicating the presence of a tetrapolar mating system. The other two collections also exhibited tetrapolar mating systems.

Specimens and tester strains (in the order A_1B_1, A_2B_2, A_1B_2, A_2B_1) used in mating studies were as follows: *ANM 492-3,12,18, ANM 495-1,4,13, ANM 511-1,5,6,7, RHP 2036-1,3,14, RHP 7880-1,2,3,7, RHP 8768-1,4,3,6* (all TENN).

Macromorphology. MEA: Radius (and means): Wk II 40–69 mm (57.3 mm), plates covered by wk IV. Mat initially silky, becoming subfelty (in RHP 2036), never farinaceous, translucent, pale yellow (2A3-3A3) to light yellow (2A4-3A5); advancing zone appressed, silky, margin even, not distinct, translucent; plug undifferentiated, becoming silky, never farinaceous, translucent to pale yellow (2A3); reverse unchanged; odor none to faintly fruity. PDA: Radius (and means): Wk II 32–63 mm (46.8 mm), plates covered by wk IV; mat subfelty, never farinaceous, translucent, white (1A1), pale yellow (2A3-4A3), yellow (3A6) or orange-yellow (4A6); advancing zone appressed, silky, occasionally plumose, narrow (1–7 mm), not distinct, translucent; plug subfelty to felty, never farinaceous, yellowish white (1A2-4A2) to yellow (3A6); reverse unchanged; odor none to faintly fruity. RHP 8768 (abnormal growth): PDA: Wk II 20–35 mm (25.8 mm), wk IV 22–42 mm (29.9 mm), wk VI 25–53 mm (32.7 mm); mat subfelty, never farinaceous, white (1A1), greyish beige (4C2), yellowish brown (5D6) or brown (6E4); advancing zone appressed to submerged, undulating, not distinct, white (1A1) to brownish orange (5C4); plug subfelty, similar to mat; reverse pale yellow (3A3), brownish orange (5C6), brown (6E8) or dark brown (6F8), odor faintly fruity.

Micromorphology. MEA: Advancing zone hyphae 1.1–5.3 μm diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; diffuse crystals absent in agar. Aerial hyphae 1–5.3 μm diam, thin-walled, clamped, infrequently branched, hyaline, contents light yellow in KOH, inamyloid; hyphal swellings absent to common, subglobose, thin-walled to rarely thick-walled, terminal or intercalary, hyaline to yellow in KOH, contents occasionally resinous; gloeocystidia common to abundant (absent in RHP 8768), frequently cylindric (23.5–135 × 2–4.5 μm), infrequently ventricose-rostrate (25.5–68 × 4–8.9 (-10) μm), rarely clavate, terminal, always basally clamped, thin-walled, rarely forked, yellow in KOH, contents resinous, partially refractive, staining dark purple in sulfobenzaldehyde; chlamydospores absent at wk II (except in RHP 8768), rare at wk IV, infrequent at wk VI, 6–11 × 4–7.5 μm (8.4 ± 1.5 × 5.7 ± 1.0), subglobose to broadly ellipsoid, terminal or intercalary, thick-walled (wall ≤ 1 μm thick), yellow in KOH, contents resinous; diffuse crystals in agar abundant (absent in RHP 8768), grain-like, globular or needle-like, refractive. Submerged hyphae 1–6 μm diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; diffuse crystals in agar few to common (absent in RHP 8768), prismatic or globular, refractive. PDA: Advancing zone hyphae same as MEA except hyphae sinuous in RHP 8768. Aerial hyphae same as MEA except hyphae sinuous and caramel brown in KOH in RHP 8768. Submerged hyphae same as MEA except hyphae occasionally sinuous in RHP 8768 and diffuse crystals in agar globular, cubodial or quartz-like, refractive. Nobles species code: 2, 3, 15, 34, 36, 37, 38, 39, 44, 50, 54, 60. Stalpers species code: 1, 2, 3, 6, 13, 14, 15, 18, 20, 24, 30, 35, 36, 38, 39, 45, 52, 53, 73, 82, 83, 85, 89, 94.

Phenoloxidase reactions tested at the margin and 1 cm from the inoculation plug for *L. angustifolius* were as follows: Wk II: On MEA, L-tyrosine (−), *p*-cresol (−), pyrogallol plus hydrogen peroxide (+), α-naphthol (− to +), syringaldazine (+); On PDA, L-tyrosine (− to +), *p*-cresol (− to +), pyrogallol plus hydrogen peroxide (+), α-naphthol (weakly + to +) at margin and (+) 1 cm from inoculation plug, syringaldazine (+); Wk VI: On MEA, similar results except α-naphthol (− to weakly +) 1 cm from inoculation plug, syringaldazine (weakly + to +) at margin; On PDA, similar results.

Specimens used in culture studies were *ANM 492, ANM 495, RHP 2036, RHP 8768* (all TENN).

Commentary. Lentinellus angustifolius is characterized macroscopically by the small (1–2.5 × 1–3.5 cm broad), yellow-brown pileus, close to crowded, narrow lamellae, and sessile habit (FIG. 1). Cultures of

Lentinellus angustifolius covered the surface of both MEA and PDA plates in four wk, formed silky to subfelty, translucent to orange-yellow colonies which were never farinaceous, produced few chlamydospores, and developed cylindric, ventricose-rostrate or clavate gloeocystidia.

Romell (1901) originally described this species as *Lentinus angustifolius* from Brazil based on two collections made by Malme in 1893. Although Romell acknowledged the close affinity of *L. angustifolius* to *L. ursinus,* he distinguished *L. angustifolius* from *L. ursinus* based on Fries' description of the lamellae in *L. ursinus* being 4–6 lin. wide. Singer and Digilio (1952) later transferred this epithet to *Lentinellus,* described the taxon in greater detail and concluded: "It was impossible to say if this is or is not a good species different from *Lentinellus ursinus.*" Similar material was collected by Singer from Florida and by Wright from Cuba (as *Lentinus castoreus* var. *pusillus* Berk. & Curt.; Singer and Digilio 1952). Based on mating studies and morphological features of the basidiomata and somatic cultures, *L. angustifolius* is a distinct species which can be adequately distinguished from *L. ursinus.*

Lentinellus vulpinus (Sow.: Fr.) Kühner & Maire, Bull Soc Mycol Fr 49:16, 1934.

Basidiomata morphology. Miller and Stewart (1971) adequately described this taxon and provided photographs and line drawings of salient microscopic features. Although minor variations in some characters were found in this study, none were significantly different from those reported by Miller and Stewart (1971) except for the presence of oleiferous hyphae which were: (i) abundant throughout pileipellis, at times terminating as solitary, cylindrical to narrowly clavate, thin-walled pileocystidia; (ii) infrequent in trama; (iii) abundant in subhymenium, terminating as pseudocystidia, and (iv) thin-walled, unclamped, rarely branched, yellowish in NH_4OH, always with resinous contents, staining dark purple in sulfobenzaldehyde.

Specimens examined. SCOTLAND. Surrey, East Horsley, Mountain Wood 2, 18 Oct 1969, *Orton 3662* (E00028786) (E). SWEDEN. UPPLAND: Uppsala, Kyrkogardsallen, 25 Aug 1945, *Seth Lundell s.n.* (UPS); Uppsala Par., Skarpan at Ekeby, 13 Sep 1981, *Nils Lundqvist 13557* (UPS); Uppsala, Stadsskogen at Skoghall, 1 Sep 1983, *S. Ryman 7246* (UPS). USA. MICHIGAN: Luce Co., Pine Stump Junction, 10 Aug 1959, *A.H. Smith 61259* (MICH); Cheboygan Co., Colonial Point Hardwoods, 26 Aug 1960, *A.H. Smith 63033* (MICH); Oakland Co., Proud Lake, 3 Sep 1966, *A.H. Smith 73305* (MICH). MINNESOTA: Beltrami Co., Webster Lake, 25 Aug 1995, *R.H. Petersen 7966* (TENN 54380).

Intracollection matings. Only one collection of *L. vulpinus* consisting of 12 SBIs was obtained in this study. Intracollection matings of these SBIs revealed a tetrapolar mating system.

Specimen and tester strains (in the order A_1B_1, A_2B_2, A_1B_2, A_2B_1) used in mating studies were *RHP 7966-4,7,10,13* (TENN).

Macromorphology. MEA: Radius (and means): Wk II 10–18 mm (13.4 mm), wk IV 34–46 mm (40.5 mm), wk VI 45–66 mm (58.6 mm); mat silky, never farinaceous, translucent; advancing zone appressed and submerged, silky and plumose, uneven, not distinct, translucent; plug undifferentiated to barely silky by wk VI, never farinaceous, translucent; reverse unchanged; odor none to faintly fruity. PDA: Radius (and means): Wk II 15–22 mm (18.9 mm), wk IV 40–44 mm (41.9 mm), wk VI 53–66 mm (57.3 mm); mat silky, never farinaceous, translucent to white (1A1); advancing zone appressed, silky, even to slightly undulating, not distinct, translucent; plug undifferentiated to subfelty by wk VI, never farinaceous, translucent to yellowish white (1A2); reverse unchanged; odor none to faintly fruity.

Micromorphology. MEA: Advancing zone hyphae 1.5–5 μm diam, thin-walled, clamped, infrequently to regularly branched, occasionally sinuous, hyaline, contents light yellow in KOH, inamyloid; aggregated crystals surrounding hyphae, globose, light yellow in KOH; diffuse crystals absent in agar. Aerial hyphae 1.1–4 μm diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; gloeocystidia abundant, cylindric, cylindro-clavate or capitulate (60–136 × 2.5–6 μm), terminal, basally clamped, thin-walled, yellow in KOH, contents resinous, partially refractive, weakly staining dark purple in sulfobenzaldehyde or not staining; chlamydospores absent; diffuse crystals absent in agar. Submerged hyphae 1.5–6 μm diam, thin-walled, clamped, infrequently to regularly branched, hyaline, contents light yellow in KOH, inamyloid; aggregated crystals surrounding hyphae, globose, light yellow in KOH; diffuse crystals in agar few to common, prismatic, slightly refractive. PDA: Advancing zone hyphae same as MEA except sinuous hyphae and aggregated crystals surrounding hyphae absent. Aerial hyphae same as MEA except diffuse crystals in agar common, globular, refractive. Submerged hyphae same as MEA except aggregated crystals surrounding hyphae absent and diffuse crystals absent in agar. Nobles species code: 2, 3, 15, 32, 36, 38, 47, 50, 54, 60. Stalpers species code: 1, 2, 3, 9, 13, 14, 15, 20, 30, 36, 39, 45, 52, 53, 73, 82, 83, 89, 94.

Phenoloxidase reactions tested at the margin and 1 cm from the inoculation plug (except at Wk II on MEA) for *L. vulpinus* were as follows: Wk II: On MEA, L-tyrosine (−), *p*-cresol (−), pyrogallol plus hy-

drogen peroxide (+), α-naphthol (+), syringaldazine (+); On PDA, L-tyrosine (weakly +), *p*-cresol (−) at margin and (weakly +) 1 cm from inoculation plug, pyrogallol plus hydrogen peroxide (+), α-naphthol (+), syringaldazine (+); Wk VI: On MEA, similar results except α-naphthol (weakly +) at margin and (−) 1 cm from inoculation plug; On PDA, similar results except L-tyrosine (−) 1 cm from inoculation plug, *p*-cresol (weakly +) at margin and (−) 1 cm from inoculation plug.

Specimen used in culture studies was *RHP 7966.*

Commentary. Lentinellus vulpinus is characterized macroscopically by the large (5–10 cm broad), white to yellowish pileus, close to crowded, broad lamellae, and short, fused stipes. Cultures of *Lentinellus vulpinus* did not cover the surface of either MEA or PDA plates after six wk, formed silky, translucent to white colonies which were never farinaceous, produced no chlamydospores, and developed only cylindric gloeocystidia.

DISCUSSION

At least three tester strains from each collection were crossed in all possible combinations except in ANM 511 and RHP 7880. Although crosses of most collections were either completely compatible or completely incompatible, a few were partially compatible. These crosses resulted in the delineation of three intersterility groups (e.g., biological species) which were identified as *L. ursinus, L. angustifolius,* and *L. vulpinus* based on morphological characters of the basidiomata and somatic cultures. A single collection of *L. vulpinus* (RHP 7966) was completely incompatible with all collections of *L. ursinus* and *L. angustifolius.* Intercollection matings within *L. ursinus* (ANM 321, ANM 473, ANM 482, ANM 491, ANM 497, ANM 508, RHP 2210, RHP 2414, ASM 8027, ASM 8109) and *L. angustifolius* (ANM 492, ANM 495, RHP 2036, RHP 8768) were compatible but intercollection matings between each of these species were completely incompatible. Additional collections of *L. angustifolius* from Austria (ANM 511) and Costa Rica (RHP 7880) were compatible amongst themselves and with North American collections of *L. angustifolius* suggesting genetic barriers to gene flow do not exist between these allopatric populations.

All collections which were self-crossed revealed a tetrapolar mating system. These results support the conclusions of Lamoure (1989) for *L. ursinus* and *L. vulpinus.* With some exceptions, intercollection matings of designated tester strains were completely compatible or completely incompatible. Although not patterned, partial compatibility usually occurred between collections from adjacent states suggesting that the same mating alleles may be operating in these populations. For example, two collections of *L. ursinus* (ASM 8027 and ASM 8109) from Michigan possessed identical alleles at the A and B loci. Since only three positive matings occurred in crosses of these collections, mating types for the three unknown SBIs of ASM 8109 were based on the assigned mating types of ASM 8027. In addition, the mating behavior of the three SBIs in ASM 8109 and three of the four tester strains in ASM 8027 was identical with all other tester strains suggesting the same mating types (i.e., same alleles) were present in each collection. Ten collections of *L. ursinus* and six collections of *L. angustifolius* displayed intercollection compatibility indicating that multiple alleles (at least 18 and 12, respectively) at the A and B loci must be operating in each species.

Culture mat analyses provided additional morphological characters which could be used for separating these taxa. All three species can be distinguished in culture by their rate of growth, color and texture of the culture mats, relative amount of chlamydospores, and shape of gloeocystidia.

Significant differences were not observed between the MEA- and PDA-grown isolates in each species except for the following: (i) the mats in *L. ursinus* were silky on MEA and subfelty on PDA; (ii) the reverse surface in *L. ursinus* was unchanged on MEA and, in some isolates, brown on PDA; (iii) the mats in *L. angustifolius* were frequently silky to rarely subfelty and translucent to light yellow on MEA and always subfelty and translucent to orange-yellow on PDA, and (iv) the submerged hyphae in *L. ursinus* and *L. angustifolius* produced prismatic crystals in the agar on MEA and cubodial to quartz-like crystals in the agar on PDA.

Miller (1971) briefly studied *L. cochleatus, L. pilatii,* and *L. ursinus* in culture and found cystidia and chlamydospores similar to those found in the present study in *L. ursinus* and *L. angustifolius.* Miller (1965) also described *L. montanus* in culture and found cystidia similar to those in *L. ursinus, L. angustifolius,* and *L. vulpinus.* As already stated by Miller (1971), gloeocystidia and/or chlamydospores appear to be unique characteristics of *Lentinellus* spp. in culture.

All monokaryons used in the intra- and intercollection matings were grown on MEA. The macromorphology of the monokaryons was identical to that of the polyspore isolates except that some isolates of *L. vulpinus* produced clavarioid basidiomata. These were similar in appearance to those reported by Miller (1971) in *L. cochleatus, L. pilatii,* and *L. ursinus.* None of the *L. ursinus* isolates in this study produced basidiomata.

The results of the phenoloxidase reactions were similar for all three species. All three produced strong reactions for peroxidase and laccase, but only weak reactions for tyrosinase, even when L-tyrosine and/or *p*-cresol were placed directly on the surface of the cultures in MEA or PDA. These data suggest that peroxidase and laccase are produced in significant quantities, whereas tyrosinase, when present, is produced in lesser quantities. All three species are identified as white-rotters since they possess all three phenoloxidase enzymes.

Similar reactions were produced by the actively growing mycelium at the margin and the older mycelium 1 cm from the inoculation plug suggesting these enzymes are not localized in any particular area of the colonies. Tests conducted at wk II and VI were similar in each species. L-tyrosine and *p*-cresol reacted similarly in the detection of tyrosinase whereas α-naphthol was more variable than syringaldazine in the detection of laccase. Although α-naphthol is laccase-specific (Marr 1979), it has been shown to produce variable results (Marr 1979). In the present study, α-naphthol was also placed directly in the MEA and PDA plates as well as on the plugs. Strong reactions were usually produced in the plates even when weak reactions were found in the plugs suggesting the quantity of mycelium is a factor when using α-naphthol. As such, α-naphthol should be placed directly in the plates if it is to be used as an indicator of laccase.

Presence of laccase and tyrosinase in *L. ursinus* was also reported by Boidin (1951). Boidin, employing methods previously described, found strong reactions for laccase using guaiacol but only weak reactions for tyrosinase using tyrosine. The presence of laccase in *L. ursinus* was also found by Piroard (1956). Employing the methods of Nobles (1948) and Boidin (1951), Piroard added gallic acid or guaiacol to malt agar to test for laccase and tyrosine or *p*-cresol to malt agar to test for tyrosinase. Although Piroard's (1956) tests determined the presence of laccase, they overlooked the small quantities of tyrosinase detected by Boidin (1951) and the present author. In this study, drop tests were used to detect the small quantities of tyrosinase present in the PDA plates.

In conclusion, three biological species with tetrapolar mating systems were identified within the *L. ursinus* complex in eastern North America. In addition to differences in basidiome morphology, culture mat analyses provided ancillary morphological characters which can be used to separate these taxa. While phenoloxidase reactions were similar for all three species, the data generated confirmed that the three taxa reported here are confirmed to be white-rot fungi.

Although three biological species within the *L. ursinus* species complex have been confirmed from eastern North America, additional aspects of this project require further study. First, since at least 18 and 12 mating alleles appear to be operating in *L. ursinus* and *L. angustifolius,* respectively, the population biology of these taxa should be investigated to determine mating allele segregation in eastern North America. Second, although one isolate of *L. angustifolius* (RHP 8768) was compatible with all other isolates of *L. angustifolius* and incompatible with all isolates of *L. ursinus* and *L. vulpinus,* the culture mat of this isolate produced a unique morphology. More specifically, the culture mat of RHP 8768 grew abnormally on PDA and was significantly different from other isolates of *L. angustifolius* on both MEA and PDA as the cultures lacked gloeocystidia and crystals but produced chlamydospores. This anomaly may have resulted from the generation of the polyspore isolate, which was from a self-cross of the opposite mating types identified in the collection rather than from the interaction of numerous germlings on the Petri plates. Finally, although results of the phenoloxidase analyses were identical on MEA and PDA, MEA-grown isolates produced no traces of tyrosinase.

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LITERATURE CITED

Boidin J. 1951. Recherche de la tyrosinase et de la laccase chez les Basidiomycètes en culture pure. Milieux différentiels. Intérêt systématique. Rev Mycol 16:173–197.

Desjardin DE. 1990. Cultural morphology of *Marasmius* species. Sydowia 42:17–87.

Ginns JH. 1974. Secondarily homothallic Hymenomycetes: several examples of bipolarity are reinterpreted as being tetrapolar. Can J Bot 52:2097–2110.

Harkin JM, Larsen MJ, Obst JR. 1974. Use of syringaldazine for detection of laccase in sporophores of wood rotting fungi. Mycologia 66:469–476.

———, Obst JR. 1973. Syringaldazine, an effective reagent for detecting laccase and peroxidase in fungi. Experientia 29:381–387.

Käärik A. 1965. The identification of the mycelia of wood-decay fungi by their oxidation reactions with phenolic compounds. Stud For Suec 31:1–80.

Kornerup A, Wanscher JH. 1967. Methuen handbook of colour. 2nd ed. London: Eyre Methuen. 242 p.

Lamoure D. 1989. Indices of useful informations for intercompatibility tests in Basidiomycetes. V. Agaricales *sensu lato*. Cryptog Mycol 10:41–80.

Largent DL. 1977. How to identify mushrooms to genus I: macroscopic features. Eureka, California: Mad River Press. 86 p.

———, Johnson D, Watling R. 1977. How to identify mushrooms to genus III: microscopic features. Eureka, California: Mad River Press. 148 p.

Marr CD. 1979. Laccase and tyrosinase oxidation of spot test reagents. Mycotaxon 9:244–276.

———. 1984. Spot tests for detection of tyrosinase. Mycotaxon 19:299–305.

———, Grund DW, Harrison KA. 1986. The taxonomic potential of laccase and tyrosinase spot tests. Mycologia 78:169–184.

Miller OK. 1965. Three new species of lignicolous agarics in the Tricholomataceae. Mycologia 57:933–945.

———. 1971. The relationship of cultural characters to the taxonomy of the agarics. In: Petersen RH, ed. Evolution in the higher basidiomycetes. Knoxville: University of Tennessee Press. p 197–208.

———, Stewart L. 1971. The genus *Lentinellus*. Mycologia 63:333–369.

Nobles MK. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rotting fungi. Can J Bot 26: 281–431.

———. 1965. Identification of cultures of wood-inhabiting hymenomycetes. Can J Bot 43:1097–1139.

Pegler DN. 1983. The genus *Lentinus*: a world monograph. Kew Bull, Addit Ser 10:1–281.

Petersen RH, Cifuentes J. 1994. Notes on mating systems of *Auriscalpium vulgare* and *A. villipes*. Mycol Res 98: 1427–1430.

Piroard MF. 1956. Recherche des phénoloxydases mycéliennes dans l'ensemble des Agaricales. Ann Univ Lyon, Ser 3, Sec C, Sci Nat 9:23–46.

Romell L. 1901. Hymenomycetes Austro-Americani in itinere Regnelliano primo collecti 1. Bihang Till K Svenska Vet-Akad Handlingar 26, Afd 3, no 16:61 p, 3 pl.

Segedin BP. 1996. A new species of *Lentinellus* (Hericiales, Lentinellaceae) and a revision of taxa attributed to *Lentinellus* in New Zealand. New Zealand J Bot 34:249–261.

Singer R, Digilio APL. 1952. Pródromo de la flora agaricina Argentina. Lilloa 25:1–461.

Staplers JA. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud Mycol 16:1–248.

———. 1996. The Aphyllophoraceous fungi II: keys to the species of the Hericiales. Stud Mycol 40:1–185.

Taylor JB. 1974. Biochemical tests for identification of mycelial cultures of basidiomycetes. Ann Appl Biol 78: 113–123.