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Systematics of the genus *Auricularia* with an emphasis on species from the southeastern United States

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Abstract: The genus *Auricularia* comprises 10–15 recognized species worldwide, and most are considered to have intercontinental to cosmopolitan distributions. Though regional field guides for the southeastern United States treat only one or two species, five species, *A. fuscossuccinea*, *A. auricula-judae*, *A. mesenterica*, *A. polytricha*, and *A. delicata*, have been recorded from the region. This study seeks to evaluate and revise current species concepts in *Auricularia* using phylogenetic and morphological methods to better understand the species occurring in the southeastern United States. Historical collections from herbaria and fresh material from the field have been examined and sequenced at two loci, ITS and *rpb2*. Phylogenetic results indicate several diverse clades are in need of taxonomic revision. Previous reports of *A. auricula-judae* in the southeastern U.S. likely represent a clade of *A. americana*. Collections identified as *A. delicata* are found to be polyphyletic and distributed in four clades. Variation in the presence or absence of a medulla layer in some species, previously a key morphological character, has made it evident that additional characters are required to reflect the genetic diversity in the genus. A

new taxonomic character, the schizomedulla, is discussed and shown to distinguish two novel species, *A. subglabra* and *A. scissa*, from the morphologically similar species *A. delicata*. A reticulate, merulioid hymenial surface can no longer be considered a character unique to *A. delicata*. Furthermore, ITS data from the voucher-specimen from which the genome of *A. delicata* was produced indicates this species is *A. subglabra*. The taxonomy and nomenclature of *Peziza nigricans* is discussed and it is shown to be the earliest priorable name for *A. polytricha sensu auct. amer.*, and the new combination *Auricularia nigricans* is proposed. We now recognize the following species of *Auricularia* from the southeastern U.S.: *A. americana*, *A. fuscusuccinea*, *A. mesenterica*, *A. nigricans*, and *A. scissa*.

Key words: Agaricomycotina, Auriculariaceae, Auriculariales, Auricularia, Costa Rica, heterobasidiomycetes, jelly fungi, neotropical fungi, southeast U.S., systematics, taxonomy.

Introduction: Field excursions in east Tennessee (southeastern United States) have resulted in the observation of abundant fruitings of a species of *Auricularia* Bull. According to regional field guides (Weber and Smith 1985, Roody 2003, Bessette et al. 2007, Binion et al. 2008, etc.), *A. auricula-judae* (Bull.: Fr.) Quél. is the only species of *Auricularia* featured from east Tennessee. However, some differences between local specimens and descriptions of *A. auricula-judae* necessitated molecular comparisons in order to verify species determinations. BLAST results showed this local *Auricularia* to be 99% similar to collections of *A. fuscusuccinea* (Mont.) Henn. from tropical Costa Rica and only 92% similar to sequences labeled *A. auricula-judae*. Using the anatomical characters established by Lowy (1951) for species delimitation, the determination of our local *Auricularia* as *A. fuscusuccinea* was confirmed. In this article we provide the first known report of *A. fuscusuccinea* from east Tennessee as well as a morphological and phylogenetic treatment of the genus in order to elucidate the disposition of *Auricularia* species richness present in the southeastern United States.

In his monograph Lowy (1952) described ten widely distributed species comprising the known members of the genus *Auricularia*. Of these ten species, Lowy reports five in the southeastern United States; *A. auricula-judae* (as *A. auricula* (L.) Underw.), *A. polytricha* (Mont.) Sacc., *A.*

fuscusuccinea, *A. delicata* (Mont.) Henn., and *A. mesenterica* (Dicks.: Fr.) Pers. According to Lowy (1952), species of *Auricularia* have cosmopolitan distributions across multiple continents, with *A. auricula-judae* being prevalent north of the Tropic of Cancer, *A. polytricha*, *A. fuscusuccinea*, and *A. delicata* distributed throughout the neotropics and paleotropics, and *A. mesenterica* being circumglobal. Since Lowy's work, it has been shown that *A. auricula-judae sensu auct. amer.* forms a separate biological species from European populations based on mating studies (Duncan and MacDonald 1967) and morphological differences in spore size (Parmasto and I. Parmasto 1987). This prompted the description of a new species from eastern Canada, *A. americana* Parm. & I. Parm. ex Audet, Boulet & Sirard, distinguished by its growth habit on conifers and basidiome size (Boulet 2003). The presence of geographically widespread species in the southeastern U.S. from either primarily tropical or temperate ranges is of biogeographical interest. Before biogeographical patterns can be investigated, however, the regional species richness must be systematically studied.

The southeastern U.S. is primarily comprised of a single ecoregion, Bailey's Humid Temperate Domain, which is mediated by a confluence of subtropical moisture and moderate temperatures without a dry season (Bailey 2004, Estill and Cruzan 1999). Several hot spots of biodiversity

and endemism have been identified in this domain, including the southern Appalachian mountains, the central basin of Tennessee, the Florida panhandle region, regions of the Gulf Coast, and central and southern Florida (Chaplin et al. 2000, Estill and Cruzan 1999). Patterns of high fungal species diversity are likely influenced by the recent advance of glaciers during the Pleistocene ice age that created glacial refugia and facilitated a convergence of previously northern distributed species with temperate and subtropical inhabiting species of the south (Chaplin et al. 2000, Estill and Cruzan 1999). Another consideration for the presence of high regional species diversity is the region's close proximity to the tropics. According to the "Out of the Tropics" (OTT) theory, the tropics can be considered both a cradle and museum, where species originate at a higher rate and biodiversity is sustained as tropical species expand their ranges into the extratropics (Jablonski et al. 2006). High levels of precipitation, moderate temperatures, and elevation gradients in the southeastern U.S., combined with the area's proximity to the tropics, form an ideal area to study species distributions, particularly the convergence of boreal, temperate, and subtropical species.

Before Lowy's monograph (1952), attempts to distinguish species of *Auricularia* were primarily based on macromorphological features such as color, hymenial and cortical configuration, hair length, size of basidiomes, and appearance of dried specimens (Barrett 1910, Kobayasi 1942, etc.). Lowy considered many of these characters to be too variable due to the influences of environmental conditions and the age of the basidiome, so he devised a classification method based on the internal stratification of hyphae and abhymenial hair length (Lowy 1951) (Fig. 1). Of particular importance to Lowy's taxonomic scheme is the presence of a strongly or weakly differentiated medulla, which he defines as a zone composed of hyphae about 6–10 μm broad, located mostly centrally and arranged parallel

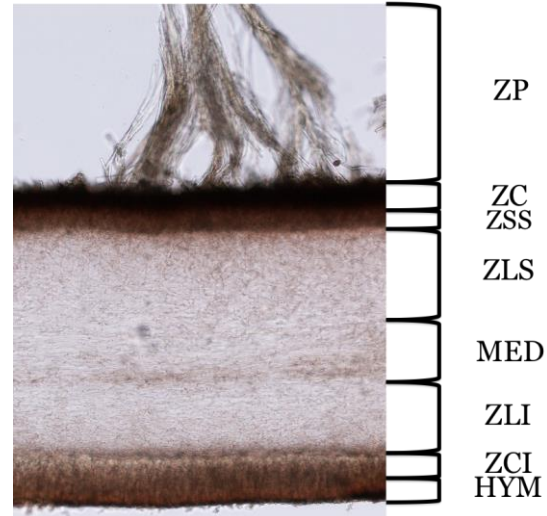


FIGURE 1. A diagram showing Lowy's (1951) classification system based on the stratified hyphal zones of a cross section with included abbreviations: ZP=Zona pilosa, ZC=Zona compacta, ZSS=Zona subcompacta superioris, ZLS=Zona laxa superioris, MED=Medulla, ZLI=Zona laxa inferioris, ZCI=Zona compacta inferioris, and HYM=Hymenium.

through the middle of the basidiome (Lowy 1951) (Fig. 1). Lowy also considered hymenial configuration to be taxonomically useful in the case of the merulioid hymenium of *A. delicata*, a character he considered unique to this taxon (Lowy 1952).

Since Lowy's monograph, progress within the study of the genus moved towards mating studies, which demonstrate limited interfertility between "microevolutionary units" within species complexes (Duncan 1972, Duncan and Macdonald 1967, Wong 1993, Wong and Wells 1987). These studies give strong support for the separation of species according to the biological species recognition criteria. Modern genetic tools give us a method for characterizing these biological differences through sequence comparison analysis. A phylogenetic study on the evolutionary relationships within the *Auriculariales* found the genus *Auricularia* to be monophyletic using ITS sequences (Weiß and Oberwinkler 2001). A study on the *Auricularia* of

Colombia added clarification to phylogenetic relationships within the genus by sampling additional species and identifying three well-supported clades (Montoya-Alvarez et al. 2011). Most recently, the genome of *A. delicata* has been sequenced as a representative sample from the *Auriculariales* and has been shown to be a white-rot species with 19 copies of fungal class II peroxidases, enzymes responsible for the degradation of lignin (Floudas et al. 2012). We seek to expand a molecular phylogeny of *Auricularia* using ITS and *rpb2* sequences to evaluate species identities in combination with morphological analysis of basidiome characters. The objectives of this study are to: (i) produce a phylogenetic framework for species recognition in *Auricularia*; (ii) circumscribe species of *Auricularia* from the southeastern U.S.; and (iii) determine which morphological and/or ecological characters are consistent with the phylogenetic assessments.

Materials and Methods

Morphological analysis

Fresh specimens of *A. fuscusuccinea* were collected from Ijams Nature Park (Knoxville, TN), and collections of *A. americana* were collected from Cumberland Gap National Historical Park (Cumberland Gap, TN) and Tellico Village (Vonore, TN) with field photos (TABLE 1). Descriptions and identification of fresh specimens were recorded following Lowy (1951, 1952). Color designations were taken from Kornerup and Wanscher (1967). Cross-sections of dried fruit bodies were mounted in 5% KOH and viewed using a Nikon Eclipse 80i light microscope. Cross-sections were photographed and measured at 4× and 10×, abhymmenial hairs at 40×, and spores and basidia at 100× in immersion oil using the NIS-Elements D3.1 (Nikon, Melville, NY) program. At least twenty measurements of abhymmenial hairs and spores were taken, and Q-values (the quotient of length by width) were calculated in specimens when sufficient features were present. All specimens

are deposited in the University of Tennessee Herbarium (TENN). Additional herbarium collections were also examined from the University of Washington Herbarium (WTU), the Farlow Herbarium (FH), the Museum of Evolution Herbarium (UPS) and the Lund University Botanical Museum (LD).

Terminology

The following terms are defined and illustrated in Fig. 1. *Medulla*: a dense, central zone of hyphae found in some species of *Auricularia*, characterized by its arrangement centrally and parallel with the surface and by the frequent presence of pigmentation. *Zona pilosa*: the sterile, abhymmenial zone composed of abhymmenial hairs when present. *Zona compacta*: the abhymmenial surface from which the hairs arise including the very dense, narrow zone of compacted hyphae that surrounds hair bases which is typically the most darkly pigmented zone in section. *Zona subcompacta*: two zones proceeding from the *zona compacta (superioris)* or the hymenium (*inferioris*) where hyphae become less dense towards the center of a section. *Zona laxa*: a zone or zones of loosely arranged hyphae in a gelatinous matrix that surround the medulla when present or comprise the central layer of section when absent. *Abhymmenial hairs*: hyphal protrusions from the cortical layer of the basidiome that are frequently tufted or gregarious and give the surface a glabrous to velutinous (1-50 μm), pubescent (51-150 μm), tomentose (151-500 μm), villose to hispid (551-1200 μm), or strigose to hirsute appearance (1201 μm and above). The following new term is introduced and illustrated. *Schizomedulla*: a medulla that splits or disassociates either in the *zona laxa superioris* or the *zona laxa inferioris* when a thin cross section is mounted in liquid (KOH). The following terms are also used but are not illustrated: *Cortical layer*: the upper surface of the basidiome opposite the hymenium.

TABLE I. Sequences used in molecular phylogenetic analysis. Bolded entries were generated for this study.

Species	GenBank ITS	GenBank rpb2	Country	State/Province	Herbarium	Collection	Date
<i>A. americana</i> 'deciduou's'	JX065152	JX065182	USA	North Carolina	TENNo49666	TFB2897	7/9/1990
<i>A. americana</i> 'deciduou's'	JX065151	JX065181	USA	Tennessee	TENNo51203	TFB4651	3/1/1992
<i>A. americana</i> 'deciduou's'	JX065146	JX065180	USA	Tennessee	TENNo61466	TFB13202	5/16/2006
<i>A. americana</i> 'deciduou's'	JX065163	JX065178	USA	Tennessee	TENNo67030	BPL116	1/5/2012
<i>A. americana</i> 'deciduou's'	JX065166	JX065179	USA	Tennessee	TENNo67029	BPL112	2/5/2012
<i>A. americana</i> 'coniferous'	JX065154	JX065177	USA	Idaho	TENNo52403	TFB5612	9/24/1992
<i>A. americana</i> 'coniferous'	DQ200918	DQ366278	USA	Oregon		PBM2295	
<i>A. auricula-judae</i> clade I	JX065174		Switzerland		TENNo50632	TFB4296	9/30/1991
<i>A. auricula-judae</i> clade I	DQ520099	DQ521419	Germany			MW446	
<i>A. auricula-judae</i> clade II	EU647879		China				
<i>A. auricula-judae</i> clade II	EU560406		China				
<i>A. auricula-judae</i> clade II	EU560401		China				
<i>A. auricula-judae</i> clade II	HQ388355		China				
<i>A. auricula-judae</i> clade II	FJ478123		China				
<i>A. auricula-judae</i> clade II	AB615232		Japan	Kochi		AFM21	
<i>A. auricula-judae</i> clade II	HQ388358		China				
<i>A. cornea</i>	JX065164	JX065185	Australia	Queensland	TENNo66990	PBM3754	2/25/2012
<i>A. cornea</i>	JX065149		Puerto Rico	Puerto Rico	TENNo49019	TFB3470	12/5/1990
<i>A. cornea</i>	HM448454		China				
<i>A. cornea</i>	HM448462		China				
<i>A. cornea</i>	HM448462		China				
<i>A. delicata</i> clade I	JX065169		Australia	Queensland	TENNo67025	CNSBlitz0012	2/20/2009
<i>A. delicata</i> clade I	JX065171		Australia	Queensland	TENNo67028	CNSBlitz0098	2/21/2009
<i>A. delicata</i> clade I	JX065165		Australia	Queensland	TENNo67027	CNSBlitz0093	2/21/2009
<i>A. delicata</i> clade I	JX065168		Australia	Queensland	TENNo67026	CNSBlitz0050	2/20/2009
<i>A. delicata</i> clade II	JX065158		Mexico	Veracruz	TENNo16963	AJS1304	11/20/1944
<i>A. delicata</i> clade II	JX065162		Mexico	San Luis Potosí	TENNo17797	AJS5896	4/21/1946
<i>A. delicata</i> clade II	JX065159		Peru	Loreto	TENNo28734	TENNo28734	7/X/1965
<i>A. delicata</i> clade II	AF291269		Costa Rica		USJ54470		
<i>A. fuscusuccinea</i>	JX065173		USA	Tennessee	TENNo63200	KF09	9/27/1978
<i>A. fuscusuccinea</i>	JX065138		Mexico	Tamaulipas	TENNo19800	LRH19800	8/X/1950
<i>A. fuscusuccinea</i>	JX065147		USA	Tennessee	TENNo22268	LRH22268	7/24/1950
<i>A. fuscusuccinea</i>	JX065143		USA	Florida	TENNo24812	POS24812	1/6/1959
<i>A. fuscusuccinea</i>	JX065148		USA	Puerto Rico	TENNo49292	TFB3463	12/6/1990
<i>A. fuscusuccinea</i>	JX065145		Mexico	Tabasco	TENNo50422	TFB4344	10/20/1991
<i>A. fuscusuccinea</i>	JX065157	JX065190	USA	Louisiana	TENNo56263	TFB9503	5/22/1997
<i>A. fuscusuccinea</i>	JX065141	JX065193	Argentina	Buenos Aires	TENNo58951	TFB10743	5/22/2001
<i>A. fuscusuccinea</i>	JX065153	JX065194	Argentina	Misiones	TENNo59094	TFB11289	5/27/2001
<i>A. fuscusuccinea</i>	JX065150	JX065192	Belize	Cayo	TENNo59847	TFB11532	6/12/2004
<i>A. fuscusuccinea</i>	JX065139	JX065189	USA	Louisiana	TENNo62651	PBM2868	8/5/2007
<i>A. fuscusuccinea</i>	JX065140	JX065186	USA	Tennessee	TENNo62782	PBM3034	8/31/2008
<i>A. fuscusuccinea</i>	JX065156	JX065188	USA	Tennessee	TENNo66036	BPL30	11/17/2010
<i>A. fuscusuccinea</i>	JX065144	JX065191	USA	Tennessee	TENNo65792	ACB111109024	11/11/2009
<i>A. fuscusuccinea</i>		JX065187	USA	Tennessee	TENNo63966	ADW0077	9/17/2008
<i>A. fuscusuccinea</i>		JX065184	Costa Rica	Guanacaste	WTU	JMB04170804	4/17/2008
<i>A. fuscusuccinea</i>	AF291270		Costa Rica			MW530	
<i>A. mesenterica</i>	JX065170		Costa Rica	San José	WTU	JMB04090802	4/9/2008
<i>A. mesenterica</i>	AF291271		Germany			FO25132	
<i>A. mesenterica</i>	AB615233		Cook Island			HE489	
<i>A. nigricans</i>	JX065176		Argentina	Misiones	TENNo59115	TFB11410	5/29/2001
<i>A. nigricans</i>	JX065172		USA	Louisiana	TENNo56825	TFB4405	3/10/1992
<i>A. nigricans</i>	JX065167		Costa Rica	San José	WTU	JMB04010803	4/1/2008
<i>A. scissa</i>	JX065175		USA	Florida	FH00301771	F64	8/5/1942
<i>A. scissa</i>	JX065160	JX065183	Dom. Rep.	La Vega	TENNo59729	TFB11193	1/5/2003
<i>A. subglabra</i>	JX065142		Costa Rica	Puntarenas	TENNo53855	TFB7868	6/19/1995
<i>A. subglabra</i>	JX065161		Costa Rica	San José	TENNo58100	TFB10405	6/19/1999
<i>A. subglabra</i>	JX065155		Costa Rica	Puntarenas	TENNo58607	TFB10499	6/21/2000
<i>A. subglabra</i>	JX524199		Costa Rica	Alajuela	TENNo57615	TFB10046	3/14/1999
<i>Eichieriella leveilleana</i>	AY509553		Canada	British Columbia			
<i>Exidia recisa</i>	AF291276		Sweden			MW315	
<i>Exidiopsis</i> sp.	AF291282		Germany			FO46291	
<i>Ramaria rubella</i>		AY786064	USA	Washington	WTU	PBM2408	10/4/2002

Molecular techniques

DNA extraction, polymerase chain reaction (PCR) and sequencing methods follow those of Birkebak et al. (2011) except for the use of a porcelain mortar and pestle to finely grind small pieces of dried basidiome material. An E.Z.N.A.[®] HP Fungal DNA Kit (Omega Bio-Tek, Norcross, GA) was used for extracting DNA from specimens more than twenty years old. PCR reactions were performed using the primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) to amplify the nuclear ribosomal ITS1-5.8S-ITS2 (hereafter referred to as ITS) barcode region. Degenerate primers b6F and b 7.1R were initially used to amplify a region of *rpb2*, a single-copy nuclear protein-coding gene (Matheny 2005). *Auricularia* specific *rpb2* primers were designed to facilitate PCR amplification: Aur-6F: 5' - GTC GGC TCG CTY TCT GC -3', and Aur-7R: 5' - GGA ATG ATR GTC GCR CAA ATG C -3'.

Sequences retrieved from the Molecular Biology Resource Facility at the University of Tennessee were assembled and annotated in Sequencher 4.9 (Gene Codes Corp, Ann Arbor, Michigan). The ITS alignment was constructed using ClustalX 2.1 (Larkin et al. 2007) from which nexus files were obtained and manually adjusted in MacClade 4.08 OS X (Maddison and Maddison 2005). Sites 75–79 and 190–193 of the ITS1 region were found to be extremely variable within all taxa and were therefore excluded before phylogenetic analysis. Finished nexus files were converted into extended Phylip format using Seaview (Gouy et al. 2010), and a maximum likelihood (ML) analysis was performed with 1000 bootstrap replicates using raxmlGUI 1.0 (Stamatakis et al. 2006, Stamatakis et al. 2008, Silvestro and Michalak 2011). The outgroups used for ITS analysis included *Exidia recisa* (Ditmar) Fr., *Exidiopsis* sp., and *Eichleriella leveilleana* (Berk. and M.A. Curtis) Burt, which have all been shown to be closely related species, but outside the ingroup, within the Auriculariales (Weiß and Oberwinkler 2001). *Ramaria rubella* (Schaeff.) R.H. Petersen was chosen as the outgroup for the

rpb2 analysis. We consider bootstrap support >70% as strong support, between 50 and 70% as moderate support, and below 50% as poor support. All sequences generated in this study are deposited in NCBI GenBank (TABLE 1).

Results

Phylogenetic results

Sixty-one ITS and twenty *rpb2* sequences were analyzed in this study. Of these, we produced forty new ITS sequences and eighteen new *rpb2* sequences. The genus *Auricularia* was recovered as monophyletic including the type species, *A. mesenterica*, a result consistent with prior studies with fewer taxa using nuclear large subunit ribosomal RNA sequences (Weiß and Oberwinkler et al. 2004) or ITS sequences (Montoya-Alvarez et al. 2011).

Twelve terminal groupings that correspond to phylogenetic species were recovered in the ML analysis of ITS data (Fig. 2A). Analysis of *rpb2* sequences recovered similar terminal groupings, from which *rpb2* data could be obtained (Fig. 2B), except for the placement of a single isolate of *A. auricula-judae*; however, the placement of this latter terminal in the *rpb2* gene tree is poorly supported by bootstrapping.

Only three species as defined by morphological features were recovered as monophyletic: *A. nigricans*, *A. fuscossuccinea*, and *A. mesenterica*. Collections labeled *A. auricula-judae*, *A. delicata*, and *A. americana* are recovered as paraphyletic or polyphyletic. Different clades within these taxa are provided unique terminal labels in Fig. 2. In the southeastern United States, four phylogenetic species were detected: *A. fuscossuccinea*, *A. americana* 'deciduous unit', *A. nigricans*, and one new species, *A. scissa*. A fifth species, *A. mesenterica*, based on morphological analysis, also occurs in the southeastern United States; however, we were unable to produce molecular data from this taxon. *Auricularia mesenterica* has been reported from Florida by Lowy (1952) and North Carolina by Curtis (1852).

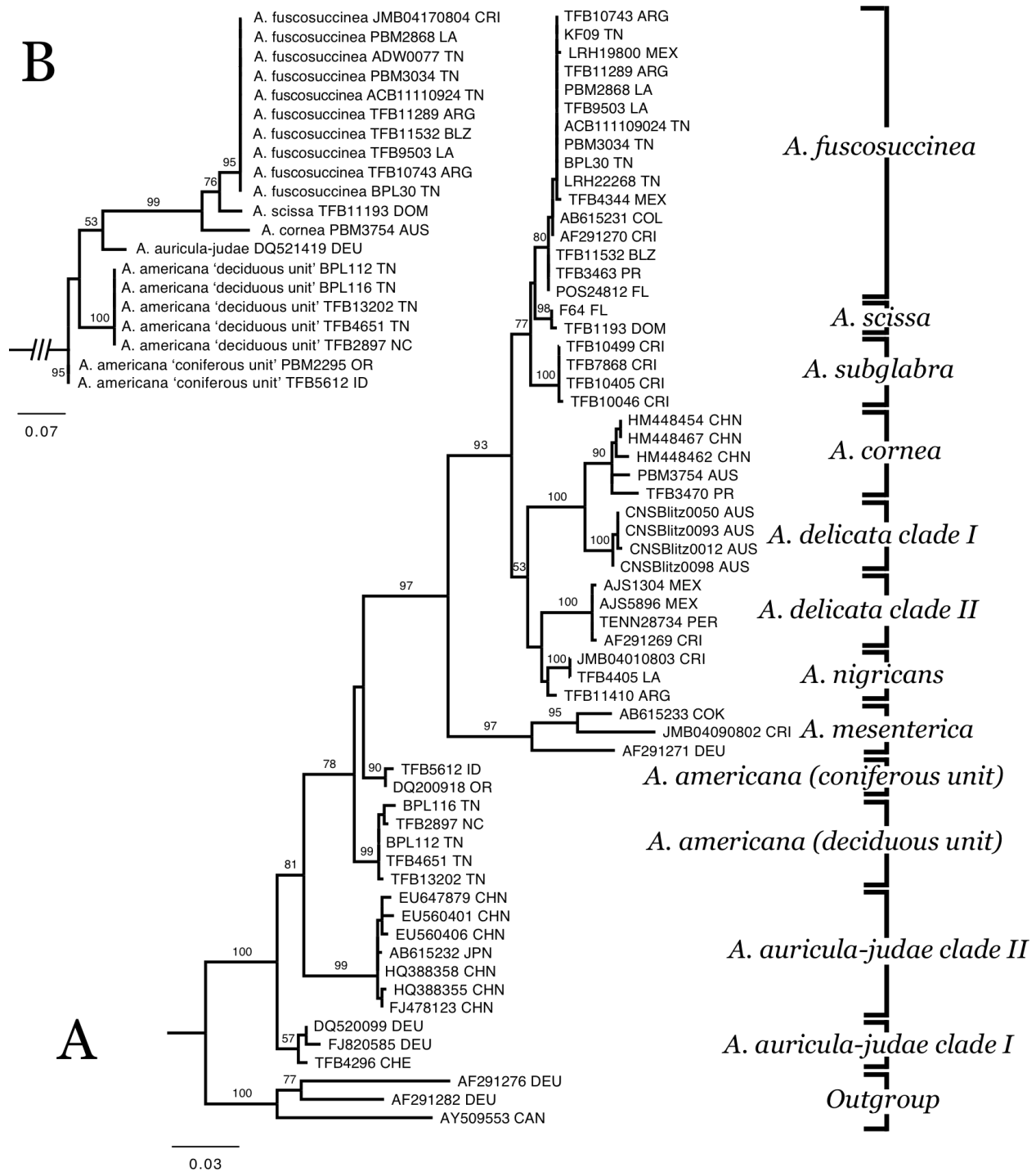


FIGURE 2. Results of maximum likelihood analysis based on ITS and *rpb2* (labeled as A and B respectively). Bootstrap values ≥ 50 are included for informative nodes and are based on 1000 bootstrap replicates. All tips are labeled with the collection number if produced for this study or with GenBank (NCBI) numbers. Tips in the ITS tree also include the country or state (if within the U.S.) abbreviation for collection locales and the clades are labeled with brackets and taxon labels. Tips in the *rpb2* tree include unique taxon labels as well as abbreviations for collection locale.

Below we present a key and a descriptive taxonomy to five species recorded from the southeastern USA. Then we present comments on an additional seven phylogenetic species recorded outside the southeastern U.S. recovered in Fig. 2, including the description of one new species from Costa Rica, *A. subglabra*.

Taxonomy

Descriptions of fruit bodies of *A. cornea*, *A. delicata*, and *A. auricula-judae* were modified and supplemented by those from Lowy (1952) as access to fresh specimens or thorough notation was lacking. Distributional and substrate records are provided as previously reported data from cited studies supplemented with additional, newly reported data.

KEY TO SPECIES OF AURICULARIA FROM THE SOUTHEASTERN UNITED STATES

1. Cortical layer tomentose, hispid to hirsute with hairs 500–2650 μm long 2
1. Cortical layer velutinous to pubescent with hairs 30–200 μm long 3
2. Cortical layer distinctly zoned with hairs alternating dark then light; hymenium meruloid and heavily lobed; effuso-reflexed and imbricate..... *A. mesenterica*
2. Cortical layer a uniform powdery drab cast when dry; hymenium mostly smooth with occasional folds or lobes; often substipitate and scattered to gregarious *A. nigricans*
3. Hymenium distinctly reticulate and meruloid; known only from southern Florida within the United States *A. scissa*
3. Hymenium mostly smooth with occasional folds; widely distributed 4
4. Medullary zone absent or weakly differentiated; yellow brown to dark tan and occasionally vinaceous brown..... *A. americana* (deciduous unit)
4. Medullary zone distinct; usually vinaceous brown to rosy brown..... *A. fuscossuccinea*

Auricularia americana Parm. & I. Parm. ex Audet, Boulet & Sirard, in Boulet, Les Champignons des Arbres de l'Est de l'Amérique du Nord: 287 (2003) : 'deciduous unit' (as per Duncan and Macdonald 1967) [Fig. 3A, B, M]
Basidiome: solitary to caespitose; substipitate to sessile; convex to cupulate to auriculiform, sometimes with a lobed margin; up to 8.5 cm broad, 1–5 mm thick; yellow brown to dark tan to vinaceous brown, 7F8 "dark brown" at its darkest and 5C6 "Pompeian yellow" at its lightest; gelatinous, tough; hymenium smooth and

lubricous; abhymenium velutinous to pubescent, dry, hairs giving a powdery drab cast.

Internal features: medulla absent; abhymenial hairs gregarious, tufted, thick-walled, ranging from 67–136 \times 4.4–6.0 μm ; clamp connections present; basidia 41–74 \times 3.0–5.3 μm , with oil guttules, sterigmata rarely observed; spores allantoid, 13.0–15.0 \times 4.6–5.90 μm , $Q = 2.35\text{--}3.0$; *zona pilosa* 67–136 μm ; *zona compacta* 20–51 μm ; *zona subcompacta superioris* 29–67 μm ; *zona laxa intermedia* 477–1807 μm ; *zona subcompacta inferioris* 102–169 μm ; hymenium 78–109 μm .

Substrate: on branches and logs of *Carya*, *Ulmus*, *Quercus*, *Fagus*, and other hardwoods (Duncan and Macdonald 1967).

Type locality: Québec, Canada.

Distribution: southeastern and midwestern U.S., Mexico.

Collections examined: U.S.A.: NORTH CAROLINA: Highlands, Blue Valley, “The Mountain” trail, 35°00’31” N, 083°13’21” W, 9 July 1990, *R.H. Petersen (TFB2897)*, TENNo49666; TENNESSEE: Cumberland Gap, Cumberland Gap National Park, Wilderness Rd. trail, 36°36’05” N, 083°40’06” W, 5 Feb 2012, *B.P. Looney (BPL112)*, TENNo67029; Gatlinburg, Great Smoky Mountains National Park, Twin Creeks trail, 35°41’10” N, 083°30’00” W, 16 May 2006, *E.B. Lickey (TFB13202)*, TENNo61466; Vonore, Tellico Village, 35°33’36” N, 084°14’10” W, 5 Jan 2012, *C. Braaten (BPL116)*, TENNo67030; Townsend, Great Smoky Mountains National Park, Abram’s Creek, Kingfisher Creek, 35°35’51” N, 083°48’20” W, 1 March 1992, *S.C. McCleneghan (TFB4651)*, TENNo51203.

Comments: The ‘deciduous unit’ of *A. americana* may be the most common species of *Auricularia* found in the southeastern U.S. It is characterized by a smooth hymenium and velutinous to pubescent cortical layer composed of abhymenial hairs that measure 60–140 µm long. It can be distinguished from its closest look-alike, *A. fuscosuccinea*, by its consistent lack of a medulla and, less reliably, by its lack of rosaceous or purple hues. The extent of its distribution outside the southeastern U.S. or overlap with the coniferous unit of *A. americana* is not known with certainty. Distinguishing the ‘deciduous unit’ from the ‘coniferous unit’, besides the difference of substrate, is the occasional presence of a medulla in the ‘coniferous unit’. This species may have a wider geographic distribution occurring patchily throughout Central and South America if a report of *A. auricula-judae* from Colombia (Montoya-Alvarez et al. 2011) indeed is

confirmed as the ‘deciduous unit’ of *A. americana*.

Auricularia fuscosuccinea (Mont.) Henn., *Bot. Jb.* 17: 492 (1893) [Fig. 3I, J, N]

Basionym: *Exidia fuscosuccinea* Mont., *Annl. Sci. Nat., Bot., sér. 2* 17: 125 (1842)

Basidiome: solitary to caespitose to clustered; often substipitate to sessile; auriculiform to petaloid; up to 6.5 cm broad, 1–4 mm thick; yellow to pigmentless at base quickly darkening to rosy vinaceous brown throughout basidiome, 9F7 “dark reddish brown” to 9E7 “oxblood red” to 8E7 “mahogany brown”; hymenium smooth, lubricous; abhymenium velutinous to pubescent with hairs reflecting 7D2 “brownish grey”.

Internal features: medulla present, often pigmented; abhymenial hairs gregarious, tufted, thick-walled, 31–167 µm × 3.8–6.8 µm; clamp connections present; basidia 45–59 × 3.0–6.5 µm, with oil guttules, sterigmata rarely observed; spores smooth, allantoid, 11.0–13.6 × 6.5–8.5 µm, Q = 1.4–2.0, with one or two large guttules; *zona pilosa* 38–136 µm; *zona compacta* 13–45 µm; *zona subcompacta superioris* 30–111 µm; *zona laxa superioris* 82–607 µm; medulla 32–361 µm; *zona laxa inferioris* 26–506 µm; *zona subcompacta inferioris* 55–224 µm; hymenium 41–86 µm.

Substrate: on branches and logs including *Liquidambar* sp. (TENNo22268), *Citrus sinensis* (TENNo24812), and *Acer negundo* (TENNo66036).

Type locality: Cuba.

Distribution: Continuous distribution in the Americas from Tennessee through Argentina; reported from Asia and Oceania, but mating studies suggest that these populations constitute a separate biological species (Wong 1993).

Collections examined: U.S.A.: FLORIDA: Altamonte Springs, 28°39’40” N, 081°23’30” W, 6 Jan 1959, *P.O. Schallert (POS24812)*, TENNo24812; LOUISIANA: Baton Rouge, Audubon Oakley House, 30°47’54” N, 091°18’30” W, 22 May 1997, *S.C. McCleneghan (TFB9503)*, TENNo56263; St. Francisville, Ouida Plantation

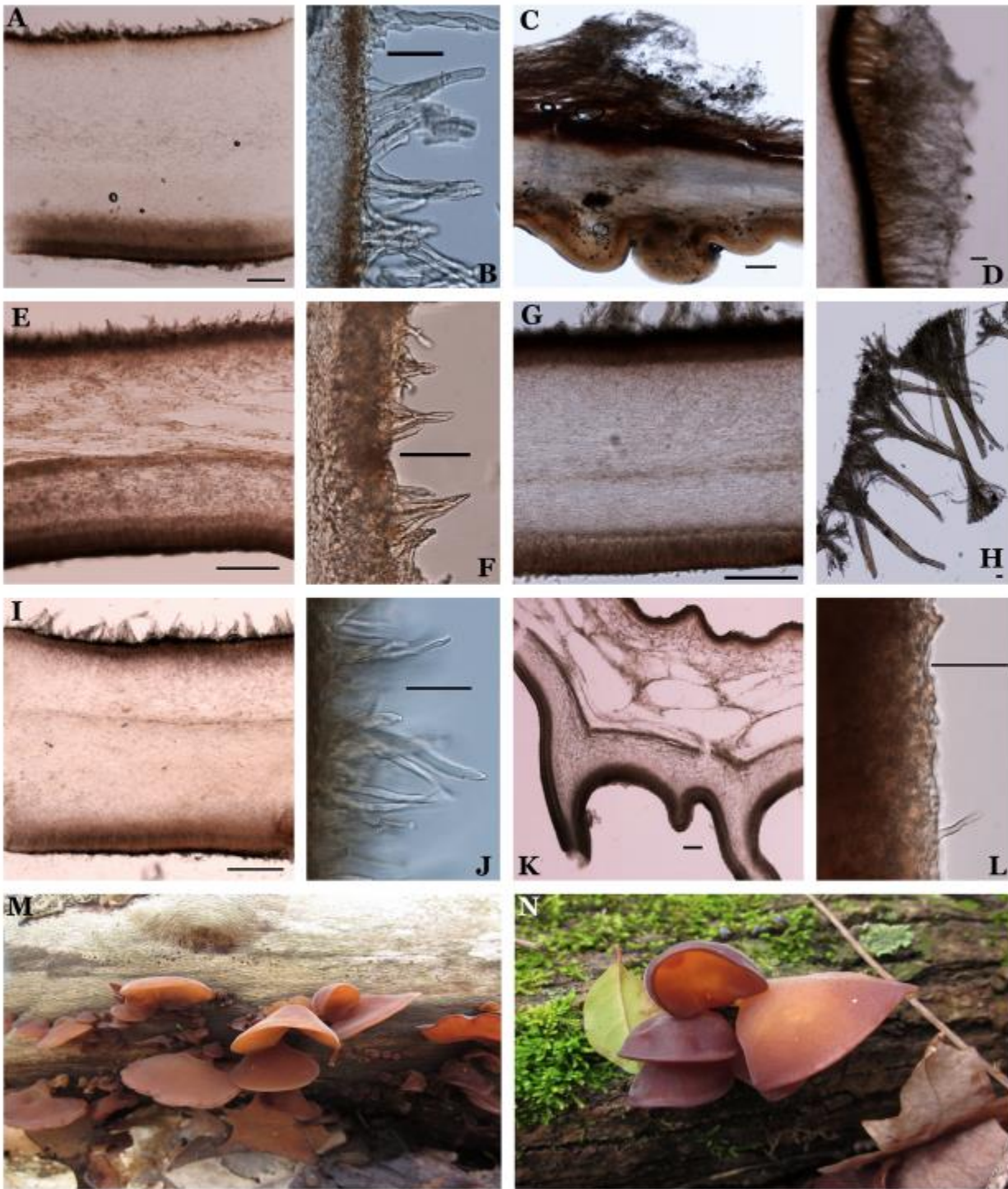


FIGURE 3. Cross-sections of basidiomes showing hyphal stratification and abhymenial hairs. Scale for full cross-sections are 200 μm and 50 μm for hair images. A. Cross-section of *A. americana* 'deciduous unit' displaying no medulla. B. Close-up of *A. americana* 'deciduous unit' abhymenial hairs. C. Cross-section of *A. mesenterica* showing no medulla. D. Pigmented hairs of *A. mesenterica*. E. Cross-section of *A. scissa* showing the schizomedulla. F. Hairs of *A. scissa* gregarious and tufted. G. Cross-section of *A. nigricans* showing a medulla. H. Separated tufts of abhymenial hair from *A. nigricans*. I. Medulla of *A. fuscossuccinea*. J. Abhymenial hairs of *A. fuscossuccinea*. K. Cross-section of *A. subglabra* showing reticulate ridges and the schizomedulla almost completely separating the section in KOH. L. Rare solitary hair of *A. subglabra*. M. Fruiting of *A. americana* 'deciduous unit'. N. Fruiting of *A. fuscossuccinea*.

30°56'49" N, 091°27'41" W, 5 Aug 2007, *P.B. Matheny* (PBM2868), TENN062651; PUERTO RICO: El Yunque, Caribbean National Forest, Big Tree trail, 18°19'37" N, 065°50'26" W, 6 Dec 1990, *R.H. Petersen* (TFB3463), TENN049292; TENNESSEE: Knoxville, Ijams Nature Center, 35°57'19" N, 083°52'06" W, 11 Nov 2009, *A. Busing* (ACB11109024), TENN065792; Knoxville, Ijams Nature Center, Tharp Trace trail, 35°57'12" N, 083°51'59" W, 17 Nov 2010, *B.P. Looney* (BPL30), TENN066036; Knoxville, Ijams Nature Center, Boardwalk trail, 35°57'23" N, 083°52'00" W, 17 Sep 2008, *A.D. Wolfenbarger* (ADW0077), TENN063966; Knoxville, Ijams Nature Center, 35°57'19" N, 083°52'06" W, 31 Aug 2008, *P.B. Matheny* (PBM3034), TENN062782; Ripley, 35°47'02" N, 089°39'46" W, 24 July 1950, *A.J. Sharp* (LRH22268), TENN022268, Memphis, Overtone Park, 35°08'51" N, 089°59'14" W, 27 Sep 1978, *K. Fones* (KF09), TENN063200; **Argentina:** Misiones, San Pedro, Parque Provincial de la Araucaria, 26°32'00" S, 054°04'00" W, 27 May 2001, *D. Krüger* (TFB11289), TENN059094; Buenos Aires, Llavallol, Lomas de Zamora, 34°47'13" S, 058°26'38" W, 22 May 2001, *K.W. Hughes* (TFB10743), TENN058951; **Belize:** Cayo District, San Antonio, Eligio Panti National Park, 17°02'57" N, 089°00'09" W, 12 June 2004, *E.B. Lickey* (TFB11532), TENN059847; **Mexico:** Tabasco, Teapa, Estacion Agronomica UNAM, 17°42'50" N, 092°56'45" W, 20 Oct 1991, *R.H. Petersen* (TFB4344), TENN050422; Tamaulipas, Gomez Farias, 23°01'45" N, 099°12'50" W, Aug 1950, *A.J. Sharp* (LRH19800), TENN019800. **Comments:** *Auricularia fuscusuccinea* is common along the Gulf Coast (Lowy 1953) but reaches its most northern range in Tennessee, where it is less common but locally abundant. Morphologically, *A. fuscusuccinea* is very similar to *A. americana* except for some reports of color differences. These two species can be distinguished microscopically by the presence of a medulla in *A. fuscusuccinea* and its absence in *A. americana* 'deciduous unit'. *Auricularia fuscusuccinea* is also only known on hardwoods,

whereas the 'coniferous unit' of *A. americana* (see below) occurs on conifer logs.

Auricularia mesenterica (Dicks.: Fr.) Pers., Mycologia Europaea 1: 97 (1822) [Fig. 3C, D] Basionym: *Helvella mesenterica* Dicks., Fasc. pl. crypt. brit. (London) 1: 20 (1785)

Basidiome: effuso-reflexed, imbricate; substipitate to sessile; up to 9 cm broad, 2–4 mm thick; upper surface strongly zonate with thin whitish and thin black bands, white zones are coarsely hirsute and black zones are strigose; texture gelatinous to rubbery; hymenium made of folded ridges forming a maze to net pattern, slightly pruinose in some areas, 22D2 "greyish blue" to 23C2 "fog blue" to 24D2 "turquoise grey", especially near margins, becoming 6D3 "café-au-lait" to 7B3 "greyish red" to 7C4 "brownish orange" to 4B4 "champagne" near reticulations.

Internal features: medulla absent; abhymenial hairs matted or clustered, frequently flattened parallel to surface, darkly pigmented yellow-brown, thick-walled, 603–2623 × 3.0–5.5 µm; clamp connections present; basidia 51–73 × 3.8–5.3 µm, with oil guttules, 3-septate, sterigmata rarely observed; spores allantoid, with one to two large guttules and an acute apiculus, 12.5–15.0 × 5.0–6.0 µm, Q = 2.1–2.8; *zona pilosa* darkly pigmented, 402–986 µm; *zona compacta* darkly pigmented, 29–38 µm; *zona subcompacta superioris* partially pigmented, 58–89 µm; *zona laxa intermedia* 464–787; *zona subcompacta inferioris* lightly pigmented, 104–114 µm; hymenium lightly pigmented, 61–69 µm.

Substrate: Hardwoods such as *Ulmus*, *Castanea*, *Fagus*, *Quercus*, and *Betula* (Lowy 1952).

Type locality: British Isles.

Distribution: Cosmopolitan distribution including: Europe, Asia (north and south), Africa, Australia, and throughout the Americas. It is reported in the southeastern U.S. from North Carolina (Curtis 1852), Florida (Lowy 1952) and Tennessee (LRH21291).

Collections examined: USA: TENNESSEE: Knoxville, Hardin Valley, 35° 55' 08" N, 084° 12' 58" W, 23 March 1952, *L.R. Hesler* (LRH21291), TENN021291; **Costa Rica:** San José, Mastatal, Rancho Mastatal, 09° 40' 25" N, 084° 22' 09" W, 9 April 2008, *J.M. Birkebak* (JMB0409200802), WTU.

Comments: *Auricularia mesenterica* is the most distinct *Auricularia* species morphologically and is more likely to be confused with bracket fungi such as *Trametes versicolor* or *Stereum ostrea* until the hymenial layer is examined, which is glabrous, gelatinous, and darkly pigmented purple to brown to black. The species has been considered cosmopolitan in distribution but rarely recorded in the southeastern U.S. Our phylogenetic results (Fig. 2) demonstrate a relatively large amount of genetic divergence at the ITS locus between geographically distant samples from the Cook Islands in the South Pacific, Costa Rica, and Germany. Despite this genetic and geographic variation the collections were recovered as monophyletic with robust support. Further taxon sampling is required to determine whether distinct geographical clusters can be recognized.

In a nomenclatural review of the genera of "Hymenomycetes," M.A. Donk (1958a) established the basis for the validity of *Auricularia* Bull. *per* Merat., with the type species as *A. mesenterica* (Dicks.) Pers., over a competing group, *Auricularia sensu* Link, where *A. auricula-judae* (Bull.) Quél. would be the type species. When article 13 of the Code allowed for the validity of unsanctioned names older than 1820, *Auricularia* Bull. became the preferable name because Bulliard is the first author to provide a description of the genus with included species (Bulliard 1791).

Auricularia nigricans (Fr.: Fr.) Birkebak, Looney, & Sánchez-García *comb. nov.* [Fig. 3G, H]
Mycobank # 803171

Basionym: *Peziza nigricans* Fr., Syst. Mycol. II: 81 (1822)

Lectotype: Swartz s.n. Acharius herbarium (LD), *hic designatus*

≡ *Peziza nigrescens* Sw., Prodr. Nov. Plant. Spec. India Occ.: 150 (1788)

≡ *Auricularia nigrescens* (Sw.) Farlow, Bibliogr. Index: 308 (1905)

≡ *Peziza nigricans* Sw., Fl. Ind. Occ.: 1938 (1806) *nom. illeg.* Art 52.1

≡ *Peziza nigricans* Fr.: Fr., Syst. Mycol. II: 81 (1822) *nom. illeg.* Art. 52.1, Art. 53.1, protected by sanctioning Art. 15

≡ *Hirneola nigricans* (Fr.: Fr.) P.W. Graff, Mycologia 9: 9 (1917) (basionym cited as *Peziza nigricans* Hook. in Kunth)

≡ *Hirneola nigricans* (Fr.: Fr.) Donk, Reinwardtia 1: 498 (1952) *comb. illeg.* Art 52.1

≡ *Hirneola nigra* Fr., Kongl. Svenska Vetenskapsakad. Handl. 1848: 147 (1849) *nom. illeg.* Art. 52.1

≡ *Lachnea nigra* (Fr.) Sacc., Syll. Fung. 8: 172 (1889) (basionym cited as *P. nigricans* Sw.: Fr.)

≡ *Auricula nigra* (Fr.) Kuntze, Rev. Gen. Pl. 2: 844 (1881) (basionym cited as *P. nigricans* Sw.: Fr.) *nom. inval.* Art. 35.1

≡ *Auricularia nigra* (Fr.) Earle, Bull. Torr. Bot. Club 26: 633 (1899) (as *Auricularia nigra* (Schwein.) Earle)

≡ *Exidia hispidula* Berk., Ann. Mag. Nat. Hist. ser. 1, 3: 396 (1839) *nom. illeg.* Art. 52.1

≡ *Hirneola hispidula* (Berk.) Berk., J. Linn. Soc., Bot. 14: 352 (1875)

≡ *Auricula hispidula* (Berk.) Kuntze, Rev. Gen. Pl. 2: 844 (1881) *nom. inval.* Art. 35.1

≡ *Auricularia hispidula* (Berk.) Farlow, Bibliogr. Index: 307 (1905)

=? *Exidia polytricha* Mont. in Bélanger, Voy. Indes Or., Bot. 2: 154 (1834)

≡ *Hirneola polytricha* (Mont.) Fr., K. svenska Vetensk-Akad. Handl. 1848: 146 (1849)

≡ *Auricularia polytricha* (Mont.) Sacc. Atti R. Inst. Veneto 6: 722 (1885)

≡ *Auricula polytricha* (Mont.) Kuntze, Rev.
Gen. pl. 2: 844 (1891) *nom. inval.* Art. 35.1

Basidiome: solitary to caespitose; substipitate; broadly to deeply convex, sometimes with an incurved margin, convoluted with age; up to 6 cm broad × 1–4 mm thick; color variable from hairs reflecting 1B2 “ash grey” to moderately brown pigmented 5C7 “oxide yellow” to darkly pigmented 5E8 “yellowish brown” and sometimes to 8E8 “oxide red” in age; rubbery; hymenium smooth, soft, light pinkish 11B3 “dull red” when young to browner 7E6 “dark Somalis” to much darker in age 7F1 “dark grey”; abhymenium densely tomentose to hispid, tufted around margins.

Internal features: medulla present, central; abhymenial hairs tufted, thick-walled, 656–1081 × 4.8–7.9 μm; clamp connections present; basidia 52–73 × 3.2–6.5 μm, 3-septate, sterigmata occasionally observed; spores allantoid, often with one large oblong guttule, 14.5–17.0 × 5.0–7.0 μm, Q = 2.1–3.0; *zona pilosa* 865–897 μm; *zona compacta* 13–45 μm; *zona subcompacta superioris* 30–111 μm; *zona laxa superioris* 82–607 μm; medulla 32–361 μm; *zona laxa inferioris* 26–506 μm; *zona subcompacta inferioris* 55–224 μm; hymenium 41–86 μm.

Substrate: branches, logs, and stumps of *Liquidambar*, *Coffea arabica*, and other hardwoods (Duncan 1972).

Type locality: Jamaica

Distribution: Distributed through the Americas and surrounding islands, with the northernmost range limit in Louisiana and the southernmost in Argentina.

Collections examined: **Jamaica:** Swartz s.n. Acharius herbarium (LD); **USA:** LOUISIANA: Baton Rouge, Steele Burden rural life museum, 30° 24' 20" N, 091° 06' 12" W, 10 Mar 1992, R.H. Petersen (TFB4405), TENN056825 (Lectotype); **Argentina:** Misiones, Iguazu, Puerto Iguazu, Macaco trail, 25° 40' 55" S, 054° 28' 40" W, 29 May 2001, D. Krüger (TFB11410), TENN059115;

Costa Rica: San José, Santa María, Parque Nacional Los Quetzales, 09° 32' 09" N, 083° 53' 19"

W, 09 Apr 2008, J.M. Birkebak
(JMBO409200802), WTU.

Comments: This is the New World taxon previously identified as the Asian taxon *A. polytricha* (see discussion below). *Auricularia nigricans* is differentiated subtly from other *Auricularia* species by its densely tomentose to hispid cortical layer. In section, hair length descriptors translate to a difference of abhymenial hairs up to 200 μm for the velutinous to pubescent appearance of *A. fuscosuccinea*, hairs between 200 and 600 μm for tomentose to villose as in *A. cornea*, and hairs starting at around 600 μm for the villose to hispid appearance of *A. nigricans*. This species is most likely to be confused with *A. cornea*, which has a shorter hair length range of 200–350 μm and is not found in the southeast U.S. *Auricularia mesenterica* has a similar hair length range but can be easily distinguished by the presence of zonations on the cortical surface and a meruloid hymenial configuration, features lacking in *A. nigricans*.

The complicated history involving the name *Auricularia nigricans* begins with Olof Swartz (1788) who published the name *Peziza nigrescens* Sw. Later Swartz (1806) cited and repeated his protologue (adding some information) but used the name *Peziza nigricans* Sw. thus creating a superfluous name based on the same type. Fries (1822) for an unknown reason referred to Swartz's species as "*Peziza nigra*", yet he cited the description of the superfluous *Peziza nigricans* Sw. from Swartz (1806). As Bulliard had previously published *Peziza nigra* Bulliard in 1783, Fries published the name *Peziza nigricans* as a *nom. nov.* for the non-existent "*Peziza nigra* Sw.," as is clarified by Fries listing the name as his own in his Index (Fries 1832). Although this name is superfluous and a homonym, it is protected as the name was published in a sanctioning work. Later, when Fries transferred this taxon to the genus *Hirneola* (1849), he "returned" to the erroneous epithet "*nigra*" thereby publishing the nomenclatural

entity *Hirneola nigra* Fr. *nom. nov.* Berkeley published the name *Exidia hispidula* Berk., citing "*Peziza nigricans* Hook. in Kunth." as a synonym, though Hooker clearly referenced Swartz (1806) and no *nomen novum* was required, thus creating another *nomen superfluum*.

All of these names are obligate synonyms of *Peziza nigricans* Fr.: Fr. as they all share one type and can be linked back to the original description of *Peziza nigrescens* Sw. (1788). This being the case, the combination of *Peziza nigricans* Fr. into *Auricularia* is necessary, as was first noted by Donk (1958b).

In his monograph Lowy (1952) makes an argument for the name *A. polytricha* (Mont.) Sacc., originally published as *Exidia polytricha* Mont. in 1834, over the older name attributed to the same fungus, published as *Peziza nigrescens* Sw. in 1788. We find that the sanctioned *Peziza nigricans* Fr.: Fr. is the appropriate name for this taxon, and this new combination of *Auricularia nigricans* is necessary to join the name with the contemporary genus. It should be noted that Lowy (1952) erroneously attributed the type locality of *A. polytricha* as Jamaica instead of its actual type locality, the peninsula of India (Bélanger et al. 1834). According to Lowy (1952), *P. nigrescens*, an obligate synonym of *P. nigricans*, represents a different fungus due to a supporting description of the author that noted the habit as "*ad terram*." While it seems unusual for a species of *Auricularia* to fruit on soil, it is, however, possible that *Auricularia* species can appear to grow "*ad terram*" from buried wood. Furthermore, this notation of habit was not included in the original description, which was published eighteen years earlier. In the original description, "*globoso-campanulata oblique, intus glabra atra extus incano-villosa; margine integro*," *globoso campanulata* indicates a growth orientation consistent, along with all other features, with our concept of this taxon (Swartz 1788). In examination of type material,

morphological features confirm this taxon to be an *Auricularia*.

Another argument that Lowy makes is that Swartz's original collection is not accessible, but Donk (1958b) later indicates that portions of Swartz's original collection are available in "Thunberg's herbarium," "Lund," and another "may be expected to be conserved at Stockholm." We have confirmed the existence of Swartz material in Thunberg's herbarium housed in the Museum of Evolution Herbarium (UPS) and in the Acharius herbarium kept at the Lund University Botanical Museum (LD), though curators at the Swedish Museum of Natural History (S) were unable to locate a third collection as was stated as a possibility by Donk. A lectotype has not previously been designated among Swartz's collections. After examination we are here designating the Lund material as the lectotype as it corresponds most closely to Swartz's elaborated description (1806) and Fries's sanctioning description (1822). The size of the material at Uppsala is larger than described in the original description, whereas the Lund material corresponds to Swartz's description of basidiome size being "... *varians a cupulae nucis Quercus ad dimidiam partem testae nucis Juglandis*" (Swartz 1806) (Fig. 4). It should also be noted that an apparent difference in hair length is visible between these collections (Fig. 4). Hairs from the Uppsala material were found to be 205–270 μm , whereas hairs in the Lund material approach 1000 μm . It appears that the material from Uppsala is conspecific with *A. cornea*, which gives an additional reason for the designation of the Lund material as lectotype as we believe it is preferable to preserve an existing name with a historical precedent of having been considered allied if not conspecific with *A. polytricha*.

Auricularia scissa Looney, Birkebak & Matheny *sp. nov.* [Fig. 3E, F]
Mycobank # 801342

Diagnosis: This species may be distinguished from *A. delicata* by the presence of a schizomedulla, from *A. subglabra* by the presence of abundant abhymenial hairs, and from all other members of the genus by having a reticulate hymenial surface.

Typus: Dominican Republic: La Vega, Jarabacoa, Los Dajaos, José Cruz, 19° 04'16" S, 070° 45'15" W, 1 May 2003, E.A. Grand (TFB11193), TENN059729.

Basidiome: solitary to caespitose; sessile to supstipitate; orbicular to cupulate to auriculiform; up to 7 cm broad × 1–3 mm thick; color variable, ranges from 4A3 “pale yellow” at the pale extreme, more commonly rosaceous 7B5 “greyish red” to 6E8 “hazel brown”; rubbery-gelatinous when fresh; hymenial surface conspicuously porose-reticulate; abhymenium velutinous to pubescent when dry, especially around point of attachment.

Internal features: schizomedulla present (occasionally faint), frequently pigmented but sometimes obscured due to splitting, not observed to split completely; abhymenial hairs gregarious, tufted, thick-walled, 43–148 μm × 2.3–5.4 μm; clamp connections present; basidia 30–44 × 3.7–4.9 μm, clavate, 3-septate, sterigmata occasionally observed; spores smooth, allantoid, 8.7–13.0 × 3.7–5.7 μm, Q = (1.6–)1.9–2.8(–3.4); *zona pilosa* 38–132 μm; *zona compacta* 15–50 μm; *zona subcompacta superioris* 9–45 μm; *zona laxa superioris* 64–135 μm; medulla 38–57 μm; *zona laxa inferioris* 52–124 μm; *zona subcompacta inferioris* 39–98 μm; hymenium 29–71 μm.

Substrate: On decaying limbs or logs.

Type locality: Dominican Republic.

Distribution: Only known from the Dominican Republic and Florida.

Collections examined: Dominican

Republic: La Vega, Jarabacoa, Los Dajaos, José Cruz, 19° 04'16" S, 070° 45'15" W, 1 May 2003, E.A. Grand (TFB11193), TENN059729 (Holotype); **USA: FLORIDA:** Sebring, Highlands Hammock State Park, 27° 27'54" N, 081° 33'00" W, 5 Aug 1942, R. Singer (F64), FH00301771.

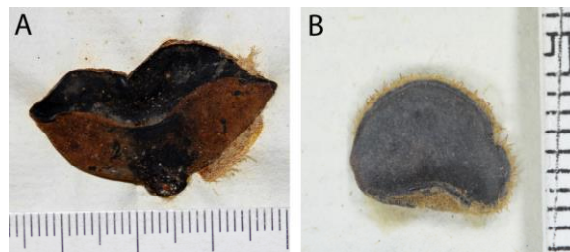


FIGURE 4. Original material of *Peziza nigricans* Fr.: Fr. collected by Olof Swartz with scale bars in millimeters. A. Specimen from the Thunberg herbarium in UPS (Photo courtesy of Dr. Mats Hjertson, Uppsala University). B. Lectotype from the Acharius herbarium in LD (Photo courtesy of Dr. Patrik Frödén, Botanical Museum, Lund University).

Comments: Only one collection of *A. scissa* is known at this time from the southeastern U.S. This collection, made by Rolf Singer in 1942 from central Florida, has been sequenced, examined and confirmed as a separate species apart from *A. delicata sensu* Lowy. The major feature that differentiates it from *A. delicata* is the presence of a schizomedulla, which is not referred to in any of Lowy's descriptions. *Auricularia scissa* is differentiated from another new species, *A. subglabra*, by the presence of abundant hairs that are usually longer than 50 μm and by its phylogenetic position (Fig. 2).

Extralimital phylogenetic species

***Auricularia americana* ‘coniferous unit’ (as per Duncan and MacDonald 1967)**

Collections examined: USA: IDAHO: Wallace, Coeur d'Alene National Fort, 47° 26'12" N, 116° 01'28" W, 24 Sep 1992, K.W. Hughes and S.C. McCleneghan (TFB5612), TENN052403.

Comments: Originally described on conifer wood, this phylogenetic species may constitute the true *A. americana* apart from the ‘deciduous unit’ of *A. americana* (Fig. 2). A confounding factor, however, is the presence of a medulla in the one examined collection of this clade, which conflicts with the original description of *A. americana* as lacking one. Duncan and Macdonald (1967) found differences in the

dimensions of basidia within the ‘coniferous unit’, which might explain this variation as representing two different species. Unfortunately, the holotype cannot be located, and the isotype (DAOM) contains but a small fragment (Scott Redhead, personal communication). Assuming conspecificity of all *A. americana* specimens on coniferous wood, the coniferous unit has been reported on *Abies balsamea*, *Picea glauca*, *Thuja occidentalis*, *Abies lasiocarpa*, *Picea engelmannii*, *Thuja plicata*, and *Abies grandis* (Duncan and Macdonald 1967, Lowy 1952). Additionally, *A. americana* has not been reported on coniferous wood from the southeastern U.S. Therefore, its distribution can be considered only in the northwestern, midwestern, and northeastern U.S. and Canada. A sequence from type material is necessary to confirm which of the two clades corresponds to *A. americana*, if any. Until then we refer to specimens collected on angiosperm wood as *A. americana* ‘deciduous unit’.

Auricularia auricula-judae (Bull.: Fr.) Quéf., *Enchir. fung.*: 207 (1886)
Basionym: *Tremella auricula-judae* Bull.: Fr., *Herb. Fr.* 9: tab. 427 (1789)

Auricularia auricula-judae clade I

Collections examined: Switzerland: Ticino, Maggia, Maggia Valley, 46° 59’00” N, 007° 05’40” E, 30 Sep 1991, *E. Horak* (TFB4296), TENN050632.

Comments: A number of features have been identified by previous studies to separate the morphologically similar *A. americana* from this European clade. Differences in the mean spore dimensions between these two taxa have been demonstrated, with *A. auricula-judae* having larger spores (Duncan and Macdonald 1967, Parmasto and I. Parmasto 1987). The criteria given for the separation of *A. americana*, however, also included differences in basidiome size, mentioned by Duncan and Macdonald (1967), as well as a consistent habit of growth on conifer wood (Boulet 2003). Fruitings of *A.*

auricula-judae in Europe are found on *Sambucus nigra* and, occasionally, other hardwoods (Duncan and Macdonald 1967).

Auricularia auricula-judae clade II

Collections examined: none.

Comments: This clade is composed of samples from east Asia (China, Japan). We have not examined any specimens that correspond to this clade. Given that the holotype of *A. auricula-judae* (Bulliard 1789) originates from Europe, we refer to the Asian group as ‘clade II’.

Auricularia cornea Ehrenb.: Fr., in Nees von Esenbeck (ed.), *Horae Phys. Berol.*: 91 (1820)
Basionym: *Exidia cornea* (Ehrenb.: Fr.) Fr., *Syst. Mycol.* II: 222 (1822)

Basidiome: solitary to gregarious; substipitate to sessile; cupulate; largest specimens about 15 cm broad, 0.8–1mm thick; hymenium smooth, shallowly venulose.

Internal features: medulla present, central; abhymenial hairs tufted, thick-walled, 166–382 × 5.0–7.7 μm; clamp connections present; basidia 52–69 × 3.2–6.0 μm, 3-septate, sterigmata rarely observed; spores allantoid, usually one large guttule, truncated hilar appendage, 13.5–17.5 × 6.0–7.5 μm, Q = 2.2–2.9; *zona pilosa* 211–336 μm; *zona compacta* 26–29 μm; *zona subcompacta superioris* 45–79 μm; *zona laxa superioris* 320–410 μm; medulla 131–135 μm; *zona laxa inferioris* 119–223 μm; *zona subcompacta inferioris* 87–143 μm; hymenium 93–99 μm.

Type locality: Marianna Islands.

Distribution: Cosmopolitan throughout Asia and also found in the Caribbean.

Collections examined: USA: PUERTO RICO: Utuado, Rio Abajo Forest Reserve, 18° 20’01” N, 066° 43’10” W, 12 May 1990, *R.H. Petersen* and *S.A. Gordon* (TFB3470), TENN049019;

Australia: Queensland, Cairns, Mt. Whitfield Conservation Park, 16° 53’05” S, 145° 44’25” E, 25 Feb 2012, *P.B. Matheny* (PBM3754), TENN066990.

Comments: Originally described from the Asian Pacific region, this fungus is widespread throughout Asia. Our findings show that it is also present in the Caribbean, which may be a result of an accidental introduction with breadfruit or another imported crop to the region sometime during the area's colonial history. *Auricularia cornea* is morphologically similar to *A. nigricans* but can be separated by its shorter hairs which are between 160–400 µm long. Wong and Wells (1987) proposed the synonymy of *A. polytricha* and *A. tenuis* with *A. cornea* based on interfertility of these taxa, but their species concepts of *A. polytricha* and *A. cornea* were erroneously based on the width of the medulla and not the abhymenial hair length. Collections ascribed to these taxa in their study most likely represent *A. cornea*. They do, however, make a good case for the synonymy of *A. tenuis* and *A. cornea* based on interfertility and the presence of a double medulla in both taxa (Wong and Wells 1987). Phylogenetic analysis of these Asian taxa will help clarify this finding.

Auricularia delicata (Fr.) Henn., Bot. Jb. 17: 492 (1893)

Basionym: *Laschia delicata* Fries, Linnaea 5: 533 (1830)

***Auricularia delicata* clade I**

Collections examined: Australia:

Queensland, Lake Tinaroo, 17° 11' 32" S, 145° 34' 39" E, 20 February 2009, collector unknown (CNSBlitz0012), TENNo60725;

Queensland, Lake Tinaroo, 17° 11' 32" S, 145° 34' 39" E, 21 February 2009, collector unknown (CNSBlitz0098), TENNo60728;

Queensland, Lake Tinaroo, 17° 11' 32" S, 145° 34' 39" E, 21 February 2009, collector unknown (CNSBlitz0093), TENNo60727;

Queensland, Lake Tinaroo, 17° 11' 32" S, 145° 34' 39" E, 20 February 2009, collector unknown (CNSBlitz0050), TENNo60726.

Comments: This clade of Australian samples forms a sister group to *A. cornea*, a species widespread throughout the Asian Pacific.

Auricularia delicata is reported throughout southeastern Asia, which could constitute the same or a different species of *Auricularia* with a porose-reticulate hymenophore. Morphological features of this species include a porose-reticulate hymenophore, lack of a medulla, and abhymenial hairs that are clustered to gregarious and over 50 µm long. A separate clade of a New World species that corresponds to *A. delicata* morphologically is discussed below (Fig. 2).

***Auricularia delicata* clade II**

Collections examined: Mexico: San Luis Potosi, Xilitla, 21° 23' 15" N, 098° 59' 20" W, 21 April 1946, A.J. Sharp (AJS5896), TENNo17797; Veracruz, Tezonapa, 18° 36' 20" N, 096° 41' 25" W, 20 Nov 1944, A.J. Sharp (AJS1304), TENNo16963; **Peru:** Loreto, Iquitos Maynas, 03° 45' 00" S, 073° 15' 00" W, July 1965, D. Griffin (TENNo28734), TENNo28734.

Comments: This clade comprises the closest match to *A. delicata sensu* Lowy, in that it has no schizomedulla, usually lacks a medulla, and has tufted hairs in the size range given by Lowy for this species (1952). Confounding this match is the persistent presence of a medulla in the Peruvian collection, a specimen determined by Lowy himself (Fig. 5). A likely explanation for this oversight is that Lowy operated under the assumption that all porose-reticulate *Auricularia* constitute *A. delicata*, and that this feature alone is enough for a positive identification. Another confounding factor in this group is the presence of a medulla in the collection AJS1304 in only some sections, whereas others yield no medulla (Fig. 5). This case highlights the need for additional morphological characters for *Auricularia* description since it is apparent that the presence/absence of a medulla is not a consistent character for all species. Type studies are also necessary to determine whether this clade constitutes genuine *A. delicata* or whether *A. delicata* even occurs outside of Africa.

Auricularia subglabra Looney, Birkebak, & Matheny sp. nov. [Fig. 3K, L]

Mycobank # 801341

Diagnosis: This species may be distinguished from *A. delicata* by the presence of a schizomedulla, from *A. scissa* by the near absence of abhymenial hairs, and from all other members of the genus by having a reticulate hymenial surface.

Typus: Costa Rica: San José, Perez Zeledon, San Gerardo Dota, Parque Nacional Los Quetzales, Cabinas Chacon, 09° 32'42" N, 083° 48'39" W, 19 June 1999, *J.L. Mata (TFB10405)*, TENN058100

Basidiome: solitary, caespitose, or clustered; substipitate to sessile; shallow cup-shaped to petaliform, occasionally lobate; up to 6.5 cm broad, 1–2 mm thick; 6D5–6D6 “sunburn” to “cinnamon brown” all over; gelatinous, rubbery, firm; hymenium meruloid, whitish, cream, light brown, tan, caramel color; abhymenial surface smooth, glabrous, shiny.

Internal features: schizomedulla present, often pigmented, splitting in either the *zona laxa superioris* or *zona laxa inferioris* in KOH, occasionally splitting entirely; abhymenial hairs solitary, infrequent, around 40 × 4 μm; clamp connections not found; basidia 33–41 × 3.6–5.1 μm, clavate, 3-septate, sterigmata occasionally observed; spores allantoid, 10.0–11.5 × 4.5–5.5 μm, Q = 2.0–2.6; *zona pilosa* lacking; *zona compacta* 58–114 μm; *zona subcompacta superioris* 27–81 μm; *zona laxa superioris* when intact 130 μm; medulla when intact 70–85 μm; *zona laxa inferioris* when intact 99–264 μm; *zona subcompacta inferioris* 24–45 μm; hymenium 56–135 μm.

Substrate: fruiting on decaying hardwoods.

Type locality: Costa Rica.

Distribution: Known only from Costa Rica.

Collections examined: **Costa Rica:** San José, Perez Zeledon, San Gerardo Dota, Parque Nacional Los Quetzales, Cabinas Chacon, 09° 32'42" N, 083° 48'39" W, 19 June 1999, *J.L. Mata (TFB10405)*, TENN058100 (Holotype); Puntarenas, Coto Brus, Hacienda la Amistad, 08° 54'52" N, 082° 46'50" W, 19 June 1995, *R.H. Petersen (TFB7868)*, TENN053855; Puntarenas,

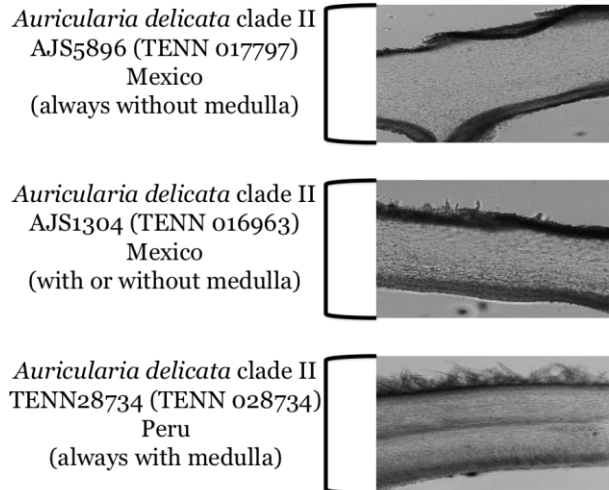


FIGURE 5. Cross-sections of basidiomes comprising the *A. delicata* clade II exhibiting variation in the presence/absence of a medulla.

Mellizas, Las Alturas Biological Station, trail to Cerro Chia, 08° 56'59" N, 082° 50'02" W, 21 June 2000, *J.L. Mata & R.H. Petersen (TFB10499)*, TENN058607; Alajuela, San Carlos, Fortuna, 10° 26'09" N, 084° 42'21" W, 14 March 1999, *J.L. Mata, R.E. Halling & R.H. Petersen (TFB10046)*, TENN057615.

Comments: *Auricularia scissa* is separated from *A. delicata* based on the presence of a schizomedulla, where the medulla layer splits partially to completely when mounted in KOH solution. Samples of *A. subglabra* form a separate clade apart from three other clades of the *A. delicata* complex (*A. delicata* I, II, and *A. scissa*) (Fig. 2). We found the presence of the schizomedulla to be consistent for all collections of this species as well as *A. scissa*, and this feature is not exhibited in any examined collections of *A. delicata* clades I and II. The splitting of the medulla most likely correlates with a micromorphological character of either the hyphae or gelatinous matrix comprising it, but the specifics of this mechanism have not been determined at this time. *Auricularia subglabra* is also distinguished by the marked lack of abhymenial hairs, with only a few short hairs present at irregular intervals throughout the basidiome, whereas *A. scissa* has hairs up to 150

μm that are occasionally tufted. The hairs of *A. subglabra* are entire and evidence of broken hairs have not been found. Additionally, *A. subglabra* is known only from montane cloud forests, a habitat in which *A. scissa* and the two *A. delicata* clades are not known to co-occur. Finally, there exists a difference in the amount of splitting observed in the schizomedulla between *A. subglabra* and *A. scissa*. *Auricularia subglabra* appears to split more readily in KOH, occasionally splitting completely apart, whereas *A. scissa* has not been observed to split completely apart. The collection (TFB10046) from which the genome of *A. delicata* was produced is included in this clade.

Discussion: A more densely sampled and robust phylogenetic analysis of *Auricularia* has produced a better understanding of both phylogenetic relationships between species as well as a framework for determining relevant morphological and ecological characters (Fig. 2). Novel clades within the *A. delicata* group have been discovered, which has led to the description of two new species based on a new taxonomic character, the schizomedulla. Also elucidated is the separation of two *A. americana* clades based on ecological specificity and phylogenetic position (Fig. 2). *Auricularia nigricans* is the earliest available name for *A. polytricha sensu auct. amer.* Five southeastern U.S. taxa have been identified, documented, and placed in a phylogenetic framework to further elucidate this region's biodiversity. More taxonomic and systematic investigation is required as collections identified as *A. americana* and *A. auricula-judae* were found to be paraphyletic and collections of *A. delicata* recovered three polyphyletic clades (Fig. 2). Additional taxon sampling will contribute further to our understanding of species relationships, but additional characters must be identified to accommodate additional diversity. As character states in the genus stand, we are forced to rely on highly variable traits and general ecological habits to separate taxa.

A revision of characters within *Auricularia* is necessary to clarify species boundaries. The same problems that Lowy found with variation of macromorphological features seem to plague his own classification at the microscopic level, especially in regards to the width of zones within the basidiomes. Though the general length of hair and presence/absence of a medulla zone seem to hold for species in some cases (Montoya-Alvarez et al. 2011, Wong 1993, Wong and Wells 1987), our study shows the presence of a medulla, Lowy's foundational character for species identification, is somewhat inconsistent and should be interpreted with caution. When mounted in KOH a distinctly stratified medullary zone of hyphae in many species of *Auricularia* is evident by the presence of slight to heavy pigmentation and by the parallel arrangement of the hyphae (Fig 1). Thicker sections will tend to exhibit a more well-defined medulla with greater pigmentation. We have observed this sort of "medullary zone" in species that, according to Lowy, are not reported to contain a medulla, including *A. americana* 'coniferous unit' and *A. delicata* clade II (Fig. 5). We would expect Lowy to have examined specimens belonging to these clades, which may be why a distinction between a weakly versus distinctly differentiated medulla has been made. For our key to southeastern U.S. taxa, we are forced to adopt the medulla as a feature because this is the only reliable character known with which to separate *A. fuscosuccinea* and *A. americana* 'deciduous unit'. Fortunately for our purposes, the presence/absence of the medulla is a consistent character in collections of southeastern U.S. taxa we have examined. Also, it should be noted that the absence of a medulla was not observed in any specimens that were described as having this character, making the absence of a medulla a viable species-level character across the genus.

The length of abhymenial hairs is consistent with descriptions as long as only the longest representative hairs are measured and special

attention is taken not to measure broken hairs, which are frequently found. Additionally, even though the width of hyphal zones are variable in section due to the size and age of basidiomes, Lowy's classification system is beneficial as a framework for describing species and may still prove a useful source of morphological characters. There may be no consistent character by which to tell species apart throughout the group, but a combination of macromorphological characters, internal characters, and geographical location can allow for reliable species determinations.

Based on our distributional data, *A. fuscusuccinea* has been reported from Louisiana, Mississippi, western Tennessee, and, now, eastern Tennessee within the southeastern U.S., though it appears to be widespread only within the Knoxville region in east Tennessee, Memphis region in west Tennessee, and Baton Rouge region of Louisiana. Very little genetic variation has been found at the ITS locus among the sampled collections ranging from Argentina to Tennessee, which may suggest a high level of gene flow among populations. Samples from Knox County constitute the northernmost report of *A. fuscusuccinea* among a contiguous distribution throughout the Americas. Collection data of *A. fuscusuccinea* fruiting times from the southeastern U.S. show that the most frequent fruiting times appear to be during the winter months, and this principally neotropical fungus can occasionally be found covered in frost. Whether *A. fuscusuccinea* is distributed along the Mid-Atlantic Coastal Plain, another area considered to be a biodiversity hotspot, has yet to be determined, but the Appalachian Mountains may act as a barrier to *A. fuscusuccinea* at its range limit due to climatic constraints (Chaplin et al. 2000, Estill and Cruzan 1999).

Species of *Auricularia* are known to be generalists on wood, but our phylogeny supports the use of ecological characters as an effective method for distinguishing species. Boulet (2003)

distinguished *A. americana* from *A. auricula-judae* by: a) its habit of growing on conifer wood and b) its smaller and thinner basidiomes than *A. auricula-judae*. In agreement with Lowy (1951) and Duncan and Macdonald (1967), we find that the size of basidiomes is highly variable due to moisture conditions as well as age and cannot reliably be used as a differentiating feature. ITS and *rpb2* analysis has revealed two distinct clades of *A. americana*, one clade comprised of specimens from the southeastern U.S. found only on hardwood and another comprised of specimens from the western U.S. found only on conifer wood. This finding supports previous mating results, which have identified reproductive barriers between these two ecological "units" of *A. americana*, though some interbreeding has been noted (Duncan and Macdonald 1967). Additionally, neither of the *A. americana* "units" have been successfully mated with the more host specific *A. auricula-judae* from Europe which is typically only found on *Sambucus nigra* (Duncan and Macdonald 1967). In order to determine which, if any, of the two *A. americana* clades comprise the "true" *A. americana*, the holotype needs to be sequenced. Morphological examination of the two clades have shown that collections of the coniferous clade exhibits a medulla, which is counter to the species description given by Parmasto & Parmasto (1987), whereas the deciduous clade's habitat is ecologically distinct from the original description of *A. americana*. In Duncan and Macdonald's (1967) study, they refer to a difference in basidial dimensions between certain members of their "coniferous" units, with members from the western U.S. having larger basidia than members of the coniferous unit from the eastern U.S. This may be evidence for two clades within the coniferous clade. Further exploration of morphology, ecology, and phylogenetic sampling of *A. americana* specimens may help to separate these different mating groups into distinct taxa.

In mating studies Duncan (1972) detected multiple “microevolutionary units” within the *A. polytricha* group, most striking of which was the complete intersterility of the only representatives from North America, a population in Louisiana, with all other Asian Pacific groups, including India, the type locality of *A. polytricha*. A different study on the mating compatibility of *Auricularia* of Hawaii (Wong and Wells 1987) reveals another complication in this species complex, as the authors have shown the presence of a bifactorial mating system and intercompatibility between Hawaiian specimens of *A. polytricha*, *A. cornea*, and *A. tenuis* (Lév.) Farl. This finding led Wong and Wells (1987) to suggest subsuming the names *A. tenuis* and *A. polytricha* under the older *A. cornea* due to nomenclatural priority. Species concepts of *A. polytricha* and *A. cornea* used by Wong and Wells (1987), however, are different than those of Lowy (1952) and Kobayashi (1981), placing an emphasis on medulla width instead of differences of abhymenial hair length. Since the width of zones is considered a highly variable character (Montoya-Alvarez 2011, Wong 1993, Wong and Wells 1986), the length of hairs cited in the Wong and Wells study (1987) indicates morphological descriptions consistent with *A. cornea*. All three “species” are intercompatible, so it is likely that they represent a single taxon. Given the robust sampling of Hawaiian specimens in their study and the results of Duncan’s (1972) mating study, the identity of this taxon is most likely *A. cornea*, which we suggest is a widespread species throughout Asia and the Caribbean (Fig. 2). Additionally, it is likely that the Wong and Wells (1986) assertion that *A. tenuis* should be synonymized with *A. cornea* as an unusual growth form is justified, but future systematic studies are needed to confirm this.

With additional sampling, results from Montoya-Alvarez et al. (2011) can be clarified by showing that the *A. nigricans* clade is sister to *A. delicata* clade II, not *A. fuscossuccinea* (Fig. 2). Type locality, mating data, and morphology allow us to

confidently determine the identity of this clade as representing *A. nigricans* and not the morphologically similar *A. cornea*. Samples from Louisiana have been shown to be intersterile with specimens from Hawaii, Micronesia, and Asia (Duncan 1972), which is represented in the phylogenetic analysis by the separation of specimens from Louisiana, Costa Rica, and Argentina from specimens in China, Australia, and Puerto Rico. Given the type locality (the Marianna Islands) of *A. cornea* and its ubiquity throughout Asia, we designate the clade that includes Asian specimens as *A. cornea*, which is a cosmopolitan species now confirmed from the American neotropics. Using traditional species concepts, these two taxa can be separated based on hair length, with *A. nigricans* having longer hairs than *A. cornea*. The presence of a distinct, long-haired *A. polytricha sensu auct. amer.* clade that is intersterile with *A. cornea* from the eastern hemisphere allows us to confidently designate the clade including Louisianan specimens as *A. nigricans*. Further research is necessary to ascertain whether cultivated strains common in Asia constitute *A. polytricha*, as has been traditionally assumed, or *A. cornea*, a common species of the region (Mengyao et al. 2008).

Sampling of collections identified as *A. delicata* throughout Central America, the Caribbean, Mexico, and Australia has led to the identified of two separate clades of *A. delicata* with robust support; *A. delicata* clade I pairing with the *A. cornea* clade and *A. delicata* clade II, which is placed as sister to *A. nigricans* with poor support. The *A. delicata* clade II corresponds most closely with Lowy’s description of *A. delicata* but because of variation in the presence/absence of a medulla and having been originally described from West Africa, we do not feel confident in assuming conspecificity. The Australian clade I probably represents a distinct Australasian *A. aff. delicata* species. Two porose-reticulate clades that pair as closely related groups to *A. fuscossuccinea* have been determined as distinct

from *A. delicata* due to the presence of a new morphological character for species delimitation called a schizomedulla. A schizomedulla is defined as a medulla zone that splits when the cross section is mounted in liquid, either in the *zona laxa superioris* or the *zona laxa inferioris*.

Auricularia subglabra is recognized as a distinct species based on genetic and distinct morphological differences from *A. fuscosuccinea* and *A. delicata*. It can be distinguished from *A. delicata* by the general absence of abhymenial hair (though solitary hairs are sometimes present) and the presence of a schizomedulla that can occasionally separate completely in KOH solution. A new species from the southeastern U.S., *A. scissa*, is also distinguished by a schizomedulla, though it does not exhibit as much splitting as *A. subglabra*. The two can be distinguished from one another based on hair length and frequency, where *A. subglabra* has single hairs or are lacking them entirely, and *A. scissa* has gregarious to tufted hairs that are longer than 50 μm . We find these characters to be consistent among all examined specimens. It should be noted that the *Auricularia delicata* genome sequenced for a recent study on the evolution of delignifying enzyme genes in saprotrophic fungi by Floudas et al. (2012) should be referred to as *A. subglabra*, not *A. delicata* (Fig. 2, see TFB10046). As a representative of the *Auriculariales*, this study illuminates the possible importance of this group as wood-decaying fungi, with the second most fungal class II peroxidases being present in this particular isolate among all thirty-one genomes analyzed.

The main challenge for the future of study in *Auricularia* is to develop reliable morphological characters to help represent the underlying genetic diversity and delimit species. Future studies may provide more sampling of specimens throughout Oceania, Asia, and Africa in order to clarify taxonomic designations and categorize the morphological variation within the genus, which

does not seem to be that extensive. Further studies should also sample from many locations within North America to examine the genetic diversity within the *A. americana* clade. As it appears that members of the *A. auricula-judae/A. americana* complex are closely related, an interesting study would be to look at the phylogeographic history of these closely related species to determine an origin of dispersal or to isolate particular vicariance events important for speciation. Other such biogeographical studies could look at the genus as a whole, as species tend to have large distributions and a large degree of conserved traits. Finally, we hope that it is now apparent that the meruloid to porose-reticulate hymenial condition in *Auricularia* can no longer be attributed as a unique character of *A. delicata* and that future studies can help identify new characters to delimit species of *Auricularia*.

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