PHYLOGENETIC RELATIONSHIPS AND REVIEW OF THE SPECIES OF *AURICULARIA* (FUNGI: BASIDIOMYCETES) IN COLOMBIA

Relaciones filogenéticas y revisión de las especies del género *Auricularia* (Fungi: Basidiomycetes) en Colombia

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**ABSTRACT**

The phylogenetic relationship of the species of the genus *Auricularia* and its allied taxa were investigated using the internal transcribed spacer (ITS) sequences of nuclear DNA. A molecular phylogenetic tree constructed using a total of 17 samples representing five species and two outgroups indicates that the species of *Auricularia* form a monophyletic group. Within the genus *Auricularia*, *A. mesenterica* is basal and the remaining *Auricularia* species form three clades; the first clade consists of *A. auricula-judae*; the second, of *A. fuscosuccinea*; and the third, of *A. polytricha*.

**Key words.** *Auricularia*, taxonomy, phylogeny, Colombia

**RESUMEN**

Las relaciones filogenéticas entre las especies del género *Auricularia* y especies cercanas se investigaron usando secuencias de ADN nuclear de la región (ITS). Se construyó un árbol filogenético molecular usando un total de 17 muestras, representando cinco especies, y dos grupos externos. Los resultados indican que las especies del género *Auricularia* conforman un grupo monofilético en donde *A. mesenterica*, se encuentra en la región basal del árbol y las otras especies estudiadas se ubicaron en tres clados. En el primer clado se ubico *A. auricula-judae*; en el segundo clado *A. fuscosuccinea*; y en el tercer clado *A. polytricha*.

**Palabras clave.** *Auricularia*, taxonomía, filogenia, Colombia.

**INTRODUCTION**

Although *Auricularia* is an easily recognized genus of edible mushrooms with a worldwide distribution, it is difficult to identify specimens to species level because of the morphological variation of the fruit-bodies due to characteristics such as color, size,
and hynenial surface which vary with temperature, humidity, sun light and position of the basidiocarp on the substrate (Kobayasi, 1981).

The genus described by Lowy (1952) based on the characteristics of nine zones of the fruit body tissue (Fig. 1), included ten species grouped in two types according to the presence or absence of a medullary layer. Type one, with a medullary layer included *A. cornea*, *A. fuscouscinea*, *A. tenuis*, *A. emini* and, *A. polytricha* and type two without a medullary layer included *A. auricula-judae*, *A. delicata*, *A. mesenterica*, *A. ornata*, and *A. peltata*. Moreover, the width of the medulla was considered at species level. Kobayasi (1981) included five new species (*A. minor*, *A. eximia*, *A. papyracea*, *A. incrassate*, and *A. hispida*) and several strains of *A. auricula-judae*, *A. polytricha* and, *A. delicata*, based on the morphological characteristics of the fruit bodies, tissue structure, the hairs on the upper surface, and the color of the hymenophore in fresh material as useful characteristics. Bandoni (1984) proposed an alternative classification of Tremellales and Auriculariales based on the characteristics of the basidia, the haploid stage of mycelia and the septal pore apparatus; however

![Diagram of hyphal zonation](image)

**Figure 1.** Diagrammatic representation of the hyphal zonation of the basidiocarps types in *Auricularia*. A. Medullated type. B. Nonmedullated type
theses characteristics have not been used to differentiate species. Recent molecular phylogenetic studies revealed new aspects of the relationship between *Auricularia* and allied taxa. WeiB and Oberwinkler (2001) investigated the phylogenetic relationships in Auriculariales and found that *Auricularia* is grouped with *Exidia*, *Exidiopsis*, and *Heterochaete*. However, the number of *Auricularia* species included in the analysis was low, and the phylogenetic relationships within the genus remains unclear. The aim of this study is to investigate the phylogenetic relationships among the species of the genus *Auricularia* in Colombia.

**MATERIALS AND METHODS**

Seventy-six specimens deposited at the Herbarium of the University of Antioquia (HUA), The National Herbarium of Colombia (COL), and the Herbarium of the University of Quindío (HUQ) and 10 collections gathered during field work in Caldas and Tolima departments of Colombia were examined. The collected specimens were described when fresh and then dried in a food dehydrator (SIGG Dorrex) at 60 °C. Light microscopy studies, were performed in the laboratory of taxonomy and ecology of fungi (TEHO), Institute of Biology, University of Antioquia.

Perpendicular sections were rehydrated in alcohol and water and mounted in \( \text{H}_2\text{O}, \text{KOH} (5\%) \) and Congo red in order to make microscopic observations and measurements of all zones (pilosa, compact, superior subcompact, intermedia laxa, medulla, inferior subcompact and hymenial) using a calibrated micrometer. For species identification the taxonomic keys of Lowy (1952) and Kobayasi (1981) were used. To understand the phylogenetic relationships within the genus, 17 sequences of *Auricularia*, including 8 previously published sequences, plus two outgroup taxa were analyzed. *Exidiopsis calcea* (AF291280) and *Exidia truncata* (AF291279) from the DNA database of Japan (DDBJ) were used as outgroups. The DNA for phylogenetic analysis was obtained from 300 mg of each *Auricularia* specimen with a Plant Genomic DNA Mini Kit (VIOGENE) used according to the manufacturers’ protocols. The isolated DNA was resuspended in TE and stored at -20°C until use. The amplification of the internal transcribed spacer region (ITS) of the nuclear DNA was performed with the following new primers: Mont-ITSF: 5’-CAC ACC TG (T/A) GCA C(C/A)(T/A) TTT CG-3’, and Mont-ITSR: 5’-CCG CT(A/G) AAG AGG CC(T/C) A (A/G)G GC-3’. Double-stranded DNA was amplified after incubation at 94°C for final extension at 72°C for 15 min. DNA was amplified by PCR in a 50\( \mu l \) reaction volume containing approximately 50 ng total DNA, 10 mM Tris-HCL buffer (pH 8.3) with 50 Mm KCL and 1.5 MgCl\(_2\), 0.2 mM of each dNTP, 1.25 units Taq DNA polymerase (TAKARA), and 0.5 \( \mu M \) of each primer. After amplification, reaction mixtures were subjected to electrophoresis in 1% low-melting-temperature agarose gels to purify amplicons. We sequenced the purified PCR products using a BigDye Terminator ver. 3.1 (Applied BioSystems) and ABI Prism 3100 Genetic Analyzer (Applied BioSystems) according to the manufacturer’s instructions. For sequencing, we used the same primers as those used for amplification. All sequences have been deposited in DDBJ/EMBL/GenBank International DNA databases (Table 1).

**Data analyses**

To construct a phylogenetic tree based on ITS sequences of *Auricularia*, sequences were assembled and manually examined for errors using FinchTV software and the amplified regions (minus the length of the primers) were aligned using CLUSTAL W (Thompson et al., 1994) in MEGA version 5 (Tamura et al., 2011) with default settings.
Phylogeny of the genus *Auricularia*

### RESULTS

Five species of *Auricularia* (*A. polytricha, A. fuscosuccinea, A. delicata, A. auricula-judae,* and *A. mesenterica*) were identified from the specimens collected in Colombia in field work or preserved in the Herbariums.

**Phylogenetic analysis**

The ITS sequences were obtained from seven samples of *Auricularia* from Colombia and Japan. The highest BLAST hits in DDBJ/EMBL/GenBank to all *Auricularia* species has > 97% similarity. The alignment of seven sequences of *Auricularia* adding to eight sequences of *Auricularia* previously published and two outgroups indicate that the length of the ITS1 and ITS2 region varied from 348 to 391 bp. The phylogenetic relationships of the species of *Auricularia* was analyzed using the models of neighbor joining, maximum parsimony and maximum likelihood.

**Phylogenetic relationships were analyzed using the neighbor-joining (NJ), Maximum parsimony (MP) and Maximum likelihood (ML) methods combined with a Bootstrap analysis involving 1000 replication rounds. The transition: transversion ratio was fixed at 2:1. Kimura’s 2-parameter method was used for the calculation of the genetic distances.**

### Table 1. list of specimens used in molecular phylogenetic analyses.

<table>
<thead>
<tr>
<th>Number</th>
<th>Species</th>
<th>Collection no.</th>
<th>Locality</th>
<th>Gen Bank accession (ITS)</th>
<th>References</th>
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<tbody>
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<td>1</td>
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<td>AFM 22</td>
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<td>FJ92587</td>
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<td></td>
<td>L 523</td>
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<td>AB615228 (This study)</td>
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<tr>
<td>5</td>
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<tr>
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<td>Yu, Z. (Unpublished)</td>
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of *A. polytricha* and *A. fuscosuccinea*. Also high bootstrap values were achieved for the cluster of *A. auricula-judae*. The phylogenetic relationships of *A. delicata* could not be significantly resolved. With the exception of *A. delicata*, the same groups obtained by neighbor-joining analysis are also present in the tree that was found applying models of maximum likelihood (Fig. 4) and maximum parsimony (not shown).

**Taxonomic key**

Key for the identification of the species of the genus *Auricularia* present in Colombia

1. Hymenophore reticulate.................. *A. delicata*
2. Cross section with medullary layer........
3. Pilose zone less than 100 µm, medullary layer less than 150 µm...... *A. fuscosuccinea*
4. Pilose zone near to 500 µm....................


Macroscopically, the members of the genus are characterized by having gelatinous, resupinate to substipitate, saprobic, and solitary to gregarious basidiocarps. Basidiocarps are of 6 to 12 cm in diameter and 1-2 mm thick; the upper (superior) surface is pilose and dark yellow to brown or reddish brown; the lower (inferior) surface smooth, rugulose to meruloid, glabrous to pruinose and concolorous with the upper surface. Microscopically, in tangential section, a hyphal zonation (pilosa, compact, superior subcompact, intermedia laxa, medulla, inferior subcompact and hymenial) is observed. The basidia are cylindrical to clavate, and transversely 3-septate. The basidiospores are inamyloid, transparent and allantoids.

**Description of the species**


*Auricularia auricula* (Hook.) Underw; Barret, Mycologia 2: 12. 1910

Basidiocarps gelatinous when fresh, yellow to dark brown, superior surface pilose, inferior surface with few folds, 3.5-6 cm wide; substipitate, 1.3-1.7 cm long, cylindrical and solid; pilose zone shows hairs of 30-150 µm long, hyaline, often with broken tips. Compact zone 20-90 µm in width. Superior subcompact zone 20-100 µm wide. Intermedia laxa 120-500 µm wide. Inferior subcompact zone 40-95 µm wide. Hymenium 60-80 µm wide; basidia 40-70 µm. Spores allantoids 16-19 µm long and 4.3-4.7 wide. Generally gregarious on dead wood. Superficially, *A. auricula-judae* resembles *A. fuscosuccinea* in color and texture, but in section it is clearly differentiated by the absence of a medulla in *A. auricula-judae* (Fig. 2). Lowy (1952) and Kobayasi (1981) describe this species with the name *A. auricula*, while other authors (Swann and Taylor, 1993) describe this species as *A. auricula-judae*. We use *A. auricula-judae*, which, according with Dr. R. G. Bandoni (personal communication) is the most accepted name worldwide.

**Collections studied**

**Phylogeny of the genus *Auricularia***


*Laschia delicata* (Fr.), Linnaea 5: 553. 1830.

Basidiocarps are gelatinous when fresh, flabelliform, orbicular, reniform, yellow to dark brown, superior surface pilose, inferior surface meruloid, reticulate, 4-8 cm; substipitate, 1.0-3.5 cm long, cylindrical and solid; pilose zone covered by hairs 20-180 µm long, hyaline, often blunt or irregularly rounded tips. Compact zone 20-110 µm wide. Superior subcompact zone 40-270 µm wide. Intermedia laxa zone 60-700 µm wide. Inferior subcompact zone 35-270 µm wide. Hymenium 40-180 µm wide; basidia 30-70 µm. Spores allantoid 9.4-12.3 µm long and 4.3 wide (Fig. 2). Generally gregarious, on wood of species of the genera *Alnus* and *Quercus*.

**Collections studied**


**Figure 2.** Cross-section of the species of *Auricularia* (left) and diagrammatic representation of the hyphal zone (right). A, B and C are the species without medulla; D and E species with medulla. A. Cross-section of *A. auricula-judae*. B. Cross-section of *A. delicata*. C. Cross-section of *A. mesenterica*. D. Cross-section of *A. fuscosuccinea*. E. Cross-section of *A. polytricha*. 
All the specimens studied showed a high variation when compared with those reported in the literature, however all specimens had a reticulate-meruloid hymenophore.


Basidiocarps are gelatinous when fresh, coriaceous when dry; orbicular, reniform, yellow translucent to dark brown, superior surface pilose, inferior surface smooth with folds, 4.6-10.3 cm; substipitate, 1.8-3.2 cm long, cylindrical and solid; pilose zone with 20-90 µm long, hyaline hairs. Compact zone 20-60 µm wide. Superior subcompact zone 10-50 µm wide. Superior laxa zone 80-200 µm wide. Medullary layer 30-110. Inferior subcompact zone 35-300 µm wide. Inferior laxa zone 40-90µm. Hymenium 70-90 µm wide; basidia 30-50 µm. spores allantoids 8-10.8 µm long and 4.2-6.5µm wide (Fig. 2). Solitary or gregarious, on wood of *Quercus sp*.

**Collections studied**

Phylogeny of the genus *Auricularia*


**4- *Auricularia polytricha* (Mont.) Sacc. Tai R. Instit. Veneto Vi 3: 722.1885.**

**Hirneola polytricha** (Mont.) Fries, K. Vet.-Akad. Handl. 1848: 146. 1849.

Basidiocarps are rubbery, gelatinous when fresh, frequently with a convex dorsal surface, dark brown to dark lilac, upper surface densely pilose, lower surface smooth, 3.5-8 cm; substipitate, 0.5-2.0 cm long, cylindrical and solid; pilose zone with 115-550 µm long, hyaline to yellow translucent hairs. Compact zone 30-80 µm wide. Superior subcompact zone 80-270 µm wide. Superior laxa zone 80-260 µm wide. Medullary layer broad 60-250. Inferior subcompact 35-300 µm wide. Inferior laxa zone 40-90 µm. Hymenium 60-80 µm wide; basidia 40-70 µm. Spores were not found in the studied collections (Fig. 2).

**Collections studied**


*Auricularia lobata* (Sommerf.) Mag. For. Naturvindesk 7: 296. 1826

Basidiocarps are rubbery when fresh, resupinate, commonly lobed, dark brown to dark lilac, superior surface concentrically zonate, densely pilose, with few folds, inferior surface smooth, 3-7 cm wide; cylindrical and solid; pilose zone with 120-560 µm long, hyaline to yellow translucent hairs. Compact zone 32-46 µm wide. Superior subcompact 140-160 µm wide. Intermedia laxa zone 320-580 µm wide. Inferior subcompact zone 138-152 µm wide. Hymenium 68-120 µm wide; basidia 32-58 µm. Spores were not found in the studied collections (Fig. 2).

**Collections studied**


**DISCUSSION**

We have studied five species of *Auricularia* from Colombia. The most common species are *A. fuscosuccinea* and *A. delicata*, while *A. mesenterica* are the scarcest. Only three specimens of *A. mesenterica* were studied from the herbarium samples. Our results support the monophyletic origin of the genus *Auricularia*. *A. mesenterica* is positioned in the most basal clade, relatively close to outgroups species.

![Figure 3. Neighbor-joining analysis of an alignment of nuclear DNA coding. Bootstrap support (> 50%) are shown in each node. The tree is rooted with *Exidiopsis calcea* and *Exidia truncata.*](image-url)
Exidiopsis calcea and Exidia truncata. *A. mesenterica* is the only resupinate species in the genus *Auricularia* and is similar to the species of *Exidia* and *Exidiopsis*. WeiB and Oberwinkler (2001) reported high similarity between species of *Exidia*, *Exidiopsis* and *Auricularia* using the 28S ribosomal large subunit and, those results were considered evidence in agreement with Bandoni’s hypothesis (1984), that the basidium of *Auricularia* species is directly derived from that of the exidioid fungi. *A. auricula-judae* is a monophyletic taxon positioned relatively close to *A. mesenterica* and although *A. mesenterica* lacks a medulla and they could be differentiated in section by differences in the sizes of the pilosa zone and laxa intermedia zone.

WeiB and Oberwinkler (2001) previously reported that *A. delicata* and *A. fuscosuccinea* occurred together in the same cluster supported by a high bootstrap values obtained by neighbor-joining analysis. However, although in our studies, *A. delicata* is positioned near to *A. fuscosuccinea* in the dendrogram obtained by neighbor-joining analysis (Fig.3) and maximum parsimony (not shown); whereas in the maximum likelihood analysis (Fig.4), *A. delicata* was positioned relatively close to *A. auricula-judae*. Although *A. delicata* is a species that lacks medulla; this is a taxon with a characteristic unique in the genus and that is the meruloid form of the inferior surface of the basidiocarps. In this study, the phylogenetic relationship of *A. delicata* could not be significantly resolved.

**Figure 4.** Maximum likelihood analysis of an alignment of nuclear DNA coding. Bootstrap support (>50%) are shown in each node. The tree is rooted with *Exidiopsis calcea* and *Exidia truncata*. 
A. fuscosuccinea is monophyletic supported by high bootstrap values and was positioned in the same cluster with A. polytricha. Both A. fuscosuccinea and A. polytricha are species with medulla that can be differentiated by thickness of the pilosa zone and medullar layer.

Mengyao et al., (2008) reported molecular diversity of A. polytricha. They found 5 groups from 19 samples of A. polytricha and reported that the different A. polytricha strains not only vary in their output, cultivation cycle, shape and taste, but also in pharmacological effects. However, the description of A. polytricha by Kobayasi (1981) differs substantially from the description by Lowy (1952), mainly in the size of the medullary layer and the length of the pilose zone. In our observations more variance occurred in the width of the hyphal zonations than that is reported in previously published accounts (Lowy, 1952; Kobayasi, 1981). Also, our studies suggest that in the genus Auricularia and mainly in A. polytricha and A. auricula-judae there could be a lot more geographical variation within these taxa than has been recognized by mycologist. (Fig. 3)

The phylogenetic tree based on neighbor-joining analysis clearly shows that in Auricularia the absence or presence of medulla is an important characteristic in the identification of species. It is evident that A. fuscosuccinea and A. polytricha are sister groups of species with a medulla. However, further studies are necessary to clarify the relationships between A. delicata and others species of the genus and the probably geographic variation between species.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Herbarium of the University of Antioquia (HUA), The National Herbarium of Colombia (COL), and the Herbarium of the University of Quindio (HUQ) for the loan of specimens. The first author would like to thank to Dennis Murphy for comments and correcting English, and to the staff of the Laboratory of Silviculture, Faculty of Agriculture, Kochi University for their invaluable help and assistance in the molecular analysis. We also thank to Dr. Ryo Arakawa for their support throughout this study.

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Phylogeny of the genus *Auricularia*


Recibido: 24/03/2010
Aceptado: 12/03/2011