

## Contribution to the study of the genus *Auricularia* (*Auriculariales*, *Basidiomycota*) in Russia

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**Abstract.** The paper covers the study of the genus *Auricularia* in Russia. The taxonomic history of the genus and problems of its morphological systematics are described in details. The evolutionary relationships of species and species complexes were revealed based on a comparative study of the nucleotide sequences of nrDNA (ITS, rpb2). The occurrence of *A. auricula-judae* only in European of Russia part was confirmed by molecular data, whereas the morphologically similar species *A. americana* had the Far Eastern distribution in Russia. In total, 7 taxa of *Auricularia* have so far been recorded from Russia: known species *A. mesenterica*, *A. minor*, *A. cornea*, *A. americana*, *A. auricula-judae*, newly described species — *A. villosula* and undescribed *Auricularia* sp. An identification key to the studied *Auricularia* species and its morphological descriptions are also provided.

**Keywords:** *Auricularia*, molecular taxonomy, morphology, phylogeny, Russia, new species, *Auricularia villosula*.

## К изучению рода *Auricularia* (*Auriculariales*, *Basidiomycota*) в России

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**Резюме.** Проведено исследование представителей рода *Auricularia* в России. Представлен очерк таксономической истории рода, рассмотрены проблемы его морфологической систематики. На основе сравнительного изучения нуклеотидных последовательностей ДНК (ITS, rpb2) изучены взаимоотношения между видами и видовыми комплексами. Выяснено, что *A. auricula-judae* имеет лишь европейское распространение, а на Дальнем Востоке обитает морфологически сходный вид — *A. americana*. Исследование показало, что на территории России встречается семь таксонов *Auricularia*: *A. mesenterica*, *A. minor*, *A. cornea*, *A. americana*, *A. auricula-judae*, описываемый в статье как новый для науки *A. villosula*, а также неописанный вид *Auricularia* sp. Для всех видов приводятся подробные морфологические описания и ключ для их определения.

**Ключевые слова:** *Auricularia*, молекулярная таксономия, морфология, филогения, Россия, новый вид, *Auricularia villosula*.

## Introduction

*Auricularia* Bull. is a cosmopolitan genus, consisting of nearly 10–15 described and accepted species (Lowy, 1952; Kobayasi, 1981; Kirk *et al.*, 2008; Looney *et al.*, 2013). Most species of this genus exhibit tropical and subtropical distribution at forest communities, and only several species inhabit temperate zone of the Northern hemisphere. All species have saprotrophic lifestyle on wood debris.

The genus was described by P. Bulliard (1785). Since 1785 more than one hundred species have been recognized in it. Currently most of them were considered as dubious species. Such species are not accepted or have been already reduced to the synonyms. Up to now *Auricularia* is considered to be a rather complicated taxonomic group, despite of its proven monophyletic status (Weiss, Oberwinkler, 2001). The problems with infrageneric taxonomy are based on high variability of morphological characters and scarcity of microstructural features.

The hyphal scheme of basidiome proposed by B. Lowy (1951) and comprising several characters, such as internal stratification of hyphae, abhymenial hair length and the presence/absence of differentiated medulla, has always been considered as the most reliable criterion for classification systems in *Auricularia*. There were several attempts to subdivide the genus into some sections at the base of these specifications (Donk, 1952; Kobayasi, 1981). However, the numerous accumulated data and special works on the investigation of the genus have shown that internal structure of basidiome, the width of hyphal zones especially, is too variable due to the environmental conditions, and the age of the basidiome as well as the time of tissue exposure in KOH (Looney *et al.*, 2013). The mating tests, ecology, geographical patterns and molecular data could serve as additional criteria to tell the species apart in this genus. As a consequence of the taxonomic characters instability, the number of species within the genus *Auricularia* remains unclear.

Before our study the exact number of *Auricularia* species occurring in Russia was not known. This number was inconsistent depending on the data of different authors (Raitviir, 1967; Govorova, 1997). Most of them were known from the Russian Far East.

The aim of this study was to investigate the phylogenetic relationships among the species of the genus *Auricularia* in Russia and to verify collections from different localities, using both morphological study and molecular genetic techniques.

## Materials and Methods

### *Morphological analysis*

Macromorphological descriptions are based on fresh specimens, herbarium collections from the Mycological Herbarium of the Komarov Botanical Institute (LE, Saint Petersburg) and Institute of Biology and Soil Science (VLA, Vladivostok) as well as color photos of basidiomata. Micromorphological characters were studied from cross-sections of dried basidiomata mounted in 5 % KOH. Cross-sections were photographed and measured at 10×, abhymmenial hairs at 10× and 20×, and spores and basidia at 40× using Zeiss AxioImagerA1 light microscope.

### *Molecular techniques*

DNA was isolated from dried specimens. The data from GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>) were used as additional information source for comparative study.

The procedure of DNA extraction completely corresponded to the manufacture protocol of the AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, California, USA). The primer pairs ITS1F-ITS4B (<http://www.biology.duke.edu/fungi/mycolab/primers.htm>) were used both for PCR and for sequencing of ITS region. For amplification and sequencing of *rpb2* locus (a single-copy nuclear protein-coding gene) the primers Aur-6F and Aur-7R (Looney *et al.*, 2013) were used.

PCR products were purified using the Fermentas Genomic DNA Purification Kit (Thermo Scientific). Sequencing was performed with an ABI model 3130 Genetic Analyzer (Applied Biosystems) using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (AB). Raw data was processed using MEGA 6 (Tamura *et al.*, 2013). The sequences were aligned with the web tool MAFFT (<http://mafft.cbrc.jp/alignment/server/>).

Phylogenetic reconstructions were performed with maximum likelihood (ML), maximum parsimony (MP) and Bayesian analysis (BA). The outgroups used for analysis of ITS data set included *Exidia recisa* (Ditmar) Fr. and *Eichleriella leveilleana* (Berk. and M. A. Curtis) Burt, which have all been shown to be closely related species nonetheless locating outside the ingroup within the *Auriculariales* (Weiss, Oberwinkler, 2001; Looney *et al.*, 2013). The outgroup used for analysis of *rpb2* data set included *Ramaria rubella* (Schaeff.) R. H. Petersen.

Maximum likelihood analysis was run in RAxML servers, v.7.2.8 (<http://phylobench.vital-it.ch/raxml-bb/index.php>), with one hundred rapid bootstrap replicates. Only the clades with a support  $\geq 65$  % were considered to be a strong support.

Maximum parsimony was performed using PAUP\*4.0.b10 (Swofford, 2002) with a heuristic search option. Characters were unordered and

weighted equally; the number of trees retained was limited to 100 (for ITS); starting trees were obtained by stepwise addition with random sequence addition and tree bisection-reconnection (TBR) branch-swapping algorithm; one tree was held at each step during stepwise addition. Parsimony bootstrap analysis was performed with 1000 replicates. Only clades with a support  $\geq 55\%$  were retained for further analysis. Gaps were treated as missing characters.

Table

Sequences used in molecular phylogenetic analysis

No	Species	GenBank ITS	GenBank RPB2	Country	Herbarium Number
1	<i>Auricularia americana</i>	JX065146	JX065180	USA, Tennessee	
2	<i>A. americana</i>	JX065163	JX065178	USA, Tennessee	
3	<i>A. americana</i>	JX065152	JX065182	USA, North Carolina	
4	<i>A. americana</i>	JX065151	JX065181	USA, Tennessee	
5	<i>A. americana</i>	JX065166	JX065179	USA, Tennessee	
6	<i>A. americana</i>	JX065154	JX065177	USA, Idaho	
7	<i>A. americana</i>	DQ200918	DQ366278	USA, Oregon	
8	<b><i>A. americana</i></b>	KJ698429	KJ698438	Russia, Far East	LE 296428
9	<b><i>A. americana</i></b>	KJ698431	KJ698439	Russia, Far East	VLA M-11352
10	<i>A. auricula-judae</i>	AF291268			
11	<i>A. auricula-judae</i>	DQ520099	DQ521419	Germany	
12	<i>A. auricula-judae</i>	JX065174			
13	<b><i>A. auricula-judae</i></b>	KJ698425		Russia, Western Caucasus	LE 254030
14	<b><i>A. auricula-judae</i></b>	KJ698426		Russia, Western Caucasus	LE 254071
15	<i>A. cornea</i>	HM448462			
16	<i>A. cornea</i>	FJ617292			
17	<i>A. cornea</i>	HM448454			
18	<i>A. cornea</i>	JX065164	JX065185	Australia	
19	<i>A. cornea</i>	FJ792587			
20	<i>A. cornea</i>	FJ617297			
21	<i>A. cornea</i>	FJ617302			
22	<b><i>A. cornea</i></b>	KJ698435		Laos	LE 269791
23	<b><i>A. cornea</i></b>	KJ698430		Russia, Far East	LE 296425
24	<b><i>A. cornea</i></b>	KJ698432		Russia, Far East	VLA M-12.828
25	<b><i>A. cornea</i></b>	KJ698424		Russia, Far East	LE 262938
26	<b><i>A. cornea</i></b>	KJ698433		Russia, Far East	VLA M-11322
27	<b><i>A. cornea</i></b>	KJ698427		Russia, Far East	LE 262998
28	<b><i>A. cornea</i></b>	KJ698428		Russia, Far East	VLA M-18.531

Table (continued)

No	Species	GenBank ITS	GenBank RPB2	Country	Herbarium Number
29	<b><i>A. cornea</i></b>		KJ698437	Russia, Far East	VLA M-15691
30	<i>A. fuscusuccinea</i>	JX065153			
31	<i>A. fuscusuccinea</i>	JX065157	JX065190	USA, Louisiana	
32	<i>A. fuscusuccinea</i>	JX065139			
33	<i>A. fuscusuccinea</i>	JX065140			
34	<i>A. fuscusuccinea</i>	JX065141	JX065193	Argentina	
35	<i>A. fuscusuccinea</i>		JX065184	Costa Rica	
36	<i>A. mesenterica</i>	AF291271			
37	<b><i>A. mesenterica</i></b>	KJ698436		Russia, Samara Region	LE 254558
38	<b><i>A. minor</i></b>	KJ698434		Russia, Far East	LE 296424
39	<i>A. nigricans</i>	JX065176			
40	<i>A. nigricans</i>	JX065172			
41	<i>A. nigricans</i>	JX065167			
42	<i>Auricularia</i> sp.	EU560401			
43	<i>Auricularia</i> sp.	EU560406			
44	<i>Auricularia</i> sp.	EU647879			
45	<i>Auricularia</i> sp.	AB615232			
46	<i>Auricularia</i> sp.	HQ388358			
47	<b><i>Auricularia</i> sp.</b>	KJ698421		Russia, Republic of Buryatia	LE 262806
48	<i>Auricularia</i> sp.	HQ388355			
49	<b><i>Auricularia</i> sp.</b>	KJ698423		Russia, Far East	LE 296433
50	<b><i>Auricularia</i> sp.</b>	KJ698422		Russia, Far East	LE 296423
51	<i>Auricularia</i> sp.	FJ478123			
52	<b><i>A. villosula</i></b>	KJ698417	KJ698440	Russia, Far East	VLA M-11291
53	<b><i>A. villosula</i></b>	KJ698418	KJ698441	Russia, Far East	LE 296422
54	<b><i>A. villosula</i></b>	KJ698419		Russia, Far East	VLA M-11348
55	<b><i>A. villosula</i></b>	KJ698420		Russia, Far East	LE 262989
56	<i>Eichleriella leveilleana</i>	AY509553			
57	<i>Exidia recisa</i>	AF291276			
58	<i>Ramaria rubella</i>		AY786064		

Note. Entries in bold are the sequences newly generated for this study.

Bayesian analysis was performed using MrBayes 3.1 (Ronquist, Huel- senbeck, 2003) software for two independent runs, each with 1 500 000 generations with sampling every 100 generations, with GTR model (for ITS) and four chains. Posterior probability (PP) value  $\geq 0.95$  is considered as significant.

The evolutionary distance between sequences was assessed using MEGA 6 with substitution model Kimura 2-parameter.

All ITS and rpb2 sequences have been deposited in GenBank; their accession numbers are given in Table.

## Results

The ITS data set consists of 55 *Auricularia* sequences and 2 sequences of an outgroup. The rpb2 data set consists of 16 *Auricularia* sequences and 1 sequence of an outgroup. For this study we produced twenty new ITS and five new rpb2 sequences. The final ITS alignment contained 574 characters (with gaps), of which 125 were parsimony informative. In the MP analysis 100 MPT were recovered (Tree Length = 427; CI = 0.6183; RI = 0.8787; RC = 0.5433). The final rpb2 alignment contained 580 characters (with gaps), of which 131 were parsimony informative. In the MP analysis 8 MPT were recovered (Tree Length = 363; CI = 0.7879; RI = 0.8127; RC = 0.6403).

The phylogenetic studies resulted in two tree-topologies for ITS and rpb2 data sets (Figs. 1b, 2). The topologies yielded from all analyses (MP, ML and BA) were the same. Eight/nine major monophyletic clades with high support were recovered in ITS tree. The terminal groupings did not vary in the analyses of the different genetic markers. Additional analysis of rpb2 region showed the same monophyletic clades in relation to the species involved.

All recovered phylogenetic lineages are characterized by distinctive morphological features (presence/absence medulla, spore size, length of abhymenial hairs).

Two ITS sequences of *Auricularia* obtained from the Russian Far East collections (LE 296428 and VLA M-11352) appeared to belong to *A. americana*-clade. The pairwise distance between sequences within this clade was 2.3 % that corresponded average level of intraspecific ITS variability in *Basidiomycota* (Nilsson *et al.*, 2008).

Four Russian Far East collections (VLA M-11348, VLA M-11291, LE 296422, LE 262989) formed distinctly separate high supported clade based on both ITS and rpb2 data sets. These collections were characterized by unique morphological features sufficient for describing a new species — *Auricularia villosula* (description is given below in taxonomical part).

All specimens with strongly pubescent basidiomata were nested in one clade — *A. cornea*. The pairwise distance between ITS sequences within this clade reaches 1.3 % that allows us to consider all members of this group as a single species.

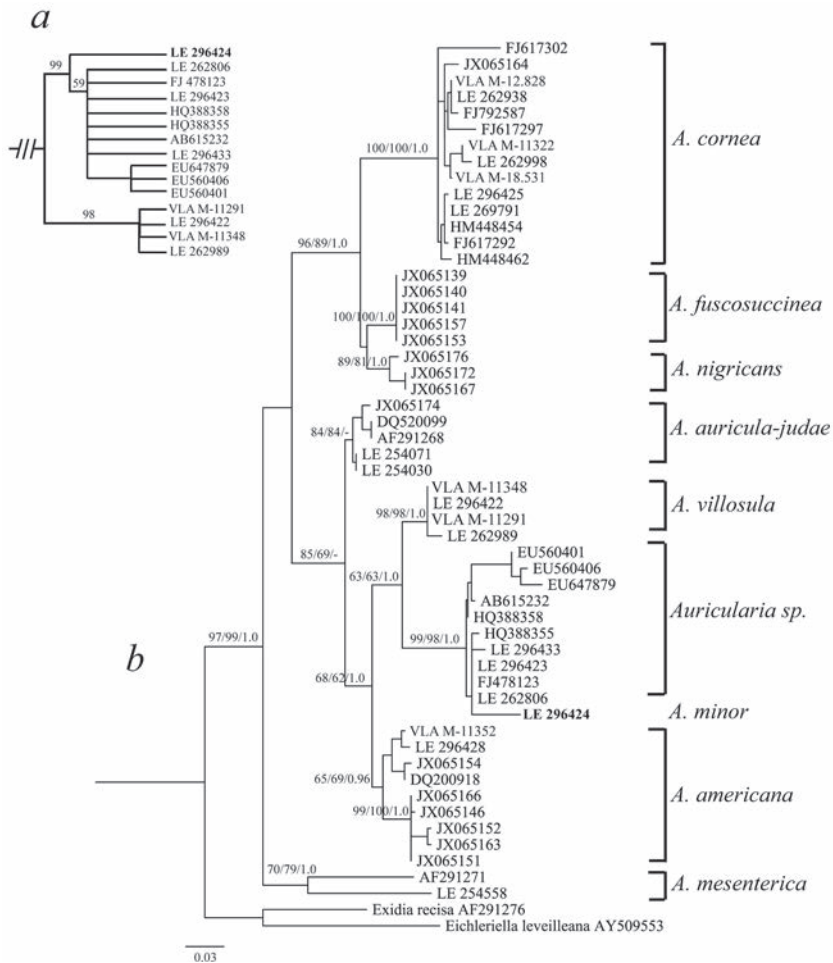


Fig. 1. Phylogenetic relationships of *Auricularia* species based on dataset of nrITS sequences.

*a* — Part of MP Bootstrap 50 % majority-rule consensus tree showing separate position of *Auricularia minor* (LE 296424); *b* — Tree inferred from ML analysis. Bootstrap values from Maximum Parsimony, Maximum Likelihood analyses  $\geq 55$  % and Posterior probability from Bayesian analysis  $\geq 0.95$  are shown over the branches (MP/ML/BA). Collections are indicated with the herbarium number (for Russian material) and GenBank accession number.

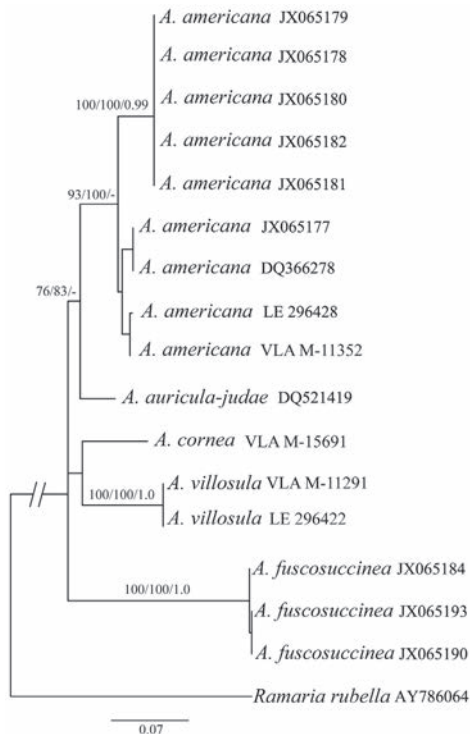


Fig. 2. Result of ML-analysis, showing phylogenetic relationships of *Auricularia* species based on RPB2 dataset.

Bootstrap values from Maximum Parsimony, Maximum Likelihood analyses  $\geq 55\%$  and Posterior probability from Bayesian analysis  $\geq 0.95$  are shown over the branches (MP/ML/BA). Collections are indicated with the herbarium number (for Russian material) and GenBank accession number.

Some collections from the Russian Far East morphologically similar to *A. auricula-judae* (Bull.) Quél. were clustered in single clade '*Auricularia* sp.'. The pairwise distance between ITS sequences within this clade was only 1.8 %.

The independent taxonomical position of *A. minor* Kobayasi was reliably confirmed with MP analysis (Fig. 1a). On ML tree this species formed a rather long branch. The phylogenetic distance between *A. minor* and '*Auricularia* sp.' clade was more than 2 %.

The European representatives of *A. auricula-judae* were characterized by isolated position from Asian species group «*A. americana* — *A. villosula* — *Auricularia* sp.» inferred from the topologies.



Seven species of *Auricularia* (*A. mesenterica* (Dicks.) Pers., *A. minor* Kobayasi, *A. cornea* Ehrenb., *A. americana* Parmasto et I. Parmasto ex Audet, Boulet et Sirard, *A. auricula-judae* (Bull.) Quél., described below *A. villosula* Malysheva and *Auricularia* sp.) were identified from the specimens collected in Russia.

#### Key to the species of the genus *Auricularia* distributed in Russia

1. Hymenium meruloid and strongly lobed ..... *A. mesenterica*.  
— Hymenium mostly smooth or with occasional folds ..... 2.
2. Cortical layer hirsute with hairs 150–500  $\mu\text{m}$  long ..... *A. cornea*.  
— Cortical layer velutinous to pubescent with hairs 50–200  $\mu\text{m}$  long ..... 3.
3. Abhymenial hairs up to 80  $\mu\text{m}$  long ..... 4.  
— Abhymenial hairs up to 200  $\mu\text{m}$  long ..... 5.
4. Basidiospores up to 15  $\mu\text{m}$  long. Medulla absent ..... *A. villosula*.  
— Basidiospores up to 11  $\mu\text{m}$  long. Medulla present ..... *A. minor*.
5. Medulla present ..... *Auricularia* sp.  
— Medulla absent or weakly differentiated ..... 6.
6. Distributed in the Russian Far East ..... *A. americana*.  
— Distributed in European part of Russia ..... *A. auricula-judae*.

***Auricularia mesenterica*** (Dicks. : Fr.) Pers., 1822, *Mycol. Eur.* 1: 97 (Plate II, 5).

≡ *Helvella mesenterica* Dicks., 1785, *Fasc. pl. crypt. brit.* 1: 20.

= *Auricularia corrugata* Sowerby, 1800, *Col. fig. Engl. Fung. Mushr.* 3: pl. 290.

= *Auricularia lobata* Sommerf., 1826, *Mag. Naturvididensk.* 6: 295.

Basidiomata effuse-reflexed, resupinate with free lobed and undulate margin, coalescing, mature specimens 2–5 mm thick and up to 15 cm wide; consistency firm-gelatinous, rubbery; superior surface distinctly concentrically zonate, densely pilose, folded, grey-ochre to brown; abhymenial hairs matted or clustered, hyaline to pigmented, thick-walled, 700–1200  $\times$  2–5  $\mu\text{m}$ ; inferior hymenial surface smooth or veined, light-brown to purple-brown, occasionally with lilac tint.

Medulla absent. Internal hyphae 2–6  $\mu\text{m}$  wide, hyaline, with clamp connections. Basidia cylindrical, 45–60  $\times$  3–5  $\mu\text{m}$ , with three septa. Basidiospores allantoid, hyaline, thin-walled, 12–15  $\times$  5–6  $\mu\text{m}$ .

Habitat: on wood of deciduous trees.

General distribution: cosmopolitan (America, Eurasia, Africa, Australia).

Specimens examined: **Russia**, Leningrad Region, Volkhov District, broadleaf forest, on fallen trunk of *Ulmus glabra*, 15.09.1999, A. E. Kovalenko, LE 208569; Samara Region, Stavropolsky District, Zhigulevsky

Nature Reserve, Shiryavenskaya valley, on a fallen trunk of deciduous tree, 17.05.2004, N. V. Psurtseva, LE 254558.

Note: This taxon is easily recognized at the base of its morphology (concentrically zonate pilose basidiome surface, veined hymenium). It cannot be confused with other species of *Auricularia*.

***Auricularia cornea*** Ehrenb. : Fr., 1820, in Nees von Esenbeck (ed.), *Horae Phys. Berol.*: 91 (Plate I, 6, 8).

≡ *Exidia cornea* (Ehrenb. : Fr.) Fr., 1822, *Syst. mycol.* 2(1): 222.

Basidiomata sessile or substipitate, solitary or caespitose, excentric, infundibuliform, saucer-shaped, with undulate margin, mature specimens 0.8–2.0 mm thick and up to 10 cm wide; consistency firm-gelatinous; when dry, thin, opaque and brittle; superior surface pilose, pale ochraceous, yellow-green, olive or pinkish when fresh; abhymenial hairs dense, often aggregate in short tufts, hyaline to pigmented, thick-walled, 150–500 × 4.3–7.8 μm; inferior hymenial surface smooth, red-brown to dark brown.

Medulla present. Internal hyphae hyaline, with clamp connections. Basidia clavate, 45–55 × 4–5 μm, with three septa. Basidiospores allantoid, hyaline, thin-walled, 11.5–14.0 × 5.0–6.5 μm.

Habitat: on wood of deciduous and coniferous trees.

General distribution: Tropical America, Africa, Eastern Asia (including Russian Far East), Australia.

Specimens examined: **Russia**, Far East, Primorye Territory, Verkhneussuriiskiy Stationary, on the branch of *Sambucus* sp., 10.08.1990, E. M. Bulakh, VLA M-11322; Marine Reserve, Gildebrand Island, on the branch of *Sambucus* sp., 03.06.1999, Bulakh, VLA M-12.828; Sakhalin Region, Moneron Island, on a fallen tree, 05.08.2001, V. Yu. Barkalov, VLA M-15691; Nadezhdinsky District, on fallen deciduous tree, 01.09.2002, Barkalov, VLA M-18.531; Ussuriysky Nature Reserve, Peishula, flood plain of Suvorovka River, mixed forest (with *Pinus koraiensis*, *Phellodendron amurense*, *Chosenia arbutifolia*), on a fallen deciduous tree, 12.08.2011, V. F. Malysheva, LE 262998; Ussuriysky Nature Reserve, Peishula, flood plain of Koryavaya River, mixed forest (with *Pinus koraiensis*, *Phellodendron amurense*, *Chosenia arbutifolia*), on a fallen deciduous tree, 16.08.2011, Malysheva, LE 262938; Sikhotealin Nature Reserve, Nechet, cedar forest with fir and deciduous trees, on a fallen deciduous tree, 16.08.2012, M. N. Gromyko, LE 296425. **Laos**, «Tat Kuangi Sl» National Park, tropical forest, on fallen wood, 01.30.2013, O. N. Ezhov, LE 269791.

Note: *A. cornea* is somewhat similar in appearance to *A. nigricans*. It differs from *A. nigricans* by less pilose surface. The most distinctive feature of *A. cornea* is olivaceous tint of its basidiomata.

Some collections previously doubtfully recognized as *A. polytricha* (Mont.) Sacc. and *A. papyracea* Yasuda appeared to belong to *A. cornea*, that was concluded from our detailed examination of their morphology and molecular data. The absence of these species at the territory of Russia was confirmed by our study.

***Auricularia villosula*** Malysheva, sp. nov. (Plate II, 2, 4).

MycoBank: MB 808546

*Recognized by yellow-brown gelatinous basidiomata, very short abhymenial hairs and the absence of medulla.*

Etymology: Refers to weak pubescence of the basidiome surface.

Holotype: **Russia**, Far East, Primorye Territory, Shkotovsky district, Bolshoy Kamen vicinities, 20.06.1991, A. G. Mikulin, LE 296422.

Basidiomata substipitate to sessile, attached to substrate by central point, solitary or caespitose, discoid or saucer-shaped, with free lobed and undulate margin, mature specimens 1–2 mm thick and up to 5 cm wide; consistency gelatinous; thin, opaque and brittle when dry; superior surface velutinous, flat or venose, yellow-brown or reddish brown when fresh, sometimes yellow-green when dry; abhymenial hairs 30–70 × 5.0–6.0 μm, irregular, hyaline, thick-walled; inferior hymenial surface glabrous, pale brown to dark brown with greenish tint.

Medulla absent. Internal hyphae thin-walled, hyaline, with clamp connections. Basidia cylindrical, 40–60 × 4.0–5.0 μm, with three septa. Basidiospores allantoid, hyaline, thin-walled, 11.7–15.3 × 4.8–6.3 μm.

Habitat: on wood of deciduous trees.

General distribution: so far known only from the Russian Far East.

Specimens examined: **Russia**, Far East, Primorye Territory, Ussuriyskiy Nature Reserve, broadleaf forest, on a fallen deciduous tree, 03.07.1990, O. K. Govorova, VLA M-11348; Ussuriyskiy Nature Reserve, mixed forest, on a fallen trunk, 30.07.1992, Govorova, VLA M-11291; Ussuriyskiy Nature Reserve, vic. Peishula Reserve station, flood plain of Suvorovka River, mixed forest (with *Pinus koraiensis*, *Phellodendron amurense*, *Chosenia arbutifolia*), on a fallen deciduous tree, 15.08.2011, Malysheva, LE 262989.

Note: The new species is morphologically similar to *Auricularia* sp., *A. auriculajudae* and *A. americana*. It differs from *Auricularia* sp. by the absence of medulla and from other species by having very short abhymenial hairs (up to 70 μm long).

***Auricularia minor*** Kobayasi, 1981, Bull. Natn. Sci. Mus., Tokyo, B 7(2): 50 (Plate I, 5, 7).

Basidiomata sessile or substipitate, solitary or caespitose, excentric, infundibuliform, saucer-shaped or earlike, with undulate margin, mature basidiomata 0.5–2.0 cm in diameter; consistency firm-gelatinous; when dry, thin, opaque and brittle; superior surface smooth or sometimes

venose, pilose, yellow-brown to dark olivaceous when fresh and yellow-green when dry; abhymenial hairs  $50\text{--}80 \times 4.5\text{--}6.5 \mu\text{m}$ , irregular, hyaline, thick-walled, without central strand; inferior hymenial surface glabrous or slightly folded, pale brown to dark brown.

Medulla present, central. Internal hyphae thin-walled, hyaline, with clamp connections. Basidia cylindrical,  $40\text{--}60 \times 3.5\text{--}5.0 \mu\text{m}$ , with three septa. Basidiospores allantoid, hyaline, thin-walled,  $7.0\text{--}11.0 \times 4.0\text{--}5.0 \mu\text{m}$ .

Habitat: on wood of deciduous trees.

General distribution: Eastern Asia (Russian Far East, Japan, Borneo, New Guinea).

Specimen examined: **Russia**, Far East, Primorye Territory, Kedrovaya Pad' Reserve, lowland forest, 10.10.1994, Govorova, LE 296424.

Note: This species is characterized by small basidiomata, short abhymenial hairs, small basidiospores and the presence of medulla.

#### ***Auricularia* sp.** (Plate II, 1, 3).

Basidiomata sessile or substipitate, solitary or caespitose, excentric, infundibuliform, saucer-shaped or earlike, with undulate margin, mature specimens 0.8–1.5 mm thick and up to 12 cm wide; consistency firm-gelatinous; when dry, thin, opaque and brittle; superior surface smooth or sometimes venose, pilose, yellow-brown to reddish brown when fresh and yellow-green when dry; abhymenial hairs  $60\text{--}200 \times 4.5\text{--}6.5 \mu\text{m}$ , irregular, hyaline, thick-walled, without central strand; inferior hymenial surface glabrous or slightly folded, pale brown to dark brown.

Medulla present, central. Internal hyphae thin-walled, hyaline, with clamp connections. Basidia cylindrical,  $40\text{--}60 \times 3.5\text{--}5.0 \mu\text{m}$ , with three septa. Basidiospores allantoid, hyaline, thin-walled,  $11.5\text{--}16.3 \times 4.2\text{--}6.0 \mu\text{m}$ .

Habitat: on wood of deciduous trees.

General distribution: Eastern Asia (China, Japan, Russian Far East), Siberia.

Specimens examined: **Russia**, Far East, Primorye Territory, Sikhote-Alin Nature Reserve, Ust'-Serebryany, flood plain forest, on fallen trunk, 04.09.1996, O. V. Morozova, LE 296433; Khankaisky district, Novo-Kachalinsk, broadleaf forest, on a fallen trunk of *Quercus* sp., 26.07.1996, A. S. Leley, LE 296423; Republic of Buryatia, Kabansky district, flood plain of Vydrina River, on a fallen trunk of *Populus* sp., 09.08.1998, Morozova, LE 262806.

Note: It is close to *A. auricula-judae* and *A. americana* but differs from both by having the medulla and from the first by different geographical distribution.

***Auricularia americana*** Parmasto et I. Parmasto ex Audet, Boulet et Sirard, 2003, in Boulet, Les Champignons des Arbres de l'Est de l'Amérique du Nord: 287 (Plate I, 1, 3).

≡ *Auricularia americana* Parmasto et I. Parmasto, 1987, *Bibl. Mycol.* 115: 137.

Basidiomata sessile or substipitate, solitary or caespitose, saucer-shaped or earlike, with lobed and undulate margin, mature specimens 1–4 mm thick and up to 8 cm wide; consistency firm-gelatinous; when dry, thin, opaque and brittle; superior surface velutinous to pubescent, yellow-brown to reddish brown when fresh, sometimes yellow-green when dry; abhymenial hairs 60–120 × 4.5–5.0 μm, irregular, hyaline, thick-walled; inferior hymenial surface, glabrous or slightly folded, pale brown to dark brown.

Medulla absent. Internal hyphae thin-walled, hyaline, with clamp connections. Basidia cylindrical, 41–74 × 3.0–5.0 μm, with three septa. Basidiospores allantoid, hyaline, thin-walled, 12.5–16.4 × 5.0–6.4 μm.

Habitat: on wood of coniferous trees.

General distribution: North America (USA, Canada), Eastern Asia (Russian Far East).

Specimen examined: **Russia**, Far East, Primorye Territory, Sikhotealin Nature Reserve, Nechet, cedar forest with fir and deciduous trees, on a fallen branch of *Pinus koraiensis*, 16.08.2012, Gromyko, LE 296428; Amur Region, Khingansky Reserve, Kundur, flood plain of Karancha River, mixed forest, 10.10.1991, Mikulin, VLA M-11352.

Note: Morphologically similar *A. auricula-judae* is characterized by slightly larger basidiospores (15.0–17.5 × 4.6–7.0 μm), habitat on deciduous wood and European distribution. Another similar species *Auricularia* sp. has a distinct medulla.

***Auricularia auricula-judae*** (Bull.) Quéél., 1886, *Enchir. fung.* (Paris): 207 (Plate I, 2, 4).

≡ *Tremella auricula-judae* Bull., 1789, *Herb. Fr.* 9: tab. 427, fig. 2.

= *Auricularia sambuci* Pers., 1822, *Mycol. eur.* (Erlanga) 1: 97.

= *Auricularia auricula* (L.) Underw., 1902, *Mem. Torrey bot. Club.* 12: 15.

= *Auricularia lactea* (Quéél.) Bigeard et H. Guill., 1913, *Fl. Champ. Supér. France*, 2.

= *Auricularia auricularis* (Gray) G. W. Martin, 1943, *Am. Midl. Nat.* 30: 81.

Basidiomata sessile or substipitate, solitary or caespitose, excentric, infundibuliform, saucer-shaped or earlike, with undulate margin, mature specimens 0.8–1.5 mm thick and up to 12 cm wide; consistency firm-gelatinous; thin, opaque and brittle when dry; superior surface smooth or sometimes venose, pilose, yellow-brown to reddish brown when fresh and yellow-green when dry; abhymenial hairs 60–200 × 4.5–6.5 μm, irregular, hyaline, thick-walled, without central strand; inferior hymenial surface, glabrous or slightly folded, pale brown to dark brown.

Medulla absent. Internal hyphae thin-walled, hyaline, with clamp connections. Basidia cylindrical,  $50\text{--}60 \times 5\text{--}6 \mu\text{m}$ , with three septa. Basidiospores allantoid, hyaline, thin-walled,  $15.0\text{--}17.5 \times 4.6\text{--}7.0 \mu\text{m}$ .

Habitat: on wood of deciduous trees.

General distribution: temperate regions of Europe (including European part of Russia and Caucasus).

Specimens examined: **Russia**, Western Caucasus, Karachaevo-Cherkessia, Teberda State Biosphere Reserve, Arkhyz site, Bukovo vicinities, fagus forest, on a fallen branch of *Fraxinus* sp., 22.08.2009, Malysheva, LE 254030; Teberda State Biosphere Reserve, vicinities of Teberda town, fagus forest, on a trunk of *Euonymus* sp., 10.08.2009, Malysheva, LE 254071.

Note: It can be distinguished from *A. americana* by larger basidiospores, habitat and geographical distribution. It differs from *Auricularia* sp. by the absence of medulla.

### Discussion

Recent extensive treatments of *Auricularia* in North America (Montoya-Alvarez *et al.*, 2011; Looney *et al.*, 2013) and China (Li *et al.*, 2014) have helped to verify in some extent the distribution and phylogenetic relationships between species, but they left open the question of taxonomical status of Asian collections of *A. auricula-judae*. In these studies conducted by A. F. Montoya-Alvarez *et al.* (2011) and B. P. Looney *et al.* (2013) the investigated Chinese and Japanese collections of *A. auricula-judae* were shown to be clearly distinct from European clade (these are 'clade I' and 'clade II' in Looney *et al.*, 2013). However, the detailed morphological studies of these East Asian collections have not been implemented by these authors. The morphological descriptions given for *A. auricula-judae* in the paper of Montoya-Alvarez and co-authors were based on collections from Colombia only, which were not involved into phylogenetic analyses and could belong to *A. americana*.

In our phylogenetic study the collections from European Russia were shown to be nested inside *A. auricula-judae* clade containing also collections from Germany and Switzerland. The collections from the Russian Far East and Siberia morphologically similar to *A. auricula-judae* appeared to be appertaining to two different clades corresponding to *A. americana* (on coniferous wood) and *Auricularia* sp. This pattern has been confirmed by two loci. The main distinguishing character of all *Auricularia* sp. specimens is the presence of medulla. In the previously cited work (Looney *et al.*, 2013) the authors designated this clade, with specimens identical to our *Auricularia* sp. as *A. auricula-judae* 'clade II' comprising specimens from East Asia (China, Japan). The taxon has been

left undescribed in that work, because the authors have not examined any specimens that correspond to this clade. Given that the holotype of *A. auricula-judae* originates from Europe, B. P. Looney *et al.* (2013) refer to the Asian group as ‘clade II’. In the present study we investigated thoroughly the morphology of the representatives of this clade from Siberia and the Russian Far East. There were no morphological differences between *Auricularia* sp., *A. auricula-judae* and *A. americana* with exception of medulla. Due to instability of such feature as the presence/absence of a medullar zone, we do not describe a new species herein, suggesting the taxon is left as a phylogenetic unit only without any supporting additional morphological characters.

The specimens with short abhymenial hairs of basidiomata and distributed at the Far East formed well supported clade in both ITS and rpb2 topologies (Figs 1, 2). We consider this clade as a new species *A. villosula*.

The representative material of two species, *A. nigricans* (Fr. : Fr.) Birkebak, Looney et Sánchez-García and *A. cornea*, has been analyzed for clarifying phylogenetic position of taxa, characterized by strong pubescence of basidiomata surface, in the general *Auricularia* phylogeny. All examined collections with strongly pubescent basidiomata appeared to be *A. cornea*. Noteworthy, some of these specimens were labeled initially as *A. tenuis* (Lév.) Farl. The problem of synonymy of *A. tenuis* to *A. cornea* has not been resolved yet. Possibly, the assumption of G. J. Wong and K. Wells (1987) about the synonymy of *A. cornea* to *A. tenuis* based on interfertility of both taxa may be true. Epitypification of *A. cornea* as well as thorough study of *A. tenuis* new collections from its type locality Borneo are needed to verify this conclusion.

The analysis of the Russian material showed the presence of some phylogeographic discrepancies within a single, morphologically uniform species complex *A. auricula-judae*. The Asian collections, including Far Eastern specimens, are represented by rather different taxa. Further studies with more sampling of specimens from Asian territory (Russia, China, Korea, Japan) could help to clarify taxonomical designations for these collections.

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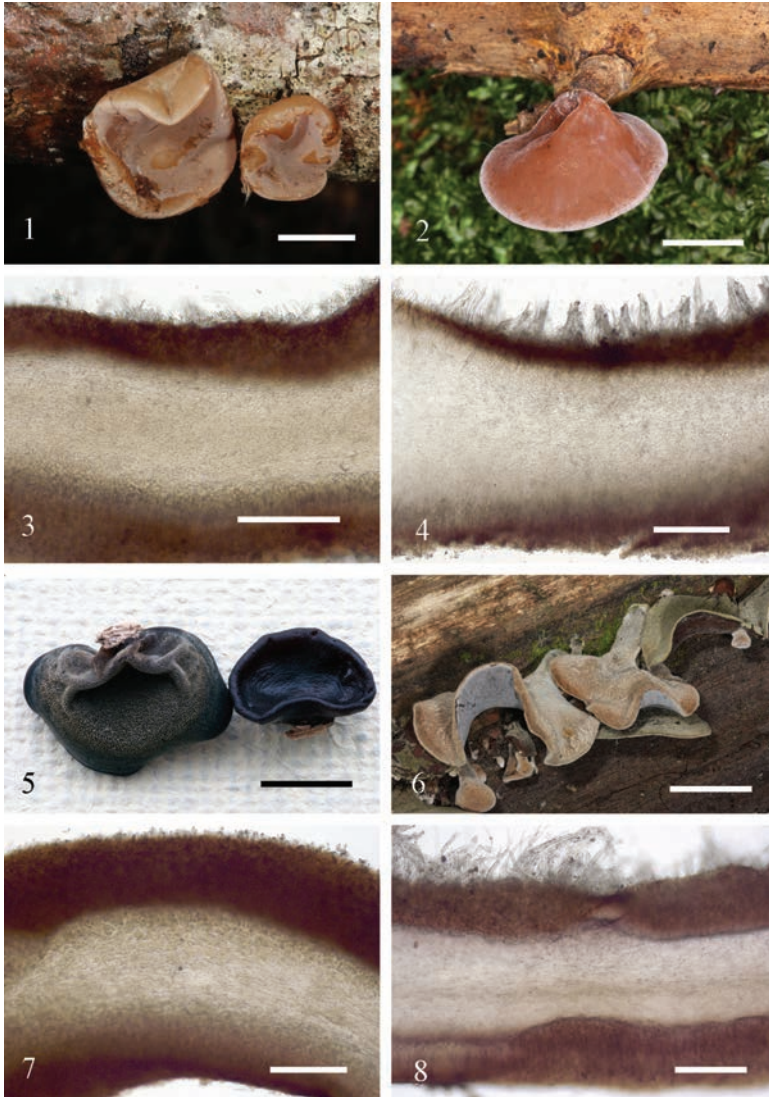


Plate I. Basidiomata and cross-sections of *Auricularia* species.

1 — basidiomata of *A. americana* (LE 296428); 2 — basidiome of *A. auricula-judae* (LE 254030); 3 — cross-section of *A. americana* without medulla (VLA M-11352); 4 — cross-section of *A. auricula-judae* without medulla (LE 254071); 5 — basidiomata of *A. minor* (LE 296424); 6 — basidiomata of *A. cornea* (LE 296425); 7 — cross-section of *A. minor* with unclear medulla (LE 296424); 8 — cross-section of *A. cornea* with medulla (VLA M-12.828). Scale bars: 1, 2, 6 — 2 cm; 3, 4, 7, 8 — 200  $\mu$ m; 5 — 0.5 cm.

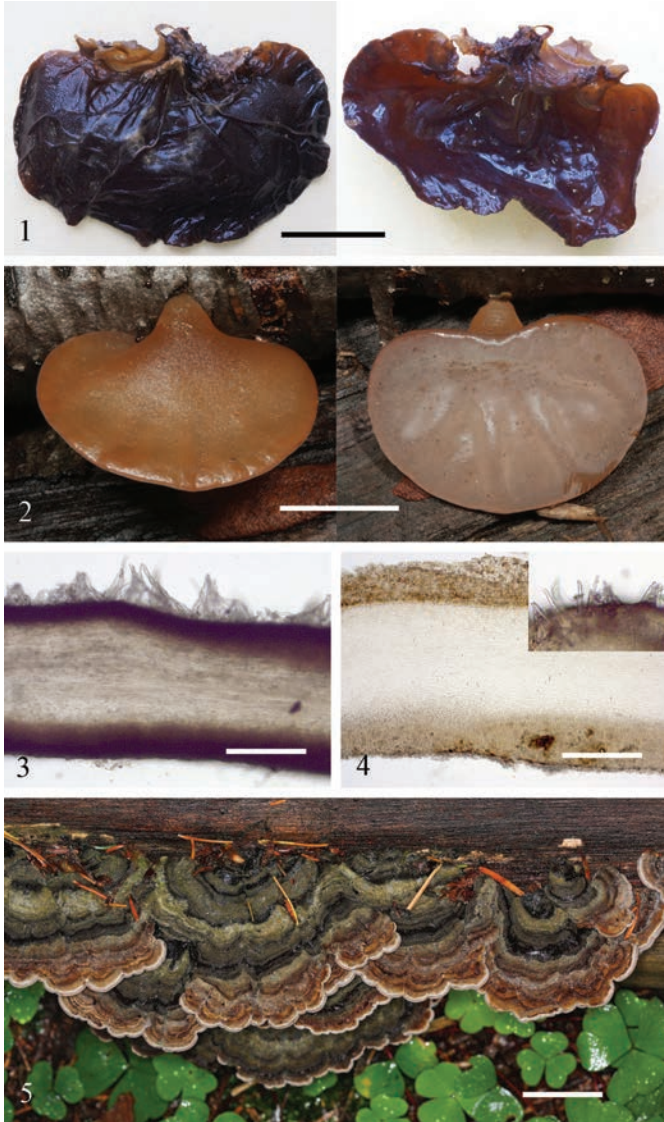


Plate II. Basidiomata and cross-sections of *Auricularia* species.

1 — basidiomata of *Auricularia* sp. (LE 262806); 2 — basidiomata of *A. villosula* (LE 262989); 3 — cross-section of *Auricularia* sp. with medulla (LE 262806); 4 — cross-section of *A. villosula* with short abhymenial hairs and without medulla (VLA M-11291); 5 — basidiomata of *A. mesenterica* (LE 254558). Scale bars: 1, 2 — 1 cm; 3, 4 — 200  $\mu$ m; 5 — 2 cm.