



Species clarification of the most important and cultivated *Auricularia* mushroom “Heimuer”: evidence from morphological and molecular data

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Abstract

Phylogenetic analysis of the *Auricularia auricula-judae* complex was carried out using ITS and nLSU ribosomal RNA gene regions, and morphology of the *A. auricula-judae* complex and related species is examined based on 33 wild collections and 10 cultivated samples worldwide. The phylogenetic analysis presented here showed that the wild and cultivated samples previously identified as *A. auricula-judae* in China are different from those from Europe (the type locality). So far no exist name for the most important Chinese *Auricularia* species is available, and thus a new species, *Auricularia heimuer*, is described and illustrated. The new species is characterized by effused-reflexed or substipitate basidiomata with fawn to reddish brown color when fresh and vinaceous gray to dark gray when dry, pilose upper surface usually with a few folds when dry, short abhymenial hairs without branching and measured as 50–150 × 4–6.5 μm, usually presence of medulla, long clavate basidia with oil guttules and measured as 40–67 × 3.0–6.5 μm, and allantoid spores measured as 11–13 × 4–5 μm. *A. auricula-judae* is not found in China, and most probably has a distribution in Europe only. In addition, *A. americana* and *A. villosula* are the first time reported in China. Both *A. heimuer* and *A. villosula* grow on angiosperm wood, while *A. americana* is found on gymnosperm wood exclusively.

Key words: Auriculariales, ITS, nLSU, phylogeny, taxonomy

Introduction

Auricularia Bull. (Auriculariaceae, Auriculariales), typified by *Auricularia mesenterica* (Dickson 1785: 20) Persoon (1822: 97), is macroscopically characterized by gelatinous, resupinate to substipitate basidiomata, dark yellow to brown or reddish brown upper surface with distinguished hairs; pallid or rosy to dark brown or black lower surface with smooth, rugulose to merulioid, glabrous to pruinose configuration (Lowy 1951, Kobayasi 1981, Montoya-Alvarez *et al.* 2011). Microscopically, it has cylindrical to clavate and transversely three-septate basidia with oil guttules and slender sterigmata, and its basidiospores are hyaline, thin-walled and allantoid (Lowy 1952, Montoya-Alvarez *et al.* 2011).

Simpleness of distinct morphological features for the genus resulted in a number of names registered under *Auricularia*. For instance, MycoBank database (<http://www.mycobank.org>) has 175 specific or infraspecific records in *Auricularia*, while the number in Index Fungorum (<http://www.indexfungorum.org>) is 165. However, many records are synonyms or have been transferred to other genera. Till 2013, about 75 names are recognized to be legitimate in the genus worldwide (Wu *et al.* 2014).

Recently, molecular studies employing multi-gene datasets have helped to clarify the relationships among *Auricularia* and related fungi (Montoya-Alvarez *et al.* 2011, Looney *et al.* 2013). On the basis of the phylogeny inferred from the internal transcribed spacer sequences of nuclear ribosomal DNA (ITS), Montoya-Alvarez *et al.* (2011) demonstrated that five *Auricularia* species [*A. mesenterica*, *Auricularia delicata* (Montagne ex Fries in Schlechtendal 1830: 533) Hennings in Wilhelm-Engelmann (1893: 492), *Auricularia fuscossuccinea* (Montagne in Milne-Edwards 1842: 125) Hennings, *Auricularia auricula-judae* (Bulliard 1789: 427) Quélet (1886: 207) and

latter is lack of medulla in cross-section (Malysheva & Bulakh 2014) and has very short abhymenial hairs [(40–80(–90) × 4.5–6.0 μm measured in present study; 30–70 × 5.0–6.0 μm in Malysheva & Bulakh (2014)](Table 2). In the dry basidiomata, *A. heimuer* has vinaceous gray to dark gray lower surface, while it is pale brown to dark brown in *A. villosula*. In addition, *A. villosula* has a wider geographic distribution in China, occurring from boreal to tropic areas, while *A. heimuer* distributes in boreal and temperate areas only.

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References

- Boulet, B. (2003) *Les Champignons des Arbres de l'Est de l'Amérique du Nord*. Publications du Québec, Québec, 744 pp.
- Bulliard, P. (1789) *Herbier de la France; ou, Collection complète des plantes indigenes de ce royaume; avec leurs propriétés, et leurs usages en médecine, vol. 9*. Chez l'auteur, Didot, Debure and Belin, Paris, 427 pp.
- Chen, C.J., Oberwinkler, F. & Chen, Z.C. (2002) *Heterorepetobasidium*, a new genus in the Auriculariales. *Mycologia* 94: 515–522.
<http://dx.doi.org/10.2307/3761785>
- Dickson, J. (1785) *Fasciculus quartus plantarum cryptogamicarum Britanniae, vol. 1*. Nabu Press, London, 34 pp.
- Duncan, E.G. & Macdonald, J.A. (1967) Micro-Evolution in *Auricularia auricula*. *Mycologia* 59: 803–818.
<http://dx.doi.org/10.2307/3757193>
- Felsenstein, J. (1985) Confidence intervals on phylogenetics: an approach using bootstrap. *Evolution* 39: 783–791.
<http://dx.doi.org/10.2307/2408678>
- Fries, E.M. (1822) *Systema Mycologicum II*. Officina Berlingiana, Berlin, 81 pp.
- Hall, T.A. (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Huang, N.L., Lin, Z.B. & Chen, G.L. (2010) *The Chinese medicinal and edible fungi*. Science Press, Shanghai, 1834 pp.
- Kobayasi, Y. (1981) The genus *Auricularia*. *Bulletin of the National Science Museum, Tokyo* 7: 41–67.
- Li, C.T., Mao, X.X. & Xu, B.J. (2013) Pulsed electric field extraction enhanced anti-coagulant effect of fungal polysaccharide from Jew's Ear (*Auricularia auricula*). *Phytochemical analysis* 24: 36–40.
<http://dx.doi.org/10.1002/pca.2376>
- Li, L., Li, J., Zou, L., Bai, S.Y., Niu, L.M. & Ma, Y.K. (2007) RAPD analysis of genetic diversity of nine strains of *Auricularia auricula* cultivated in Heilongjiang Province. *Journal of Forestry Research* 18: 136–138.
<http://dx.doi.org/10.1007/s11676-007-0027-7>
- Li, L.J. (1984) Two new species of the genus *Auricularia* from China. *Acta Mycologica Sinica* 4: 149–154.
- Li, L.J. (1987) A study of the *Auricularia* from Hainan Island. *Journal of Wuhan Botanical Research* 5: 43–48.
- Li, L.J. & Liu, B. (1985) A new species of *Auricularia*. *Journal of Shanxi University* 1: 56–58.
- Looney, B., Birkebak, J. & Matheny, P.B. (2013) Systematics of the genus *Auricularia* with an emphasis on species from the southeastern United States. *North American Fungi* 8: 1–25.
<http://dx.doi.org/10.2509/naf2013.008.006>
- Lou, L.H., Zhu, H.Z., Tang, H.G. & Lou, R.J. (1992) A preliminary study of the various species of *Auricularia*. *Edible Fungi of China* 11: 30–32.
- Lowy, B. (1951) A morphological basis for classifying the species of *Auricularia*. *Mycologia* 43: 351–358.
<http://dx.doi.org/10.2307/3755598>
- Lowy, B. (1952) The genus *Auricularia*. *Mycologia* 44: 656–692.
- Malysheva, V.F. & Bulakh, E.M. (2014) Contribution to the study of the genus *Auricularia* (Auriculariales, Basidiomycota) in Russia. *Novosti Sistematiki Nizshikh Rastenii* 48: 164–180.
- Milne-Edwards, P.M. (1842) *Annales des sciences naturelles, vol. 17*. Paul Renouard, Paris, 392 pp.
- Montoya-Alvarez, A.F., Hayakawa, H., Minamya, Y. & Fukuda, T. (2011) Phylogenetic relationships and review of the species of

- Auricularia* (Fungi: Basidiomycetes) in Colombia. *Caldasia* 33: 55–66.
- Nylander, J.A.A. (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Parmasto, E. & Parmasto, I. (1987) Variation of basidiospores in the Hymenomycetes and its significance to their taxonomy. *Bibliotheca Mycologica* 115: 1–168.
- Persoon, C.H. (1822) *Mycologia europaea I*. I. Palmius, Erlangae, 358 pp.
- Petersen, J.H. (1996) Farvekort. *The Danish Mycological Society's colour-chart*. Foreningen til Svampekundskabens Fremme, Greve. 6 pp.
- Posada, D. & Crandall, K.A. (1998) Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
<http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Quélet, L. (1886) *Enchiridion Fungorum in Europa Media et praesertim in Gallia Vigentium*. Paris, 352 pp.
<http://dx.doi.org/10.5962/bhl.title.47025>
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
<http://dx.doi.org/10.1093/bioinformatics/btg180>
- Schlechtendal, D.F.L.von (1830) Ein Journal für die Botanik in ihrem ganzen Umfange. *Linnaea* 5: 533.
- Shi, Y.L., Xin, X.L., Zhang, C.Y. & Li, A.M. (2006) Effects of *Auricularia auricula* Underw polysaccharide on sport capacity of living organism. *Chinese Journal of Clinical Rehabilitation* 10: 106–108.
- Swofford, D.L. (2002) *PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10*. Sinauer Associates, Massachusetts.
- Teng, S.C. (1939) *High fungi of China*. National Institute of Zoological Botanical Academy, Beijing, 614 pp.
- Teng, S.C. (1963) *Fungi of China*. Science Press, Beijing, 808 pp.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.
<http://dx.doi.org/10.1093/nar/25.24.4876>
- Weiß, M. & Oberwinkler, F. (2001) Phylogenetic relationships in Auriculariales and related groups – hypotheses derived from nuclear ribosomal DNA sequences. *Mycological Research* 105: 103–415.
<http://dx.doi.org/10.1017/S095375620100363X>
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: *PCR Protocols: A guide to methods and applications*. Academic Press, New York, pp. 315–322.
<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wilhelm-Engelmann, V. von (1893) *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie, vol. 17*. Missouri Botanical Garden Press, Leipzig, 592 pp.
- Wu, F., Yuan, Y., Liu, H.G. & Dai, Y.C. (2014) *Auricularia* (Auriculariales, Basidiomycota): a review of recent research progress. *Mycosystema* 33: 198–207.
<http://dx.doi.org/10.13346/j.mycosystema.130282>
- Yang, X.M. (1988) *Chinese mushroom cultivation*. Agriculture Press, Beijing, 584 pp.
- Zeng, W.C., Zhang, Z., Gao, H., Jia, L.R. & Chen, W.Y. (2012) Characterization of antioxidant polysaccharides from *Auricularia auricula* using microwave-assisted extraction. *Carbohydrate polymers* 89: 694–700.
<http://dx.doi.org/10.1016/j.carbpol.2012.03.078>
- Zhang, G.Y., Zhao, L.Y., Liu, Y., Wang, B.G., Cao, L.K. & Sun, H.W. (2005) Study on nutritional value of *Auricularia auricula*. *Journal of Jilin University (Medicine Edition)* 31: 220–222.
- Zhang, R.Y., Hu, D.D., Gu, J.G., Hu, Q.X., Zuo, X.M. & Wang, H.X. (2012) Development of SSR markers for typing cultivars in the mushroom *Auricularia auricula-judae*. *Mycological Progress* 11: 587–592.
<http://dx.doi.org/10.1007/s11557-011-0798-2>
- Zhou, L.W. & Dai, Y.C. (2013) Phylogeny and taxonomy of poroid and lamellate genera in the Auriculariales (Basidiomycota). *Mycologia* 105: 1219–1230.
<http://dx.doi.org/10.3852/12-212>