



Suillus marginielevatus, a new species and *S. triacicularis*, a new record from Western Himalaya, Pakistan

SAMINA SARWAR^{1*}, MALKA SABA², ABDUL N. KHALID² & BRYN M. DENTINGER³

¹ Department of Botany, Lahore College for Women University, Lahore

² Department of Botany, University of the Punjab, Lahore, 54590, Pakistan

³ Mycology Section, Jodrell Labs, Royal Botanic Gardens Kew, Richmond Surrey, UK

Correspondence to *: S. Sarwar; SAMINA_BOLETUS@YAHOO.COM

Abstract

Suillus marginielevatus sp. nov. and *S. triacicularis* are reported from Himalayan moist temperate forests of Pakistan in association with conifers. Morphologically *S. marginielevatus* is close to *S. sibiricus*, *S. granulatus* and *S. intermedius* but it can be distinguished by uplifted pileus margin and curved stipe with no ring at all stages. Phylogenetic analysis of internal transcribed spacer (ITS) region showed that this species form separate clade from other closely related *Suillus* species. *Suillus triacicularis* represents a new record for Pakistani mycobiota. Field photographs of fresh basidocarps and line drawings of microcharacters are provided along with phylogenetic tree.

Keywords: conifers, morphology, phylogeny, subtropical

Introduction

Suillus Gray (1821: 646) is a large genus in the Boletales with about 170 known species characterized by fleshy, pored fruiting bodies with a slimy pileus, a stipe with glandular dots and sometimes a ring, wide pore openings and smooth spores (Bessette *et al.* 2000, Kuo 2004). *Suillus* spp. are important ectomycorrhizal associates of conifers in temperate and boreal regions (Singer 1986, Wu *et al.* 2000). Recent studies have shown that the species concepts in *Suillus* need to be re-examined (Kretzer *et al.* 1996, Kretzer & Bruns 1999). Molecular markers are a useful tool for species identification in combination with morphological analysis (Sen 1990, Zhou *et al.* 2001). Many scientists have done molecular and phylogenetic analysis of *Suillus* species and have discussed their genetic diversity and biogeographic pattern (Kretzer *et al.* 1996, Wu *et al.* 2000, Manian *et al.* 2001, Bruns *et al.* 2010). Until, twelve (12) species of *Suillus* have been reported from Himalayan moist temperate region of Pakistan (Ahmad 1962, Shibata 1992, Murakami 1993, Iqbal & Khalid 1996, Razaq 2007, Niazi 2008, Sarwar *et al.* 2011, Sultana *et al.* 2011, Sarwar & Khalid 2014). These forests extend along the whole length of the outer ranges of the Himalayas between the subtropical pine forests and the sub-alpine meadow vegetation (Hussain & Ilahi 1990).

Main scientific contribution of this article is description of a new species for science, *S. marginielevatus* and a new record from Pakistan, *S. triacicularis* B. Verma & M.S. Reddy (2014). The second contribution is assessment of phylogenetic relationship in genus *Suillus* based on internal transcribed spacer (ITS) region of ribosomal DNA sequence. This region has been sequenced from a broad range of samples belonging to the genus *Suillus* (Kretzer *et al.* 1996, Kretzer & Bruns 1997, Wu *et al.* 2000, Manian *et al.* 2001).

Materials and Methods

Basidiomes were collected during the rainy season (June–August, 2010) and macromorphological characteristics were recorded in the field. Specimens were studied by a light microscope with magnification up to (100X) in the laboratory. In the description of spore dimensions, the first value corresponds to the range of the lengths and widths, the values

References

- Ahmad, S. (1962) Further contributions to the fungi of Pakistan. II. *Biologia* 8: 123–150.
- Bessette, A., Roody, W.C. & Bessette, A.R. (2000) *North American Boletes: a color guide to the fleshy pored mushrooms*. Syracuse University Press, New York, 396 pp.
- Bruns, T.D., Grubisha, L.C., Trappe, J.M., Kerekes, J.F. & Vellinga, E.C. (2010) *Suillus quiescens*, a new species commonly found in the spore bank in California and Oregon. *Mycologia* 102: 438–446.
<http://dx.doi.org/10.3852/09-149>
- Dentinger, B.T.M., Margaritescu, S. & Moncalvo, J.M. (2010) Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematic of mushrooms. *Molecular Ecology Resources* 10: 628–633.
<http://dx.doi.org/10.1111/j.1755-0998.2009.02825.x>
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32 (5): 1792–1797.
<http://dx.doi.org/10.1093/nar/gkh340>
- Gray, S.F. (1821) *A natural arrangement of British plants. Vol. 1*. Baldwin, Cradock and Joy, London, 824 pp.
- Hussain, F. & Ilahi, I. (1990) *Ecology and vegetation of Lesser Himalayas, Pakistan*. Jadoon Printing Press, Peshawa, 185 pp.
- Iqbal, S.H. & Khalid, A.N. (1996) Material for the fungus flora of Pakistan. I. Check list of Agarics, their distribution and association with the surrounding vegetation. *Science International (Lahore)* 8: 51–64.
- Jackson A.B. (1938) *Pinus wallichiana*. In: *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew. Vol. 1938. No. 2*. H.M. Stationery office, London, pp. 85.
- Jukes, T.H. & Cantor, C.R. (1969) Evolution of protein molecules. In: Munro, H.N. (Ed.) *Mammalian protein metabolism*. Academic Press, New York, pp. 21–132.
<http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7>
- Kretzer, A. & Bruns, T.D. (1997) Molecular revisitation of the genus *Gastrosuillus*. *Mycologia* 89: 586–589.
<http://dx.doi.org/10.2307/3760995>
- Kretzer, A. & Bruns, T.D. (1999) Use of atp6 in fungal phylogenetics: An example from the Boletales. *Molecular Phylogenetics and Evolution* 13: 483–492.
<http://dx.doi.org/10.1006/mpev.1999.0680>
- Kretzer, A.M., Li, Y., Szaro, T. & Bruns, T.D. (1996) Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications. *Mycologia* 88: 776–785.
<http://dx.doi.org/10.2307/3760972>
- Krombholz, J.V. von (1846) *Naturgetreue Abbildungen und Beschreibungen der Schwämme*. 10: 1–28.
- Kuntze, O. (1898) *Revisio Genera Plantarum. 3 (2)*. Arthur Felix, Leipzig, 535 pp. [in German]
- Kuo, M. (2004) The genus *Suillus*. Available from: <http://www.mushroomexpert.com/suillus.html> (accessed 1 November 2004)
- Manian, S., Sreenivasaprasad, S., Bending, G.D. & Mills, P.R. (2001) Genetic diversity and interrelationships among common European *Suillus* species based on ribosomal DNA sequences. *FEMS Microbiology Letters* 204: 117–121.
<http://dx.doi.org/10.1111/j.1574-6968.2001.tb10873.x>
- Murakami, Y. (1993) *Larger fungi from Pakistan*. In: Nakaike, T. & Malik, S. (Eds.) *Cryptogamic Flora of Pakistan. Vol. 2*. Pakistan Science Foundation, Islamabad, pp. 105–147.
- Niazi, A.R. (2008) *Biodiversity of Ectomycorrhizas in Conifers from Himalayan Moist Temperate Forests of Pakistan*. PhD thesis, Department of Botany, University of Punjab, Lahore, Pakistan. [unkown pagination]
- Presl, J. (1846) Wšobecný rostl. *Praha* 2: 1917.
- Pritsch, K., Boyle, H., Munch, J.C. & Buscot, F. (1997) Characterization and identification of black alder ectomycorrhizas by PCR/RFLP analyses of the rDNA internal transcribed spacer (ITS). *New Phytologist* 137: 357–369.
<http://dx.doi.org/10.1046/j.1469-8137.1997.00806.x>
- Razaq, A. (2007) *Taxonomic Studies on Basidiomycota from Northern Areas of Pakistan*. PhD thesis, University of Karachi, Karachi, 318 pp.
- Richter, D.L. & Bruhn, J.N. (1993) Mycorrhizal fungus colonization of *Pinus resinosa* A it. transplanted on northern hardwood clearcuts. *Soil Biology and Biochemistry* 25: 355–369.
[http://dx.doi.org/10.1016/0038-0717\(93\)90135-X](http://dx.doi.org/10.1016/0038-0717(93)90135-X)
- Roussel, H.F.A. (1796) *Flore du Calvados et terrains adjacents, composée suivant la méthode de Jussieu*. L.-J. Poisson, Caen, 268 pp.
- Santana, B.O., Lodge, D.J., Baroni, T.J. & Both, E.E. (2007) Boletes from Belize and the Dominican Republic. *Fungal Diversity* 27: 247–416.

- Sargent, C.S. (1897) *The Silva of North America* 11: 9.
- Sarwar, S. & Khalid, A.N. (2014) Diversity and phylogeny of genus *Suillus* (*Suillaceae*, *Boletales*) from coniferous forests of Pakistan (Asia). *International Journal of Agriculture & Biology* 16: 489–497.
- Sarwar, S., Hanif, M., Khalid, A.N. & Guinberteau, J. (2011) Diversity of Boletes in Pakistan, focus on *Suillus brevipes* and *Suillus sibiricus*. In: Savoie, J.M., Foulongne, M.O., Largeteau, M. & Barroso, G. (Eds) *Proceedings of the Seventh International Conference on Mushroom Biology and Mushroom Products. Vol. 1*. INRA, France, pp. 123–133. Available from: http://www.wsbmp.org/Previous_Conference_7.html. (accessed 7 October 2011)
- Sen, R. (1990) Intraspecific variation in 2 species of *Suillus* from Scots pine (*Pinus sylvestris* L.) forests based on somatic incompatibility and isozyme analysis. *New Phytologist* 114: 607–616.
<http://dx.doi.org/10.1111/j.1469-8137.1990.tb00431.x>
- Shibata, H. (1992) Higher Basidiomycetes from Pakistan. In: Nakaïke, T. & Malik, S. (Eds.) *Cryptogamic Flora of Pakistan. Vol. 1*. Pakistan Science Foundation, Islamabad, pp. 145.
- Singer, R. (1945) The Boletineae of Florida with notes on extralimital species. II. The Boletaceae (Gyroporoideae). *Farlowia* 2: 223–303.
- Singer, R. (1973) Notes on bolete taxonomy. *Persoonia* 7 (2): 313–320.
- Singer, R. (1986) *The Agaricales in modern taxonomy. 4th Edition*. Koeltz Scientific Books, Koenigstein, 981 pp.
- Snell, W.H., Dick, E.A. (1956) Notes on boletes: IX. *Mycologia* 48: 302–310.
<http://dx.doi.org/10.2307/3755478>
- Sultana, K., Rauf, C.A., Riaz, A., Naz, F., Irshad, G. & Hauque, M.I. (2011) Checklist of agarics of Kaghan valley. *Pakistan Journal of Botany* 43: 1777–1787.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 1731–1739.
<http://dx.doi.org/10.1093/molbev/msr121>
- Thiers, H.D. (1975) *California Mushrooms. A field guide to the boletes*. Hafner Press, New York, 261 pp.
- Verma, B. & Reddy, M.S. (2014) *Suillus triacicularis* sp. nov., a new species associated with *Pinus roxburghii* from northwestern Himalayas, India. *Phytotaxa* 162 (3): 157–164.
<http://dx.doi.org/10.11646/phytotaxa.162.3.4>
- White, T.J., Bruns, T.D., Lee, S. & Taylor, J. (1990) Amplification and Direct sequencing of fungal ribosomal RNA genes for Phylogenetics. *PCR Protocol, a Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.
- Wu, Q.X., Mueller, G.M., Lutzoni, F.M., Huang, Y.Q. & Guo, S.Y. (2000) Phylogenetic and biogeographic relationships of eastern Asian and eastern North American disjunct *Suillus* species (Fungi) as inferred from nuclear ribosomal RNA ITS sequences. *Molecular Phylogeny and Evolution* 17: 37–47.
<http://dx.doi.org/10.1006/mpev.2000.0812>
- Zhou, Z.H., Miwa, M. & Hogetsu, T. (2001) Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal *Suillus grevillei* populations. *New Phytologist* 149: 339–348.
<http://dx.doi.org/10.1046/j.1469-8137.2001.00029.x>