



On the taxonomic identity of a fungal morph used in traditional medicine in Kerala State, India

K. P. DEEPNA LATHA¹, SHEEBA VELUTHOOR² & PATINJAREVEETIL MANIMOHAN^{1*}

¹Department of Botany, University of Calicut, Kerala, 673 635, India

²Corevalleys Herbal Technologies, Calicut, Kerala, 673 027, India

*Corresponding author: pmanimohan@gmail.com

Abstract

A hypogean fungus, referred to in the local Malayalam language as nilamanga (meaning hypogean mango) is often used by traditional healers and tribal people in Kerala State, India as a cure for an assortment of ailments. Taxonomic identity of this fungal morph has long intrigued mycologists starting from M. J. Berkeley who provisionally named it as *Sclerotium stipitatum* Berk. & Curr. in 1860. Its unique morphology and total lack of spores of any kind defied proper identification. Morphological examinations revealed that a nilamanga specimen that we obtained recently from Kerala was indistinguishable from Berkeley's *Sclerotium stipitatum* currently preserved at Kew herbarium. Molecular phylogenetic methods unequivocally proved that the nilamanga specimen was *Xylaria acuminatilongissima*, a termite associated species first reported from Taiwan. The hypogean origin of nilamanga specimens indicate that they could very well be growing on abandoned subterranean termite nests. The sterile structure can be considered as a morphologically variable, multihyphal aggregated sclerotial stage of the fungus that can remain dormant or quiescent when the environment is unfavourable.

Key words: medicinal mushroom, *Xylaria*, traditional medicine, nilamanga, *Sclerotium stipitatum*

Introduction

A very interesting hypogean fungus, referred to in the local Malayalam language as nilamanga (meaning hypogean mango) and in Tamil as puttumanga (meaning mango of the termitorium) is often used by traditional healers and tribal people in Kerala State, India as a cure for an assortment of ailments especially stomach ailments (Shortt 1867, Balakrishnan & Kumar 2001). It is obtained by chance while digging soil for some other purposes and remarkably, is often obtained from the foundations of old houses when they are dismantled and this feature further added to the mystery surrounding this fungus. The identity of this remarkable medicinal fungus has long intrigued mycologists starting from Berkeley (1860) who examined the material transmitted to him from the former Travancore (southern Kerala) and provisionally named it as *Sclerotium stipitatum* Berk. & Curr. (1860: 93). Currey & Hanbury (1860) and Cooke (1888) supported Berkeley's view that it could be sclerotial stage of some fungus. Dennis (1961) and Rogers *et al.* (2005) indicated that it could be a *Xylaria*. The reason for the inability to identify its exact taxonomic position was the total lack of spores of any kind. Moreover, the sterile morph has a unique morphology quite unlike that of most commonly encountered fungi. Nilamanga specimens are often almost spherical and of the size of a table tennis ball with a smooth blackish rind and a whitish, somewhat hard, homogeneous interior. Owing to this morphology and the hypogean occurrence, they often recall some tuberous roots.

Recently, we obtained specimens of this material from a local healer and also Berkeley's (1860) material (*Sclerotium stipitatum*, holotype: K(M) 125991) on loan from Kew Herbarium. The results of our studies on these materials are presented here.

phylogenetic methods unequivocally prove that the recently collected nilamanga specimen is *X. acuminatilongissima* (Xylariaceae, Xylariales, Ascomycota, Fungi), a termite associated species.

Acknowledgments

We are thankful to Dr Firdous Iqbal of Changampally Vaidya Bhavan, Tirunavaya, Kerala for providing specimens of nilamanga for this study. We are also thankful to the staff of Kew (Mycology) Herbarium for arranging loan of holotype of *Sclerotium stipitatum*. KPDL acknowledges support from the Kerala State Council for Science, Technology and Environment (KSCSTE) in the form of a PhD fellowship (Grant No. 001/FSHP/2011/CSTE).

References

- Balakrishnan, V. & Kumar, N.A. (2001) 'Nilamanga' (*Sclerotium stipitatum*?) - A rare termite fungal sclerotia with medicinal properties known among the tribal and rural communities of Kerala. *Ethnobotany* 13: 9–14.
- Batra, L.R. & Batra, S.W.T. (1979) Termite-fungus mutualism. In: Batra, L.R. (Ed.) *Insect-fungus symbiosis: nutrition, mutualism and commensalism*. Allanheld, Osmun, New York, pp. 117–163.
- Berkeley, M.J. (1843) Notices of fungi in the Herbarium of the British Museum. *Annals and Magazine of Natural History* 10: 369–384. <http://dx.doi.org/10.1080/03745484309445244>
- Berkeley, M.J. (1860) On two tuberiform vegetable productions from Travancore. *Transactions of the Linnean Society of London* 23: 91–92. <http://dx.doi.org/10.1111/j.1096-3642.1860.tb00121.x>
- Cooke, M.C. (1883) On *Xylaria* and its allies. *Grevillea* 11(59): 81–94.
- Cooke, M.C. (1888) *Fungi; their nature, influence, and uses*. D. Appleton and Co., New York, 207 pp. <http://dx.doi.org/10.5962/bhl.title.55880>
- Currey, F. & Hanbury, D. (1860) Remarks on *Sclerotium stipitatum*, Berk. et Curr., *Pachyma cocos*, Fries, and some similar productions. *Transactions of the Linnean Society of London* 23: 93–97. <http://dx.doi.org/10.1111/j.1096-3642.1860.tb00122.x>
- Dennis, R.W.G. (1961) Xylarioideae and Thamnomycetoideae of Congo. *Bulletin du Jardin Botanique de l'État à Bruxelles* 31: 109–154. <http://dx.doi.org/10.2307/3667330>
- De Notaris, G. (1853) Micromicetes italici novi vel minus cogniti, decas 6-8. *Memorie della Reale accademia delle scienze di Torino* 13: 95–126.
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1791–1797. <http://dx.doi.org/10.1093/nar/gkh340>
- Fries, E.M. (1851) Novae symbolae mycologicae, in peregrinis terris a botanicis danicis collectae. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* 1: 17–136.
- Heim, R. (1977) *Termites et champignons-Les champignons termitophiles d'Afrique Noire et d'Asie meridionale*. Société Nouvelle des Éditions Boubée, Paris. 207 pp.
- Hsieh, H.-M., Ju, Y.-M. & Rogers, J.D. (2005) Molecular phylogeny of *Hypoxyylon* and closely related genera. *Mycologia* 97: 914–923. <http://dx.doi.org/10.3852/mycologia.97.4.844>
- Hsieh, H.M., Lin, C.R., Fang, M.J., Rogers, J.D., Fournier, J., Lechat, C. & Ju, Y.-M. (2010) Phylogenetic status of *Xylaria* subgenus *Pseudoxyllaria* among taxa of the subfamily Xylarioideae (Xylariaceae) and phylogeny of the taxa involved in the subfamily. *Molecular Phylogenetics and Evolution* 54: 957–969. <http://dx.doi.org/10.1016/j.ympev.2009.12.015>
- Izumitsu, K., Hatoh, K., Sumita, T., Kitade, Y., Morita, A., Gafur, A., Ohta, A., Kawai, M., Yamanaka, T., Neda, H., Ota, Y. & Tanaka, C. (2012) Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience* 53: 396–401. <http://dx.doi.org/10.1007/S10267-012-0182-3>
- Ju, Y.-M. & Hsieh, H.-M. (2007) *Xylaria* species associated with nests of *Odontotermes formosanus* in Taiwan. *Mycologia*. 99(6): 936–957. <http://dx.doi.org/10.3852/mycologia.99.6.936>
- Ju, Y.-M., Rogers, J.D., San Martín, F. & Granmo, A. (1998) The genus *Biscogniauxia*. *Mycotaxon* 66: 1–98.

- Klotzsch, J.F. (1832) Mycologische Berichtigungen. *Linnaea* 7: 193–204.
- Ko, H.J., Song, A., Lai, M.N. & Ng, L.T. (2011) Immunomodulatory properties of *Xylaria nigripes* in peritoneal macrophage cells of Balb/c mice. *Journal of Ethnopharmacology* 138: 762–768.
<http://dx.doi.org/10.1016/j.jep.2011.10.022>
- Kuntze, O. (1891) *Revisio generum plantarum*, A. Felix, Leipzig, 636 pp.
- Lee, J.S., Ko, K.S. & Jung, H.S. (2000) Phylogenetic analysis of *Xylaria* based on nuclear ribosomal ITS1-5.8S-ITS2 sequences. *FEMS Microbiology Letters* 187: 89–93.
[http://dx.doi.org/10.1016/S0378-1097\(00\)00181-6](http://dx.doi.org/10.1016/S0378-1097(00)00181-6)
- Li, S.F. & Wen, H.A. (2008) Antioxidant activities of bioactive components from *Xylaria gracillima* in submerged culture. *Mycosystema* 27(4): 39–47.
- Lu, X.L., Ma, Z.Z. & Ding, R.R. (1993) The modulating effects of Se or *Xylaria nigripes* polysaccharide on the activity of LICC. *Immunological Journal* 9(1): 13–16. [in Chinese]
- Ma, Z.Z., Zhang, S.Y., Chen, W.R., Li, D.L. & Ding, R.R. (1989) The effects of *Xylaria nigripes* polysaccharide on the activity of macrophage in mice. *Immunological Journal* 5(2): 13–16. [in Chinese]
- Peršoh, D., Melcher, M., Graf, K., Fournier, J., Stadler, M. & Rambold, G. (2009) Molecular and morphological evidence for the delimitation of *Xylaria hypoxylon*. *Mycologia* 101: 256–268.
<http://dx.doi.org/10.3852/08-108>
- Petch, T. (1906) The fungi of certain termites nests. *Annals of the Royal Botanic Gardens of Peradeniya* 3: 4–67.
- Petch, T. (1913) Termite fungi: a résumé. *Annals of the Royal Botanic Gardens of Peradeniya* 5: 303–341.
- Rogers, J.D. & Samuels, G.J. (1986) Ascomycetes of New Zealand 8. *Xylaria*. *New Zealand Journal of Botany* 24: 615–650.
<http://dx.doi.org/10.1080/0028825X.1986.10409947>
- Rogers, J.D., Ju, Y.-M. & Lehmann, J. (2005) Some *Xylaria* species on termite nests. *Mycologia* 97: 914–923.
<http://dx.doi.org/10.3852/mycologia.97.4.914>
- Sands, W.A. (1969) The association of termites and fungi. In: Krishna, K. & Weesner, F.M. (Eds.) *Biology of termites*. Vol. I. Academic Press, New York, pp. 495–524.
<http://dx.doi.org/10.1016/B978-0-12-395529-6.50020-9>
- Shortt, J. (1867) An account of *Sclerotium stipitatum* (Berk. et. Curr.) of Southern India. *The Journal of the Linnean Society (Botany)* 9: 417–419.
<http://dx.doi.org/10.1111/j.1095-8339.1867.tb01305.x>
- Song, A., Ko, H.J., Lai, M.N. & Ng, L.T. (2011) Protective effects of Wu-Ling-Shen (*Xylaria nigripes*) on carbon tetrachloride-induced hepatotoxicity in mice. *Immunopharmacology and Immunotoxicology* 33: 454–460.
<http://dx.doi.org/10.3109/08923973.2010.534100>
- Stadler, M., Hawksworth, D.L. & Fournier, J. (2014) The application of the name *Xylaria hypoxylon*, based on *Clavaria hypoxylon* of Linnaeus *. *IMA Fungus* 5: 57–66.
<http://dx.doi.org/10.5598/imafungus.2014.05.01.07>
- 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: 2731–2739.
<http://dx.doi.org/10.1093/molbev/msr121>
- Visser, A.A., Ros, V.I.D., De Beer, Z.W., Debets, A.J.M., Hartog, E., Kuyper, T.W., Læssøe, T., Slippers, B. & Aanen, D.K. (2009) Levels of specificity of *Xylaria* species associated with fungus-growing termites: a phylogenetic approach. *Molecular Ecology* 18: 553–567.
<http://dx.doi.org/10.1111/j.1365-294X.2008.04036.x>
- White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR Protocols: a guide to methods and applications*. Academic Press, Inc, San Diego, California, pp. 315–322.
<http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wood, T.G. & Thomas, R.J. (1989) The mutualistic association between Macrotermitinae and *Termitomyces*. In: Wilding, N., Collins, N.M., Hammond, P.M. & Webber, J.F. (Eds.) *Insect-fungus interactions*. Academic Press, London, pp. 69–92.
<http://dx.doi.org/10.1016/B978-0-12-751800-8.50009-4>
- Wu, G.F. (2001) A study on DPPH free-radical scavengers from *Xylaria nigripes*. *Acta Microbiologica Sinica* 41(3): 363–366. [in Chinese]
- Zhao, Z., Li, Y., Chen, H., Huang, L., Zhao, F., Yu, Q., Xiang, Z. & Zhao, Z. (2014) *Xylaria nigripes* mitigates spatial memory impairment induced by rapid eye movement sleep deprivation. *International Journal of Clinical and Experimental Medicine* 7: 356–362.