MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF BRACKET MACRO FUNGI IN BRGY. PUGARO, BALUNGAO, PANGASINAN

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ACCEPTANCE SHEET

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ABSTRACT

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Bracket macrofungi are non-timber forest inhabitants that play crucial ecological roles and perform enormous economic advantages for mankind. Hence, the study characterized the bracket macrofungi by both morphological and molecular approaches using both internal transcribed spacer (ITS) sequences and large subunit LSU rDNAs primers. The genomic DNA was extracted using cethyl-trimethyl ammonium bromide (CTAB) method and diluted 1:100 by means of sterilized distilled water and quantified using Multiskan FC Microplate Photometer.

BLAST analysis of the nucleotide sequences of the six bracket macrofungi revealed that *Gyrodontium sacchari* with 99%, *Xylaris papulis* with 100%, *Stereopsis radicans* with 91%, *Polyporus* sp. with 99%, *Phlebia* sp. with 93%, and *Ganoderma lucidum* with 100% pair wise identity with samples deposited in Genbank (NCBI). Six of the collected bracket macrofungi were identified up to species level.

Keywords: bracket macrofungi, DNA, rDNA-ITS, rDNA-LSU, BLAST

LITERATURE CITED

Avin, F. A., Bhassu, S., & Sabaratnam, V. (2013). A simple and low-cost technique of DNA extraction from edible mushrooms examined by molecular phylogenetics. *Research on Crops*, *14*(3), 897-90.

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P., & Kauserud, H. (2010). ITS as an environmental DNA barcode for fungi: an in-silico approach reveals potential PCR biases. *BioMed Central Microbiology, 10*, 189. Retrieved from https://doi.org/10.1186/1471-2180-10-189

Boa, E. (2004). Wild edible fungi: A global overview of their use and importance to people. In M. Iqbal (Ed.), *Trade restrictions affecting international trade in non-wood forest products* (2nd ed., pp. 41-49). Rome: Italy: FAO.

Case, R. J., Boucher, Y., Dahllof, I., Holmstrom, C., Doolittle, W. F., & Kjelleberg, S. (2007). Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. *Applied and Environmental Microbiology*, *73*(1), 278-288. Retrieved from https://doi:10.1128/AEM.01177-06

Das, K., & Aminuzzaman, F. M. (2017). Morphological and ecological characterization of xylotrophic fungi in mangrove forest regions of Bangladesh. *Journal of Advances in Biology and Biotechnology, 11*(4), 1-15. Retrieved from https://doi: 10.9734/JABB/2017/30971

Das, S. K., Mandal, A., Datta, A. K., Gupta, S., Paul, R. Saha, A., ... Dubey, P. K. (2013). Nucleotide sequencing and identification of some wild mushrooms. *The Scientific World Journal,* 1-7. Retrieved from http://dx.doi.org/10.1155/2013/40319

De Castro, M. E. G., & Dulay, R. M. (2015). Macrofungi in multistorey agroforestry systems in Mt. Makiling forest reserve, Los Baños, Laguna, Philippines. *Journal of Chemical, Biological and Physical Sciences, 5*(2), 1646-1655.

De Leon, A. M., Kalaw, S. P., Dulay, R. M., Undan, J. R., Alfonso, D., Undan, J. Q. & Reyes, R. G. (2016). Ethnomycological survey of the Kalanguya indigenous community in Caranglan, Nueva Ecija, Philippines. *Current Research in Environmental & Applied Mycology, 6*(2), 61-66. Retrieved from https://doi10.5943/cream/6/2/1

De Leon, A. M., Luangsa-ard, J. J. D., Karunaratham, S. C., Hyde, K. D., Reyes, R. G. & Dela Cruz, T. E. (2013). Species listing, distribution, and molecular identification of macrofungi in six Aeta tribal communities in Central Luzon, Philippines. *Mycosphere, 4*(3), 478-494. Retrieved from https://doi10.5943/mycosphere/3/2/9

Dela Cruz, T. E. E., Dagamac, N. H. A., Torres, J. M. O., Santiago, K. A. A., & Yulo, P. R. J. (2013). Status review of microbiology researches in the Philippines 2000-2012. *Mycology*, 61-68

Dijk, H. V., Onguene, N. A., & Kuyper, T. W. (2003). Knowledge and utilization of edible mushrooms by local populations of the rain forest of South Cameroon. *Ambio, 32(1),* 19-23

Franca, L. T. C., Carrilho, E., & Kist, T. B. L. (2002). A review of DNA sequencing techniques. *Quarterly Reviews of Biophysics, 35*(2), 169–200. Retrieved from https://doi.org/10.1017/S0033583502003797

Geng, Y., Zhu, S., L. Zhenming, L., Xu, H., Shi, J. S., & Xu, Z. H. (2014). Anti-inflammatory activity of mycelial extracts from medicinal mushrooms. *International Journal of Medicinal Mushrooms, 16*(4), 319-325. Retrieved from https://doi.10.1615/IntJMedMushroom

Hoa, N. X., Trang, L. T. H., Hieu, H. T., Loan, L. T. T., Ngan, L. T. M., & Ho, P. T. (2017). Isolation and study on pure culture of wild edible mushrooms collected from provinces in the southeast region of Vietnam. *Journal of Science and Technology, 55*(1), 122-133. Retrieved from https://doi.10.15625/2525-2518/55/1A/12389

Hyde, K. D., Abd-Elsalam, K., & Cai, L. (2010). Morphology: Still essential in a molecular world. *Mycotaxon*, 114, 439-451. Retrieved from https://doi: 10.5248/114439

Ibrahim, R. I. H. (2011). A modified CTAB protocol for DNA extraction from young flower petals of some medicinal plant species. *Gene conserve, 10*(40), 165-182

Kapoor, P., & Sharma, B. M. (2014). Studies on different growth parameters of Ganoderma lucidum. *International Journal of Science, Environment and Technology, 3*(4), 1515-1524

Kress, W. J., & Erickson, D. L. (2008). DNA barcodes: Genes, genomics, and bioinformatics. Paper presented at the Department of Botany, National Museum of Natural History, Smithsonian Institution: Washington, DC. *Vol. 105,* 2761–2762. Retrieved from https://doi.org/10.1073/pnas.0800476105

Lazo, C. R. M., Kalaw, S. P., & De Leon, A. M. (2015). Ethnomycological survey of macrofungi utilized by Gaddang communities in Nueva Vizcaya, Philippines. *Current Research in Environmental & Applied Mycology, 5*(3), 256-262. Retrieved from https:// doi10.5943/cream/5/3/8

Lee, S. V., & Bahaman, A. R. (2012). Discriminatory Power of Agarose Gel Electrophoresis in DNA Fragments Analysis. In Magdeldin, S. (Ed.), *Handbook of Gel Electrophoresis-Principles and Basics* (4th ed., pp. 41-57). Putra University: Malaysia.

Lee, Y., Han, S. S., & Jeong, I. S. (2002). Taxonomical characteristics of Xylaria spp. collected from Malaysia. *Mycobiology, 30*(4), 193–196.

Lindeqiust, I. L., Niedermayer, T. H., & Julich W. D. (2005). The pharmacological potential of mushroom. *Evidence-based Complementary and Alternative Medicine, 2*(3), 285-299. Retrieved from https://doi: 10.1093/ecam/neh107

Lopez, A. B., Aquino, J. D., Undan, J. Q., Waing, K. G., & Undan, J. R. (2016). Molecular identification and phylogeny of some wild microscopic fungi from selected areas of Jaen, Nueva Ecija, Philippines. *Advances in Environmental Biology, 10*(12), 153-158.

Novotny, V., Drozd, P., Miller, S. E., Kulfan, M., Janda, M., Basset, Y., & Weiblen, G. D. (2006). Why are there so many species of herbivorous insects in tropical rainforests? *Science, 313*, 1115-1118. Retrieved from https://doi10.1126/science.1129237

Nuñez M., & Ryvarden, L. (1995). Polyporus (Basidiomycotina) and related genera. Oslo, Norway: *Synopsis Fungorum, 10*, 1–85.

Okhouya, J. A., Akpaja, E. O., Osemwegie, O.O., Oghenekaro, A. O., & Ihayere, C. A. (2010). Nigeria Mushrooms: Underutilized non-wood forest resources. *Journal of Applied Science and Environmental Management, 14*(1), 43-54.

Porter, T. M. & Golding, G. B. (2011). Are similarity- or phylogeny-based methods more appropriate for classifying internal transcribed spacer (ITS) metagenomic amplicons? *The New Phytologist, 192*(3), 775-782. Retrieved from https://doi:10.1111/j.1469-8137.2011.03838.x

Powledge, T. M. (2004). The polymerase chain reaction. *Advances in Physiology Education, 28*, 44-50. Retrieved from https://doi: 10.1152/advan.00002.2004

Priyamvada, H., Akila, M., Singh, R. K., Ravikrishna, R., Verma, R. S., Philip, L., … Gunthe, S. S. (2017). Terrestrial macrofungal diversity from the tropical dry evergreen biome of Southern India and Its potential role in aerobiology. *PLoS One, 12*(1). Retrieved from https://doi.org/10.1371/journal.pone.0169333

Rai, M., Tidke, G., & Wasser, S. P. (2005). Therapeutic potiential of mushrooms. *International Centre for Cryptogamic Plants and Fungi, 4*(4), 246-257.

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: A primer for the natural products research community. *Journal of Natural Products, 80*, 756–770. Retrieved from https://doi: 10.1021/acs.jnatprod.6b01085

Rakeman, J. L., Bui, U., Lafe, K., Chen, Y. C., Honeycutt, R. J., & Cookson, B. T. (2005). Multilocus DNA sequence comparisons rapidly identify pathogenic molds. *Journal of clinical microbiology, 43*(7), 3324-33. Retrieved from https://doi:10.1128/JCM.43.7.3324-3333.2005

Rhoades, J. (2018). Tree Bracket Fungus – Learn About Prevention and Removal of Bracket Fungus. Retrieved from https://www.gardeningknowhow.com/ornamental/fungus-lichen/tree-bracket-fungus.html

Roberts, P., & Evans, S. (2013). *The Book of Fungi*, Lewes, United Kingdom: Ivy Press.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, V. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. In Janzen, D. H (Ed.), *Fungal Barcoding Consortium*: Vol. 109. (pp. 6241-6246). Philadelphia, PA: University of Pennsylvania Press.

Shujiang, L., Tianhui, Zhu., Guanghai, L., & Hanmingyue, Z. (2012). Diversity of macrofungal community in Bifeng Gorge: The core giant panda habitat in China. *African Journal of Biotechnology, 11*(8), 1970-1976. Retrieved from https://doi: 10.5897/AJB11.3232

Sibounnavong, P., Cynthia, C. D., Kalaw, S. P., Reyes, R. G., & Soytong, K. (2008). Some species of macrofungi at Puncan, Carranglan, Nueva Ecija in the Philippines. *Journal of Agricultural Technology, 4*(2), 105-115.

Sjokvist, E., Larsson, E., Eberhardt, U., Ryvarden, L., & Larsson, K. H. (2012). Stipitate stereoid basidiocarps have evolved multiple times. *Mycologia, 104*(5), 1046-1055. Retrieved from https://doi:10.3852/11-174

Sotome, K., Akagi, Y., Lee, S. S., Ishikawa, N. K., & Hattori, T. (2013). Taxonomic study of *Favolus* and *Neofavolus* gen. nov. segregated from *Polyporus* (Basidiomycota, Polyporales). *Fungal Diversity, 58*, 245–266. Retrieved from https://doi10.1007/s13225-012-0213-6

Strobel, G. A. (2002). Microbial gifts from rainforests. *Canadian Journal Plant Pathology, 24*(1), 14-20. Retrieved from https://doi.org/10.1080/07060660109506965

Tripathy, S. K., Maharana, M., Ithape, D. M., Lenka, D., Mishra, D., ... Raj, K. R. R. (2017). Exploring rapid and effiecient protocol for isolation of fungal DNA. *International Journal of Current Microbiology and Applied Sciences, 6*(3), 951-960. Retrieved from https://doi.org/10.20546/ijcmas.2017.603.113

Ukwuru, M. U., Muritala, A., & Eze, L. U. (2018). Edible and Non-Edible Wild Mushrooms: Nutrition, Toxicity and Strategies for Recognition. *Journal of Clinical Nutrition and Metabolism, 2*(2).

Undan, J. Q., Alfonso, D. O., Dulay, R. M., De Leon, A. M., Kalaw, S. P., et al. (2016). Molecular identification and phylogeny of different macrofungi in Mt. Bangkay, Cuyapo, Nueva Ecija, Philippines based on ITS nrDNA region. *Advances in Environmental Biology, 10*(12), 35-42.

Valenzuela, R., Raymundo, T., Decock, C., & Esqueda, M. (2012). Aphyllophoroid fungi from Sonora, Mexico 2: New records from Sierra de Alamos–Rio Cuchujaqui Biosphere Reserve. *Mycotaxon, 122*, 51-59. Retrieved from https://dx.doi.org/10.5248/122.51

Ware, M. (2017). What is the nutritional value of mushrooms? Medical News Today. Retrieved February 23, 2017, from https://www.medicalnews today.com/articles/278858.php

Yanxin, H. E., Wangbao, W. U., & Nengshu, L. I. (2016). A checklist of macrofungi in Yaoluoping Nature Reserve, Anhui. *Journal of Resources and Ecology, 7*(2), 144-150. Retrieved from https://doi:10.5814/j.issn.1674-764x

Zain, M. E., Razak, A. A., El-Sheikh, H. H., Soliman, H. G., & Khalil, A. M. (2009). Influence of growth medium on diagnostic characters of Aspergillus and Penicillium species. *African Journal of Microbiology Research, 3*(5), 280-286.

Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Bioactivities and health benefits of mushrooms mainly from China. *Molecules, 21*(7), (2-16). Retrieved from https://doi:10.3390/molecules21070938