**Xylaria sp. The Candle Snuff Fungus from West Java**

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**ABSTRACT**

Candle snuff fungus belongs to Xylaria group. Generally, Xylaria has a form like stick or candle or elongated fruit of shapes. Xylaria is classified into Ascomycota within Xylariaceae. This study found one species of candle-shaped mushroom in IPB University. This study aimed to identified and characterized the specimen using molecular and morphological data. The specimen was collected and preserved into FAA solution and deposited into Herbarium Bogoriense as BO 24426. Molecular analyses using Large Subunit as a region for amplification showed that the BO 24426 was classified into Xylaria sp. This species closes to Xylaria consociata. The stromata were erected, unbranched, and tapered to the apex. The texture was rigid and hard. Ascus bore 8 ascospores. The ascospores were fusiform or bean-shaped and smooth. The morphological observations supported molecular identification of BO 24426 as Xylaria sp. Other genes were needed to ensure the exact species of Xylaria.

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**Introduction**

Mushroom is a macro-fungus that belongs to Ascomycota and Basidiomycota (Brundrett et al., 1996). Some mushrooms show the unique characters as the unusual shapes. In Indonesia, many unique shapes of mushrooms are found as a specific name of genus, such as puffball for *Calvatia* (Hermawan & Putra, 2018, 2021), Cannonball for *Sphaerobolus* (Hermawan & Maulana, 2020), goblet for *Trichaleurina* (Hermawan, Amelya, et al., 2020), fan for *Telephora* (Hermawan, Imaningsih, et al., 2020), coral for *Ramaria* (Hermawan, Imaningsih, et al., 2020), trumpet for *Cantharellus* (Hermawan, Imaningsih, et al., 2020), microphone-like for *Lysurus* (Hermawan et al., 2021), and parasol for *Chlorophyllum* (Hermawan, Imaningsih, et al., 2020). Others unique mushroom is *Xylaria*.

The *Xylaria* is famous with name of the dead men’s finger or the candle snuff fungus. *Xylaria* contains 819 name records (Index Fungorum, 2021). *Xylaria* is classified into Xylariales within Pezizomycotina (Mycobank, 2021). The traits of this genus are known as saprobiic and also endophytic fungi. *Xylaria* as an endophyte lives inside the plant tissue without causing any disease symptoms for the plant (Petrini, 1991). A special trait of *Xylaria* also emerges from nest of termites (Rogers et al., 2005). This study found the *Xylria* stromata on the ground (without substrate). It indicates the possibility of the trait as symbiont with insect.

Traditional classification and identification of fungi has relied upon microscopic features, colony characteristics on media and also biochemical reactions (Sutton & Cundell, 2004). Sometimes the traditional method
cannot ensure the name of species, especially for Xylaria. The Xylaria are difficult to identify and classify using only the stromata characteristics (Whalley, 1996). Some researchers in Xylaria study using the molecular comprehensive for identifying the species (Ju et al., 2009; Ma et al., 2013; Okane et al., 2008). In Indonesia, study of Xylaria is usually conducted using only morphological character (Kristin et al., 2020). The molecular study is important to make sure the name of species and will be supported using morphology. The internal transcribed spacer (ITS) region is a good region to identify the fungi (Brandt et al., 2005; White et al., 1990). But, other region such as Large Subunit is also needed to make stronger analyses in fungi. For Xylariaceae, the sequences that popular to be analyzed are Large Subunit region (Okane et al., 2008), some ITS sequences are rarely available in GenBank. Therefore, this study used molecular study for Large Subunit sequences.

Materials and Methods

Mushrooms Sampling Site

The stromata were collected in August 2019 and located in the Arboretum of IPB University. The fungus was found as a Xylaria stroma based on the morphology. The stroma was collected, documented, and observed the morphological characters. The observation was conducted in the mycology laboratory of Biology Department, Mathematics and Natural Sciences, IPB University, Indonesia. The apothecium was preserved in FAA (Seshagirirao et al., 2016) and deposited into Herbarium Bogoriense Indonesia.

Morphological Observation

The morphological data of stromata were observed and documented to confirm the genus as Xylaria. The data would be used to support the molecular analyses. The observation was conducted using an Olympus stereo and binocular microscope cs22LED. The features of macro-and micro-morphology such as asci and ascospores. The observations were about

Molecular Identification

The stromata were identified using molecular analyses. The genomic DNA was extracted using the protocol as in (Hermawan, Amelya, et al., 2020). The genomic DNA quality and quantity were verified using a nanodrop spectrophotometer. The amplification was used Large Subunit (LSU) as forward LR0R (5’-GTA CCC GCT GAA CTT AAG C-3’) and reverse LR5 (5’-ATC CTG AGG GAA ACT TC-3’) primers. PCR amplification was performed in 40 µL total reaction containing 12 µL ddH2O, 2 µL of 10 pmol of each primer, 20 µL PCR mix from 2X Kappa Fast 2G, and 4 µL 100 ng template DNA. Amplification used a Thermoline PCR. The PCR condition was set as follows: initial denaturation at 94 ℃ for 2 minutes, followed by 30 cycles of denaturation at 94 ℃ for 45 seconds, annealing at 56 ℃ for 1 minute, and extension at 72 ℃ for 1 minute. Then final extension was set at 72 ℃ for 10 minutes. The amplicons were estimated on 1 % agarose gels and visualized by the Gel DocTM XR system. PCR products were sent to the 1st Base Malaysia for sequencing.

Phylogenetic Analyses

The sequence was deposited in GenBank. This sequence, Xylaria species in (Okane et al., 2008), some Xylaria species from GenBank, and Vamsapriya bambusicola (outgroup) were constructed into phylogenetic tree (Table 1). Sequences were aligned using Clustal X Ver. 2.1 software and saved as PHYLIP format files. All sequences were aligned using 600 base pairs of the ITS region. The phylogenetic tree of Randomized Axelerated Maximum Likelihood (RAxML) Black Box was generated on CIPRES (Stamatakis, 2014). Bootstrap analyses with 1000 replicates assessed the phylogenetic tree. Bootstrap (BS) ≥ 50 was shown on the branch.
Table 1. Collection code, species, GenBank accession numbers of Internal Transcribed Spacer used in this study.

| Species              | Collection code | GenBank acc. no |
|----------------------|-----------------|-----------------|
| Xylaria allantoidea  | BCC 1340        | AB376730        |
| Xylaria anisopleura  | BCC 17352       | AB376732        |
| Xylaria apiculata    | BCC 1136        | AB376700        |
| Xylaria arbuscula    | BCC 1156        | AB376703        |
| Xylaria arbuscula    | RHP 21          | MT215561        |
| Xylaria arbuscula    | CBS 126416      | MH875561        |
| Xylaria aristata     | BCC 1229        | AB376716        |
| Xylaria aristata     | BCC 1260        | AB376722        |
| Xylaria badia        | BCC 1171        | AB376705        |
| Xylaria badia        | BCC 1190        | AB376711        |
| Xylaria bambusicola  | BCC 22739       | AB376809        |
| Xylaria bambusicola  | BCC 23628       | AB376821        |
| Xylaria bambusicola  | MFLUCC 11-0606  | KU863148        |
| Xylaria bambusicola  | BCC 23627       | AB376820        |
| Xylaria coccophora   | BCC 1085        | AB376688        |
| Xylaria consociata   | BCC 18196       | AB376733        |
| Xylaria cubensis     | BCC 1321        | AB376729        |
| Xylaria cubensis     | BCC 1144        | AB376701        |
| Xylaria cubensis     | BCC 1303        | AB376725        |
| Xylaria cubensis     | BCC 11027       | AB376683        |
| Xylaria cubensis     | BCC 1219        | AB376715        |
| Xylaria curta        | BCC 1007        | AB376681        |
| Xylaria curta        | BCC 1151        | AB376702        |
| Xylaria enteroleuca  | CBS 128357      | MH876349        |
| Xylaria escharoidea  | BCC 23279       | AB376818        |
| Xylaria escharoidea  | BCC 23634       | AB376882        |
| Xylaria fejeensis    | BCC 1115        | AB376696        |
| Xylaria grammica     | IHI A82         | MK408621        |
| Xylaria grammica     | BCC 1002        | AB376679        |
| Xylaria grammica     | BCC 1170        | AB376704        |
| Xylaria grammica     | 5084            | JQ862608        |
| Xylaria hypoerythra  | BCC 22968       | AB376812        |
| Xylaria hypoxylon    | AFTOL-ID 51     | AY544648        |
| Xylaria hypoxylon    | CBS 126417      | MH875562        |
| Xylaria hypoxylon    | DM1153          | MT773340        |
| Xylaria hypoxylon    | DSM 108379      | MK577428        |
| Xylaria juruensis    | BCC 1232        | AB376717        |
| Xylaria juruensis    | BCC 1086        | AB376689        |
| Xylaria juruensis    | BCC 1083        | AB376687        |
| Xylaria juruensis    | BCC 1263        | AB376723        |
| Xylaria juruensis    | BCC 1234        | AB376719        |
| Xylaria juruensis    | BCC 1233        | AB376718        |
| Xylaria laevis       | BCC 1182        | AB376709        |
| Xylaria longipes     | 19GCAS018       | MW077324        |
| Xylaria longipes     | CBS 148.73      | MH872351        |
| Xylaria longipes     | CBS 347.37      | MH867427        |
| Xylaria longipes     | DSM 107183      | MK408619        |
### Table 1. Continue

| Species                  | Collection code | GenBank acc. no | LSU  |
|--------------------------|-----------------|-----------------|------|
| Xylaria mellissii        | BCC 1005        | AB376680        |      |
| Xylaria mellissii        | BCC 1186        | AB376710        |      |
| Xylaria multiplex        | BCC 1177        | AB376707        |      |
| Xylaria multiplex        | BCC 1036        | AB376684        |      |
| Xylaria obovata          | BCC 1053        | AB376685        |      |
| Xylaria obovata          | BCC 18718       | AB376737        |      |
| Xylaria obovata          | BCC 1100        | AB376690        |      |
| Xylaria papulis          | BCC 22966       | AB376811        |      |
| Xylaria phyllocharis     | BCC 1352        | AB376731        |      |
| Xylaria phyllocharis     | BCC 1065        | AB376686        |      |
| Xylaria piperiformis     | BCC 22987       | AB376814        |      |
| Xylaria polymorpha       | CBS 162.22      | MH866242        |      |
| Xylaria psidii           | BCC 1199        | AB376713        |      |
| Xylaria psidii           | BCC 1127        | AB376698        |      |
| Xylaria schweinitzii     | BCC 1013        | AB376682        |      |
| Xylaria schweinitzii     | BCC 1001        | AB376678        |      |
| Xylaria sicula           | CBS 401.58      | MH869355        |      |
| Xylaria sp.              | BO 24426        | MW433688        |      |
| Xylaria sp.              | BCC 1181        | AB376708        |      |
| Xylaria sp.              | BCC 1133        | AB376699        |      |
| Xylaria sp.              | BCC 1288        | AB376724        |      |
| Xylaria tuberoides       | BCC 18361       | AB376736        |      |
| Xylaria xylarioides      | CBS 127883      | MH876177        |      |
| Xylaria xylarioides      | CBS 128018      | MH877980        |      |
| Vamsapriya bambusicola   | MFLUCC 11-0577  | KM462836        |      |

### Results and Discussion

#### Specimen

Two stromata were found that grew on the ground without substrate (Figure 1a). Sexual morphology: Stromata look like greyish candle snuff with whitish powder covered stromata from upper (Figure 1a-b). The stromata were erected, unbranched, and 6.5-9.1 cm of height. The stromata were tapered to the apex of stromata. The texture was rigid and hard. Asci were appeared inside the stromata. Ascus contains 8 ascospores (Figure 1c). The ascospore has oil globular inside, 1 or 2 oil globules appeared in the corner of ascospore, fusiform or bean-shaped and smooth (free of ornament). The size was 12.2–13.2 x 5.0–5.9 µm (Figure 1d). Specimen examined: Landscape Arboretum of IPB University, BO 24426, Rudy Hermawan. GenBank Submission: LSU: MW433688.

*Xylaria* has the unique morphology as a candle, spoon and finger (Kirk et al., 2008). Then, Fournier et al. (2018) described the updated morphology of *Xylaria* species as in pulvinate to depressed-spherical shape. *Xylaria* BO 24426 has the morphology with candle snuff with whitish powder covering the stromata. The whitish powder does not contain spore or other sexual structure. The spore as ascospore is inside the stromata part.

Rogers and Samuels (1986) described *Xylaria* species has dark carbonaceous stromata and pigmented ascospores as dark color with a germ slit. *Xylaria* BO 24426 showed the dark ascospores. The ascospores was produced inside the ascus with 8 spores. Germ slit character is really hard to be observed. The observation should use the advanced microscope with the high magnification of lens. the observation of BO 24426 has not successful observed the germ slit characteristic.
The ascospore shape of BO 24426 has fusiform or bean-shaped. Other species of *Xylaria* have many shapes such as ellipsoid slightly inequilateral with broadly to narrowly rounded ends (as *X. berteroi*), ellipsoid-inequilateral to sub-oblong with broadly rounded ends (as *X. alboareolata*) etc. (Fournier et al., 2018). Based on our BO 24426, the ascospores also have the oil globules inside the ascospores.

**Molecular work**

The phylogenetic tree showed that the specimen BO 24426 was classified as *Xylaria* sp. with 41% bootstrap value (Figure 2) making other clades from *Xylaria consociata*. Okane et al. (2008) mentioned the *X. consociata* as a saprobic *Xylaria*. Whereas the *Xylaria* sp. BO 24426 is not saprobic *Xylaria*. According to Figure 2, BO 24426 has a different line length with the *Xylaria consociata*. This line was indicated that the evolutionary based on their sequences is different. It can be showed that *Xylaria* BO 24426 is not *Xylaria consociata*. In Okane et al. (2008) also mentioned some of *Xylaria* as *Xylaria* sp. The region of gene amplification for molecular identification within *Xylaria* species sometimes can’t show the strong identification. As a single gene or region in this study, BO 24426 only can be identifying until the Genus name.

Based on this phylogenetic tree of *Xylaria* BO 24426, the species can be assumed as a new species or unidentified. The BO 24426 is assumed as a new species because the BO 24426 makes other clade among other species. Or it can also be assumed as unidentified species. The other gene regions are needed to complete the differences among the species and make the perfect phylogenetic tree. This additional work will ensure the BO 24426 as a new species or not.

The other functional genes for identification are Internal Transcribed Spacer, Actin, Small Sub Unit, etc. Schoch et al. (2012) mentioned the ITS region is the most useful for identification and also as a general gene for identification among fungal species. The existence of ITS as a general gene in *Xylaria* is not really helpful to be found in NCBI or GenBank. Okane et al. (2008) had used the LSU as additional gene for making the phylogenetic tree among *Xylaria* species. The phylogenetic was enough clearly to distinguish the species among *Xylaria* species.

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**Figure 1. Morphology of *Xylaria* BO 24426 (A; B) Stromata on substrate; (C) ascus; (D) ascospores. Scale bars: (A) 10 cm; and (C; D) 10 µm.**
Figure 2. *Xylaria* BO 24426 phylogenetic tree based on the LR0R/LR5 region using RAxML. Bootstrap (BS)≥50 was shown on the branch. The *Xylaria* BO 24426 must be in bold.

Conclusion

*Xylaria* BO 24426 has been identified as *Xylaria* sp. with 41% BS value and closed to *X. consociata*. The stromata look like greyish candle snuff with whitish powder covering stromata. As a single gene identification using LSU region is not enough and strong to identify BO 24426 into a species among *Xylaria*. 

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Moreover, BO 24426 has potential as a new species, because the clade of them showed the branch length that separated with other species.

References
Brandt, B. W., Zwaan, B. J., Beekman, M., Westendorp, R. G. J., & Slagboom, P. E. (2005). Shutting Between Species for Pathways of Lifespan Regulation: A Central Role for the Vitellogenin Gene Family? *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 27(3), 339–346. https://doi.org/10.1002/bies.20161

Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (1996). *Working with Mycorrhizas in Forestry and Agriculture*. ACIAR Monograph 32.

Fournier, J., Lechat, C., & Courtecuisse, R. (2018). The Genus *Xylaria* sensu lato (Xylariaceae) in Guadeloupe and Martinique (French West Indies) I. Taxa with penzigioid stromata. *Ascomycete.Org*, 10(4), 131–176. https://doi.org/10.25664/art-00239

Hermawan, R., Amelya, M. P., & Julia, Z. R. (2020). *Trichaleurina Javanica* from West Java. *Jurnal Mikologi Indonesia*, 4(2), 175–181. https://doi.org/10.46638/jmi.v4i2.85

Hermawan, R., Fusvita, L., Nugraha, N. H., & Amelya, M. P. (2021). Morphological Characteristic and Phenetic Relationship of *Lysurus* periphragmoides Collected from West Java. *Jurnal Biodjati*, 6(1), 102–110. https://doi.org/10.15575/biodjati.v6i1.10724

Hermawan, R., Imaningsih, W., & Badruzaufari. (2020). Mushrooms Assumed as Ectomycorrhizal Fungi on South Kalimantan Serpentine Soil. *Jurnal Mikologi Indonesia*, 4(1), 149–155. https://doi.org/10.46638/jmi.v4i1.71

Hermawan, R., & Maulana, I. (2020). *Sphaerobolus stellatus*: Cannonball Mushroom from West Java. *Jurnal Mikologi Indonesia*, 4(2), 218–222. https://doi.org/10.46638/jmi.v4i2.86

Hermawan, R., & Putra, I. P. (2018). *Calvatia pyriformis*: A New Record in Indonesia. *Jurnal Sains Dan Teknologi*, 1(2), 26–29. https://doi.org/10.31764/justek.vXiY.3737

Hermawan, R., & Putra, I. P. (2021). *Calvatia rugosa*: Epigeous Puffball Mushroom Reported from West Java. *Science Education and Application Journal*, 3(1), 1–6. https://doi.org/10.30736/seaj.v3i1.331

Index Fungorum. (2021). *Xylaria*. http://www.indexfungorum.org/names/names.asp

Ju, Y.-M., Hsieh, H.-M., Vasilyeva, L., & Akulov, A. (2009). Three new *Xylaria* species from Russian Far Eas. *Mycologia*, 101(4), 548–553. https://doi.org/10.3852/08-188

Kirk, P. M., Cannon, P. F., Minter, D. W., & Stalpers, J. A. (2008). *Ainsworth and Bisby’s Dictionary of the Fungi*. CABI Publishing.

Kristin, R., Rahmwati, R., & Mukarлина, M. (2020). Inventarisisi Jamur Makroskopis Filum Ascomycota di Kawasan Universitas Tanjungpura Pontianak Kalimantan Barat. *Jurnal Protobiont*, 9(1), 36–40. http://dx.doi.org/10.26418/protobiont.v9i1.40555

Ma, H.-X., Vasilyeva, L., & Li, Y. (2013). The genus *Xylaria* (Xylariaceae) in the south of China—6. A new *Xylaria* species based on morphological and molecular characters. *Phytotaxa*, 147(2), 48–54. http://dx.doi.org/10.11646/phytotaxa.147.2.2

Mycobank. (2021). *Xylaria*. https://www.mycobank.org

Okane, I., Toyama, K., Nakagiri, A., Suzuki, K., Srikitikulchai, P., Sivichai, S., Hywel-Jones, N., Potcharoen, W., & Læssøe, T. (2008). Study of endophytic *Xylariaceae* in Thailand: Diversity and taxonomy inferred from rDNA sequence analyses with...
sapropes forming fruit bodies in the field. *Mycoscience, 49*(6), 359–372. https://doi.org/10.1007/S10267-008-0440-6

Petrini, O. (1991). *Fungal Endophytes of Tree Leaves*. In: Andrews J.H., Hirano S.S. (eds) *Microbial Ecology of Leaves*. Brock/ Springer Series in Contemporary Bioscience.

Rogers, J. D., Ju, Y.-M., & Lehmann, J. (2005). Some Xylaria species on termite nests. *Mycologia, 97*(4), 914–923. https://doi.org/10.1080/15572536.2006.11832783

Rogers, J. D., & Samuels, G. J. (1986). Ascomycetes of New Zealand 8. Xylaria. *New Zealand Journal of Botany, 24*(4), 615–650. https://doi.org/10.1080/0028825X.1986.110409947

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., & Consortium, F. B. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of The National Academy of Sciences of The United States of America, 109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

Seshagirirao, K., Harikrishanaik, L., Venumadhav, K., Nanibabu, B., Jamir, K., Ratnamma, B. K., Jena, R., & Babarao, D. K. (2016). Preparation of Herbarium Specimen for Plant Identification and Voucher Number. *Roxburghia, 6*(1–4), 111–119.

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England), 30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sutton, S. V. W., & Cundell, A. M. (2004). Microbial Identification in the Pharmaceutical Industry. *Pharmaceutical Forum, 30*(5), 1884–1894. http://www.microbiologynetwork.com

Whalley, A. J. S. (1996). The xylariaceous way of life. *Mycological Research, 100*(8), 897–922. https://doi.org/10.1016/S0953-7562(96)80042-6

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. *PCR Protocols: A Guide to Methods and Applications*, 315–322. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1