Jurnal Mikologi Indonesia Vol 4 No 2 (2020): 175-181

JMI Mikoina

Available online at: www.jmi.mikoina.or.id

Jurnal Mikologi Indonesia

ISSN: 2579-8766 Online

Trichaleurina javanica from West Java

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Hermawan R, Amelya MP, Julia ZR. 2020 - *Trichaleurina javanica* from West Java. Jurnal Mikologi Indonesia *4*(2), 175-181. doi:10.46638/jmi.v4i2.85

Abstract

Trichaleurina is a fleshy mushroom with goblet-shaped within Pezizales. Many genera have a morphology similar to *Trichaleurina*, such as *Bulgaria* and *Galiella*. Some previous reports had been described fungi like *Trichaleurina* as *Sarcosoma*. Indonesia has been reported that has *Trichaleurina* specimen (the new name of *Sarcosoma*) by Boedijn. This research aimed to obtain, characterize, and determine the *Trichaleurina* around IPB University. Field exploration for fungal samples was used in the Landscape Arboretum of IPB University. Ascomata of *Trichaleurina* were collected, observed, and preserved using FAA. The specimen was deposited into Herbarium Bogoriense with collection code BO 24420. The molecular phylogenetic tree using RAxML was used to identify the species of the specimen. Morphological data were used to support the species name of the specimen. Specimen BO 24420 was identified as *Tricahleurina javanica* with 81% bootstrap value. Molecular identification was supported by the morphological data, such as the two oil globules and the size of mature ascospores.

Keywords – goblet-shaped fungi – Herbarium Bogoriense – Pyronemataceae

Introduction

Trichaleurina is a genus built by Rehm (1903) and known by other researchers since a publication of a valid genus by Rehm (1914). The genus is classified as Pezizales (Mycobank, 2020). According to Kirk (2008), Pezizales contains 199 genera and 1683 species. *Trichaleurina* has four species, i.e., *T. celebica*, *T. javanica*, *T. polytricha*, and *T. tenuispora* (Index Fungorum, 2020). Despite Index Fungorum (2020) recorded four species, the last publication (Carbone et al., 2013) mentioned only two species in their phylogenetic tree based on the molecular study.

Trichaleurina is included in Pyronemataceae (Rhem, 1903). The genus is not popular to study in this era, compared with the *Bulgaria* and *Galiella* as the same Pyronemataceae. Perry et al. (2007) had been studied the phylogenetics of the family Pyronemataceae, but they did not mention *Trichaleurina* in their phylogenetic tree. The knowledge of *Trichaleurina* is not often mentioned compared with other genera that have similar characters of morphology, i.e., *Bulgaria*, *Galiella*, and *Sarcosoma*. *Trichaleurina* had been reported for distribution in India (Patel et al., 2018). Specifically, the study mentioned *T. javanica*. The exploration of the genera happened in Indonesia (Boedijn, 1932), i.e., *Sarcosoma orientale* noted as a Java

Dikirimkan 28 Juli 2020, Diterima 1 November 2020, Terbit online 15 Desember 2020
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collection. Then, Carbone & Agnello (2015) suggested the name becomes *Galiella* based on their herbarium analyses.

Among *Bulgaria*, *Galiella*, and *Trichaleurina*, the morphology of apothecium is similar. They have differences in the ornamentation and the spore, especially of their molecular. Carbone et al. (2013) studied and explained more about *Bulgaria*, *Sarcosoma*, and *Trichaleurina*. To distinguish the differences among the genera, a molecular study is needed to solve this problem.

Materials and Methods

Fungal Sampling

The apothecia were explored in January 2020 and located in the Arboretum of IPB University. The exploration was focused on the goblet mushroom for *Trichaleurina*. The apothecia were 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 (Kottapalli et al., 2016) and deposited into Herbarium Bogoriense, LIPI, Indonesia.

Morphological Observation

The morphological data of apothecia were observed and documented to confirm the species and 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 excipular cells, paraphyses, asci, and ascospores. The observations were about the shape, size, and ornamentation, then compared with the other publication of the species within *Trichaleurina*.

Molecular Identification

The apothecia were identified using molecular analyses. The sterile part of apothecium was extracted to get genomic DNA. 500 µL CTAB-buffer (cetyl-trimethyl ammonium bromide) was added into apothecium paste and incubated at 65°C for 30 min. Then, 500 µL of chloroform isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 15 min. The supernatant was collected. 500 µL of phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 12,000 rpm for 10 min. The supernatant was collected, and 50 µL 2M NaOAc (Sodium Hypo-Acetate) and 500 µL absolute ethanol were added. This mixture was stored at -20°C overnight, then centrifuged at 15,000 rpm for 30 min. The supernatant was removed. 300 µL of 70 % ethanol was added and centrifuged at 10,000 rpm for 5 min. The supernatant was removed, and the DNA sample was in the pellet. The pellet was air-dried using a Speed Vacuum on 30°C for 30 min. The pellet was re-suspended in 50 µL of TE–buffer and 10 µL 1 mg/mL of RNAse. Then, the sample was incubated at 37°C for 10 min. RNAse was then deactivated by incubating the mixture at 70°C for 10 min. 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°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. Then final extension was set at 72°C 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, 7 sequences of *T. tenuispora*, 3 sequences of *T. javanica*, 3 sequences of *Galiella*, 4 sequences of *Bulgaria*, and *Geastrum saccatum* (outgroup) were used for phylogenetic tree reconstruction (Table 1). Sequences were aligned using Clustal X Ver. 2.1 software and saved as PHYLIP format files. 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. The taxa used in this study

Collection code	Species	GenBank acc. no
		LSU
Voucher TNM F10376 TYPE	*Trichaleurina tenuispora*	NG_059958
Voucher TNM F17974	*Trichaleurina tenuispora*	KF418261
Voucher TNM F4705	*Trichaleurina tenuispora*	KF418258
Voucher 420526MF0708	*Trichaleurina tenuispora*	MH668019
Voucher 420526MF0945	*Trichaleurina tenuispora*	MH668020
Voucher TNS F 31213	*Trichaleurina tenuispora*	KF418265
Voucher TNM F17898	*Trichaleurina tenuispora*	KF418260
Isolate HK022 Voucher HKAS 88981	*Trichaleurina javanica*	MG871326
Voucher TNM F8917	*Trichaleurina javanica*	KF418266
Voucher TUR A 198583	*Trichaleurina javanica*	KF418269
Voucher BO 24420	*Trichaleurina javanica*	MT732395
Strain WZ0111	*Galiella amurensis*	AY789267
AFTOL ID 129	*Galiella rufa*	FJ176869
CBS 912.72	*Galiella rufa*	MH878298
AFTOLN ID 916	*Bulgaria inquinans*	DQ470960
CBS 118.31	*Bulgaria inquinans*	MH866601
CBS 145.55	*Bulgaria inquinans*	MH868960
CBS 315.71	*Bulgaria inquinans*	MH877810
CBS 223.49	*Geastrum saccatum*	MH868038

Results

Specimen description:

Saprobic on the rotten wood (Figure 1a). **Sexual morphology**: Apothecium looks like globet- or cup-shaped (Figure 1b). The immature apothecium is more or less cylindric; wrinkled on the upper part; very dark grayish cyan of skin of apothecium; and hairy. The mature apothecium is exactly goblet-shaped with very dark grayish cyan on the surface; hairy; upper part of apothecium concave with soft orange; inside of apothecium fleshy gelatinous-rubbery and tough (Figure 1c); 2.3–3.0 cm in diameter; 3.2–3.6 cm in height. The hair 25–100 × 5–10 µm, brownish cylindrical cell, rough surface's cell, septate cell, and apically blunt (Fig 1d). Paraphyses hyphae 490 µm in length, septate, filiform, longer than ascus (Figure 1e). Excipular cells crowded, septate (Figure 1f). Asci 8-spored, long tapering base, cylindrical, 400–420 × 12–18 µm (Figure 1g). Ascospore ellipsoid or occasionally nearly subfusiform, mostly 2-4 oil globules inside, 18–31 × 15–17 µm. (Figure 1h). **Asexual morphology**: Undetermined.

Specimen examined: Landscape Arboretum of IPB University, BO 24420, Rudy Hermawan, and Mega Putri Amelya. **GenBank Submission:** LSU: MT732395. **Molecular work.** The phylogenetic tree showed that the specimen BO 24420 was classified as *T. javanica* with 81% bootstrap value (Figure 2). Among *Trichaleurina, Bulgaria*, and *Galiella* were separated.

Figure 1. Morphology of *Trichaleurina javanica*. (a-b) apothecia on substrate; (c) slicing of apothecia; (d) cylindrical hair; (e) apically swollen paraphyses; (f) excipular cells; (g) asci; (h) ascospores. Scale bars: (a-c) 3 cm; (d-f) 50 µm; (g) 100 µm; and (h) 30 µm.

Discussion

Trichaleurina has been reported for India's distribution (Patel et al., 2018), whereas Indonesia has not been reported yet. But the other genus with similar morphology, namely *Galiella*, has been reported for *G. celebica* in Saktu Island, Kepulauan Seribu, North Jakarta (Noverita et al., 2018). The identification was based on the morphology of apothecium. *Trichaleurina javanica* has a synonym by *Galiella javanica* (Mycobank, 2020). According to both morphologies, they have similar of apothecium seems like goblet-shaped. Another genus that has the similar morphology of apothecia as *Trichaleurina* is *Bulgaria*. The similar apothecia of them make a worried if the identification is only based on the morphology. The case had happened when some apothecia seem like goblet-shaped had been found in Indonesia by Boedijn (1932).

Further study corrects the specimens (Carbone et al., 2013). The apothecia found in the same place have two different genera, i.e., *Bulgaria* and *Trichaleurina*. Carbone et al. (2013) mentioned that the other specimens were based on the mature spore size. Identification of the goblet-shaped mushroom such as *Bulgaria*, *Galiella*, and *Trichaleurina* should be supported by the molecular study or specifically intensive morphology.

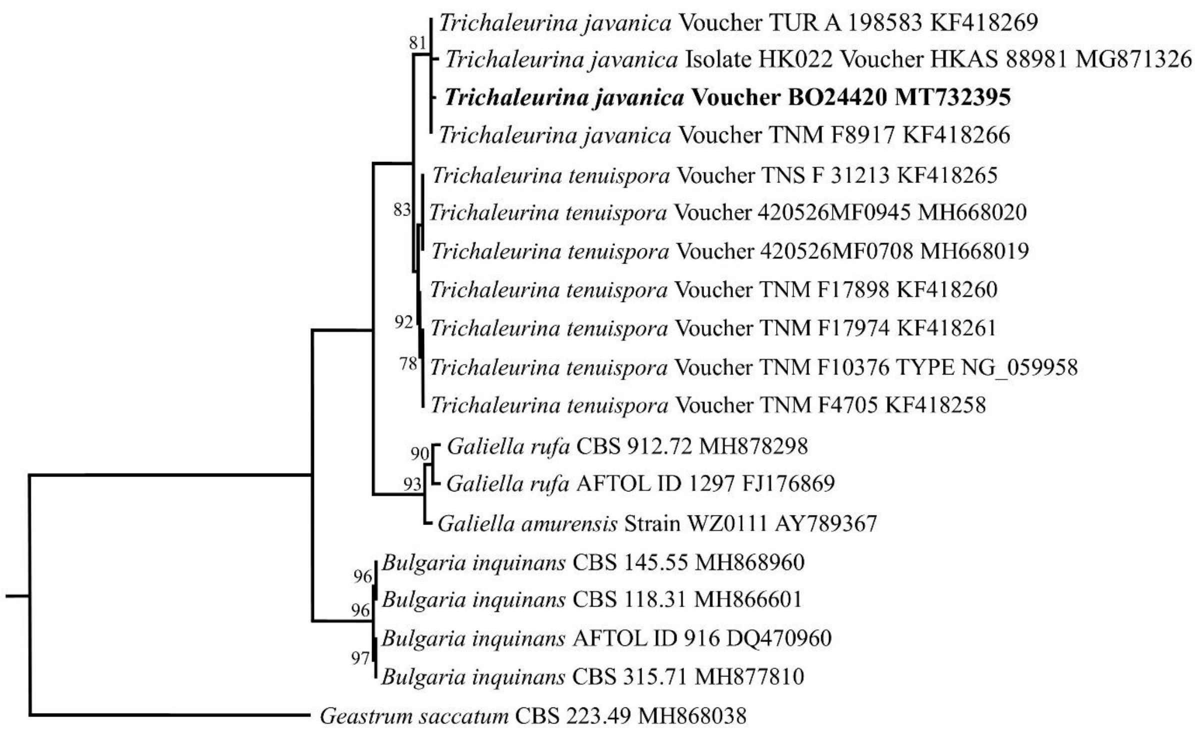

Figure 2. *Trichaleurina javanica* phylogenetic tree based on the LR0R/LR5 region using RAxML. Bootstrap (BS)≥50 was shown on the branch. The BO 24420 must be in bold.

According to the last publication about their molecular study, *Trichaleurina* has two species, i.e., *T. javanica* and *T. tenuispora*. The microscopic morphology that important to identify *Trichaleurina* in species is mature ascospores. *T. javanica* has 18–31 × 15–17 µm, whereas *T. tenuispora* has 27–40 × 12–15 µm. The size of *T. tenuispora* in size is bigger than *T. javanica*. Additionally, the inside appearance of ascospores among them is distinctly different. *T. javanica* has mostly two oil globules, whereas *T. tenuispora* has up to four mostly. The characters of *T. tenuispora* are cited in Carbone et al. (2013).

Trichaleurina is classified in Pezizales. The specific character is the operculate ascus, which is generally opened by rupturing to form a terminal or operculum (Hansen & Pfister, 2006). Then, it is classified into Pyronemataceae. Pyronemataceae is a family that was built by Corda in 1842 (Index Fungorum, 2020). Based on Hansen & Pfister (2006), this family is not monophyletic on their phylogenetic tree using the Neighbour Joining method. The phylogenetic tree was using a large combined small subunit to the identification. Other families are bore in the clade, i.e., Ascodesmidaceae and Glaziellaceae. The member of this family is changed along with the latest research published. Rifai (1968) mentioned only a single genus in this family, namely *Pyronema*. The character in this family is described by Apothecia minute, orange to red, ivory, scattered to gregarious, at first conical-shaped of apothecium, expanding to turbinate, with or without delicate hairs on the operculum part, asci operculate, 8-spored and paraphyses filiform (Rifai, 1968). Then, Eckblad (1968) classified 21 genera into Pyronemataceae and 49 genera by Korf (1973), one of the genera is *Trichaleurina*.

Identification using RAxML has a result that the specimen of BO 24420 is classified as *T. javanica*. This identification is supported by the specimen's specific morphology, such as the size and number of oil globules of ascospores. The bootstrap value is 81 % in the *T. javanica* clade. The phylogenetic tree in Figure 2 also showed the separation among *Bulgaria*, *Galiella,* and *Trichaleurina*. This phylogenetic tree is monophyletic. *Trichaleurina javanica* has an anamorph culture, namely *Kumanasamuha* (Carbone et al., 2013). The genus is built and described by P.Rag. Rao & D. Rao (Rao & Rao, 1964). But the species name is not built yet. The anamorph is only similar morphologically with *Kumanasamuha sundara* but different by shorter conidiophores and fertile lateral branches, and smaller conidia (Carbone et al., 2013).

The goblet-shaped mushrooms such as *Bulgaria*, *Galiella,* and *Trichaleurina* are not popular to report the potency. Only *Galiella* has been reported for edible in Sarawak (Abdullah & Rusea, 2009), identified as *G. rufa*, known as 'mata kerbau' (buffalo eyes) in local people. Other reports for other genera have not available yet for the edible category. Matias et al. (2016) successfully reported the angiogenic and anti-tumor potential of *Trichaulerina* mushroom. This potency is also supported for knowing of the secondary metabolite identification. Sogan et al. (2018) identified the secondary metabolite from *T. celebica,* which contains alkaloids, fatty acids, triterpenes, sterols, steroids, anthraquinones, anthrones, and steroids. Altogether, *T. celebica* has promising bioactivities which can be utilized in the nutraceutical or pharmacological industry

References

Abdullah, F. & Rusea, G. (2009). Documentation of inherited knowledge on wild edible fungi from Malaysia. *Blumea, 54*, 35-38. https://doi.org/10.3767/000651909X475996

Boedijn, K. B. (1932). The genus *Sarcosoma* in Netherlands India. *Bulletin du Jardin Botanique de Buitenzorg, 3e série, 12,* 273-279.

Carbone, M. & Agnello, C. (2015). Studies in *Galiella* (Ascomycota, Pezizales). III. The identity of *Sarcosoma orientale* and *Wolfina papuana*. *Ascomycete.org., 7,* 232-238.

Carbone, M., Yei-Zeng, W. & Chun-Lin, H. (2013). Studies in *Trichaleurina* (Pezizales). Type studies of *Trichaleurina polytricha* and *Urnula philippinarum*. The status of *Sarcosoma javanicum*, *Bulgaria celebica*, and *Trichaleurina tenuispora sp. nov*., with notes on the anamorphic genus *Kumanasamuha*. *Ascomycete.org.*, *5*, 137-153.

Eckblad, F. E. (1968). The genera of the Operculate Discomycetes. A reevaluation of their taxonomy, phylogeny, and nomenclature. *Nytt Magasin for Botanik,* 15*,* 1-191.

Hansen, K. & Pfister, D. H. (2006). Systematics of the Pezizomycetes - the operculate Discomycetes. *Mycologia, 96*, 1029-1040. https://doi.org/10.1080/15572536.2006.-11832631

Index Fungorum. (2020). www.indexfungorum.org (Accessed April 12, 2020).

Kirk, P. M., Cannon, P. F., Minter, D. W. & Stalpers, J. A. (2008). *Dictionary of the Fungi* (10^{th} edition). CAB International.

Kottapalli, S., Krishna, H., 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*, 111-119.

Korf, R. P. (1973). Discomycetes and Tuberales. In: Ainsworth GC, Sparrow FK, Sussman AS, eds. *The Fungi: an Advanced Treatise IV*. p 249–319. Academic Press.

Matias, C., Maslang, J., Bungihan, M., Saludarez, M., Liday, D., Villanueva, H., Balonquita, M. & Mariñas, M. (2016). *Isolation of the Cytotoxic, Angiogenic, and Anti-tumor compounds from Trichaluerina celebica* (Undergraduate Thesis). Saint Mary's University.

Mycobank. (2020). *Trichaleurina javanica*. http://www.mycobank.org/name/Trichaleurina%-20javanica&Lang=Eng (Accessed April 12, 2020).

Noverita, Nabilah, Siti, F.Y. & Yudistari. (2018). Macrofungi on Saktu Island Kepulauan Seribu Jakarta Utara and their potential. *Jurnal Mikologi Indonesia, 2*, 16-29. https://doi.org/10.46638/jmi.v2i1.38

Patel, R. S., Vasava, A. M. & Rajput, K. S. (2018). Distribution of *Trichaleurina javanica* (Rehm) M. Carbone, Agnello & P. Alvarado (Chorioactidaceae) in India. *Plant Biosystem - An International Journal Dealing with All Aspects of Plant Biology*, 1-4. https://doi.org/10.1080/11263504.2018.1461704

Perry, B. A., Hansen, K. & Pfister, D. H. (2007). A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). *Mycological Research, 3*, 549-571. https://doi.org/10.1016/j.mycres.2007.03.014

Rao, P. R. & Rao, D. (1964). *Kumanasamuha* a new genus of Dematiaceae. *Mycopathologia, 22,* 330-334. https://doi.org/10.1007/BF02049066

Rehm, H. (1903). Die Discomyceten-Gattung *Aleurina* Sacc. *Annales Mycologici,* 1*,* 514-516.

Rehm, H. (1914). Ascomycetes Philippinenses VI –Communicati a clar. C. F. Baker. *Leaflets of Philippine Botany, 6*, 2257-2281.

Rifai, M. A. (1968). The Australasian Pezizales in the Herbarium of the Royal Botanic Gardens Kew. *Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen, Afd Natuurk, Tweede Sect, 57,* 1-295.

Sogan, M. M., Maslang, J. A. L. & Dulay, R. M. R. (2018). Myco-chemicals and teratogenic activity of wild mushroom *Trichaleurina celebica* from Mt. Palali, Quezon, Nueva Vizcaya, Luzon Island, Philippines. *The CLSU International Journal of Science & Technology, 2*, 17-23. https://doi.org/10.22137/ijst.2018.v3n2.03

Stamatakis, A. (2014). RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics. http://bioinformatics.oxfordjournals.-org/ content/early/2014/01/21/ bioinformatics.btu033.abstract.