



# Taxonomy and phylogeny of *Smaragdiniseta musae* sp. nov. and *Albifimbria verrucaria* (Hypocreales, Stachybotryaceae) on *Musa* from Thailand

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## Abstract

## Background

*Smaragdiniseta musae* is introduced as a leaf-based novel saprobic species from *Musa*. Multi-gene phylogenetic analyses of internal transcribed spacer (ITS), RNA polymerase II second largest subunit (rpb2) and  $\beta$ -tubulin (tub2) data support the taxonomic placement of the new collection in *Smaragdiniseta* (Hypocreales, Stachybotryaceae). The novel species is characterised by cup-shaped sporodochia covered by numerous peripheral setae and simple hyaline, guttulate conidia produced by the ultimate branches (phialides) of conidiophores.

## New information

This is the first report of *Smaragdiniseta* from Thailand and on Musaceae. In addition, we report *Albifimbria verrucaria* for the first time from Thailand, based on morpho-molecular evidence.

## Keywords

one new species, banana, fungi, host, hyaline conidia, Musaceae, myrothecium-like, saprobes

## Introduction

Crous et al. (2014) established Stachybotryaceae and Lombard et al. (2016) further revised the family, based on phenotypic characteristics and molecular analyses of LSU, ITS, rpb2, cmdA, tef1 and tub2 sequences. These arguments were accepted in the recent classifications of Sordariomycetes by Hyde et al. (2020) and Wijayawardene et al. (2020). The polyphyletic nature of *Myrothecium* (Chen et al. 2016) was further justified by Lombard et al. (2016) who established 13 new genera in Stachybotryaceae with myrothecium-like morphology viz. *Albifimbria*, *Capitofimbria*, *Dimorphiseta*, *Gregatothecium*, *Inaequalispora*, *Myxospora*, *Neomyrothecium*, *Paramyrothecium*, *Parvothecium*, *Smaragdiniseta*, *Striaticonidium*, *Tangerinosporium* and *Xenomyrothecium*. *Smaragdiniseta* was established to accommodate *S. bisetosa* (= *Myrothecium bisetosum*), which is characterised by cup-shaped sporodochia with straight, hyaline, smooth-walled peripheral setae (Rao and De Hoog 1983, Lombard et al. 2016). The setae grow rapidly from sporodochia and are soon covered by a weft of emerald green, echinulate marginal hyphae (Rao and De Hoog 1983). The sexual morph of *Smaragdiniseta* is yet to be determined.

The taxonomic placement of *M. bisetosum* was doubted by Rao and De Hoog (1983) as it is morphologically resembling *Sarcopodium* by the formation of setae (Ehrenberg 1818). However, Rao and De Hoog (1983) distinguished *M. bisetosum* from *Sarcopodium* by the macroscopic colour of the conidiomata. They interpreted the new collection that was similar in morphology to *Sarcopodium* and *Myrothecium*. In the phylogenetic analyses of Lombard et al. (2016), *M. bisetosum* formed a monophyletic lineage sister to *Albifimbria* that was well separated from *Myrothecium*. *Smaragdiniseta* remains monospecific (Index Fungorum 2022, Cooper and Kirk 2022) and no additional taxonomic work has been conducted since Lombard et al. (2016).

In Lombard et al. (2016), the ex-neotype strain of *Myrothecium verrucaria* formed another highly supported monophyletic clade in Stachybotryaceae. *Albifimbria* was introduced to classify this lineage and *M. verrucaria* was synonymised and typified under *Albifimbria*. In addition, *A. lateralis*, *A. terrestris* and *A. viridis* were introduced as novel taxa (Lombard et al. 2016). *Albifimbria* is characterised by the production of verrucose setae around the sporodochia (Lombard et al. 2016). In addition, some *Albifimbria* species bear funnel-

shaped mucoid appendages in conidia (Lombard et al. 2016). Currently, four species of *Albifimbria* are listed in Index Fungorum (Cooper and Kirk 2022).

We are studying the saprobic fungi associated with *Musa* spp. from Thailand with the intention of providing a better understanding of their taxonomy, based on both morphology and phylogeny (Samarakoon et al. 2020a, Samarakoon et al. 2020b, Samarakoon et al. 2021a, Samarakoon et al. 2021b). This study is aimed at documenting two myrothecium-like taxa in Stachybotryaceae isolated from the dead leaves of *Musa*. Based on morphological illustrations, descriptions and phylogenetic analyses, we introduce one of our collections as *Smaragdiniseta musae* sp. nov. from Mae Sai, Chiang Rai, Thailand. This is the second species in *Smaragdiniseta* which further validates the taxonomic establishment of Lombard et al. (2016) and breaks the monotypic nature of the genus. In addition, we report *Albifimbria verrucaria* on *Musa* sp. as a new country record to Thailand.

## Materials and methods

### Sample collection, morphological studies and isolation

Dead leaves of *Musa* with characteristic sporodochia were collected from Thailand from January to October 2019. Specimens were transferred to the laboratory in small cardboard boxes. Fungi were observed using a Motic SMZ 168 series microscope (Motic Asia, Kowloon, Hong Kong). Conidiomata were mounted on glass slides in tap water and lactoglycerol for examination and photomicrography. The specimens were further observed using a Nikon ECLIPSE 80i compound microscope (Nikon Instruments Inc., Melville, NY, USA) and photographed using a Canon 550D digital camera (Canon Inc., Ota, Tokyo, Japan). Measurements were taken with the aid of Tarosoft (R) Image Frame Work programme. More than 10 measurements were made for the structures. The images were further arranged using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolations for the samples were conducted according to Senanayake et al. (2020). Germinated conidia were individually transferred to potato dextrose agar (PDA) plates and incubated at 25°C. Colony characters were examined after two weeks. Dried herbarium specimens were deposited in the Mae Fah Luang University Herbarium (Herb. MFLU), Chiang Rai, Thailand. Living cultures in PDA were deposited in the Culture Collection of Mae Fah Luang University (MFLUCC). Facesoffungi numbers (Jayasiri et al. 2015) and MycoBank numbers (<http://www.Mycobank.org>) were received for the isolates. The illustrations and descriptions were submitted to the GMS MICROFUNGI ([gmsmicrofungi.org](http://gmsmicrofungi.org)) database (Chaiwan et al. 2021). The finalised alignment and tree were submitted to Zenodo (<https://zenodo.org/record/6867700#.Ytghz3ZBzIU>).

### DNA extraction, PCR amplification and sequencing

DNA was extracted from the mycelium of 14 days-old cultures. The mycelium was crushed using a plastic pestle and DNA was extracted using Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, P.R. China) following the manufacturer's guidelines.

Three gene regions; viz. internal transcribed spacer (ITS), partial  $\beta$ -tubulin (*tub2*) and partial second largest subunit of the DNA-directed RNA polymerase II (*rpb2*), were amplified using ITS5/ITS4 (White et al. 1990), Bt2a and Bt2b (Glass and Donaldson 1995) and fRPB2-5f/fRPB2-7cR (Liu et al. 1999), respectively.

Polymerase chain reaction (PCR) was conducted using the following protocol. The total volume of the PCR reaction was 25  $\mu$ l and comprised 12.5  $\mu$ l of 2  $\times$  Power Taq PCR MasterMix (a premix and ready-to-use solution, including 0.1 Units/ $\mu$ l Taq DNA Polymerase, 500  $\mu$ M dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCL pH 8.3, 100 mM KCl, 3 mM MgCl<sub>2</sub>, stabiliser and enhancer), 1  $\mu$ l of each primer (10 pM), 2  $\mu$ l genomic DNA and 8.5  $\mu$ l of deionised water. The total reaction comprised 35 cycles. The annealing temperatures were according to Lombard et al. (2016) and Samarakoon et al. (2021b). The amplified PCR fragments were sent to TsingKe Biological Technology (Beijing) Co., China for sequencing. DNA sequence data obtained were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>).

### Sequence alignment

Newly-generated sequence data of different gene regions were subjected to BLAST searches using BLASTn in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to retrieve similar sequences. The results and initial morphology indicated that our strains belong to Stachybotryaceae (Hypocreales). The collection numbers for these similar sequences (Table 1) were then downloaded from GenBank, based on BLASTn results and Lombard et al. (2016). Single gene alignments were made by MAFFT v. 7.036 (<http://mafft.cbrc.jp/alignment/server/large.html>, Katoh et al. 2019) using the default settings and later refined as necessary using BioEdit v. 7.0.5.2 (Hall 1999).

Table 1.

Names, culture collection numbers and their respective GenBank accession numbers of the Stachybotryaceae taxa that have been subjected to phylogenetic analyses. Type strains are superscripted with T and new collections are indicated in bold black.

Species	Strain	GenBank Accession Numbers		
		ITS	rpb2	tub2
<i>Albifimbria lateralis</i>	CBS 117712 <sup>T</sup>	<a href="#">KU845881</a>	<a href="#">KU845919</a>	<a href="#">KU845957</a>
<i>A. terrestris</i>	CBS 109378	<a href="#">KU845882</a>	<a href="#">KU845920</a>	<a href="#">KU845958</a>
<i>A. terrestris</i>	CBS 126186	<a href="#">KU845883</a>	<a href="#">KU845921</a>	<a href="#">KU845959</a>
<i>A. terrestris</i>	CBS 127838	<a href="#">KU845884</a>	<a href="#">KU845922</a>	<a href="#">KU845960</a>
<i>A. verrucaria</i>	CPC 30056	<a href="#">KU845885</a>	<a href="#">KU845923</a>	<a href="#">KU845961</a>
<i>A. verrucaria</i>	CBS 328.52 <sup>T</sup>	<a href="#">KU845886</a>	<a href="#">KU845924</a>	<a href="#">KU845962</a>
<i>A. verrucaria</i>	CBS 188.46	<a href="#">KU845888</a>	<a href="#">KU845926</a>	<a href="#">KU845964</a>
<b><i>A. verrucaria</i></b>	<b>MFLUCC 22-0017</b>	<a href="#">ON563487</a>	NA	<a href="#">ON586153</a>
<i>A. viridis</i>	CBS 449.71 <sup>T</sup>	<a href="#">KU845898</a>	<a href="#">KU845936</a>	<a href="#">KU845974</a>

Species	Strain	GenBank Accession Numbers		
		ITS	rpb2	tub2
<i>A. viridis</i>	CBS 127346	<a href="#">KU845899</a>	<a href="#">KU845937</a>	<a href="#">KU845975</a>
<i>Alfaria caricicola</i>	CBS 113567 <sup>T</sup>	<a href="#">KU845983</a>	<a href="#">KU846001</a>	<a href="#">KU846014</a>
<i>Alf. terrestris</i>	CBS 168.97	<a href="#">KU845987</a>	<a href="#">KU846005</a>	<a href="#">KU846018</a>
<i>Capitofimbria compacta</i>	CBS 111739 <sup>T</sup>	<a href="#">KU846287</a>	<a href="#">KU846349</a>	<a href="#">KU846404</a>
<i>Dimorphiseta terrestris</i>	CBS 127345 <sup>T</sup>	<a href="#">KU846314</a>	<a href="#">KU846375</a>	<a href="#">KU846431</a>
<i>Fusarium sambucinum</i>	CBS 146.95	<a href="#">KM231813</a>	<a href="#">KM232381</a>	<a href="#">KM232078</a>
<i>Gregatothecium humicola</i>	CBS 205.96 <sup>T</sup>	<a href="#">KU846315</a>	<a href="#">KU846376</a>	<a href="#">KU846432</a>
<i>Inaequalispora prestonii</i>	CBS 175.73 <sup>T</sup>	<a href="#">KU846316</a>	<a href="#">KU846377</a>	<a href="#">KU846433</a>
<i>Myrothecium inundatum</i>	CBS 196.74 <sup>T</sup>	<a href="#">KU846451</a>	NA	<a href="#">KU846532</a>
<i>M. simplex</i>	CBS 582.93 <sup>T</sup>	<a href="#">KU846456</a>	NA	<a href="#">KU846537</a>
<i>Myxospora aptrootii</i>	CBS 101263 <sup>T</sup>	<a href="#">KU846458</a>	<a href="#">KU846496</a>	<a href="#">KU846539</a>
<i>Neomyrothecium humicola</i>	CBS 310.96 <sup>T</sup>	<a href="#">KU846467</a>	<a href="#">KU846505</a>	NA
<i>Paramyrothecium acadense</i>	CBS 123.96	<a href="#">KU846288</a>	<a href="#">KU846350</a>	<a href="#">KU846405</a>
<i>Pa. breviseta</i>	CBS 544.75 <sup>T</sup>	<a href="#">KU846289</a>	<a href="#">KU846351</a>	<a href="#">KU846406</a>
<i>Pa. cupuliforme</i>	CBS 126167 <sup>T</sup>	<a href="#">KU846290</a>	<a href="#">KU846352</a>	<a href="#">KU846407</a>
<i>Pa. foeniculicola</i>	CBS 331.51 <sup>T</sup>	<a href="#">KU846292</a>	<a href="#">KU846354</a>	<a href="#">KU846409</a>
<i>Pa. foliicola</i>	CBS 419.93	<a href="#">KU846293</a>	<a href="#">KU846355</a>	<a href="#">KU846410</a>
<i>Pa. humicola</i>	CBS 127295 <sup>T</sup>	<a href="#">KU846295</a>	<a href="#">KU846356</a>	<a href="#">KU846412</a>
<i>Pa. nigrum</i>	CBS 116537	<a href="#">KU846296</a>	<a href="#">KU846357</a>	<a href="#">KU846413</a>
<i>Pa. parvum</i>	CBS 142.42	<a href="#">KU846297</a>	<a href="#">KU846358</a>	<a href="#">KU846414</a>
<i>Peethambara sundara</i>	CBS 521.96	<a href="#">KU846470</a>	<a href="#">KU846508</a>	<a href="#">KU846550</a>
<i>Septomyrothecium maraitiense</i>	MUCL 47202 <sup>T</sup>	NA	<a href="#">KU846510</a>	NA
<i>Smaragdiniseta bisetosa</i>	CBS 459.82 <sup>T</sup>	<a href="#">KU847229</a>	<a href="#">KU847281</a>	<a href="#">KU847319</a>
<b><i>S. musae</i></b>	<b>MFLUCC 22-0015<sup>T</sup></b>	<a href="#">ON563485</a>	<a href="#">ON586151</a>	<a href="#">ON572191</a>
<b><i>S. musae</i></b>	<b>MFLUCC 22-0016</b>	<a href="#">ON563486</a>	<a href="#">ON586152</a>	<a href="#">ON572192</a>
<i>Striaticonidium brachysporum</i>	CBS 131.71	<a href="#">KU847230</a>	<a href="#">KU847282</a>	<a href="#">KU847320</a>
<i>Tangerinosporium thaltricola</i>	CBS 317.61 <sup>T</sup>	<a href="#">KU847243</a>	NA	<a href="#">KU847333</a>
<i>Virgatospora echinofibrosa</i>	CBS 110115	<a href="#">KU847244</a>	<a href="#">KU847293</a>	<a href="#">KU847334</a>
<i>Xenomyrothecium tongaense</i>	CBS 598.80 <sup>T</sup>	<a href="#">KU847246</a>	<a href="#">KU847295</a>	<a href="#">KU847336</a>
<i>Xepicula crassiseta</i>	CBS 392.71 <sup>T</sup>	<a href="#">KU847247</a>	<a href="#">KU847296</a>	<a href="#">KU847337</a>

Abbreviations of culture collections; **CBS**: CentraalbureauvoorSchimmeldcultures, Utrecht, The Netherlands, **CPC**: Working collection of Pedro Crous housed at CBS, **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand, **MUCL**: Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada, NA: Sequence data are not available in GenBank.

### Phylogenetic analyses

Maximum Likelihood (ML) trees were generated using the RAxML-HPC2 on XSEDE (8.2.8) (Stamatakis et al. 2008, Stamatakis 2014) in the CIPRES Science Gateway platform (Miller et al. 2010) with GTR+I+G model of evolution for single and multi-gene alignments. Bootstrap supports were gained by running 1000 pseudo-replicates. Maximum Likelihood (ML) bootstrap values equal to or greater than 60% are given above each node of the phylogenetic tree (Fig. 3).

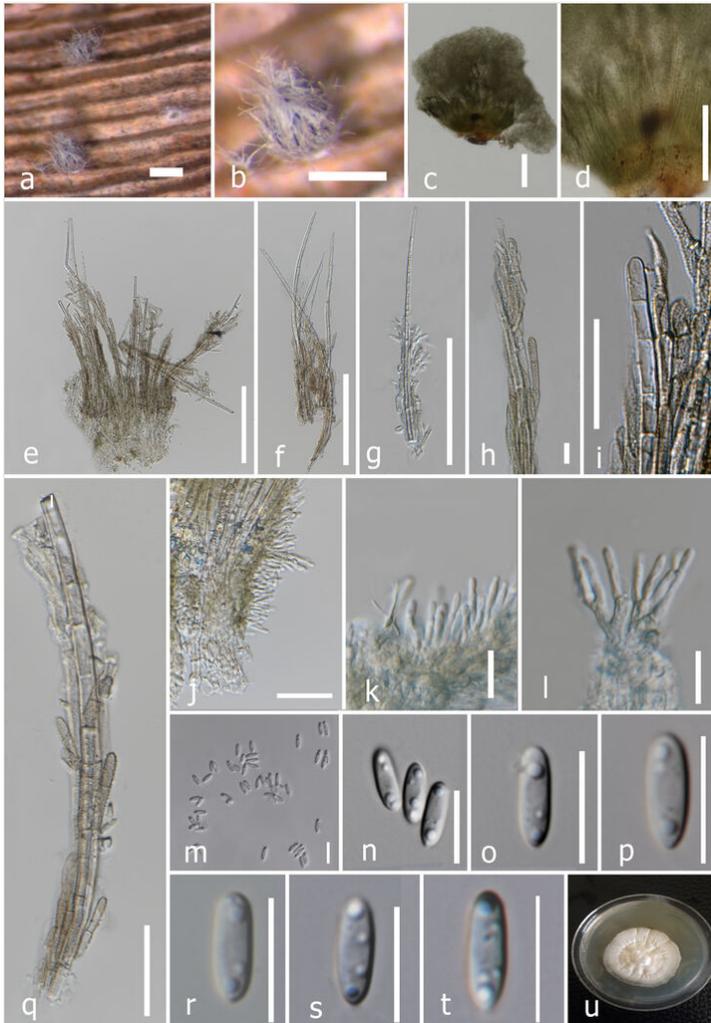


Figure 1. [doi](#)

*Smaragdiniseta musae* (MFLU 22-0047, holotype) **a, b** sporodochia on the host; **c, d** cupulate sporodochia; **e-i, q** setae and marginal hyphae; **j-l** attachments of conidiophores and phialides; **m-t** conidia; **u** colony on PDA after 8 weeks. Scale bars: 400  $\mu\text{m}$  (**a, b**), 100  $\mu\text{m}$  (**c, d, i, q**), 50  $\mu\text{m}$  (**e-h**), 20  $\mu\text{m}$  (**j**), 10  $\mu\text{m}$  (**k-t**).

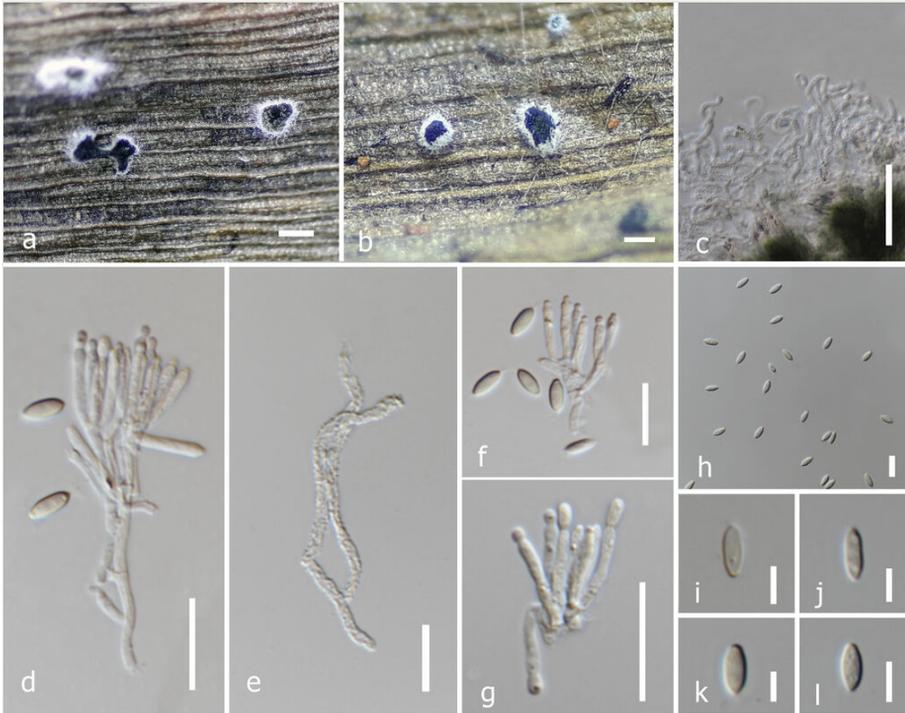


Figure 2. [doi](#)

*Albifimbria verrucaria* (MFLU 22-0048) **a, b** sporodochia on the host; **c, e** marginal hyphae; **d, f, g** conidiophores and phialides; **h-l** conidia. Scale bars: 400  $\mu\text{m}$  (**a, b**), 100  $\mu\text{m}$  (**i-j**), 50  $\mu\text{m}$  (**c**), 10  $\mu\text{m}$  (**d-h**), 5  $\mu\text{m}$  (**i-l**).

A Bayesian analysis was conducted with MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001) to evaluate posterior probabilities (Rannala and Yang 1996, Zhaxybayeva and Gogarten 2002) by Markov Chain Monte Carlo sampling (MCMC). Two parallel runs were conducted, using the default settings and with the adjustments as follows; four simultaneous Markov chains were run for 2,000,000 generations and trees were sampled every 100<sup>th</sup> generation and 20000 trees were obtained. The first 4,000 trees of the burn-in phase were discarded. The remaining 16,000 trees were taken for calculating PP in the majority rule consensus tree. Branches with Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are written above each node of the multigene tree (Fig. 3). The trees were displayed with FigTree v.1.4.0 (Rambaut 2011) and re-arranged in Microsoft PowerPoint.

## Taxon treatments

### *Smaragdiniseta musae* Samarakoon & Chomnunti, sp. nov.

- MycoBank [MB844095](https://www.mycobank.org/record/MB844095)
- FoF 10846 <https://www.facesoffungi.org/?s=FoF10846>

## Material

### Holotype:

- a. scientificName: *Smaragdiniseta musae* Samarakoon & Chomnunti; kingdom: Fungi; phylum: Ascomycota; class: Sordariomycetes; order: Hypocreales; family: Stachybotryaceae; taxonRank: species; genus: *Smaragdiniseta*; specificEpithet: *musae*; scientificNameAuthorship: Samarakoon & Chomnunti; continent: Asia; country: Thailand; stateProvince: Chiang Rai; locality: Mae Sai; year: 2019; month: June; day: 16; habitat: Terrestrial; occurrenceRemarks: Found on a dead leaf of *Musa* sp.; recordNumber: BNS264; recordedBy: Binu C. Samarakoon; disposition: Living cultures: MFLUCC 22-0015 and MFLUCC 22-0016; associatedSequences: GenBank MFLUCC 22-0015: [ON563485](#) (ITS), [ON586151](#) (rpb2), [ON572191](#) (tub2); MFLUCC 22-0016: [ON563486](#) (ITS), [ON58615](#)(rpb2), [ON572192](#) (tub2); identifiedBy: Binu C. Samarakoon; institutionID: MFLU; collectionID: MFLU 22-0047

## Description

Saprobic on dead leaves of *Musa* sp. **Sexual morph:** Undetermined. **Asexual morph:** Sporodochia 0.2–0.35 mm diam., cup-shaped, scattered, solitary, circular with an entire margin, initially emerald green, later becoming black, composed of hyaline, erect conidiophores, surrounded by filamentous white marginal hyphae and setae at periphery. Fungal hyphae arranged in parallel like a palisade layer, compacted, verrucose, olivaceous-brown or hyaline, sometimes warticulate, septate, branched, irregularly thick-walled, with ultimate hyphal cell rounded at apex and flat at base; hyphal cells: 10–14 × 0.8–2.5 µm ( $\bar{x}$  = 13.3 × 1.6 µm, n = 30). Aggregated hyphae hyaline or emerald green when immature, tan brown at maturity. Setae fast growing from marginal hyphae, numerous, aggregating as a pale grey brush, elongated, straight or slightly curved, tapering towards apex, hyaline, septate, unbranched, apiculate at apex, sometimes caudate, truncate or rounded, swollen to globose to ovate or rounded at base, setae wall 0.4–1.5 µm ( $\bar{x}$  = 0.8 µm, n = 30) thick, often smooth, sometimes rough with hyaline acellular coatings, (60–)90–160(–250) × 0.8–2.8 µm ( $\bar{x}$  = 126.4 × 1.6 µm, n = 30); septa 4–8 µm apart. Marginal hyphae often coiling or growing around the setae or projecting out, forming a wefty cover around setae, 95–125 µm ( $\bar{x}$  = 106 µm, n = 30) wide. Conidiophores 4–14 × 1.3–2.5 µm ( $\bar{x}$  = 12.9 × 1.7 µm, n = 30), macronematous, hyaline, smooth, thin-walled, arising from sub hyaline or pale brown, with slightly thickened and swollen basal cells, often with a narrow truncate base, wider in the middle, tapering to rounded at apex, 3–4 × 5–6 µm ( $\bar{x}$  = 3.5 × 5.5 µm, n = 10), septate, rarely unbranched, mostly branched and with 2–3 conidiogenous sub-branches at each node. Conidiogenous cells phialidic, rough or thin-walled, rod-shaped, elongated or ovate, 4–9 × 1–3 µm ( $\bar{x}$  = 6.8 × 1.9 µm, n = 20), without a collarette, pinching off simple conidia at the apex of each phialide. Conidia simple, hyaline, smooth, thin-walled, elliptic or slightly ovate, rounded at one end and acute at other end, with two distinct guttules at vertical ends, sometimes with 3–5 guttules, 6–10 × 2–3.5 µm ( $\bar{x}$  = 8.3 × 2.7 µm, n = 10).

**Culture characteristics.** Conidia germinating on PDA after 48 hours, germ tubes being produced from the acute end. Colonies growing on PDA reaching 20 mm diam. after 2 weeks in light conditions at 25°C, mycelium mostly immersed, not slimy, cottony, pinkish-white, dense in the middle and comparatively sparse at the periphery. Radially and unevenly striated, colonies have a slightly wrinkled appearance from the top. The formation of sporodochia was not observed in mature colonies.

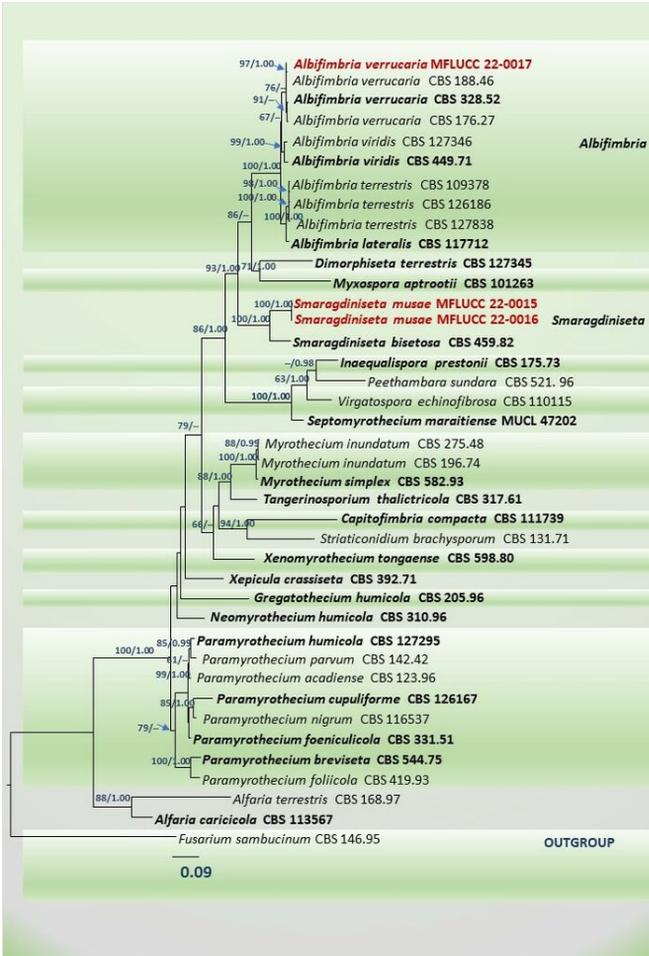


Figure 3. [doi](#)

Maximum Likelihood tree revealed by RAxML analyses of ITS, rpb2 and tub2 sequence data of selected genera of Stachybotryaceae, showing the phylogenetic position of *Albifimbria verrucaria* (MFLUCC 22-0017) and *Smaragdiniseta musae* (MFLUCC 22-0015, MFLUCC 22-0016). ML bootstrap supports ( $\geq 60\%$ ) and Bayesian posterior probabilities ( $\geq 0.95$  BYPP) are given above the nodes, respectively. The tree is rooted with *Fusarium sambucinum* (CBS146.95) (Nectriaceae). Strains generated in this study are indicated in bold red. Ex-type strains are indicated in bold black. The scale bar represents the expected number of nucleotide substitutions per site.

## Etymology

The species epithet reflects the host genus, *Musa*.

## Notes

Based on BLASTn search results of ITS, tub2 and rpb2 sequence data, *Smaragdiniseta musae* (Fig. 1) showed a high percentage identity (ITS = 97.23%, tub2 = 89.16% and rpb2 = 91.10%) without gaps to *S. bisetosa* (CBS 459.82). In the multigene phylogeny, *S. musae* clustered with *S. bisetosa* in having strong statistical support (100% ML, 1.00 BYPP) (Fig. 3). The base pair comparison of ITS, tub2 and rpb2 of our new taxon revealed 3.14% (17/540), 12.36% (35/283) and 9.34% (63/674) nucleotide differences with *S. bisetosa*. Besides, *S. musae* differs from *S. bisetosa* by the conidial morphology. The conidia of *S. musae* have two distinct guttules at the vertical poles. In addition, some conidia bear minute guttules at the centre. However, the taxonomic illustration and description of *S. bisetosa* did not indicate the guttule formation in the conidia (Rao and De Hoog 1983). Moreover, the conidia of *S. bisetosa* are obclavate, narrowly ellipsoidal or rod-shaped, whereas our new taxon has elliptic or slightly ovate-shaped conidia with a rounded top and an acute base. Both ends of the conidia of *S. bisetosa* are rounded or sometimes are found with a truncate base (Rao and De Hoog 1983). We have not observed a truncate base in the conidia of *S. musae*. Furthermore, the marginal hyphae of *S. musae* often coil or grow around the setae, but have never overgrown. The marginal hyphae always reach around 95–125 µm of the setae and terminate at a point. According to the description of Rao and De Hoog (1983), in *S. bisetosa*, the marginal hyphae always covered the entire setae. Our new collection is similar in morphology to the other genera in Stachybotryaceae in having conidiophores where the ultimate branches become phialides (Lombard et al. 2016). This feature phenotypically justifies the placement of our new collection in Stachybotryaceae. Based on distinct morphological characteristics and strong statistical support from our molecular phylogeny, *Smaragdiniseta musae* is, therefore, herein introduced as a new species on *Musa* sp. from Chiang Rai Province, Thailand. This is the first report of *Smaragdiniseta* on Musaceae from Southeast Asia. In addition, *S. musae* is the second taxon that is being described in this genus.

## *Albifimbria verrucaria* (Alb. & Schwein.) L. Lombard & Crous

- Lombard, Houbraken, Decock, Samson, Meijer, Réblová, Groenewald & Crous, *Persoonia* 36: 177 (2016) <https://doi.org/10.3767/003158516X691582>
- MycoBank [MB815927](https://www.mycobank.org/MB815927)
- FoF 04192 <https://www.facesoffungi.org/?s=FoF04192>

## Material

- a. scientificName: *Albifimbria verrucaria* (Alb. & Schwein.) L. Lombard & Crous; kingdom: Fungi; phylum: Ascomycota; class: Sordariomycetes; order: Hypocreales; family: Stachybotryaceae; taxonRank: species; genus: *Albifimbria*; specificEpithet: *verrucaria*; scientificNameAuthorship: (Alb. & Schwein.) L. Lombard & Crous; continent: Asia;

country: Thailand; stateProvince: Chiang Rai; municipality: Mae Sai; year: 2019; month: June; day: 25; habitat: Terrestrial; occurrenceRemarks: Found on a dead leaf of *Musa* sp.; recordNumber: BNS292; recordedBy: Binu C. Samarakoon; disposition: Living culture: MFLUCC 22-0017; associatedSequences: GenBank: MFLUCC 22-0017: [ON563487](#)(ITS), [ON586153](#)(tub2); identifiedBy: Binu C. Samarakoon; institutionID: MFLU; collectionID: MFLU 22-0048

## Description

Saprobic on dead leaves of *Musa* sp. **Sexual morph:** Undetermined. **Asexual morph:** Sporodochia cupulate or discoid, scattered or gregarious, having irregular or rounded outline composed of white marginal hypha, with conidial mass flattened or convex, pale olivaceous-green at an immature stage, black and shiny at maturity,  $10\text{--}18 \times 0.8\text{--}3 \mu\text{m}$  ( $\bar{x} = 12.3 \times 2.4 \mu\text{m}$ ,  $n = 20$ ). Stroma rarely well-developed, usually with a thin layer of isodiametric or elongated hyaline cells  $15\text{--}25$  ( $\bar{x} = 16.8 \mu\text{m}$ ,  $n = 10$ ). Setae: not observed. Marginal hyphae hyaline, usually verrucose, septate, curling and coiling, some branched, rounded or blunt at apex,  $1.5\text{--}4$  ( $\bar{x} = 3.3$ ,  $n = 20$ ) in diam. Conidiophores arising from a thin stromatic layer, hyaline, smooth,  $30\text{--}48 \times 1\text{--}2 \mu\text{m}$  ( $\bar{x} = 42.2 \times 1.7 \mu\text{m}$ ,  $n = 30$ ) septate, branching repeatedly, forming 2–4 branches at each level, with ultimate branches becoming phialides, which give rise to numerous conidia, conidiophores sometimes also arising from the hyphae. Phialides hyaline, rough-walled  $30\text{--}48 \times 1\text{--}2 \mu\text{m}$  ( $\bar{x} = 42.2 \times 1.7 \mu\text{m}$ ,  $n = 30$ ), 3–7 in a whorl, closely packed in a dense parallel layer, cylindrical, hyaline, collate at the base, rounded or acute at apex, sometimes slightly tapering towards apex,  $8\text{--}16 \times 1\text{--}3.5 \mu\text{m}$  ( $\bar{x} = 12.2 \times 1.8 \mu\text{m}$ ,  $n = 30$ ). Conidia broadly fusiform, always pointed at one end, mostly truncate or rounded at the other end, hyaline, sometimes sub hyaline, smooth,  $5\text{--}9.5 \times 2\text{--}3.5 \mu\text{m}$  ( $\bar{x} = 7.6 \times 3.0 \mu\text{m}$ ,  $n = 30$ ).

**Culture characteristics.** Conidia germinated on PDA after 12 hours. Colonies growing on PDA reaching 40 mm diam. after 2 weeks in the light conditions at 25°C, mycelium is mostly immersed, not slimy, cottony, white, dense in the middle and comparatively sparse at the periphery, fast-growing. Sporodochia formed after 12 days at the centre as a black uneven ring.

## Notes

Based on BLASTn search results of ITS, tub2 and rpb2 sequence data, our strain (MFLUCC 22-0017) showed a high similarity (ITS = 100% tub2 = 100% and rpb2 = 99%), excluding gaps to *Albifimbria verrucaria* (CBS 188.46). In the multigene phylogeny, MFLUCC 22-0017 grouped with *A. verrucaria* strains with strong statistical supports (97% ML, 1.00 BYPP) (Fig. 3). Morphologically, our collection (Fig. 2) is similar to the descriptions of Tulloch (1972) and Lombard et al. (2016). *Albifimbria verrucaria* has previously been reported from *Musa* sp. as a saprobe in Venezuela (Dennis 1970). This is the first report of *Albifimbria* from Thailand. MFLUCC 22-0017 is the first saprobic *A. verrucaria* strain found in Thailand. In addition, this is the second report of *Albifimbria* on Musaceae.

## Analysis

### Phylogenetic analyses

The combined ITS (1–599), rpb2 (604–1281) and tub2 (1286–1596) gene alignment was composed of 40 sequences that represented some of the selected taxa in Stachybotryaceae. The best scoring RAXML tree is presented (Fig. 3) with a final ML optimisation likelihood value of -13217.698. The matrix had 714 distinct alignment patterns with 15.47% of undetermined characters or gaps. Estimated base frequencies were: A = 0.239962, C = 0.278798, G = 0.255871, T = 0.225369; substitution rates AC = 1.609807, AG = 6.303653, AT = 1.525739, CG = 1.563045, CT = 10.838331, GT = 1.0; proportion of invariable sites I = 0.479596; gamma distribution shape parameter  $\alpha$  = 0.654983. All trees (ML and BI) resulting from the multi-gene alignment were equal in topology, without notable differences from Lombard et al. (2016). *Smaragdiniseta musae* (MFLUCC 22-0015, MFLUCC 22-0016) formed an independent lineage sister to *S. bisetosa* (CBS 459.82) (ML = 100%, BYPP = 1.00) with strong statistical support. In addition, MFLUCC 22-0017 grouped with *Albifimbria verrucaria* (CBS 188.46) (ML = 97%, BYPP = 1.00), respectively. RAXML trees generated from single gene alignments of ITS, rpb2 and tub2 sequences were attached as supplementary data (Suppl. material 1).

## Discussion

*Smaragdiniseta* has been previously documented as a saprobe only from terrestrial habitats (Rao and De Hoog 1983). There were no reports on pathogenic and endophytic lifestyles or sexual morphs that represent the genus. *Smaragdiniseta* was only discovered in India, while no other reports have been published on the occurrence worldwide. *Albifimbria* was subsequently discovered to show all three nutritional modes viz. as endophytes (Li et al. 2020, Wei et al. 2022), as pathogens (Gilardi et al. 2020, Herman et al. 2020, Rehman et al. 2021, Sharma et al. 2021) and as saprobes (Tulloch 1972). However, *Albifimbria* has mostly been discovered from plant hosts in terrestrial habitats (Tulloch 1972). In addition, the genus has also been reported in human blood (Masetti et al. 2020) and soil (Gurung et al. 2019, Murgia et al. 2019). Several genera of Stachybotryaceae, such as *Albifimbria*, *Memnoniella*, *Myrothecium* and *Stachybotrys*, are capable of producing bioactive compounds (Pervez et al. 2015, Wang et al. 2015, Li et al. 2020, Sun et al. 2020).

*Albifimbria verrucaria* has been reported as a plant pathogen that causes stem necrosis and leaf spots on various crops, such as *Glycine latifolia* (Herman et al. 2020), leafy vegetables (Matić et al. 2019, Rehman et al. 2021), ornamental crops (Matić et al. 2019) and tomato (Gilardi et al. 2020). In addition, *A. verrucaria* was also reported as an antagonistic agent on grapevine pathogens (Li et al. 2020). Additionally, *A. verrucaria* has been applied as a bio-pesticide to many weeds and nematodes (Assaf 2020). *Albifimbria verrucaria* can produce many lytic enzymes (viz. lipase, protease and kinase) which can degrade the cuticles of insects and, thus, can be used as an insecticide (Assaf 2020, Weaver et al. 2021). Chemical screening of *Smaragdiniseta* isolates has not been

conducted so far and still, the profiles remain undiscovered. Hence, apart from the taxonomic treatments, the chemical profiles of these genera also can be investigated as they are excellent sources of secondary metabolites. In addition, *A. verrucaria* was reported as a human pathogen causing keratomycosis (Moreno-Flores et al. 2020, Liu et al. 2021). Hence, there are opportunities for taxonomists to conduct sampling, isolation and identification of these hidden taxa from various hosts and provide baseline data for future research work.

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## Supplementary material

### Suppl. material 1: Maximum Likelihood trees

**Authors:** Binu C. Samarakoon

**Data type:** Phylogenetic

**Brief description:** Maximum Likelihood trees revealed by RAxML analyses ITS, btub and rpb2 single gene regions

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