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Additions to *Memnoniella* Species (Stachybotryaceae) Associated with *Bidens pilosa* and *Chromolaena odorata* (Asteraceae) in Northern Thailand and Their Potential Antibacterial Properties

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Abstract

Memnoniella species were isolated from *Bidens pilosa* and *Chromolaena odorata* in northern Thailand. Maximum likelihood and Bayesian inference analyses of the combined dataset of ITS, *tef1-α*, *rpb2* and *tub2* were conducted to confirm their taxonomic placement within *Memnoniella*. Updated phylogenetic analyses, together with detailed descriptions, and illustrations of the isolates are also presented. Based on the morphology and multi-gene phylogeny, *Memnoniella chromolaenae*, *M. oblongispora*, and *M. longistipitata* are synonymized under *M. levispora.* Additionally, we introduced *M. asteracearum* (MFLUCC 25-0166) as a new species and reported a new host record of *M. levispora.* This study also provides preliminarily evaluation of the antibacterial activity of *Memnoniella* species against three bacterial pathogens: *Bacillus subtilis* (TISTR 1248), *Staphylococcus aureus* (TISTR Y4b), and *Escherichia coli* (TISTR 527), revealing the strongest activity observed against *B. subtilis* (TISTR 1248). In addition, our study contributes to expand the understanding of weed-associated saprobic fungal diversity by documenting *Memnoniella* species on *Bidens pilosa* and *Chromolaena odorata.*

Keywords – 1 new species – 1 new host record – Ascomycota – Preliminary screening – multi-gene phylogeny

Introduction

Bidens pilosa and *Chromolaena odorata*, both belonging to the Asteraceae family, are significant, economically important, and widespread weeds (Xu et al. 2017, Mitra & Mukherjee 2018). These weeds are known for their ability to spread across various regions (Kriticos et al. 2005, Bairwa et al. 2010), often disrupting native plant communities and thriving in environments such as roadsides, agricultural lands, and disturbed areas (Zungsontiporn et al. 2007, Mapook et al. 2020, Htet et al. 2023, 2024). The interaction between fungi and weed communities has become a topic of significant interest, particularly as researchers increasingly explore the use of fungal pathogens for biological weed control (Hasan & Ayres 1990, Elango et al. 1993, Daba et al. 2021). However, the biodiversity of saprobic fungi associated with weeds remains underexplored, with

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only a few studies addressing it (Li et al. 2017, Mapook et al. 2020, Li et al. 2020, Htet et al. 2023, 2024).

Memnoniella, a genus within Stachybotryaceae (Hypocreales, Hypocreomycetidae, Sordariomycetes), was first introduced by Höhnel (1923). Morphologically, *Memnoniella* shares characteristics similar to *Stachybotrys.* This genus, once considered synonymous with *Stachybotrys*, has been the subject of taxonomic confusion that researchers have since discussed and clarified (Wang et al. 2015a). Subsequently, a study conducted by Lombard et al. (2016) on the Stachybotryaceae indicated that species of *Memnoniella* were found to cluster in a well-supported clade distinct from the *Stachybotrys* based on the combined cmdA, ITS, *rpb*2, *tef*1- *α* and *tub*2 sequence data. Additionally, *M. echinata* is also epitypified as the type (Lombard et al. 2016). Several new species and records were continuously described from *Memnoniella* (Li et al. 2003, Prasad et al. 2003, Lombard et al. 2016, Zheng et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Samarakoon et al. 2021, Yeh et al. 2023, Tian et al. 2024). Furthermore, Wang et al. (2015b) explored the chemical and bioactive secondary metabolites produced by *Memnoniella* species. *Stachybotrys* and *Memnoniella* species can produce a wide range of bioactive secondary metabolites, with almost 200 identified compounds which exhibit important biological activity and medicinal potential (Wang et al. 2015b).

Memnoniella species are characterized by macronematous, mononematous, unbranched conidiophores, phialidic conidiogenous cells with conspicuous collarettes, and unicellular, aseptate, smooth to verrucose conidia arranged in dry chains or slimy masses (Prasad et al. 2003, Lombard et al. 2016, Zheng et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Samarakoon et al. 2021, Yeh et al. 2023). The genus is cosmopolitan, with global reports across Asia, Europe, Africa, and the Americas, and occurs in diverse substrates including soil, plant material, paper, and human-associated environments (Lombard et al. 2016, Du et al. 2025). Currently, 35 species are listed in Index Fungorum (2026) and 26 species in Species Fungorum (2026).

In this study, we aimed to investigate *Memnoniella* species associated with *Bidens pilosa* and *Chromolaena odorata* in northern Thailand through detailed morphological characterization and multi-gene phylogenetic analyses, and to preliminarily evaluates their antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Our findings will add to the fungal diversity knowledge of *Memnoniella* species on *Bidens pilosa* and *Chromolaena odorata* from northern Thailand.

Material and Methods

Isolation and Identification

The specimens were collected from the dead stems of *Bidens pilosa* and *Chromolaena odorata* in Mueang Chiang Rai and Theong districts, Chiang Rai Province, Thailand. The specimens were examined using a Motic SMZ 168 Series microscope (Motic Asia, Hong Kong). Single spore isolation was conducted on malt extract agar (MEA), following the methods of Senanayake et al. (2020). Spore germination was observed within 24 hours, after which the spores were transferred to fresh MEA plates and incubated at room temperature. Colony characteristics were observed and recorded after 10 days. All micro-morphological characteristics were examined using a Nikon ECLIPSE 80i compound microscope (Nikon, Japan) equipped with a Canon 550D digital camera (Canon, Japan). Measurements of fungal structures were taken using Tarosoft Image Framework (v 0.9.7). Adobe Photoshop CS6 Extended (v 10.0) was used to edit and prepare photo plates (Adobe Systems, USA). Specimens were deposited in the herbaria of Mae Fah Luang University (Herb. MFLU), while living cultures were maintained in the Mae Fah Luang University culture collection (MFLUCC). Faces of Fungi (FoF) numbers and Index Fungorum (IF) numbers were registered according to the guidelines provided by Jayasiri et al. (2015) and Index Fungorum (2026). Additionally, species descriptions were submitted to the GMS Microfungi web database (Chaiwan et al. 2021). Species identification was confirmed following the recommendations outlined by Chethana et al. (2021) and Maharachchikumbura et al. (2021).

DNA extraction, PCR amplification and Sequencing

Forty-days old fungal mycelium was scraped off and transferred to a 1.5-mL microcentrifuge tube. DNA was extracted using the E.Z.N.A.® Tissue DNA Kit (Omega Biotek Inc., Georgia) according to the manufacturer's instruction. DNA amplification was performed by polymerase chain reaction (PCR) using the primer pairs as follows: internal transcribed spacer (ITS: ITS5 & ITS4; White et al. 1990), elongation translation factor-1 alpha (*tef1- α*, 728F & EF2; Carbone & Kohn 1999), RNA polymerase II subunit (*rpb2:* 5F & 7cR; Liu et al. 1999), and β-tubulin (*tub2:* Bt2a & Bt2b; Glass & Donaldson 1995). The final volume of the PCR reaction was 25 µl, containing 2 µl of DNA template, 1.5 µl of each forward and reward primer, 12.5 µl of MasterMix and 8.5 µl of ddH_2O. The PCR thermal cycling conditions for *tef1-α*, *rpb2* and *tub2* were as follows: initial denaturation at 94 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30s, annealing at 58 °C for 90s, elongation at 72 °C for 90 s, and a final extension at 72 °C for 10 min. The PCR thermal cycling program for ITS was processed by initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30s, elongation at 72 °C for 90 s, and a final extension at 72 °C for 10 min. The PCR products were checked on 1% agarose gel electrophoresis. Purification and sequencing of the PCR products was carried out at a commercial sequencing provider (Solgent Co., Ltd, Thailand), using the same primers as described above.

Sequence alignment and phylogenetic analyses

Analyzed sequences were obtained from the GenBank based on recent publications (Zheng et al. 2019, Mapook et al. 2020, Phukhamsakda et al. 2020, Samarakoon et al. 2021, Yeh et al. 2023, Du et al. 2025) and the Blastn search results. All sequences used in this study are listed in Table 1. Phylogenetic analyses were conducted using the combined ITS, *tef1-α*, *rpb2* and *tub2* sequence data. DNA sequence data were aligned using the MAFFT v. 7 online tool (http://mafft.cbrc.jp/alignment/server; 2016), and the combined sequences were manually adjusted through MEGA v. 6.0. Manual gap adjustments were done to improve the alignment, and ambiguously aligned regions were also excluded. The resulting combined sequence matrix contained a total of 2105 base pairs.

RAxML and Bayesian analyses were carried out on the CIPRESS Science Gateway Portal (http://www.phylo.org) (Miller et al. 2010). Maximum likelihood analysis was performed by RAxML-HPC v.8 (Stamatakis 2014) with rapid bootstrap analysis, followed by 1000 bootstrap replicates and the GTRGAMMA substitution model. MrBayes was used to perform BI analysis on XSEDE 3.2.7 (Ronquist et al. 2012), with trees sampled at every 1000^{th} generation during the 5,000,000-generation run of four concurrent Markov chains. The first 25% of the trees were removed as part of the burn-in phase, and calculations for the posterior probability were made for the remaining 75% of the trees (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002). The phylogenetic tree was displayed using Fig Tree v1.4.0 (Rambaut 2012) and was modified in Microsoft Office PowerPoint v. 2013.

Preliminary screening of antibacterial activity

The antibacterial potential of isolated strains was determined using the agar plug diffusion method (Balouiri et al. 2016). Three bacterial organisms were used to investigate this potential: Gram-positive *Bacillus subtilis* (TISTR 1248) and *Staphylococcus aureus* (TISTR Y4b), and Gram-negative *Escherichia coli* (TISTR 527). Ampicillin was used as the positive control to compare the inhibition zones of our strains (Alam et al. 2019). Following the protocols of Mapook et al. (2020) and Htet et al. (2023), the three bacterial test organisms were incubated and grown on nutrient agar for 24 hours. Subsequently, 2–3 loops of each bacterial test organism were inoculated to nutrient broth and incubated in a shaking incubator (140 rpm) at 37 °C for 24 hours. Sterile Mueller-Hinton (MH) agar media was used for antibacterial activity testing. Microbial suspensions were prepared by cell counting (6.7×10^5 cells/ml) and added to the sterile agar media prior to solidification. Fungal mycelium plugs from our isolates were then transferred to solid medium plates and

incubated for 24 to 48 hours. The antibacterial activity was evaluated by measuring and recording the zones of inhibition in millimeters (mm).

Results

Phylogenetic analyses

The dataset comprised 51 taxa from Stachybotryaceae and included four *Brevistachys* isolates (CBS 141058, CBS 141057, CBS 397.73, and CBS 696.73) as the outgroup. Maximum likelihood (ML) analyses and Bayesian Inference (BI) of the combined dataset were performed to determine the placement of our new isolates. The tree topology of ML and BI (not shown) was similar. The best-scoring RAxML tree with a final likelihood value of -14541.329906 is shown in Figure 1. RAxML analysis yielded 1081 distinct alignment patterns, with 35.65% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.230466, C = 0.285570, G = 0.261614, T = 0.222349; substitution rates: AC = 1.051962, AG = 3.896367, AT = 1.221785, CG = 1.005178, CT = 6.518191, GT = 1.000000; gamma distribution shape parameter α = 0.234062.

In our phylogenetic analyses, *Memnoniella* species formed an independent clade and were divided into four subclades (A, B, C, and D) in the phylogenetic tree (Fig. 1). Our three isolates are represented by two species within the genus (Fig. 1). Our isolate MFLUCC 25-0166 formed a sister clade to *M. ellipsoidea* isolates (CBS 136199, CBS 136200 and CBS 136201) with 92% ML and 0.84 BYPP support in Clade A. Our isolates, MFLUCC 25-0167 and MFLUCC 25-0165 clustered with the clade that comprises of *M. chromolaenae*, *M. oblongispora*, *M. longistipitata*, and *M. levispora* with 100% ML and 1.00 BYPP support (clade C).

Taxonomy

Memnoniella asteracearum Htet, A. Mapook &, K. W. T. Chethana, sp. nov. Fig 2.

Index Fungorum number: IF904382, Facesoffungi number: FOF17968

Holotype – MFLU 25-0084

Etymology – Name reflects the host plant family “Asteraceae”, from which this species was found.

Saprobic on dead stems of *Bidens pilosa* (Asteraceae). **Sexual morph:** Undetermined. **Asexual morph:** Colonies superficial, erect, scattered on the host substrate. *Mycelium* partly superficial and partly immersed. *Conidiophores* 120–150 × 7.5–8.5 µm ($\overline{x}$ = 130 × 8.1 µm, n = 5), macronematous, mononematous, erect, simple, straight or flexuous, unbranched, smooth, thick-walled, septate, hyaline to pale brown, bearing conidiogenous cells at the apex. *Conidiogenous cells* 8–12 × 5–10 µm ($\overline{x}$ = 11 × 6 µm, n = 5), phialidic, monophialidic, discrete, determinate, terminal, obovate, smooth, hyaline to pale brown. *Conidia* 9–13 × 5–7 µm ($\overline{x}$ = 11.1 × 5.5 µm, n = 20), acrogenous, aseptate, ellipsoidal, olivaceous brown to dark brown, verrucose, 1–2 guttules on the surface, thick-walled, rounded at both ends.

Culture characteristics – Conidia germinating on MEA within 24 hours, reaching 34 mm after 10 days at room temperature, irregular, filamentous, concentric, pale yellow, flat, transparent, slightly wrinkled on the surface, irregular, concentric, yellow to pale pink in reverse surface.

Material examined – Thailand, Chiang Rai Province, Mueang Chiang Rai District, on dead stems of *Bidens pilosa* (Asteraceae), 12 May 2023, Zin Hnin Htet, BP-HMS-2 (MFLU 25-0084, holotype), ex-type culture MFLUCC 25-0166.

Notes – In our phylogenetic analyses, our isolate (MFLUCC 25-0166) clustered sister with *M. ellipsoidea* (CBS 136199, CBS 136201 and CBS 136200) with 92% ML and 0.84 BYPP support (Fig 1). When comparing morphology, our isolate shares similar morphological characteristics features with the type strain of *M. ellipsoidea* (CBS 136201), such as macronematous, mononematous, straight or flexuous, unbranched conidiophores bearing phialidic conidiogenouse cells at the apex and acrogenous, aseptate, ellipsoidal conidia.

Table 1. GenBank accession numbers used in this study.

Species name	Isolates	GenBank accession numbers				References
		ITS	*rpb2*	*tub2*	*tef1- α*	
Brevistachys globosa	**CBS 397.73**	**KU846037**	**KU846073**	**KU846084**	**KU846100**	**Lombard et al. (2016)**
Brevistachys lateralis	**CBS 141058**	**MK442572**	**MK442689**	**MK442661**	**MK442727**	**Crous et al. (2019)**
Brevistachys ossiformis	CBS 696.73	KU846044	NA	KU846107	NA	Lombard et al. (2016)
Brevistachys variabilis	CBS 141057	NR_153620	NA	NA	NA	Lombard et al. (2016)
Memnoniella alishanensis	**MFLUCC 20-0168**	**MW114372**	**NA**	**MW148278**	**NA**	**Tennakoon et al. (2021)**
Memnoniella alishanensis	UESTCC 23.0159	PP831946	PP855580	PP838882	PP838852	Du et al. (2025)
Memnoniella alishanensis	CGMCC 3.25611	PP831947	PP855581	PP838883	PP838853	Du et al. (2025)
Memnoniella alishanensis	CGMCC 3.25612	PP831948	PP855582	PP838884	PP838854	Du et al. (2025)
Memnoniella asteracearum sp. nov.	MFLUCC 25-0166	PV345720	PX120472	PX246142	PV366413	This study
Memnoniella brunneoconidiophora	CBS 109477	KU846138	KU846192	KU846243	KU846218	Lombard et al. (2016)
Memnoniella brunneoconidiophora	**CBS 136191**	**KU846139**	**KU846193**	**KU846244**	**KU846219**	**Lombard et al. (2016)**
Memnoniella celtidis	**MFLUCC 20-0040**	**MW114374**	**NA**	**MW148280**	**NA**	**Tennakoon et al. (2021)**
Memnoniella celtidis	NCYUCC 19-0326c	MW114375	NA	MW148281	NA	Tennakoon et al. (2021)
Memnoniella chiangmaiensis	**MFLU 24-0303**	**PQ498098**	**NA**	**NA**	**NA**	**Sun et al. (2025)**
Memnoniella chromolaenae	MFLUCC 17-1507	MT214371	NA	NA	NA	Mapook et al. (2020)
Memnoniella cnidiicola	**CGMCC 3.25686**	**PP831951**	**PP855585**	**PP838887**	**PP838857**	**Du et al. (2025)**
Memnoniella cnidiicola	UESTCC 23.0167	PP831952	PP855586	PP838888	PP838858	Du et al. (2025)
Memnoniella dichroa	CBS 526.50	KU846140	KU846194	NA	KU846220	Lombard et al. (2016)
Memnoniella dichroa	**ATCC 18913**	**AF081472**	**NA**	**NA**	**NA**	**Haugland & Heckman (1998)**
Memnoniella echinata	CBS 304.54	KU846143	KU846197	NA	NA	Lombard et al. (2016)
Memnoniella echinata	CBS 343.50	KU846144	KU846198	KU846246	NA	Lombard et al. (2016)
Memnoniella echinata	**CBS 216.32**	**KU846142**	**KU846196**	**KU846245**	**KU846222**	**Lombard et al. (2016)**
Memnoniella ellipsoidea	CBS 136199	KU846150	KU846204	KU846252	KU846230	Lombard et al. (2016)
Memnoniella ellipsoidea	CBS 136200	KU846151	KU846205	KU846253	KU846231	Lombard et al. (2016)
Memnoniella ellipsoidea	**CBS 136201**	**KU846152**	**KU846206**	**KU846254**	**KU846232**	**Lombard et al. (2016)**

Table 1. Continued.

Species name	Isolates	GenBank accession numbers				References
		ITS	*rpb2*	*tub2*	*tef1- α*	
Memnoniella guttulatispora	**UESTCC 23.0164**	PP831954	PP855587	PP838890	PP838859	Du et al. (2025)
Memnoniella humicola	**CBS 463.74**	**KU846154**	**KU846208**	**NA**	**KU846234**	**Lombard et al. (2016)**
Memnoniella levispora	Memno0407	KF626494	NA	NA	NA	Santos et al. (2015)
Memnoniella levispora	Menlev3308	KF626495	NA	NA	NA	Santos et al. (2015)
Memnoniella levispora	MFLUCC 20-0189	MW477993	NA	MW480236	NA	Samarakoon et al. (2021)
Memnoniella levispora	MFLUCC 25-0165	PV345722	NA	NA	NA	This study
Memnoniella levispora	MFLUCC 25-0167	PV345723	PX120474	PX246149	PV366415	This study
Memnoniella. longistipitata	**ATCC 22699**	AF081471	NA	NA	NA	**Haugland & Heckman (1998)**
Memnoniella longistipitata	CBS 136197	KU846155	KU846209	KU846256	KU846235	Lombard et al. (2016)
Memnoniella mori	**MFLUCC 18-1640**	**MW114377**	**NA**	**MW148283**	**NA**	**Tennakoon et al. (2021)**
Memnoniella nilagirica	MFLUCC 15-0660	KU760374	KU760394	NA	NA	Lin et al. (2016)
Memnoniella oblongispora	MFLUCC 17-2064	MT310665	MT394724	NA	MT394676	Phukhamsakda et al. (2020)
Memnoniella oblongispora	MFLUCC 15-1074	KU760376	KU760396	KY124127	NA	Lin et al. (2016)
Memnoniella oenanthes	CBS 388.73	KU846156	KU846210	NA	NA	Lombard et al. (2016)
Memnoniella oenanthes	**ATCC 22844**	**AF081473**	**NA**	**NA**	**KU846236**	**Haugland & Heckman (1998)**
Memnoniella pseododichroa	**BCRC FU31689**	**ON692522**	**LC714856**	**LC714861**	**LC714858**	**Yeh et al. (2023)**
Memnoniella pseododichroa	BCRC FU31700	ON692523	LC714857	LC714862	LC714859	Yeh et al. (2023)
Memnoniella pseudonilagirica	**CBS 136405**	**KU846157**	**KU846211**	**KU846257**	**NA**	**Lombard et al. (2016)**
Memnoniella putrefolia	CBS 136171	KU846159	KU846213	KU846259	NA	Lombard et al. (2016)
Memnoniella putrefolia	**CBS 101177**	**KU846158**	**KU846212**	**KU846258**	**KU846239**	**Lombard et al. (2016)**
Memnoniella reniformis	**UESTCC 23.0170T**	**PP831959**	**PP855592**	**PP838895**	**PP838864**	**Du et al. (2025)**
Memnoniella reynoutriae	**CGMCC 3.25615T**	**PP831960**	**PP855593**	**PP838894**	**PP838865**	**Du et al. (2025)**
Memnoniella reynoutriae	UESTCC 23.0169	PP831961	PP855594	PP838896	PP838866	Du et al. (2025)
Memnoniella sinensis	**YMF 1.05582**	**MK773576**	**MK773575**	**MK773574**	**NA**	**Zheng et al. (2019)**
Memnoniella verrucosispora	**CGMCC 3.25614**	**PP831962**	**PP855595**	**PP838898**	**PP838867**	**Du et al. (2025)**
Memnoniella verrucosispora	UESTCC 23.0168	PP831963	PP855596	PP838899	PP838868	Du et al. (2025)

* Remarks: NA (not available). Ex-type isolates are bold. The newly generated sequences are indicated in blue color.

However, our isolate (MFLUCC 25-0166) differs from *M. ellipsoidea* in having longer conidiophores (120–150 × 7.5–8.5 µm vs. 55–140 × 4–8 µm), larger conidiogenous cells (8–12 × 5–10 µm vs. 8–12 × 4–6 µm), and larger conidia (9–13 × 5–7 µm vs. 8.5–9.5 × 4.5–5.5µm). The base pair difference between our isolate and *M. ellipsoidea* (CBS 136201) revealed ITS: 1.8% (10/553), *rpb2*: 1.9% (14/720), *tef1*-α: 5.1% (22/427), *tub2*: 0.3% (1/313). Therefore, we introduced *Memnoniella asteracearum* as a new species found on *Bidens pilosa* (Asteraceae) in northern Thailand.

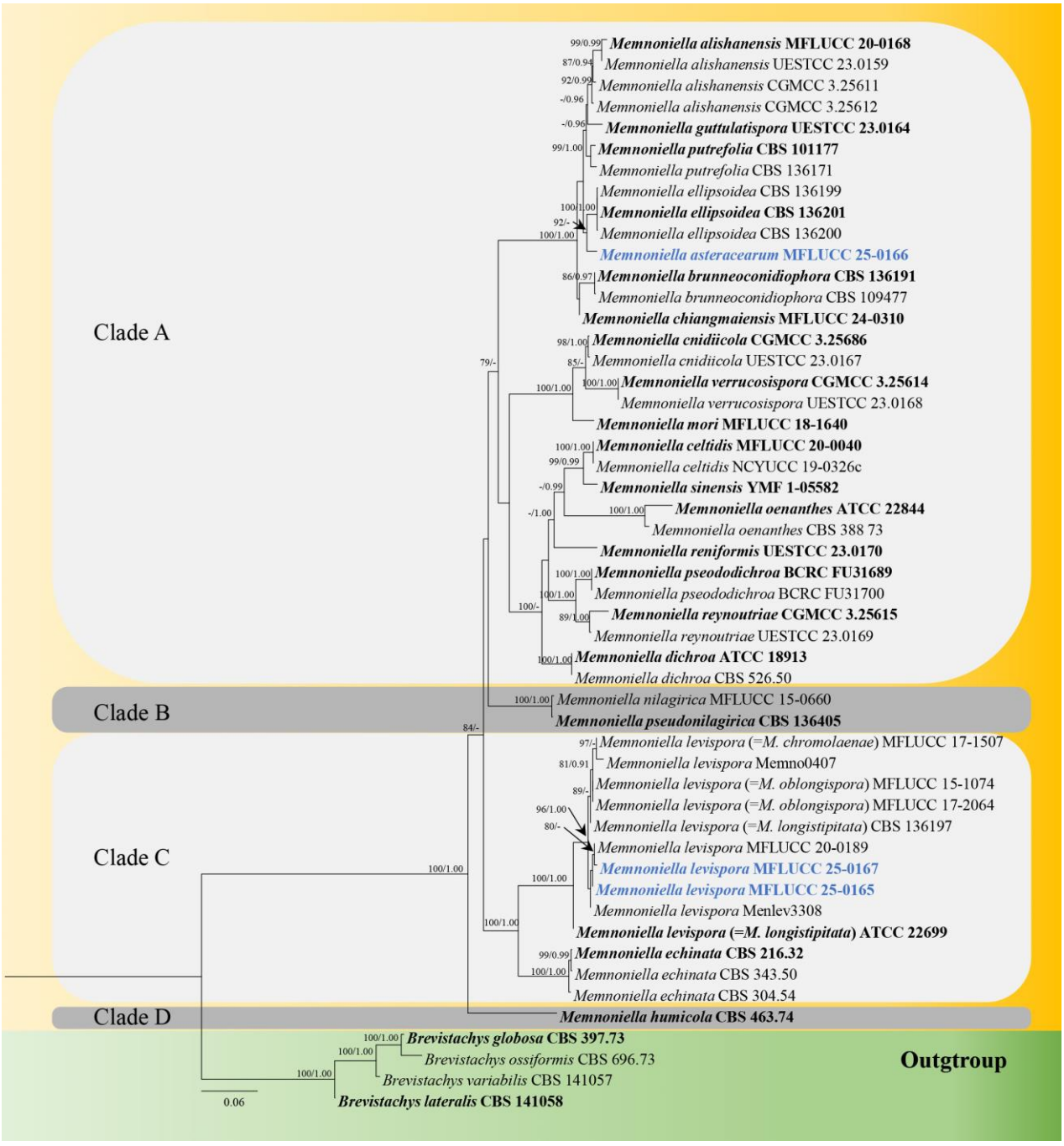


Fig. 1 – Phylogram generated from maximum likelihood analysis based on the combined dataset of ITS, *tef*1-α, *rpb*2 and *tub*2 sequence data. Bootstrap support values for ML equal to or greater than 75% and BYPP equal to or greater than 0.90 are given at the nodes. Newly generated sequences are in blue and type species are in **bold**.

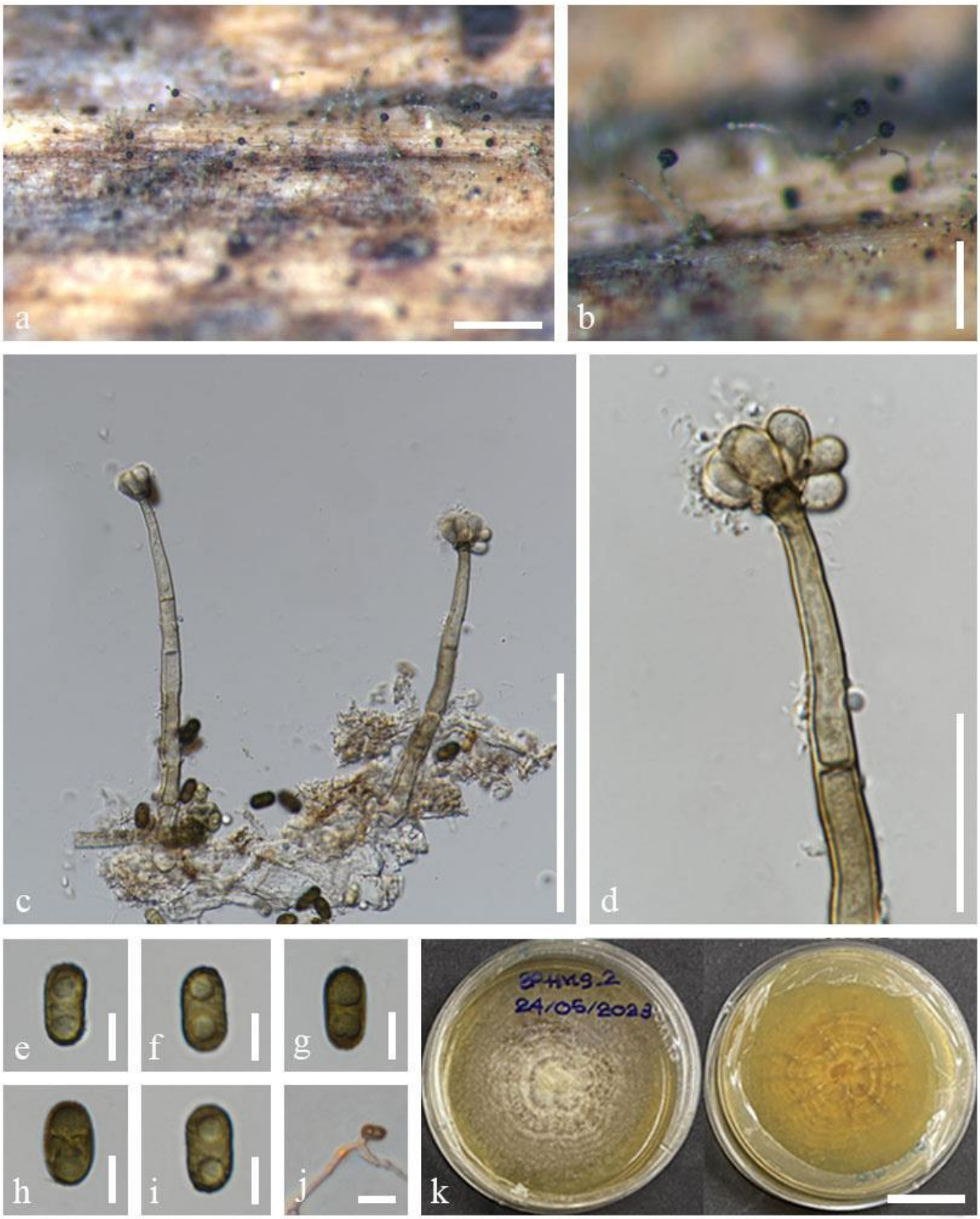


Fig. 2 – *Memnoniella asteracearum* (MFLU 25-0084, holotype). a, b Appearance of colonies on host substrate. c, d Conidiophores with conidiogenous cells. e–i Conidia. j A germinating conidium. k Culture on MEA. Scale bars: a = 200 µm, b = 100 µm, c = 50 µm, d = 30 µm, e–i = 5 µm, j = 10 µm, k = 1 mm.

Memnoniella levispora Subram., J. Indian Bot. Soc. 33: 40 (1954) Fig 3

= *Memnoniella longistipitata* Li, Yang, Haugland & Vesper, Mycotaxon 85: 254 (2003)

= *Memnoniella oblongispora* C.G. Lin, McKenzie, Yong Wang bis & K.D. Hyde, Mycosphere 7(9): 1280 (2016)

= *Memnoniella chromolaenae* Mapook & K. D. Hyde, Fungal Diversity 101: 135 (2020)

Index Fungorum number: IF552085, Facesoffungi number: FOF09573

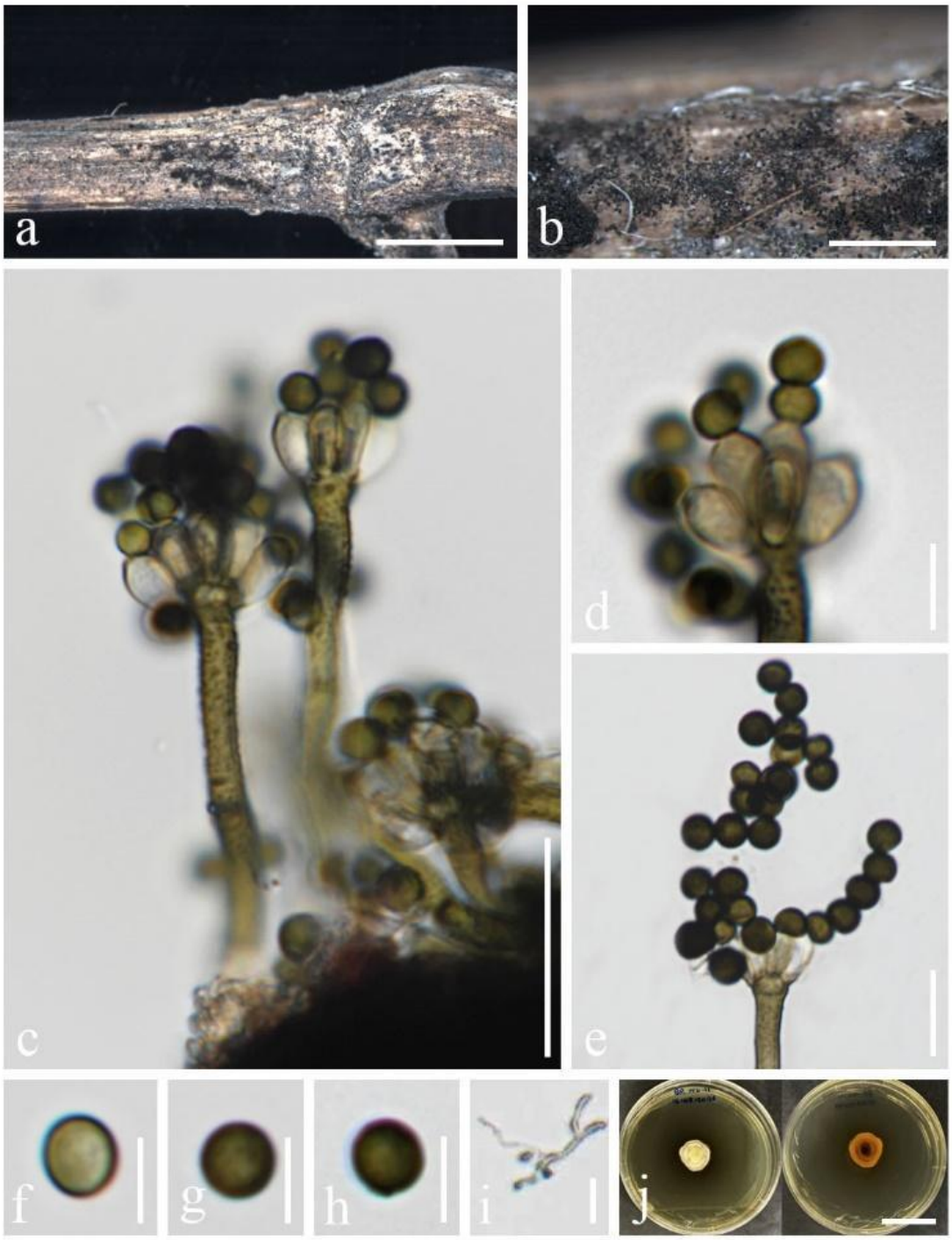


Fig. 3 – ***Memnoniella levispora*** **(MFLU 25-0086, new host record).** a, b Appearance of colonies on the host substrate. c–e Conidiophores and conidiogenous cells. f–h Conidia. i A germinating conidium. j Culture on MEA. Scale bars: a, b = 500 µm, c = 20 µm, d–i = 5 µm, j = 10 mm.

Saprobic on dead stems of *Bidens pilosa* (Asteraceae). **Sexual morph**: undetermined. **Asexual morph**: *Colonies* black and velvety on the substrate surface. *Conidiophores* 40–65 × 3–5 µm ($\overline{x}$ = 51.9 × 4 µm, n = 5), macronematous, mononematous, often simple, erect, straight or flexuous, irregularly or sympodially branched, rough, thick-walled, septate, olivaceous brown, bearing a crown of phialides at the apex. *Conidiogenous cells* 6–7 × 3–5 µm ($\overline{x}$ = 6.4 × 4.1 µm, n = 5), monophialidic, discrete, determinate, terminal, globose to subglobose, sometimes ellipsoidal, smooth, hyaline to olive-brown. *Conidia* 4–6 × 4–6 µm ($\overline{x}$ = 5.6 × 4.9 µm, n = 20), unicellular, simple, catenate, acrogenous, globose to subglobose, aseptate, olivaceous-grey to black, smooth, thick-walled, formed 5-10 conidia in long chains.

Culture characteristics – Conidia germinating on MEA within 24 hours, reaching 30 mm after 10 days at room temperature, irregular, entire, concentric, flat, white, changed to red on the culture media (MEA); irregular, concentric, pink to red in the reverse surface.

Material examined – Thailand, Chiang Rai Province, Nang Lae District, on dead stems of *Bidens pilosa* (Asteraceae), 3 August 2023, Zin Hnin Htet, BP-NL-11 (MFLU 25-0086), living culture MFLUCC 25-0165; *ibid*., Mueang Chiang Rai District, on dead stems of *Chromolaena odorata* (Asteraceae), 12 May 2023, Zin Hnin Htet, CO-DP-1 (MFLU 25-0085), living culture MFLUCC 25-0167.

Known host and distribution – on *Morus* (Moraceae) in India (Subramanian 1954), on *Musa* sp. (Musaceae) in China (Samarakoon et al. 2021), on *Oryza sativa* (Poaceae) in Cuba (Barrios & Pérez 2005), on *Roystonea regia* (Arecaceae) in Cuba (Arnold 1986), on *Sanchezia* sp. (Acanthaceae) in India and Pakistan (Gond et al. 2013), on *Tectona grandis* (Lamiaceae) in Thailand (Doilom et al. 2017), on unknown host in Japan (Li et al. 2003), on *Chromolaena odorata* (Asteraceae) in Thailand (Mapook et al. 2020), on *Quercus* sp. (Fagaceae) in Thailand (Lin et al. 2016), on *Clematis subumbellata* (Ranunculaceae) in Thailand (Phukhamsakda et al. 2020), on *Bidens pilosa* (Asteraceae) in Thailand (this study).

Notes – Our isolates MFLUCC 25-0165 and MFLUCC 25-0167 clustered with other isolates of *Memnoniella levispora* (MFLUCC 20-0189 and Menlev3308) with 100% ML and 1.00 BYPP support (Fig 1). Morphologically, our isolates are similar to *M. levirspora* in having straight or flexuous, macronematous, mononematous conidiophores, bearing phialidic conidiogenous cells, and spherical to subspherical conidia (Table 2). Comparisons of ITS, *tef1- α*, *rpb2*, and *tub2* sequences between phylogenetically related species revealed no significant base pair differences between species (Table 3). Thus, we identify our two isolates as *M. levispora*, found on the Asteraceae weeds *Bidens pilosa* and *Chromolaena odorata* in northern Thailand, with *B. pilosa* representing a new host record.

Preliminary study of antibacterial activity

Our study investigated the potential antibacterial activity of *Memnoniella asteracearum* and *M. levispra* using three bacterial test organisms: *Bacillus subtilis* (TISTR 1248), *Escherichia coli* (TISTR 527) and *Staphylococcus aureus* (TISTR Y4b). *Memnoniella asteracearum* (MFLUCC 25-0166) showed antibacterial activity against *B. subtilis* (TISTR 1248) with a 20 mm inhibition zone, observable as a partial inhibition, when compared with the positive control (15 mm), a 14 mm inhibition against *E. coli* (TISTR 527), observable as a complete inhibition when compared with the positive control (30 mm), and a 16 mm inhibition zone against *S. aureus* (TISTR Y4b), observable as a complete inhibition, when compared to positive control (25 mm). *Memnoniella levispora* (MFLUCC 25-0167) showed antibacterial activity against *B. subtilis* (TISTR 1248) with a 15 mm inhibition, observable as a partial inhibition when compared with the positive control (15 mm) and a 13 mm inhibition zone against *S. aureus* (TISTR Y4b), observable as a complete inhibition when compared with the positive control (25 mm) and no inhibition zone is detected for *E. coli* (TISTR 527) (Fig. 4). However, both fungal species showed a smaller inhibition zone than the positive control, ampicillin.

Discussion

Our study presented a synopsis of *Memnoniella* species (Table 4) and summarizes the morphology, host occurrence, and geographic distribution of species in *Memnoniella*. The species are predominantly recorded from plant-derived substrates, especially decaying leaves and wood, with hosts, representing a wide range of plant families (Lombard et al. 2016). Geographically, most species are distributed in tropical and subtropical regions, particularly in Asia, with China and Thailand showing the highest number of records. Additional occurrences from South America, Europe, and other regions indicate a broad but uneven global distribution of the genus (Lombard et al. 2016, Mapook et al. 2022, Du et al. 2025).

Table 2. Morphological comparison of *M. levispora* and *M. chromolaenae*, *M. oblongispora*, *M. longistipitata*

Species		Conidiophores	Conidiogenous cells	Conidia	References
M. chromolaenae	*Colonies* of dry conidial chains	Effuse colonies on the host, dark brown to black, powdery. Conidiophores are macronematous, mononematous, 40–85 × 2.5–4 µm, septate, erect, or flexuoux, unbranched, hyaline at the base, becoming dark grey to black towards the apex.	Bearing 6 conidiogenous cells at the apex of conidiophores, 5.5–7.5 × 3–4.5 µm, phialidic, discrete, ellipsoid or obovoid to clavate or reniform, smooth, subhyaline.	Catenate, acrogenous, globose to subglobose, aseptate, olivaceous-grey to black, long chain conidia, 3–4.5 × 3.5–4.8 µm.	Mapook et al. (2023)
	Colonies of slimy conidial chains (NA)				
M. oblongispora	*Colonies* of dry conidial chains	Effuse conies on the host, dark brown, hairy. Conidiophores are macronematous, mononematous, 30–70(–120) × 3–6 µm, septate, erect, or flexuoux, unbranced, hyaline at the base, becoming brown towards the apex.	Bearing a crown of conidiogenous cells at apex, 5–10 × 2.5–3.5 µm, monophialidic, discrete, oblong, pale brown.	Acrogenous, globose, aseptate, olivaceous, brown to dark brown, long chain conidia, 3–5 × 2.5–4.5 µm.	Phukhamsakda et al. (2020)
	Colonies of slimy conidial chains	Colonies on MEA. Conidiophores are macronematous, mononematous, 32–125 × 4–7 µm, septate, erect, or flexuoux, unbranched, hyaline at the base, becoming olive grey towards the apex	Bearing a crown of conidiogenous cells at the apex, 8–17 × 4.8–5.6 µm, monophialidic, discrete, pyriform, obovate, ellipsoidal, clavate or reniform, brown.	Acrogenous, spherical, oblong, aseptate, guttulate, aggregated in large, slimy, black and glistening heads, 8–14 × 4.5–7 µm.	
M. longistipitata	*Colonies* of dry conidial chains	Colonies on MEA. Conidiophores are very long, distinct, single, occasionally in groups, determinate, erect, or flexuous, unbranched, 260-–460 × 3.6–4.7 µm, hyaline at first and becoming olivaceous.	Bearing 3-9 conidiogenous cells at the apex, obovoid to ellipsoidal to clavate, light olivaceous, 9.7–10.2 × 4.7–5.5 µm, with noticeable collarettes.	Catenate, acrogenous, globose to subglobose, aseptate, hyaline at first and becoming dark olivaceous or black conidia, 5.8–8.5 × 6.3–8.3 µm.	Li et al. (2003)

Table 2. Continued.

Species		Conidiophores	Conidiogenous cells	Conidia	References
	Colonies of slimy conidial chains	NA	NA	Acrogenous, oblong or ovoid, aseptate, aggregate in slimy conidial chains, dark olivaceous, 10.6–11.9 × 4.8–5.7 µm.	
M. levispora	*Colonies* of dry conidial chains	Effuse colonies on the host, powdery, black. Conidiophores are macronematous, mononematous, 49–54 × 3–4 µm, septate, erect or flexuous, unbranched, hyaline at the base, becoming dark grey to black towards the apex.	Bearing a crown of conidiogenous cells at the apex, 6–8 × 3–5 µm, monophialidic, discrete, clavate, ellipsoidal or broadly fusiform, pigmented.	Catenate, acrogenous, globose, aseptate, blackish brown to black, long chain conidia, 4–6 × 4–6 µm.	Doilom et al. (2017)
	Colonies of slimy conidial chains (NA)				

Table 4. The number of polymorphic nucleotide differences between *M. levispora* MFLUCC 20-0189 and *M. chromolaenae*, *M. oblongispora*, *M. longistipitata* (without gaps)

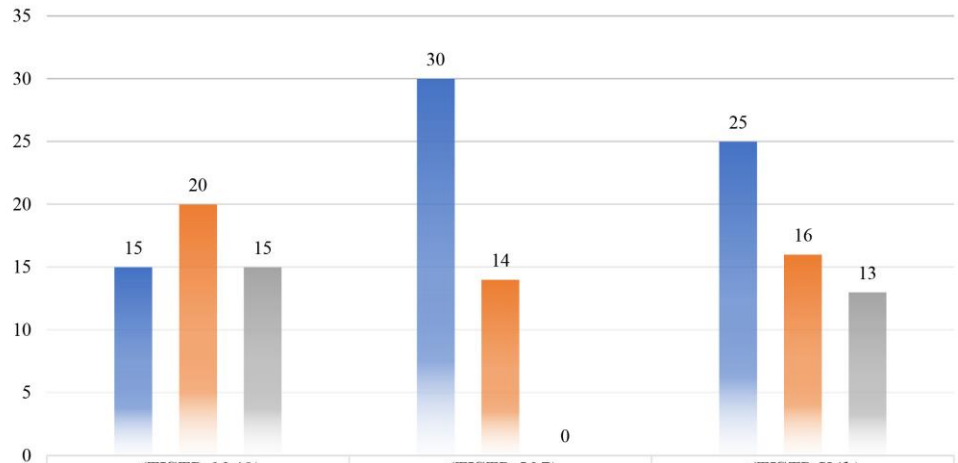
Species	ITS (553 bp)	*tef1-α* (427 bp)	*rpb2* (720 bp)	*tub2* (313 bp)
M. chromolaenae (MFLUCC 17-1507)	1	NA	NA	NA
M. oblongispora (MFLUCC 15-1074)	0	NA	NA	5
M. longistipitata (ATCC 22699)	3	NA	NA	NA
M. levispora (MFLUCC 25-0167)	4	15	3	4
M. levispora (MFLUCC 25-0165)	0	3	NA	0

Table 4. Morphology, host occurrence and geographic distribution of species in *Memnoniella.*

Species	Host/substrate	Conidiophores	Conidiogenous cells	Conidia	Location	References
M. alishanensis	*Macaranga tanarius* (Euphorbiaceae), *Morus australis* (Moraceae)	100–150 × 3–5 µm	7–10 × 3.5–5 µm	10–12 × 5–6 µm	Taiwan	Tennakoon et al. (2021)
M. brunneoconidiophora	From a decayed leaf of an unknown plant	30–45 × 3–5 µm	7–11 × 3–5 µm	(6–)7.5–8.5(–9) × (3–)3.5–4.5(–5) µm	Venezuela	Lombard et al. (2016)
M. celtidis	*Celtis formosana* (Cannabaceae)	100–140 × 5–6.3 µm	7.8–9.4 × 3.6–5 µm	9–11 × 4.5–6 µm	Taiwan	Tennakoon et al. (2021)
M. cnidiicola	*Cnidium monnieri* (Apiaceae)/ *Gynostemma pentaphyllum* (Cucurbitaceae)	180–236 × 11–16 µm	11–18 × 5.5–9 µm	9–14 × 5–8 µm	China	Du et al. (2025)
M. cunninghamiae	*Cunninghamia lanceolata*	70–250 × 2 4.5 µm	7.5–13.5 × 3–5 µm	3–5.5 × 2–3 µm	China	Tian et al. (2024)
M. dichroa	*Musa acuminata* (Musaceae), *Ilex aquifolium* (Aquifoliaceae)	up to 120 µm long	8 × 2 µm	10–12 × 4–5 µm	England, Thailand, Netherland	Photita et al. (2003)
M. echinata	*Macaranga tanarius* (Euphorbiaceae), *Triticum saltivum* (Poaceae)	40–100 × 4–6 µm	7–10 × 2–5 µm	3–6 × 3–5 µm	Canada, Indonesia, Japan, Solomon Islands, Netherlands	Lombard et al. (2016)
M. ellipsoidea	*Bromelia* sp. (Bromeliaceae), *Cananga odorata* (Annonaceae)	55–140 × 4–8 µm	8–12 × 4–6 µm	(8–)8.5–9.5 (–10) × (4–)4.5–5.5(–6) µm	Nepal, Thailand	Lombard et al. (2016)
M. guttulatispora	*Alsophila spinulosa* (Cyatheaceae)	115–240 × 10–14 µm	11–15 × 6–8 µm	9–13 × 5–7 µm	China	Du et al. (2025)
M. humicola	From soil under *Elaeis guineensis* (Arecaceae)	35–70 × 4–6 µm	6–9 × 2–4 µm	(5–)5.5–6.5(–7) × 2–3 µm	Suriname	Lombard et al. (2016)
M. levispora	*Musa* sp. (Musaceae), *Morus* sp. (Moraceae), *Oryza sativa* (Poaceae), *Roystonea regia* (Arecaceae), *Sanchezia* sp. (Acanthaceae), *Tectona grandis* (Lamiaceae)	43.6–60 × 2.5–4.7 µm	4–6.9 × 2.3–3.1 µm	2.5–4.3 × 1.5–3.6 µm	China, Cuba, India, Pakistan, Thailand	Samarakoon et al. (2021)

Table 4. Continued.

Species	Host/substrate	Conidiophores	Conidiogenous cells	Conidia	Location	References
M. levispora (=*M. oblongispora*)	*Quercus* sp. (Fagaceae), *Clematis subumbellata* (Ranunculaceae)	*Colonies* on the natural substrate 30–70(–120) × 3–6 µm	5–10 × 2.5–3.5 µm	3–5 × 2.5–4.5 µm	Thailand	Phukhamsakda et al. (2020)
M. levispora (=*M. chromolaenae*)	*Chromolaena odorata* (Asteraceae)	40–85 × 2.5–4 µm	5.5–7.5 × 3–4.5 µm	3–4.5 × 3.5–4.8 µm	Thailand	Mapook et al. (2020)
M. levispora (=*M. longistipitata*)	Unknown host	(170–) 260–460(–610) × (3.2–)3.6–4.7(–4.9) µm	9.7–10.2 × 4.7–5.5 µm	5.8–8.5 × 6.3–8.3 µm	Japan	Li et al. (2003)
M. mori	*Morus australis* (Moraceae)	70–110 × 4–6.5 µm	9–12 × 4–6 µm	10–13 × 5–8 µm	Taiwan	Tennakoon et al. (2021)
M. nilagirica	Decaying wood of an unknown plant	185–350 × 9–22 µm	13–20 × 3.5–11.5 µm	18–23 µm	Thailand	Lin et al. (2016)
M. oenanthes	*Euphorbia tirukalli* (Euphorbiaceae), *Ficus* (Moraceae)	120–180 × 6–9 µm	12–21 × 4–7 µm	reniform or ellipsoidal conidia, sometimes attenuated at the base	India, Thailand	Wang et al. (2015a)
M. pseudonilagirica	*Ceiba pentandra* (Bombacaceae)	150–300 × 9–15 µm	13–20 × 6–11 µm	(14–)18–22(–23) × (12–)17–21(–22) µm	Nepal	Lombard et al. (2016)
M. putrefolia	decayed leaf of Melastomataceae	50–90 × 5–9 µm	10–14 × 3–7 µm	(8–)9–11 × (4–)4.5–5.5(–6) µm	Brazil	Lombard et al. (2016)
M. pseodochroa	*Angiopteris lygodiifolia* (Marattiaceae)	(135–)176–243(–255) µm	(13–)14–16(–17) × 5–7(–8) µm	(13–)14–15.5(–17) × 6–7 µm (n=30) in R. Kirschner 5198, (11–)13–15(–16) × 6–7.5(–8) µm (n=30) in R. Kirschner 5373	Taiwan	Yeh et al. (2023)
M. reniformis	*Dryopteris* sp. (Dryopteridaceae)	164–255 × 7–16 µm	10–14 × 3–5 µm	9.5–12 × 5–6 µm	China	Du et al. (2025)
M. reynoutriae	*Reynoutria japonica* (Polygonaceae)	136–246 × 12–20 µm	15–17 × 5–6 µm	12–16 × 6.5–9 µm	China	Du et al. (2025)
M. sinensis	Unknown host	50–113×4.3–6.0 µm	9.5–12.5×4.9–7.2 µm ($\overline{x}$ =11.0×6.3 µm, n=20)	(9.5–)10.1–13.4 (–15.0) ×5.1–8.1(8.4) µm	China	Zheng et al. (2019)



	(TISTR 1248) *Bacillus subtilis*	(TISTR 527) *Escherichia coli*	(TISTR Y4b) *Staphylococcus aureus*
	Zone of inhibition (mm); Ampicillin (+)		
Poaitive control	*15*	*30*	*25*
Memnoniella asteracearum (MFLUCC 25-0166)	*20*	*14*	*16*
M. levispora (MFLUCC 25-0167)	*15*	*0*	*13*

Fig. 3 – Preliminary antibacterial activity result of isolated strains in this study.

Memnoniella species necessitates the use of molecular data for precise taxonomic identification (Wang et al. 2015a, Lombard et al. 2016). The morphological differences between *Memnoniella* and its related genus *Stachybotrys* have been discussed, particularly in terms of conidia production (Li et al. 2003, Wang et al. 2015a). *Stachybotrys* produces slimy conidia while *Memnoniella* produces dry conidia in chains (Li et al. 2013). Zuck (1946) suggested that isolates developing both types of conidia were intermediates between *Stachybotrys* and *Memnoniella*, making them important for understanding the evolutionary relationships between these two genera.

Some *Memnoniella* species (Clade C) exhibited both types of conidial development, such as *M. echinata*, *M. longistipitata* and *M. oblongispora* (Zuck 1946, Li et al. 2003, Phukhamsakda et al. 2020). Therefore, the presence of species with only dry conidial chains or both conidial types within Clade C raised questions, making these species difficult to distinguish and resulting in taxonomic confusion. For example, Lin et al. (2016) introduced *M. oblongispora*, characterized by slimy conidia production based on the phylogenetic analyses, showing it clustered with *M. longistipitata*. In a subsequent study, Phukhamsakda et al. (2020) identified new isolates of *M. oblongispora* capable of producing both conidia types. *Memnoniella oblongispora* was originally described as distinct from *M. longistipitata* by Lin et al. (2016), who did not observe its ability to produce an additional conidial form. Phukhamsakda et al. (2020) later confirmed the occurrence of both conidial types. The taxonomic placement of the clade C was unstable since some studies excluded *M. levispora* from their analyses (Phukhamsakda et al. 2020, Mapook et al. 2020, Yeh et al. 2023), while others included *M. levispora* but left out *M. oblongispora* (Samarakoon et al. 2021). Our study included all possible *Memnoniella* species to resolve phylogenetic placement of the isolate in Clade C. There were a few differences in the gene sequences of these species, which may simply represent variations within the same species (Table 2). However, the available of sequences from type strains is insufficient, which is the reason for having poorly resolved branches in Clade C. After a thorough examination of the morphology and phylogeny, we propose that *M. chromolaenae*, *M. oblongispora*, and *M. longistipitata* should be combined under *M. levispora* based on nomenclatural priority.

Additionally, the present study provides a preliminary evaluation of the antibacterial activity of *M. asteracearum* (MFLUCC 25-0166) and *M. levispora* (MFLUCC 25-0167) against

representative Gram-positive and Gram-negative bacteria. The strongest antibacterial activity was observed against *B. subtilis*, especially for *M. asteracearum*, which produced an inhibition zone of 20 mm. This finding suggests that metabolites produced by *Memnoniella* species may be more effective against Gram-positive bacteria. The relatively higher susceptibility of *B. subtilis* and *Staphylococcus aureus* compared to *Escherichia coli* is consistent with previous studies, which have shown that Gram-negative bacteria often exhibit increased resistance due to the presence of an outer membrane that limits the penetration of antimicrobial compounds (Zhou et al. 2023). In contrast, the simpler cell wall structure of Gram-positive bacteria may facilitate greater sensitivity to fungal secondary metabolites (Lelario et al. 2018).

Memnoniella asteracearum inhibited all three tested bacterial species, whereas *M. levispora* showed more limited activity, with no detectable inhibition against *E. coli*. This interspecific variation in antibacterial activity may reflect differences in secondary metabolite profiles, biosynthetic gene clusters, or metabolite concentrations between the two *Memnoniella* species. Such variability has been widely reported among closely related fungal taxa and highlights the importance of species-level screening when evaluating antimicrobial potential (Takahashi et al. 2021).

Despite the observed antibacterial effects, both *Memnoniella* species produced smaller inhibition zones than the positive control, ampicillin, indicating that their antibacterial activity is weaker than that of a standard broad-spectrum antibiotic. However, the detected inhibitory activity supports the hypothesis that *Memnoniella* species are capable of producing bioactive compounds with antibacterial properties. These findings warrant further investigation, including optimization of culture conditions, solvent extraction of secondary metabolites, determination of minimum inhibitory concentrations (MICs), and chemical characterization of the active compounds.

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Data Availability

All of the data that support the findings of this study are available in the main text

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