

## ***Metarhizium tawauensis* (Clavicipitaceae, Hypocreales), a new entomopathogenic fungus from Sabah, northern Borneo, Malaysia**

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### **Abstract**

*Metarhizium* Sorokin (Clavicipitaceae, Hypocreales) is a widely studied entomopathogenic fungi. Currently, *Metarhizium* has expanded to 92 species worldwide. The rainforests of Malaysia provide ideal ecological conditions that support high fungal diversity. In Malaysia, previous studies on entomopathogenic fungi (EPF) primarily focused on their applications as biological control agents, with limited attention to systematics and taxonomy. However, to date, studies on detailed systematics and phylogeny are still lacking in this region. During the entomopathogenic fungi survey in Sabah, Malaysia, an adult hemipteran host was collected, partially buried in soil near orchid *Plocoglottis plicata* (Roxb.) Ormerod. A new species, *Metarhizium tawauensis* (BORH/F03657 and BORH/F03658), is introduced based on detailed morphological observations, scanning electron microscopy (SEM) and is further supported by phylogenetic analyses of a concatenated ITS–TEF-1 $\alpha$ –RPB2 dataset. This study expands the known diversity of *Metarhizium* and provides essential taxonomic and phylogenetic baseline data for the newly described species, *M. tawauensis*, from Sabah, Malaysia. Future studies should focus on extensive surveys and multilocus genomic analysis to better understand the diversity, distribution and potential biocontrol applications.

**Key words:** novel taxon, Clavicipitaceae, Hypocreales, morphology, and phylogenetic analysis

### **Introduction**

*Metarhizium* (Clavicipitaceae, Hypocreales, Hypocreomycetidae, Sordariomycetes) represents one of the most significant genera within the clavicipitoid fungi, a morphologically and ecologically diverse lineage extensively investigated for its role in insect biological control (Yang *et al.* 2025, Wijayawardene *et al.* 2022, Wei *et al.* 2022, Hyde *et al.* 2024). Phylogenetic studies have revealed a complex evolutionary history within the genus, highlighting adaptive diversification that underpins its broad ecological distribution and versatility (Kobmoo *et al.* 2024, Kepler *et al.* 2014).

This genus was originally described by Metchnikoff (1879) from infected larvae of *Anisoplia austriaca* (Coleoptera) in Russia, where it was associated with the “green muscardine disease” in its asexual morph (Zimmermann *et al.* 1995). A comprehensive taxonomic revision by Tulloch (1976) reduced the genus to two species, *Metarhizium anisopliae* (Metschn.) Sorokin and *Metarhizium flavoviride* W. Gams & Rozsypal, recognizing intraspecific varieties within *M. anisopliae* (Metschn.) Sorokin based primarily on conidial dimensions. Earlier taxa, including *M. album* (Petch 1931) and *M. brunneum* (Petch 1934), were subsequently synonymized under *M. anisopliae* (Metschn.) Sorokin. Later, Rombach *et al.* (1986, 1987) refined species delimitation using morphological traits including conidial and

phialide structure, the formation of prismatic conidial columns, and the presence or absence of subhymenial zones. The advancement of molecular phylogenetics, initially employing ITS sequence data and later multigene datasets, led to the recognition of several phylogenetic lineages and the elevation of previously defined varieties to species rank (Kim *et al.* 2025, Würf and Schmidt 2025, Kepler *et al.* 2012, Luangsa-ard *et al.* 2017). Subsequent studies demonstrated that certain taxa formerly assigned to *Metarhizium* were phylogenetically distinct and reassigned to other clades, underscoring the critical role of integrative systematics in resolving species boundaries (Mongkolsamrit *et al.* 2020).

Species of *Metarhizium* are globally distributed and exhibit diverse asexual morphologies and life cycle strategies (Yapa *et al.* 2025, Luangsa-ard *et al.* 2017). The asexual morph is characterized by branched conidiophores producing green to brown conidia (Lin *et al.* 2025, Chen *et al.* 2022), whereas the sexual morph forms stromata that are whitish-greenish yellow to green, bearing partially or completely immersed perithecia (Kobmoo *et al.* 2025, Kepler *et al.* 2014). Members of the genus display broad host ranges, parasitizing more than 200 insect species across 42 families and eight orders including Orthoptera, Coleoptera, Hemiptera, Diptera, Hymenoptera, and Lepidoptera as well as mites and nematodes (Chen *et al.* 2018a, 2018b, Nishi & Sato 2019, Mongkolsamrit *et al.* 2020, Thanakitpipattana *et al.* 2020). Many taxa are currently accommodated within the *Metarhizium anisopliae* species complex, with certain species exhibiting host specificity, such as *M. acridum* (Driver & Milner) J.F. Bisch., S.A. Rehner & Humber and *M. gryllicicola* Khons., Thanakitp. & Luangsa-ard (Vega *et al.* 2012, Thanakitpipattana *et al.* 2020).

Asia is considered a major centre of diversity for *Metarhizium*, with numerous species documented from China and Japan (Guo *et al.* 1986, Chen *et al.* 2023, Li *et al.* 2023). According to Index Fungorum (<https://www.indexfungorum.org/Names/Names.asp>, accessed March 11, 2026), the genus *Metarhizium* consists of 92 accepted species. Despite the favorable ecological conditions of tropical Southeast Asia for entomopathogenic fungi, regional surveys remain limited. In Malaysia, particularly in Sabah, investigations into *Metarhizium* diversity are still scarce, notwithstanding the region's high insect biodiversity and extensive forest ecosystems. Previous records of *Metarhizium* from Malaysia are extremely limited and are mostly associated with non-insect substrates; for example, *Metarhizium carneum* was reported from speleothem surfaces in Balambangan Cave, Sabah (Wasti *et al.* 2024).

*Metarhizium* species are regarded as environmentally sustainable alternatives to chemical pesticides and are widely implemented in integrated pest management programs (Hussien *et al.* 2025, Radha *et al.* 2025, Yao *et al.* 2025). Commercial formulations of *M. anisopliae* have been successfully deployed in Brazil, Iran, Japan, Thailand, and the USA (Jackson & Jaronski 2008, Ghayedi & Abdollahi 2013, Thongkaewyuan & Chairin 2018). Consequently, accurate species delimitation through integrative taxonomy is essential to ensure efficacy, host specificity, and biosafety in biocontrol applications (Bischoff *et al.* 2009, Luangsa-ard *et al.* 2017, Chen *et al.* 2018).

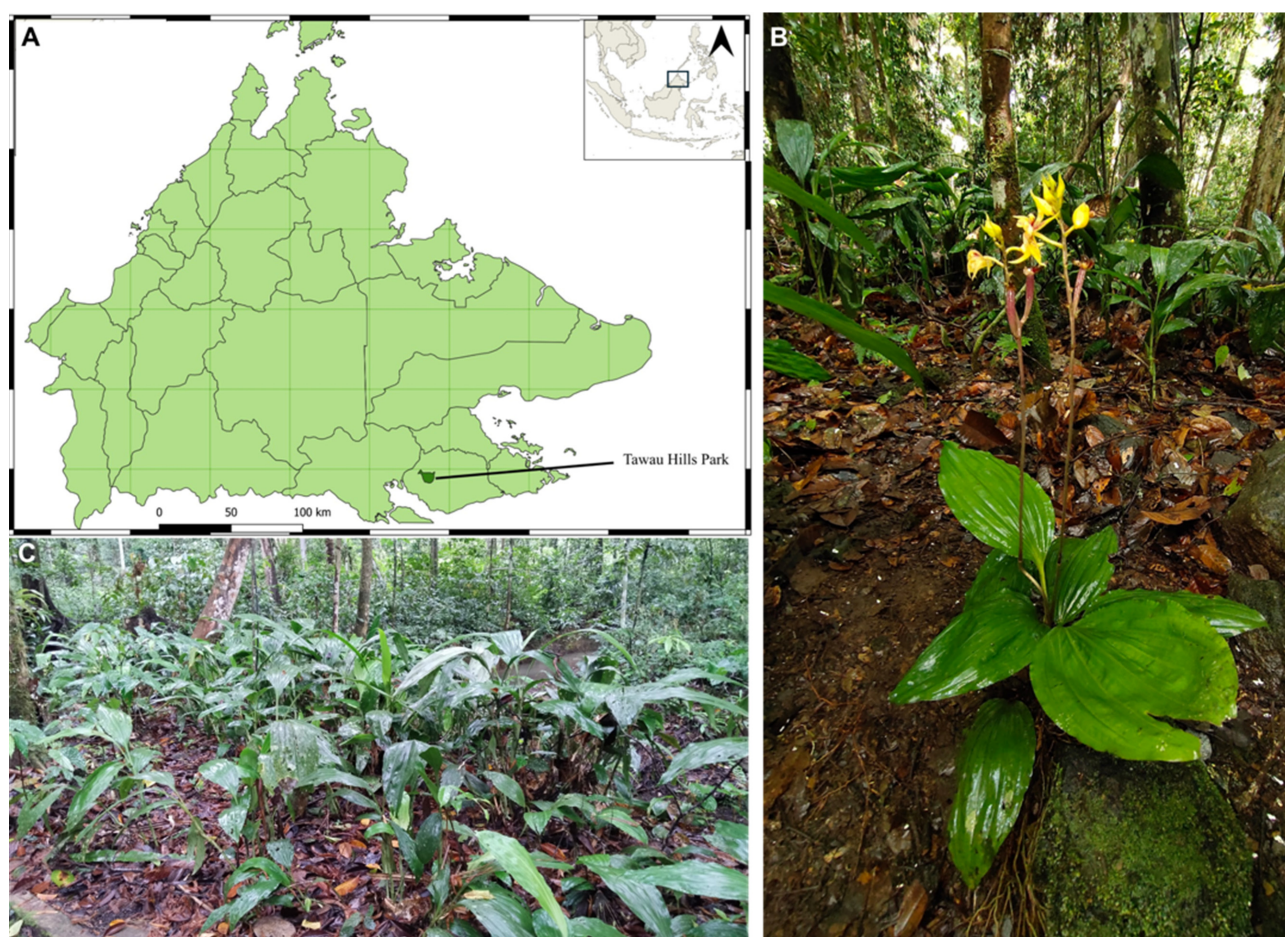
Tawau Hills Park is a 27,972-ha protected area in Sabah, Malaysia, containing lowland dipterocarp forest, which transitions into thick, damp mossy forest at higher altitudes. The Botanical Garden at Tawau Hills Park is an ex-situ conservation facility established in 1999 and opened to the public in 2006 (Sabah Parks, 2024). The garden features seven themed zones and an orchid collection of more than 150 species (Sabah Parks, 2024; MyBIS, 2024).

In the present study, we introduce *Metarhizium tawauensis*, a novel entomopathogenic fungus associated with Hemiptera collected from Sabah, Malaysia. This study utilizes morphological and phylogenetic evidence to describe the new species, further resolving the taxonomic complexity of *Metarhizium* in Southeast Asia.

## Materials and methods

### *Sampling sites and sample collection*

Two fresh entomopathogenic fungal specimens with their hosts were collected from the Botanical Garden, Tawau Hills Park, Tawau (N 04°39.916', E 118°28.841'), Sabah, Malaysia, at an elevation of 287 m, buried in soil near orchid *Plocoglottis plicata* (Roxb.) Ormerod, on 20 January 2023 (FIGURE 1). The host insect was identified in the field as an adult hemipteran based on gross morphological features; however, due to the absence of diagnostic taxonomic characters and specimen preservation constraints, identification to a lower taxonomic level was not possible. Important collection information was recorded. Micromorphological features, including synnemata, conidiophores, phialides, and conidia, were documented using a compound microscope (Leica DM2500 LED, Germany), which was operated at magnifications up to ×1000 (Senanayake *et al.* 2020).



**FIGURE 1.** (A) Study site map showing the sampling locality. (B–C) Natural habitat of the species at the collection site, partially buried in soil near orchid *Plocoglottis plicata* (Roxb.) Ormerod in the Botanical Garden, Tawau Hills Park, Tawau, Sabah, Malaysia.

### Morphological observation

Macromorphological characteristics and associated data, including host location in the field, stromatal color, shape, and perithecial orientation, were examined using a dissecting microscope (Olympus SZ61). For micro-morphological observations on the host, including perithecia, asci, ascus tip, and ascospores, a small portion of stroma from the host was mounted in 1% Congo red dye, and then a compound microscope (Leica DM2500 LED, Germany) was used at magnifications up to  $\times 1000$ . Measurements were taken from 15 structures for each character (Yang *et al.* 2025, da Costa Silva *et al.* 2025). For scanning electron microscopy (SEM), samples were initially fixed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.2) and kept overnight at 4 °C. After fixation, the specimens were rinsed three times in the same buffer and subsequently post-fixed in 1% osmium tetroxide for 1 h at 4 °C. The samples were then dehydrated through 70% ethanol and dried using a CO<sub>2</sub> critical-point dryer. The dried materials were mounted onto aluminium stubs with carbon adhesive tape, sputter-coated with an approximately 10 nm thick layer of gold–palladium, and observed under a scanning electron microscope at an accelerating voltage of 5–10 kV (Güerri-Agulló *et al.* 2010).

For culture isolation, a small portion of fresh tissue from the host was extracted, sterilized using 70% ethanol for 3 times, rinsed with water, dried on sterilized filter paper and transferred onto potato dextrose agar (PDA), and then incubated at 25 °C. For cultural characterization, isolates were regrown on potato dextrose agar (PDA) and incubated at 25 °C under alternating light and dark conditions to attain pure cultures. Cultures were examined for colony morphology, growth rate, pigmentation, and the size and shape of phialides and conidia (Mongkolsamrit *et al.* 2020). Colony colors of fresh specimens and PDA-grown cultures were described and standardized using the Methuen Handbook of Color (Kornerup & Wanscher 1963).



### DNA Extraction, PCR and Sequencing

Genomic DNA was extracted from freshly grown mycelium obtained from pure culture and directly from host stromata using the E.Z.N.A.® Plant/Fungal DNA Kit, following the manufacturer's instructions. The extracted total genomic DNA was stored at -20 °C until further use. The internal transcribed spacer region (ITS) was amplified using primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.* 1990). The translation elongation factor 1-alpha gene (*TEF-1α*) region was amplified using primers 983F (5'-GGTGACGGTGACCTTGATGGCACG-3') and 2218R (5'-AAACAGGGTGGTGACCGTGATGGGTTG-3') (Rehner and Buckley 2005). The second largest subunit of RNA polymerase II gene (RPB2) was amplified using primers RPB2\_F1 (5'-CAAACCGAAGGCAGCGAGAC-3') and RPB2\_F76 (5'-CCTGATGACCGTGATCACTT-3'), and the PCR products were sequenced.

Polymerase chain reaction (PCR) was performed in a total reaction volume of 50 µl containing 22 µl of 2× Taq PCR StarMix with Loading Dye (GenStar), 20 µl of double-distilled water, 2 µl of each primer, and 4 µl of DNA template. Amplifications were carried out using a Bio-Rad T100 Thermal Cycler (Singapore) under the following conditions: an initial denaturation at 94 °C for 3 min; followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 50 s, and extension at 72 °C for 45 s for ITS; and 33 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 50 s, and extension at 72 °C for 1 min for *TEF-1α*; and 33 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 50 s, and extension at 72 °C for 1 min for RPB2; with a final extension at 72 °C for 10 min (Rehner and Buckley 2005, Bischoff *et al.* 2009). The PCR products were purified and sequenced by Apical Scientific Sdn. Bhd, Selangor, Malaysia, using the same primer pairs. The obtained sequences were manually edited to remove ambiguous regions using BioEdit v.7.0.5.3 (Hall *et al.* 2011). The resulting ITS, *TEF-1α* and RPB2 sequences were deposited in GenBank, and accession numbers are provided in Table 1.

**TABLE 1.** List of Specimens and their GenBank Accession numbers used in this study.

| Species                      | Strain                | ITS       | <i>Tef-1α</i> | <i>RPB2</i> | References                         |
|------------------------------|-----------------------|-----------|---------------|-------------|------------------------------------|
| <i>Metarhizium acridum</i>   | ARSEF7486             | NA        | EU248845      | EU248925    | Bischoff <i>et al.</i> (2009)      |
| <i>M. acrididarum</i>        | HQ331446              | PX411451  | NA            | NA          | Unpublished                        |
| <i>M. album</i>              | ARSEF2082             | NA        | DQ522352      | DQ522452    | Sung <i>et al.</i> (2007)          |
| <i>M. alvesii</i>            | LSB 91                | NA        | KY007614      | KY007613    | Lopes <i>et al.</i> (2018)         |
| <i>M. alvesii</i>            | LSB 87                | PP150490  | NA            | NA          | Lopes <i>et al.</i> (2018)         |
| <i>M. anisopliae</i>         | ARSEF7487             | HQ331446  | DQ463996      | DQ468370    | Bischoff <i>et al.</i> (2009)      |
| <i>M. anisopliae</i>         | CBS13071              | MT078884  | MT078845      | MT078918    | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. argentinense</i>       | CEP424                | NA        | MF966624      | MF966626    | Gutierrez <i>et al.</i> (2019)     |
| <i>M. atrovirens</i>         | TNMF10184             | JN049882  | NA            | NA          | Kepler <i>et al.</i> (2012)        |
| <i>M. brachyspermum</i>      | IFM 65744             | NR_198026 | NA            | NA          | Unpublished                        |
| <i>M. brittlebankisoides</i> | GZAAS5                | KC561835  | KC561899      | NA          | Unpublished                        |
| <i>M. baoshanense</i>        | BUM634                | KY264173  | KY264170      | KY264184    | Chen <i>et al.</i> (2018)          |
| <i>M. baoshanense</i>        | CCTCCM2016589         | KY264172  | KY264169      | KY264183    | Chen <i>et al.</i> (2018)          |
| <i>M. bibionidarum</i>       | CBS64867              | NA        | LC126075      | LC125923    | Nishi <i>et al.</i> (2017)         |
| <i>M. bibionidarum</i>       | NBRC112661            | NA        | LC126076      | LC125924    | Nishi <i>et al.</i> (2017)         |
| <i>M. biotecnense</i>        | BCC51812 <sup>T</sup> | MN781878  | MN781693      | MN781792    | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. biotecnense</i>        | BCC51813              | MN781879  | MN781694      | MN781793    | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. blattodeae</i>         | MY00896               | HQ165697  | HQ165678      | HQ165638    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. brasiliense</i>        | ARSEF2948             | NA        | KJ398809      | NA          | Kepler <i>et al.</i> (2014)        |
| <i>M. culicidarum</i>        | BCC2673               | NA        | MN781706      | MN781753    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. culicidarum</i>        | BCC7625               | NA        | MN781705      | BCC7625     | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. candelabrum</i>        | BCC29224              | NA        | MN781708      | MN781804    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. candelabrum</i>        | BCC31660              | NA        | MN781709      | MN781805    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. chaiyaphumense</i>     | BCC28262              | NA        | QHW05239      | QHW05295    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. chaiyaphumense</i>     | BCC28241              | NA        | QHW05240      | QHW05340    | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. cicadae</i>            | BCC48696              | NA        | QHW05259      | QHW05356    | Lin <i>et al.</i> (2025)           |

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TABLE 1. (Continued)

| Species                   | Strain                   | ITS      | <i>Tef-1a</i> | <i>RPB2</i> | References                             |
|---------------------------|--------------------------|----------|---------------|-------------|----------------------------------------|
| <i>M. clavatum</i>        | BCC84543                 | NA       | QNJ99315      | QHW05345    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. cylindrosporum</i>  | CBS 25690                | NA       | AHW83607      | AHW83418    | Li <i>et al.</i> (2010)                |
| <i>M. cylindrosporum</i>  | YFCC 970                 | NA       | XAT91544      | XAT91459    | Li <i>et al.</i> (2010)                |
| <i>M. dianzhongense</i>   | KUNCC 10809              | NA       | WZP33353      | WZP33347    | Wei <i>et al.</i> (2024)               |
| <i>M. dianzhongense</i>   | KUNCC 10810              | NA       | WZP33354      | WZP33348    | Wei <i>et al.</i> (2024)               |
| <i>M. eburneum</i>        | BCC79267                 | NA       | NA            | QHW05291    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. eburneum</i>        | BCC79252                 | NA       | QHW05238      | QHW05292    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. ellipsoideum</i>    | BCC53509                 | NA       | QHW05270      | QHW05365    | Unpublished                            |
| <i>M. ellipsoideum</i>    | BCC49285                 | NA       | QHW05269      | QHW05364    | Unpublished                            |
| <i>M. ellipsoideum</i>    | BCC12847                 | NA       | QHW05271      | QHW05366    | Unpublished                            |
| <i>M. flavoviride</i>     | CBS12565                 | NA       | ABE68823      | AKP21149    | Unpublished                            |
| <i>M. formicae</i>        | SY09321                  | PV082703 | PV171223      | PV171167    | Unpublished                            |
| <i>M. frigidum</i>        | ARSEF 4124               | NA       | ABE68825      | QCF59683    | Bischoff <i>et al.</i> (2006)          |
| <i>M. fusoideum</i>       | BCC53130                 | NA       | QHW05254      | QHW05351    | Unpublished                            |
| <i>M. fusoideum</i>       | BCC28246                 | NA       | QHW05255      | QHW05352    | Unpublished                            |
| <i>M. gaoligongense</i>   | CCTCCM2016588            | NA       | ARB09015      | ARB09021    | Unpublished                            |
| <i>M. gaoligongense</i>   | YFCC 824                 | NA       | XAT91408      | XAT91579    | Unpublished                            |
| <i>M. gryllidicola</i>    | BCC82988                 | NA       | QNJ99327      | QGX86513    | Thanakitpipattana <i>et al.</i> (2020) |
| <i>M. gryllidicola</i>    | BCC53857                 | NA       | QNJ99325      | QGX86537    | Thanakitpipattana <i>et al.</i> (2020) |
| <i>M. guniujiangense</i>  | RCEF880                  | NA       | UQS49349      | UQS49382    | Chen <i>et al.</i> (2022)              |
| <i>M. guniujiangense</i>  | RCEF6758                 | NA       | UQS49354      | UQS49405    | Chen <i>et al.</i> (2022)              |
| <i>M. huainamdangense</i> | BCC44270                 | NA       | QHW05268      | QHW05363    | Zhao <i>et al.</i> (2025)              |
| <i>M. huainamdangense</i> | BCC32190                 | NA       | QHW05266      | QHW05313    | Zhao <i>et al.</i> (2025)              |
| <i>M. huainamdangense</i> | BCC7672                  | NA       | QHW05267      | QHW05362    | Zhao <i>et al.</i> (2025)              |
| <i>M. indigoticum</i>     | TNSF18553                | NA       | JF416010      | JF415992    | Kepler <i>et al.</i> (2012)            |
| <i>M. indigoticum</i>     | TNSF18554                | NA       | JF416011      | JF415993    | Kepler <i>et al.</i> (2012)            |
| <i>M. kalasinense</i>     | BCC53582                 | KC011179 | KC011189      | NA          | Luangsa-ard <i>et al.</i> (2017)       |
| <i>M. koreanum</i>        | ARSEF2039                | NA       | KJ398806      | NA          | Kepler <i>et al.</i> (2014)            |
| <i>M. koreanum</i>        | BCC27998                 | MN781903 | MN781700      | MN781797    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. koreanum</i>        | BCC30455                 | MN781904 | MN781701      | MN781798    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. lepidiotae</i>      | ARSEF7488                | NA       | EU248865      | EU248945    | Bischoff <i>et al.</i> (2009)          |
| <i>M. lymantriidae</i>    | BUM818                   | OM955147 | OM988196      | OM988188    | Chen <i>et al.</i> (2023)              |
| <i>M. lymantriidae</i>    | KUNCC4991 <sup>T</sup>   | OM955148 | OM988197      | NA          | Chen <i>et al.</i> (2023)              |
| <i>M. macrosemae</i>      | RCEF6696                 | MW718317 | MW723113      | MW723164    | Chen <i>et al.</i> (2022)              |
| <i>M. macrosemae</i>      | RCEF6729                 | MW718319 | MW723115      | MW723165    | Chen <i>et al.</i> (2022)              |
| <i>M. majus</i>           | MDOAE201                 | MZ749667 | NA            | NA          | Bischoff <i>et al.</i> (2009)          |
| <i>M. majus</i>           | CG644                    | AY781677 | NA            | NA          | Bischoff <i>et al.</i> (2009)          |
| <i>M. majus</i>           | ARSEF1914                | NA       | EU248868      | EU248948    | Bischoff <i>et al.</i> (2009)          |
| <i>M. megapomponiae</i>   | BCC25100 <sup>T</sup>    | MN781906 | MN781702      | MN781799    | Mongkolsamrit <i>et al.</i> (2020)     |
| <i>M. minus</i>           | ARSEF1099                | NA       | KJ398799      | KJ398706    | Kepler <i>et al.</i> (2014)            |
| <i>M. minus</i>           | ARSEF2037                | NA       | DQ522353      | DQ522454    | Sung <i>et al.</i> (2007)              |
| <i>M. multisynnematum</i> | HKAS 132148 <sup>T</sup> | PQ423680 | NA            | PQ569906    | Yang <i>et al.</i> (2025)              |
| <i>M. multisynnematum</i> | HKAS 132273              | PQ423681 | PQ569876      | PQ569907    | Yang <i>et al.</i> (2025)              |
| <i>M. multisynnematum</i> | GZCC 24-0206             | PQ423682 | PQ569877      | PQ569908    | Yang <i>et al.</i> (2025)              |
| <i>M. multisynnematum</i> | GZCC 24-0196             | PQ423683 | PQ569878      | PQ569909    | Yang <i>et al.</i> (2025)              |
| <i>M. neoanisopliae</i>   | CBS 289                  | NA       | YDQ79375      | YDQ79376    | Kobmoo <i>et al.</i> (2024)            |

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TABLE 1. (Continued)

| Species                      | Strain                          | ITS             | <i>Tef-1a</i>   | <i>RPB2</i>     | References                         |
|------------------------------|---------------------------------|-----------------|-----------------|-----------------|------------------------------------|
| <i>M. niveum</i>             | BCC52400 <sup>T</sup>           | MN781907        | MN781685        | MN781785        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. nornnoi</i>            | BCC19364                        | MN781891        | MN781696        | NA              | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. nornnoi</i>            | BCC25948 <sup>T</sup>           | MN781892        | MN781697        | NA              | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. novozealandicum</i>    | ARSEF4661                       | NA              | KJ398811        | NA              | Kepler <i>et al.</i> (2014)        |
| <i>M. novozealandicum</i>    | ARSEF4674                       | NA              | KJ398812        | NA              | Kepler <i>et al.</i> (2014)        |
| <i>M. ovoidosporum</i>       | BCC29223                        | NA              | QHW05272        | QHW05318        | Unpublished                        |
| <i>M. ovoidosporum</i>       | BCC32600                        | NA              | QHW05273        | QHW05319        | Unpublished                        |
| <i>M. owariense</i>          | YFCC 989                        | NA              | XAT91634        | XAT91634        | Unpublished                        |
| <i>M. pemphigi</i>           | CG645                           | NA              | UIX35933        | XSS91252        | Unpublished                        |
| <i>M. phasmatodeae</i>       | BCC49272                        | NA              | QNJ99329        | QGX86515        | Yang <i>et al.</i> (2025)          |
| <i>M. phasmatodeae</i>       | BCC2841                         | NA              | QHW05237        | QHW05338        | Yang <i>et al.</i> (2025)          |
| <i>M. phasmatodeae</i>       | HKAS 132219                     | NA              | XLM45491        | NA              | Yang <i>et al.</i> (2025)          |
| <i>M. phuwiangense</i>       | BCC 78206                       | NA              | QHW05275        | QHW05321        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. phuwiangense</i>       | BCC85068                        | NA              | QHW05276        | QHW05369        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. phuwiangense</i>       | BCC85069                        |                 | QHW05277        | QHW05370        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. pinghaense</i>         | CBS25790                        | NA              | BAI82521        | WOR78954        | Unpublished                        |
| <i>M. pinghaense</i>         | RCEF6288                        | NA              | AKM28585        | AKP21151        | Unpublished                        |
| <i>M. purpureonigrum</i>     | BCC89247 <sup>T</sup>           | MN781725        | MN781771        | MN781817        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. prachinense</i>        | MY06677                         | NA              | AOR81580        | NA              | Luangsa-ard <i>et al.</i> (2020)   |
| <i>M. prachinense</i>        | MY06601                         | NA              | AOR81579        | NA              | Luangsa-ard <i>et al.</i> (2020)   |
| <i>M. purpureum</i>          | BCC82173                        | MN781919        | MN781722        | MN781815        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. purpureum</i>          | BCC82642 <sup>T</sup>           | MN781918        | MN781723        | MN781816        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. purpureum</i>          | BCC83548                        | MN781920        | MN781724        | NA              | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. puerense</i>           | YFCCMP 9458                     | NA              | XHC76169        | XHC76173        | Ma <i>et al.</i> (2024)            |
| <i>M. puerense</i>           | YFCCMP 9459                     | NA              | XHC76170        | XHC76174        | Ma <i>et al.</i> (2024)            |
| <i>M. pupicola</i>           | DY05341                         | PV082705        | PV171225        | NA              | Chen <i>et al.</i> (2025)          |
| <i>M. putuoense</i>          | HMAS285457 <sup>T</sup>         | NA              | OQ980403        | NA              | Li <i>et al.</i> (2023)            |
| <i>M. putuoense</i>          | HMAS285458                      | NA              | OQ980404        | NA              | Li <i>et al.</i> (2023)            |
| <i>M. reniforme</i>          | ARSEF429                        | DQ069284        | HQ165690        | HQ165650        | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. rileyi</i>             | CBS80671                        | AY624205        | EF468787        | EF468937        | Sung <i>et al.</i> (2007)          |
| <i>M. rileyi</i>             | NBRC8560                        | HQ165711        | HQ165688        | NA              | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. robertsii</i>          | ARSEF4739                       | NA              | EU248848        | EU248928        | Bischoff <i>et al.</i> (2009)      |
| <i>M. samlanense</i>         | BCC17091 <sup>T</sup>           | HQ165707        | HQ165686        | HQ165646        | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. samlanense</i>         | BCC39752                        | MT078880        | MN781695        | MN781794        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. sulphureum</i>         | BCC36585                        | NA              | MN781686        | MN781786        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. sulphureum</i>         | BCC36592 <sup>T</sup>           | NA              | MN781687        | MN781787        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. sulphureum</i>         | BCC39045                        | MK632037        | MK632066        | MK632147        | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. synnematis</i>         | XCH L12                         | KJ845877        | NA              | NA              | Unpublished                        |
| <b><i>M. tawauensis</i></b>  | <b>BORH/F 03657<sup>T</sup></b> | <b>PZ055849</b> | <b>PZ096247</b> | <b>PZ466281</b> | <b>This study</b>                  |
| <b><i>M. tawauensis</i></b>  | <b>BORH/F 03658</b>             | <b>PZ055850</b> | <b>PZ096248</b> | <b>PZ466282</b> | <b>This study</b>                  |
| <i>M. viride</i>             | CBS65971                        | HQ165714        | HQ165692        | HQ165652        | Luangsa-ard <i>et al.</i> (2017)   |
| <i>M. viridulum</i>          | CSC23C0002                      | PQ787829        | PQ805234        | NA              | Unpublished                        |
| <i>M. viridulum</i>          | CSC23C0001                      | PQ787828        | PQ805233        | NA              | Unpublished                        |
| <i>Papiliomyces puniceum</i> | KUNCC 4992 <sup>T</sup>         | OM955150        | OM988198        | OM988190        | Li <i>et al.</i> (2023)            |
| <i>P. longiclavatus</i>      | GZUH YC20061403                 | MZ702080        | MZ955880        | OM419142        | Zhang <i>et al.</i> (2023)         |

Notes: “NA” shows no data in GenBank, while the newly generated sequences are in bold, and the type strains are indicated with superscript T.

### Sequence alignment and Phylogenetic construction

The newly generated DNA sequences (2ITS, 2 *TEF-1 $\alpha$*  and 2 RPB2) in this study were first checked for ambiguous sequences using BioEdit v.7.1 (<https://bioedit.software.informer.com/7.1>) (Hall 2004). The ITS, *TEF-1 $\alpha$*  and RPB2 sequences obtained in this study, together with their corresponding GenBank accession numbers, are provided in Table 1. The final concatenated (ITS, *TEF-1 $\alpha$* , and RPB2) dataset comprised 109 taxa and included six newly generated sequences representing two isolates of the proposed new species (2 ITS, 2 *TEF-1 $\alpha$* , and 2 RPB2 sequences), along with representative sequences of related taxa obtained from GenBank. The alignment consisted of 4,997 nucleotide positions, including 1,875 distinct patterns, 1,451 parsimony-informative sites, 372 singleton sites, and 3,174 constant sites. The concatenated datasets were constructed to improve resolution among closely related taxa and to support species-level identification. All sequence alignments were performed using MUSCLE v. 3.6 (Edgar 2004). Two isolates of *Papiliomyces* (*P. puniceum* (KUNCC 4992) and *P. longiclavatus* (GZUH YC20061403) were used as the outgroup following Yang *et al.* (2025) due to their close phylogenetic affinity but clear distinction from *Metarhizium*. To determine the most appropriate nucleotide substitution model for Bayesian phylogenetic analysis, model selection was performed using JModelTest2 on XSEDE v2.1.6 (Darriba *et al.* 2012). The GTR+G model was selected as the optimal evolutionary model and implemented in MrBayes. The corresponding parameters applied in the analysis were: lset nst = 6, rates = gamma, prset revmatpr = fixed (1.0000, 1.7639, 1.0000, 1.0000, 3.1308, 1.0000), and shapepr = fixed (0.725) (Song *et al.* 2023). Bayesian Inference (BI) was conducted using MrBayes v3.2.7a (Ronquist and Huelsenbeck 2003) to estimate posterior probabilities for phylogenetic relationships. Two independent runs, each consisting of four Markov chains, were performed for 5,000,000 generations, with trees sampled every 1,000 generations. The resulting trees were used to generate consensus phylogeny and assess branch support. Bootstrap support (BS) values of  $\geq 70\%$  and Bayesian posterior probabilities (PP) of  $\geq 0.95$  were regarded as indicating strong statistical support for inferred clades. Phylogenetic trees were visualized using FigTree v1.4.4 (<https://evomics.org/resources/software/molecular-evolution-software/figtree/>) and edited with Adobe Illustrator v27.6.1. The sequence alignments used for phylogenetic analyses have been submitted to TreeBASE (<https://www.treebase.org>) (submission ID: 32531).

## Results

### Phylogeny

The phylogenetic relationships of *Metarhizium* species were inferred using Maximum Likelihood (ML) and Bayesian Inference (BI) analyses based on a concatenated dataset of ITS, *TEF-1 $\alpha$* , and RPB2 sequence data (Figure 2). Both analytical methods produced highly similar topologies; therefore, only the ML phylogram is presented, with ML bootstrap support values (MLBS  $\geq 70\%$ ) and Bayesian posterior probabilities (PP  $\geq 0.95$ ) indicated at the nodes. The phylogeny included representative species of *Metarhizium* and related taxa. Species of *Metarhizium* formed a well-supported clade, with most taxa resolved as distinct lineages.

The ITS and *TEF-1 $\alpha$*  phylogenetic trees both show the two novel isolates from this study to be closely related to *M. phuwiangense* Luangsa-ard, Mongkols., Himaman, Thanakitp. & Samson (SUPPL. Figures 1 and 2). However, in the *TEF-1 $\alpha$*  phylogeny, *M. reniforme* was presented as a sister clade to the novel species, with both forming a sister clade to *M. phuwiangense* Luangsa-ard, Mongkols., Himaman, Thanakitp. & Samson. This represents the primary topological difference between the two analyses.

In the concatenated phylogenetic analysis, the two isolates obtained in this study, BORH/F03657 and BORH/F03658, grouped together in a strongly supported clade (ML = 100%, PP = 1.00). This clade was recovered as sister to *Metarhizium phuwiangense* (BCC 85069 and BCC 85068) with strong support (ML = 100%, PP = 1.00). The *Metarhizium tawauensis* clade formed an independent lineage within the genus and was separated from other closely related species. Nearby taxa in the phylogeny included *M. baoshanense*, *M. dianzhongense*, *M. formicae*, *M. fusoidium*, and *M. macrosemiae*. The placement of BORH/F03657 and BORH/F03658 was consistent in both ML and BI analyses, and all major nodes associated with the lineage received high statistical support.





**FIGURE 2.** Phylogenetic tree from RAxML analysis consisting of combined ITS, *TEF-1α* and RPB2 sequence data. The values at the nodes show ML bootstrap proportions (ML ≥ 70%) and posterior probabilities (PP ≥ 0.95). The tree was rooted with two isolates of *Papiliomyces* (*P. puniceum* (KUNCC 4992) and *P. longiclavatus* (GZUH YC20061403)). Newly generated strains from this study are indicated in bold.

## Taxonomy

***Metarhizium tawauensis* Shahbaz, M & Sathiya Seelan, J.S *sp. nov.* (Fig. 3A–L)**

*Mycobank number*: MB862554

*Etymology*:—Referring to the collection site “Tawau”, in Sabah, Malaysia.

*Holotype*:—MALAYSIA. Sabah: Botanical Garden, Tawau Hills Park, Tawau, (N 04°39.916' E 118°28.841' ), elevation 287 m, partially buried in soil near orchid *Plocoglottis plicata* (Roxb.) Ormerod, 20 January 2023, Muhammad Shahbaz and Jaya Seelan Sathiya Seelan (BORH/F 03657, holotype!).

*Description*:—*Sexual morph*: Stromata solitary, erect, arising directly from an adult Hemipteran host partially buried in soil. The host was medium-sized, approximately 12–18 mm long and 5–8 mm wide, dark brown to black (6F5–7F8). Stromata slender, cylindrical to slightly clavate, 18–35 mm long, 0.6–1.2 mm wide, dark brown to blackish-brown (6F6–7F8); surface rough due to embedded perithecia. Fertile portion occupying the upper region of the stroma, 6–12 mm long, densely packed with perithecia, giving a granular appearance. *Perithecia* completely immersed, globose to subglobose, dark brown (6F5), 180–260 × 120–210 µm ( $\bar{x}$  = 220 µm,  $n$  = 15), densely arranged within stromatal tissue (Figure. 3D). *Asci* cylindrical to narrowly clavate, thin-walled, hyaline to pale yellowish (3A3), 120–180 × 6–8 µm ( $\bar{x}$  = 150 × 7 µm,  $n$  = 15), arising from the basal hymenial layer and arranged perpendicular to the stromatal surface (Figure. 3E). *Ascus tip* rounded to slightly thickened, lacking a distinct apical apparatus (Figure. 3F). *Ascospores* filiform, hyaline, smooth-walled, multiseptate, tapering towards the ends, 90–140 × 1.0–1.8 µm ( $\bar{x}$  = 115 × 1.4 µm,  $n$  = 15) (Figure. 3G).

Asexual morph on natural host: Not observed.

*Asexual morph in culture*: *Colonies* on PDA slow-growing, reaching 25–35 mm diam. after 14 days at 25 °C; colony surface circular, compact, velvety to slightly cottony, white to pale cream (1A1–4A3), with a slightly raised center and entire, smooth margins (Figure. 3H). Reverse pale yellow to light orange (3A3–5A5) centrally, becoming lighter toward the margins, without diffusible pigment in the medium (Figure. 3I). Vegetative hyphae hyaline, smooth-walled, septate, 1.5–2.5 µm wide ( $\bar{x}$  = 2.0 µm,  $n$  = 15), irregularly branched. *Phialides* cylindrical to slightly lageniform, hyaline, smooth-walled, arising singly or in small clusters from aerial hyphae, 8–14 × 2–3 µm ( $\bar{x}$  = 11 × 2.5 µm,  $n$  = 15), with short, narrow necks. *Germinating conidia* hyaline, producing one or occasionally two germ tubes from terminal or subterminal regions, germ tubes slender, smooth-walled, and developing into vegetative hyphae (Figure 3K). *Conidia* produced singly or occasionally in short chains from phialides, globose to subglobose, smooth-walled, hyaline to pale greenish (29A3), 3.0–4.5 × 1.5–2.2 µm ( $\bar{x}$  = 3.75 × 1.85 µm,  $n$  = 15), with rounded ends and uniform morphology (Figure 3L).

*Material examined*: Malaysia, Sabah, Tawau, alt. 287 m, N 04°39.916' E 118°28.841', 20 January 2023, collected by Muhammad Shahbaz & Jaya Seelan Sathiya Seelan, BORH/F03657 (holotype).

*Additional material examined*: Malaysia, Sabah, Tawau, alt. 287 m, N 04°39.916' E 118°28.841', 20 January 2023, collected by Muhammad Shahbaz and Jaya Seelan Sathiya Seelan, BORH/F03658.

Known distribution:—Tawau, Sabah, Malaysia (This study)

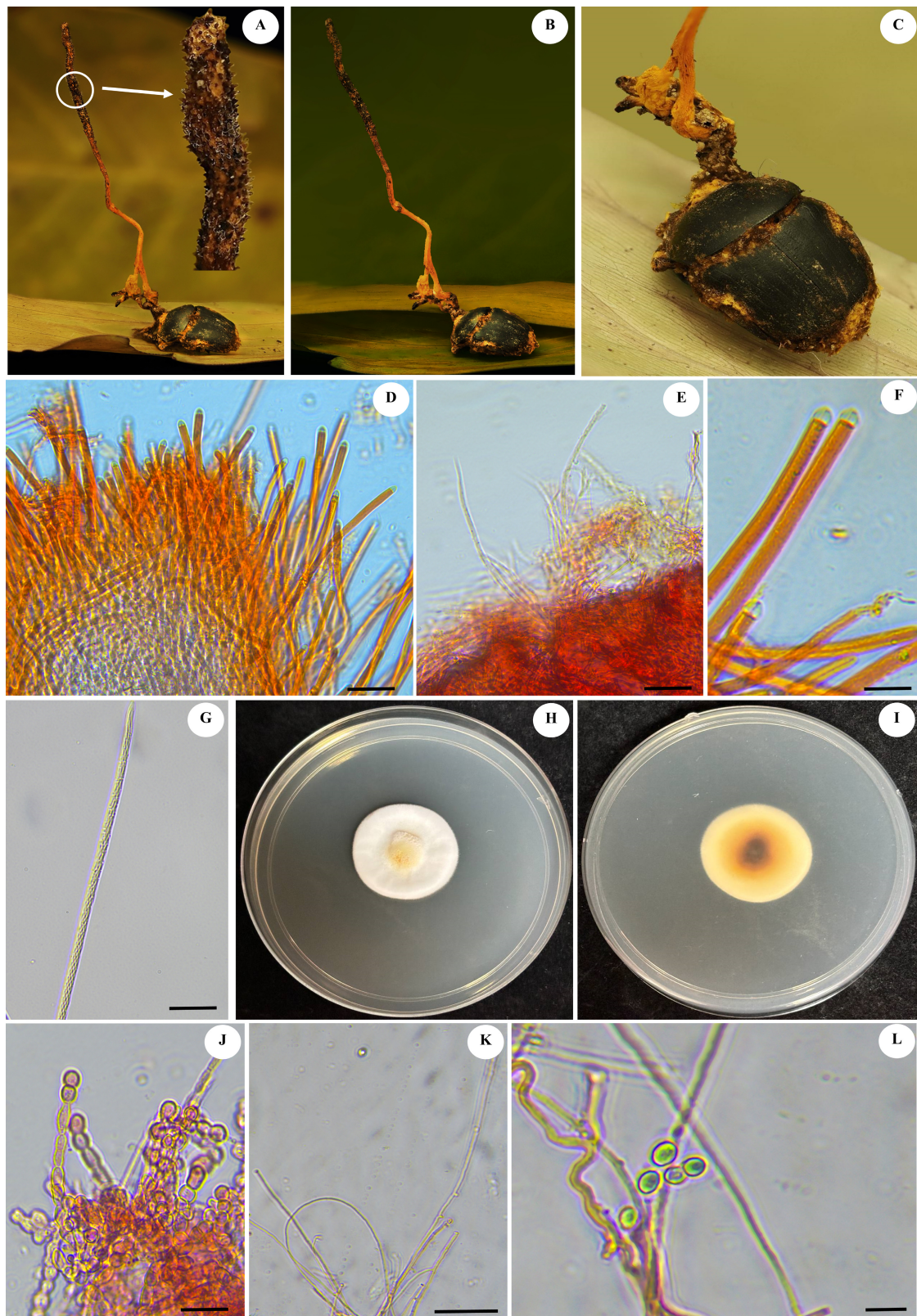
Known hosts:—Adult Hemipteran host partially buried in the soil.

*Remarks*: Morphologically, our new collection (BORH/F03657 and BORH/F03658) is most similar to *M. phuwiangense*, with some differences. The collections from this study (BORH/F03657 and BORH/F03658) are solitary, unbranched, slender, and dark brown to blackish, measuring approximately 18–35 mm in length, with a cylindrical to slightly clavate fertile region. In contrast, Thailand isolates *Metarhizium phuwiangense* Luangsa-ard, Mongkols., Himaman, Thanakitp. & Samson (BCC85068, BCC85069 and BCC78206) associated with adult Coleopteran which typically produces yellowish to yellow-orange stromata that are shorter, stouter, and frequently branched. Thus, stromatal arrangement (solitary versus branched), together with differences in size and coloration, clearly distinguishes the Sabah isolate from the Thailand species. Furthermore, the collection from this study (BORH/F03657 and BORH/F03658) was collected from adult Hemiptera buried in the soil, similar to *Metarhizium reniforme* (ARSEF429).

The culture of Sabah isolate produces phialides arranged singly along hyphae, similar to *M. phuwiangense* (BCC85068, BCC85069, and BCC78206), but differs from *M. reniforme* (ARSEF429) by lacking the distinctly thin-necked phialides of that species. It produces predominantly solitary conidia, occasionally occurring in short chains, similar to *M. phuwiangense*, and forms greenish, globose to subglobose conidia comparable in colour to *M. reniforme*, although lacking the characteristic reniform conidia of the latter. In terms of colony characteristics, the Sabah isolate forms a white colony with abundant conidial production, whereas *M. phuwiangense* (BCC85068, BCC85069, and



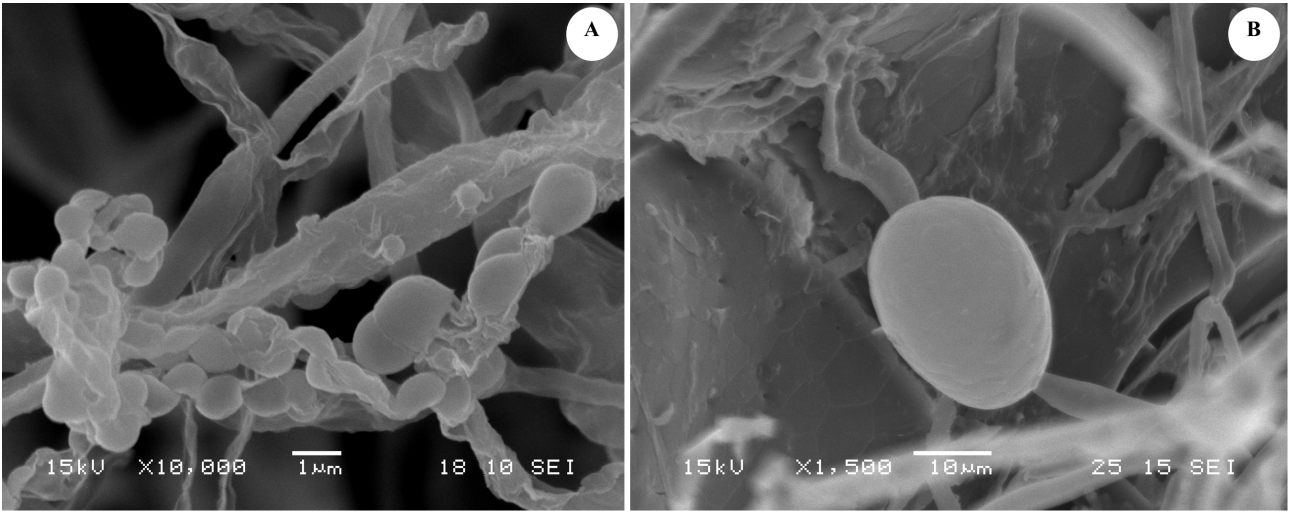
BCC78206) typically shows pale green–white mycelium. On the basis of phylogenetic analysis, our new collections (BORH/F03657 and BORH/F03658) form a distinct clade with ML= 100% and PP= 1 and appeared as a sister clade of *Metarhizium phuwiangense* (BCC85068, BCC85069 and BCC78206) and *Metarhizium reniforme* (ARSEF429) from Thailand respectively. Thus, the new collections were identified as *M. tawauensis*, representing the first report of this species.



**FIGURE 3.** *Metarhizium tawauensis* (Holotype BORH/F03657). **A** and **B**. Stromata arising from host, **C**. Closer view of host, **D**. *Perithecia*, **E**. Asci, **F**. Ascus tip, **G**. Ascospores, **H**. Culture front colony on PDA, **I**. Culture reverse colony on PDA, **J**. Phialides, **K**. Germinating conidia, **L**. Conidia. Scale bars D–G, J–L = 20  $\mu$ m.



Scanning electron microscopic (SEM) observations revealed conidia smooth-walled, globose to subglobose, borne singly at the apices of phialides, with a smooth surface lacking conspicuous ornamentation (Figures 4A–B).



**FIGURE 4.** Scanning electron microscopic (SEM) observations. A. Phialides bearing conidia, and B. Conidium.

**TABLE 2.** Comparison of the sexual morph and culture characteristics of the new species *Metarhizium tawauensis* with closely related *Metarhizium* species.

| Species                | Host             | Stromata (mm)                                           | Perithecia (µm)                           | Asci (µm)               | Ascospores (µm)               | Phialides (µm)           | Conidia (µm)            | References                         |
|------------------------|------------------|---------------------------------------------------------|-------------------------------------------|-------------------------|-------------------------------|--------------------------|-------------------------|------------------------------------|
| <i>M. flavum</i>       | Coleoptera larva | Pale yellow to olive yellow, 15 (Long)                  | 500–650 × 270–330                         | 280–320 × 5–6           | 200–315 × 1.5–2               | (7–)8–10 × (2–)2.5–3     | (7–)7.5–9.5 (–10) × 2–3 | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. phuwiangense</i> | Coleoptera adult | Orange brown, 15 (long) 1.5–2 (wide)                    | 320–470 × 180–300                         | 100–271 ×3–5            | 94–107 × 1                    | 5–7(–10) × (2–)2.5–3(–4) | (6–)6.5–8(–10) × 3–4    | Mongkolsamrit <i>et al.</i> (2020) |
| <i>M. putuoense</i>    | Coleoptera Larva | Pale yellow to light brown 25–60 (long) and 2–4 (broad) | (420–)450–500(–530) × (220–)250–300(–330) | (190)210–260(280) × 5–7 | (170–)190–240(–260) × 1.0–1.5 | 7–11 × 1.5–3.0           | 6–7 × 3–4               | Li <i>et al.</i> (2023)            |
| <i>M. reniforme</i>    | Orthoptera       | NA                                                      | NA                                        | NA                      | NA                            | 2.5 ± 0.4 × 2.3 ± 0.2    | 4.5 ± 0.7 × 2.2 ± 0.4   | Kalkar <i>et al.</i> (2006)        |
| <i>M. tawauensis</i>   | Hemiptera adult  | Dark brown 18–35 (long) 0.6–1.2 (wide)                  | 180–260 × 120–210                         | 120–180 × 6–8           | 90–140 × 1.0–1.8              | 8–14 × 2–3               | 3.0–4.5 × 1.5–2.2       | This Study                         |

### Discussion

This study introduces one new species of the genus *Metarhizium* from Malaysia based on morphological and molecular assessments. The new species, *Metarhizium tawauensis* (BORH/F03657 and BORH/F03658), represents a significant addition to the diversity of the Clavicipitaceae family and provides the first baseline data for this specific lineage within Sabah, Malaysia. Morphologically, *M. tawauensis* is similar to *M. flavum*, *M. phuwiangense*, and *M. putuoense*. However, several distinct features differentiate it from its closely related species. Among these species, *M. phuwiangense* produces orange-brown stromata and larger perithecia (320–470 × 180–300 µm) while *M. tawauensis* exhibits dark brown stromata and smaller perithecia. Additionally, *M. putuoense* is characterized by longer stromata (25–60 mm) and larger perithecia (420–)450–500(–530) × (220–)250–300(–330) µm). *M. flavum* appears morphologically close in having stromata arising from Coleopteran hosts while *M. tawauensis* (BORH/F03657 and BORH/F03658), possesses stromata associated with Hemipteran adults. *M. flavum* has larger perithecia (500–650 × 270–330 µm) than *M. tawauensis*. The asci of *M. tawauensis* (120–180 × 6–8 µm) are also shorter.. *M. tawauensis* produces filiform, hyaline, smooth-walled, multiseptate ascospores that are comparatively longer than those of *M. phuwiangense*, but smaller than

those of *M. flavum* and *M. putuoense*. The ascospores are also notably more slender than those of *M. flavum* and *M. putuoense*, but slightly broader than *M. phuwiangense* (Table 2)

From the asexual morph perspective, *M. tawauensis* is distinguished by its phialides ( $8\text{--}14 \times 2\text{--}3 \mu\text{m}$ ) and comparatively smaller conidia ( $3.0\text{--}4.5 \times 1.5\text{--}2.2 \mu\text{m}$ ). In contrast, *M. flavum*, *M. phuwiangense*, and *M. putuoense* produce larger conidia. These micromorphological differences provide additionally diagnostic characters supporting our specimen as a new species. The conidia size of *M. tawauensis* is also clearly distinct from *M. reniforme*, which has shorter phialides and differently shaped conidia (Table 2).

Phylogenetic analyses support the novel species as a distinct lineage and shows as a sister clade to *M. phuwiangense* and *M. reniforme*. In the ITS phylogeny, the isolates (BORH/F03657 and BORH/F03658) from this study form a well-supported clade (ML=100, PP=1) sister to *M. phuwiangense* (SUPPL. Figure.1); *M. reniforme* appears separate from the novel species sequences because ITS alone often has lower resolution at the species level, while *TEF-1 $\alpha$*  provides higher phylogenetic signal for closely related *Metarhizium* taxa (Kepler *et al.* 2014). The *TEF-1 $\alpha$*  tree shows the isolates form a well-supported clade (ML=100, PP=1) sister to *M. reniforme* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones but both species form sister clade to *M. phuwiangense* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones (SUPPL. Figure.2). The concatenated ITS-*TEF-1 $\alpha$* -RPB2 phylogeny integrates three loci, positioning the new isolates in a well-supported distinct clade (ML=100, PP=1) sister to *M. phuwiangense* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones and both species form sister clade to *M. reniforme* (Figure 2). These results demonstrate a robust, well-supported, distinct phylogenetic lineage, justifying recognition of the isolates as *Metarhizium tawauensis* sp. nov., representing the first report of this species.

Ecologically, *M. tawauensis* was collected from adult Hemiptera, whereas closely related species such as *M. flavum* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones, *M. putuoense* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones are associated with Coleopteran larva and *M. phuwiangense* (Samson & H.C. Evans) Luangsa-ard, Boucias & Hywel-Jones infects adult Coleopteran. The occurrence of *M. tawauensis* in Sabah, Malaysia further supports its adaptations to tropical Southeast Asia.

## Conclusion

The introduction of *Metarhizium tawauensis* sp. nov. is based on distinct morphological features and phylogenetic analyses which differentiate from closely related species within the genus *Metarhizium*. This study provides the first report of *M. tawauensis* expanding *Metarhizium* diversity.

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## Authorship contribution statement

**MUHAMMAD SHAHBAZ, JAYA SEELAN SATHIYA SEELAN, ZAINUDDIN BALUAT, and ELYSE YANG MEEI LING:** Fieldwork and sample collection. **MUHAMMAD SHAHBAZ and JAYA SEELAN SATHIYA SEELAN:** Conceptualization and formal analysis. **MUHAMMAD SHAHBAZ, JAYA SEELAN SATHIYA SEELAN and IBRAHEM GHANI WASTI:** Original manuscript writing, figures, initial review, and editing. **JAYA SEELAN SATHIYA SEELAN and IBRAHEM GHANI WASTI:** Critical review. **JAYA SEELAN SATHIYA SEELAN:** Supervision and final validation.

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**Data availability:** the nucleotide sequences have been deposited in the NCBI GenBank database (<https://submit.ncbi.nlm.nih.gov/subs>). The accession numbers will be made publicly available upon publication of this article.

## Declarations

**Competing interests:** The authors state no conflict of interests.

**Ethical approval:** current research work does not require any ethical approval.

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