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Metarhizium indicum, a new species of entomopathogenic fungus infecting leafhopper, *Busoniomimus manjunathi* from India

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ABSTRACT

A new species of entomopathogenic fungus, *Metarhizium indicum,* which derives its species epithet after its Indian origin is reported here. The fungus was found to cause natural epizootics in leafhopper (*Busoniomimus manjunathi*) infesting *Garcinia gummi-gutta* (Malabar tamarind), an evergreen spice tree native to South and Southeast Asia, known for its use as a culinary flavourant, dietary supplement and traditional remedy for various human ailments. The fungus was found to cause more than 60% mortality in field collected insects. The identity of the new species was established based on its distinct morphological characteristics and multi-gene sequence data analyses. Phylogenetic analyses using internal transcribed spacer region (ITS), DNA lyase (APN2) and a concatenated set of four marker genes [translation elongation factor 1-alpha (TEF), β-tubulin (BTUB), RNA polymerase II largest subunit (RPB1) and RNA polymerase II second largest subunit (RPB2)] along with marked differences in nucleotide composition and genetic distance unambiguously support our claim that the present fungus infecting *Garcinia* leafhopper is a new addition to the genus *Metarhizium*.

1. Introduction

Entomopathogenic fungi (EPF) belonging to the genus *Metarhizium* Sorokin (Hypocreales: Clavicipitaceae) are well known generalist insect pathogens with worldwide distribution. First reported as "green muscardine disease" (Metschnikoff, 1879) from Russia, now the genus encompasses several recognized species infecting insects belonging to diverse orders and documented from various climatic conditions ranging from tropics to temperate regions of the world (Bischoff et al., 2009; Driver et al., 2000; Kepler et al., 2014; Mongkolsamrit et al., 2020). Various biocontrol products based on *Metarhizium* are available in the market and are being used effectively to manage many insect pests worldwide, including Brazil, Iran, India, Japan, Thailand, and the USA (Kumar et al., 2019; Mongkolsamrit et al., 2020).

Earlier descriptions of *Metarhizium* species relied mainly on morphological traits which, in general, overlap among the species of this genera resulting in underreporting of several cryptic species. With the advent of molecular tools, the diversity of this genus was uncovered and many cryptic species were elevated as new species and a number of new taxa were added to the repository of *Metarhizium* across the world indicating its evolution and diversity. Several new species have been added to the list of valid species in the past two decades and at present the *Metarizhium* lineage contains more than 60 species, and the list is fast growing with various new additions reported from the Asian continent (Bidochka and Small, 2005; Mongkolsamrit et al., 2020; Yamamoto et al., 2020). In fact, Asia has been suggested as the origin of genetic diversity of *Metarhizium* lineage with several teleomorphs reported from this region (Bidochka and Small, 2005).

Though one third of the global fungal diversity exists in India with more than 27,000 reported fungal species (Manoharachary et al., 2005), taxonomic work on EPF, especially those belonging to that of *Metarhizium* lineage is very minimal, considering the diverse soil and climatic conditions of this sub-continent. Except for a lone report of *M. globosum* as a new species from this country, the other reported fungi of this lineage from India are the commonly reported *M. anisopliae*, *M. pingshaense* and *M. rileyi* (Bischoff et al., 2009; Mongkolsamrit et al., 2020; Senthil Kumar et al., 2021) highlighting that India still remains untapped of its potential with respect to exploitation of EPF belonging to *Metarhizium* lineage. This further underscores the need for rigid taxonomic evaluation of the reported species using modern molecular tools

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Table 1
Morphological features of representative *Metarhizium* spp. infecting hemipteran hosts.

Taxon	Countries reported	Conidia (µm)	Phialides (µm)	Shape of conidia	Colony colour	Reference
M. brasiliense	Brazil	3.0–10.0 × 2.0–3.0	7.0–10.0 × 1.5– 2.0	Ovoid to cylindrical	Cream to pale yellow	Kepler et al. (2014)
M. owariense	Japan	7.0–10.0 × 4.0–5.0	5.0–8.0 × 3.0–4.0	Ovoid, ellipsoid	Greenish yellow	Kepler et al. (2014)
M. cylindrosporum	China, Japan, Taiwan	3.0–8.0 × 2.0–3.0; 14.0–22.0 × 3.0–4.0	4.0–7.0 × 2.0–3.0	Ovoid, subglobose and cylindrical	Pale yellow	Kepler et al. (2014)
M. viridulum	Taiwan, Thailand	7.0–13.0 × 3.0–4.0	5.0–9.0 × 3.0–5.0	Ellipsoid, cylindrical	Pale yellow	Kepler et al. (2014)
M. album	Indonesia, Philippines	3.0–6.0 × 1.5–2.5	10.0–12.5 × 2.0–3.5	Ovoid to ellipsoid	Pale brown	Rombach et al. (1987)
M. album	Sri Lanka, Thailand	5.0–6.0 × 1.5–2.0	5.0–14.0 × 1.5–2.0	Ellipsoid, cylindrical	Pale brown	Mongkolsamrit et al. (2020)
M. indicum	**India**	**5.5–9.3 × 1.8–2.3 (media) 3.5–5.5 × 1.0–2.0 (host)**	**5.5–9.0 × 2.0–2.5 (media) 5.0–8.5 × 1.5–2.5 (host)**	**Ellipsoid, cylindrical**	**Brown to dark brown**	**This study**

and to intensity explorations for identifying cryptic and unreported species to unravel the hidden diversity of *Metarhizium* in India.

In the present study, we report an EPF, *Metarhizium indicum* sp. nov., infecting a leafhopper, *Busoniomimus manjunathi* (Viraktamath & Viraktamath), on Malabar tamarind or Brindle berry (*Garcinia gummi-gutta* (L.) N. Robson). The leafhopper was first reported as a pest of mango (*Mangifera indica*) in India (Viraktamath and Viraktamath, 1985). *Garcinia gummi-gutta* is an evergreen spice tree native to India, Sri Lanka, Nepal, and also cultivated in the subtropical regions of China, Malaysia, and Philippines (Orwa et al., 2009). Apart from its use as a souring condiment in curries, it is also used in ayurvedic medicines and found to exhibit medicinal properties such as hypolipidaemic, antidiabetic, anti-inflammatory, anticancer, anthelmintic, anticholinesterase and hepatoprotective activities (Semwal et al., 2015). Very few insect pests have been reported to infest *Garcinia* trees, of which, the leafhopper *B. manjunathi* is a serious pest, which can cause wilting of branches to complete drying of the plant. At present, chemical insecticides are recommended and no biological control options are available to manage this pest (Sivakumar et al., 2013). The entomopathogen reported here is a first report on the natural occurrence of an EPF on these insects. We characterized the fungus based on its morphological traits and multigene analyses to support our claim to recognize this taxon as a hitherto unreported species.

2. Materials and methods

2.1. Collection and isolation of the fungus

Dead leafhoppers wholly covered with fungal mycelium and adhered to leaves of *Garcinia* plants collected from the research farm of Indian Council of Agricultural Research-Indian Institute of Spices Research (ICAR-IISR), Kozhikode, Kerala (11°17′59.81″N, 75°50′30.76″E) during September 2014 were used for isolation of the fungus. The fungus from the cadavers was isolated using Potato Dextrose Agar (PDA) amended with 0.05% chloramphenicol in Petri dishes (17 × 100 mm) under aseptic conditions in a laminar air flow chamber. The plates were incubated at 25 ± 1 °C for 10 days in a biochemical oxygen demand (BOD) incubator, and the fungus was further purified by sub-culturing. Pure cultures of the fungus were established on PDA without antibiotics by plating serially diluted conidial suspension of the fungus and by arbitrarily selecting one colony forming unit (CFU), which was used for further studies (Senthil Kumar et al., 2016). Ex-type culture was deposited in National Fungal Culture Collection of India (NFCCI WDCM 932), MACS Agharkar Research Institute, Pune, Maharashtra, India, as NFCCI 5467, and a single infected specimen preserved in dried state was deposited at the Entomopathogenic Fungal Repository of ICAR-IISR, Kozhikode as holotype with accession number IISR-EPF-01.

2.2. Disease prevalence

The epizootic capacity of the fungus was studied under laboratory conditions using field collected insects as described earlier (Senthil Kumar et al., 2011) with modifications. Briefly, 75 live adult hoppers collected from the field, every month from November 2017 to January 2018 were transferred to three plastic containers (4 L) at the rate of 25 insects per container and secured with nylon mesh. Fresh twigs of *Garcinia* placed in a conical flask (50 ml) with water, secured with cotton plug were provided to insects as feed. Observation on insect mortality was recorded up to 10 days from the date of collection to calculate the cumulative monthly mortality. Dead insects after surface sterilization with 0.5% sodium hypochlorite followed by three rinses in sterile distilled water were transferred to Petri dishes lined with moist filter paper for sporulation, and the cadavers were examined under a light microscope to confirm the cause of death. The whole setup was incubated under ambient room temperature conditions (28–32 ± 2 °C, 60–70% relative humidity at natural photo phase).

2.3. Identification of the fungus

2.3.1. Morphological characterization

To study the colony characters of the fungus, 6 mm disc of the fungus (grown on PDA) was inoculated on various growth media, including Czapek yeast autolysate agar (CYA), creatine sucrose agar (CREA), oatmeal agar (OA), yeast extract sucrose agar (YES), potato dextrose agar (PDA) and quarter strength Sabouraud dextrose agar with yeast extract (SDAY/4) and incubated at 25 ± 2 °C for 7 days. Macroscopic features such as growth and appearance of the colony, pigmentation on reverse of the media were recorded. Micro-morphological features of conidiophores, conidiogenous cells, and conidia of the fungus grown on culture media, and from field collected infected insects were recorded using a ZEISS Axio Imager 2 compound microscope (Carl Zeiss, Oberkochen, Germany) with a digital camera control unit AxioCam MRc 5 (Carl Zeiss, Oberkochen, Germany). Conidia and conidiophores were mounted in lactic acid cotton blue and measured using the AxioVision v. 4.8 software, with 30 measurements per structure. The Methuen Handbook of Colour was followed to determine the colour codes given in the description (Kornerup and Wanscher 1978).

2.3.2. DNA extraction, PCR and sequencing

DNA was extracted from mycelium and conidia (~500 mg) scraped from 21 days old culture grown on full strength SDAY medium using fungal genomic DNA extraction Kit (Chromous Biotech, India) following the manufacturer's instructions. PCRs were performed to amplify the internal transcribed spacer region gene (ITS) with primers ITS4 and ITS5 (White et al., 1990), partial β-tubulin gene (TUB) with primers bt2a and bt2b (Glass and Donaldson, 1995), partial translation elongation factor

1α gene (TEF) with primers 983F and 2218R (Rehner and Buckley, 2005), RNA polymerase II largest subunit (RPB1) with primers RPB1a and RPB1-VH6R (Hofstetter et al., 2007; Stiller and Hall, 1997), RNA polymerase II second largest subunit (RPB2) with primers RPB2_5F and fRPB2-7cR (Liu et al., 1999) and DNA lyase (APN2) with primers APN_clavF2 and APN_clavR8 (Kepler et al., 2014). PCR reactions were carried out with 10–20 ng of template DNA in volumes of 25 µl containing 2.5 µl of 10 × PCR buffer, 2.0 µl of dNTPs (2.5 mM each), 0.75 µl each of (20 µM) forward and reverse primer pairs, 0.2 µl of 5 units/µl *Taq* DNA polymerase (Sigma, USA) after making the remaining volume with PCR grade water (Himedia, India). The PCR conditions for ITS, TUB and TEF were as described by Senthil Kumar et al. (2016) without modifications, whereas for RPB1 & 2 and APN2 the conditions were as described by Senthil Kumar et al. (2021). The amplified bands were excised and purified using Sigma GelElute™ Gel Extraction Kit (Sigma, India) as per manufacturer's protocol, and the products were sent to SciGenom Labs Private Ltd., Kochi, India, for bi-directional sequencing using the same set of PCR primers in ABI 3730 XL DNA Analyzer. Sequences were manually trimmed using BioEdit software and subjected to BLAST (Basic Local Alignment Search Tool) search to identify sequences deposited in GenBank that had significant homology. The partial sequences of ITS gene and that of TEF, TUB, RPB1, RPB2 and APN2 were deposited in GenBank with accession numbers KY393104 and KY411140–KY411144, respectively.

2.3.3. Phylogenetic analyses

Reference sequences of ITS, RPB1, RPB2, TEF, TUB and APN2 of taxonomically recognized species of *Metarhizium* used in earlier studies (Kepler et al., 2014; Mongkolsamrit et al., 2020) retrieved from the GenBank (Supplementary Table 1) were initially aligned using ClustalW multiple alignment tool in BioEdit software. Subsequently, alignments were trimmed to the length of IISR-EPF-01 sequences. The partial gene sequences of RPB1, RPB2, TEF and TUB were concatenated into a single matrix containing 3244 aligned characters using SequenceMatrix (Vaidya et al., 2011). A partition homogeneity test was performed to assess incongruences among these three gene sets with PAUP* version 4.0a149 (Swofford, 2002). The test indicated no incongruence between RPB1, RPB2, TEF and TUB genes. Phylogenetic tree for the concatenated set was generated with RAxML (Stamatakis, 2014) based on a combined RPB1, RPB2, TEF and TUB sequence data for the genus *Metarhizium* with 1000 bootstrap replicates. Bayesian phylogenetic inference for the concatenated set was also performed using MrBayes 3.2.7 (Ronquist and Huelsenbeck, 2003). Analysis was run over 10 million generations, with tree sampling every 100 generations; the first 25% of trees were discarded prior to consensus tree calculation. Similarly, the ITS and APN2 sequences were aligned separately with reference sequences of *Metarhizium* spp. retrieved from GenBank and ML trees were constructed with RAxML as described above. *Pochonia chlamydosporia* var. *chlamydosporia* (Goddard) Zare et Gams (Metacordycipitaceae) was used as outgroup in all the analyses.

2.4. Scanning electron microscopy (SEM)

Field collected sporulated insect cadavers dehydrated in a desiccator for 5 days at room temperature were used for SEM studies. The specimens were mounted on carbon stubs, gold sputtered for 20 s and directly viewed under a Scanning Electron Microscope (Hitachi SU6600) as described earlier (Senthil Kumar et al., 2016).

2.5. Statistical analysis

Insect mortality data was subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) and mean comparisons were done by Tukey's honest significant difference (HSD) test ($\alpha = 0.05$) in GraphPad Prism® Version 7.0 for Windows, GraphPad Software, La Jolla, California USA.

Fig. 1. *Garcinia* leafhopper, *Busoniomimus manjunathi* infected by *Metarhizium indicum*. Scale bar = 5 mm.

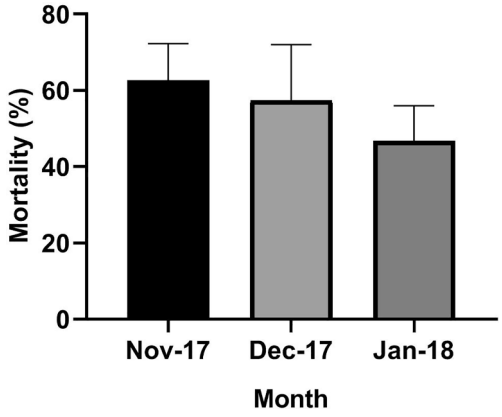

Fig. 2. Natural incidence of *Metarhizium indicum* in *Garcinia* leafhopper, *Busoniomimus manjunathi* during November 2017 – Januray 2018. Bars indicate per cent average mortality of field collected insects 10 days post collection. Error bars represent mean ± SE of three replicates.

3. Results

3.1. Disease prevalence

Groups of dead mummified leafhoppers, adhering to the undersurface of *Garcinia* leaves, completely covered by fungal mycelium was first observed during April 2012. Sporulated hoppers appeared to be white (5A1) to light brown (5D4) in colour with numerous coremium-like structures, arising from the surface, typical to the genus of *Metarhizium* infecting hoppers (Fig. 1). More than 60% of the insects collected from the field during November 2017 died due to fungal infection, which showed a declining trend during December 2017 and January 2018 (Fig. 2). However, no significant difference in epizootics was recorded among the months ($F = 0.50$; $df = 2, 6$; $P = 0.63$). Dead insects sporulated within a week after death, and microscopic examination of the fungus from sporulated cadavers confirmed the infection by the present strain of fungus.

3.2. Morphological identification

3.2.1. Taxonomy

Metarhizium indicum Senthil Kumar, Jacob, Devasahayam & Rajeshkumar, ***sp. nov.***

MycoBank MB 844381.

Etymology: The species epithet *indicum* refers to the Indian origin of the fungus.

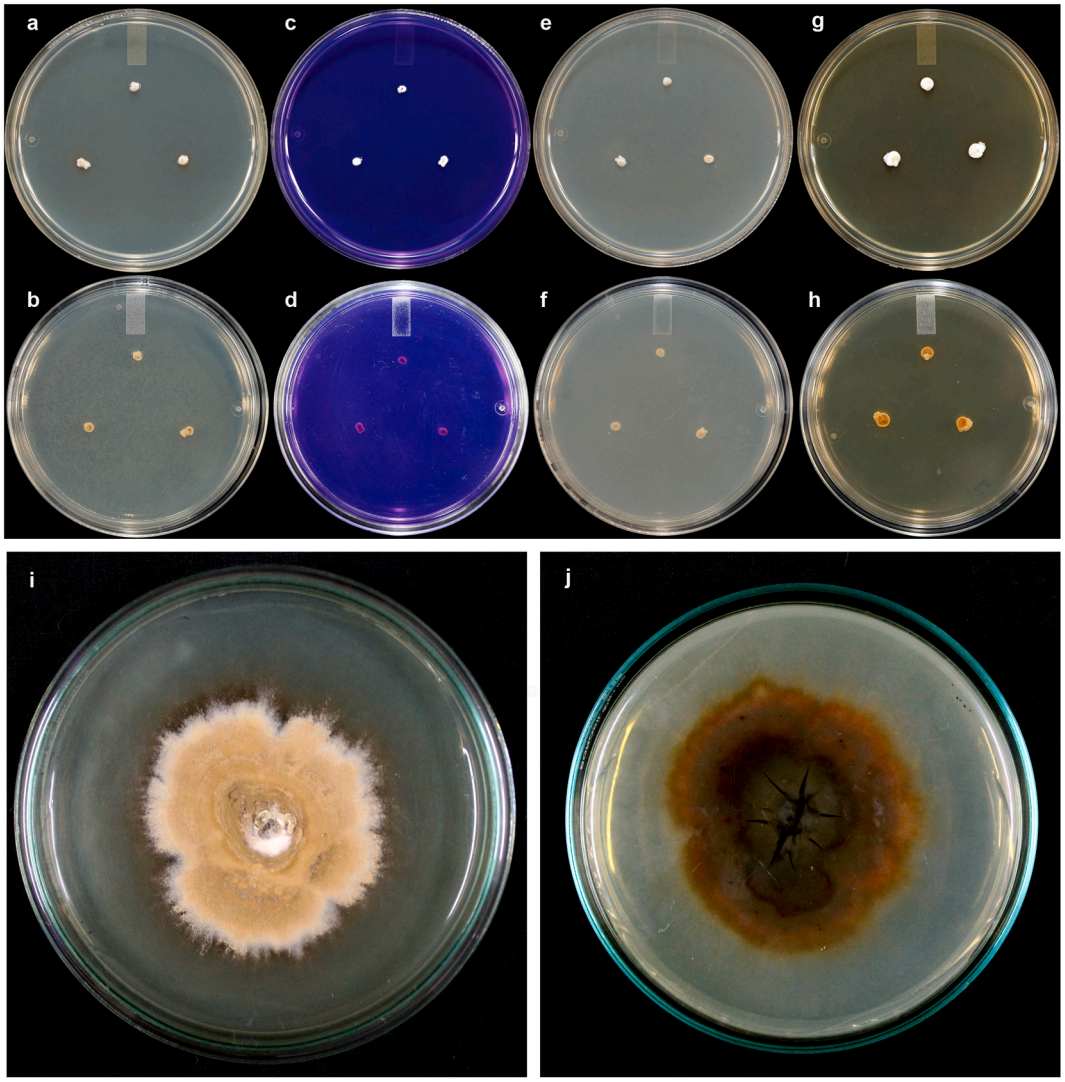

Fig. 3. *Metarhizium indicum* (IISR-EPF-01, culture ex-type NFCCI 5467). Colonies after 7 d at 25 ± 2 °C on (a & b) CYA obverse and reverse. (c & d) CREA obverse and reverse. (e & f) OA obverse and reverse. (g & h) YES obverse and reverse. (I & j) mature colony on SDAY/4 obverse and reverse.

Colonies on CYA at 25 ± 2 °C; after 7 days, 4.0–5.0 mm in diameter, velutinous, white (5A1), reverse light brown (5D4) (Fig. 3 a & b). *Colonies* on CREA at 25 ± 2 °C; after 7 days, 3.5–5.0 mm in diameter, velutinous, white (5A1), reverse reddish brown (8E6), acid production absent (Fig. 3 c & d). *Colonies* on OA at 25 ± 2 °C; after 7 days, 4.0–4.5 mm in diameter, velutinous, white (5A1), reverse light brown (5D4) (Fig. 3 e & f). *Colonies* on YES at 25 ± 2 °C; after 7 days, 5.0–8.0 mm in diameter, velutinous to floccose, white (5A1), reverse light brown to brown (6D4 to 6E4) (Fig. 3 g & h). *Colonies* on SDAY/4 or PDA, at 25 ± 2 °C; after 7 days, slow growing, 1.0–2.0 mm, 2.0–3.0 mm in diameter respectively, velutinous, white (5A1), reverse light brown (5D4). Colonies gradually turned dark brown after sporulation; colony reverse become yellow brown (5E8) to brown (5F8) upon maturation with the media turning yellow (Fig. 3 i & j). The colour codes were determined using Kornerup and Wanscher (1978).

Asexual morph: *Conidiophores* simple or dichotomously branched smooth walled, long. *Phialides* are solitary, and directly on the conidiophores, ampulliform, with a narrow short collula, 5.0–8.5 × 1.5–2.5 µm on infected hoppers and 5.5–9.0 × 2.0–2.5 µm on SDAY/4. *Conidia* mostly ellipsoid or cylindrical, tips obtuse, zero septate, smooth-walled, 3.5–5.5 × 1.0–2.0 µm on infected hoppers and (5.3–) 5.5–9.3 × 1.8–2.3 µm (Mean: 7.02 × 2.09 µm) on SDAY/4 (Fig. 4 a-e, Table 1).

Sexual morph: Undetermined.

Holotype: IISR-EPF-01, a single infected hopper, collected by C. M. Senthil Kumar on 23 September 2014, and deposited as dry specimen at the Entomopathogenic Fungal Repository of ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India.

Ex-type culture: NFCCI 5467, deposited at National Fungal Culture Collection of India, MACS Agharkar Research Institute, Pune, Maharashtra, India.

Type host: Leaf hopper, *Busoniomimus manjunathi* (Viraktamath & Viraktamath) (Idiocerinae: Cicadellidae: Hemiptera) infesting *Garcinia gummi-gutta* (L.) N. Robson.

Type locality: India, Kerala State, Kozhikode District (11°17′59.81″N, 75°50′30.76″E).

Known distribution: Kozhikode District, Kerala State, India.

GenBank Numbers: ITS-KY393104; TEF1-KY411140; TUB2-KY411141; RPB1-KY411142; RPB2-KY411143 and APN2-KY411144.

Notes: *Metarhizium indicum* fits well with the key diagnostic characters of the genus *Metarhizium,* having conidial chains adhering laterally in to cylindrical mass with *en masse* conidia colourless to pale brown. Conidia in the type species, *Metarhizium anisopliae* is (3.5–) 5–8 (–9) × 2.5–3.5 µm and in *M. anisopliae* var. *major* with conidia 10.0–14.0 (–18.0) µm long. However, in the new species *M. indicum,* conidia are slightly larger and the conidial width is smaller than the type species *M. anisopliae.* Similarly, length of the conidia is found smaller in the new

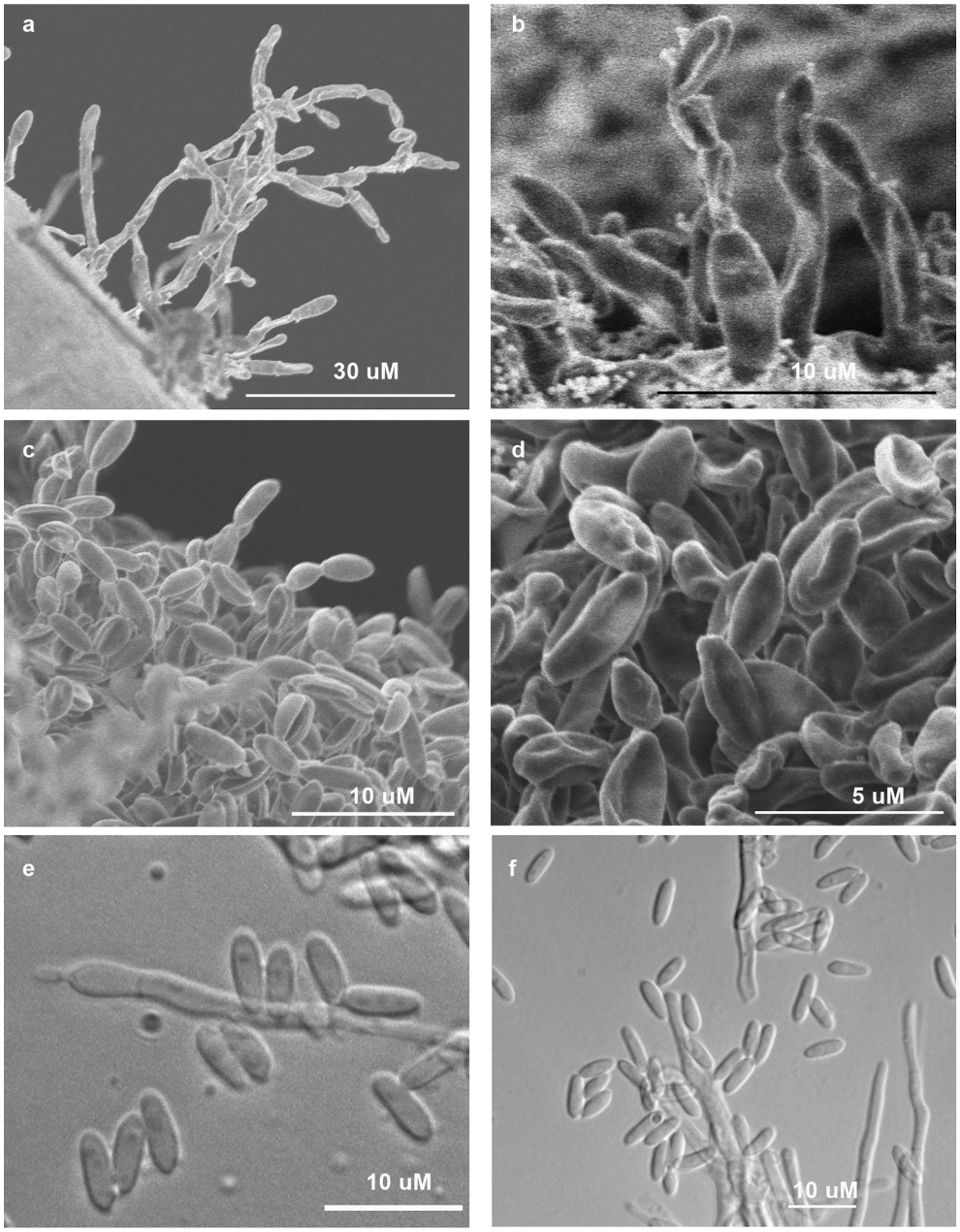

Fig. 4. *Metarhizium indicum* (IISR-EPF-01, culture ex-type NFCCI 5467). Scanning electron micrographs showing (a & b) development of phialides and conidia on host (c & d) conidia on insect host and light micrograph of (e) phialide with developing conidia, and (f) conidia on SDAY/4 media. Scale bar: a = 30 μm; b, c, e, f = 10 μm; d = 5 μm.

species, *M. indicum* compared to *M. anisopliae* var. *major*.

3.3. Molecular characterization

PCR amplification of partial ITS, TEF1, TUB2, RPB1, RPB2 and APN2 for the present strain yielded amplicons of size 615 bp, 824 bp, 315 bp, 1043 bp, 1029 bp and 990 bp respectively. BLAST homology search results indicated that the reported fungus shared more similarity with *M. album* type strains. The ITS sequence of the current strain shared 90% similarity with *M. album* type strain ARSEF 1942 (HM055452). However, the 3′ TEF sequence did not show similarity with any of the reported type strains of *M. album*. The TUB2 sequence was 93.4 and 92.4% identical to *M. album* strains ARSEF 2082 (KJ398579) and ARSEF 1942 (KJ398572), respectively, whereas the RPB1 and RPB2 sequences shared 95% similarity with *M. album* ARSEF 2082 strain sequences (KJ398617 and KJ398715), and the APN2 sequence was only 90% similar to the *M. album* ARSEF 2082 strain (KJ398519). Comparison of different sequenced regions of *M. indicum* with *M. album* indicated that there were 31 base substitutions, 17 deletions and 12 nucleotide insertions in ITS gene and 21 base substitutions, 2 deletions and 3 nucleotide insertions in TUB gene. Whereas, in the case of RPB1, RPB2, TEF and APN2 gene regions there were 45, 51, 43 and 97 base substitutions respectively without any indels (Supplementary Table 2; Supplementary Fig. 1).

There was topological congruence in RAxML and Bayesian phylogenetic trees, and hence only the former tree is presented here. Phylogenetic analyses with concatenated nucleotide sequences of RPB1, RPB2, TEF and TUB placed the new species as a sister clade with *M. album* strains with a strong statistical support (BS 100) (Fig. 5). Further analysis using ITS and APN2 sequences separately also

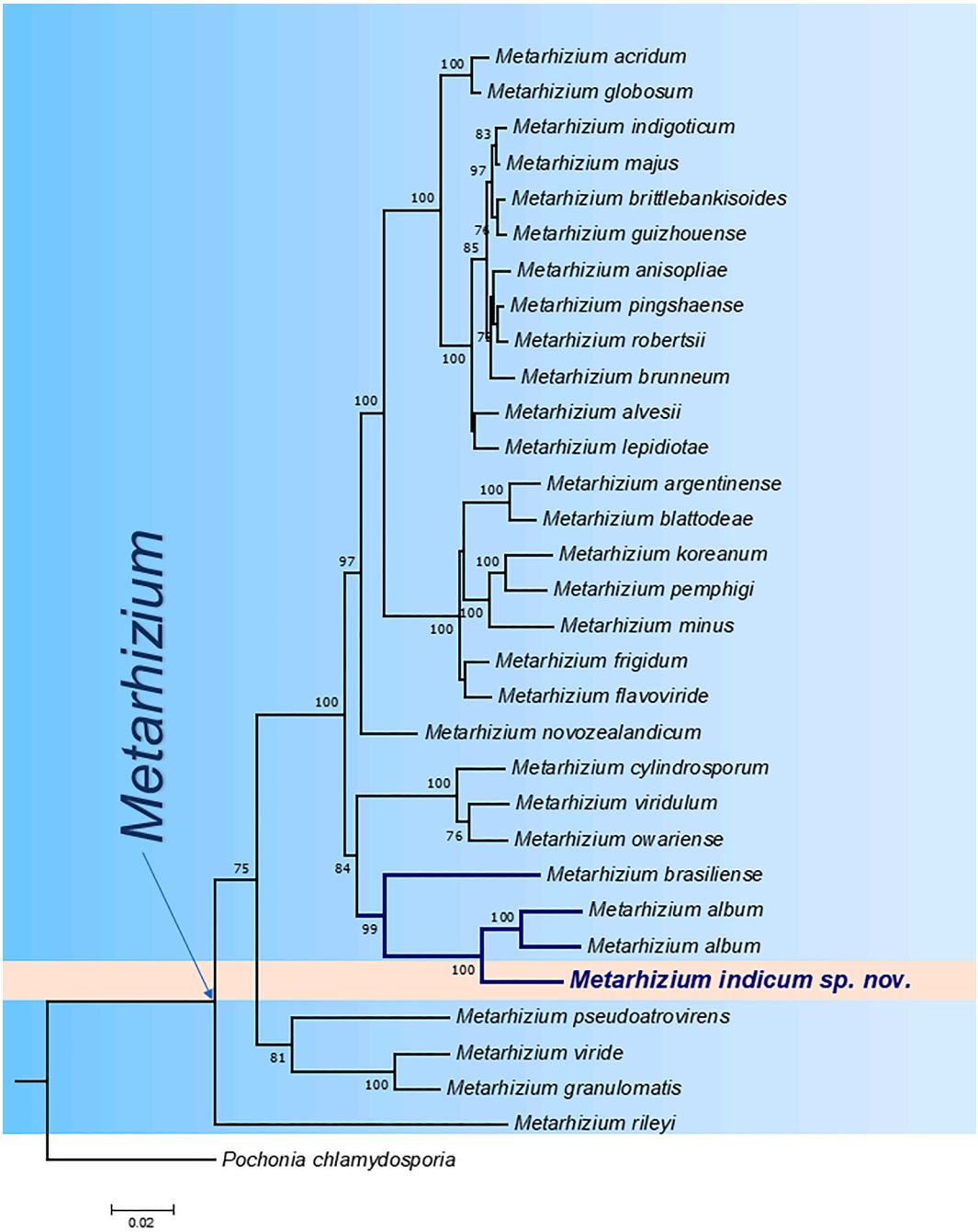

Fig. 5. Phylogram generated from RAxML analysis (1000 replicates) based on a combined RPB1, RPB2, TEF and TUB sequence data for the genus *Metarhizium* with a final likelihood value of −15829.781411. The matrix had 933 distinct alignment patterns with 5.06% undetermined characters and gaps. Estimated base frequencies were as follows: A = 0.249921, C = 0.209147, G = 0.295497, T = 0.245435; substitution rates: AC = 1.314018, AG = 4.030014, AT = 0.927226, CG = 0.946324, CT = 10.633573, GT = 1.000000; gamma distribution shape parameter α = 1.866905. Bootstrap support values for ML greater than or equal to 70% are given above the nodes. The tree is rooted to *Pochonia chlamydospora*. The new taxon is shown in bold and blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

confirmed that the present strain is unique and undescribed, and formed a sister clade to *M. album* with strong bootstrap values of 100% and 99%, respectively (Supplementary Figs. 2 and 3).

4. Discussion

The current system of reporting new species of hypocrealean entomopathogens encourages the use of genomic characters, considering the view that morphological characters alone are not adequate to identify an organism up to species level (Montalva et al., 2016). Several species of the genus *Metarhizium* have overlapping morphological features, which make it difficult to distinguish them based on morphological traits alone (Bischoff et al., 2009; Driver et al., 2000; Rehner and Buckley, 2005) with few exceptions (Bischoff et al., 2009; Iwasaki et al., 2019; Rombach et al., 1987). Accordingly, multigene analyses of *Metarhizium* species complex in the recent past had exposed the presence of cryptic species in this group and had resulted in description of several new species (e.g., Bischoff et al., 2009; Gutierrez et al., 2019; Lopes et al., 2018; Mongkolsamrit et al., 2020; Montalva et al., 2016).

In the present study, we report a new species, *Metarhizium indicum,*

infecting leafhopper, *Busoniomimus manjunathi*. The fungus is distinctly different from its closest related species, *M. album*, which is prevalent among hoppers in Indonesia, Philippines, Sri Lanka and Thailand (Petch, 1931). *Metarhizium album* is characterized by its pale brown colony and presence of cylindrical to ellipsoid or ovoid and smaller conidia compared to its related species (Rombach et al., 1987). The Sri Lankan isolate of *M. album* have smaller conidia (3.0–4.0 × 1.8 μm) and were reported to be ovoid to oblong oval in shape, whereas those reported from Thailand were found to be cylindrical to ellipsoidal in shape and were larger in size (5.0–6.0 × 1.5–2.0) compared to those of Sri Lankan isolates. In contrast, the conidia of *M. indicum* are larger in size and ellipsoid to cylindrical in shape, when compared to all reported isolates of *M. album*. Moreover, *M. album* was never reported from India and in general, geographical endemism is also considered as a basis to propose species status in fungi (Bischoff et al., 2009; Roets et al., 2008; Zhao et al., 2008).

Molecular analyses indicate the closeness of our strain with *M. album* suggesting that the hopper pathogens form a well-supported monophyletic lineage. However, there are marked differences in nucleotide composition and genetic distance as revealed by the BLASTn analyses among the proposed new species and its closest strain, *M. album*. These features along with its distinct morphological traits unambiguously support our claim that the present fungus is a novel species of *Metarhizium*. Further studies will focus on testing the infectivity and host range of this fungus against other hopper pests and major globally distributed insect pests for exploring the possibility to develop it as a biocontrol agent against various crop pests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2023.107919.

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