Asian Journal of Mycology 5(2): 59–69 (2022) ISSN 2651-1339

www.asianjournalofmycology.org **Article**

Doi 10.5943/ajom/5/2/5

Meliola crotalariae sp. nov. (Ascomycetes, Meliolales) from Malabar Wildlife Sanctuary in Kerala State, India

Lini KM

Department of Botany, St. Thomas College, Kozhencherry-689103, Kerala, India. email: linikmathew1985@gmail.com

Lini KM 2022 – *Meliola crotalariae* sp. nov. (Ascomycetes, Meliolales) from Malabar Wildlife Sanctuary in Kerala State, India. Asian Journal of Mycology 5(2), 59–69, Doi 10.5943/ajom/5/2/5

Abstract

A novel species, *Meliola crotalariae* is introduced herewith morphological evidence. It was collected from infected leaves of *Crotalaria verrucosa* (Fabaceae) in the Malabar Wildlife Sanctuary of Kozhikode district in Kerala State (India). The novel taxon is characterized by having alternate to unilateral and only 2% opposite appressoria with shorter mycelial setae (420 µm). This study provides a detailed description and illustrations with morphological comparisons of related *Meliola* species.

Keywords – Ascomycetes – Black Mildew fungi – *Crotalaria verrucosa* – Fabaceae – Meliolaceae

Introduction

During a survey and study of black mildew-causing fungi in the Western Ghat's forests of Malabar Wildlife Sanctuary in Kerala State, the plant *Crotalaria verrucosa* (Fabaceae) were seen infected with a fungus. Microscopic observations of this fungus revealed that it belongs to the Meliolaceae. The fungus revealed appressoriate, black mycelium with mycelial setae, phialides and globose perithecia. Microscopic examinations of the infected plants revealed that it is a hitherto undescribed species of the genus *Meliola* Fries, hence, this note.

Black mildews are a group of black colonies forming parasitic fungi, belonging to several taxonomic groups, namely Meliolales, Asterinales, and *Schiffnerula* and its anamorphic forms (Hofmann 2009). They are distinct from the saprophytic sooty moulds. Sooty moulds grow on the secretions of insects or nectar produced by the glands of plants and are not host-specific (Hosagoudar 2008b). However, black mildews are not associated with mealy bugs, and the majority of them are obligatory biotrophs, yet a few are necrotrophs (Hosagoudar 1996). They considered host specific with a very narrow host range (Florence 2004). Hence, the identity of the host plant, preferably up to the species level, needs to be known for the correct identification of these fungi.

Fungi play a major role in the establishment and maintenance of the ecosystem and the development of mutualistic relationships with many other organisms. Many species rely on specific plants for food and habitat, and the destruction of plant diversity affects microbial diversity. In many instances, it is possible for the species to extinction much before they have been discovered. Fungi are important components of biodiversity in tropical forests, knowledge about the taxonomy and biology of tropical fungi has immediate relevance to the control of harmful interactions and the harnessing of useful fungal activities for human welfare, and this implies the need for fungal taxonomists. Therefore, this work becomes the manual for the identification of the foliicolous fungi

Submitted 9 August 2022, Accepted 14 November 2022, Published 6 December 2022
Corresponding Author: Lini K. Mathew – e-mail – linikmathew1985@gmail.com

of the area (Kapoor 1967, Cooke 1880).

Black mildews are obligate biotrophs, belonging to Sordariomycetes, Dothideomycetes and Hyphomycetes. Black colony-forming, parasitic fungi placed under Armatellaceae, Asterinaceae, Dysrhynchaceae, Englerulaceae, Lembosiaceae, Meliolaceae, Meliolinaceae and a few genera of hyphomycetes are referred black mildews (Hosagoudar 1996, 2008a, 2012, 2013b, Hosagoudar & Archana 2012, Hosagoudar & Sabeena 2011, 2014, Thimmaiah et al. 2013). The families Meliolaceae and Armatellaceae of Meliolales are perithecial fungi, accommodated under Sordariomycetes (Hongsanan et al. 2015, Hyde et al. 2020). Asterinaceae and Lembosiaceae include thyriothecioid ascomycetes classified in Asterinales in Dothideomycetes (Hongsanan et al. 2020). Dysrhynchaceae, Englerulaceae, and Meliolinaceae are families of uncertain position placed in Dothideomycetes (Hongsannan et al. 2020).

History of Classification of Meliolales

The order Meliolales encompasses seven genera (Stevens 1927, 1928). Hosagoudar (1996, 2008a, 2013a) followed Hansford (1961), and subsequently elaborated the classification and included nine genera, namely *Armatella, Basavamyces, Amazonia, Ectendomeliola, Appendiculella, Meliola, Asteridiella, Irenopsis* and *Prataprajella* under Meliolales. The order comprises Meliolaceae and Armatellaceae (Hosagoudar 2003, Hongsanan et al. 2015, Hyde et al. 2020).

Martin (1941) proposed Meliolaceae without a Latin diagnosis and Hansford (1946) later validated the description of the taxa. Hosagoudar (2003) introduced the family Armatellaceae. Lumbsch & Huhndorf (2010) considered 26 genera under Meliolales. Hongsanan et al. (2015) re-examined specimens from 17 genera of Armatellaceae and Meliolaceae. They accepted *Armatella* in Armatellaceae, and *Amazonia, Appendiculella, Asteridiella, Cryptomeliola, Endomeliola, Irenopsis* and *Meliola* were retained in the family Meliolaceae. *Haraea, Prataprajella, Hypasteridium, Pauahia, Leptascospora, Ceratospermopsis, Ticomyces, Ectendomeliola, Metasteridium, Urupe, Ophiociliomyces, Ophioirenina, Ophiomeliola, Parasteridium, Pleomeliola, Pleomerium* and *Xenostigme* were treated as doubtful genera, hence, segregated to Ascomycetes genera *incertae sedis*. Selkirk (1975) accommodates the fossil Meliolales in Meliolinites after the discovery of *Meliola spinksii* Dilcher and *M. anfractus* Dilcher on Eocene specimens by Dilcher (1965).

The genus *Meliola* was proposed by Fries (1825) and the description was amended by Bornet (1851). The type species is *Meliola psidii* Fr. (Fries 1830). The detailed studies of the genus were provided by Gaillard (1892), Beeli (1920) with digital formula for comparisons between genera and species, Stevens (1927, 1928), Hansford (1961, 1963), Katumoto & Hosagoudar (1989), Hosagoudar et al. (1997a), and Hosagoudar (2008a, 2013a). Stevens (1927, 1928) recommended the systematic placement of Meliola under the tribe Meliolineae within Perisporiales but Nannfeldt (1932) included Meliolaceae in Ascoloculares. Later Meliolaceae, Dothideaceae and Erysiphaceae were placed in Myriangiales (Hansford 1946). Viewing Meliolaceae as bitunicate, Eriksson (1981) and Hawksworth et al. (1983) treated Meliolaceae under Dothideales contrary to Yarwood (1973), who considered Meliolaceae as unitunicate Pyrenomycetes because of its close resemblance to Erysiphaceae or the powdery mildews. The placement is justified because of the parasitic nature of both taxa, and similarities in morphology, including the presence of appressoria on mycelium and melanised ascomata. These conflicts in classifications were due to the confusion in confirming Meliolaceae as unitunicate or bitunicate. The phylogenetic analysis later confirmed Meliolaceae are unitunicate Pyrenomycetes but are not close relatives of Erysiphaceae (Saenz & Taylor 1999). *Meliola* is the largest genus of Meliolaceae, and the accommodation of the genus in the family is supported by molecular data (Saenz & Taylor 1999, Pinho et al. 2014).

Phylogeny of Meliolales

Limited molecular phylogenetic studies have been conducted in Meliolaceae. Molecular data of only 25 species are available (Zeng et al. 2017) even though 3064 epithets are listed in Index Fungorum (Jayawardena et al. 2020). As per the latest revisions, the molecular data of only 20

confirmed species of Meliolaceae are available in the GenBank (Hyde et al. 2020). Successful protocol for culturing the fungus is not yet developed because of their obligate parasitic nature (Maharachchikumbura et al. 2015). The thick cell wall, presence of dark pigment on the wall and non-culturable nature of the fungi makes DNA isolation difficult in black mildew (Justavino & Piepenbring 2007). However, there are few successful attempts to amplify DNA directly from Ascomata have been published (Saenz & Taylor 1999, Justavino & Piepenbring 2007, Vitoria et al. 2010, Jaklitsch & Voglmayr 2012, Maharachchikumbura et al. 2015, Hongsanan et al. 2015).

Saenz & Taylor (1999), by analysing 18S rDNA sequences from ascomata, concluded that Meliolales is a member of Pyrenomycetes with a close resemblance to Sordariales. This conclusion is in accordance with Luttrell (1989) that stated the close relationship of Meliolaceae to Pyrenomycetes due to the development of thin-walled, unitunicate asci from the basal hymenium. Saenz & Taylor (1999) located the phylogenetic position of *Meliolina* among bitunicate ascomycetes. Hence, *Meliolina* was found to be distantly related to Meliolaceae. Therefore, it was resolved that the powdery mildew are not Pyrenomycetes and are not a close relative of Meliolaceae. Saenz et al. (1994) analysed the 18S rRNA gene and predicted that Erysiphaceae evolved earlier than Pyrenomycetes. The fossil records of Pyrenomycetes are available only from the Miocene but that of *Meliola* are obtained from Eocene onwards. This seems contrary to Saenz et al. (1994), but it might be due to the better preservation of Meliolaceae members because of the presence of melanin in the mycelium. Powdery mildew lack melanin in mycelium, but its presence is confirmed in ascomata (Saenz & Taylor 1999).

Phylogenetic studies indicated that Meliolales formed a distinct clade in Sordariales (Saenz & Taylor 1999). Zhang et al. (2006) also support the systematic placement of Meliolales in the class Sordariomycetes due its close similarities in morphology. Phylogenetic analyses of the 28S rDNA sequence confirmed Meliolales as a monophyletic order within Sordariomycetes (Pinho et al. 2012). Phylogeny inferred from the 28S rDNA is ambiguous, hence, increased taxon sampling by the selection of additional genes will improve the resolution of the clade (Miller & Huhndorf 2005, Tang et al. 2007). Pinho et al. (2012) viewed the phylogenetic position of Meliolales as uncertain. Lumbsch & Huhndorf (2010) placed Meliolaceae in the subclass Meliolomycetidae under Sordariomycetes, which was justified by Pinho et al. (2012), Justavino et al. (2015), and Maharachchikumbura et al. (2015) based on phylogenetic analyses using RPB2, LSU, SSU and TEF sequence data. Being distantly related to Dothideomycetes, Meliolaceae was excluded from the former taxa (Hyde et al. 2013, Wijayawardene et al. 2014). The *Meliola* was confirmed to be polyphyletic, belonging to Sordariomycetes (Hyde et al. 2020, Marasinghe et al. 2020, Zeng et al. 2020, Jayawardena et al. 2020).

Materials & Methods

Study Area

The Malabar Wildlife Sanctuary is a part of the Nilgiri Biosphere Reserve of the Western Ghats, a biodiversity hotspot, located in Chakkittappara and Koorachundu revenue villages of Quilandy Taluk in Kozhikode district, Kerala State. It lies between 11° 75’ and 11° 76‘ north and 76° 20’ and 75° 38’ east, the forests lie on the Northwest slopes of the Western Ghats contiguous with the forests of Ladysmith Reserved Forests and Kurichiar mala of Kalpetta Forest Range of South Wayanad Forest Division.

Morphological observation

Infected plant parts (twigs with living leaves of *Crotalaria verrucosa*, Fabaceae) were collected from Peruvannamuzhy, Malabar Wildlife Sanctuary, Kozhikode, Kerala, India, (Coordinates: 11°35′0″N 75°49′0″E / 11.58333°N 75.81667°E / 11.58333), photographed (plant habit and fungal infections), evaluated and brought to the laboratory.

In the laboratory, samples were divided into two sets: one for the preparation of microscopic slides and the other for preserving as herbarium specimens. For herbarium preparation, the infected

parts were dried by the usual pressing method between thick blotting papers using a wire press. After drying, they were examined carefully under a zoom stereomicroscope (Magnus) to study colony characteristics and avoid colonies with hyperparasites. The nail polish technique (Hosagoudar & Kapoor 1985) was adopted to study the morphological and structural characters of fungi. A drop of transparent nail polish was applied and carefully thinned with the help of a fine brush or a glass rod without disturbing the selected colonies and kept in a dust-free chamber for it to get dried. After drying, a thin, colourless film flipped off with slight pressure on the opposite side of the leaves and just below the colonies in the case of soft host parts. In the case of hard host parts, the flip eased off with the help of a razor or scalpel. The lifted flip was mounted directly in dibutyl phthalate polystyrene xylene (DPX), labelled and dried.

Detailed taxonomic description of the specimen written by studying the micro-morphological characters using different magnifications of a binocular compound microscope Olympus (CX21iLED) with MagVision image analyzer software for the final confirmation of the identity of foliicolous fungi. Biometric data is based on at least 10 measurements of micro-morphological structures and given the minimum to maximum values of 10 measurements. The colour photographs are made with a Magcam DC10 CMOS camera of 10 megapixels, and illustrations are provided as plates with legends. Using appropriate reference literature, isolates were identified and assigned to respective genera and species.

The novel taxon was identified based on previous literature (various books, monographs, reviews and indices) (Hosagoudar 2008a, 2013a, Hosagoudar et al. 2012, 2013, Thomas 2015). The type specimen was deposited in the regional herbarium Mar Thoma College Herbarium, Tiruvalla (MTCHT), Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvanathapuram (TBGT). The new taxon is linked with Index Fungorum databases as explained in Index Fungorum (2022).

Results

Meliola crotalariae sp. nov. Lini K. Mathew (**Fig. 1**)
Etymology: The specific epithet is based on the host genus. Known only from the present locality.

Figs 1, 2

Description to the species

Coloniae epiphyllae, tenues vel subdensae, ad 3mm diam diam. Hyphae rectae vel subrectae vel undulatae, opposite vel irregulariter subacutis vel laxe ramosae, laxe vel dense reticulatae, cellulae 15 –37 x 4–6 µm. Appressoriis intermixtae alternatae vel unilateralis, de II% contrarium, antrorsa vel retrorsis vestiti, usque ad curvam recta, recurva, 9–15 µm longae; cellulae cylindraceae vel cuneatae 2-4 longae; cellulae apicales globosae vel subglobosae, rectae vel leniter curvulae, integrae, 8–14 x 6–9 µm. Phialides paucae, appressoriis, alternatae vel oppositae, ampulliformes, 10–18 x 5–9 µm. Setae circum dispersa Peritheciis tot simplicibus rectis flexuosae, acutae vel obtusae ad apicem usque 420 µm longae. Perithecia dispersa, globosa, usque ad 200 µm diam; ascosporae filiformes vel ellipsoideis, IV-septatae, constrictae ad septa, 33–38 x 12–17 µm.

Colonies epiphyllous, thin to subdense, up to 3 mm in diameter, confluent. *Hyphae* straight to undulate, branching opposite to irregular at subacute to wide angles, loosely to closely reticulate, cells 15–37 × 4–6 µm. *Appressoria* alternate to unilateral, about 2% opposite, antrorse to retrorse, straight to curved, recurved, 9–15 µm long; stalk cells cylindrical to cuneate, 2–4 µm long; head cells globose to subglobose, straight to slightly curved, entire, 8–14 × 6–9 µm. *Phialides* few, mixed with appressoria, alternate to opposite, ampulliform, 10–18 × 5–9 µm. *Mycelial setae* scattered to grouped around perithecia, numerous, simple, straight to flexuous, acute to obtuse at the tip, up to 420 µm long. Sexual morph: *Ascomata* scattered, globose, up to 200 µm in diameter. *Ascospores* cylindrical to ellipsoidal, 4–septate, constricted at the septa, 33–38 × 12–17 µm.

Materials examined: India, Kerala: Kozhikode, Malabar Wildlife Sanctuary, Peruvannamuzhy, on the leaves of *Crotalaria verrucosa* (Fabaceae), 26 December 2014, Lini K. Mathew, MTCHT 55 (Holotype), TBGT 6934 (Isotype).

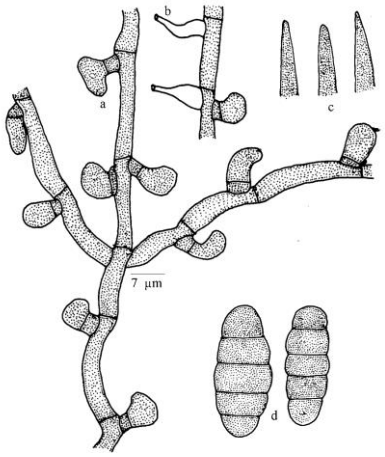

Fig. 1 – ***Meliola crotalariae***. a. appressoriate mycelium. b. Phialides. c. Mycelial setae. d. Ascospores

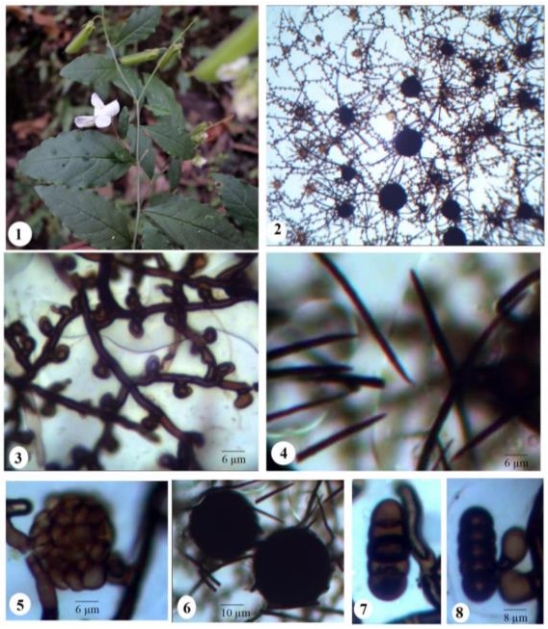

Fig. 2 – ***Meliola crotalariae***. 1. Infected leaves of *Crotalaria verrucosa* (Fabaceae). 2. Coloies with perithecia. 3. appressoriate mycelium with Phialides. 4. Apical portion of the mycelial setae. 5. Developing perithecium. 6. Mature perithecia. 7. Ascospore. 8. Germinating ascospore

Discussion

The detailed study of the fungal flora of Uganda was conducted by Hansford (1937) who reported 92 species from Meliolales. Hansford (1961) published a monograph on Meliolaceous fungi with descriptions of 1814 species associated with vascular plants, including 332 angiosperms families. This is a monumental work, that included all known species of Meliolales at that time. Hansford (1963) further published elaborate additions to the descriptions and illustrations of Meliolales.

Meliolaceae from tropical America and South America have been studied by Stevens (1927, 1928), Chacon & Cruz (1999), Justavino & Piepenbring (2007), Macedo et al. (2010) and Pinho et al. (2012, 2013, 2014). From Brazil, *ca.* 240 species were reported (Justavino & Piepenbring 2007)

and from Panama, *ca.* 100 species reports are available (Stevens 1927, 1928, Justavino & Piepenbring 2007). Pinho et al. (2014) extensively studied Meliolaceous fungal diversity in the Amazon rainforest in Brazil, used 28S rDNA nucleotide sequences to analyse phylogenetic relationships of Meliolales, and published the first record of molecular sequence for *Irenopsis*. From members of the Euphorbiaceae in Brazil, thirteen species of Meliolaceous fungi were reported. *Irenopsis vincensii* was proposed as the new name for the black mildew associated with *H. brasiliensis* (Hansford 1961, Pinho et al. 2012). *Meliola centellae* Pinho & O. L. Pereira, the record of the first black mildew fungus associated with Apiaceae was described and illustrated by Pinho et al. (2012). The authors also introduced a method to extract DNA from *Meliola*. New species, *Appendiculella lozanellae* Rodr. Just. & M. Piepenbr. and *Appendiculella chiriquiensis* Rodr. Just. & M. Piepenbr. were the first reports of *Appendiculella* infecting members of Cannabaceae and Sapindaceae, respectively (Justavino & Piepenbring 2007) in western Panama. Molecular data of 18S and 28S rDNA for *A. lozanellae* were the first published sequence data for the genus *Appendiculella*. At present, sequence data are only available for three species in *Appendiculella* (Marasinghe et al. 2020).

The diversity of Meliolaceae in China is *ca.* 345 species (Justavino & Piepenbring 2007). Meliolaceae of Hainan province (Hu & Lu 1989), Guangdong province (Hu 1990) and Yunnan Province (Song & Li 2003, 2004) were explored in detail. The highest number of Meliolaceae members were reported from India (Justavino & Piepenbring 2007). Hosagoudar (2008a) consolidated reports of Meliolaceae from India and published records of 612 species, including *Meliola* and infra-specific taxa (453), *Asteridiella* (73), *Appendiculella* (10), *Irenopsis* (31), *Prataprajella* (2) *Amazonia* (28), *Armatella* (13), and single species of *Basavamyces* and *Ectendomeliola*. Later 123 species, belonging to *Amazonia* (3), *Appendiculella* (1), *Irenopsis* (8), *Asteridiella* (22), *Meliola* (88) and *Ectendomeliola* (1), biotrophic on 120 host plants of 49 families of vascular plants were added (Hosagoudar 2013a). The attempt to culture ascospores of *Meliola in vitro* in media containing KNO_3, Na_2HPO_4 and $(NH4)_2SO_4$ was attempted by Bal (1919). The germinated ascospores failed to continue growth after formation of few appressoria. The ascospores failed to germinate in media prepared with beef broth, peptone and host extract. *In vivo* and *in vitro* attempts to germinate ascospore of *Asteridiella* Hansford (1961), culture of macerated colonies and ascospores of *Meliola* sp. (Goos 1974), hanging drops method to culture *M. jasminicola* (Thite 1975), attempt to germinate ascospores of *M. argentina* and *M. palmicola* on agar medium (Goos 1978) and *in vito* culture and germination of ascospores of *Meliola* sp. (Hosagoudar & Udaiyan. 1993) were reported unsuccessful.

Indian context of Meliolales

The investigations on Meliolalaceous fungi in India were pioneered by Cooke (1880, 1884) and reported two species, namely *Meliola densa* Cooke and *M. zigzag* Berk. & Curt. from Belgaum, Karnataka. Other earlier works include that of Sydow (1913), Sydow & Sydow (1914), reports of Meliolalaceae from Bengal by Bal & Dutta (1922) and fungal reports of South India published by Hansford & Thirumalachar (1948). Subsequent studies in Meliolaceae in India were undertaken by Kapoor (1967) and Kar & Maity (1970, 1971). Extensive investigations were carried out further by Hosagoudar & Goos (1990), Hosagoudar & Abraham (1998a & b), Hosagoudar (1996, 2006a, b, c, 2008a, 2013a), Hosagoudar & Agarwal (2006, 2008), Hosagoudar & Jacob Thomas (2009a, 2010), Hosagoudar & Riju (2013), Hosagoudar et al. (2011a, 2013a, 2014), Hosagoudar & Sabeena (2014), and Sabeena et al. (2017, 2018).

Several new species of *Meliola* from Peppara and Neyyar Wildlife Sanctuaries of Kerala state were reported (Hosagoudar & Abraham 1998b, Hosagoudar & Thomas 2010). Hosagoudar (2006c) gave a detailed account of the biogeographical distribution of 533 taxa belonging to Meliolaceae from India. The existence of 488 taxa reported in the Western Ghats and the species distribution could not be located in the Trans Himalayas, Deserts, Semi-Arid regions and Coastal vegetations of India. Hosagoudar & Riju (2013) identified fungi belonging to 6 genera, *Amazonia* (3), *Armatella* (4), *Asteridiella* (12), *Irenopsis* (1), *Meliola* (47), *Prataprajella* (1) from the Silent Valley National

Park, Palghat, Kerala. Meliolaceae of the Wayanad district of Kerala was studied by Hosagoudar & Sabeena (2014) and reported 128 species in *Amazonia* (6), *Appendiculella* (1), *Armatella* (6), *Asteridiella* (12), *Irenopsis* (7) and *Meliola* (96). Extensive surveys on Meliolaceous fungal flora were carried out in the Palode Forest Range of Kerala (Hosagoudar & Archana 2012), the Shendurney Wildlife Sanctuary of Kerala (Thomas 2015) and the Malabar Wildlife Sanctuary in Kerala (Mathew 2018).

Hosagoudar et al. (2014) reported several new species of Meliolaceae from the Andaman Islands. *Asterediella anaxagoreae, Asteridiella colocasiae, Irenopsis andamanica, Meliola andamanica, M. canarifolia, M. chukrasiicola, M. harrietensis, M. myristicacearam, M. parishiae, M. savarkarii and M. ternstroemiicola* were reported as new species from the Andaman Islands. Sabeena et al. (2017) added a few records to the Meliolales of the Andaman Islands. Later, Niranjan & Sarma (2018) published a checklist of fungi from the Andaman and Nicobar Islands, consisting of 47 species from Meliolaceae. Dubey & Pandey (2019) explored the biodiversity of Meliolaceae in the Konkan region of Maharashtra, India.

The present species, *Meliola crotalariae* was collected from infected leaves of *Crotalaria verrucosa* (Fabaceae) (Plate: 1, Fig. 1). It can be compared with *Meliola stizolobii* Hansf. and Deight. on *Crotalaria* sp. from Sudan and *Meliola desmodii-laxiflori* Deight. var. *crotalariae* Deight on *Crotalaria anagyroides* from Malaya, which were the only *Meliola* species infecting the host genus *Crotalaria* with Beeli formula 3133.3223 and 3111.4223, respectively (Hansford 1961, Hosagoudar 1996, 2008a, 2013a). *Meliola crotalariae* has the Beeli formula of 3113.3222 (Table 1**)**.

Table 1 Synopsis table of morphologically similar species.

Species name	**Beeli formula**	**Morphological characters**				
		Colonies	**Mycelium**	**Appressoria**	**Mycelial setae**	**Ascospores**
M. crotalariae	3113.3222	epiphyllous, up to 3 mm in diameter, confluent	straight to undulate	alternate to unilateral, about 2% opposite, head cells globose to subglobose, straight to slightly curved, entire, 8–14 × 6–9 µm	simple, straight to flexuous, acute to obtuse at the tip, up to 420 µm long.	cylindrical to ellipsoidal, 4-septate, constricted at the septa, 33–38 × 12–17 µm
M. stizolobii	3133.3223	epiphyllous, subdense, velvety, to 5 mm diameter.	sub straight to undulate	alternate or to 20% opposite, head cells globose to widely piriform, entire 8–14 × 9–14 µm	numerous, scattered over mycelium, straight, to 800 µm, apex 2–4-dentate to 10 µm, furcate to 15 µm, the branches cristate-dentate.	oblong, obtuse, 4–septate, slightly constricted, 31–39 × 12–14 µm.
M. desmodii-laxiflori var. crotalariae	3111.4223	Colonies, caulicolous, dense, velvety, upto 5 mm diameter	Hyphae substraight to sinuous,	alternate, antrorse. head cells entire, subglobose, oblong or ovate, straight or bent, 11–15 × 11–15 µm.	numerous, scattered, straight, simple, acute, to 700 µm long	oblong to slightly cuneate, obtuse, 4-septate, slightly constricted, 30–42 × 12–14 µm.

Meliola stizolobii differs from *Meliola crotalariae* in having 20% opposite appressoria with longer, dentate to furcate mycelial setae (800 µm). *Meliola desmodii-laxiflori* var. *crotalariae* differs from *Meliola crotalariae* in having caulicolous colonies and longer mycelial setae (Hansford 1961, Hosagoudar, 1996, 2008a, 2013a; Hosagoudar & Abraham 1998, Kar & Bhattacharyya 1982). Based on the host specificity, Beeli formula and morphological features, *M. crotalariae* can be accommodated into a new species.

Acknowledgements

I thank the Department of Botany, Mar Thoma College, Tiruvalla, Registrar, MG Uiversity, Kottayam and KSCSTE, Govt. of Kerala, for providing facilities. Forest Department of Kerala is thankfully acknowledged for granting permission to conduct the field study.

References

Bal SN, Dutta AC. 1922 – Few species of *Meliola* growing on different host plants. *Journal of the Department of Science, University of Calcutta* 4, 1–14.

Bal SN. 1919 – *Meliola* growing on *Phoenix sylvestris* and *Citrus medica* (var. *acida*). *Journal of Department of Science, University of Calcutta* 1, 1–4.

Beeli M. 1920 – Note sur le Genre *Meliola*. *Bulletin du Jardin Botanique Bruxelles* 7(1), 89–160.

Bornet E. 1851 – Etudes sur l'organisation des especes qui composent le genre *Meliola*. *Annales des Sciences Naturelles, Botanique Ser*. III (16), 257.

Chacon S, Cruz F. 1999 – Descripcion de 13 nuevos registros de mildius negros (Meliolales) del estado de Veracruz, Mexico. *Revista mexicana de micologia* 15, 23–26.

Cooke MC. 1880 – Fungi of India. *Grevillea* 8(47), 93–96.

Cooke MC. 1884 – Some exotic fungi. *Grevillea* 12(61), 37–39.

Dilcher DL. 1965 – Epiphyllous fungi from Eocene deposits in Western Tennessee, U.S.A. *Palaeontographica Abstracts B* 116, 1–54.

Dubey R, Pandey AD. 2019 – Statistical analysis of foliicolous fungal biodiversity of Konkan region, Maharashtra, India, A novel approach. *Plant Pathology and Quarantine* 9(1), 77–115. Doi 10.5943/ppq/9/1/9

Eriksson O. 1981 – The families of bitunicate ascomycetes. *Nordic Journal of Botany* 1(6), 1–209. Doi 10.1111/j.1756-1051.1981.tb01167.x

Florence EJM. 2004 – *Biodiversity Documentation of Kerala part II, Microorganism (Fungi).* Kerala Forest Research Institute, Hand Book no.17, 1–293.

Fries EM. 1825 – Systema orbis. *Vegetabilis* 2, 1–373.

Fries EM. 1830 – Eclogae fungorum, praecipue ex herbarus germanorum de scriptorum. *Linnaea* 5, 497-553

Gaillard A. 1892 – Le genre *Meliola*. *Bulletin de la Societe Mycologique de France* 8, 176–188.

Goos RD. 1974 – A scanning electron microscope and *in vitro* study of *Meliola palmicola*. *The Proceedings of the Iowa Academy of Sciences* 81, 23–27.

Goos RD. 1978 – Field and laboratory studies of meliolaceous fungi in Hawaii. *Mycologia* 70, 996–1006.

Hansford CG, Thirumalachar MJ. 1948 – Fungi of South India. *Farlowia* 3, 285–314.

Hansford CG. 1937 – Contributions towards the fungus flora of Uganda– I. The Meliolineae of Uganda. *Botanical Journal of the Linnean Society* 51, 265–284.

Hansford CG. 1946 – The foliicolous Ascomycetes and their parasites and associated fungi. *Mycological Papers* 15,1–240

Hansford CG. 1961 – The Meliolineae. *Sydowia Annales Mycologici, Ser. II, Beihefte* 2, 400–420.

Hansford CG. 1963 – Iconographia meliolinearum. *Beihefte zur Sydowia* 5, 1–285.

Hawksworth DL, Sutton BC, Ainsworth GC. 1983 – *Ainsworth and Bisby's Dictionary of the Fungi*. 7th *edn*. Commonwealth Mycological Institute, Kew, Surrey, England, Pp. 1–445.

Hofmann TA. 2009 – *Plant parasitic Asterinaceae and Microthyriaceae from the Neotropics (Panama)*. Germany, 407 pp.

Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN. et al. 2020 – Refined families of Dothideomycetes, orders and families *incertae sedis* in Dothideomycetes. *Fungal Diversity* 105, 17–318. Doi 10.1007/s13225-020-00462-6

Hongsanan S, Tian Q, Persoh D, Zeng XY. et al. 2015 – Meliolales. *Fungal Diversity* 74(1), 91–141. Doi 10.1007/s13225-015-0344-7

Hosagoudar VB, Abraham TK. 1998a – New and interesting Meliolaceae members from Kerala, *Indian Phytopathology* 51, 58–61.
Hosagoudar VB, Abraham TK. (1998b) Some Meliolaceae from Kerala, India. *Sydowia* 50, 14–20.
Hosagoudar VB, Agarwal DK. 2006 – *Ectendomeliola*, a new genus of the family Meliolaceae. *Indian Phytopathology* 59 (1), 98–100.
Hosagoudar VB, Agarwal DK. 2008 – *Taxonomic Studies of Meliolales, Identification Manual*. International Book Distributors, Dehra Dun, India, 1–263.
Hosagoudar, V.B. and Archana, G.R. (2012) *Foliicolous Fungal Flora of Palode Forest Range, Thiruvananthapuram, Kerala*. Sadguru publications, India, 1–328pp.
Hosagoudar VB, Goos RD. 1990 – Meliolaceous fungi from the State of Kerala, India-II. The genus *Meliola*. *Mycotaxon* 37, 217–272.
Hosagoudar VB, Thomas J. 2009a – Meliolaceae of Kerala, India-XXXII. *Indian Journal of Science and Technology* 2 (6), 22–24. Doi 10.17485/ijst/2009/v2i6.6
Hosagoudar VB, Thomas J. 2010 – Interesting foliicolous fungi from southern Western Ghats of Kerala, India. *Journal of Applied and Natural Science* 2(1), 102–105.
Hosagoudar VB, Riju MC. 2013 – Foliicolous fungi of Silent Valley National Park, Kerala, India. *Journal of Threatened Taxa* 5(3), 3701–3788. Doi 10.11609/JoTT.o3302.01-88
Hosagoudar VB, Sabeena A. 2011 – A black mildew fungus, *Schiffnerula azadirachtae* sp. nov. (Ascomycota, Englerulaceae) from Kerala, India. *Journal of Threatened Taxa* 3(3), 1620–1621.
Hosagoudar VB, Sabeena A. 2014 – Foliicolous fungi of Wayanad District in Kerala State, India. *Journal of Threatened Taxa* 6(7), 5909–6052. Doi 10.11609/jott.o3658.5909-6052
Hosagoudar VB, Udaiyan K. 1993 – Indian Meliolales. *Indian Review of Life Sciences* 13, 61–82.
Hosagoudar VB. 1996 – *Meliolales of India*. Botanical Survey of India, Calcutta pp. 366.
Hosagoudar VB. 2003 – *Armatellaceae*, a new family segregated from the Meliolaceae. *Sydowia* 55(2), 162–167.
Hosagoudar VB. 2006a – Meliolaceous fungi on economically important plants in India-III, on wild edible plants. *Zoos´ Print Journal* 21(10), 2425–2438.
Hosagoudar VB. 2006b – Additions to the fungi of Kerala-IV. *Zoos´ Print Journal* 21(11), 2475–2478.
Hosagoudar VB. 2008a – *Meliolales of India.* Vol. II. Botanical Survey of India, Calcutta, 155–354 pp.
Hosagoudar VB. 2008b – *Foliicolous fungal flora of Peppara and Neyyar wildlife sanctuaries in Kerala state*. Balavidya Ganapathy Educational and Charitable Trust, Sundharakkottai, Mannargudi, 144–145pp.
Hosagoudar VB. 2012 – Asterinales of India. *Mycosphere* 2 (5), 615–852. Doi 10.5943/*Mycosphere* /3/5/9
Hosagoudar VB. 2013a – Meliolales of India. Vol. III. *Journal of Threatened Taxa* 5(6), 3993–4068. Doi 10.11609/jott.o3307.3993-4068
Hosagoudar VB. 2013b – *Hand book of black mildews*. Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1-217 pp.
Hosagoudar VB, Kapoor JN. 1985 – New technique of mounting meliolaceous fungi. *Indian Phytopathol.* 38, 548 – 549.
Hosagoudar VB, Abraham TK, Puspangadan P. 1997a – *The Meliolineae - A Supplement.* Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala, India, 1–201 pp.
Hosagoudar VB, Thomas J, Agarwal DK. 2011a – Additions to black mildews of India. *Taprobanica* 3(1), 42–43.
Hosagoudar VB, Mathew SP, Babu D. 2014 – Foliicolous fungi of Andaman Islands, India. *Journal of Threatened Taxa* 6(2), 5447–5463. Doi 10.11609/jott.o3652.5447-63
Hosagoudar VB, Thimmaiah CJ, Jayashankara M, Sabeena A. 2013a – Black mildews (Ascomycetes) from southern Western Ghats of Peninsular India with description of 14 new species. *Journal of Threatened Taxa* 5(2), 3661–3669. Doi 10.11609/JoTT.o3262.3661-69

Hosagoudar VB. 2006c – Biogeographical distribution of Meliolaceae members in India. *Zoos´ Print Journal* 21(12), 2495–2505.
Hu H. 1990 – A preliminary study of Meliolaceae in Guangdong Province II. *Acta Mycologica Sinica* 9, 269–281.
Hu YX, Lu DJ. 1989 – The Meliolaceae of Hainan province. *Acta Mycologica Sinica* 8, 180–190.
Hyde KD, Jones EBG, Liu JK, Ariyawansa, H. et al. 2013 – Families of Dothideomycetes. *Fungal Diversity* 63,1–313.
Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bao DF, Bhat DJ. et al. 2020 – Refined families of Sordariomycetes. *Mycosphere* 11(1), 305–1059. Doi 10.5943/*Mycosphere* /11/1/7
Index fungorum. Available from, http,//www.indexfungorum.org (Accessed on February 8 2022).
Thomas J. 2015 – Report of the major research project on *Diversity and distribution of Black Mildew causing fungi in Shendurney Wildlife Sanctuary, Kerala*. University Grants Commission, New Delhi, India, 1–130 pp.
Jaklitsch WM, Voglmayr H. 2012 – Phylogenetic relationships of five genera of Xylariales and *Rosasphaeria* gen. nov (Hypocreales). *Fungal Diversity* 52, 75−98. Doi 10.1007/s13225-011-0104-2
Jayawardena RS, Hyde KD, Chen YJ, Papp V. et al. 2020 – One stop shop IV, taxonomic update with molecular phylogeny for important phytopathogenic genera, *Fungal Diversity* 103, 76–100. Doi 10.1007/s13225-020-00460-8
Justavino DR, Piepenbring M. 2007 – Two new species of *Appendiculella* (Meliolaceae) from Panama. *Mycologia* 99, 544–552.
Justavino DR, Kirschner R, Piepenbring M. 2015 – New species and new records of Meliolaceae from Panama. *Fungal Diversity* 70, 73–84.
Kapoor JN. 1967 – Notes on Indian Meliolineae. *Indian Phytopathology* 20, 151–160.
Kar AK, Maity MK. 1970 – Pyrenomycetes of West Bengal (India) –I. *Nytt Magasin for Botanik* 17, 81–90.
Kar AK, Maity MK. 1971 – Additions to the Pyrenomycetes of West Bengal (India). *Nova Hedwigia* 21, 279–285.
Kar AK, Bhattacharyya. 1982 – Records of some Pyrenomycetes from India. *Indian Phytopathol*. 35, 39 – 46.
Katumoto K, Hosagoudar VB. 1989 – Supplement to Hansford's The Meliolineae Monograph. *Journal of Economic and Taxonomic Botany* 13, 615–635.
Lumbsch HT, Huhndorf SM. 2010 – Myconet volume 14 Part One. Outline of Ascomycota-2009. Part two. Notes on Ascomycete systematics. Nos. 4751–5113. *Fieldiana Life Earth Sciences* 1, 1–64.
Luttrell ES. 1989 – Morphology of *Meliola floridensis*. *Mycologia* 81, 192–204.
Macedo DM, Pinho DB, Barreto RW, Cuda JP, Pereira OL. 2010 – Black mildew fungi (Meliolaceae) associated with *Schinus terebinthifolius* (Brazilian pepper tree) in Brazil. *Mycotaxon* 114, 429–437. Doi 10.5248/114.429
Maharachchikumbura SSN, Hyde KD, Jones EBG, McKenzie EHC. et al. 2015 – Towards a natural classification and backbone tree for Sordariomycetes. *Fungal Diversity* 72, 199–301.
Marasinghe DS, Boonmee S, Hyde KD, Xie N, Hongsanan S. 2020 – Morpho-molecular analysis reveals *Appendiculella viticis* sp. nov. (Meliolaceae). *Phytotaxa* 454 (1), 45–54.
Martin GW. 1941 – Outline of the fungi. *Studies in Natural History University Iowa* 18, 192–204.
Mathew LK. 2018 – *Foliicolous Fungal Flora of Malabar Wildlife Sanctuary in Kerala State*. Ph.D. Thesis. Mahatma Gandhi University, Kottayam, India, vi+55–328pp.
Miller NA, Huhndorf SM. 2005 – Multi-gene phylogenies indicate ascomal wall morphology is a better predictor of phylogenetic relationships than ascospore morphology in the Sordariales (Ascomycota, Fungi). *Molecular Phylogenetics and Evolution* 35, 60–75. Doi 10.1016/j.ympev.2005.01.007
Nannfeldt JA. 1932 – Studien uder die morphologie und systematik der nicht-lichenisierten, inoperculaten Discomyceten. *Nova Acta Regiae Societatis Scientiarum Upsaliensis, Ser*. IV

(8), 1–368.
Niranjan M, Sarma VV. 2018 – A check-list of fungi from Andaman and Nicobar Islands, India. *Phytotaxa* 347(2), 101–126. Doi 10.11646/*Phytotaxa* .347.2.1
Pinho, D.B., Firmino, A.L., Ferreira-Junior, W.G. and Pereira, O.L. (2012) An efficient protocol for DNA extraction from Meliolales and the description of *Meliola centellae* sp. nov. *Mycotaxon* 122, 333–345.
Pinho DB, Firmino AL, Ferreira-Junior WG, Pereira OL. 2013 – New Meliolaceae from the Brazilian Atlantic Forest 2, species on host families Annonaceae, Cecropiaceae, Meliaceae, Piperaceae, Rubiaceae, Rutaceae and Tiliaceae. *Mycologia* 105(3), 697–711. Doi 10.3852/12-163
Pinho D.B, Junior JH, Firmino AL, Junior BTH. et al. 2014 – Reappraisal of the black mildews (Meliolales) on *Hevea brasiliensis*. *Tropical Plant Pathology* 39(1),89–94.
Sabeena A, Biju H, Thomas J, Biju CK, Mathew SP. 2017 – Three new Foliicolous Ascomycetes from Andaman Islands, India. *Bio Bulletin* 3(1), 74–79.
Sabeena A, Hosagoudar VB, Divaharan V. 2018 – Foliicolous fungi on medicinal plants in Thiruvananthapuram District, Kerala, India. *Journal of Threatened Taxa* 10(3), 11470–11479. Doi 10.11609/jot.3761.10.3.11470-11479
Saenz GS, Taylor JW, Gargas A. 1994 – 18S rRNA gene sequences and supraordinal classification of the Erysiphales. *Mycologia* 86, 212–216.
Saenz GS, Taylor JW. 1999 – Phylogenetic relationships of *Meliola* and *Meliolina* inferred from nuclear small subunit rRNA sequences. *Mycological Research* 103(8), 1049–1056.
Selkirk DR. 1975 – Tertiary fossil fungi from Kiandra, New South Wales. *Proceedings of the Linnean Society of New South Wales* 100, 70–94.
Song B, Li TH. 2003 – Six taxa of Meliolaceae from Yunnan province in HKAS, China. *Mycotaxon* 87, 421–424.
Song B, Li TH. 2004 – Interesting taxa of Meliolaceae in HMAS, China. *Mycotaxon* 90, 129–132.
Stevens FL. 1927 – The Meliolineae- I. *Annales Mycologici* 25(5-6), 405–469.
Stevens FL. 1928 – The Meliolineae- II. *Annales Mycologici* 26(3-4), 165–383.
Sydow H. 1913 –Beitrage zur kenntnis der pilzflora des sudlichen ostindiens-I. *Annales Mycologici* 11, 326–330
Sydow H, Sydow P. 1914 – Nova Fungorum species-XIII. *Annales Mycologici* 12, 195–204.
Tang AMC, Jeewon R, Hyde KD. 2007 – Phylogenetic utility of protein (RPB2, beta-tubulin) and ribosomal (LSU, SSU) gene sequences in the systematics of Sordariomycetes (Ascomycota, Fungi). *Antonie Van Leeuwenhoek* 91, 327–349. Doi 10.1007/s10482-006-9120-8
Thimmaiah CJ, Hosagoudar VB, Jayashankar M. 2013 – Black mildews of Kodagu, Karnataka, India. *Journal of Threatened Taxa* 5(16), 5021–5180. Doi 10.11609/JoTT.o3657.5021-180
Thite AN. 1975 – Ascospore germination in *Meliola jasminicola*. *Indian Phytopathology* 28, 94–96.
Vitoria NS, Bezerra JL, Gramacho KP. 2010 – A simplified DNA extraction method for PCR analysis of *Camarotella* spp. *Brazilian Archives of Biology and Technology* 53, 249–252
Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL. et al. 2014 – Naming and outline of Dothideomycetes– 2014. *Fungal Divers*ity 69(1), 1–55.
Yarwood CE, 1973 – Pyrenomycetes: Erysiphales. In: The Fungi. An Advanced Treatise. G.C. Ainsworth, F.K., Sparrow & A.S. Sussman (eds.) Academic Press, New York (4A) 71–86
Zeng XY, Jeewon R, Hongsanan S, Hyde KD, Wen TC. 2020 – Unravelling evolutionary relationships between epifoliar Meliolaceae and angiosperms. *Journal of Systematics and Evolution* 60(1), 23–42.
Zeng XY, Zhao JJ, Hongsanan S, Chomnunti P. et al 2017 – A checklist for identifying Meliolales species. *Mycosphere* 8(1), 218–359. Doi 10.5943/*Mycosphere* /8/1/16
Zhang N, Castlebury LA, Miller AN, Hundorf SM. et al. 2006 – An overview of the systematics of the Sordariomycetes based on a four-gene phylogeny. *Mycologia* 98, 1076–1087.