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Global Diversity of *Prillieuxina* (Asterinales, Asterinaceae): Addition of a new species from India

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ABSTRACT

This study reports a novel *Prillieuxina* species infecting *Actinodaphne bourdillonii* (family Lauraceae) from the Azhutha forest range of Periyar Tiger Biosphere Reserve, Kerala, India. The species' novelty is established based on host specificity, as no prior reports of *Prillieuxina* on *Actinodaphne* in the Lauraceae family exist from India. The parasitic link between fungus and host plants involves intricate co-evolutionary programs, explaining the observed host specificity in biotrophs. Despite Lauraceae's ancient association with black mildews, *Prillieuxina*'s limited affiliation suggests a need for further biochemical and genetic investigations into this unique host-pathogen interaction.

Keywords: Ascomycetes, Black Mildew fungi, Dothideomycetes, Fungi, Coevolution, Host specificity

INTRODUCTION

The Western Ghats traverse the western coastline of Southern India, stretching from 08°19'08"–21°16'24" N to 72°56'24"–78°19'40" E, with a north-south span of 1,490 km. They have a minimum width of 48 km and a maximum width of 210 km, with a total area of 136,800 km² (CEPF 2007). This Mountain range extends through the states of Gujarat, Maharashtra, Goa, Karnataka, Kerala, and Tamil Nadu, with a singular interruption known as the Palghat Gap, which spans 30 km in northern Kerala. The Western Ghats have some of the world's most distinctive animals, flora, and fungi. Compared to other hotspots, it has the largest human population density (exceeding 300 individuals/km²), complicating conservation efforts (Molur, 2009). The Western Ghats have experienced a reduction of about 50% in forest cover since the early 1900s, and this trend persists due to heightened fragmentation and encroachments. Efforts must be undertaken to investigate the biodiversity of the Western Ghats, and documentation is essential for their protection.

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Asterinaceae consists of obligate, biotrophic ascomycetes that associate with leaves of various vascular plants in tropical and subtropical regions around the world. The extensive proliferation of black colonies obstructs light penetration and diminishes chlorophyll levels in leaves, consequently impairing the photosynthetic efficiency of plants. Research indicates that when *Asterina congesta* infects a sandal tree, the host generates increased levels of cyclic compounds, including proline, indicative of considerable stress. The genus, *Prillieuxina* Arn., is an ascomycetous black mildew-causing fungus belonging to the family Asterinaceae under the order Asterinales of class Dothideomycetes. They develop ectophytic non-appressoriate mycelia (Hofmann, 2009) on the phylloplane of the compatible host plant through the germination of uniseptate ascospores formed within bitunicate meiosporangia, which grow within dimidiate ascomata known as perithecia, dehiscing stellately on maturity.

The genus *Prillieuxina* is represented by 76 species as foliicolous associates on various host families. Hosagoudar (2012) consolidated reports of 265 species belonging to Asterinales, comprising 12 species of *Prillieuxina* from India, of which nine species are new records to India. The systematic works on thyriothecioid ascomycetes of Kerala were extensively studied during the past decade (Chandraprabha et al. 2011;

Hosagoudar & Sabeena 2014; Sabeena et al. 2018, 2020). The Lauraceae family, an ancient plant group, is categorised under the subclass Magnoliidae and mostly inhabits tropical and subtropical climates, particularly in Asia and the tropical Americas. This family mainly comprises trees and shrubs, excluding the parasitic vine species *Cassytha*. This family possesses considerable economic importance, with several species employed in diverse sectors such as food, lumber, medicines, and perfumes. Ethnobotanically, the plants of this family have been employed to remedy various ailments (Damasceno et al. 2019). The Lauraceae family has a long history of fungal associations. Fossil data indicate that Lauraceae has been a prevalent host for black mildew fungus from the early Cenozoic era. Members of Lauraceae have a tropical and subtropical range, predominantly found in Asian and American rainforests, with around 67 genera and over 2500 species. This study reports a new species of *Prillieuxina* infecting *Actinodaphne bourdillonii* Gamble, a plant from the Lauraceae family, found in the Azhutha forest range within the Periyar Tiger Biosphere Reserve in Kerala, India.

MATERIALS AND METHODS

Infected plant specimens were meticulously gathered from the Moozhikkal Section of the Periyar Tiger Reserve in Kerala, and observations were documented concerning their pathogenicity, infection characteristics, and colony morphology, among other details. In the field, each plant specimen was gathered individually in plastic bags. The specimens were meticulously pressed and desiccated between blotting sheets. Upon confirming their desiccation, they were stored in the manifold or butter paper folders. The host was recognised through the flora. For microscopic examination in the laboratory, the standard nail polish procedure developed by Hosagoudar and Kapoor (1985) was employed to see the complete colony in its natural state. A drop of high-quality, clear nail polish was placed in the selected colonies and meticulously thinned using a small brush without disrupting the colonies. Upon completely drying the nail polish on the colonies, a thin, colourless film or layer was produced, securely encapsulating the colonies. A drop of DPX will be applied on a transparent slide, and the flip will be evenly distributed. One or two drops of DPX were applied to the flip, followed by a clean cover glass placement. Gentle pressure on the cover glass expels the excess DPX, which can be removed after drying. The slides were labelled and positioned in a dust-free room for 1-2 days to facilitate drying. These permanent slides were then utilised for additional research. By examining the micro-morphological characteristics, a thorough taxonomic description was prepared. Microscopic examinations were conducted using an Olympus (CX21iLED) binocular compound microscope equipped with MagVision image analyzer software, and microphotographs were captured using a CMOS digital camera. The standard literature was used to make morpho-taxonomic determinations (Hosagoudar, 2012). After each collection, the materials were deposited in the Mar Thoma College Tiruvalla Herbarium (MTCTH), Kerala, India.

RESULTS

Prillieuxina actinodaphnicola Jacob Thomas & Susan Kuriakose sp. nov. **Fig. 1.**

Myco Bank No.: MB 858772
Etymology: Named after the host genus.

Key to the genera

Thyriothecia orbicular, dehisce stellately at the center…….…… Asterinaceae
1. Appressoria present……..… 2
1. Appressoria absent or very rarely bserved……..…7
7. Appressoria formed only around the Stomata……*Symphaster*
7. Appressoria not formed……..…*Prillieuxina*

Colonies are mostly hypophyllous, rarely amphigenous, dense, velvety, up to 2 mm in diameter, and confluent. Hyphae straight to sub-straight, branching irregularly, loosely to closely reticulate, cells 5-30 µm long and up to 2 to 2.2 µm broad. Appressoria absent. Thyriothecia develop all along the hyphae, more or less orbicular, dark brown, up to 150 µm in diameter; many thyriothecia join together marginally, dehisce stellately at the center, margin fringed, fringed hyphae from the margin join with other thyriothecia and form a plate of thyriothecia, but keep individuality; asci are few to many per thyriothecia, globose, octosporous, up to 45 µm in diameter; ascospores are conglobate, brown, 1-septate, constricted at the septum.

Type: India, Kerala, Moozhikkal Section, Azhutha Range, Periyar Tiger Reserve, on the leaves of *Actinodaphne bourdillonii* Gamble (Lauraceae), 24/01/2021, MTCHT 700 (holotype), collected by Susan Kuriakose.

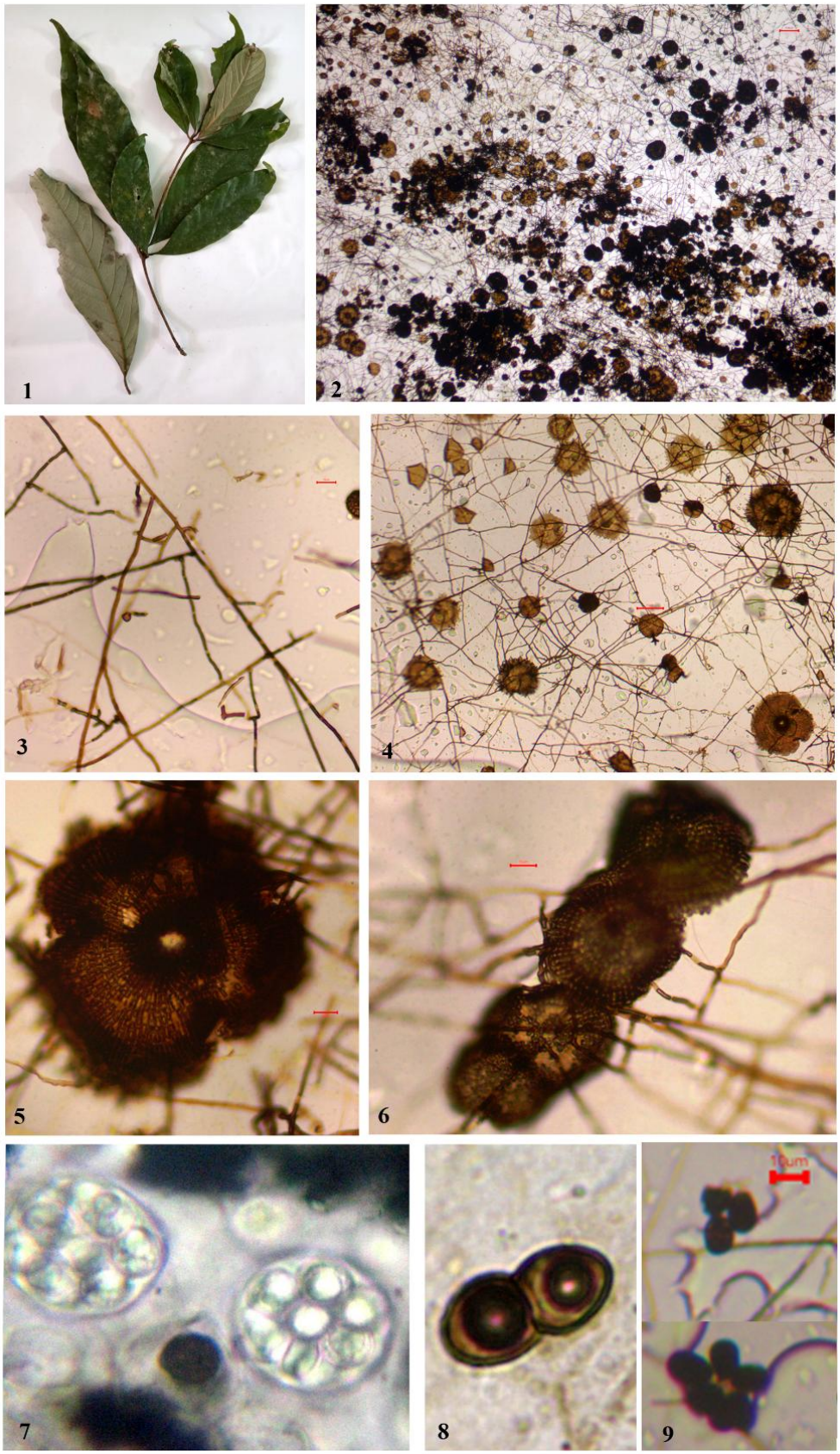

Figure 1. *Prillieuxina actinodaphnicola* sp.nov. 1. Infected leaves of *Actinodaphne bourdillonii* Gamble, 2. Fungal colony with thyriothecia, 3. Branched mycelium, 4. Fungal colony with thyriothecia and Pycnia, 5. Dehiscing thyriothecium, 6. Connate thyriothecia, 7. Asci, 8. Ascospore, 9. Pycnidiospores.

Table 1. Documented global occurences of *Prillieuxina*

S.N.	Taxon	Host/Host Family	Coverage
1	*Prillieuxina acokantherae*	*Acokanthera spectabilis, Acokanthera venenata,* and *Carissa arduina*	Gauteng
2	*Prillieuxina aeglicola*	*Citrus aurantifolia*	India
3	*Prillieuxina amazonica*	*Remijia amazonica*	Amazonas
4	*Prillieuxina amboinensis*	*Cordyline terminalis*	Maluku
5	*Prillieuxina anamirtae*	*Anamirta cocculus*	Philippines
6	*Prillieuxina antioquensis*	*Miconia theaezans*	Colombia
7	*Prillieuxina aporosae*	*Aporosa cardiosperma*	India
8	*Prillieuxina aquifoliacearum*	*Ilex denticulate*	India
9	*Prillieuxina ardisiae*	*Ardisia humilis*	Jawa
10	*Prillieuxina argyreiae*	*Argyreia*	India
11	*Prillieuxina baccharidincola*	*Baccharis*	Sao Paulo
12	*Prillieuxina burchelliae*	*Burchellia capensis*	Western Cape Province
13	*Prillieuxina calami*	*Calamus*	Philippines
14	*Prillieuxina capizensis*	*Leucosyke capitellata*	Philippines
15	*Prillieuxina cinchonae*	*Cinchona pubescens*	Costa Rica
16	*Prillieuxina citricola*	*Citrus aurantifolia*	India
17	*Prillieuxina clavispora*	*Alyxia monilifera*	Philippines
18	*Prillieuxina conocephali*	*Conocephalus suaveolens*	Jawa
19	*Prillieuxina creberrima*	*Premna*	Philippines
20	*Prillieuxina cryptocaryae*	*Cryptocarya glaucescens*	Queensland
21	*Prillieuxina cylindrotheca*	*Eugenia*	Sao Paulo
22	*Prillieuxina diaphana*	*Solanum manucaling*	Philippines
23	*Prillieuxina dichapetali*	*Dichapetalum geloniodes*	India
24	*Prillieuxina d*iospyri	*Diospyros malabarica*	India
25	*Prillieuxina dipteridis*	*Dipteris conjugata*	Jawa
26	*Prillieuxina dipterocarpi*	*Dipterocarpus vernicifluus*	Philippines
27	*Prillieuxina dissiliens*	*Elaeodendron croceum*	Western Cape Province
28	*Prillieuxina distinguenda*	*Ixora philippinensis*	Philippines
29	*Prillieuxina dysoxyli*	*Dysoxylum cumingianum*	Philippines
30	*Prillieuxina elaeagni*	*Elaeagnus kologa*	India
31	*Prillieuxina garciniae*	*Garcinia imberti*	India
32	*Prillieuxina gracilis*	*Derris diadelpha*	Philippines
33	*Prillieuxina hippeastri*	*Hippeastrum*	Puerto Rico
34	*Prillieuxina hiugensis*	*Ilex latifolia*	Japan
35	*Prillieuxina humboldtiae*	*Humboldtia*	India
36	*Prillieuxina humiriae*	*Humiria floribunda*	Amazonas
37	*Prillieuxina hydnocarpi*	*Hydnocarpus falcatus*	Philippines
38	*Prillieuxina ilicicola*	*Ilex odorata*	Jawa
39	*Prillieuxina inconspicua*	*Chilianthus arboreus*	Southern Africa
40	*Prillieuxina intensa*	*Pisonia*	New Zealand
41	*Prillieuxina ixorae*	*Ixora ferrea*	Puerto Rico
42	*Prillieuxina ixoricola*	*Ixora*	Ghana
43	*Prillieuxina ixorigena*	*Ixora coccinea*	India
44	*Prillieuxina jasmini*	*Jasminum flexile*	India
45	*Prillieuxina loranthi*	*Loranthus leytensis*	Philippines
46	*Prillieuxina luzonensis*	*Shorea polysperma*	Philippines
47	*Prillieuxina mabae*	*Maba sandwicensis, M. hillebrandii*	Hawaiian Is.
48	*Prillieuxina malabarensis*	*Pothos scandens*	India
49	*Prillieuxina manaosensis*	Annonaceae	Amazonas
50	*Prillieuxina melastomacearum*	Melastomataceae	Puerto Rica
51	*Prillieuxina memecyclonis*	*Memecylon umbellatum*	India
52	*Prillieuxina microchita*	*Nothopanax pyriformis*	Sabah
53	*Prillieuxina microspila*	*Leucosyke capitellata*	Maluku

54	*Prillieuxina mimusopis*	*Mimusops obovata*	Eastern Cape Province
55	*Prillieuxina obesa*	*Canarium villosum*	Philippines
56	*Prillieuxina parameriae*	*Parameria barbata*	Philippines
57	*Prillieuxina pavettae*	*Pavetta tomentosa*	Kerala
58	*Prillieuxina phoradendri*	*Phoradendron*	Puerto Rica
59	*Prillieuxina polyalthiae*	*Polyalthia longifolia*	India
60	*Prillieuxina pterigotae*	*Pterygota alata*	India
61	*Prillieuxina pterocelastri*	*Pterocelastrus variabilis*	Southern Africa
62	*Prillieuxina pumila*	*Horsfieldia gigantifolia*	Philippines
63	*Prillieuxina ramuligera*	*Microdesmis casearifolia*	Philippines
64	*Prillieuxina rhaphiostylidis*	*Rhaphiostylis beninensis*	Togo
65	*Prillieuxina saginata*	*Pinanga*	Philippines
66	*Prillieuxina santiriae*	*Santiria nitida*	Philippines
67	*Prillieuxina sinensis*	*Sabia gracilis*	China
68	*Prillieuxina stuhlmannii*	*Ananas sativus*	Tanzania
69	*Prillieuxina systema-solare*	*Banksia marginata*	Tasmania
70	*Prillieuxina tarennae*	*Tarenna flava*	India
71	*Prillieuxina tecleae*	*Teclea natalensis*	South Africa
72	*Prillieuxina tetracerae*	*Tetracera indica*	Jawa
73	*Prillieuxina tijibodensis*	*Rotten leaves*	Jawa
74	*Prillieuxina venusta*	*Anaxagorea luzonensis*	Philippines
75	*Prillieuxina winteriana*	*Rollinia*	Brazil
76	*Prillieuxina woodiana*	*Cryptocarya woodii*	South Africa

(Source: https://www.indexfungorum.org**)**

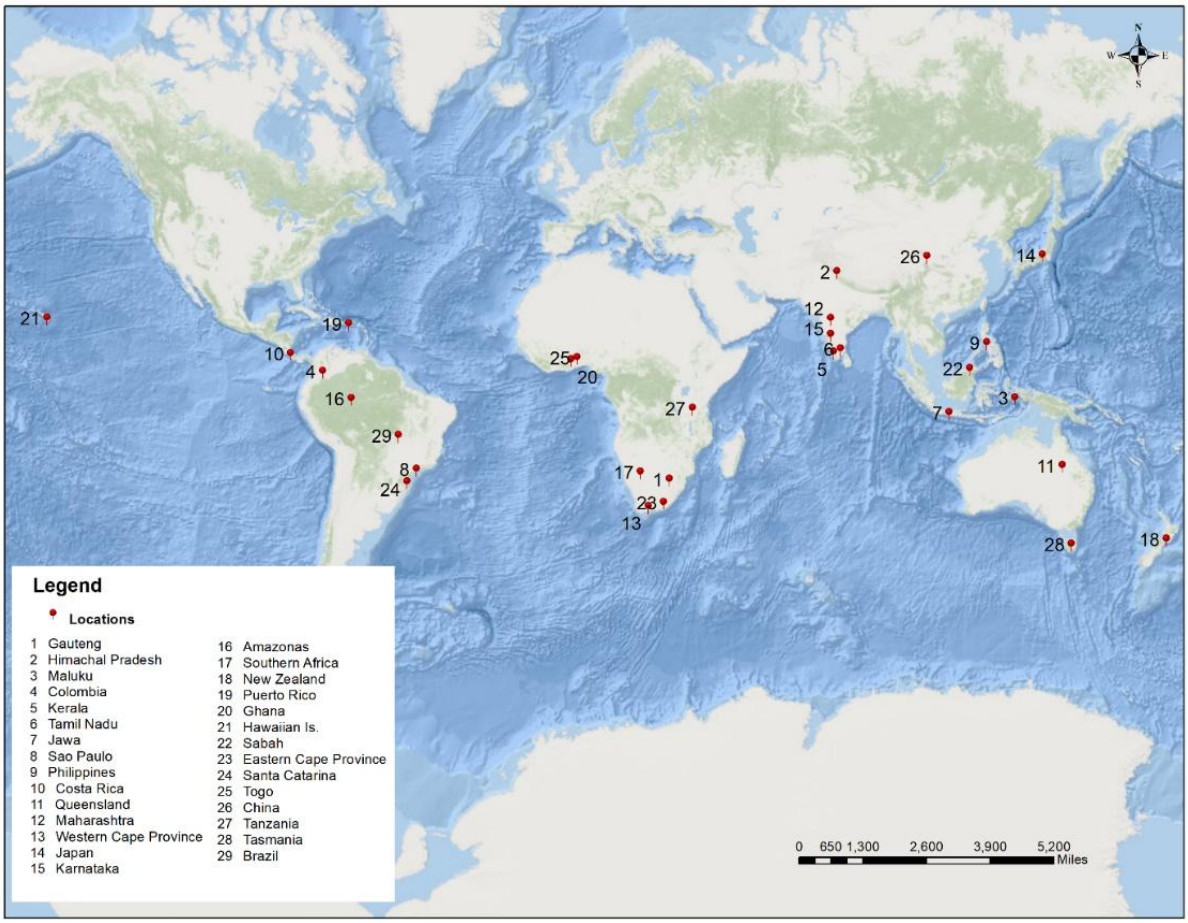

Figure 2. Map showing global distribution of *Prillieuxina*

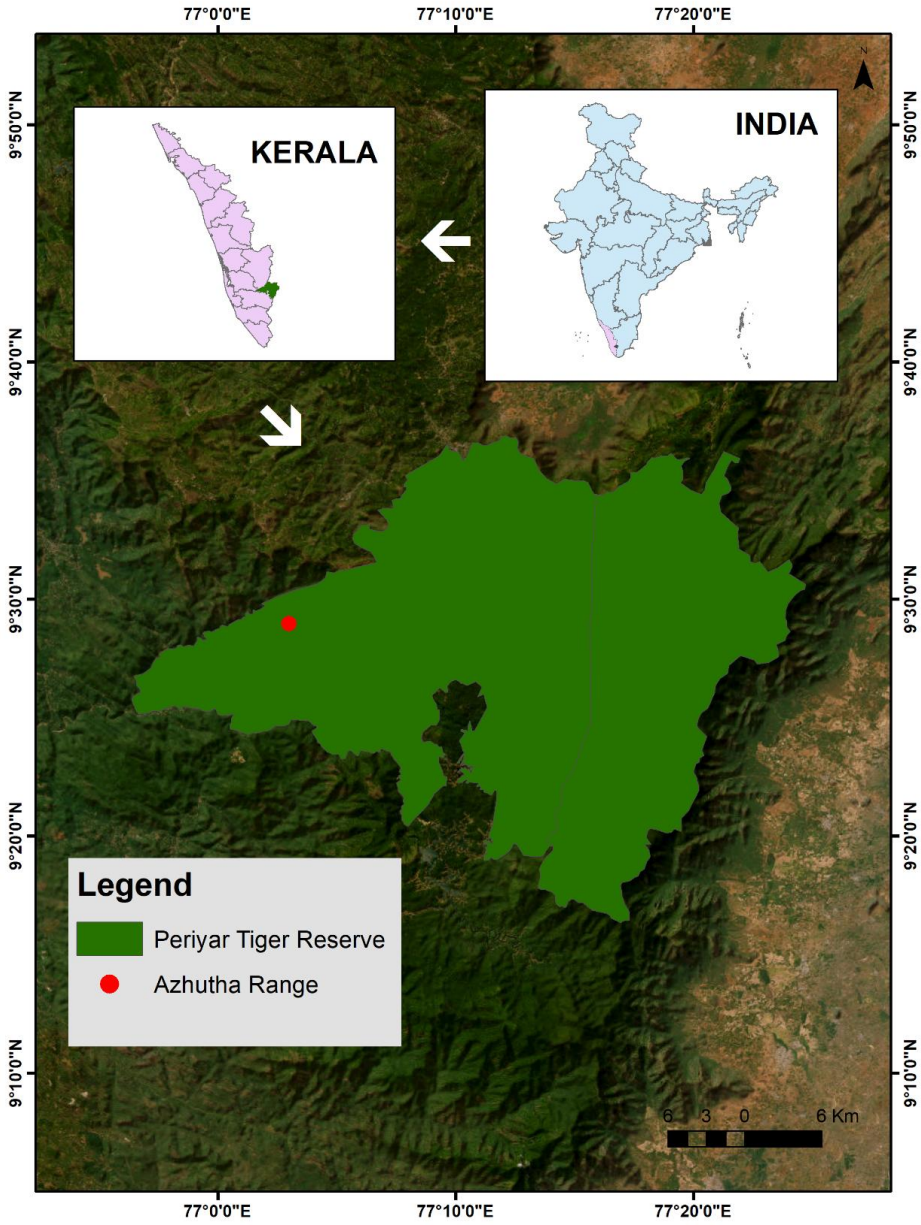

Fig. 3. Map of study area

DISCUSSION

The genus *Prillieuxina* Arn. is an ascomycetes black-mildew-causing fungi named after the French botanist Edouard Ernest *Prillieux* (Marasinghe et al. 2023). Molecular phylogenetic data suggest that *Prillieuxina* is a primitive genus coming under the family Asterinaceae (Asterinales, Dothideomycetes) (Guatimosim et al. 2015). Morpho taxonomic characteristics such as the absence of appressorium and setae, epiphytic nature of colonies, etc., justify the same. The family Asterinaceae is polyphyletic in origin. With bare minimum adaptations, *Prillieuxina* has flourished in many Angiosperm families. *Asterina cryptocaryae* Cooke is known on this host family. After examining the holotype, Stevens & Ryan transferred it to the genus *Prillieuxina* because of the lack of appressoria.

The majority of species in the Asterinaceae family are host, genus, or family-specific, as they rely only on live leaves for survival. *Asterina* species thrive on the leaves of around 106 plant species, primarily found in tropical climates (Hofmann et al., 2010; Hosagoudar & Abraham 2000) (Fig. 2). Their host associations justify the majority of *Asterina* species. The species status is mainly established based on host species, genus, and family specificity.

The present study identifies a novel foliicolous fungal species infecting *Actinodaphne* from the Lauraceae family in the Moozhikkal Section of the Periyar Tiger Reserve in Kerala. The novelty of the fungal species is established based on host species specificity, as there is no previous report of

Prillieuxina on *Actinodaphne* in the Lauraceae family (Table 1) from India (Fig.3). Therefore, this should be a new species of *Prillieuxina* based on morphology and host specificity.

CONCLUSION

Fungal diversity is believed to be intricately linked to host diversity. The parasitic link between fungus and Host plants arises from intricate developmental programs and synchronized signalling mechanisms in both the pathogen and its host (Hahn & Mendgen, 2001). To overcome host plant resistance, these fungal parasites develop various structural and chemical components, while plants devise various defence mechanisms against fungal attack. This leads to a co-evolutionary conflict between infections and prospective hosts, perhaps elucidating the host specificity seen in biotrophs (Ferreira et al. 2006). Only one species of the genus *Prillieuxina* is known among Lauraceae members (*Prillieuxina woodiana* on *Cryptocarya woodii*). The genus *Prillieuxina* has little affiliation with the Lauraceae family, although the family contains many foliicolous fungal companions dating back to the early Cenozoic era. This should be investigated in terms of biochemical interactions and genetic underpinnings

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