Species of Laboulbeniales fungi parasitic on some common Indian insects: Isolation, visualization and characterization by Scanning Electron Micrographs (SEM)

Anupama Shukla
Department of Botany, Acharya Narendra Dev College, University of Delhi, New Delhi-110019, India
Email: anupamashukla@andc.du.ac.in

Article Info
https://doi.org/10.31018/jans.v16i1.5347
Received: December 7, 2023
Revised: January 20, 2024
Accepted: January 30, 2024

How to Cite

Abstract
The Laboulbeniales are a group of lesser-known fungi. They occur as ectoparasites on the exoskeleton of various arthropod species, mainly beetles, cockroaches, ants, dipterans and a few millipede genera appearing as hairs/bristles coming out of their cuticle. They are small, non-mycelial fungi forming compact multicellular thalli, starting from a two-celled sheathed ascospore which continues to envelop the mature thallus. The fungi, being obligate, spend their entire life cycle on their insect hosts. The present study attempted to locate the Laboulbeniales fungi in the common insects of India, isolate them and identify them based on morphology. Different species of insects; Carabid beetles - Stenolophus sp., Bembidion sp., Black ants - Camponotus sp., and Earwigs - Labidura riparia; were collected from different habitats of damp garden soil, river banks, light sources and entomological collection museum situated as different sites New Delhi. The insects were scrutinized for the presence of fungi through light and scanning electron microscopy. The morphological features of each fungus were recorded, and the species were characterized. The studies showed the presence of Laboulbenia anaplogenii and L. stenolophi on the legs of Stenolophus sp.; L. egens on Bembidion sp. legs; L. camponoti on the wings of black ants and Dimeromyces anisolabis on the legs of earwigs. The identification was made primarily based on the size, shape and structure of basal foot, receptacles, antheridia and foot of the thallus or it’s remnant scar found on the insect cuticle. This is the first Indian report of conducting scanning imaging of the Laboulbeniales fungi.

Keywords: Fungi, Laboulbeniales, Scanning study, Thallus

INTRODUCTION

In the middle of the 19th century, a few strange minute organisms were observed on the beetles (Anonymous, 1849). Initially, they were thought to be parasitic plants, worms, or even red algae, but the foundational studies (Thaxter, 1895; 1896; 1899; 1908; 1931) established these organisms as fungi. Among these, the fungi belonging to the order Laboulbeniales were found to be common ectoparasites. The members of the order Laboulbeniales, commonly called beetle hangers or laboulbs, which belong to the class Ascomycetes are characterized by the lack of a mycelium throughout their life cycle and the formation of a specialised type of peritheciun (Reboleira et al., 2018). The reduced hyphal system, called thallus, may be monocious or dioecious in these fungi. The fungus has carpogonium as the female sexual organ and the antheridia as the male sexual organs. The fertilization leads to the formation of perithecia on the monocious thallus or the female thallus in dioecious species. They produce the ascospores which germinate to form a new thallus. The ascospores are transmitted to new hosts by direct contact (De Kesel, 1993; 1995; 1996). Thus, these fungi do not have any anamorphic stage and a mycelium is never formed. These distinctive features of Laboulbeniales clearly set them apart from other fungi, and developmental studies have connected these cryptic fungi to ascomycetes (Blackwell et al., 2020).

The Laboulbeniales fungi are unique and significant as they occur as obligate ectoparasites on the cuticle of various arthropod hosts (Santamaria et al., 2006). They can develop on the superficial integuments of a wide range of hosts, mainly the insects, flies, cockroaches,
earwigs, ants, mites, termites, millipedes (Santamaria et al., 2014; 2017; 2018) and biting lice, mallophaga (Meola and Devaney, 1976). Although these are obligate ectoparasites, they have not been observed to cause any harm to their insect hosts, though they may reduce the size of the hosts (Csősz et al., 2021). The mode of nutrition is not very clear yet, as there have been only a few reports of haustorial structures penetrating into the insect body cavity (Meola and Devaney, 1976; Reboleira, 2021).

Some genera (like Laboulbenia) have numerous species on numerous hosts with most species showing no specificity and occurring on a large number of unrelated hosts showing specificity to a varying degree. Specificity of the fungus may be for the host, sex of host and/or position-on-host (Benjamin, 1971). Some fungi are found restricted to a single species of host insect or a certain position on the host insect and thus appear to be as limited in their host range as the obligate fungi parasitizing angiosperms. Some species occur on body parts of both sexes of their hosts, which come in contact during copulation (Enghoff, 2017). On the millipedes, the fungal thalli are found on both male and female legs and can be transmitted by copulatory behaviour (Reboleira et al., 2018).

To date, as per our present knowledge, limited studies have been carried out regarding the isolation, identification and characterization of Laboulbeniales fungi from the insect cuticle from India (Kaur et al., 1993; Kaur and Mukerji, 1995; 1996a; b; Pathak and Mukerji, 1997; Narang et al., 2023) The present study was an attempt in this direction and has characterized these fungi through Scanning Electron Microscopy (SEM) with structural details.

**MATERIALS AND METHODS**

Collection and fixation of fungi

The study was conducted on four species of insects, comprising carabid beetles (Order – Coleoptera), winged ants (Order – Hymenoptera), and earwigs (Order – Dermaptera). The insects collected included *Stenolophus* sp., *Bembidion* sp., *Camponotus* sp., and *Labidura riparia*. These were collected from different sources, viz., damp soil from the garden of the University of Delhi, bank of the river Yamuna at Wazirabad, light sources like fluorescent tube lights and bulbs, museum entomological collections from Indian Agriculture Research Institute, PUSA, New Delhi. The study isolated fungi from four insect species belonging to the orders Coleoptera, Hymenoptera and Mallophaga. The insects included two species of carabid beetles: *Stenolophus* sp. and *Bembidion* sp.; winged male black ant- *Camponotus* sp. and the earwig - *Labidura riparia*.

The insect parts infested with the fungus were excised carefully using ultra-fine tweezers, needles and forceps. The excised parts were transferred to the vials containing 70% alcohol and preserved. The fixation of the preserved parts was held in 2% Osmium tetraoxide (OsO₄) in water overnight.

Preparation, mounting and visualization of fungal specimens through Scanning Electron Microscope

The fixed parts of the specimens were washed with water. They were passed through the graded series of water-acetone mixture (30%, 50%, 70%, 80%, 90%, 95% and finally 100%) for dehydration. The specimens were left in each alcohol grade for at least an hour. Post-exposure to pure acetone, the specimen parts were air-dried.

The specimens were then mounted on the SEM sample holders (stubs) using the conductive carbon paint. The stubs were covered with double-sided tape and put in a specimen holder. Lastly, the specimens were coated with gold to increase conductivity and prevent electron beam damage. Each part was then examined under the SEM. Photographs were taken on an Ilford FP4 Plus film. The specimens were identified and various parts and characteristic features were recorded (Tragust et al., 2016).

**Ethical approval:** For this type of study, no Ethical approval is required.

**RESULTS AND DISCUSSION**

In the present study, five species of fungi were located on the legs and wings of the insects namely *Stenolophus*, *Bembidion Camponout*, *Labidura riparia*, which were identified based on their characteristic features (Table 1) using the literature (Thaxter, 1895; 1896; 1899; 1908; 1931). The observations revealed the presence of two genera of the fungi: *Laboulbenia and Dimeromyces*. Among these, *Dimeromyces* was found on the legs of earwigs, while *Laboulbenia* was located on the wings of male black ants and legs of carabid beetles. Different parts and morphological features of each fungus were observed through Scanning electron microscope.

**Morphological features of collected fungi Laboulbenia anaplogenii**

The mature thallus of *L. anaplogenii*, located on the carabid beetle, *Stenolophus* sp., consisted of a basal, conical foot, a five-layered receptacle with the layers I, II, III and V being single-celled while layer IV multicellular. The layer IV formed a projection beyond the Vth layer, which led to a bunch of flask-shaped antheridia and branches of appendages. The perithecium arose from layer II of the receptacle (Figs. 1 a-c).
Laboulbenia egens
The SEM micrographs shown in Fig. 2 a and b present the morphological features of Laboulbenia egens located on the legs of carabid beetle, Bembidion sp. The thalli occurred often singly and rarely in groups of two/three. It had a conical, pointed foot at its base, which led to the receptacle. The receptacle had single-celled lower tiers of the receptacle having long, thin, rectangular cells. The perithecium was large, angular in shape, with an acute apex. The inner margin (towards the receptacle) was straight, while the outer margin was convex, showing two punctations on the outer surface. The primary appendages were short and arose from a band-like thick insertion cell (Fig. 2a).

Laboulbenia stenolophi
The thalli of the L. stenolophi fungus were located on the legs of the carabid beetle, Stenolophus sp. Unlike L. egens which shows three thalli, it showed many thalli in various stages of development (Figs. 3 a-c). It exhibited the typical features of the genus, including a basal foot and five-layered receptacle, with each layer single-celled except the fourth, which had two cells. The fourth layer was observed to form a slight projection laterally. Also visible on the insect cuticle were ring-like structures indicating the area where the fungal thalli were attached (Reboleira et al 2021) (Fig. 3b). Perithecium was lateral, and originated from the second layer of the receptacle. Perithecium was typically obovate with an acute and blunt apex. A slight constriction was found below the apex. The tip was hyaline, and the subterminal portion was blackened. There were four to five primary appendages which were long and multicellular. Antheridia were formed at the base of the appendages.

Laboulbenia camponoti
Another species L. camponoti, was observed on the wings of a male black ant, Camponotus (Figs. 4 a, b). The fungus was monoecious, with the male sexual apparatus represented by a single antheridium (Fig. 2d, shown by an arrow) arising from the primary appendage of the receptacle and the perithecium formed on the receptacle. The thallus was comparatively very small in size. The perithecium was oblong with a pointed tip, outer margin convex, and inner margin straight and 2/3rd of its length was not joined to any cell of the receptacle.

Dimeromyces anisolabis
The fifth species of fungus, Dimeromyces anisolabis, was observed on the legs of the earwig, Labidura riparia. The fungus was found dioecious, having separate male and female thalli attached by the small conical foot. The male thallus, was smaller, consisting of receptacle and two compound antheridia. The compound antheridia were stout, flask-shaped, and the discharge tube had a pointed tip and was bent anteriorly (Fig. 5c). The female thallus was bigger with 8-9 layered receptacle, each layer single-celled. The receptacle had a ba-
sal and a distal part. The basal part had oblique septa between the cells, as in male thalli, while the distal part was cylindrical with straight, horizontal septa between bulging cells. Perithecium was generally single, but two were also seen, formed laterally on the third layer of the receptacle. It was elongated in shape. The male and the female thalli were found developing intermingled on the insect part (Figs. 5 a-c).

The present study explored the occurrence of parasitic Laboulbeniomycetes fungi on a few species of insects. Laboulbeniomycetes are minute in size and non-pathogenic, to a large extent, and have therefore been largely ignored by mycologists. Despite being relatively understudied, these fungi have certain unique features, which is why they are considered a model group for studies in ecosystem health. According to the general opinion, they do not cause bodily harm to their hosts, though some studies held with ants, ladybirds, and millipedes have shown a negative impact on colony health, reduced mobility or movement (Haelewaters et al., 2020a). These fungi have also been seen to cause increased mortality in North American ladybirds and enhanced susceptibility to other pathogenic fungi (Haelewaters et al., 2020a). Thus, they may be of specific danger for reduced population size, range restriction, loss of genetic diversity, and total species extinction of insects in a changing climate (Thomas, 2000; Warren et al., 2021). Consequently, it has been recommended that Laboulbeniales may be possibly utilized as indicators of ecosystem health (Kaishian, 2021).

The Laboulbeniales fungi are obligatory in nature, and are host-specific, with even specific positions on the hosts. In this present study, Laboulbeniales fungi of Laboulbenia and Dimeromyces species have been isolated from carabid beetles, male ant and earwigs. In Massachusetts, USA, Haelewaters et al. (2019) reported...
13 species of Laboulbeniales parasites on carabid beetles, which included *L. anoplogenii*, *L. casnoniae*, *L. clivinalis*, *L. egens*, *L. filifera*, *L. flagellata*, *L. inflata*, *L. macrotheca*, *L. pedicellata*, *L. terminalis*, *L. variabilis*, *L. vulgaris* and *Peyritschiella geminata*. Earlier studies have isolated various other taxa of fungi from different insects. In South America, four species of *Prolixandro- myces*; *P. anseris*, *P. tritici*, *P. blackwelliae*, and *P. bro- melicola*, were recorded for the first time on heteropter- ans (Kaishian and Weir, 2018).

The present SEM study showed the occurrence of Laboulbeniales on specific parts of the insects studied, such as legs and wings. Earlier studies by rotational SEM have shown that a few species occur only in par-
particular positions on their hosts, which is mostly related to the host's copulatory behaviour and also helps in fungal transmission (Reboleira, 2018). Since their discovery, they have been seen to be attached strongly to the surface of the insect, i.e. the cuticle, which was also observed in the present study. The parasitic fungi derive nutrition from insects, though by different means in different species. They may obtain their requirements from absorption or by mere contact with the living tissues. Some species are attached only to the insect cuticle, while others form penetrating structures which enter deep into the body cavity or remain near the surface (Tragust et al., 2016). The species, devoid of penetrating structures, may derive nutrition from the cuticle itself as the cuticle in the insects is dynamic and has micro-channels through which wax is extruded, which is probably being used by the fungi. Alternately, it may use the resources on the cuticle. According to Boucias and Pendland (1991), plant and microbial flora components, habitat, faecal matter, etc., are often seen on the insect cuticle. Nutrition can also be gained from the environment in which the hosts are found. In India, limited studies of these fungi have been carried out so far (Kaur et al., 1993; Kaur and Mukerji, 1996; Pathak and Mukerji, 1997; Kaur and Mukerji, 2006; Narang et al., 2023). The present study scrutinized these fungi using the high-resolution scanning electron microscope and emphasized their morphology. The images showed the fungal thalli in various stages of development and in different positions on the hosts. The attaching organ or the foot of fungi, a triangular, black coloured structure, was also visible through which they are attached to the hosts and does not form a penetrating structure. The appendages and antheridia were also clearly seen. Tragust et al. (2016) have also reported the attachment of Laboulbeniales on the integument of host ants, Lasius neglectus, Myrmica scabrinodis, Camponotus sylvaticus, and Messor wasmannii via a hoof-like foot structure. They also confirmed the lack of penetration structures, similar to my study, by the absence of a fungal penetration pore below the fungal thallus dislodged from the host ants. However, these results contradict the earlier notions that all Laboulbeniales species may produce at least minute penetration structures for obtaining resources from hosts (Benjamin, 1971). Nevertheless, the proposition that secretions from numerous exocrine glands might provide needed resources in ants may help provide nutrition to these fungi (Billen, 2009). As per reports, approximately 2325 species of 145 genera have been reported (Haelewaters, 2020b), although this number is underrated as an estimated number of 40,000 members of this order is present (Weir and Hammond, 1997).

Conclusion

The present study reports the occurrence of different ectoparasitic Laboulbeniales fungi on the cuticle of the legs/wings of carabid beetles, black ants, and earwigs. This is the first such Indian report documenting the characterisation of the Laboulbeniales fungi on insects via scanning imaging. The fungi were identified based on their morphological features, particularly the structure of thallus, which varied in different fungi species. It also showed that not all members of the group form penetrating structures; instead, some may derive their nutrition from insect cuticular secretions or via their appendages. Such studies carry immense taxonomic significance. However, despite India's tremendous potential to conduct studies on this unexplored group of fungi to enhance biodiversity data, limited studies have been carried out in this area. Future studies are, thus, recommended to explore these fungi and discuss their nutrition and phylogenetic status.

ACKNOWLEDGEMENTS

The author wishes to thank the Principal, Acharya Narendra Dev College and Professor Sarita Kumar for their support and encouragement.

Conflict of interest

The author declare that she has no conflict of interest.

REFERENCES

