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Observations on *Ophiocordyceps nutans* in the Western Ghats

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ABSTRACT

Species of *Cordyceps* are the endoparasitic fungi infect insect pests and other arthropods throughout the world. They are well known for value-added metabolites and traditionally used for medicinal purposes. The teleomorphic entomophagous fungus *Ophiocordyceps nutans* associated with sap-sucking pentatomid stinkbug *Halyomorpha halys* of the Western Ghats of India. Regular observations for the last six years during the southwest monsoon in forest locations of different altitudinal ranges revealed occurrence of *O. nutans* only in two high altitude forests ranging from 845-935 m asl with temperature fluctuation between 21 and 24°C. This fungus was constantly associated with the stinkbug *H. halys* infecting the bark of one of the common tree species *Cassine glauca*. The bark of *C. glauca* consists of endophytic fungus *Hymenostilbe nutans* (anamorphic state of *O. nutans*). It has been predicted that the phytophagous *H. halys* will ingest *H. nutans* in turn become host for its teleomorphic state *O. nutans* leading to completion of life cycle. It is likely *Ophiocordyceps sinensis* of the Himalayas and *O. nutans* of the Western Ghats have evolved simultaneously with adaptation to high altitude cool and warm climatic conditions, respectively. The *O. nutans* serve as a potential biocontrol agent as it has the capacity to parasitize up to 22 species of stinkbugs threatening agriculture and silviculture. This tripartite trophic relationship among entomopathogenic fungi, tree species and stinkbugs serves as a model to explore the intricacies of relationships, biotechnological potentials and conservation strategies.

Key words: Medicinal fungi, *Cordyceps*, *Hymenostilbe*, *Halyomorpha*, *Cassine*; tripartite association.

INTRODUCTION

Cordyceps (Ascomycetes: Hypocreales) (sac- or club-fungi) represents more than 500 species endoparasitic on insects and other arthropods in varied climatic conditions prevailing in six continents (Stensrud et al. 2005; Sung et al. 2007). Based on the phylogenetic analysis of multiple genes, the Clavicipitaceae has been split into three families (Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae) and five genera (*Cordyceps*, *Elaphocordyceps*, *Metacordyceps*, *Ophiocordyceps* and *Tyrannicordyceps*) (Sung et al. 2007; Kepler et al. 2012).

Although *Cordyceps* serving as indigenous prestigious traditional Chinese medicine over 2000 years (Zhu et al. 1998a, b) they are still untapped source of many natural products. In spite of parasitizing and killing a wide range of insects, the fruit bodies of *Cordyceps* are immensely valued in traditional/folk/herbal medicines (Dong et al. 2015). In the recent past, significant strides have been made on the novelty of some metabolites produced by the *Cordyceps*. The cordycepin (3′-deoxyadenosine) is one of the key metabolites possess wide spectrum of biological activities especially useful in cancer therapy, modulating immune system, to induce apoptosis, to treat hyposexuality and to combat hyperlipidemia (Shin et al. 2003; Holliday and Cleaver 2008; Park et al. 2010; Rathor et al. 2014; Jiraungkoorskul and Jiraungkoorskul 2016; Wen et al. 2017). However, structurally versatile bioactive compounds are also derived from a variety of *Cordyceps* species (e.g. cordyanhydrides, cordypyridones, cordytropolone, cycloaspeptide, epicoccin, gliocladicillin, trichocladinol, trichothecanes and spirotenuipesines) (Isaka et al. 2005; Zhang et al. 2012; Lo et al. 2013).

Description of the entomophagous fungus *Ophiocordyceps* dates back to the early nineteenth century (Petch 1931). Patouillard (1887) reported *Ophiocordyceps nutans* for the first time from Japan followed by reports from other regions (China, Costa Rica, India, Korea, Nepal, New Guinea and Taiwan) (Shimizu 1994; Sung 1996; Karun and Sridhar 2013a). *Ophiocordyceps* constitutes the largest genus of *Cordyceps* consisting up to 200 species, which is the biggest amongst the entomopathogenic genera under Hyprocreales (Sung et al. 2007; Johnson et al. 2009; Quandt et al. 2014; Wen et al. 2014; Sanjuan et al. 2015). The perfect-imperfect connection between *O. nutans* and *Hymenostilbe nutans* was established by Hywel-Jones (1995) and Sasaki et al. (2008) ascertained *H. nutans* also serves as a potential biocontrol agent. Even though the Western Ghats of India is one of the important hotspots of biodiversity, investigations on the entomophagous fungi are scanty (Bhagwath et al. 2005; Juliya et al. 2012; Karun and Sridhar 2013a). The aim of this commentary is to provide a brief outline about the *O. nutans* recovered from the Western Ghats.

TYPE LOCALITY

On regular macrofungal forays during the southwest monsoon in the Western Ghats forests of Karnataka (forest reserve, shola forest, sacred grove and coffee agroforest), for the first time during June-August 2012 an enotmopathogenic fungus with bright-orange fruit bodies associated with fallen brownish-green marmorated pentatomid stinkbugs *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) was seen in the basins of the tree species *Cassine glauca* (Rottb.) O. Kuntze (Celasraceae) (Karun and Sridhar 2013a, b; Karun and Sridhar, 2016). On detailed macroscopic and microscopic examinations, it was identified as *Ophiocordyceps nutans* (Pat.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora. Detailed description has been provided in Karun and Sridhar (2013a). The coffee agroforest in B'Shettigeri region near Virajpet, Kodagu District of Karnataka (75.52°E, 12.7°N) is the type locality. For the last five years during the wet season (June-August) its occurrence was consistent in this type locality with an average density of 4-5 fruit bodies per square meter. However, fruit bodies were also found consistently in low density (1/5m^2) under the groves of *C. glauca* for the last five years in a mixed jungle of Makutta region of the Western Ghats.

HOST

The evergreen tree species *C. glauca* is economically valuable and it has wide distribution in India, Indo-China, Indonesia, Malay, Myanmar, Philippines, Sri Lanka and Thailand (Pullaiah 2006). This naturally distributed tree species serve as shade tree in coffee plantations and coffee agroforests of the Western Ghats of India (Fig. 1). Cultivation *C. glauca* as shade tree confer several advantages in reduction of attack of stinkbugs *H. halys* to nearby rice and horticultural crops. The extent of damage caused to *C. glauca* denotes the strong ability of stinkbugs in spite of harboring the anamorphic antagonistic endophyte *Hymenostilbe nutans* Samson & H.C. Evans (Sasaki et al. 2008). However, ingestion of *H. nutans* through bark tissue is catastrophic to the stinkbugs leading to death followed by emergence of teleomorphic *O. nutans* (Fig. 2, 3).

The invasive pentatomid stinkbug *H. halys* originated from Japan, China and Korea (Hoebeke and Carter, 2003). It is an agricultural pest damaging several crops like apples, cherry, green beans, peaches, soybeans, pears and raspberries worldwide (McPherson 2017). Besides bark, these stinkbugs are also feed on fruits and inflorescence of *C. glauca*, which harbor endophytic *H. nutans* (Karun and Sridhar 2013a, b). The *H. halys* may be depending on host tree *C. glauca* or its endophyte *H. nutans* to acquire sterols (Svoboda and Weirich 1995). However, the question remains whether the endophytic *H. nutans* really controls or supports *H. halys* to complete its lifecycle? It is likely the symbiotic *H. nutans* supply ergosterol to *H. halys* for its metamorphosis.

DISCUSSION

Sasaki et al. (2008) evaluated morphological and molecular differences of fruit bodies of *O. nutans* sampled from nine host species of hemipterans and found not much variability. They studied *O. nutans* of three host families of hemipterans include Acanthosomatidae (*Acanthosoma denticaudum*, *A. forficula*, *A. haemorrhoidale angulatum* and *A. labiduroides*), Pentatomidae (*Elasmucha putoni*, *Lelia decempunctate*, *Pentotoma jamonica* and *P. rufipes*) and Utostyulidae (*Urostylis annulicornis*). Molecular approaches help connecting the speciation of *Ophiocordyceps* along with its host within an ecological niche (Sanjuan et al. 2015). The uniqueness of *Ophiocordyceps* relies on single-

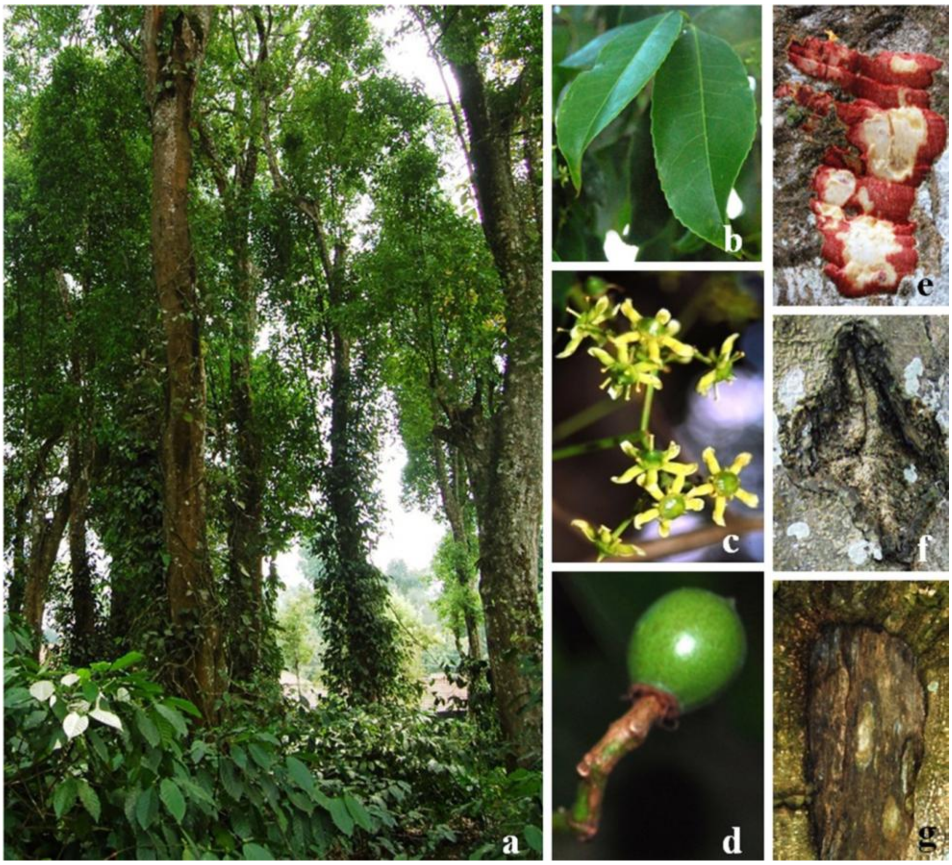

Fig. 1. Grove of *Cassine glauca* trees in coffee agroforest near the Virajpet in the Western Ghats (a), leaves (b), inflorescence (c), developing fruit (d), white pith with dark-reddish bark (e), distortion of bark by stink bugs (*Halyomorpha halys*) (f) and severe damage of bark and partly interior by stink bugs (g).

Fig. 2. Accumulated dead stink bugs (*Halyomorpha halys*) on the floor of *Cassine glauca* grove (a), dorsal view of dead stink bugs (b), ventral view of dead stink bugs (c), immature and maturing wiry fruit bodies of *Ophiocordyceps nutans* emerging from stink bugs (d, e) and two mature stromata of *O. nutans* (f).

host- and multi-host-dependence. Such clades of *Ophiocordyceps* are of immense value in biocontrol of phytophagous insects. The *O. nutans* is known to be parasitic on nearly 22 species of stinkbugs threatening agriculture and silviculture and thus serve as a powerful biocontrol agent (Sasaki et al., 2008). Evans et al. (2011) considered *Ophiocordyceps unilateralis* as a keystone species in Brazilian tropical forests owing to its complex and wide host range. They also considered *Ophiocordyceps* as a flagship genus for reassessment of fungal diversity and cautioned the erosion of mycodiversity owing to rapid loss of tropical forest ecosystems.

The high priced medicinal fungus *O. sinensis* has been reported from the high altitude alpine Himalayas of India (3000-5000 m asl) with its optimum growth temperature 18°C (Zhu et al. 1998; Sharma 2004; Shrestha et al. 2010; Lo et al. 2013; Xia et al. 2017). Our observations for the last six years showed consistent occurrence of *O. nutans* at an altitudinal range of 845-935 m asl of Virajpet-Makutta forest range in the Western Ghats during southwest monsoon (June-August). The temperature during this period fluctuates between 21 and 24°C. Interestingly, the *Ophiocordyceps formosana* preferred to inhabit broadleaf forests at about 1500-1800 m asl in Taiwan (Wang et al., 2015). In spite of investigations on macrofungi in the Western Ghats forests of Karnataka (forest reserve, shola forest and sacred grove), *O. nutans* occurred only in the type locality coffee agroforest of the B'Shettigeri of Virajpet-Makutta range (Karun and Sridhar 2016). However, Bhagwath et al. (2005) and Juliya et al. (2012) also recorded *Cordyceps* sp. in the coffee agroforests and moist deciduous forests the Western Ghats. As evident in Himalayan *O. sinensis* adapted to high altitude cold regions, the *O. nutans* might prefer high altitude warm regions of the Western Ghats. It is likely *O. sinensis* and *O. nutans* have simultaneously evolved in the Indian Subcontinent to adapt to high altitude cold and high altitude warm climatic conditions prevailing in the Himalayas and Western Ghats, respectively (Xia et al. 2017). It is also possible that there will be latitude-dependent convergent evolution of these entomopathogenic fungi adapted to infect high altitude insect populations. Future molecular phylogenetic studies on the anamorphic and teleomorphic stages of these entomopathogenic fungi shed more light on their adaption to environmental conditions and coevolution with insects.

The 'host relatedness' and the 'host habitat' are the two hypotheses put forwarded by Brooks and McLennan (1991) on *Ophiocordyceps*. The former hypothesis connects to isolation leading to cospeciation, while the latter relates to occupation of a niche by distantly related hosts. Interestingly, connection has been established between truffles and cicadas (Nikoh and Fukatsu 2000; Spatafora et al. 2007) as well as among fungi, grasses and insects (Kepler et al. 2012). Similarly, we also found a connection between *Polyporus* sp. and cicada in grassland of Southwest India (Sridhar et al. 2017).

The flagship species is generally defined as high profile fascinating species having significant ecological roles with cultural associations (Dietz et al. 1994; Caro and O'Doherty 1999). The designation to a fungus as flagship species symbolizes threats and necessity of enforcement of conservation strategies. *Ophiocordyceps sinensis* being a flagship species of global concern demands conservation, sustainable harvest and protection of vegetation for conservation (Sharma 2004; Negi et al. 2006, 2015; Winkler 2008; Cannon et al. 2009; Cannon 2011; Zhang et al. 2012 ; Sigdel et al. 2017). Based on the first report in Western Ghats, Karun and Sridhar (2013a) proposed to consider *O. nutans* as a flagship species of the Western Ghats with concern about conservation and medicinal benefits.

OUTLOOK

There are many potential endophytic and entomopathogenic fungi need innovative approaches to harness their biotechnological potential. In view of plethora of value-added metabolites, dead stinkbugs *H. halys* possessing *O. nutans* will serve as valuable pool of metabolites. Recent advances in production of cordycepin from *Cordyceps* (Wen et al. 2017) and antioxidant activity of polysaccharide obtained from mycelia of anamorphic *Hirsutella* (Liu et al. 2014) through fermentation are strongly encouraging to utilize entomopathogenic fungi for therapeutic purposes. Assessment of cultured anamorphic and teleomorphic fungi for desired metabolites are the issues of prime importance. More insights on the pattern of association of stinkbugs with anamorphic or teleomorphic stages of fungi will also facilitate rearing stinkbugs to harvest fungi to harness valuable metabolites. The pentatomid stinkbug *Cyclopelta siccifolia* Westwood (Heteroptera: Pentatomidae) is a common stinkbug on several tree species (*Erythrina indica*, *Pongamia glabra* and *Robinia pseudoacacia*) in the Western Ghats (Rudresh and Hosetti 2013). Observations are warranted to follow any chances of infection of stinkbug *C. siccifolia* by the *O. nutans* or its anamorphic state *H. nutans*.

Live tissues of many plants (e.g. foliage and bark) harbored by a variety of horizontally or vertically transmitted fungi gained importance recently in evaluation of bioactive potential rather than their role in control of phytophagous insects or plant growth promotion. Many fungi have either anamorphic or teleomorphic phases in host plant species or in insect pathogen alternates to complete asexual and or sexual life cycles. Occurrence of

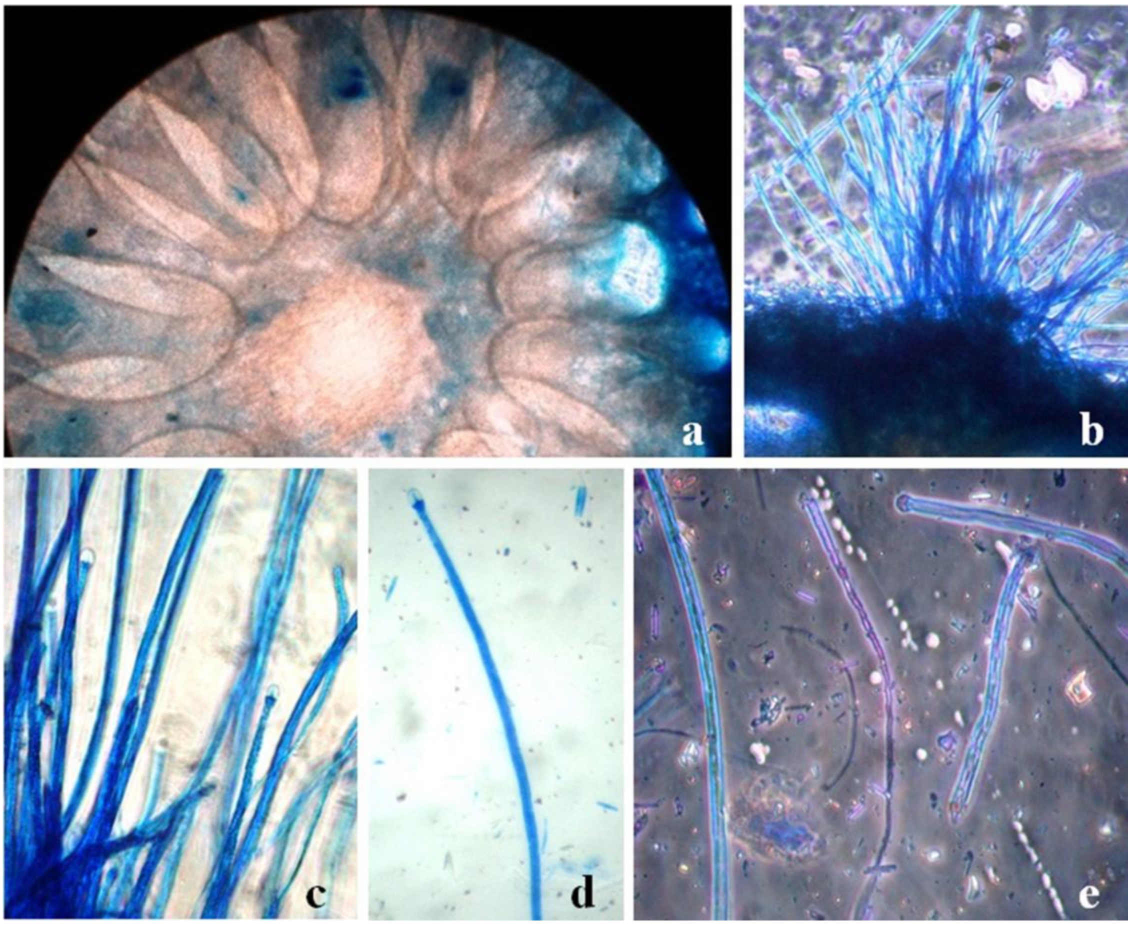

Fig. 3. Microscopic view showing cross section of fruit body of *Ophiocordyceps nutans* showing arrangement of overlapping perithecia (a), emerging asci (b), maturing asci with bulbous tips (c), immature and mature (packed with spores) asci showing bulbous tips and some released spores (d, e).

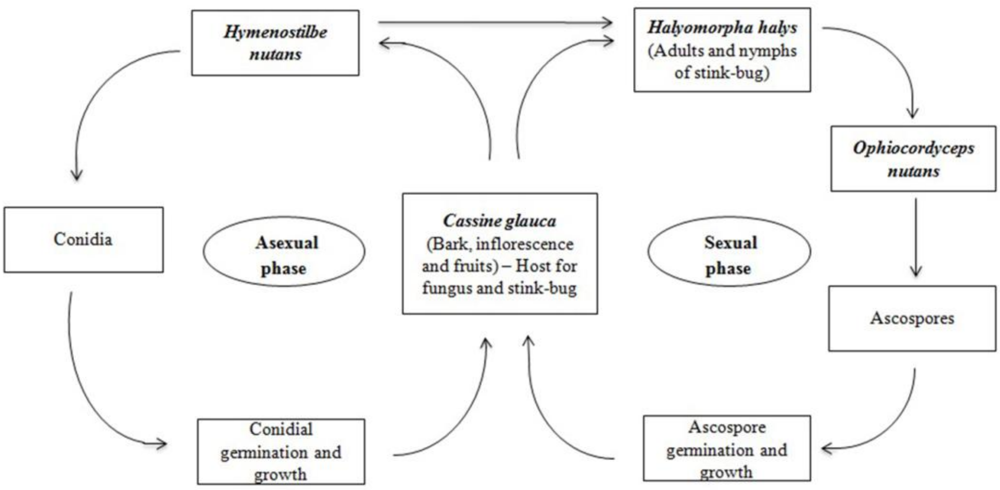

Fig. 4. Schematic representation of sexual and asexual life cycles of *Ophiocordyceps nutans*.

anamorphic *H. nutans* with tree species *C. glauca* and teleomorphic *O. nutans* with stink bug *H. halys* is one of the typical examples of shift of fungal lifestyle (Fig. 4). Association of anamorphic fungus *H. nutans* with tree species *C. glauca* and teleomorphic fungus *O. nutans* with stink bug *H. halys* provides ample opportunities to understand the tripartite relationships and interactions. The fungal endophytism is not restricted to only live plant tissues as it extends to dead leaf or bark litter which determines the pattern of decomposition and mineralization. Further, many hither to unknown endophytic fungi associated with plant species need appraisal about lifestyle to confirm their role in plant protection and plant growth promotion. Tripartite relationship among entomopathogenic fungi (*Hymenostilbe / Ophiocordyceps*), tree species (*Cassine*) and stinkbugs (*Halyomorpha*) provide an excellent platform to understand the evolutionary consequences in the Western Ghats of India.

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