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Discovery of a sooty mould scavenging beetle, *Leiochrinus nilgirianus* Kaszab (Coleoptera: Tenebrionidae) on coconut palms infested by the invasive rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hiemiptera: Aleyrodidae)

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Abstract Scavenging action of *Leiochrinus nilgirianus* Kaszab (Coleoptera: Tenebrionidae) on sooty mould deposition on coconut palms due to heavy honeydew production by the invasive rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), is reported for the first time from Kerala, India. Adult beetles, averaging 2.07 ± 0.9 / leaflet, with a maximum of five beetles per leaflet, and immature stages were generally confined to the abaxial of the palm leaves during daytime, but were found feeding on sooty mould during morning hours in damp conditions. The specially aided adaptive leg features of *L. nilgirianus* with bristle-like hairs on the undersurface of tarsomeres probably aid in proper adhesion on sooty mould laden plant surface and well developed tarsal claws that perhaps assist swift movement on fungus-laden leaflets. On an average one adult beetle could clear 1–2 mm^2 sooty mould laden area in a period of one minute. *In situ* habitat conservation of *L. nilgirianus* would help to reduce the sooty mould at no cost in the most natural and eco-friendly manner avoiding chemical management options and other expensive methods.

Keywords Mycophagous insect · Ecosystem service · Spiralling whitefly · *Cocos nucifera*

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The coconut palm (*Cocos nucifera* L.) is a versatile crop in the Indian economy providing food and livelihood security to more than 12 million people in the country and an important source for industry and tourism. During 2016, gradient outbreak of rugose spiralling whitefly (*Aleurodicus rugioperculatus* Martin), an invasive pest of Neotropical origin, was first reported on coconut palm from Pollachi, Tamil Nadu and Palakkad, Kerala. It was found to feed and breed profusely from the under surface of the palm leaves, numbering more than 10 live colonies in a leaflet (Josephrajkumar et al. 2016; Mohan et al. 2016; Shanas et al. 2016; Selvaraj et al. 2016; Srinivasan et al. 2016; Sundararaj and Selvaraj 2017). As rugose spiralling whitefly (RSW) is a highly polyphagous invasive species, a biosecurity alarm was sounded to monitor its spread and extent of damage caused. Though RSW initially created panic by its expansive mode of ovipositional damage in different crops including banana, bird of paradise, custard apple, jack, *Heliconia* sp., *etc.,* it could not sustain feeding on other crops successfully compared to coconut and relatively to some extent, on banana, which are its most favoured host plants. *Encarsia guadeloupae* Viggiani, an aphelinid

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parasitoid of spiralling whitefly (*Aleurodicus dispersus* Russell) fortuitously introduced in India in the late 1990s and well established in South India, turned out to be a very effective parasitoid of *A. rugioperculatus* as well. It parasitized *A. rugioperculatus* to an extent of 60% and kept the pest under check not allowing it to flare up in any of the South Indian States from where RSW has been recorded so far (Josephrajkumar et al. 2016; Shanas et al. 2016).

Though RSW could not cause greater economic damage, it produced enormous quantities of honeydew on coconut and other intercrops in the palm system, resulting in heavy deposition of sooty mould (*Leptoxyphium* sp., Family: Capnodiaceae) on affected plants. On coconut, sooty mould deposition was confined to the upper surface of coconut leaves including midrib. Sooty moulds are black-coloured fungi that grow as secondary to infestation by honeydew producing insects such as aphids, whiteflies, and Coccoidea and have no interrelationship with the host plants associated with. Presence of black sooty mould is one of the characteristic symptoms of feeding damage by RSW and indicated the presence of RSW in the palm system. This unsightly black coating of sooty mould on the palm leaves hindering photosynthesis was a matter of concern that attracted research attention. Spraying 1% starch solution was found effective in flaking out the sooty mould during summer season (Mohan et al. 2016). In the normal course, sooty mould was expected to be washed away by monsoon showers which had rather limited impact initially. However, we observed the gradual disappearance of sooty mould on the leaves of RSW infested palms and made intensive efforts to document the reason for this swift disappearance of sooty mould. A study was undertaken to ascertain the causes for this reduction in sooty mould on RSW-infested palms. In this paper, we report a tenebrionid beetle, *Leiochrinus nilgirianus* Kaszab, as a sooty mould scavenger on coconut with notes on its diagnosis based on morphological and molecular characterization and its biology.

Methodology

Observations were undertaken during morning hours (06.30–07.30 h IST) of August 2017 at the coconut experimental plot of ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam (9° 8'N latitude; 76° 30′E longitude), Kerala, South India on sooty mould infected palm leaflets by randomly choosing 15 leaflets to document the factors responsible for the disappearance of sooty mould and compared with healthy palms. Indeterminate tenebrionid beetles and their life stages found on sooty mould infested leaves were collected and preserved. This insect was morphologically identified and molecular characterization using cytochrome c oxidase (*COI)* gene was also done. Total DNA was isolated from freshly emerged grubs by CTAB method. Primers LCO 14905'-GGTCAACA AATCATAAAGATATTGG-3' HCO 2198:5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al. 1994) synthesized from Eurofins Genomics India Pvt. Ltd. in salt free status were used for amplification of mitochondrial cytochrome c oxidase subunit I (*COI*) gene. Polymerase Chain reaction (PCR) was performed as per Sambrook and Russell (2001) with few modifications. All the PCR amplifications were carried out in Techne Flexigene thermal cycler. PCR was carried out with an initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min 30 s. Final extension at 72 °C for 10 min was given for end filling. The products were analyzed in 1.0% agarose gel. The amplicons obtained were purified from each reaction mixture using PCR purification kit from Qiagen. The purified products were sequenced at Scigenom labs Pvt. Ltd., Kochi, Kerala. Feeding potential of the insect on sooty mould was also assessed by measuring the area consumed by the insect in square millimetre using graph paper plotting.

Results and discussion

A close examination of the sooty mould tainted coconut leaves led to the identification of a fungus feeding tenebrionid beetle and all its life stages. The beetle was identified as *Leiochrinus nilgirianus* Kaszab 1946 (Coleoptera: Tenebrionidae: Diaperinae: Leiochrinini) by Dr. Wolfgang Schawaller (retired), Stuttgart Museum, Germany. The voucher specimens are deposited at the ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, and Natural History Museum, Stuttgart, Germany. This beetle has a body shape and colour pattern resembling lady beetles (Coccinellidae) with swift mobility and limited flying ability. These beetles were generally confined to the abaxial surface of the

palm leaves during daytime, but were found feeding on sooty mould during morning hours (06.30–07.30 h IST) in damp conditions, averaging 2.07 ± 0.9 / leaflet, with a maximum of five beetles per leaflet. They were found to be light shy and photo-sensitive, preferring concealed habitats upon influx of sunlight and converging at the concealed corners on the palm leaflets. Occurrence of *L. nilgirianus* coincided with the southwest monsoon phase generating a humid and damp environment conducive to its development. These beetles were only observed on sooty mould covered leaves of coconut and sooty mould laden non-hosts such as custard apple (*Annona squamosa*) and *A. muricata,* indicating their preference to sooty mould, rather than the respective plants. Due to their feeding, the black sooty mould deposits were gradually and eventually completely cleansed and the leaves became bright green, reviving their photosynthetic efficiency. Our observations indicated on an average one adult beetle could clear 1–2 mm^2 sooty mould laden area in a period of one minute.

Adult and immature larvae of *L. nilgirianus* were found to feed mainly on moist sooty mould and not on dried sooty mould, for which early morning wetness prevailing during the monsoon phase was so crucial. Members of the tribe Leiochrinini, to which *L. nilgirianus* belongs, are usually restricted to humid habitats feeding from under rotten woods and or barks, especially on mosses and algae (Löbl et al. 2008; Schawaller 2016). Occurrence of *L. nilgirianus* was earlier reported from Peechi-Vazhani wildlife sanctuary in Kerala, India (Mathew et al. 2005) and the species was originally described from specimens collected from Goa, India (Kaszab 1946). This beetle is often found on wet trunks of silver oak, erythrina and other shade trees used as live support for black pepper and vanilla in the plantations of South India (Mathew et al. 2005). This nature of feeding is specially aided by adaptive leg features including the presence of bristle-like hairs on the undersurface of tarsomeres that probably aid in proper adhesion on the sooty mould deposition and well developed tarsal claws that probably assist swift movement on fungus-laden leaflets. A maximum of five beetles could be observed on the same leaflet in the ecological bioengineering experiment garden at Kayamkulam which favoured the ecological guild for the defender as well as scavenging beetles. This is the first report of the occurrence of a tenebrionid beetle scavenging on sooty mould fungus in palm ecosystem.

Life stages

The eggs of *L. nilgirianus* (Fig. 1b) are oval, smooth, shiny and bright pinkish to purplish and are laid in clusters on the undersurface of the palm leaflets. Upon eclosion after 2–3 days, a thin white membranous film is found covering the emerging grub, which is shed within 3–5 h. The freshly emerged larvae (Fig. 1c) are initially transparent and gradually get melanized and turn dark brown-black (Fig. 1d, e). The larvae are onisciform (Fig. 1f) and could roll into a sphere during locomotion (Fig. 1g) and could also be stretched like a cone. The intersegmental membrane of these grubs is elastic to accomplish this unique pattern of locomotion. The larvae are confined to the abaxial surface of leaflets during day time and probably move to the adaxial surface for feeding on the sooty mould during night hours and early morning before sunrise. Mature, final instar larvae nearing pupation turn paler with the dorso-lateral parts creamy yellow (Fig. 1h), which is quite prominent during locomotion. Pupation takes place on the abaxial surface of the palm leaflets and the pupae (Fig. 1i) are exarate and creamy yellow with black patches. Adult beetles are creamy yellow on emergence (Fig. 1j) and gradually turn ferrugineous (Fig. 1k). Adult beetles are characterized by: strongly convex and hemispherical body, head fully concealed under pronotum, antenna (Fig. 1l) yellowish brown, composed of 11antennomeres, antennomeres 5–11 often dark brown to black, pronotum anteriorly rounded, legs with penultimate tarsomeres dilated, lobed beneath, and distinctly broader than apical tarsomere. It resembles other known species of *Leiochrinus* from South India and can be conclusively identified only based on the male genitalia.

Molecular characterization

Molecular characterization of the beetle was done to facilitate its easy identification as there is no taxonomic expertise available on Indian Tenebrionidae. DNA was isolated from grubs and mitochondrial cytochrome c oxidase (*COI*) gene of *L. nilgirianus* was amplified at 710 bp. Nucleotide sequence of *COI* gene of *L. nilgirianus* (GenBank No. MG212503) showed 80% identity with *Diaperis boleti* (L.) (Tenebrionidae: Diaperinae) [GenBank acc. No. KJ962024.1]. The amino acid sequence derived from the *COI* gene

Fig. 1 **Life stages and adult features of *L. nilgirianus*: a**. Adult beetles; **b**. Purplish eggs in groups; **c**. Emerging neonates; **d**. First instar larvae; **e**. Second instar larvae; **f**. Larva with extended body; **g**. Larva rolled into a sphere; **h**. Final-instar larva; **i**. Pupa; **j**. Freshly emerged adult; **k**. Ferrugineous adult; **l**. Antennae; **m**. Bristles on tarsomeres; **n**. Tarsal claws

sequence of *L. nilgirianus* showed about 99% similarity to *Leiochrinini* sp. 2 ACP-2013 (Tenebrionidae: Diaperinae, GenBank acc. No. AHU86945.1) submitted from the Natural History Museum, London, UK.

In general, ecosystem services provided by insects include food for wildlife, pest destruction, crop pollination, scavenging, *etc.* which is estimated around $57 billion in the United States of America alone (Losey and Vaughan 2006). Noteworthy scavenging activities observed in insects are termites breaking down wood, springtails decomposing dead leaves and carrion beetles and fly maggots feeding on dead animals (Pedigo and Rice 2014). Scavenger caterpillars (*e.g.*, *Pyroderces rileyi* (Walsingham), Cosmopterygidae) are known to feed on dead matter and sooty mould on orange and lemon trees (Dreistadt 2012). Psocids (Insecta: Psocodea) are commonly known to feed on a variety of mildews, mould and dead insects in damp and humid environments and we also observed heavy incidence of unidentified psocids on rugose spiraling whitefly infested coconut and banana (Poorani and Thanigairaj 2017). Many families and genera of beetles are associated with sooty moulds including Derodontidae (found in temperate South America, New Zealand and Australia) (Hava 2006), Cyclaxyridae and Metaxinidae (families endemic to New Zealand), Nitidulidae (*e.g., Hisparonia* Kirejtshuk, a genus endemic to New Zealand), Mycetophagidae (*e.g.*, *Triphyllus* Dejean, one species associated mainly with sooty moulds), and Melandryidae (*e.g.*, *Doxozilora* Broun, a genus endemic to New Zealand) (Gimmel et al. 2009). Adults and larvae of *Agapytho* Broun (Agapythidae) have been

found on sooty mould growing on the exudates of Margarodidae (Hemiptera) that infest *Nothofagus* trees (Leschen and Lawrence 2010). Members of the tribe Leiochrinini are generally confined to moist and humid habitats feeding on mosses and algae (Schawaller 2016) and *Leiochrinus nilgirianus* seems to have a more diversified diet that includes sooty mould. The present instance appears to be unique in which sooty mould deposition is totally cleared by an insect scavenger. Consumption of sooty mould deposition on palm leaflets by insects, thereby improving photosynthesis and rejuvenating palm health on a large scale, is one of the first instances of beneficial scavenging activity reported in any economic crops so far.

In addition to flaking out the sooty mould fungus by spraying 1% starch solution, which is currently recommended, conserving *L. nilgirianus* is a good strategy to maintain the health of coconut plantations. In a nutshell, the parasitoid, *E. guadeloupae* and the scavenger beetle, *L. nilgirianus* appear to have considerably reduced the pestiferous potential of *A. rugioperculatus* on coconut in Kerala conditions. Conservation of *E. guadeloupae* by limited or zero pesticide usage coupled with *in situ* preservation of scavenger beetles appears to be a very effective strategy that would help in controlling *A. rugioperculatus* and in clearing sooty mould from coconut palms at no cost in the most natural and eco-friendly manner avoiding chemical management options and other expensive methods.

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