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## Non-native Neotropical nesting whitefly, *Paraleyrodes minei* Iaccarino on coconut palms in India and its co-existence with Bondar’s nesting whitefly, *Paraleyrodes bondari* Peracchi

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**Field occurrence of the exotic neotropical nesting whitefly, *Paraleyrodes minei* Iaccarino in association with Bondar’s nesting whitefly, *Paraleyrodes bondari* Peracchi on coconut leaflets is reported from Kerala, India. These coconut palms were previously infested by the rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin, which was reported from Kerala and Tamil Nadu during 2016. *P. minei* closely resembles *P. bondari*, but is devoid of the oblique grey bands on the wings and it constructs loosely woven, woolly wax nests. Female *P. minei* are white, but males are smoky grey. Cockhead-like male aedeagus with two thin appendixes projected downwards is the unique feature for species-level identification of *P. minei*. Detection of three non-native whiteflies of neotropical origin infesting coconut palms in India within a span of two years suggests their simultaneous introduction. Invasive potential of *P. minei* due to its polyphagous nature and short lifecycle calls upon strict policy frameworks in exchange of planting materials. Domestic quarantine should be strictly enforced in the country to avoid spread of this pest to other coconut-growing regions.**

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WHITEFLIES are polyphagous sucking pests of global significance in consequence of their direct feeding damage, as vectors of plant diseases as well as inducing secondary deposits of sooty moulds on leaf surfaces by honeydew production, thus impairing photosynthesis<sup>1,2</sup>. Most of the whitefly introductions have been from the New World into the Old World during the past 30–40 years<sup>3</sup>. Arrival of New World members of Aleurodicinae into the Old World for the first time is always a matter of concern for quarantine authorities as well as agricultural communities, as their entry would upset biodiversity. So far, 443 species of whitefly from 63 genera are known to attack more than 320 host plants from India<sup>4</sup>.

The invasive rugose spiralling whitefly (*Aleurodicus rugioperculatus* Martin) which was reported on coconut palms from Palakkad (Kerala) and Pollachi (Tamil Nadu) during 2016, still persists in many parts of peninsular India and has further spread up to Assam in the North East<sup>5</sup>. The pestiferous potential of *A. rugioperculatus* has been greatly subdued in peninsular India at present by conservation biological control using the aphelinid parasitoid, *Encarsia guadeloupae* Viggiani and *in situ* preservation of the sooty mould scavenger beetle, *Leiochrinus nilgirianus* Kaszab<sup>6</sup>. The pesticide-holiday approach advocated by ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI) in the bio-suppression of *A. rugioperculatus* in India is considered remarkable, because of which ecologically safe methods involving conservation of the natural enemy guild as well as the scavenger beetle niche could be deployed for containing the rugose whitefly.

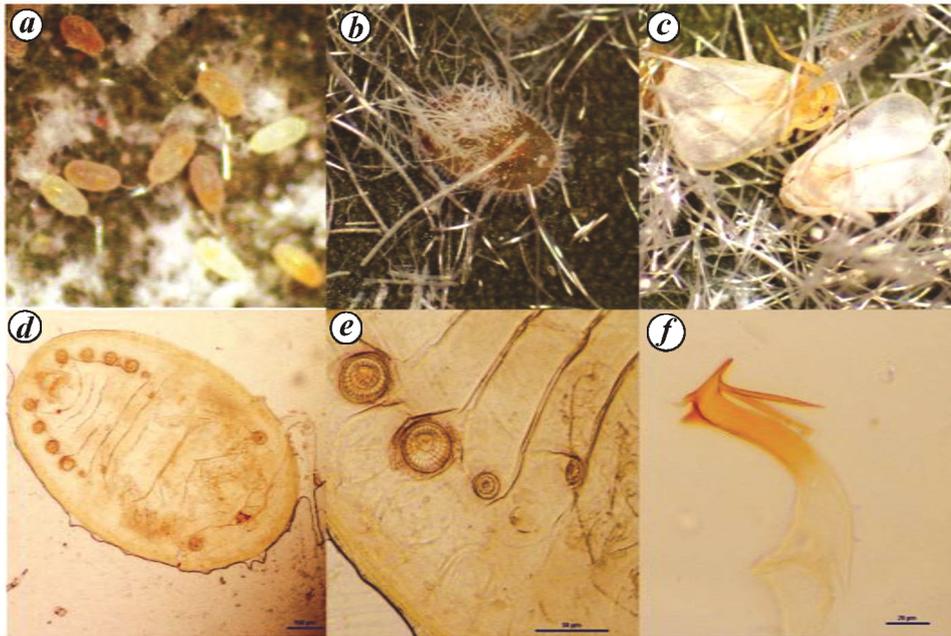
During December 2018, we found an unknown whitefly species, smaller in size (1.07 mm) than the rugose whitefly with conspicuous oblique grey bands and unique woolly wax nests on the abaxial palm leaflets, which was later identified as Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi (Josephraj Kumar *et al.*, communicated) from Kayamkulam, Kerala, peninsular India (9.1748°N, 76.5013°E). On close examination of some other leaflets, we found another whitefly species as small as *P. bondari*, but devoid of oblique grey bands, and it was found to construct rather loose woolly wax nests not as conspicuous as that of *P. bondari*. This whitefly was also found feeding exclusively on coconut leaflets previously infested by *A. rugioperculatus* and coexisted with *P. bondari* in the same coconut garden. Based on morphological identification confirmed by *COI* sequencing, here we report the occurrence of yet another neotropical exotic nesting whitefly, *Paraleyrodes minei* Iaccarino from Kayamkulam, with critical notes on its diagnosis.

The egg-laying pattern of adult nesting whiteflies, size and antennal features of the adult, architecture of the nest and physical features of the nymphal stages were

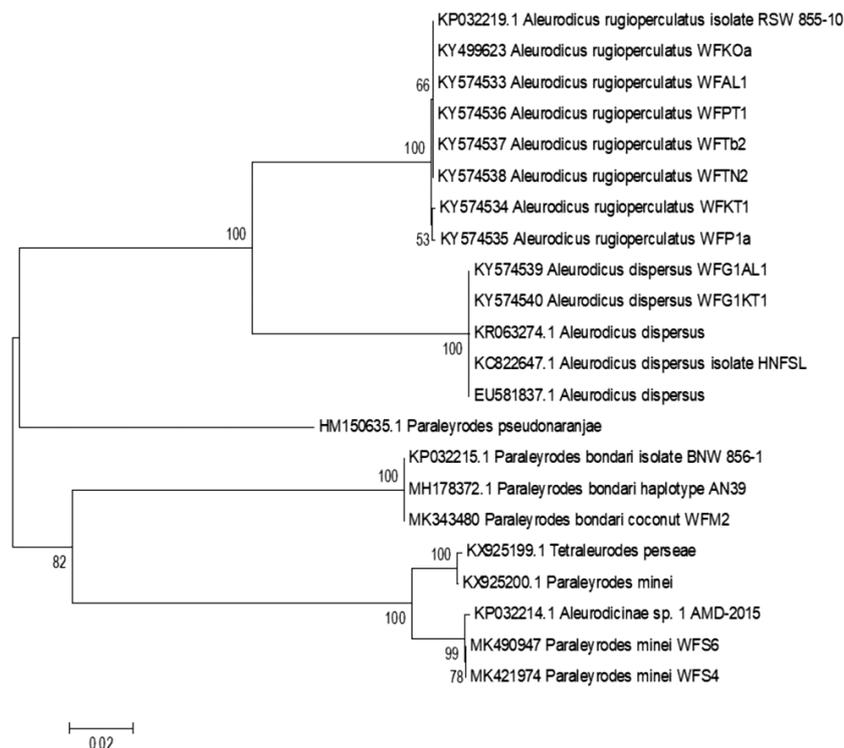
recorded. Permanent slide mounts of male genitalia and the puparia were made and examined using a stereomicroscope (LEICA EZ4W) and a trinocular research microscope (Nikon Eclipse Ni) as described by Martin<sup>3</sup> to confirm the identity.

Since molecular characterization is more important for species-level identification of nesting whitefly, genomic DNA was isolated from the adult whitefly identified morphologically. Primers LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198: 5'-TAAACCTCAGGGTGACCAAAAAATCA-3' synthesized (Eurofins Genomics India Pvt Ltd, Bengaluru) in salt-free status were used for amplification of mitochondrial cytochrome c oxidase subunit I (*COI*) gene<sup>7</sup>. All the polymerase chain reaction (PCR) amplifications were carried out in a thermal cycler (Techne Flexigene, England)<sup>8</sup>. 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 sec. Final extension was at 72°C for 10 min for end-filling. The products were analysed in 1.0% agarose gel. The amplicons obtained were purified from each reaction mixture using a PCR purification kit (Qiagen, Germany). The purified products were then sequenced (AgriGenome Labs Pvt Ltd, Kochi).

Adult whiteflies are small in size (1.07 mm) and construct relatively less-denser woolly wax nest than that of *P. bondari*. Male whiteflies have smoky grey wings with whip-like three-segmented antenna that are orange-tinged (Figure 1c). Female whiteflies are relatively white with a grey blotch at the terminal region, antenna four-segmented with the swollen second segment. Occurrence of cream-coloured egg clusters with short stalks that turn slight pinkish upon eclosion, and flat creamy-yellow nymphs with prominent fibreglass strands from the dorsum, are some characteristic features for the identification of *P. minei* which were observed in the specimens studied. Puparial characters include one larger cephalic pore and four abdominal compound pores (Figure 1d) about 33–35 µm, and outer ring with ovoid cellular facets giving the appearance of stylized flower petals. The last four abdominal compound pores are associated with the following simple discoidal pores: compound pore no. 3 has two or three discoidal pores, compound pore no. 4 has two, and compound pore nos 5 and 6 have one each. Two to three discoidal pores are associated with the two reduced abdominal pores which are half the size of the larger abdominal pores (Figure 1e) and comprise 8–9 flower petal-like facets<sup>3,9</sup>. The tongue-like lingula is extended beyond the posterior margin vasiform orifice with two pairs of apical setae. The operculum partially covers the lingula and the vasiform orifice. Male claspers have a strong nail and the aedeagus has a characteristic cock-head-shaped apex comprising three short appendixes located on the upper and posterior surface, and two long and thin appendixes projected downwards (Figure 1f)



**Figure 1.** Morphological identity of *Paraleyrodes minei*. *a*, Stalked eggs of *Paraleyrodes minei*; *b*, Puparium with fibreglass-like strands; *c*, Male and female adult *P. minei*; *d*, Characteristic cephalic and abdominal compound pores on stained puparium; *e*, Normal and reduced compound pores with flower-petal like facets; *f*, Cock-head-shaped male aedeagus.



**Figure 2.** Phylogenetic tree of nesting and spiralling whiteflies characterized from India.

beneath the short anterior appendix<sup>9-11</sup>. Based on these morphological features, the whitefly species was identified as *P. minei*.

At a glance, *P. minei* and *P. bondari* could be distinguished by the absence or presence of oblique grey bands on the wings as well as imperfect nest architecture;

however, the male aedeagus structures along with molecular characterization are extremely important for identifying the species. The features and location of compound pores, structure of vasiform orifice and lingula, and characters in life stages are not considered as diagnostic features in the identification of *Paraleyrodes* spp. *P. minei* Iaccarino and *P. bondari* Peracchi have been identified based on the taxonomic keys on puparium and male genitalia characters at ICAR-CPCRI, Regional Station, Kayamkulam and confirmed by molecular characterization of *COI* gene<sup>3</sup>. The voucher specimens of *P. bondari* Peracchi and *P. minei* Iaccarino after confirmation are deposited in ICAR-National Bureau of Agricultural Insect Resources (NBAIR) repository as NBAIR/Hem-1/2019 and NBAIR/Hem-W8/2019 respectively, along with their respective GenBank accession numbers.

The *COI* sequences of *Paraleyrodes* sp. under study showed 100% identity with the Aleurodicinae sp. 1AMD-2015 (GenBank accession no. KP032214) reported from Florida, USA in 2011 (Figure 2)<sup>12</sup>. Based on adult morphology, puparial characters and male genitalia, the species identity was confirmed as *P. minei*. Hence, the nucleotide sequence was deposited as *P. minei* in GenBank (accession no. MK421974. MK490947), though it showed only 93% identity with *P. minei* (GenBank accession no. KX925200) in the existing database.

Female adults have broad and swollen abdomen with well-developed wax plates, whereas the male abdomen is narrow becoming pointed at the apex and the wax glands are not well developed. Female adults lay stalked eggs in clusters on the lower surface of leaflets bending inwards towards the leaf surface deposited around loosely woven, woolly wax nest (Figure 1 a). The emerging mobile crawlers are creamish, sub-elliptical with flocculent wax on the dorsum extending from thorax to abdomen. On locating a proper feeding site, the crawlers shed their appendages, remain fixed, become flat and creamish, producing a fringe of short hyaline wax rods rising from the dorsum with fibreglass-like wax rods (Figure 1 b). Not greatly varying from *P. bondari* puparium, the cephalic pore and six abdominal pores with flower petal-like facets could be seen clearly through the stereomicroscope. *P. minei* feeds from abaxial leaf surface and produces honeydew which in turns leads to sooty mould deposits interfering with photosynthetic efficiency of the palms. In certain leaflets, we could also observe the presence of sooty mould scavenger beetle, *Leiochrinus nilgirianus*.

Although *P. minei* was first described from Syria on citrus in 1990, all *Paraleyrodes* spp. are considered native of the neotropical region (Iaccarino)<sup>10</sup>. It was reportedly first noticed in San Diego, California, USA in 1984. Later it was recorded from Belize, Guatemala, Mexico, Puerto Rico (neotropical region), Bermuda, California, Florida, Texas (Nearctic region), Lebanon, Morocco, Spain, Syria, Turkey (Palaeartic region), Benin

(Ethiopian region) and Hong Kong (Oriental region)<sup>3</sup>. The occurrence of *P. bondari* and *P. minei* was reported on coconut in Hawaii during 1971 and 1990 respectively, in port interceptions<sup>13</sup>. Among *Paraleyrodes* spp., *P. bondari* and *P. minei* are extremely mobile and have been reported from different regions of the world. This constitutes only the second instance of the occurrence of *P. minei* in Asia/the Oriental region. It was recorded from Kasaragod as well as Kayamkulam in Kerala.

Though coconut is not the primary host in other countries, *P. minei* could sustain and complete its biology successfully on the same. We observed the pest population varied from 3 to 30 nest colonies on many coconut leaflets and primarily did not cause any economic damage. However, with further increase in pest population and sooty mould deposits, it may bring forth economic setbacks. We could also record *P. minei* from *Psidium guajava* (guava) as well as from the ornamental plant, *Heliconia* sp. More than 25 susceptible plant hosts have been recorded from other countries so far<sup>13</sup>.

According to the competition exclusion principle or Gause's law, two species competing for the same limiting resource cannot coexist at constant population values. However, we observed simultaneous coexistence of *P. bondari* and *P. minei* in most of the coconut leaflets as a rare exception. This synchrony of mutual survival of two species indicates some intrinsic interaction among them, and calls for a detailed study. The emergence of nesting whiteflies at the receding phase of rugose spiralling whitefly suggests simultaneous introduction of these species, wherein the nesting whitefly group emerged into prominence after the natural parasitism of *A. rugioperculatus*. Advent of nesting whiteflies as a cryptic species along with rugose spiralling whitefly cannot be ruled out, as there are reports of coexistence of the nesting whitefly, *P. minei* and the woolly whitefly, *Aleurothrixus floccosus* (Maskell) on citrus from Greece<sup>14</sup>. Some lady beetle grubs were recorded in our observations on *P. minei*-infested coconut leaflets.

The appearance of three exotic whiteflies within a span of two years on the coconut system highlights the increased bio-security risks arising from uncontrolled exchange of plant material and warrants strengthening of quarantine. The exchange of planting materials through liberalized trade and transboundary movement of such materials without proper pest risk analysis has to be dealt with strict reforms and policy frameworks. Surfacing of sucking pest complex and climate change are intrinsically related and possibly similar weather conditions prevailing in the native range of these whiteflies and in Kerala could be another reason for their successful establishment. The abundance of these non-native insects in India indicates good survival of these exotic species in the country in a short time-period. Bionomics and bio-geographical distribution of the pest in India on varied hosts and its feeding impact in different locations on a wide array of host

plants need further studies to understand its pestiferous nature.

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