Research Article

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# New report on the invasive Bondar's Nesting Whitefly (*Paraleyrodes bondari* Peracchi) on oil palm in India

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## Abstract

This communication is the new report of the neotropical invasive Bondar's Nesting Whitefly (BNW) , *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae)incidence in oil palm in India. A typical feature of BNW infestation is the presence of woolly wax nests on the abaxial surface of oil palm leaflets. The nesting whitefly population was observed to increase phenomenally on oil palm and within a year ie., from 2021 to 2022, a 100 per cent palm infestation was observed. During this period the intensity per palm increased by 24.49 per cent and per leaf increased by 63.28 per cent. Analysis of the partial mitochondrial cytochrome oxidase subunit 1 (*CO1*) sequences from adult specimens indicated 100% nucleotide identity with Bondar's Nesting Whitefly from coconut.

***Keywords:*** India, Oil palm, Bondar's Nesting Whitefly, *Paraleyrodes bondari*, invasive

## Introduction

Oil palm (*Elaeis guineensis* Jacq.) has earned the sobriquet "The Golden Palm" as it is the most versatile and productive oil crop with its origins in West Africa. The palm is likely to play a major role in augmenting the supply of vegetable oil in the world. Two kinds of edible oils are obtained from oil pam: palm oil and palm kernel oil. In India, oil palm is being cultivated in 13 states covering about 3,15,000 hectares under irrigated conditions, with potential states being Andhra Pradesh, Karnataka, Tamil Nadu and Bihar. In Andhra Pradesh, oil palm is grown in 0.15 million hectares with a production of about 1.1 million tonnes (Selvaraj *et al*. 2019).

The presence of Rugose Spiraling Whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Sternorrhyncha: Aleyrodidae) in India in coconut plantations was reported across Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh States in 2016-2017 (Shanas *et al*. 2016, Sundararaj and Selvaraj 2017). Along with coconut, the other preferred host of the RSW was observed to be oil palm. The first incidence of RSW on oil palm in Andhra Pradesh State was observed in 2016 in Kalavalapalli village (16.9482 ° N, 8l.6398 °E) of West Godavari district. Yield losses of up to 45 per cent were reported (Kalidas 2019).

Three years after the appearance of the RSW *ie.,* during June 2020, the presence of neotropical

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invasive Bondar's Nesting Whitefly (BNW), *Paraleyrodes bondari* Peracchi was observed in the same village on the lower surface of coconut leaflets. By December 2020, the incidence was observed on five-years-old Deli x Nigeria oil palm plantations adjacent to the infested coconut plantation (Fig. 1) and by February 2022 hundred per cent infestation of BNW on oil palm was observed. The first incidence of BNW in India and its detailed description was reported on coconut palms from Kerala State by Josephrajkumar *et al*. (2019). This study was carried out with an objective to study the incidence of BNW in oil palm in the state of Andhra Pradesh, assessment of its severity and its molecular characterization.

## Materials and methods

### Assessment of severity of the infestation

The incidence and intensity of the whiteflies in the observational field was calculated as detailed below;

**1.** Per cent palms infested: No. of palms infested by the whiteflies /Total number of palms in the garden

**2.** Intensity per palm: Number of leaves infested by the whiteflies /Total leaves per palm

**3.** Intensity per leaf: Number of leaflets infested by the whiteflies /Total leaflets per leaf.

The severity of BNW was assessed on the lower three fronds per palm based on the following criteria, *i.e*. low: 0-10 live adult nests /leaflet; moderate: 11-20 live adult nests/leaflet; severe: >20 live adult nests /leafletp

### Molecular characterization

The adult whiteflies were collected and preserved in 70% ethanol for species confirmation through molecular studies. Genomic DNA was extracted from the samples using HiPurA™ Insect DNA Purification Kit (Hi-Media, India) following the manufacturer's instruction. The extracted DNA

**Fig 1a**. Nesting whitefly colony

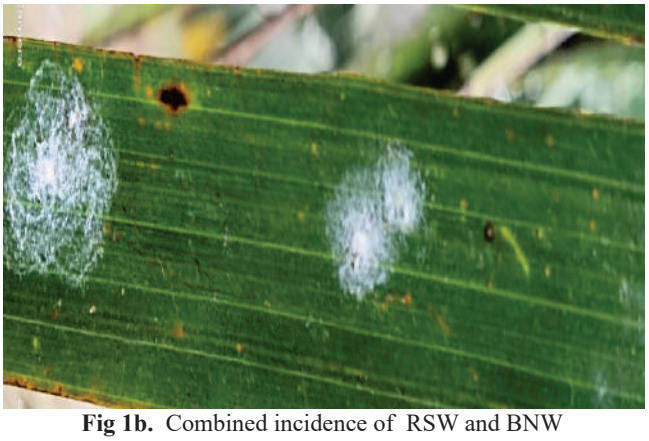

**Fig 1b.** Combined incidence of RSW and BNW

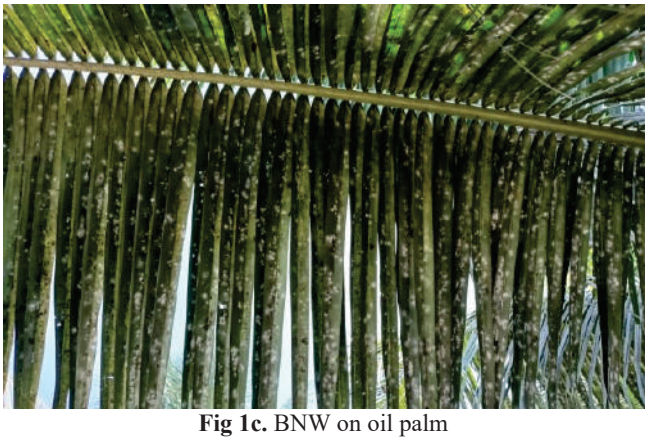

**Fig 1c.** BNW on oil palm

**Fig 1d.** Severe incidence of BNW on oil palm

**Fig. 1: Incidence of Bondar's nesting whitefly in oil palm**

was eluted in 200 µL elution buffer. The quality and quantity of extracted DNA were tested using the spectrophotometer and 1.2 % agarose gel electrophoresis, and the DNA samples were stored at -20°C for further use. The DNA sequence from mitochondrial *CO1* was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGAT ATTGG-3′) and HC02198 (5′- TAAACTTCAG GGTGACCAAAAAATCA-3′) (Folmer *et al*., 1994). Amplification was carried out in a 20 µL reaction volume which contained 2 µL of 10X Buffer [10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin], 0.8 µL dNTPs (0.25 mM each), 3 µL of the forward and reverse primer's (2µM each) , 0.3 µL of 3U *Taq* DNA polymerase (Genei Laboratories Pvt. Ltd., India) and 35 ng DNA. The PCR cycling condition consisting of an initial denaturation at 94ºC for 2 min followed at 35 cycles of denaturation at 94ºC for 30 sec, annealing at 50 ºC for 1 min and extension at 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. After amplification, the PCR products were separated on 1.2% agarose gel in 1X TBE buffer by electrophoresis stained with ethidium bromide. The gel visualized in a gel documentation system. The PCR product was purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel Inc). The purified PCR fragments were sequenced at the sequencing facility at Bionivid Technology [P] Ltd., Bengaluru, India. Each sample was subjected to three sets of sequencing reactions using the forward and reverse primers for high accuracy. The forward and reverse sequence of *CO1*, obtained after sequencing, was aligned using BioEdit (Hall, 1999). Each nucleotide sequence obtained was compared with its related species using the BLASTn tool (http://blast.ncbi.nlm.nih.gov/), and the sequence was deposited in NCBI (https://www.ncbi.nlm.nih.gov/).

Nucleotide sequences of the *COI* from diverse whitefly species were retrieved from the NCBI in FASTA format (Table 1). Multiple sequence alignments were performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/), and phylogenetic trees were constructed by using neighbour-joining (NJ) algorithm in MEGAX software (https://www.megasoftware.net/ ) with 1000 replications for bootstrap analysis (Kumar *et al*., 2016).

## Results and discussion

Genus *Paraleyrodes* is physically much smaller than most other aleurodicines; puparium with 5 or 6 compound pores in which the anterior 1 or 2 pairs much smaller than the remaining 4 abdominal pairs and the cephalic pair; thorax with two pairs of cicatrices and a pair of submedian setae; outer submargin with a row of 14 pairs of hair-like setae. Adults with all wing veins unbranched; females have 4 articulated antennal segments; males have only 3 articulated antennal segments and complex aedeagal apices. The larvae and puparia secrete long waxy filaments that often

**Table 1: List of nucleotide sequences of *COI* retrieved from GenBank**

| No. | Accession Number | Species | Location | Reference |
|---|---|---|---|---|
| 1. | MW488198.1 | *Paraleyrodes bondari* | Tamil Nadu | Banumathi *et al*., 2021 |
| 2. | MW041899.1 | *Paraleyrodes bondari* | Karnataka | Shivaji Thube *et al*., 2020 |
| 3. | MK343480.1 | *Paraleyrodes bondari* | Kerala | Josephrajkumar *et al*., 2019 |
| 4. | KP032215.1 | *Paraleyrodes bondari* | Florida | Dickey *et al*., 2015 |
| 5. | HM150635.1 | *Paraleyrodes pseudonaranjae* | Hainan, China | Zhu *et al*., 2016 |
| 6. | MW488186.1 | *Paraleyrodes minei* | Tamil Nadu | Banumathi *et al*., 2021 |
| 7. | MW047062.1 | *Paraleyrodes minei* | Karnataka | Shivaji Thube *et al*., 2020 |
| 8. | KF595126.1 | *Paraleyrodes pseudonaranjae* | Beijing, China | Zhang *et al*., 2013 |
| 9. | KP032221.1 | *Paraleyrodes pseudonaranjae* | Florida | Dickey *et al*., 2015 |
| 10. | KF059955.1 | *Aleuronudus melzeri* | Colombia | Ovalle *et al*., 2013 |
| 11. | KP032222.1 | *Metaleurodicus cardini* | Florida | Dickey *et al*., 2015 |
| 12. | KP032214.1 | *Aleurodicinae sp.* | Florida | Dickey *et al*., 2015 |
| 13. | KX925199.1 | *Tetraleurodes perseae* | Mexico | Palacios *et al*., 2020 |
| 14. | JQ340180.1 | *Aleuroclava indicus* | Shanghai, China | Shi *et al*., 2012 |
| 15. | JQ340181.1 | *Aleuroclava indicus* | Shanghai, China | Shi *et al*., 2012 |
| 16. | KY574534.1 | *Aleurodicus rugioperculatus* | Kottayam, Kerala, India | Chandrika Mohan *et al*., 2017 |

form an annulus surrounding the feeding insects. Adults remain inside a nest like mealy wax and females usually secrete so much wax around them while ovipositing and hence the members of this genus are appropriately known as "nesting whiteflies" (Martin, 2004).

This whitefly is very small (< 1.0 mm) and has conspicuous X-shaped oblique grey bands on the wings. The nymphs and adults are present in nesting chambers of woolly wax resemble a bird's nest (Fig. 2). The adult whitefly laid stalked eggs, and the nymphs are flat with fibre glass like projections from the dorsum. In the present investigations, the co-existence of *P. bondari* and *A. rugioperculatus* was observed in the same leaflets of oil palm and combined incidence of both whiteflies was recorded in the infested garden. Co-existence of *P. bondari* and *A. rugioperculatus* on coconut palms was earlier reported by Josephrajkumar ( 2019) and Vidya *et al*. (2019) indicating probable simultaneous introduction of both these pests from the New World.

**Assessment of severity of the infestation**

As BNW was observed to be actively feeding on oil palm detailed studies on its incidence and intensity were taken up to assess the build up of BNW on oil palm . In January 2021, about 24.77 per cent of infestation of BNW was observed and within a month, it increased to 57.33 in the observational garden. Further, the intensity per palm increased from 54.76 to 58.20 per cent, but a drastic increase in intensity per leaf was observed, with an increase from 26.17 to 79.35 per cent (Table 1).The data on incidence of BNW was again collected in January 2022 by which period an increased infestation of

**Fig 2a.** BNW egg

**Fig 2b.** Nymph of BNW

**Fig 2c.** Pupa of BNW

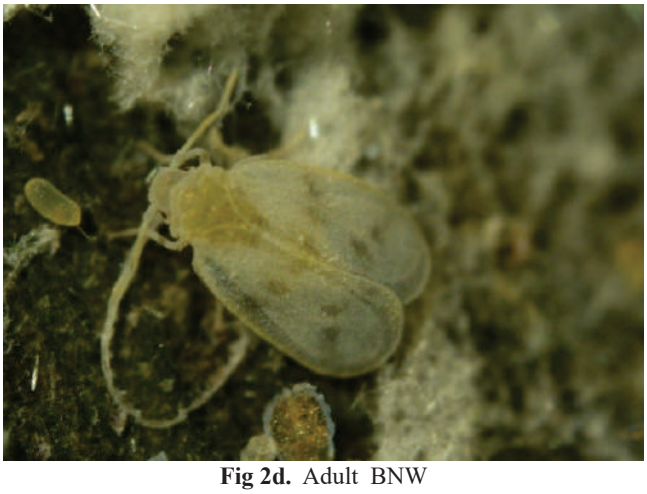

**Fig 2d.** Adult BNW

**Fig. 2: Life stages of Bondar's nesting whitefly**

**Fig. 3:** ***Apertochrysa astur*** **larva feeding on egg's of Bondar's nesting whitefly**

85.50 per cent was observed which reached 100 percent by February 2022 . The intensity per palm ranged above 70 per cent while intensity per leaf ranged above 80 percent in 2022 indicating suitability of oil palm as host to BNW. However, no parasitisation by *E. guadelopuae* was observed on this whitefly but natural feeding by predator *Apertochyrsa astur* was recorded (Fig. 3). It was reported by Vidya *et al*. **(**2019) that the population of *P. bondari* was more (8.04 nymphs per 30 cm leaflet) than that of *A. rugioperculatus* (4.4 nymphs per 30 cm leaflet) on coconut. She further reported the other host crops of BNW are Subabul (*Leucaena leucocephala*) , *Tetraleurodes acacia* , *Morinda citrifolia* , *Dialeurodes kirkaldyi* and *Cinnamomum verum*.

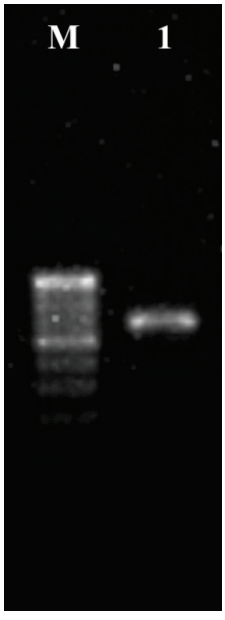


**Fig. 4. Representative gel showing PCR amplification of the *COI* gene from Bondar's nesting whitefly collected from oil palm. Lane M: 100 bp ladder; Lane 1: Amplification of Bondar's nesting whitefly DNA**

## Molecular characterization

A partial mitochondrial *CO1* gene (~ 700 bp) sequence of adult whiteflies collected from West Godavari Andhra Pradesh was amplified using the primers (Fig. 4). The sequences obtained were subjected to a BLAST search, and sequences were confirmed to be *COI* and exhibited 98 per cent similarity with *P. bondari* isolate Coconut cytochrome c oxidase subunit I (*CO1*) gene (accession number: MW488198.1). The sequence generated in the present study has been deposited in NCBI (GenBank accession :MW704277). By analysing the phylogenetic tree (Fig. 5), it was observed that *P. bondari* has a closer evolutionary relationship with *P. pseudonaranjae,* whereas, *Aleuroclava indicus* was distantly placed.

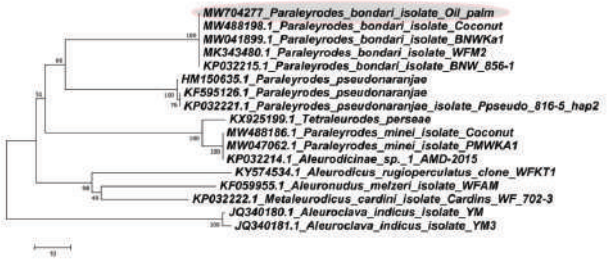


**Fig. 5: Phylogenetic tree of Bondar's nesting whitefly constructed based on the neighbour-joining (NJ) method. Bootstrap values are displayed at each node.**

Documentation of the presence of this invasive species in Andhra Pradesh and its association with oil palm imparts an immense momentum for a country wide survey to ascertain the host range, distribution pattern and damage assessment of this important species. This would prove decisive step towards comprehending the threat to oil palm posed by BNW and designing suitable IPM strategies to manage this pest.

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