

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF TWO EXOTIC WHITEFLY SPECIES, *Aleurodicus rugioperculatus* MARTIN AND *Paraleyrodes bondari* PERACCHI, INVADING COCONUT (*Cocos nucifera* L.) PLANTATIONS OF TAMIL NADU, INDIA

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ABSTRACT

Morphological and molecular studies of invasive insect pests are vital for their precise identification, monitoring, and management. These studies provide insights into their morphology, genetic diversity, and evolutionary adaptations, which are crucial for developing effective control measures. Two invasive whiteflies, *Aleurodicus rugioperculatus* Martin and *Paraleyrodes bondari* Peracchi, were found to be causing significant damage to coconut plantations in Tamil Nadu, leading to considerable losses for farmers. In light of this, a survey was conducted during 2020 and 2021 to assess the incidence of these two whitefly species (*A. rugioperculatus* and *P. bondari*) in coconut (*Cocos nucifera* L.) plantations across Tamil Nadu, India. Nymphs and adults were collected from coconut fields and morphologically identified through live photographs and slide-mounted specimens. Morphological identification revealed distinctive traits for each species. Pupae of *A. rugioperculatus* exhibited a rugose operculum with a triangular lingula, compound pores featuring a dagger-like process, and smaller compound pores on segments VII and VIII. Adults displayed irregular light brown bands on their wings, while smaller males had a long, pincer-like structure at the end of the abdomen. In contrast, *P. bondari* was distinguished by flower-petal-like ovoid facets on the puparium's abdominal compound pores, and adults showed "X"-shaped oblique greyish stripes on their wings. Further molecular confirmation was achieved through polymerase chain reaction (PCR) amplification and sequencing of the mitochondrial Cytochrome Oxidase subunit I (COI) gene. A 650 bp fragment was successfully amplified, and the resulting sequences showed a 100% match with available sequences in GenBank, confirming the species identities of *A. rugioperculatus* and *P. bondari*. Phylogenetic analysis of the COI gene sequences, conducted using MEGA-11 software and the maximum likelihood method, produced a dendrogram clustering the specimens into a single clade with distinct sub-lineages corresponding to different survey locations. The characterization of *A. rugioperculatus* and *P. bondari* is essential for their management in coconut plantations. DNA barcoding and COI gene sequencing ensure accurate identification, while phylogenetic analysis reveals their invasion pathways and genetic diversity.

Keywords: *Aleurodicus rugioperculatus*, coconut, molecular conformity, morphological characters, *Paraleyrodes bondari*, whiteflies.

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INTRODUCTION

Coconut (*Cocos nucifera* L. Arecaceae) is a vital crop in India, often referred to as the 'tree of heaven' or 'kalpavriksha' due to its immense significance in human life. Globally, coconut is cultivated across 12.257 million

hectares, yielding 66,674 million nuts annually, with a productivity of 5,440 nuts per hectare. In India, it is grown over 2,153.74 hectares, producing 19,309.90 million nuts with a productivity of 8,966 nuts per hectare (CDB, 2022). Coconut plantations are susceptible to various insect pests, with 547 insect and mite species recorded infesting coconut palms throughout the year,

including 118 exotic species (Abhishek and Dwivedi, 2021). Among these, sucking pests particularly invasive whiteflies pose a significant threat to coconut production. India is home to 440 whitefly species belonging to 63 genera (Dubey and Sundararaj, 2015), with bio-invasions contributing to nearly 25% of economic losses due to invasive pests, weeds, and diseases (Mohan *et al.*, 2017). Whiteflies have recently emerged as a major concern for coconut farmers in India. The Rugose Spiraling Whitefly (*Aleurodicus rugioperculatus* Martin) was first identified in 2004 in Belize, Central America (Martin, 2004), followed by reports from South Florida in 2009 (Stocks and Hodges, 2012). In India, it was initially observed in 2016 in Kerala (Shanas *et al.*, 2016), Karnataka (Selvaraj *et al.*, 2017), and Tamil Nadu (Srinivasan *et al.*, 2016), rapidly spreading across South Indian coconut plantations. Another invasive species, the Bondar's Nesting Whitefly (*Paraleyrodes bondari* Peracchi), was first reported in Kayamkulam, Kerala, in 2018 and has since become a significant pest in coconut plantations. *P. bondari* is known to infest more than 25 host plants, causing extensive damage in coconut-growing regions (Josephraj Kumar *et al.*, 2019) and 25 plant species from 19 families found in southern Tamil Nadu (Suriya *et al.*, 2024). The nymphs and adults of whiteflies feed on the sap from the underside of leaves, causing honeydew to be released onto the upper leaf surfaces. This sticky substance fosters the growth of *Capnodium* fungus, which gives affected plants a black, sooty appearance. Additionally, it hinders photosynthesis, leading to physiological imbalances, leaf malformations, premature nut drop, and restricted growth, ultimately lowering nut production. (Chandrika *et al.*, 2016).

Selvaraj *et al.* (2017) investigated the intensity of damage and incidence of invasive whiteflies in Tamil Nadu, Kerala, Andhra Pradesh, and Karnataka, reporting considerable variation in infestation severity. Coconut palms suffered 40-60% damage, while banana leaves experienced 25-40% damage. *Aleurodicus rugioperculatus* caused significant losses (20-35%) in coconut crops, particularly in coastal regions such as Mangalore and Udupi. In Jashore, a 2019 study reported infestation rates between 46.66% and 68.33%, with the highest level (68.33%) in Magura Sadar Upazila (Dutta *et al.*, 2019). In Tamil Nadu, Coimbatore district recorded 62.86% infestation between August 2017 and February 2019, while higher rates were observed in 2019-2020, particularly in Tirunelveli (75.4%) and Kanyakumari (75.8%) (Elango *et al.*, 2019; Alagar *et al.*, 2020). A field survey conducted from December 2020 to August 2021 in Tamil Nadu's southern districts found the highest whitefly incidence in Kanyakumari (56.30%) and the lowest in Tenkasi (48.83%) (Suriya *et al.*, 2023).

Currently, four whitefly species *A. rugioperculatus*, *P. bondari*, *Paraleyrodes minei*, and *Aleurotrachelus atratus* are considered coexisting pests

of coconut, making species identification a challenging task. The primary objective of this study is to achieve accurate identification of whiteflies through morphological characterization using taxonomic keys and to confirm species identity using molecular markers. Precise identification is crucial as it forms the foundation for implementing effective management strategies.

MATERIALS AND METHODS

Morphological identification of whiteflies: A total of 10 to 15 whitefly puparia samples were collected from coconut gardens in southern Tamil Nadu and preserved in vials containing 70% ethanol. The preserved specimens were then prepared for slide mounting following the procedure described by Sirisena *et al.* (2013). The preparation process began with soaking the whitefly puparia overnight in a 10% potassium hydroxide (KOH) solution at room temperature. After soaking, the specimens were transferred to water, where their body contents and any residual KOH were removed by rinsing with tap water. For permanent mounting, the specimens were stained with acid fuchsin for 12 to 24 hours to enhance the visibility of internal structures. Following staining, they were rinsed in 80% alcohol and then transferred to 95% isopropanol for 15 minutes to dehydrate the cuticle. Next, the specimens underwent a dewaxing process in which lipids were dissolved by immersing them in histoclear phenol for 5 minutes. They were then rinsed in 95% isopropanol to remove any remaining dissolved waxes. To further clear the cuticle, the specimens were soaked in clove oil for 10 minutes. Finally, each puparium was placed in a drop of Canada balsam at the center of a slide, covered with a cover slip, and allowed to dry for permanent mounting. The mounted specimens were then examined for morphological identification using a LEICA SAPO image analyzer and LAS V4.13 software, with the aid of the morphological key developed by Martin (2004) for Rugose spiraling whitefly and key given by (Josephraj Kumar *et al.*, 2019) was used for Bondar's nesting whitefly.

Molecular characterization of coconut whiteflies: Genomic DNA from coconut whiteflies was extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Bossier *et al.*, 2004). The quality of the extracted DNA was assessed through 0.8% agarose gel electrophoresis. For molecular characterization, the cytochrome oxidase-I (COI) gene region was amplified using polymerase chain reaction (PCR). COI gene-specific primers LCO1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGA CCAAAAA ATCA-3') (Folmer *et al.*, 1994) were used for amplification. The PCR reaction mixture (50 μ L) consisted of 25 μ L of

master mix, 4 μ L each of forward and reverse primers, 6 μ L of genomic DNA (25-30 ng), and 15 μ L of sterile water. The thermal cycling conditions included an initial denaturation at 97°C for 6 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes, with a final extension at 72°C for 7 minutes. PCR products were analyzed by gel electrophoresis on a 1% agarose gel and visualized under the Gel Documentation System (Gel LUMINAX). The amplified products were then sequenced using the Sanger dideoxy method at Bioserve Biotechnologies Private Limited, Hyderabad.

Phylogenetic tree construction: The mtCOI sequences of *A. rugioeperculatus* and *P. bondari* were annotated and manually curated using the DNA MAN program before being submitted to GenBank under accession numbers OK037183, OK040507, OK042272, OK042851, OK632470, and OK632478. Sequence similarity was assessed by comparing the obtained sequences with reference sequences available in GenBank (NCBI, Bethesda, USA).

Phylogenetic analysis was performed using all available mtCOI sequences from GenBank, along with those generated in this study, incorporating *Aleurodicus dispersus* and *Paraleyrodes minei* as out-group sequences. Sequence refinement was conducted using the BioEdit Sequence Alignment Editor (Version 7.0.5.3), followed by multiple sequence alignment with Clustal W (Thompson *et al.*, 1994) in MEGA-X software. A phylogenetic dendrogram was constructed using the Neighbor-Joining method with 1000 bootstrap replicates in MEGA-11, ensuring robust evolutionary relationship analysis.

Protein Sequence Retrieval and Homology Modeling for *A. rugioeperculatus* and *P. Bondari*: The protein sequences of cytochrome c oxidase subunit I (COX-I) from *Aleurodicus rugioeperculatus* and *Paraleyrodes bondari* were retrieved from NCBI in FASTA format, along with their respective accession numbers. A homology model was generated using SWISS-MODEL, an automated platform for protein structure prediction based on sequence homology (Waterhouse *et al.*, 2018). The predicted protein models were validated using the SAVESv6.0 service (<https://saves.mbi.ucla.edu/>) to ensure structural accuracy. To assess the stereochemical quality of the models, Ramachandran plots were generated using PROCHECK, following the methodology outlined by Laskowski *et al.* (1993). This validation process confirmed the reliability of the structural models for both whitefly species.

RESULTS

Morphological identification of *A. rugioeperculatus* and *P. Bondari*: *A. rugioeperculatus* was identified through slide-mounted specimens. The puparium of this species features two small compound pores on both the seventh and eighth abdominal segments, each accompanied by a dagger-like projection. It also exhibits a corrugated (rugose) operculum, a reticulated dorsal cuticle, and a distinct lingual apex, distinguishing it from *A. dispersus* puparium. The species is characterized by a pointed lingula and rugosity on the operculum's surface (Fig. 1). The eggs are creamy yellow and laid in a concentric spiral pattern by females on the underside of leaves. This whitefly undergoes five developmental stages. The first instar, or crawler, is the only mobile immature stage. Nymphs vary in color from pale to golden yellow, with a single broader fluff at the posterior end, producing dense cottony wax and long, thin sticky filaments. Adult whiteflies resemble small moths, measuring 2.27–2.59 mm in length, nearly three times larger than common whiteflies. They can be identified by irregular light brown bands across the middle of the forewings, along with a third spot near the distal end of the forewings. Males are smaller than females and possess a long, pincer-like structure at the end of their abdomen (Fig. 1).

The puparium of *P. bondari* was used for slide mounting. Compared to other *Aleyrodidae* species, this puparium contains 5–6 compound pores, with one or two smaller pairs in the anterior region and four larger pairs in the abdominal and cephalic regions. The thoracic region features 5–6 compound pores (33–35 μ m), each encircled by a chitinous spine rim. Six pairs of abdominal compound pores are present from segments III to VIII. The nymphal instars exhibit seven to eight stylized, flower-petal-shaped ovoid facets on the abdominal compound pores. The last four abdominal compound pores contain varying numbers of simple discoidal pores: the third has two to three, the fourth has two, and the fifth and sixth each have one (Fig. 2). Eggs are laid in woolly wax nests on the underside of leaves. Young nymphs are transparent, while older nymphs are enclosed in flocculent wax and covered with fibrous rods. Adult *P. bondari* is distinguished by "X"-shaped, oblique, greyish stripes on its wings and the formation of unique woolly wax nests on the underside of leaves (Fig. 2).

Molecular characterization of *A. rugioeperculatus* and *P. Bondari*: The COI marker was used to identify coconut whitefly species (Fig. 3). PCR products were sequenced using Sanger's dideoxy sequencing technique and submitted to GenBank with accession numbers listed in Table 1. The nucleotide sequences of *A. rugioeperculatus* and *P. bondari* were retrieved from the NCBI database and aligned using the BioEdit Sequence Alignment Editor (version 7.0.5.3). A dendrogram was

constructed in MEGA 11 to determine sequence similarities with other known sequences. The amplicon sizes for *A. rugioperculatus* isolates from Kanyakumari, Tirunelveli, Thoothukudi, and Tenkasi were 529 bp, 569 bp, 629 bp, and 499 bp, respectively. For *P. bondari*, the amplicon sizes identified in the Kanyakumari and Tirunelveli isolates were 658 bp and 652 bp, respectively.

Phylogenetic analysis for *A. rugioperculatus* and *P. Bondari*: In this study, the *A. rugioperculatus* and *P. bondari* sequences generated exhibited 99% similarity to previously deposited COI gene sequences of the same species. The 37 mtCOI gene sequences obtained showed 99–100% similarity with reference *A. rugioperculatus* and *P. bondari* sequences. Phylogenetic analysis, performed using high-quality mtCOI sequences from NCBI (Fig. 4), demonstrated strong bootstrap support (100%) in branching values. Sequences of *A. rugioperculatus* from Indian coconut plantations, specifically Tirunelveli (OK042272) and Kanyakumari (OK037183), were closely related, along with other *A. rugioperculatus* populations from India. The *A. rugioperculatus* isolates from Tenkasi (OK040507) and

Thoothukudi (OK042851) showed a strong phylogenetic relationship with the Indian areca nut population (OP024188) (Fig. 4).

For *P. bondari*, the sequences from Kanyakumari coconut plantations (OK632470) closely resembled those from *Bhendi* (MW488187) in India. However, *P. bondari* populations from Tirunelveli (OK632478) formed distinct single clades within the phylogenetic tree and did not exhibit close relatedness to any other sequences.

Structural modelling and validation of target proteins in *A. rugioperculatus* and *P. Bondari*: The three-dimensional structures of target proteins from *A. rugioperculatus* and *P. bondari* were generated using the SWISS-MODEL web server (Fig. 5). The quality and validity of the predicted models were assessed using Ramachandran plot analysis (Fig. 6), which showed that most residues fell within the allowed regions (Table. 2). This indicates high structural accuracy and reliability. The analysis confirms the integrity of the models, supporting their potential use in further studies or applications.

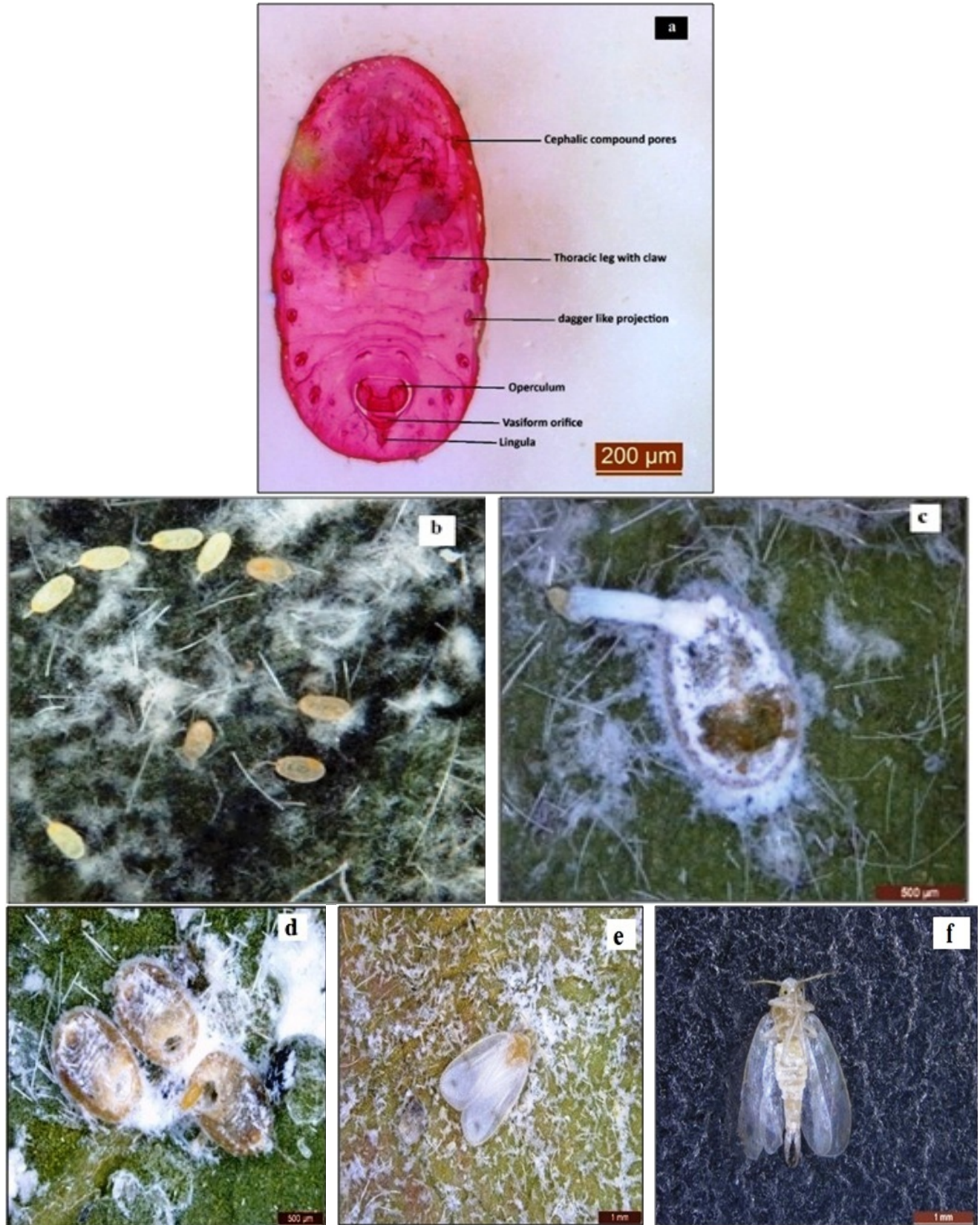
Table 1. Genbank accession numbers of *Aleurodicus rugioperculatus* and *Paraleyrodes bondari*.

S. No	Genbank Accession No	Organism	Location	Host plant
1	OK037183	<i>Aleurodicus rugioperculatus</i>	Kanyakumari	Coconut
2	OK040507	<i>Aleurodicus rugioperculatus</i>	Tenkasi	Coconut
3	OK042272	<i>Aleurodicus rugioperculatus</i>	Tirunelveli	Coconut
4	OK042851	<i>Aleurodicus rugioperculatus</i>	Thoothukudi	Coconut
5	OK632470	<i>Paraleyrodes bondari</i>	Kanyakumari	Coconut
6	OK632478	<i>Paraleyrodes bondari</i>	Tirunelveli	Coconut

Table 2. Ramachandran plot statistics of the *A. rugioperculatus* and *P. bondari*, cytochrome c oxidase subunit I, partial protein target. *.

Protein Target (COI*, partial)	Ramachandran Plot Statistics			
	Residues in most favored regions (%)	Residues in additional allowed regions (%)	Residues in generously allowed regions (%)	Residues in disallowed regions (%)
OK037183 (<i>A. rugioperculatus</i>)	94.8%	4.6	0.7	0.0
OK042851 (<i>A. rugioperculatus</i>)	93.9%	5.6	0.6	0.0
OK042272 (<i>A. rugioperculatus</i>)	93.9%	5.5	0.6	0.0
OK040507 (<i>A. rugioperculatus</i>)	95.1%	4.2	0.7	0.0
OK632470 (<i>P. bondari</i>)	95.2%	4.8	0.0	0.0
OK632478 (<i>P. bondari</i>)	95.1%	4.9	0.0	0.0

*COI, cytochrome c oxidase subunit I.



a. Mounted pupa, b. eggs, c. nymph, d. pupa, e. adult female, f. adult male

Fig 1. Morphological identification of rugose spiralling whitefly, *Aleurodicus rugioeperculatus*



a. mounted pupa, b. woolly wax nest, c. eggs, d. nymph, e. pupa, f. adult
Fig 2. Morphological identification of bondar's nesting whitefly, *Paraleyrodes bondari*

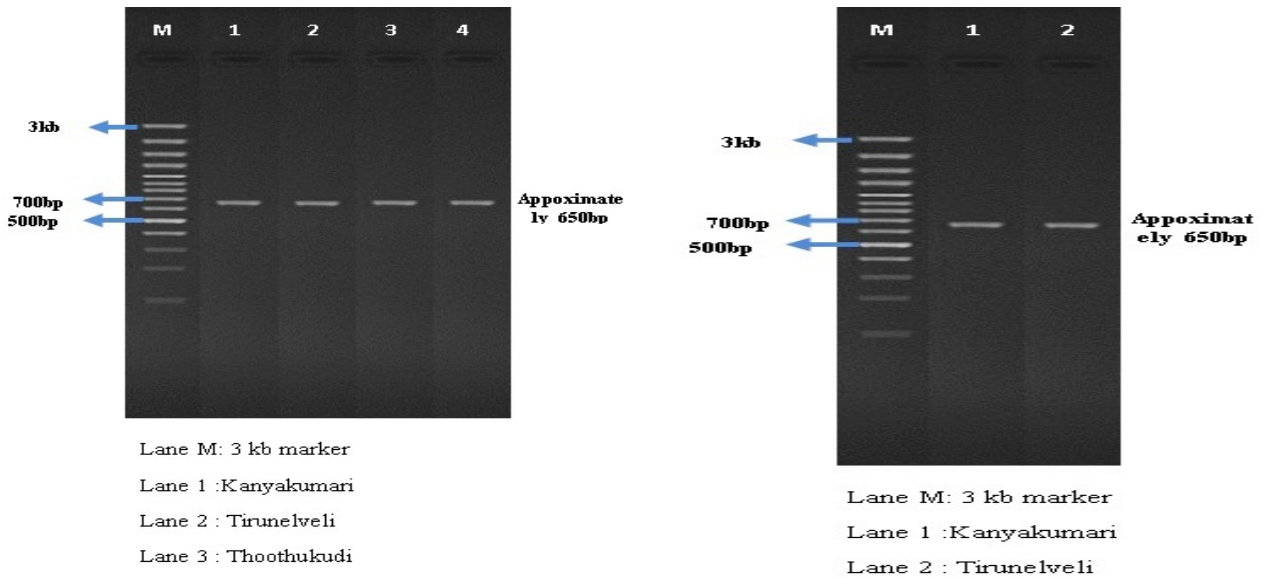


Fig 3. PCR- Gel images of rugose spiralling whitefly, *Aleurodicus rugioperculatus*, bondars nesting whitefly, *Paraleyrodes bondari*

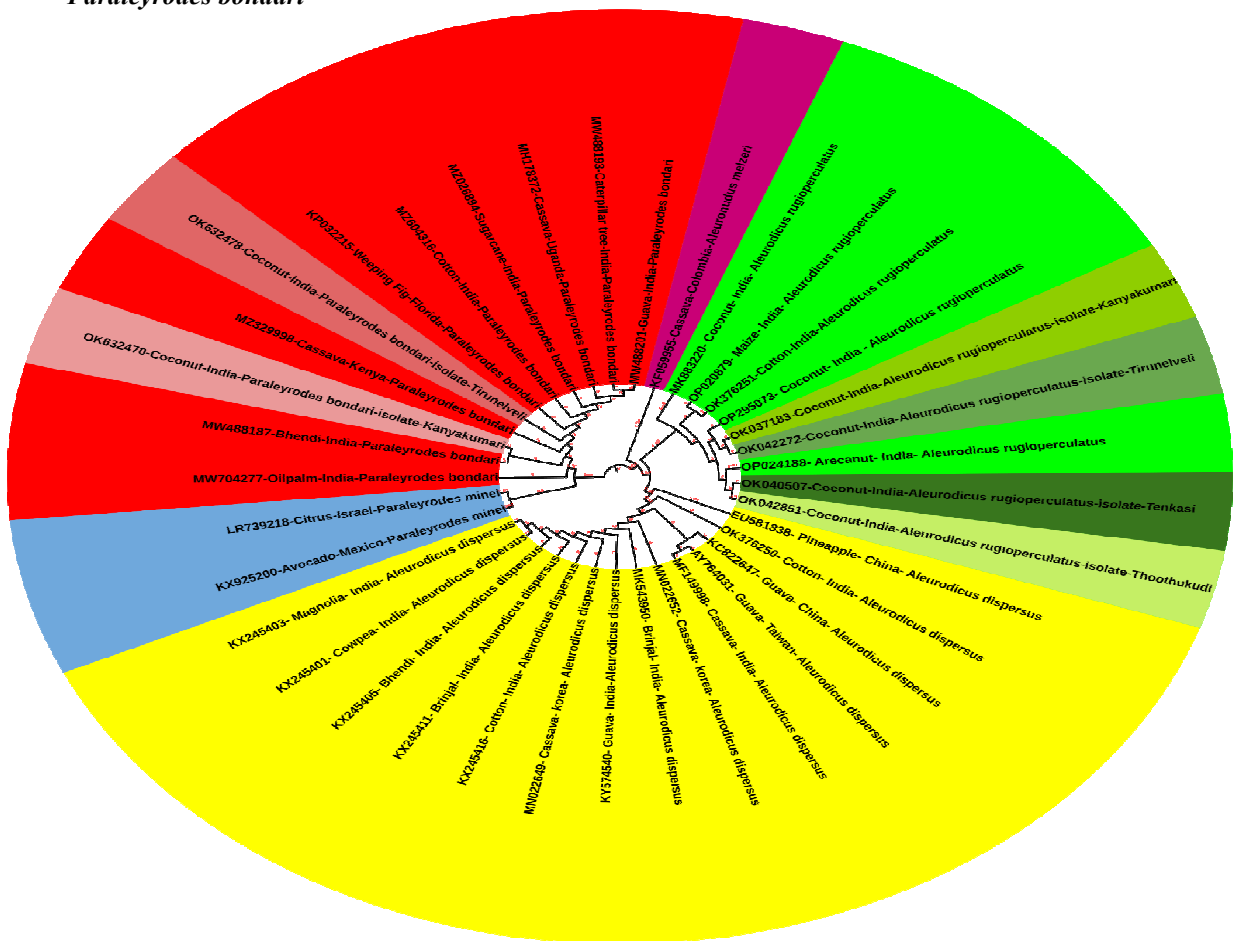


Fig 4. Phylogeny tree of rugose spiralling whitefly, *Aleurodicus rugioperculatus* and bondar’s nesting whitefly, *Paraleyrodes bondari*

- a. *A. rugioperculatus* (Kanyakumari) OK037183, b. *A. rugioperculatus* (Tenkasi) OK040507, c. *A. rugioperculatus* (Tirunelveli) OK042272, d. *A. rugioperculatus* (Thoothukudi) OK042851, e. *P. bondari* (Kanyakumari) OK632470, f. *P. bondari* (Tirunelveli) OK632478

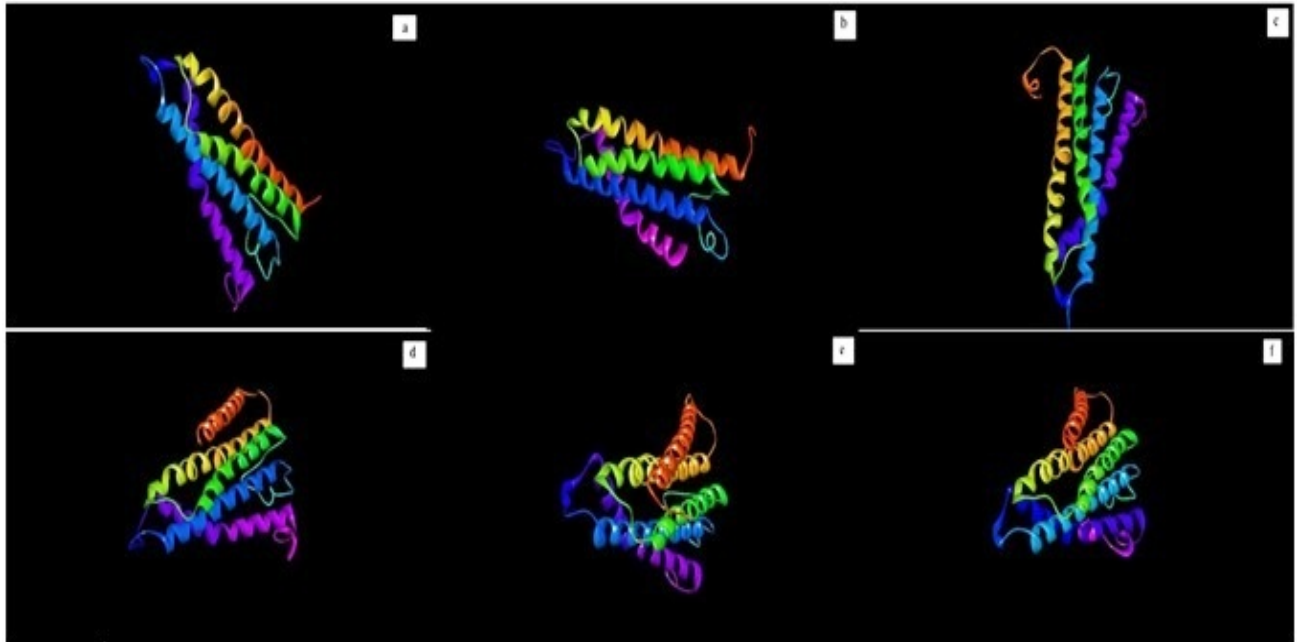
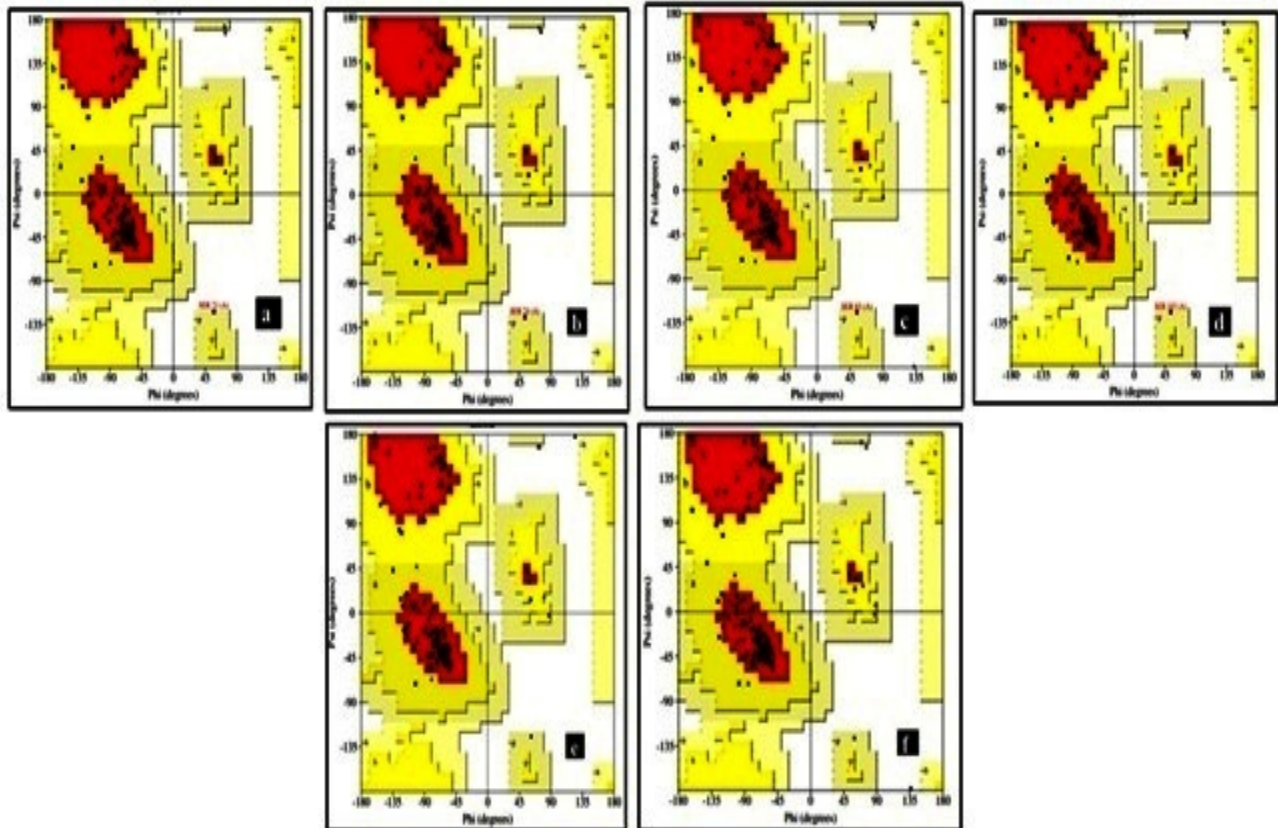


Fig 5. Three-dimensional structure of the target proteins from *A. rugiopectulatus* and *P. bondari*



a. *A. rugiopectulatus* (Kanyakumari) OK037183, b. *A. rugiopectulatus* (Tenkasi) OK040507, c. *A. rugiopectulatus* (Tirunelveli) OK042272, d. *A. rugiopectulatus* (Thoothukudi) OK042851, e. *P. bondari* (Kanyakumari) OK632470, f. *P. bondari* (Tirunelveli) OK632478

Fig 6. Ramachandran plot analysis of the *A. rugiopectulatus* and *P. bondari*, cytochrome c oxidase subunit I, partial protein target

DISCUSSION

Morphological characterization of *A. rugioperculatus* and *P. bondari*: Morphological characterization plays a crucial role in identifying insect species. In our study, *A. rugioperculatus* was distinguished by the presence of a rugose operculum with a triangular lingula, compound pores with dagger-like processes, smaller compound pores in the VII and VIII abdominal segments, and a single broader fluff at the posterior end of the nymphs. These characteristics align with descriptions provided by Martin (2004) and Srinivasan *et al.* (2016), confirming its identification. Additional studies by Saneera *et al.* (2023) and Selvaraj *et al.* (2017) further support these findings, detailing morphological features of *A. rugioperculatus* in India. Logeshkumar *et al.* (2024) and Singh *et al.* (2022) also documented similar diagnostic traits, including compound pores with dagger-shaped axial processes and a rugose operculum. Moreover, Veeramani *et al.* (2024) reported the first occurrence of *A. rugioperculatus* in the cotton ecosystem of Tamil Nadu, highlighting its distinct morphological traits. Jethva *et al.* (2020) and Madushani and Sirisena (2024) reported the first occurrence of *A. rugioperculatus* on coconut plantations in Gujarat, India. The eggs are smooth, elliptical, and translucent, laid in a spiral pattern under leaves and fruits, and covered in wax. The nymphal stage consists of five instars, with the first instar (crawler stage) being the only mobile form. As development progresses, nymphs become immobile, oval, and convex, forming dense clusters covered in wax. The puparium is distinguished by a quadrate operculum with a wrinkled ("rugose") texture and an apically acute lingula extending slightly short of the posterior margin. These traits confirm the identity of *A. rugioperculatus* across different life stages, aiding in accurate identification and management.

Similarly, *P. bondari* was identified based on distinct morphological traits. Martin (2004) described the presence of seven to eight stylized, flower-petal-shaped ovoid facets on the abdominal compound pores, a key distinguishing feature. Josephraj Kumar *et al.* (2019) noted variations in the number of discoidal pores across the last four abdominal compound pores, aiding in identification: the third compound pore contains two to three discoidal pores, the fourth has two, while the fifth and sixth each contain one. Vidya *et al.* (2019) emphasized the importance of these traits in differentiating *P. bondari* from other whitefly species. Our study confirms that the observed morphological characteristics of *A. rugioperculatus* and *P. bondari* align with previous literature, providing robust identification criteria for these whitefly species.

Molecular characterization of *A. rugioperculatus* and *P. bondari*: In our study, molecular identification revealed variations in amplicon sizes among different

geographic isolates from southern Tamil Nadu, with sizes of 529 bp in Kanyakumari, 569 bp in Tirunelveli, 629 bp in Thoothukudi, and 499 bp in Tenkasi. These differences may suggest local genetic diversity or regional adaptations. Our study closely aligned with the nucleotide sequence of *Aleurodicus rugioperculatus* (Rugose Spiraling Whitefly, RSW), which was deposited in the GenBank database under accession number OP295073. Comparative sequence analysis showed over 98% similarity with previously submitted sequences, including OP0295073, OP414609, and MK883219, indicating strong genetic relationships among different populations (Logeshkumar *et al.*, 2024). Veeramani *et al.* (2024) also confirmed a high level of genetic similarity among collected *A. rugioperculatus* specimens, supporting its molecular identification. The COI gene sequences obtained in this study exhibited 99.9% identity with an *A. rugioperculatus* sequence (KP032219) from Florida, USA (Dickey *et al.*, 2015). Furthermore, the DNA barcode generated showed 99.9% identity with sequences from coconut (*Cocos nucifera*), including MK883218, MK883219, MK883220, MK926750, and MK926751, as reported by Pradhan *et al.* (2021). These findings suggest a high degree of genetic uniformity of *A. rugioperculatus* across various host plants and geographic regions. Selvaraj *et al.* (2017) reported a COX-I gene sequence of 658 bp for *A. rugioperculatus*.

Our study identified amplicon sizes of 658 bp and 652 bp in isolates from Kanyakumari and Tirunelveli, confirming species identity while indicating slight geographic variations. The COX-I sequences of *P. bondari* from different regions exhibited high nucleotide similarity. Kenyan isolates showed a close match with Indian isolates from guava (MW488201) and coconut (MW488198), with similarities of 99.84% and 100%, respectively. A 632 bp COX-I sequence from Uganda (MH178372) displayed 100% similarity to a Florida isolate (KP032215), suggesting minimal genetic divergence. Similarly, *P. bondari* sequences from India (MK333262) demonstrated 100% similarity with those from Uganda and Florida, indicating genetic homogeneity across continents. The present study closely aligns with *P. bondari* partial mitochondrial COX-I sequences reported by Josephraj Kumar *et al.* (2019) and Vidya *et al.* (2019), which measured 675 bp and 621 bp, respectively. Sadhana *et al.* (2021) further confirmed this genetic consistency, with COX-I sequences from *P. bondari* on cotton (MZ604316) showing over 99.9% identity with previously deposited sequences. Specifically, their sequence had 100% query coverage and identity with a *P. bondari* COX-I barcode from Indian coconuts (MK343480), supporting species identification and genetic stability. Sankarganesh *et al.* (2025) analyzed mitochondrial COX-I gene variations in *A. rugioperculatus* across India, reporting amplified sequences ranging from 684 to 691 bp (GenBank:

ON739017, MT542036, and MK159741). The study revealed significant genetic variation influenced by geographic factors, highlighting the importance of ongoing molecular surveillance of this invasive whitefly.

Overall, the COX-I gene proves to be a reliable molecular marker for species identification and phylogenetic studies of *A. rugioperculatus* and *P. bondari*. The high genetic similarity among isolates from different regions suggests low differentiation within these species, supporting COX-I sequencing as a tool for global tracking and comparison of whitefly populations.

Conclusion: This study confirms the presence of two invasive whitefly species, *A. rugioperculatus* and *P. bondari*, on coconut leaflets in Tamil Nadu, posing a major threat to coconut cultivation. Combining taxonomic and molecular techniques enabled accurate identification, underlining the value of integrated approaches for monitoring. These polyphagous pests spread rapidly across various plant species, increasing the risk of widespread infestations. Severe outbreaks have already caused significant economic losses, highlighting the need for urgent management. An Integrated Pest Management (IPM) strategy featuring biological control agents, cultural practices, and botanical insecticides should be prioritized. Regular monitoring, early detection, and awareness among growers and stakeholders are essential. Collaborative action among researchers, policymakers, and farmers is key to developing sustainable control measures.

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Authors' contributions: SS, GP & VS- Wrote the manuscript. NB & JS- Designed the research study and helped with revisions of the article. All authors read and approved the final manuscript.

Data availability: The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: The study reviewed the occurrence of the pest in a region and does not use any human and animal subjects and connotes no ethics approval.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interest

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REFERENCES

- Abhishek, T.S and S.A. Dwivedi (2021). Review on integrated pest management of coconut crop. Int. J. Entomol. Res. 6: 115-120.
- Alagar, M., K. Rajamanikam, S. Chinnadurai, A. Yasmin and H.P. Maheswarappa (2020). Surveillance, assessment of infestation, biology, host range of an invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin and status of its natural enemies in Tamil Nadu. J. Entomol. Zool. Stud. 8(3): 2041-2047.
- Bossier, P., W. Xiaomei, F. Catania, S. Dooms, G. Van Stappen, E. Naessens and P. Sorgeloos (2004). An RFLP database for authentication of commercial cyst samples of the brine shrimp *Artemia* spp. (International Study on Artemia LXX). Aquaculture 231(1-4): 93-112. <https://doi.org/10.1016/j.aquaculture.2003.11.001>
- CDB (2022). Area, Production & Productivity of Coconut in India. Coconut Development Board. <http://www.coconutboard.nic.in/stat.html>
- Chandrika, M., A. Josephraj Kumar, H. Vinayaka, V. Krishnakumar, P.B. Renjith, A.S. Anjali and S. Chowdappa (2016). Gradient out break and bio-suppression of spiralling whitefly in coconut gardens in South India. Ind. Coconut J.59(8): 9-12.
- Dickey, A.M., I.C. Stocks, T. Smith, L. Osborne and C.L. McKenzie (2015). DNA barcode development for three recent exotic whitefly (Hemiptera: Aleyrodidae) invaders in Florida. Fla. Entomol. 98(2): 473-478. <https://doi.org/10.1653/024.098.0213>
- Dubey, A.K and R. Sundararaj (2015). A new combination and first record of the genus *Aleurothrixus* Quaintance and Baker (Hemiptera: Aleyrodidae) from India. Biosystematica. 9(1/2): 23-28.
- Dutta, N. K., D. Sarker, K. Begum, M.A. Sarker, M.I. Islam and M.M. Rahman (2019). First record of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* martin (hemiptera: Aleyrodidae) in Bangladesh with its host range and status as coconut pest. Bangladesh J. Entomol. 29 (2):73-83.
- Elango, K., S. Nelson, S. Sridharan, V. Paranidharan and S. Balakrishnan (2019). Biology, distribution and host range of new invasive pest of India coconut rugose spiralling whitefly *Aleurodicus rugioperculatus* Martin in Tamil Nadu and the status of its natural enemies. Int. J. Agric. Sci.11(9):8423-8426.
- Folmer, O., W.R. Hoeh, M.B. Black and R.C. Vrijenhoek (1994). Conserved primers for PCR amplification of mitochondrial DNA from

- different invertebrate phyla. *Mol Mar Biol Biotechnol.* 3(5):294-299.
- Jethva, D. M., P.S. Wadaskar and A.V. Kachot (2020). First report of rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) on coconut in Gujarat, India. *J. Entomol. Zool. Stud.* 8(2): 722-725.
- Josephraj Kumar, A., C. Mohan, M. Babu, A. Krishna, V. Krishnakumar, V. Hegde and P. Chowdappa (2019). First record of the invasive Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi on coconut from India. *Phytoparasitica.* 47(3): 333-339. <https://doi.org/10.1007/s12600-019-00741-2>
- Laskowski, R.A., M.W. MacArthur, D.S. Moss, J.M. Thornton and Procheck (1993). A program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr.* 26: 283-291. <https://doi.org/10.1107/S002188982009944>
- Logeshkumar, P., R. Nalini, A. Josephraj Kumar, P. Chandramani, M.L. Mini, R.D. Singh and M. Murugan (2024). Insights into the incidence, severity and morphometrics of the invasive Rugose Spiralling Whitefly *Aleurodicus rugioperculatus* Martin in coconut belts of Tamil Nadu, India. *Int. J. Trop. Insect Sci.* 44(6): 2783-2801. <https://doi.org/10.1007/s42690-024-01359-6>
- Madushani A, M. A and U.G.A.I. Sirisena A (2024). Identification of whitefly species (Hemiptera: Aleyrodidae) invaded coconut palms in Sri Lanka. *J. Trop. Crop Sci.* 11(1): 19-32. <https://doi.org/10.29244/jtcs.11.01.19-32>
- Martin, J. H (2004). Whiteflies of Belize (Hemiptera: Aleyrodidae). Part 1 introduction and account of the subfamily Aleurodicinae Quaintance & baker. *Zootaxa.* 681(1): 1-119. <https://doi.org/10.11646/zootaxa.681.1.1>
- Mohan, C., A. Josephraj Kumar, M. Babu, P.S. Prathibha, V. Krishnakumar, V. Hegde and P. Chowdappa (2017). Invasive rugose spiralling whitefly on coconut. *Technical Bulletin*, 117: 16.
- Pradhan, S.K., A.N. Shylesha, K. Selvaraj and B.V. Sumalatha (2021). Distribution, Host Range and status of Invasive Rugose Spiralling Whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) in Karnataka. *Agric. Res.* 11(3): 499-505. <https://doi.org/10.1007/s40003-021-00593-5>
- Sadhana, V., K. Senguttuvan, M. Murugan, N. Manikanda Boopathi and N. Sathiah (2021). First record of Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae), occurrence and infestation in the cotton ecosystem of Tamil Nadu, India. *J. Pharma Innovation.* 10(10S): 1278-1284.
- Saneera, E., S. Raguraman, M. Kannan, A. Josephraj Kumar and S. Jeyarani (2023). Microscopy based morphological characterization of rugose spiraling whitefly, (*Aleurodicus rugioperculatus* Martin) -an exotic pest on coconut in India. *Microsc. Res. Tech.* 86(5): 529-538. <https://doi.org/10.1002/jemt.24292>
- Sankarganesh, E (2025). Mitochondrial COI Gene Sequences Based Molecular Variation in Rugose Spiraling Whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) Species from Different Geographic Locations of India. *Adv Earth & Env Sci.* 5(4): 1-8.
- Selvaraj, K., Sundararaj, R., Venkatesan, T., Ballal, C. R., Jalali, S.K., Gupta, A., Mrudula, H.K (2017). Potential natural enemies of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin in India. *J. Biological Control.* 30(4): 236-239.
- Shanas, S., J. Job, T. Joseph and G. Anju Krishnan (2016). First report of the invasive rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) from the old world. *Entomon.* 41(4): 365-368. <https://doi.org/10.33307/entomon.v41i4.227>
- Singh, S., D. Samant, M.R. Sahoo, K. Kishore, D. Jinger and G.C. Acharya (2022). Invasion and escalation of *Aleurodicus rugioperculatus*: An alarming pest in east coast region of India. *Indian J. Agric. Sci.* 92(8): 1029-1032. [10.56093/ijas.v92i8.120353](https://doi.org/10.56093/ijas.v92i8.120353)
- Sirisena, U.G.A.I., G.W. Watson, K.S. Hemachandra and H.N.P. Wijayagunasekara (2013). A modified technique for the preparation of specimens of Sternorrhyncha for taxonomic studies. *Trop. Agric.* 24(2): 139 – 149.
- Srinivasan, T., P.A. Saravanan, A. Josephraj Kumar, S. Sridharan and P.M. David (2016). Invasion of the Rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera Aleyrodidae) in Pollachi tract of Tamil Nadu, India. *Madras Agric. J.* 103(10-12): 349-353.
- Stocks, I.C. and G. Hodges (2012). The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, a new exotic whitefly in South Florida (Hemiptera: Aleyrodidae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, *Aleurodicus rugioperculatus*, pest-alert. pdf (Accessed 7 March, 2017).
- Suriya, S., G. Preetha, N. Balakrishnan and J. Sheela (2023). Seasonal incidence, population dynamics and morphometric traits of exotic coconut whiteflies in southern Tamil Nadu. *J. Hortic. Sci.* 18(1):216-22. <https://doi.org/10.24154/jhs.v18i1.2167>

- Suriya, S., G. Preetha, N. Balakrishnan and J. Sheela (2024). Host Plants of Invasive Whiteflies-Rugose Spiralling Whitefly, *Aleurodicus rugioperculatus* Martin and Bondar's Nesting Whitefly, *Paraleyrodes bondari* Peracchi. Indian J. Ecol. 51(4): 883-890.<https://doi.org/10.55362/IJE/2024/4325>
- Thompson, J. D., D.G. Higgins and T.J. Gibson (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22): 4673-4680.<https://doi.org/10.1093/nar/22.22.4673>
- Veeramani, S., S. Kannan, M. Marimuthu, Y. Chandran, R. Thirumalaiyandi, C.N. Ravikumar and J. Mannu (2024). First record of rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae), its occurrence and infestation in the cotton ecosystem of Tamil Nadu, India. Int. J. Trop. Insect Sci. 44(2): 537-544.<https://doi.org/10.1007/s42690-024-01181-0>
- Vidya, C.V., R. Sundararaj, A.K. Dubey, H. Bhaskar, M. Chellappan and M.K. Henna (2019). Invasion and establishment of Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae) in Indian mainland and Andaman and Nicobar Islands. Entomon. 44(2): 149-154. <https://doi.org/10.33307/entomon.v44i2.443>
- Waterhouse, A., M. Bertoni, S. Bienert, G. Studer, G. Tauriello and R. Gumienny (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46 (W1):W296-W303.<https://doi.org/10.1093/nar/gky427>.