Morpho-molecular characterization and genetic diversity of the invasive rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) across agro-ecological zones of Bangladesh

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Abstract

The invasive rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) has emerged as a serious threat to coconut and other crops in Bangladesh. First recorded in 2019, the RSW has rapidly dispersed nationwide, however its details morpho-molecular characterization and genetic diversity have remained largely unexplored. The present study provides the first comprehensive nationwide morpho-molecular identification and genetic diversity analysis of RSW collected from infested coconut palms (*Cocos nucifera* L.) across all thirty agro-ecological zones (AEZs) of Bangladesh. Species identification was confirmed using morphological characteristics and DNA barcoding of the mitochondrial cytochrome c-oxidase subunit I (mtCOI) gene. Morphological examination confirmed key diagnostic features of *A. rugioperculatus*, including a convex puparium with a single straight tail-like projection and greyish-brown irregular bands on adult forewing, clearly distinguishing it from the morphologically similar species, *A. disperses.* Analysis of 615 bp of the mtCOI gene from thirty individuals (one per AEZ) revealed complete genetic uniformity across all sampled locations. A single haplotype was detected ($h = 1$) with zero haplotype diversity ($Hd = 0$), zero nucleotide diversity ($\pi = 0$) and no segregating site ($S = 0$). The nucleotide composition showed a strong AT bias (76.1%) with negative AT-skew (-0.1966). Phylogenetic analysis using the Maximum Likelihood method placed all Bangladeshi isolates within a single monophyletic clade with strong bootstrap support, clustering closely with invasive lineages reported from the India and the United States. The absence of detectable genetic variation across all sampled agro-ecological zones strongly suggests a recent founder event, likely involving a single introduction followed by rapid clonal expansion. The findings of this study—particularly the complete genetic homogeneity observed across all AEZs, provide important baseline information for the development of region-specific monitoring programs and predictive outbreak models under changing climatic conditions, and support the implementation of precise and targeted integrated pest management (IPM) strategies.

Introduction

Whiteflies (Hemiptera: Aleyrodidae) are tiny, polyphagous sap-feeding insects that pose a persistent global threat to agricultural productivity through direct feeding, the excretion of honeydew that promotes sooty mold on leaves, and the transmission of economically important plant diseases (David, 2012; Sani et al. 2020). Over the past few years, at least three invasive whitefly species- rugose spiralling whitefly (RSW) (*Aleurodicus rugioperculatus* Martin), Bondar's nesting whitefly (BNW) (*Paraleyrodes bondari* Peracchi), and nesting whitefly (NW) (*Paraleyrodes pseudonaranjae* Martin) have been recorded in Bangladesh (Dutta et al, 2019; Das et al. 2026). Among them, the RSW has emerged as a serious threat to agricultural systems in tropical and subtropical regions worldwide due to its polyphagous nature. Native to Belize and other parts of Central America and the Caribbean (Martin, 2004), this species has rapidly expanded its geographic range over the past two decades, establishing invasive populations in Florida, the USA, in 2009 (Stocks & Hodges, 2012), India in 2016 (Shanas et al. 2016), and subsequently in several Southeast Asian countries (Josephrajkumar et al. 2020). The pest's high reproductive potential, broad host range encompassing over 118 plant species across 43 families and ability to cause

direct feeding damage as well as indirect damage through sooty mold formation have contributed to its successful invasion and economic impact (Kumar et al. 2013; Francis et al. 2016; Sani et al. 2020).

In Bangladesh, the RSW was first recorded in 2019 on coconut palms in the southwestern region (Dutta et al. 2019). Since then, the pest has rapidly dispersed across the country, causing severe infestation in coconut-growing areas (Khan, 2022; Das et al. 2023). Coconut is a cash crop in Bangladesh, supporting rural livelihoods, providing food security and contributing significantly to the national economy. Bangladesh ranks 12th globally in coconut production, with over 442,000 metric tons produced annually from approximately 25,000 hectares (FAOSTAT, 2022; BBS, 2021). The rapid spread of RSW therefore poses a substantial threat to the sustainability of coconut cultivation and the livelihoods that depend upon it.

Accurate species identification is a critical first step in understanding invasion dynamics and developing effective pest management strategies. Although, the RSW was initially recorded in Bangladesh in 2019, the identification was based on preliminary observations without detailed morphological description or molecular validation (Dutta et al. 2019), whitefly taxonomy is notoriously complex due to phenotypic plasticity, cryptic species diversity and presence of morphologically similar congeners that frequently co-occur on the same host plants (Shanas et al. 2016; Josephrajkumar et al. 2019; Josephrajkumar et al. 2020; Saneera et al. 2023; Selvaraj et al. 2024; Das et al. 2026). For instance, *Aleurodicus dispersus* (spiraling whitefly), which shares many morphological features similar to *A. rugioperculatus*, is also present in Bangladesh and infests common host plants. Therefore, reliance only on morphological traits alone may therefore lead to misidentification, hampering accurate risk assessment and devising effective species-specific management interventions. These challenges underscore the necessity of integrating molecular approaches to achieve accurate species identification which is critical for understanding ecological adaptation, invasion dynamics, and designing effective pest management strategies across diverse agro-ecological zones.

DNA barcoding based on mitochondrial cytochrome c oxidase subunit I (mtCOI) gene sequencing has emerged as a powerful tool for resolving taxonomic ambiguities, confirming species identity, and characterizing genetic diversity in many invasive insect populations (Hebert et al. 2003, 2004). Beyond species, mtCOI barcoding enables the assessment of population genetic structure, the inference of invasion pathways, and the detection of founder effects—all of which are critical for understanding the evolutionary dynamics of invasive species (Ghosh et al. 2017; Firake et al. 2021; Prabhulinga et al. 2021). Despite the economic importance of RSW in Bangladesh, no molecular characterization or population genetic assessment of this invasive pest has been conducted to date, representing a significant knowledge gap.

Although a small country, Bangladesh is characterized by 30 distinct agro-ecological zones (AEZs), each with unique climatic, edaphic, and agronomic features that may influence pest population dynamics and dispersal patterns. The extent of genetic variation among the populations of A. rugioperculatus across Bangladesh remains unknown. Thus, understanding the genetic diversity and population structure of *A.*

rugioperculatus from the whole country will be helpful in predicting future spread, identifying potential source populations, and designing region-specific management strategies.

The present study was therefore undertaken (i) to confirm the species identity of *A. rugioperculatus* in Bangladesh through integrated morphological and molecular (mtCOI) approaches; ii) to assess the genetic diversity and population structure of RSW across 30 AEZs of Bangladesh, and iii) to elucidate their phylogenetic relationships between Bangladeshi populations and global invasive lineages. We hypothesized that, given the recent invasion of RSW in Bangladesh, populations would exhibit low genetic diversity consistent with a founder effect, and that all Bangladeshi isolates would cluster closely with invasive populations from neighboring countries. The findings of this study provide the first nationwide morpho-molecular characterization and genetic assessment of RSW in Bangladesh, establishing a critical baseline for future monitoring and supporting the development of sustainable, targeted pest management strategies to protect coconut production and rural livelihoods.

Materials and methods

Sample collection

Whitefly samples were collected from coconut palms (*Cocos nucifera* L.) across 30 agro-ecological zones (AEZs) of Bangladesh during June 2024 to May 2025 (Fig. 1 & Table S1). At each sampling location, five coconut palms were selected based on the presence of characteristic egg spirals, puparia and adult whitefly infestations. For morpho-molecular analyses, adult whiteflies were collected using two distinct approaches: (i) individuals designated for morphological examination were preserved in dry Falcon tubes (n = 5 per palm; 25 per location), and (ii) individuals intended for molecular analysis were immediately preserved in 95% ethanol (n = 5 per palm; 25 per location). Additionally, egg spirals and puparia were collected from the same host plants and stored in dry Falcon tubes for further examination. All samples were promptly placed in portable cool boxes containing ice packs to maintain sample integrity during transportation and were subsequently carried to the laboratory of the Department of Entomology, Bangladesh Agricultural University. Dry-preserved specimens were stored at 4°C for morphological examination whereas ethanol-preserved specimens were maintained at − 20°C until DNA extraction. The same standardized sampling protocol was consistently followed across all AEZs.

Morphological characterization

Key morphological traits, including egg laying pattern, eggs and puparial characteristics and adult morphological features of the collected whiteflies were examined using a zoom stereomicroscope (SM-1TS-144S-10M, AmScope, USA). Diagnostic identification of rugose spiralling whitefly was based on the characteristic spiraling oviposition patterns accompanied by dense cottony wax deposits, distinct puparial morphology and the presence of irregular greyish-brown bands on the forewings of adults. These features were considered critical for distinguishing *Aleurodicus rugioperculatus* from other

closely related whitefly species, following the taxonomic descriptions of Martin (2004) and Shanas et al. (2016).

Molecular characterization

DNA extraction, PCR amplifications and gel electrophoresis

For molecular analysis, ethanol-preserved specimens (n = 90; 3 specimens per AEZ) were subjected to DNA extraction and PCR amplification following prior morphological confirmation as rugose spiralling whitefly examined under a zoom stereomicroscope. Genomic DNA was extracted from adults of RSW collected from the 30 AEZs of Bangladesh using the FavorPrep^{TM}Tissue Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) in accordance with the manufacturer's directions and the DNA concentration was measured through a nanodrop spectrophotometer. A fragment of approximately 700bp of the mitochondrial cytochrome c-oxidase subunit I (mtCOI) gene was amplified by utilizing the universal primers (LCO1490, 5'-ggtcaacaaatcataaagatattgg-3' and HC02198: 5'- taaacttcagggtgaccaaaaaatca-3' (Folmer et al. 1994). PCR amplification was carried out following the thermal cycling conditions described by Das et al (2026). All PCR amplifications produced distinct amplicons of approximately 700 bp, as verified by agarose gel electrophoresis. From these, thirty representative PCR products (n = 30, 1 per AEZ) were selected for DNA sequencing to confirm species identity. Voucher specimens were deposited in the Central Insect Museum of the Department of Entomology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, for future references.

Sequence processing, identification, phylogenetic analysis and genetic diversity

Amplicons were purified using the FavorPrep™ GEL/PCR Purification Kit and submitted to Macrogen (Seoul, South Korea) for sequencing. The resulting sequence chromatograms were meticulously scrutinized and manually edited using FinchTV software v1.4.0 (Geospiza Inc.) to generate high-quality sequence reads. Taxonomic identification was carried out by querying the sequence against the GenBank database using the BLAST (http://www.ncbi.nlm.nih.gov). According to BLAST homology results using COI gene fragments, whitefly species were identified. For a comparative phylogeographic study, reference COI sequences of the *A. rugioperculatus* from other countries were retrieved from GenBank (Table 1). Following multiple sequence alignments, evolutionary relationships were inferred using the maximum likelihood algorithm based on the Kimura 2-parameter model, and a phylogenetic tree was constructed using 1000 bootstrap replicates in MEGA 12.

Multiple sequence alignment was performed using the ClustalW algorithm implemented in the *msa* package (Bodenhofer et al. 2015) in R v4.3.0. The alignment was trimmed to a common overlap region of 615 bp to ensure data consistency across all samples. Reference COI sequences of *A. rugioperculatus from* other countries and the outgroup *Paraleyrodes bondari* (PV596240.1) were

retrieved from GenBank (Table 1). Evolutionary relationships were inferred using the maximum likelihood (ML) algorithm based on the Tamura-Nei (1993) model with 1000 bootstrap replicates, implemented in MEGA 12 (Kumar et al. 2016; Tamura et al. 2021). Initial trees were obtained via Neighbor-Joining and Maximum Parsimony, with the tree having the superior log-likelihood selected for the final analysis.

Genetic diversity indices, including the number of haplotypes (h), haplotype diversity (*Hd*), nucleotide diversity (π), and segregating sites (*S*), were calculated using the *pegas* package (Paradis, 2010) in R. Nucleotide composition (AT content and AT-skew) was characterized from the aligned sequences. Kimura 2-parameter (K2P) genetic distances were computed using the *ape* package (Paradis & Schliep, 2019) for comparison with global reference sequences. A haplotype network was constructed using the TCS method in *pegas* to visualize relationships among haplotypes (Paradis, 2010).

Table 1
List of nucleotide sequences of the COI gene retrieved from GenBank to construct a phylogenetic tree

No.	NCBI Accession Number	Species	Location
1–30	PV612625.1–PV612652.1 PX397040.1–PX397041.1.	*Aleurodicus rugioperculatus*	Bangladesh (This study)
31	KP032219.1	*Aleurodicus rugioperculatus*	USA (Florida)
32	KY574533.1	*Aleurodicus rugioperculatus*	India (Kerala)
33	MK159738.1	*Aleurodicus rugioperculatus*	India (Karnataka)
34	OQ835715.1	*Aleurodicus rugioperculatus*	India (West Bengal)
35	PV596240.1	*Paraleyrodes bondari*	Bangladesh (Outgroup)

Results

Morphological identification

Morphological examination of egg, puparium and adult confirmed the identity of the collected specimens as *Aleurodicus rugioperculatus.* Comparison with the morphologically similar species *Aleurodicus dispersus* (spiralling whitefly) revealed key diagnostic features distinguishing the two taxa (Fig. 3; Table 2). The puparium of the *A. rugioperculatus* was convex oval, with a single, straight, and prominent tail-like projection extending beyond the operculum (Fig. 2C, Fig. 3C and Table 2), whereas *A. dispersus* possesses a convex puparium with two prominent tail-like projections (Fig. 3F, Table 2). The adults were white, robust, measuring 2.3–2.5 mm in body length, with characteristic greyish-brown mottling or irregular bands on the forewings (Fig. 2D, 3A). By comparison, *A. dispersus* adults are smaller (1.8–2.3 mm), slender, uniformly white, and lack wing markings (Fig. 3D, Table 2). The eggs of both

species are smooth, stalkless, elliptical, and creamy white to yellow. Both species deposit eggs in characteristic spiral patterns covered with dense white cottony wax masses. No consistent differences in egg morphology were observed between the two species (Fig. 2A& 2B, 3B and 3E). These morphological characters reliably distinguish *A. rugioperculatus* from its sympatric congener *A. dispersus.*

Table 2
Comparative morphological features of *A. rugioperculatus* and *A. dispersus*

Features	A. *rugioperculatus*	A. *dispersus*
Adult size	2.3–2.5 mm	1.8–2.3 mm
Markings on wings	Irregular greyish-brown bands on both of the forewings	Pure white; forewings without markings
Egg laying pattern	Spiral structure with dense white waxy masses	Spiral structure with dense white waxy masses
Puparium	Convex and oval, with a single prominent tail-like projection	Convex with two prominent tail-like projections

Genetic diversity and population structure

A fragment of approximately 700 bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene was successfully amplified from all whitefly samples collected across the 30 AEZs of Bangladesh. Following alignment and trimming, a final dataset of 615 bp was generated for each of the 30 samples. Sequence analysis revealed complete genetic monomorphism across the nationwide survey. All 30 Bangladeshi isolates shared 100% nucleotide identity, representing a single haplotype (h = 1). Consequently, haplotype diversity (Hd = 0.000), nucleotide diversity (π = 0.000000), and segregating sites (S = 0) were all zero (Table 3). Nucleotide composition analysis showed a strong AT bias (76.1%), with Thymine (45.5%) and Adenine (30.6%) being the most abundant bases. The AT-skew was − 0.1966, indicating a slight bias toward Thymine (Table 3).

Table 3
Summary of genetic diversity parameters for *A. rugioperculatus* across 30 agro-ecological zones in Bangladesh

Parameter	Value
Number of sequences (n)	30
Agro-ecological zones sampled	30
Alignment length (bp)	615
Number of haplotypes (h)	1
Haplotype diversity (*Hd*)	0.000
Nucleotide diversity (π)	0.000000
Segregating sites (*S*)	0
AT content (%)	76.1
AT-skew	−0.1966

Molecular confirmation and phylogenetic analysis

BLAST analysis confirmed species identity, with all isolates showing 100% sequence similarity to previously reported *A. rugioperculatus* sequences. A total of thirty sequences generated in this study were deposited in GenBank under accession numbers PV612625.1–PV612652.1 and PX397040.1–PX397041.1. Phylogenetic analysis using the maximum likelihood method (Fig. 4) demonstrated that all 30 Bangladeshi isolates formed a single monophyletic clade with 79% bootstrap support, clustering with reference sequences from the USA (Florida) and India (Kerala, Karnataka and West Bengal). The Tamura-Nei genetic distance matrix (Table S2) revealed 0% divergence among all *A. rugioperculatus* sequences, confirming genetic homogeneity across all geographic regions. The outgroup *Paraleyrodes bondari* (PV596240.1) showed approximately 23.07% divergence from the *A. rugioperculatus* clade, clearly separating the two genera. The TCS haplotype network (Fig. S1) further supported these findings, collapsing all 30 Bangladeshi isolates together with the USA and Indian reference sequences into a single dominant node (H1), with no mutational steps separating these populations. This network configuration provides additional evidence for a recent shared ancestry and clonal expansion across the invaded range.

Discussion

The RSW was first recorded in Bangladesh on coconut palm in 2019 based on preliminary morphological observation, which lacked detailed diagnostic characterization and were not validated by molecular analysis (Dutta et al. 2019). Since then, the species has rapidly dispersed throughout Bangladesh and infested a wide range of host plants (Khan, 2022; Das et al. 2023). The present study therefore, provides

the first nationwide morpho-molecular characterization and genetic assessment of RSW across all 30 agro-ecological zones (AEZs) of Bangladesh. By integrating classical taxonomy with mitochondrial DNA barcoding, this study establishes a baseline for an invasive species that has rapidly transitioned from a localized introduction to a nationwide agricultural threat within a five-year period.

The convergence of morphological markers specifically the unique single, straight puparial tail-like projection and the characteristic greyish-brown forewing banding (Shanas et al. 2016) with 100% mtCOI gene sequence identity provides a robust diagnosis of *A. rugioperculatus*. In the South Asian context, where the morphologically similar *A. dispersus* frequently co-occurs on the same host plants (e.g., *Cocos nucifera* L), such dual-layered validation is essential (Josephrajkumar et al. 2020; Das et al. 2026). This integrated approach helps resolve taxonomic ambiguity, which often constrains the effective deployment of species-specific biological control agents such as the parasitoid *Encarsia guadeloupae*, known for its high host specificity (Josephrajkumar et al. 2020; Kolanthasamy et al. 2023).

The most notable finding of this study is the absence of detectable genetic variation ($Hd = 0.000$; $\pi = 0.000$) among the 30 sampled individuals. Although the sampling design (one individual per AEZ) limits the assessment of within-population variation, the 100% sequence identity across a 615 bp mtCOI fragment over a broad geographic range (~ 500 km) indicates a high degree of genetic similarity and suggests clonal expansion of the maternal lineage. This pattern is consistent with a founder event, potentially originating from a small propagule (Nei et al. 1975). The observed identity with sequences from West Bengal (India) and Florida (USA) indicates a shared invasive lineage. However, the absence of representative sequences from the native range (Central America/Caribbean) limits definitive inference regarding the invasion pathway (Martin, 2004; Chandrika Mohan et al. 2018). The lack of segregating sites, despite the rapid life cycle of whiteflies (Saranya et al. 2021), further suggests that expansion has occurred rapidly enough that detectable mitochondrial mutations have not accumulated (Brower, 1994).

The transition from a southwestern occurrence in 2019 (Dutta et al. 2019) to a widespread nationwide presence by 2024–2025 represents a rapid expansion. While wind-assisted flight likely contributes to local spread (Elango et al. 2020; Khan, 2022), the geographic ubiquity documented here is consistent with human-mediated dispersal (Sushil et al. 2023), a phenomenon also documented in India (Pradhan et al. 2020; Selvaraj et al. 2024). The internal trade of infested coconut seedlings and ornamental plants may have acted as a primary carrier for the pest's "leap-frog" movement across AEZs (Das et al. 2023). This finding highlights a potential vulnerability in domestic phytosanitary surveillance and suggests the need for stricter regulation of host plant movement.

The observed genetic homogeneity has important implications for pest management. On one hand, the lack of detectable population structure suggests that biological control strategies may be broadly effective nationwide. Natural enemies such as *Encarsia guadeloupae* and *Isaria fumosorosea* (Saranya et al. 2021; Sandeep et al. 2022), if effective in one region, are likely to perform similarly across other AEZs. On the other hand, this uniformity also presents risks. If insecticide resistance evolves in any population, the absence of genetic barriers could facilitate rapid spread of resistance alleles throughout

the country (Raymond et al. 1991; Chakim & Dewi Kristini, 2024). These findings emphasize the importance of implementing integrated pest management (IPM) strategies that prioritize biological control and reduce reliance on chemical pesticides.

Several limitations should be acknowledged. The mtCOI marker reflects only maternal lineage and does not capture nuclear genetic variation or recombination (Hebert et al. 2003; Dong et al., 2021; Sankarganesh et al. 2025). In addition, sampling a single individual per AEZ limits inference about within-population diversity. The observed AT-rich composition (76.1%) and negative AT-skew are typical of insect mitochondrial genomes (Cameron et al. 2014) but do not provide insight into adaptive variation (Kumar et al. 2022; Wei et al. 2010). Future research employing high-resolution genomic tools, such as single nucleotide polymorphisms (SNPs) or whole-genome sequencing (WGS), is needed to determine whether adaptive variation exists beyond mitochondrial uniformity (Muliyar Krishna et al. 2025). Expanded sampling across time and host plants, including banana and guava (Rao et al. 2018; Khan, 2022; Das et al. 2023), will further improve understanding of the invasion dynamics and evolutionary trajectory of this species.

In conclusion, the RSW has successfully colonized all sampled AEZs of Bangladesh, with no detectable mtCOI variation among the sampled individuals. These findings are consistent with a recent founder event followed by rapid geographic expansion. This study establishes a critical baseline for national biosecurity and supports the development of targeted, sustainable management strategies to mitigate the impact of this invasive pest.

Declarations

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Authorship contribution: MSR: Sample collection, morphology studyand manuscript revision; **GD:** Fund acquisition, study conception and design, morphological study, interpretation of results, final manuscript preparation; **MTTH:** Molecular study and manuscript revision; **MMU**: Manuscript revision; **KMGD**: Genetic diversity analysis, graph preparation, manuscript revision;

MMR: Molecular study.

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Figures

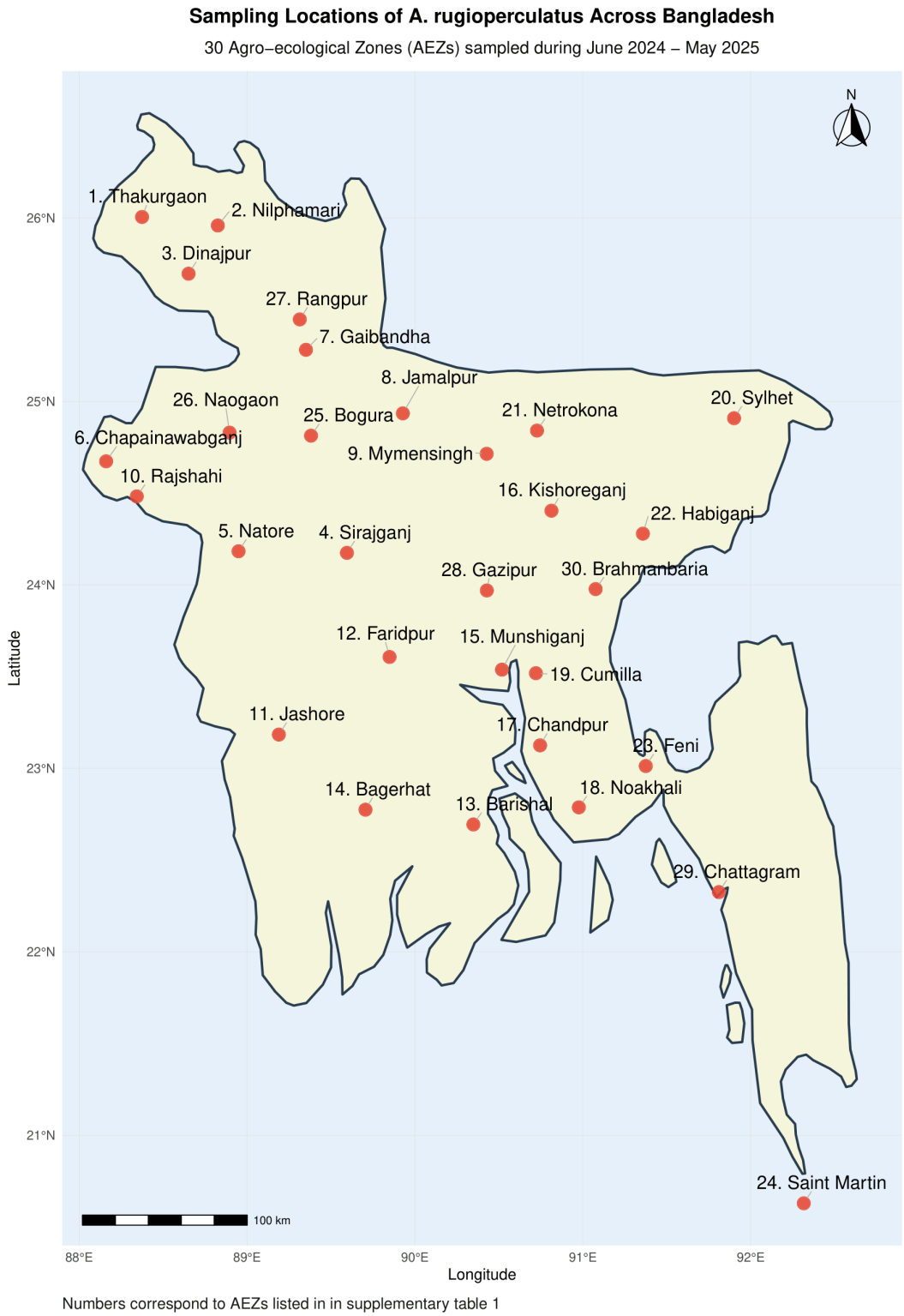

Figure 1

Sampling locations of *A. rugioperculatus* across 30 agro-ecological zones (AEZs) in Bangladesh. Samples were collected from coconut palms (*Cocos nucifera* L.) during June 2024 to May 2025. Numbers correspond to AEZs listed in Supplementary Table 1, followed by district names. Red circles indicate sampling sites.

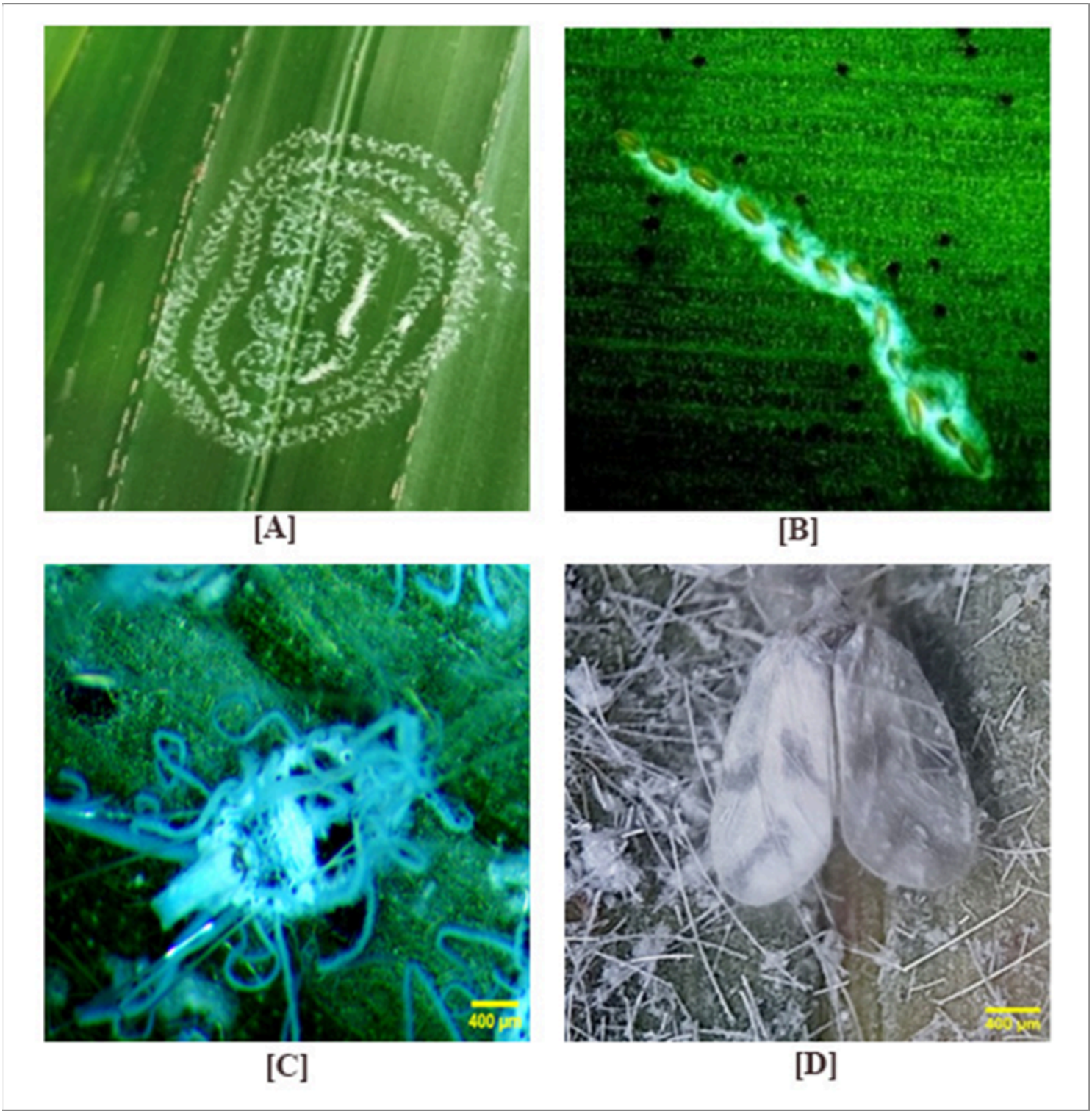

Figure 2

Morphological characteristics of *A. rugioperculatus*. (A) Egg spiral on coconut leaf, (B) Eggs embedded in cottony wax masses, (C) Convex puparium with a single prominent tail-like projection, and (D) Adult with greyish-brown irregular bands on the forewings.

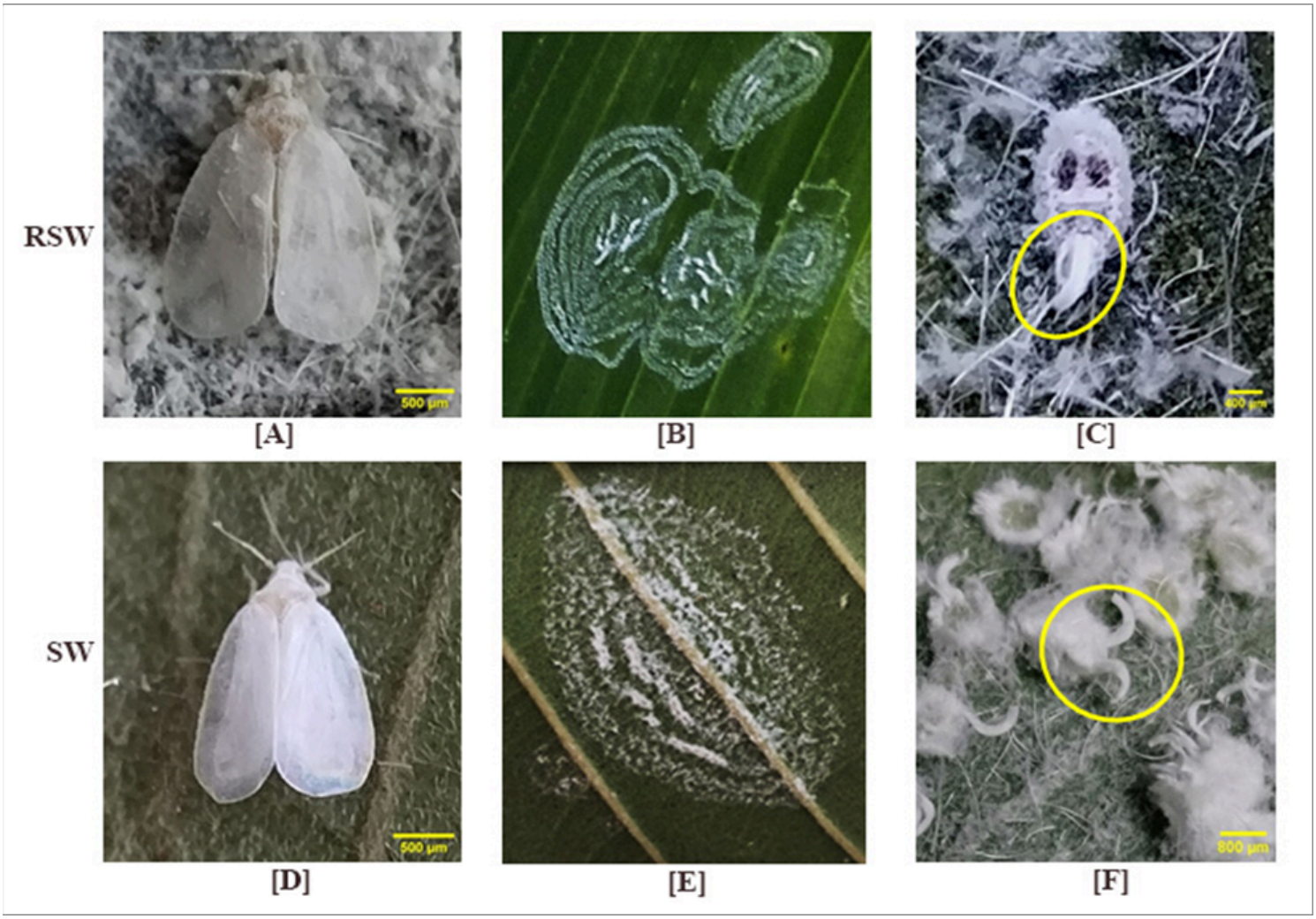

Figure 3

Comparative morphology of rugose spiralling whitefly (RSW, *Aleurodicus rugioperculatus*) and spiralling whitefly (SW, *Aleurodicus dispersus*). RSW (Top row: A-C): (A) Adult with greyish-brown mottling and irregular bands on the forewings; (B) Egg spiral and eggs are embedded in cottony wax masses; (C) Puparium with a single tail-like projection (yellow circle). SW (Bottom row: D-F): (D) Adult pure white without markings on the forewings; (E) Egg spiral and eggs are embedded in cottony masses; (F) Puparium with two tail-like projections (yellow circle).

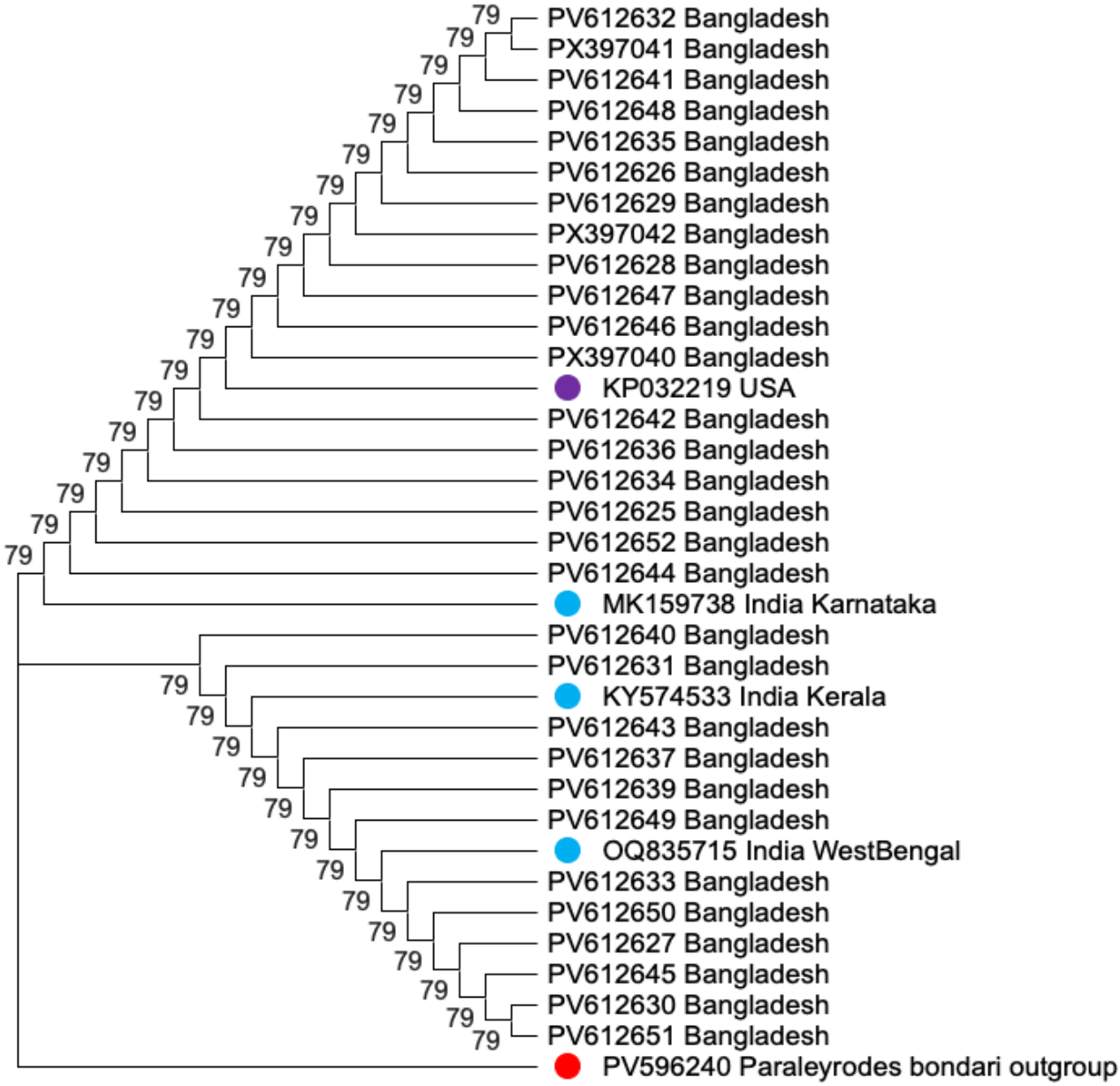

Figure 4

Maximum likelihood phylogenetic tree of *A. rugioperculatus* based on 615 bp of the mitochondrial COI gene. The tree was constructed using the Maximum Likelihood method with 1,000 bootstrap replicates in MEGA12 [4.5]. Bootstrap support values (≥50%) are shown at nodes. The Tamura-Nei (1993) model [3] was used for distance calculation, with initial trees obtained via Neighbor-Joining and Maximum Parsimony. The analysis included 35 sequences (615 bp) and was rooted with *Paraleyrodes bondari* (PV596240_Outgroup_Pbondari) as outgroup. Labels indicate accession numbers and geographic origin.

Supplementary Files

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- Supplementarymaterials.docx