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RESEARCH ARTICLE

Assessment of Mealybug (Hemiptera: Pseudococcidae) Species Diversity in Different Host Plants from Selected Locations in Sri Lanka Using Morphological and Molecular Methods

K.W.I. Sathsarani, S. Terensan*, and K. Gajapathy

Highlights

- Ten mealybug species were identified across three districts in Sri Lanka using morphological and molecular methods
- Some mealybug species infested multiple crops, showing a broad host range
- *Phenacoccus solani* and *Phenacoccus manihoti* were recorded in Sri Lanka for the first time, expanding the known diversity
- Phylogenetic analysis confirmed species identification and revealed distinct evolutionary relationships among genera

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Assessment of Mealybug (Hemiptera: Pseudococcidae) Species Diversity in Different Host Plants from Selected Locations in Sri Lanka Using Morphological and Molecular Methods

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Abstract

Mealybugs are economically significant, sap-sucking insects causing substantial damage to crops. Although some studies exist, few have combined classical morphological and molecular approaches with an assessment of their economic significance. This study aimed to identify mealybug species based on morphology, analyze their host preference, and confirm the identification using DNA markers. Mealybug samples were collected from randomly selected fields from Kilinochchi, Jaffna, and Monaragala districts in Sri Lanka. Morphological identification was done using taxonomic keys. PCR amplification was performed for the D3 region of the 28S rDNA. A phylogenetic tree was constructed using the Maximum-Likelihood method with the Kimura 2-parameter model and the Gamma distribution. Ten mealybug species, representing seven genera, were identified from different districts and host plants, including *Phenacoccus solenopsis*, *Ferrisia virgata, Coccidohystrix insolita, Planococcus minor, Phenacoccus solani, Planococcus lilacinus, Phenacoccus manihoti, Paracoccus marginatus, Pseudococcus viburni, and Rastrococcus mangiferae*. Notably, *P. solenopsis, F. virgata, P. minor, P. lilacinus,* and *P. marginatus* were found to infest multiple host plant species, indicating their broader host preference. *P. solani* and *P. manihoti* are reported for the first time from Sri Lanka. Molecular data validated the morphological identification, and phylogenetic analysis revealed distinct clades representing separate genera exhibiting monophyletic and paraphyletic relationships among them. Field observations indicated a frequent association between mealybugs and ants, where *Oecophylla smaragdina* and *Technomyrmex albipes* were observed on *P. lilacinus*-infested guava trees. Furthermore, natural enemies of mealybugs, such as mealybug destroyers, were observed in 20% (n=6) of the fields. This comprehensive study provides valuable insights into mealybug species diversity, host preference, and insect associations, which are critical for developing effective integrated pest management strategies tailored for specific regions and host plants.

Keywords: Insect pest; Host preference; Insect associations; DNA markers; Phylogenetic analysis

INTRODUCTION

Mealybugs (Hemiptera: Pesudococcidae) are small insects (Pacheco da Silva et al*.,* 2014), exhibiting a wide-ranging feeding proclivity encompassing vegetables, fruits, and ornamental plants (Muniappan et al., 2009). These sedentary insects inflict substantial damage on plants by deliberately extracting plant fluids and secreting honeydew. The latter serves as a substrate for the development of sooty mold, resulting in the formation of a black, cottony wax on plant surfaces (McKenzie, 2023). Additionally, these are the vectors for viral diseases (Sether et al., 1998; Sforza et al., 2003). Often, they are associated with different species of ants, where ants consume honeydew secreted by mealybugs, and they suppress the natural enemy of mealy bugs (Sether et al., 1998).

Traditionally, chemical management has been the predominant approach for mealybug control. However, the frequent utilization of insecticides has led to adverse consequences for the natural enemies of mealybugs, particularly when employing broad-spectrum insecticides. Furthermore, the emergence of insecticide resistance has compromised the efficacy of certain chemical treatments. Concerns pertaining to low selectivity and human toxicity have resulted in diminished acceptance and usage of specific insecticides, prompting numerous countries to adopt sustainable pesticide use programmes and regulations

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(Franco et al., 2016). Despite the apparent inefficacy of chemical insecticides, their presence is detectable within the barks, fruits, and bases of leaf petioles, as mealybug stages are typically ensconced in a waxy coating that obstructs the reach of pesticides (Mani et al., 2011). This confluence of factors poses a considerable economic threat to farmers, necessitating specialized management approaches. To achieve this goal, it is imperative to undertake a comprehensive study of insect diversity, a factor that exhibits considerable variation across different geographical regions.

The extensive taxonomic diversity is underscored by the presence of over 2000 known species distributed across approximately 290 genera, solidifying mealybugs as the second-largest family within the Superfamily: Coccoidea. In Sri Lanka, a total of 38 mealybug species have been reported based on the morphological parameters (Prishanthini & Vinobaba, 2009). However, species identification presents a formidable challenge due to the morphological similarities observed in both adult and nymphal stages (Hosseini & Hajizadeh, 2011; Abd-Rabou et al., 2012). Despite external resemblances, each mealybug species exhibits distinctive biological and physical characteristics. In practice, identification often relies on female adults due to the rarity of male specimens (Sether et al., 1998). As a response to the challenges associated with morphological identification, molecular species identification is gaining prominence in diagnostic and ecological studies, especially for insects, where morphological identification proves arduous or time-consuming (Saccaggi et al., 2008). Numerous studies have employed DNA markers to accurately discern mealybug species originating from different geographical regions (Pieterse et al., 2010; Abd-Rabou et al., 2012; Palma-Jiménez et al., 2016; Dewer et al., 2018).

Therefore, it is crucial to conduct a holistic examination of the ecosystem of the mealybugs, encompassing hosts, species, and natural enemies, as this comprehensive approach is instrumental in formulating a management plan tailored to the needs and characteristics of specific regions. This study was conducted to assess the diversity and damage patterns of mealybugs on different host plants in selected districts of Sri Lanka, using both morphological and molecular approaches.

MATERIALS AND METHODS

Sample collection

Samples of mealybugs were collected from 27 randomly selected locations across fields in the dry zone districts, such as Kilinochchi, Jaffna, and Monaragala districts of Sri Lanka. Each location was chosen based on the abundance of mealybugs and the immediate visibility of the damage, with each sampling site containing a minimum of 20 and a maximum of 72 mealybugs on average. During the collection process, samples were taken from the aerial parts of host plants, and all the insects associated with the selected plant parts were collected and recorded. The specimens were collected and stored in polythene bags with small pinholes, with each bag marked with the GPS coordinates, host plant name, date, and sample number. The samples were temporarily stored in a shaded area before being transported to the laboratory at the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna. Upon arrival at the laboratory, the bags were placed at 4 ^{0}C in the refrigerator for 10-15 minutes to immobilize and kill the mealybugs for morphological identification. After identification, the samples were preserved at -20 ^{0}C in a freezer for molecular studies. Infestation level of the host plants was recorded based on the parameters described by Prishanthani & Vinobaba (2009).

Morphological identification

The morphology of the mealybugs was observed using a dissection microscope (×80) (Olympus AX 80, Japan). Mealybug morphological identification was conducted following a published taxonomic key (McKenzie, 1967; Prishanthini & Vinobaba, 2009). Detailed drawings of each species, highlighting their identification features, were created by hand after the identification process.

DNA extraction, PCR, and phylogenetic tree analysis

The ethanol precipitation method (Milligan, 1998) was employed to extract the DNA. One mealybug was placed in each of the 1.5 mL microcentrifuge tubes. The mealybug was homogenized in 50 µL homogenization buffer, and it was ground well using a micro pestle. After the grinding, cells were lysed by using 50 µL of lysis buffer (0.05% SDS, Tris buffer pH 8.3, 0.5% EDTA, 5M NaCl). Then the lysed solution was incubated at 70 ^{0}C for 15 minutes in a heat block (Labnet, USA), and it was allowed to cool to room temperature before adding 15 µL of 8 M Potassium Acetate. After that, the content was incubated in ice for 30 minutes. Then it was centrifuged at 14000 rpm for 10 minutes. Then, the supernatant was transferred to the new centrifuge tube, and DNA precipitation was done by adding 200 µL of absolute ethanol. This was placed at -20 ^{0}C overnight. Then centrifugation was done at 14000 rpm for 10 minutes to precipitate the DNA pellets. The supernatant was pipetted out. After that, DNA pellets were washed using 70 % ethanol (400 µL) and centrifuged at 14000 rpm for 5 minutes. Then 70% ethanol was removed by pipetting out, and DNA pellets were dried 50^{0}C in a heat block for 15 to 20 minutes. The DNA was rehydrated using 50 µL of nucleus-free water and stored at -20 ^{0}C. DNA quantification was performed on the final extract for each specimen of each species using a NANODROP 1000 Spectrophotometer (Thermo Scientific, USA).

PCR was conducted using D3 universal primers; D3A 5´-GAC CCG TCT TGA AAC ACG GA-3´ and D3B 5´-TCG GAA GGA ACC AGC TAC TA-3´ (Simon et al., 1991). PCR was performed with a total of 25 µl reaction mix with 1.0 µl each primer (100 µM of D3A and D3B),12.5µl of 2X master mix, 0.1 U Taq DNA polymerase, 4.5 µl DNA, with the remaining volume made with nucleotide-free water. Thermal cycling conditions were set at an initial denaturation of 95 ^{0}C for 5 minutes, followed by 30 cycles of denaturation for 30 seconds at 95 ^{0}C, annealing for 30 seconds at 55 ^{0}C, extension for 1 minute at 72 ^{0}C followed by a final extension for 7 minutes at 72 ^{0}C.

The product was visualized for successful amplification using a 1.5% agarose gel stained with Ethidium bromide. PCR amplicons obtained from species collected widely were sent to Macrogen Inc. (Geumcheon-gu, Seoul, Korea) for Sanger sequencing. Nucleotide sequences of genes from both strands were edited by using Finch TV, and edited sequences were aligned using the Clustal W programme in MEGA 11 (Tamura et al*.,* 2021). Edited individual sequences were submitted to GenBank to get the Accession numbers. Edited sequences were used to search for matching sequences using the Blastn tool. Matched sequences were retrieved in FASTA format and aligned.

The best DNA model for maximum likelihood tree construction was found using MEGA 11 (Tamura et al*.,* 2021) for the complete alignment. A phylogenetic tree was constructed using the maximum likelihood method, and a test of phylogeny was done using the bootstrap method (n=100). The substitution model selected was the Kimura-2 parameter model with a Gamma distribution. The cut-off value of the condensed tree was set at 80%.

RESULTS

Distribution and host preference

Ten mealybug species were collected from a range of economically significant crops, including *Abelmoschus esculentus* (okra), *Solanum lycopersicum* (tomato), *Solanum torvum* (turkey berry), *Solanum melongena* (brinjal), *Coffea* sp. (coffee), *Manihot esculenta* (cassava), as well as fruit crops such as *Psidium guajava* (guava), *Carica papaya* (papaya), *Ananas comosus* (pineapple), and *Annona aquamosa* (soursop), along with ornamental plants like *Hibiscus* (shoe flower) and *Acalypha wilkesiana* (copperleaf), and *Sanchezia* sp. (zebra plant) as listed in Table 1.

In Kilinochchi, mealybugs were found infesting Okra, tomato, turkey berry, shoe flower, copperleaf, guava, cassava, and zebra plant. The predominant species identified in Kilinochchi was *P. solenopsis* Tinsley. In Monaragala, mealybugs were observed on turkey berry, guava, brinjal, papaya, pineapple, and soursop, with various species including *P. solenopsis*, *Pl. lilacinus* Cockerell*,* and *Pa. marginatus* Wiliams and Willink. The Jaffna district exhibited infestations on brinjal, guava, turkey berry, coffee, and cassava, predominantly by *Fe. virgate* Cockerell, *Pl. minor* Maskell, and *P. solani* Ferris*.*

Mealybug infestations were particularly severe on guava, brinjal, turkey berry, and certain ornamental species, with the association of more than two species (Figure 1).

Among the infested crops, more serious damage was observed in guava, which was more than 60% (Figure 2).

P. solenopsis was the most prevalent species, displaying a high degree of host preference and infesting multiple crop species, including okra, tomato, turkey berry, and shoe flower. *F. virgata* and *P. minor* were also common and infested guava, brinjal, and coffee. Additionally, species such as *C. insolita* Green, *P. solani*, *P. lilacinus*, *Ph. manihoti* Matile-Ferrero, *P. marginatus*, and *Ps. viburni* Signoret were recorded on specific host plants in distinct geographical locations (Table 1).

Field observation of infestation and ant association

The infestation level of mealy bug species is particularly higher in guava, cassava, ornamental plants, and papaya crops. Among them, guava plants were severely infested by

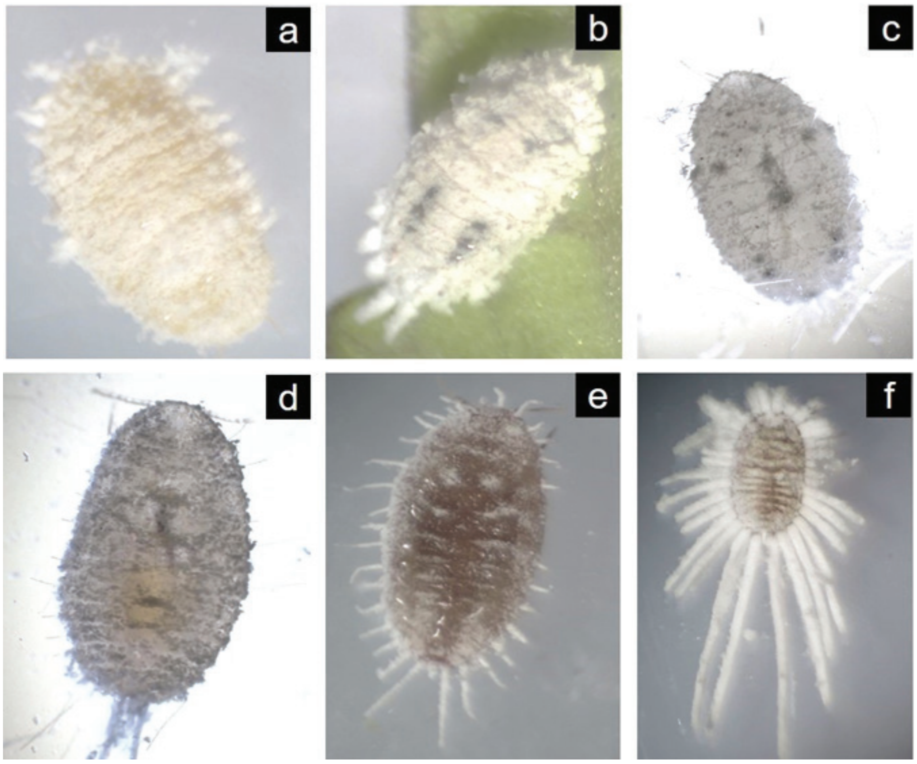

Figure 1: The identified mealybug species from different host plants: a) *Phenacoccus manihoti*, b) *Phenacoccus solenopsis*, c) *Coccidohystrix insolita*, d) *Planococcus minor*, e*) Phenacoccus solani*, f) *Rastrococcus mangiferae*

Table 1: Diversity of mealybug species and their host associations in three districts of Sri Lanka: Kilinochchi (KN), Jaffna (JF), and Monaragala (MN)

Code	District	Longitude	Latitude	Host plant	Collection date	Species	Infestation level
KN 14	Kilinochchi	9.319170	80.396921	*Abelmoschus esculentus*	14/3/2022	*Phenacoccus solenopsis*	Medium
KN 16	Kilinochchi	9.318510	80.397201	*Solanum lycopersicum*	14/3/2022	*Phenacoccus solenopsis*	Low
KN 17	Kilinochchi	9.3164758	80.399670	*Solanum torvum*	14/3/2022	*Phenacoccus solenopsis*	Medium
MN 09	Monaragala	6.749849	81.309824	*Solanum torvum*	4/4/2022	*Phenacoccus solenopsis*	Medium
KN 02	Kilinochchi	9.367743	80.366559	*Hibiscus*	29/03/2022	*Phenacoccus solenopsis*	High
KN 05	Kilinochchi	9.320061	80.401766	*Acalypha wilkesiana*	3/30/2022	*Ferrisia virgata*	Medium
JF 01	Jaffna	9.696239	80.032601	*Solanum melongena*	3/22/2022	*Ferrisia virgata*	Medium
JF 02	Jaffna	9.696186	80.032690	*Solanum melongena*	3/22/2022	*Ferrisia virgata*	Medium
JF 03	Jaffna	9.696231	80.032999	*Solanum melongena*	3/22/2022	*Ferrisia virgata*	High
KN 03	Kilinochchi	9.367615	80.36655	*Psidium guajava*	03/29/2022	*Ferrisia virgata*	High
JF 07	Jaffna	9.695863	80.033120	*Psidium guajava*	3/22/2022	*Ferrisia virgata*	High
MN 02 a	Monaragala	6.749714	81.309365	*Psidium guajava*	4/4/2022	*Ferrisia virgata*	High
JF 05 a	Jaffna	9.696114	80.033062	*Solanum melongena*	3/22/2022	*Planococcus minor*	Medium
JF 04	Jaffna	9.696385	80.033177	*Solanum melongena*	3/22/2022	*Coccidohystrix insolita*	Low
JF 06 b	Jaffna	9.696019	80.032935	*Solanum melongena*	3/22/2022	*Planococcus minor*	Low
JF 08	Jaffna	9.695384	80.033416	*Solanum torvum*	3/22/2022	*Planococcus minor*	Incidental
JF 11	Jaffna	9.695554	80.034015	*Coffea sp*	3/22/2022	*Planococcus minor*	Medium
MNH 01	Monaragala	6.866508	81.353649	*Solanum melongena*	5/2/2022	*Phenacoccus solani*	Low
MN 02 b	Monaragala	6.749714	81.309365	*Psidium guajava*	4/4/2022	*Planococcus lilacinus*	High
KN 15	Kilinochchi	9.315760	80.395943	*Manihot esculenta*	14/03/2022	*Paracoccus marginatus*	High
JF 09	Jaffna	9.695384	80.032611	*Manihot esculenta*	3/22/2022	*Phenacoccus manihoti*	High
JF 10	Jaffna	9.696079	80.032712	*Manihot esculenta*	3/22/2022	*Phenacoccus manihoti*	High
MN 03	Monaragala	6.749689	81.309322	*Manihot esculenta*	4/4/2022	*Phenacoccus manihoti*	High
MN 08	Monaragala	6.749585	81.309774	*Carica papaya*	4/4/2022	*Paracoccus marginatus*	High
MN 06	Monaragala	6.749738	81.309645	*Ananas comosus*	4/4/2022	*Planococcus lilacinus*	High
MN 01	Monaragala	6.749535	81.309740	*Annona aquamosa*	4/4/2022	*Pseudococcus viburni*	Medium
KN 01	Kilinochchi	9.316074	80.398841	*Sanchezia sp.*	3/28/2022	*Rastrococcus mangiferae*	High

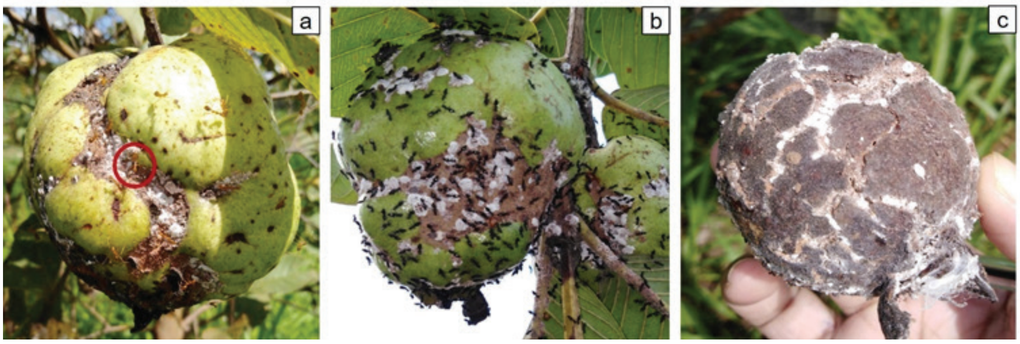

Figure 2: The interaction between mealybugs and ants on guava fruit highlights different ant species associated with mealybug infestations and the resulting damage caused by severe infestations. a) Mealybugs associated with *Oecophylla smaragdina*, b) Mealy bug associated with *Technomyrmex albipes* c) severely infested fruit

the mealybugs in all studied districts. Ants were frequently observed in association with mealybug infestations. *Oecophylla smaragdina* (red ant) was notably found in high numbers in association with *P. lilacinus* mealybug infestations on guava trees. Its infestation level was observed as high. Additionally, *Technomyrmex albipes* (black ant) was observed in the vicinity of *P. lilacinus* mealybug-infested guava trees. A natural enemy of mealy bug, called mealybug destroyer, was observed in 20% of the studied fields, particularly in the Moneragala District.

Molecular confirmation and phylogenetic tree

After the PCR, two distinctly sized amplicons were observed in the agarose gel, with one at around 350bp and the other at 450bp length (Figure 3). *F. virgata* in the *Acalypha wilkesiana* (Croton) plant has resulted in an amplicon with around 350bp compared to 450bp amplicons derived from the same species from the other plants (Figure 3c). Some samples have resulted in two bands at 350 and 450 bp (Figure 3a,3b, and 3d), and they were not considered for sequencing.

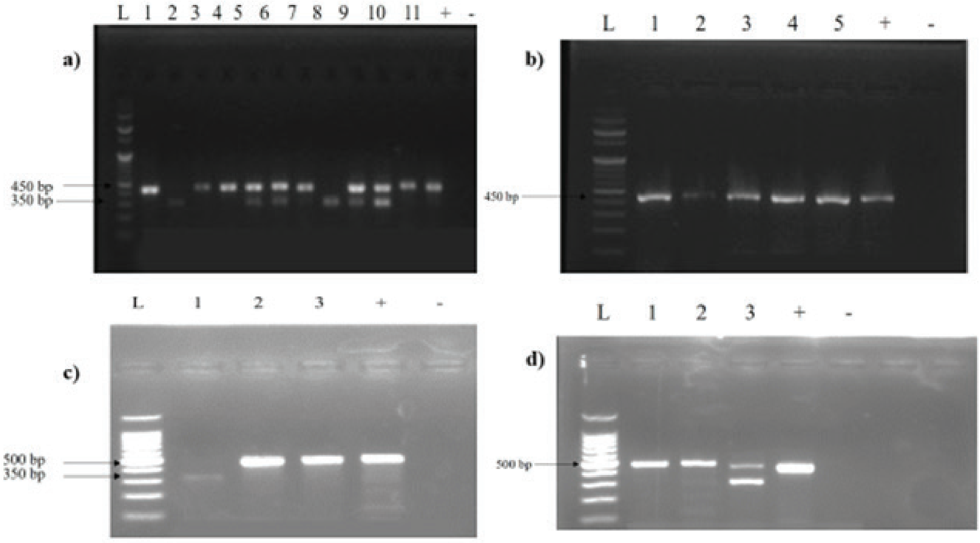

Figure 3: Agarose gel (1.5%) analysis of PCR amplicons from DNA extracted from mealybug species from different host plants using the D3 universal primer pair. a) L-Ladder (100 bp), Lane 1-*Phenacoccus solenopsis*, Lane 2- *Ferrisia virgata*, Lane 3-*Planococcus minor*, Lane 4-*Planococcus minor,* Lane 5-*Phenacoccus solani,* Lane 6-*Planococcus lilacinus*, Lane 7-*Phenacoccus manihoti*, Lane 8-*Paracoccus marginatus*, Lane 9-*Planococcus lilacinus*, Lane 10-*Pseudococcus viburni*, Lane 11-*Rastrococcus mangiferae*, Lane 12-possitive control, Lane 13- negative control. b) L-Ladder (100bp), Lane 1-*Phenacoccus solenopsis* in *Abelmoschus esculentus*(okra) plant, Lane 2- *Phenacoccus solenopsis* in *Solanum melongina* (brinjal) plant, Lane 3- *Phenacoccus solenopsis* in *Solanum torvum* (turkey berry) plant, Lane 4- *Phenacoccus solenopsis* in *Solanum torvum* (turkey berry) plant, Lane 5- *Phenacoccus solenopsis* in *Hibiscus* plant, Lane 6-Possitive control, Lane 7-negative control. c) L-Ladder (100bp), Lane 1-*Ferrisia virgata* in *Acalypha wilkesiana* (Croton) plant, Lane 2- *Ferrisia virgata* in *Solanum melongina* (brinjal) plant, Lane 3- *Ferrisia virgata* in *Psidium guajava* (Guava), Lane 4- positive control, Lane 5-negative control. d) L-Ladder (100bp), Lane 1- *Planococcus minor* in *Solanum melongina* (brinjal) plant, Lane 2- *Planococcus minor* in *Solanum torvum* (turkey berry) plant, Lane 3- *Planococcus minor* in *coffea* sp.(coffee) plant, Lane 4-possitive control, Lane 5-negative control

The GenBank accession numbers are ON787833 to ON787846. From the BLASTn search, it was found that the identification based on morphology was correct, except for two samples. The sequence of one sample (Accession Number ON787841) identified as *P. solenopsis* was found to match closely with *P. giganteus*. Two unidentified specimens were identified as *P. minor* and *P. viburni* based on the search.

The p-distance values showed high variations in the sequences (319 bp after ambiguous data were deleted using the pair-wise deletion method), as illustrated below in Table 2.

The maximum variation was found between *F. virgata* and *P. minor* in pineapple, with 54.4%. The lowest variation was observed between *P. marginatus* and *P. lilacinus* (5.1%).

On average, the p-distance value between species was 21%. The most suitable method for creating the phylogenetic tree was the Kimura-2 parameter model with Gamma distribution as per the lowest BIC in MEGA 11.0. The phylogenetic tree was divided into multiple distinct clades, as shown in Figure 4. *P. minor* and *P. lilacinus* form two sister clades. Two other distinct clades were *P. solenopsis* and *R. mangifera.* The other identified species form paraphyletic groupings. One species identified as *Planococcus* sp. (Accession Number ON787846) was extremely isolated from the other *Planococcus* species, and it was later considered an unidentified species.

Table 2: Estimates of Net Evolutionary Divergence between Groups of Sequences. The number of base differences per site from estimation of the net average between groups of sequences is shown. This analysis involved 14 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 319 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021)

	Rastrococcus mangiferae	*Planococcus sp.*	*Planococcus minor*	*Planococcus lilacinus*	*Phenacoccus solenpsis*	*Phenacoccus*	*Paracoccus marginatus*	*Ferrisia virgata*
Rastrococcus mangiferae								
Planococcus sp.	0.5438							
Planococcus minor	0.1641	0.5090						
Planococcus lilacinus	0.1648	0.5166	0.0271					
Phenacoccus solenopsis	0.1056	0.4898	0.0990	0.0890				
Phenacoccus	0.2283	0.5373	0.2340	0.2183	0.1001			
Paracoccus marginatus	0.2491	0.5410	0.1750	0.1753	0.1670	0.2727		
Ferrisia virgata	0.1603	0.5217	0.0544	0.0510	0.0737	0.2092	0.1393	
Psudococcus viburni	0.3206	0.5870	0.2752	0.2723	0.2516	0.3452	0.3584	0.2577

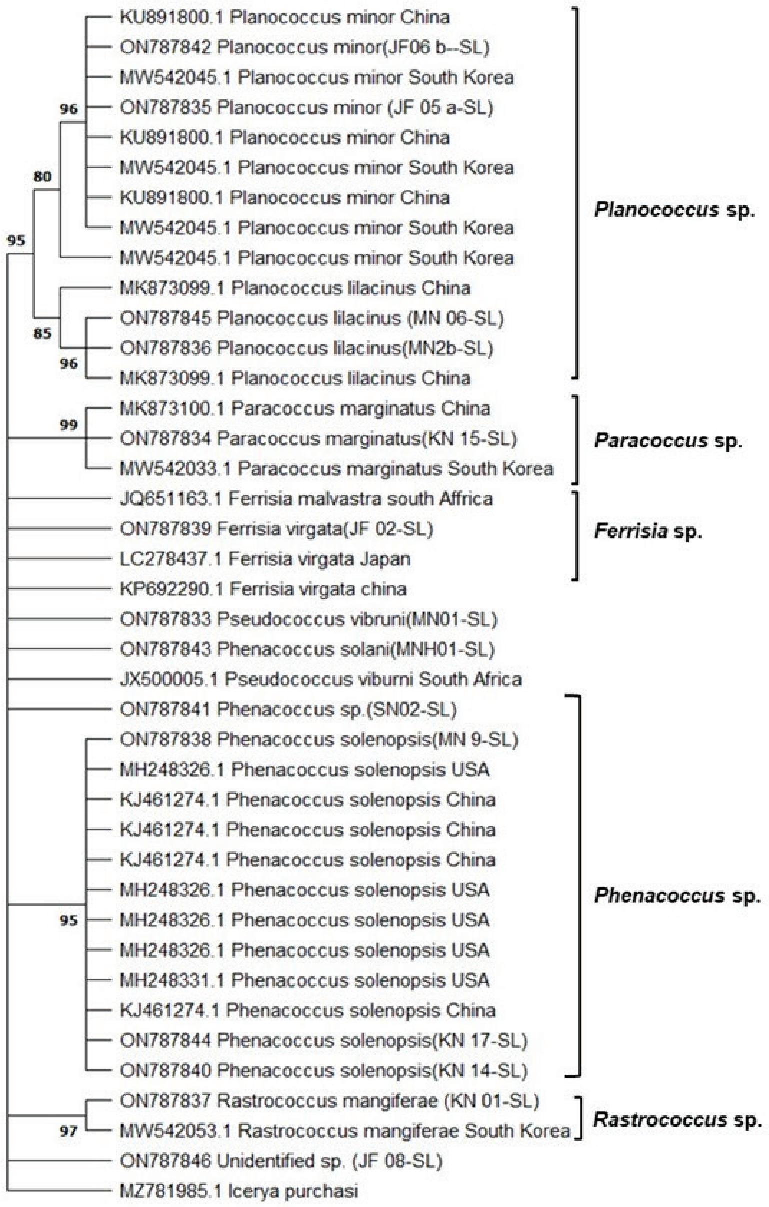

Figure 4: Maximum-likelihood tree (MEGA 11) with bootstrap support (100 replicates) illustrating the clustering pattern of mealybugs using partial 28s rDNA sequences (319 bp)

DISCUSSION

Distribution and host preference

Mealybugs pose significant challenges to agricultural productivity globally due to their diverse host preferences and ability to cause economic damage to a wide range of crops and ornamental plants. In our study conducted across three districts in Sri Lanka (Kilinochchi, Monaragala, Jaffna), we investigated the distribution and host preferences of mealybug species infesting economically important crops and ornamentals. Our findings revealed a diverse array of mealybug species associated with various host plants, aligning with previous research emphasizing the role of host plant preferences in shaping mealybug distribution patterns.

The district-wise analysis highlighted distinct infestation patterns, with cotton mealy bug, *P. solenopsis,* emerging as a predominant species infesting crops like okra, tomato, and brinjal in Kilinochchi. This species has been reported in 26 plant species, including families of Solanaceae and Malvaceae in the Batticaloa district of Sri Lanka (Prishanthini & Vinobaba 2009). Similarly, Monaragala exhibited a varied composition of mealybug species infesting crops such as turkey berry, guava, and pineapple, including species like *P. lilacinus* and *P. marginatus.* In the case of guava trees in Monaragala, *P. lilacinus* has been identified as the causal agent of all the identified sample while in the Jaffna District, *F. virgate* was the causal agent. *P. marginatus* is the primary pest of papaya in Sri Lanka (Sirisena et al., 2013), while the species is reported from Cassava in our study.

This is the first report for the species *P. solani* and *P. manihoti. P. solani,* a polyphagous species, is native to North America. This species can be a considerable threat to the agriculture sector (Huang et al., 2020). *P. manihoti,* a native South American species, is a serious pest affecting the cultivation of cassava. This was first detected in Asia in Thailand in 2008 (Parsa et al*.,* 2012).

Particular concerns observed were the severe infestations observed on guava, brinjal, and turkey berry, with guava exhibiting significant damage levels exceeding 60%. This highlights the urgent need for effective pest management strategies to mitigate yield losses and economic impacts associated with mealybug infestations. Species-specific behaviors of predominant mealybug species like *P. solenopsis* and *F. virgata* underscore their adaptability and wide host range, necessitating targeted management approaches integrating biological control and integrated pest management tactics.

Field observation of damage pattern and ant association

The infestation levels of mealybug species were notably higher in certain crops and ornamental plants, with guava, cassava, and papaya crops showing particularly elevated levels of infestation. This observation aligns with previous studies highlighting the susceptibility of these crops to mealybug infestations in other parts of Sri Lanka except the studied locations (Galanihe et al., 2010; Sirisena et al*.,* 2013). Furthermore, the severity of infestation on guava trees across all studied districts underscores the economic significance of mealybugs as pests in agricultural settings. The observed high infestation levels on guava trees highlight the vulnerability of this crop to mealybug damage. Mealybugs can cause direct damage to guava trees by feeding on sap and secreting honeydew, leading to leaf curling, wilting, and reduced fruit quality. The presence of ants with mealybugs on infested plants is significant because it often indicates a mutualistic relationship. Ants protect mealybugs from predators in exchange for the sweet honeydew the mealybugs produce, which can worsen infestations and hinder pest control efforts. But the presence of natural enemies, such as the mealybug destroyer, underscores the potential role of biological control in regulating mealybug populations.

Molecular confirmation and phylogenetic tree

The amplicons that resulted in a double band in the gel were considered contaminated, and they were not used for sequencing reactions. Sequencing of *P. manihoti* was not done due to the lack of sample numbers. The clustering pattern was mostly in concurrence with the morphology-based species separation. *P. solani* and *P. viburni* have shown close proximity in the tree. This might be due to the lack of samples available for analysis, which resulted in lower resolution in the separation of clades or improper morphological identification. Genera *Phenacoccus* and *Rastrococcus* were isolated from all the other genera, which form a monophyletic clade within them. The paraphyletic relationship between *F. malvastra* from South Africa (Accession number JQ651163.1) with the other two *F.* species, along with the genera like *Pseudococcus* and *Paracoccus,* was also an interesting observation, as most other genera formed monophyletic clades. Grouping based on geographical differences was less prominent except for *P. minor* from South Africa (Accession Number MW542045.1) and *P. lilacinus* from China (Accession Number MK873099.1), which were isolated from their clades.

CONCLUSION

This study assessed the diversity and host associations of mealybugs in selected dry-zone districts of Sri Lanka, integrating morphological and molecular tools for accurate identification. The findings confirm that several economically important mealybug species occur across multiple host plants, demonstrating their polyphagous nature and potential threat to diverse crops. For example, *P. solenopsis, P. minor*, *F. virgata,* and *P. lilacinus* were found on more than one host plant. The frequent association with ants and the presence of natural enemies highlight the ecological interactions that may influence their spread and management. The variation between the DNA sequences of species was very high. The phylogenetic tree constructed using the maximum-likelihood method was divided into multiple groups representing monophyletic and paraphyletic relationships among the genera. Overall, the results emphasize the importance of early detection, integrated pest monitoring, and the use of both morphological and molecular approaches for effective management of mealybugs in Sri Lanka.

DECLARATION OF CONFLICT OF INTEREST

Authors declare no conflict of interest

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AUTHOR CONTRIBUTION

All authors have accepted responsibility for the content of the manuscript, reviewed all results, and approved the final version. Conceptualization, Data collection, Statistical analysis, Supervision, Writing the first draft, Supervision, data interpretation, Manuscript editing and reviewing, Project administration: **S.T**; Supervision, data interpretation, Manuscript editing and reviewing: **K.G;** Methodology, Data interpretation, writing first draft; **K.W.I.S.**

DATA AVAILABILITY STATEMENT

The DNA sequence data are available in GenBank with specific accession numbers as illustrated in the manuscript.

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