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DNA Barcoding Indicates that Ecological Networking with Symbiotic Ants and Host Plants Shapes the Mealybug Diversity in Agricultural Fields in Southern Taiwan

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Mealybug (Hemiptera: Coccomorpha: Pseudococcidae) diversity in agroecosystems can be shaped by multi-trophic interactions involving host plants and mutualistic symbiotic ants, yet the mealybug species composition and the evolutionary drivers of ant symbiosis and the host plant communities remain understudied in Taiwan. Morphological analyses were performed to characterize diagnostic morphological features and identify mealybug and ant specimens. We used DNA barcoding and phylogenetic approaches to identify mealybugs and their associated ants from Southern Taiwan to assess whether their associations are more consistent with tight coevolution or opportunistic ecological networking. We examined 30 mealybug-ant-host plant triads from agricultural fields in Southern Taiwan (Chiayi and Kaohsiung). We used cytochrome *c* oxidase subunit I (*COI*) sequences as a barcoding gene. BLASTn results identified six mealybug and four ant species across the 30 sampled triads. Phylogenetic reconstruction and ASAP species delimitation aligned with BLAST results, resolving six mealybug species (*Planococcus minor*, *Dysmicoccus brevipes*, *Dysmicoccus neobrevipes*, *Paracoccus marginatus*, *Phenacoccus madeirensis*, and *Maconellicoccus hirsutus*) and four ant species (*Pheidole megacephala*, *Tetramorium wroughtonii*, *Technomyrmex albipes*, and *Tetraponera* sp., which exhibits 94.32% *COI* similarity with *Tetraponera allaborans*). The ant-mealybug assemblages were dominated by generalist taxa. Furthermore, the analyses for co-phylogeny (Procrustes SS, $m^2 = 0.899$, $p = 0.771$) and Shannon diversity (*H'*; Mealybug diversity associated with ants (0.9557–1.4942); ant diversity associated with mealybugs (0.5004–1.3297)) suggest that the ant-mealybug symbiosis in our study area is not strictly structured by coevolution but is more consistent with opportunistic associations. These findings highlight the importance of ecological networking in shaping mealybug diversity in

Southern Taiwan and provide a molecular baseline for future community-level and network analyses.

Keywords: Hemipteran agriculture pests, Cophylogeny, *COI* gene species delimitation, Ecological adaptation, Symbiosis

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BACKGROUND

Mealybug (Hemiptera: Coccomorpha: Pseudococcidae) is a notorious agricultural pest infesting a variety of crops worldwide, which causes significant economic damage by feeding on plant sap, weakening the host plants, and reducing crop yields (Ahmed et al. 2023; Kansiime et al. 2023). Mealybug excretes honeydew, which promotes the growth of sooty mold, further harming the plants (Schulze-Sylvester et al. 2021). The previous studies have reported that more than 21 ant species worldwide coexist with mealybugs in a mutualistic symbiosis (Cheng et al. 2015; Feng et al. 2015; Ho and Khoo 1997; Ivens et al. 2018; Jahn et al. 2003; Jahn and Beardsley 2000; Krishnan et al. 2014; Yadav et al. 2026). The ants usually protect the mealybugs from natural predators and shelter them (Charles et al. 2010; Lapolla and Schneider 2023). In exchange, ants harvest honeydew from mealybugs as a nutrient source (Feng et al. 2015; Lapolla and Schneider 2023). Moreover, mealybugs also act as vectors of plant viruses. They were reported to transmit viruses of the genera *Ampelovirus*, *Closterovirus*, *Badnavirus*, and *Vitivirus*, causing plant diseases and crop losses (Ahmed et al. 2023). Those factors make mealybugs a serious concern in agricultural systems, particularly in regions with favorable climatic conditions, e.g., Southeast Asian Tropical Areas. We aim to investigate the possible evolutionary drivers that assemble the mealybug communities and their diversities in Southern Taiwan, where we observed rapid mealybug population growth.

The economic losses caused by mealybugs have been reported in several countries. The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (William and Granara, 1992), is among the most economically damaging invasive pests globally. In Kenya, infestations have led to average papaya yield losses of approximately 57%, causing household economic losses of about US$3,009 per hectare annually and national losses estimated at US$29.8 million per year (Kansiime et al. 2023). In India, classical biological control of this species generated substantial

economic benefits, with annual gains estimated between US$121 million and US$309 million and a five-year net present value ranging from US$524 million to US$1.34 billion (Myrick et al. 2014). The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Tinsley, 1898) has caused cotton yield reductions of 30–60% in South Asia (Fand and Suroshe 2015). Similarly, in Cameroon, increased banana rejection due to mealybug infestation following pesticide restrictions resulted in a sharp rise in financial losses from approximately US$3,100 to US$7,600 per month while also damaging export reputation (Becke et al. 2024). While the economically important crops, e.g., papaya and banana, in Southern Taiwan are also vulnerable to mealybugs, it is important to understand the ecological and evolutionary factors that drive the abundance and diversity of the mealybug pests while simultaneously considering their symbiotic ants and host plants.

In Taiwan, research and reports about mealybugs are very limited. Over the past 10 years, only three publications have addressed mealybugs in Taiwan, including a report of a new invasive species *Pseudococcus dendrobiorum* Williams (Williams, 2004) by Chen et al. (2015), a record of mealybug on Pineapple plantations (Huang and Wang 2016), and the efficacy test of a novel insecticide against *Planococcus citri* Risso (Cox, 1989) by Lin and Huang (2017). The collection of mealybug specimens at the Taiwan Agricultural Research Institute (TARI) reported that there are 72 species of mealybug found in Taiwan (Chen et al. 2012) based on a morphological study. Despite this taxonomic documentation, comprehensive information on the ecological aspects of these species, including their host plants, natural enemies, symbiotic ants, and genetic data, remains understudied. We found only 48 genetic records of mealybugs from Taiwan in the NCBI database (accessed in January 2026), including 17 records of 16S rRNA, 18 records consisting of a single contiguous sequence containing partial 18S rRNA, ITS, and 5.8S rRNA regions, and 13 records of *COI* (NCBI, 2026). Those sequences are often simply being used for molecular species identification.

Our goal was to determine whether different mealybug species are linked to particular symbiotic ants across sampling sites. We have surveyed the mealybugs and their associated ants in the farms in Alishan Township, Chiayi County, and Namasia District, Kaohsiung, in Southern Taiwan (Fig. 1). Based on our field survey, we observed that mealybugs coexist with ants within their colonies in a mutualistic relationship. For species identification, we conducted morphological characterization to identify the sample to the lowest taxonomic level. Thus, we used a DNA barcoding approach for species confirmation that is widely applied in ants (Hebert et al. 2003; Hebert and Gregory 2005) and mealybugs identification (Abd-Rabou et al. 2012; Oliveira et al. 2023; Puig et al. 2021; Wang et al. 2016; Xi et al. 2024).By using cophylogenetic analysis, we evaluated whether these associations reflect long-term coevolutionary history via co-cladogenesis,

or are the results of more recent host-shift events attributed to the ecological adaptation of local plant communities. We hypothesize that our studied mealybugs are generalists to their ants and host plants. Therefore, the signal of co-cladogenesis of ant species and mealybugs in our study areas will not be a significant factor of assembling the mealybug communities in Southern Taiwan.

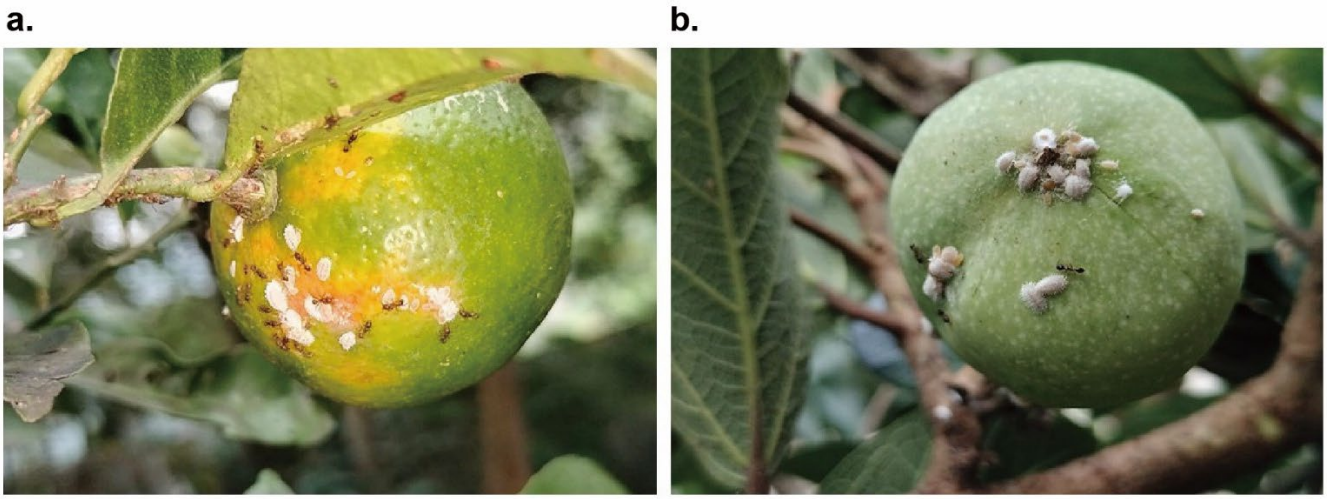

Fig. 1. Examples of coexistence of mealybugs and ants on the host plants in Southern Taiwan. (a) *Planococcus minor* and *Tetramorium wroughtonii* on *Citrus* sp. fruit (Namasia district, Kaohsiung City). (b) *Planococcus minor* and *Pheidole megacephala* on a *Ficus pumila* fruit (Alishan Township, Chiayi County).

MATERIALS AND METHODS

Taxon sampling

We collected mealybug and ant samples from neighboring agricultural sites in the Namasia District, Kaohsiung City, and the Alishan Township, Chiayi County, Taiwan (Table S1). We chose sampling sites based on a previous field survey of mealybug presence around that area conducted in April 2023. We surveyed crops, including orchards, vegetables, and ornamental plants, that were potential hosts for mealybugs and the symbiotic ant (Wang et al. 2019). Sampling was conducted from October to November 2023. We used wood sticks to collect mealybugs and ants directly from infested plants. Afterward, we preserved them in 99.5% ethanol and stored them at -20°C for further work.

Morphological Characterization and Molecular Confirmation of mealybug and ant samples

We also preserved the samples in 75% ethanol for morphological observation. Prior to molecular analyses, morphological identification was performed for all mealybug and ant specimens. Mealybug specimens were slide-mounted according to Kosztarab and Kozár (1988) in the Laboratory of Plant Protection, University of Bengkulu, Indonesia, and examined under a binocular dissection microscope (LEICA EZ4HD). Diagnostic morphological characters were compared with taxonomic keys to identify the specimens based on the standards set by Williams (2004) and Zarkani et al (2023). For ant samples, we examined them using a Leica S APO Stereo Microscope. We followed identification guide to the ant genera (Bolton, 1995). Because several mealybug and ant species exhibited highly similar morphological characteristics and some specimens lacked sufficient diagnostic features for unambiguous species-level identification, *COI* gene sequencing was used to verify and confirm species identities for one individual from each host plant. Therefore, the species designations reported in this study are based on molecular identification results.

DNA extraction and PCR amplification

Molecular analysis was conducted on adult female mealybug specimens and the symbiotic ants. These were selected from 30 independent tripartite combinations; each combination consisted of an adult mealybug, its symbiotic ant, and the host plant. One representative mealybug sample per combination, i.e., 30 total, was selected for molecular analysis. The ant samples were grouped into four morphotypes based on their morphological characteristics. One representative sample from each ant morphotype was selected for molecular analysis (Table S2). No molecular data was obtained for the host plants, however their identities were recorded for the species diversity pattern analysis.

We extracted genomic DNA from the whole bodies of each sample using the Maxwell RSC Blood DNA Kit (Promega, USA) according to the manufacturer's protocol (Promega Corporation 2018). We homogenized the sample with lysis buffer and Proteinase K, then incubated it at 56 °C for 12 hours. Afterward, the lysates were processed, and we performed DNA extraction using the Maxwell RSC Instrument. Finally, we assessed DNA concentrations using the QuantiFluor dsDNA System. We amplified a 649-bp segment of the cytochrome oxidase I (*COI*) barcoding region from mealybug samples using the primer pairs: PcoF1 (5'-CCTTCAACTAATCATAAAAATATYAG-3') and LepR1 (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (Hebert et al. 2003; Park et al. 2011). For ant samples, we used the specific primers LCO1-1490.F (5'-GGTCAACAAATCAT AAAGATATTGG-3') and HCO1-2198.R (5'-TAAACTTCAGGGTGA CCAAAAAATCA-3') to

amplify *COI* (Folmer et al. 1994). First, we prepared the PCR reaction mixture for each sample, which contained template DNA, nuclease-free water, KAPA HiFi Hotstart Ready Mix, and both forward and reverse primers. The cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 98°C for 20 s, annealing at 48°C for 15 s, and extension at 72°C for 45 s, and final extension at 72°C for 3 min and hold at 4ºC for 5 min (Wang et al. 2016). The amplified products were visualized on a 1.5 % agarose gel. Sanger sequencing was performed on an ABI 3730XL sequencer by Genomics Co., Ltd. (New Taipei City, Taiwan). We performed the de novo assembly of the *COI* sequences using Geneious Prime (v2025.0.1) (Kearse et al. 2012). After trimming from both ends and confirming no internal stop codons within the target fragments, via translation using the invertebrate mitochondrial genetic code, since internal stop codons should not appear in genes that could be expressed as amino acid sequences, we uploaded our sample sequences to the NCBI database (Table S2).

Phylogenetic analysis and species delimitation

We performed BLASTn (v2.17.0) (Altschul et al. 1990) through the NCBI online portal (http://blast.ncbi.nlm.nih.gov/Blast.cgi, NCBI 2026) to evaluate sequence similarity against the GenBank database; the top-hit sequences with the highest similarity were retrieved as reference sequences (Table S2) (Johnson et al. 2008). We aligned our sequences and the reference sequences (Table S3) using MAFFT (v7.475) (Katoh et al. 2002). We identified the best-fit model (GTR+G+I) for both mealybug and ant datasets using ModelFinder in IQ-TREE (v2.3.6) (Minh et al. 2020). We conducted phylogenetic analysis using BEAST (v1.10.4) (Drummond et al. 2007; Hill and Baele 2019, Suchard et al. 2018). Bayesian phylogenetic inference was performed using two independent MCMC runs: $1x10^7$ generations for ants and 2×10^8 generations for mealybugs, with trees and parameters sampled every 1×10^4 generations. Convergence and sampling adequacy were assessed in Tracer (v1.7.2) (Rambaut et al. 2018). The first 10 % of samples were discarded as burn-in to estimate the posterior distributions of trees and model parameters, ensuring that all parameters reached effective sample sizes (ESS > 200). We summarized the maximum clade credibility (MCC) trees from both the mealybug and ant datasets using TreeAnnotator (v1.10.4) (Drummond et al. 2012), with posterior probability values at the nodes. Finally, we visualized the summary tree in FigTree (v1.4.4) (Rambaut 2009).

We conducted species delimitation using Assemble Species by Automatic Partitioning (ASAP) (Puillandre et al. 2021), a genetic-distance-based method that identifies putative species from DNA barcode sequences (Muster et al. 2021; Oliveira et al. 2023; Schattanek-Wiesmair et al.

2024). After confirming the species identification, the relationships among mealybugs, ants, and their host plants were visualized in a network plot to describe the connections among the species.

Cophylogenetic relationship and diversification analysis

The cophylogenetic relationship between mealybugs (hosts) and their associated ants (symbionts) was analyzed using the Procrustean Approach to Cophylogeny (PACo) implemented in R with the paco (v0.4.2) (Hutchinson et al. 2017) and ape (v5.8) packages (Paradis and Schliep 2019). Phylogenetic trees based on *COI* gene sequences were converted into cophenetic distance matrices and combined with a binary host-symbiont association matrix derived from field observations. The analysis used the Cailliez correction and 1×10^3 permutations to assess the significance of the global fit between host and symbiont phylogenies. Lower Procrustean residuals indicate stronger phylogenetic congruence (coevolution), whereas higher Procrustean residuals suggest weaker associations or host-switching events (Hutchinson et al. 2017). We visualized the results as a residual heatmap using the ggplot2 (v3.5.1) (Villanueva and Chen 2019)

We evaluated the Shannon Diversity Index (*H'*) of the symbiotic associations using the vegan package v.2.6.4 (Oksanen et al. 2022). The analysis was conducted from two primary perspectives: Ant-based Diversity: For each symbiotic ant species, we calculated the Shannon diversity of its associated mealybug species and host plants. Mealybug-based Diversity: For each mealybug species, we determined the diversity of its associated symbiotic ant and host plants. We constructed bar charts with ggplot2 to compare ant-associated and mealybug-associated diversity patterns.

RESULTS

To investigate mealybug-ant-plant interactions in Southern Taiwan, we surveyed mealybug-infested plants across two neighboring districts and collected samples from 21 host plant species. Common host plants included *Hibiscus rosa-sinensis* (Chinese hibiscus), *Coffea* sp. (coffee), *Citrus* sp. (citrus), *Carica papaya* (papaya), *Annona montana* (mountain soursop), *Ficus pumila* (creeping fig), *Pouteria caimito* (abiu), and *Dimocarpus longan* (longan), whereas the complete list of host plants is provided in table S1. Mealybugs and their tending ants were collected from each host plant to be examined in our laboratory. Based on morphological characterization, the specimens were identified belonging to five mealybug genera (*Planococcus*, *Dysmicoccus*, *Phenacoccus*, *Maconellicoccus,* and *Paracoccus*) (Table 1; Fig. S1) and four ant species belonging to four genera

(*Pheidole*, *Technomyrmex*, *Tetramorium*, and *Tetraponera* (Table 2; Fig. S2). For species-level identification, we continued further analysis using *COI* sequences.

Table 1. Diagnostic morphological characters used for genus-level identification of mealybug samples

No.	Diagnostic Character	Specimen A	Specimen B	Specimen C	Specimen D	Specimen E
1	Presumed genus	*Planococcus*	*Dysmicoccus*	*Paracoccus*	*Phenacoccus*	*Maconellicoccus*
2	Body shape	elongate oval to broadly oval	broadly oval	oval	oval	oval
3	Antenna segments	8 segments	8 segments	8 segments	9 segments	9 segments
4	Translucent pores	Present on hind coxa and hind tibia, absent on hind femur	Absent on hind coxa, present on hind tibia hind femur	Present hind coxa, absent on hind tibia and hind femur	Absent hind coxa and hind tibia, present on hind femur	Absent on hind coxa, present on hind tibia and hind femur
5	Claw denticle	Absent	Absent	Absent	Present	Absent
6	Anal lobe cerarii	Two conical setae	Two conical setae	Two conical setae + 2 auxiliary setae	Three lanceolate setae + one small seta	Two conical setae
7	Ostiole	Anterior and posterior pairs present	Anterior and posterior pairs present	Anterior and posterior pairs present	Both anterior and posterior pairs present	Well developed
8	Circulus	Oval, single	divided	Broad and divided	Mushroom-shaped	Quadrate to oval, divided
9	Ventral setae	flagellate	short/flagellate	Flagellate	Flagellate	Flagellate
10	Cerarii number	18 pairs	17 pairs	17 pairs	18 pairs	4 pairs
11	Cerarii distribution	Entire body margin	Entire body margin	Entire body margin	Entire body margin	Posterior body margin/abdomen
12	Trilocular pores	Widely distributed on dorsal and ventral surfaces	Widely distributed on dorsal and ventral surfaces	Widely distributed on dorsal and ventral surfaces	Widely distributed on dorsal and ventral surfaces	Widely distributed on dorsal and ventral surfaces
13	Multilocular disc pores	multilocular disc pores are primarily restricted to the venter (underside) of the abdomen	Present mainly on ventral abdominal segments	Present on ventral surface	Present mainly on ventral abdominal segments	Present, especially on ventral abdomen
14	Oral collar tubular ducts	situated on venter and abdomen	Present only on venter	present	Present, primarily on ventral surfaces	Present
15	Oral rim tubular ducts	Absent	Absent	Present on dorsum	Absent	Present on dorsum and margin on venter
16	Dorsal tubular ducts	Present	Absent	Present	Absent	Present
17	Dorsal setae	Flagellate	short and longer setae are present	Short and stout	Short and lanceolate	Slender and Flagellate
18	Anal ring	Well developed, bearing six setae	Well developed, bearing six setae	Well developed, bearing six setae	Well developed, bearing six setae	Well developed, bearing six setae
19	cerarii shapes	Two conical setae	2 to 4 conical setae	Two conical setae	Two conical setae	Two conical setae
20	Anal Lob bar	Present	Present	Present	Absent	Present

Note: Specimens examined were *Planococcus* (EEG2687, EEG2688, EEG2691, EEG2692, EEG2695, EEG2699, EEG2707, EEG2708, EEG2709, EEG2710, EEG2712, EEG2715, EEG2717, and EEG2719), *Dysmicoccus* (EEG2689, EEG2690, EEG2693, EEG2694, EEG2696, EEG2697, EEG2711, and EEG2714), *Paracoccus* (EEG2700, EEG2702, EEG2704, EEG2705, EEG2713, and EEG2718), *Phenacoccus* (EEG2698), and *Maconellicoccus* (EEG2701).

Table 2. Diagnostic morphological characters used for genus-level identification of ant specimens

No.	Diagnostic Character	Specimen A (*Pheidole*)	Specimen B (*Tetramorium*)	Specimen C (*Technomyrmex*)	Specimen D (*Tetraponera*)
1	Body size (minor worker)	2.1 mm	2.2 mm	2.5 mm	5 mm
2	Major and minor worker dimorphism	Present	Absent	Absent	Absent
3	Body color	Reddish-brown	Yellowish-brown	Blackish body; tarsi are yellow-white	Blackish-brown body; tarsi are yellow-white
4	Body shape	Compact	Compact and robust	Slender	Very slender and elongate
5	Head shape	Oval	Subrectangular	Oval	Elongate-subrectangular
6	Antennae segments	12	12	12	12
7	Antennal club	Present (3 segments)	Present (3 segments)	Absent	Absent
8	Antennal scape	Moderate	Short	Long, surpassing posterior head margin	Moderate
9	Mesosoma shape	Convex and compact	Robust and compact	Slender and smooth	Narrow and elongated
10	Mesosoma sculpture	Smooth	Strongly sculptured (rugose)	Smooth and shiny	Smooth and polished
11	Propodeal spines	two small spines	two sharp spines	Absent	Absent
12	Petiole	Present	Well-developed	Very reduced	Narrow and distinct
13	Postpetiole	Present	Present	Absent	Present
14	Gastral tergites	4	4	5	4
15	Waist segments	2	2	1	2
16	Body pilosity	Sparse erect hairs	Sparse erect hairs	Sparse hairs	Sparse fine hairs

Note: Specimens examined were *Pheidole* (EEG2720, EEG2721, EEG2733, EEG2735, EEG2751, EEG2752, and EEG2756), *Tetramorium* (EEG2732, EEG2734, EEG2738, EEG2739, EEG2740, EEG2744, EEG2745, and EEG2746), *Technomyrmex* (EEG2722, EEG2728, EEG2729, EEG2730, EEG2731, EEG2736, EEG2737, EEG2742, EEG2747, EEG2748, EEG2750, EEG2753, EEG2754, and EEG2755), and *Tetraponera* (EEG2743).

COI sequences and BLAST result

We obtained a 663 bp *COI* alignment from 30 mealybug samples. BLASTn results showed high *COI* sequence similarity (> 98%) between 30 mealybug samples and their corresponding reference sequences (Table S2). Based on morphological diagnostic data and *COI* data, among the mealybugs, 14 samples are *Planococcus minor* Maskell (Cox, 1989) six are *P. marginatus* William and Granara, five are *Dysmicoccus brevipes* Cockerell (William and Granara, 1992), and three are *Dysmicoccus neobrevipes* Beardsley (Beardsley, 1959). In addition, one individual is closely related to *Phenacoccus madeirensis* Green (Williams, 2004) and one to *Maconellicoccus hirsutus* Green (Williams, 2004) in the phylogenetic analysis (Fig. 2a). The results from mealybug samples support six species-level identifications.

Molecular identification of four morphotypes of ants based on a 687 bp *COI* alignment confirmed that each representative ant was identified as *Pheidole megacephala* Fabricius (Bolton, 1994), *Tetramorium wroughtonii* Forel (Fisher and Bolton, 2016), and *Technomyrmex albipes* Smith (Bolton, 2007). However, due to a lower *COI* similarity (94.32%) with *Tetraponera allaborans*

Walker (Ward, 2001), we identified sample EEG2743 as a cryptic *Tetraponera* sp. in this study (Table S2).

Phylogenetic analyses and ASAP species delimitations

The BEAST phylogenetic tree exhibits six distinct clades corresponding to six mealybug species (Fig. 2; posterior probabilities (PP) range from 0.998 to 1 for each clade). Furthermore, the ASAP species delimitation analysis identified the best model (Rank: 1, ASAP score: 2.5, subsets: 7) with seven species, including the outgroup. As with the BLASTn results, our ASAP results independently identified six mealybug species, *M. hirsutus*, *P. madeirensis*, *D. neobrevipes*, *P. marginatus*, and *P. minor* (Fig. 2a), with samples in each species forming a monophyletic clade.

When using 11 species (including four species showing the highest BLAST similarity to our samples and seven closely related reference species) of ants in the BEAST analyses, our collected ant samples are closely related to four known species, *P. megacephala*, *T. albipes*, *T. wroughtonii*, and *T. allaborans*, with high support (PP = 1.00 for each clade) (Fig. 2b). The ASAP species delimitation analysis indicates that our ant samples comprise four species (Rank: 1, ASAP score: 2.5, subsets: 12), which agrees with the four-species scenario in the BLASTn results (Fig. 2b).

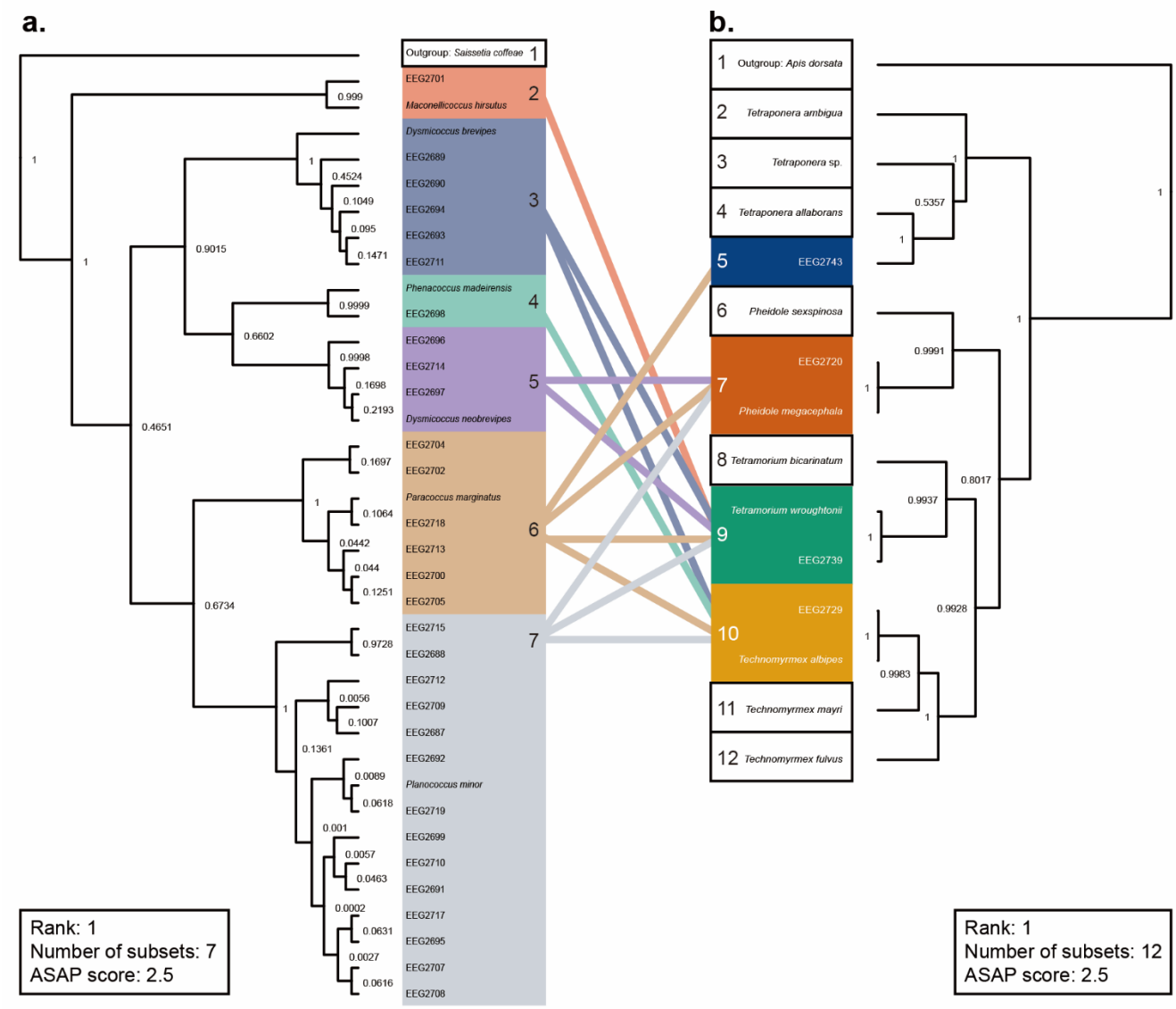

Fig. 2. Phylogenetic relationships and species delimitation in mealybugs and symbiotic ants. (a) Mealybug phylogenetic tree and species delimitation. Background colors indicate the Rank 1 partition identified by the ASAP (Assemble Species by Automatic Partitioning) analysis. The colors represent the same species subset: Pink (EA977C): *Maconellicoccus hirsutus*; Slate Blue (818DAF): *Dysmicoccus brevipes*; Light Green (8ECABB): *Phenacoccus madeirensis*; Pale Purple (AC98C8): *Dysmicoccus neobrevipes*; Tan (D9B590): *Paracoccus marginatus*; and Light Gray (CDD3D9): *Planococcus minor*. (b) Ant phylogenetic tree and species delimitation. Background colors indicate the Rank 1 partition identified as follows: Dark Blue (004180): *Tetraponera* sp.; Orange (CB5D17): *Pheidole megacephala*; Emerald Green (0F976F): *Tetramorium wroughtonii*; and Yellow (DE9B13): *Technomyrmex albipes*. The connecting lines between the two trees represent observed symbiotic relationships. The color of each line corresponds to the mealybug taxon.

Procrustes Approach to Cophylogeny (PACo)

The PACo analyses demonstrated that the ant-mealybug associations are driven by a mutualistic ecological network rather than adaptive coevolution (Procrustes SS, $m^2 = 0.899$, $p =$

0.771; Fig. 3a). Among the four ant species, the Individual Procrustes Residuals (IPR) range from 0.2104 to 0.2825, indicating a generalist property. *Tetramorium wroughtonii* (n = 8) exhibited the highest and most concentrated IPR values (Q1 = 0.270, Median = 0.280, Q3 = 0.282); followed by *T. albipes* (n = 14, Q1 = 0.251, Median = 0.254, Q3 = 0.257); *P. megacephala* (n = 7) showed a broader variation (Q1 = 0.219, Median = 0.228, Q3 = 0.252, Fig. 3b). In contrast, *Tetraponera* sp. (n = 1) has high mealybug specificity with *P. marginatus* IPR = 0.0750 (Fig. 3a). This may indicate local phylogenetic congruence; however, this could also be attributed to a small sample size Notably, the symbiotic pair consisting of *T. wroughtonii* (n = 8) and the host *M. hirsutus* (n = 1) exhibited the highest IPR (0.4062), representing a phylogenetic incongruence and suggesting that ant species frequently undergo host-shifting events.

In the interaction network (Fig. 3c), the mealybug *P. minor*, occurring in 14 samples, was identified as the dominant species in the study area, exhibiting a wide range of ecological associations across 10 host plant species and forming symbiotic relationships with three ant species (*P. megacephala*, *T. albipes*, and *T. wroughtonii*). *M. hirsutus* and *P. madeirensis* were represented by a single sample each: *M. hirsutus* was only associated with the *T. wroughtonii* (n = 8) on the *H. rosa-sinensis* (Malvaceae) (n = 2); while *P. madeirensis* interacted with *T. albipes* (n = 9) on *Tithonia diversifolia* (Asteracease) (n = 3), suggesting fewer common occurrences and less specialization with the symbiotic ant. Furthermore, across the 30 combinations, 21 distinct host plants were recorded, highlighting the ability of mealybugs to utilize a broad range of plant resources (Fig. 3c, Table S1).

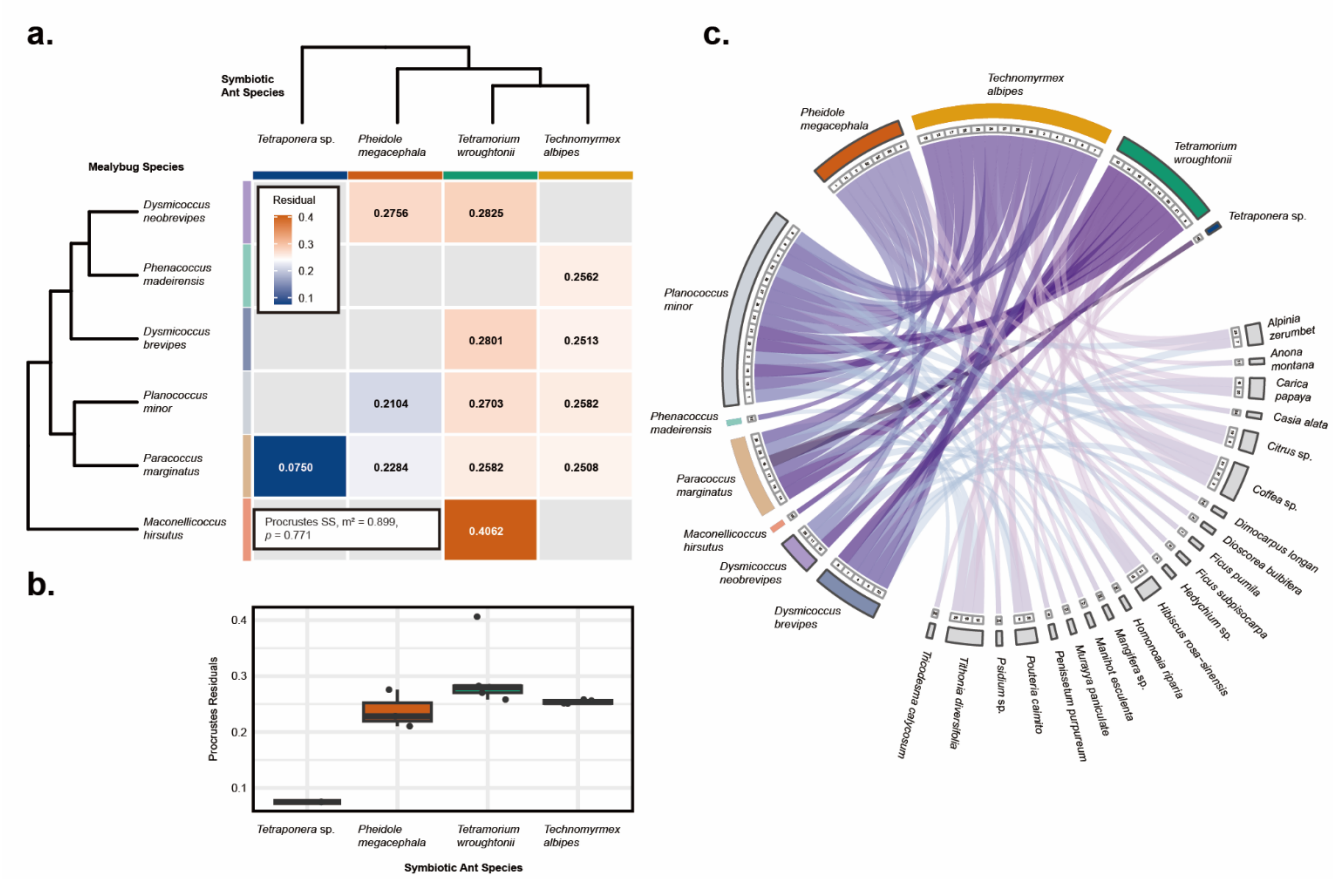

Fig. 3. Cophylogenetic analysis of mealybugs and ants. (a) Heatmap of Procrustes residuals showed phylogenetic congruence between the mealybug (host) and ant (symbiont) phylogenies. Cells represent the residuals for each interaction. The stronger phylogenetic congruence indicates low residuals in blue, and the weaker phylogenetic congruence indicates high residuals in orange. (b) Boxplot of residuals displays the Procrustes residuals grouped by symbiotic ant taxa. (c) The interaction network visualizes the associations between host plants, mealybugs, and ants. The outer ring represents the taxa, and the inner ring displays individual interaction sets by numbers. The light blue ribbons indicate plant–mealybug interactions, and light purple ribbons represent plant–ant interactions. The interactions between mealybugs and ants are shown by dark purple ribbons, which represent the host-symbiotic network.

Species diversity pattern of mealybug, ant, and host plant

While low Shannon diversity indices (H') indicated specialization, the diversity indices were calculated for the 30 tripartite combinations to reflect the ecological specialization (host mealybug and symbiotic ant) and the resources (host plants) across the species (Fig. 4). Regarding the mealybugs: *P. minor* was associated with three ant species ($H' = 1.0346$) and exhibits the highest host plant diversity, with 12 species ($H' = 2.4410$). *P. marginatus* exhibited the most diverse ant associations, with all four ant species ($H' = 1.3297$), while occurring on four host plant species ($H' = 1.3297$). *D. brevipes* was found in association with two ant species ($H' = 0.5004$) across five host plant species ($H' = 1.6094$). *D. neobrevipes* interacted with two ant species ($H' = 0.6365$) and was

distributed across three host plant species (H' = 1.0986) (Fig. 4a). Most mealybug species exhibited high ecological flexibility, being associated with multiple ant species and host plants. The only exceptions were *M. hirsutus* and *P. madeirensis*, which were each represented by a single association record. Among the ants: *T. albipes* was associated with three host mealybug species (H' = 1.1710) and the highest plant diversity (H' = 2.3420) across 11 host plants. *T. wroughtonii* interacted with six host mealybug species (H' = 1.4942) and occupied seven host plant species (H' = 1.9062)**.** Additionally, *P. megacephala* was associated with three mealybug species (H' = 0.9557) and distributed across seven host plant species (H' = 1.9459). Similarly, our ant sample serves as the generalist species, demonstrating high ecological flexibility through its multiple associations with mealybug species and host plants (Fig. 4b).

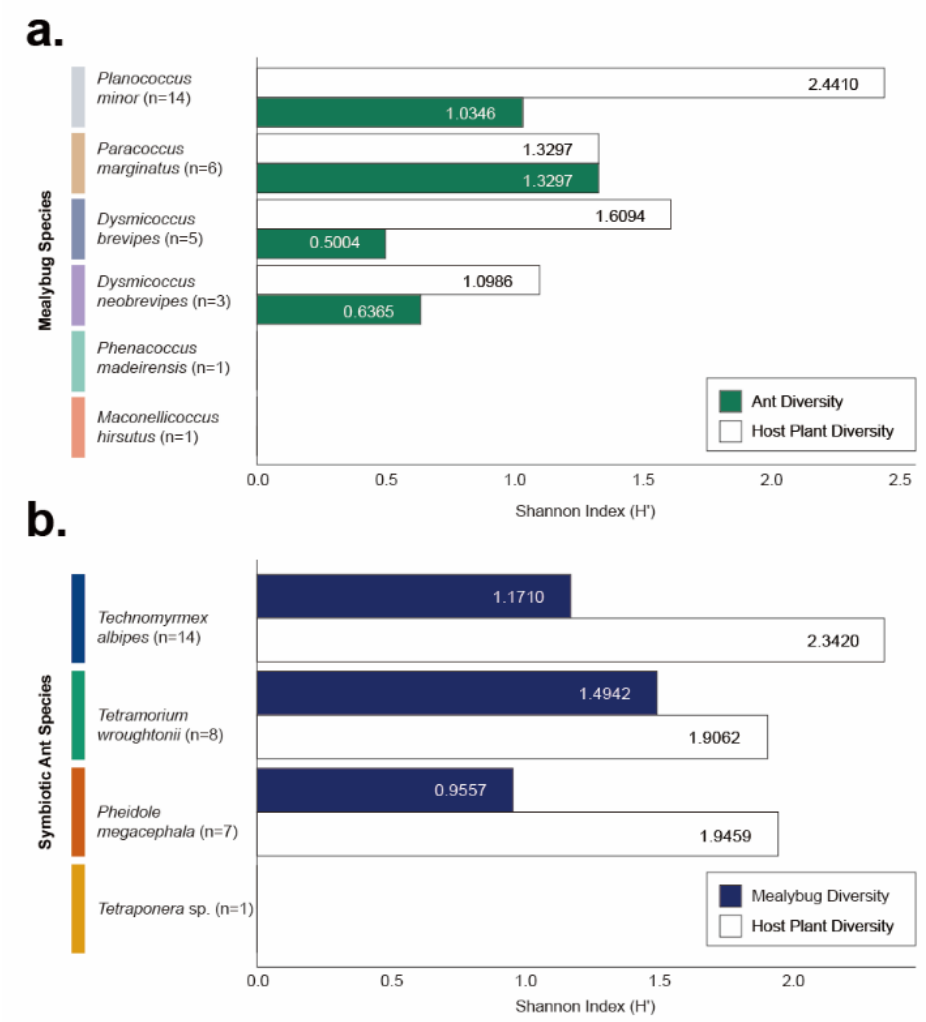

Fig. 4. Diversity analysis of mealybug–ant–host plant networks using Shannon indices (H'). (a) Shannon diversity index grouped by mealybug species. The barplot illustrates the diversity of associated symbiotic ants (Green) and host plants (white) for each mealybug taxon. (b) Shannon diversity index grouped by ant species. The barplot illustrates the diversity of associated host mealybug species (Blue) and host plants (white) for each ant taxon.

DISCUSSION

Our *COI* DNA barcoding results identified six mealybug species and four associated ant species (Fig. 2 and Table S1), confirming previously reported taxa in Taiwan while adding new distributional records of some mealybugs and ants from Southern Taiwan. All of the mealybugs in this study are globally recognized as invasive species (Mdellel et al. 2019; Mwanauta et al. 2021; Spodek et al. 2016; Wang et al. 2019). *P. minor* was the most dominant mealybug species in the study area, infesting numerous host plants. Other species are also infesting multiple host plants and are reported to be polyphagous insects, posing potential risks to agricultural areas (Wong et al. 2014; Wang et al. 2016). We found three ant species associated with multiple mealybug species (*P. megacephala*, *T. wroughtonii*, and *T. albipes*) (Fig. 2), confirming their association status as mealybug-tending ants in Taiwan. Notably, *Tetraponera* sp. (similar to *T. allaborans*) is newly recorded as a mealybug-associated species, representing the first documented case of this interaction worldwide and highlighting the need for further research into its ecological and behavioral dynamics. The diversity analysis revealed clear differences in host-use patterns among mealybugs (Fig. 4a), with *P. minor* identified as the most generalist species exploiting a wide range of host plants, while the others appeared to be likely more specialists infesting fewer host plants. Cophylogenetic analysis (Fig. 3a) further showed non-significant evolutionary congruence between mealybugs and ants, suggesting that their opportunistic associations, which are ecologically driven networking and mutualisms, are not driven by a specific coevolutionary history. Overall, this study expands the understanding of mealybug, ant, and host plant associations in Taiwan.

More than 72 mealybug species have been documented in TARI (Chen et al. 2011), including five examined in the present study. Chen et al. (2011) reported the presence of *P. minor* from Taipei and Pingtung. *P. marginatus* has been reported from multiple regions in Taiwan (Chen et al. 2011). Similarly, *P. madeirensis* was first recorded by Yeh et al. (2006) and has been found in most of the agricultural lands in Taiwan. *M. hirsutus* has been documented from Chiayi, Penghu, Taipei, and Tainan. The present study adds new distributional records of *P. minor*, *P. madeirensis*, and *M. hirsutus* from Kaohsiung City, thereby expanding the known geographic ranges of these species in Taiwan. *D. brevipes* has been reported from Taipei, Chiayi, Kaohsiung, and Pingtung (Chen et al. 2011). The existence of *D. neobrevipes* was already confirmed by Wong et al. (2014), which infested more than 53 families of host plants, including pineapple, one of the main fruits cultivated in Southern Taiwan. Our study confirmed the existence of this species in Kaohsiung.

In association with mealybugs, the ant species *P. megacephala* is an invasive species native to Africa and was introduced globally in the late nineteenth century, including to Taiwan (Liu et al. 2022). *Technomyrmex albipes* was reported by Yamane et al. (2018), who identified this species as newly introduced to Taiwan based on specimens collected in Nantou and Taitung. Hsu et al. (2025) also reported that outbreaks of *T. albipes* occurred during the summer in Southern Taiwan. In contrast, *T. wroughtonii* was classified as a native species in Taiwan, according to the Taiwan Biodiversity Network website (TBN, 2024). *T. wroughtonii* was reported to occur in Taipei by Tsai et al. (2009), and *Tetraponera* sp. that is closely related to *T. allaborans* needs to be examined in the future whether it is a new species or subspecies. All ant species in our study were already known to occur in Taiwan. Our study adds new distributional records of *P. megacephala* and *T. wroughtonii* from Kaohsiung City.

Planococcus minor is the most dominant species in the sampling area, yet there is no report of crop damage of this species in Taiwan. We found that *P. minor* infested 14 host plants (Fig. 3c), including the major fruit plant, mango. These findings align with a previous study in China (Wang et al. 2016), which identified *P. minor* as a major pest in tropical and subtropical agricultural ecosystems due to its high adaptability and symbiotic relationships with ants and host plants. *P. minor* has been reported infesting 196 genera of plants worldwide, encompassing more than 200 plant species (Zarkani et al. 2023), and the losses caused by this pest in crop plantations can reach 80-100% in coffee and cacao nurseries (Puspitasari et al. 2023). Notably, coffee and cacao are increasingly important crops in Southern Taiwan.

Dysmicoccus brevipes and *D. neobrevipes* are commonly known as pink pineapple mealybugs and are well-known pests of pineapple, grapevine, and sugarcane (Ahmed et al. 2023; Bertin et al. 2010; Moreno et al. 2023). We found that they are on three and five host plants respectively (Fig. 3c). *P. marginatus* is a highly polyphagous species, which was first recorded in Taiwan on papaya (Chen et al. 2011) and was found on five host plants in this study. It is reported to infest more than 200 plant species, including several economically important crops (Finch et al. 2021). *M. hirsutus* was found in one host plant in this study. However, it was reported to infest more than 330 host plants worldwide, including a large proportion of ornamental species (Chong et al. 2015), and has caused approximately US$1.6 billion economic losses in the USA (Ranjan 2006). They are distributed in more than 34 countries, underscoring their high invasion potential (Chong et al. 2015).

Phenacoccus madeirensis was first recorded in Taiwan in 2006 and expanded rapidly throughout the island (Yeh et al. 2006). We only found it associated with one host plant (Fig. 3c). However, it has been recognized as an invasive species with a broad host range, comprising 57 host

plant species (Williams 2004). Therefore, its limited occurrence in our sampling should be interpreted with caution. Given its broad host range and ecological adaptability, *P. madeirensis* has the potential to establish interactions with a wider range of host plants and mutualistic ants than observed in the present study.

Lai and Chang (2007) reported that ten ant species, including *P. megacephala* in this study, were associated with the mealybug *M. hirsutus* in Pingtung. We observed *P. megacephala* associating with three different mealybug species: *D. neobrevipes*, *P. marginatus*, and *P. minor* in our study areas. *P. megacephala*, *T. wroughtonii*, and *T. albipes* have been recognized as mealybug-tending species in earlier studies (Hata et al. 1992; Jahn and Beardsley 2000; Sether et al. 1998). Other than mealybugs, *P. megacephala* has also been reported to engage in a symbiotic relationship with the green scale insect *Coccus viridis* (Bach 1991). Similarly, *T. wroughtonii* was known to facilitate the spread of certain aphid species through protective mutualism against natural predators (Siddiqui et al. 2019). However, its association with mealybugs has not been documented in Taiwan until this study.

Reports of *Tetraponera* species coexisting with mealybugs are rare. However, *Tetraponera* sp. (similar to *T. allaborans)* in our study is newly recorded as a mealybug-tending species in Taiwan. Only one study has reported other *Tetraponera* sp. co-occurring with mealybugs on bamboo (Klein et al. 1992). Therefore, our findings confirm that *T. wroughtonii* and *Tetraponera* sp. are also associated with mealybugs. Further studies are necessary to elucidate the ecological interactions and behavioral mechanisms underlying these ant-mealybug relationships.

The observed diversity patterns suggest that an opportunistic, generalist ant–mealybug mutualism is shaped by local ecological conditions, rather than by tightly specialized associations. In this system, more abundant ant species tend to *COI*ncide with higher mealybug partner diversity and associated plant diversity, e.g., ant species *T. albipes* (n = 14) associated with three mealybugs ($H' =$ 1.1710) and 11 plants ($H' =$ 2.3420). Similarly, the mealybug *P. minor* (n = 14) is associated with three ant species ($H' =$ 1.0346), which illustrates a highly ecological-driven association among those abundant species (Fig. 3c and Fig. 4). This decoupling of the specialized association is biologically plausible because ant attendance is often shaped by resource distribution and local community structure, rather than host heterogeneity alone. Overall, the pattern indicates that variation in ant diversity is more closely linked to the composition of mealybug and plant communities, which is consistent with broader evidence that ant-hemipteran mutualisms are flexible and context-dependent (Stadler and Dixon 2005; Blüthgen et al. 2006; Zhang et al. 2012).

Our results of the cophylogenetic analysis support the ecological-driven association hypothesis because no strong phylogenetic congruence between mealybugs and ants was found,

which rejects the hypothesis of long-term coevolutionary association among our studied species (Fig. 3). This pattern aligns with previous studies showing that mutualisms between sap-sucking insects and ants are shaped more by ecological context than by shared evolutionary history (Blüthgen et al. 2006). Notably, we have the first discovery of the coexistence of *Tetraponera* sp. associated with mealybugs in this study. The association between *P. marginatus* and *Tetraponera* sp. showed weak phylogenetic co-divergence (note that it is based on a small sample size), suggesting a possible local congruence in coevolutionary association. This first discovery thus highlights the need for further, larger-scale research on the mealybug-ant association in Taiwan.

CONCLUSIONS

This study successfully identified and analyzed mealybug and ant species using the DNA barcoding approach. Six mealybug species and four ant species were recorded. Most mealybug and ant species exhibited generalist associations, while cophylogenetic analysis suggested that their interactions are not driven by strict coevolution but are instead by ecological driver. However, this study was limited by a relatively small sampling area and a low number of collected samples, which may not fully represent the diversity and complexity of mealybug-ant associations across Taiwan. Therefore, further studies covering wider geographic regions, more mealybugs, ants, and host plant species are desired. Seasonal sampling is recommended to better understand the ecological dynamics of these mutualistic interactions.

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Author contributions: Apriza Hongko Putra and Yong-Chao Su designed the experiments. Cheng-Yu Wu conducted the data analysis, visualization of the results, and assisted with writing.

Andriyanto helped with the laboratory experiments. Apriza Hongko Putra and Yong-Chao Su wrote the manuscript. All authors have read and approved the final manuscript.

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

Availability of data and materials: Sequences generated in the study were deposited into the GenBank database (accession numbers in Table S2).

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Supplementary materials

Table S1. (download)

Table S2. (download)

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