Northward geographic diversification of a kleptoparasitic spider *Argyrodes lanyuensis* (Araneae, Theridiidae) from the Philippine Archipelago to Orchid Island

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Abstract
Oceanic islands are unique geographic systems that promote local adaptations and allopatric speciation in many of their highly endemic taxa. This is a common case in the Philippine Archipelago, where numerous unrelated taxa on islands have been inferred to have diversified in isolation. However, few cases have been reported in invertebrates especially among parasitic organisms. Here, we tested for biogeographical structure in novel populations of the "generalist" kleptoparasitic spider, *Argyrodes lanyuensis* Yoshida, Tso & Severinghaus, 1998 in the Philippines. Results showed that, in addition to Orchid/Lanyu Island, this species has a wide geographic distribution in the Philippine Archipelago. The estimated divergence time of this lineage using the mitochondrial cytochrome oxidase 1 (mt-CO1) suggests that this species diverged ca. 3.12 MYA, during the Pliocene. Two reciprocal monophyletic clades were elucidated in *A. lanyuensis*, but with limited differentiation across Pleistocene Aggregate Island Complex (PAIC) boundaries and modern-day islands. However, in our analyses of morphological variation, we identified two phenotypically differentiated units in males (Orchid Island, Taiwan + Luzon, Philippine PAIC populations vs. Palawan + West Visayan + Mindanao PAIC populations). We infer that this species diverged in the southern portion of the Philippine Archipelago and only recently colonized Orchid Island. Our study provides new information on the extensive distribution of *A. lanyuensis* outside Orchid Island, Taiwan, but we documented a very limited geographically associated genetic variation. Our study points to behavioral phenomena such as foraging behavior as essential contributor to the evolutionary process of species diversification, in contrast to the traditionally invoked geographic drivers of divergence.

KEYWORDS
Araneae, biogeography, distribution pattern, molecular phylogenetics
**INTRODUCTION**

Oceanic island chains usually host high levels of endemic terrestrial biodiversity because of strong geographic isolation, which promotes the partitioning of their fauna and flora (Gillespie, 2007; Lomolino et al., 2010). Dispersal plays an important mechanism in the process of diversification of taxa in an oceanic island (De Queiroz, 2005; Gillespie et al., 2012). Once a historical oceanic island emerged above the surface of the ocean, it is then available for colonization of taxa from distant land areas (Cowie & Holland, 2006). However, effective colonization to oceanic islands particularly by terrestrial biota depends on several factors, such as climatic conditions, wind speed variation, local adaptation, sizes of islands, distance to source biota, and geographic boundary fluctuations. (Leihy & Chown, 2020; Lomolino et al., 2010; Whittaker et al., 2007). All of which may contribute to the historical subdivision of populations on oceanic islands.

The Philippine Archipelago is known as one such highly partitioned case: a dynamic, highly fragmented geographical template. It consists of more than 7,000 oceanic islands situated at a unique location—spanning portions of the Australasian and Asian faunal regions (Brown & Diesmos, 2009; Brown et al., 2013; Lohman et al., 2011). It hosts substantial genetic structure, both within species and among highly differentiated lineages (Brown et al., 2016; Hosner et al., 2014; Siler, Oaks, et al., 2012; Su et al., 2014; Wood et al., 2020). The subdivision of populations, species, and even higher taxa have been hypothesized to be the result of dynamics current and historical geographic processes of the archipelago (Hall, 1998, 2002; Yumul et al., 2009). With the relatively clear understanding of the geographic boundaries, and dynamic nature of their corresponding geological history, reassessment of species diversity and mechanisms of diversification has been explored comprehensively in multiple clades (Hosner et al., 2014; Linkem et al., 2010; Siler, Jones, et al., 2012; Weinell & Brown, 2017). This resulted in the identification of localized evolutionary trends and many instances of allopatric speciation following bouts of dispersal (Barley et al., 2020; Brown et al., 2016; Oaks et al., 2019; Siler et al., 2010). It is intuitive to consider that pronounced subdivision of the Philippine Islands might cause or be related to diversification, presumably resulting in the formation of new endemic species once their ancestors invaded relatively isolated islands (Heaney, 1985; Inger, 1954). However, whether such species continued to expand their range via recent dispersals among islands has rarely been reported (but see Brown et al., 2010; Siler et al., 2014).

The Philippines is located approximately 390 km south of Taiwan, but Orchid/Lanyu Island (Taiwan) and the Batanes and Babuyan island groups (Philippines) span the intervening seas with a series of small island chains (Figure 1a). Initially documented on Orchid/Lanyu Island, the kleptoparasitic spider, *Argyrodes lanyuensis* (Figure 1b), has been considered endemic to this small island since it was described in 1998 (Yoshida et al., 1998). However, our recent sampling of argyrodidne spiders in the Philippines has revealed the occurrence of *A. lanyuensis* in at least six of the archipelago's islands (Figure 1a). We used this species to assess whether a strongly subdivided geographic system (the oceanic portion of the archipelago) would be effective in generating pronounced geographical structure in genetic variation among populations of this kleptoparasitic spider. The foraging behavior of the subfamily Argyrodidinae is remarkable in that they rely on either araneophagy or kleptoparasitism—or sometimes both—as their main feeding strategy (Cobbold & Su, 2010; Vollrath, 1979; Whitehouse, 2011). *Argyrodes lanyuensis* is closely related to the Philippine endemic *A. tripunctatus* Simon 1877, and two Australasian species, *A. nasutus* Pickard-Cambridge 1880, and *A. rainbow* Roewer 1942 (Su & Smith, 2014). Based on the first reports of Yoshida et al. (1998), *A. lanyuensis* forages prey items and silk from the webs of a wide range of orb-weaving spider hosts, that is, *Nephila*, *Gasteracantha*, and *Cyrtophora*. Aside from orb-weaving hosts, it was also observed to hunt prey items and consume silk on *Achaearanea* (Theridiidae) host. Thus, an ecological “generalist” kleptoparasites like *A. lanyuensis* tend to have high tolerance on a wide array of spider hosts than ecological “specialist” kleptoparasites which specifically utilize one species/genus of orb-weaving hosts (e.g., *A. fisiifrons* and *A. minaceus* kleptoparasites). Since most of the geographic variability, biogeography, and individual species distributions of Arygrodidinae on oceanic islands have not been fully characterized, we focused on *A. lanyuensis* as a fitting representative of ecological “generalist” kleptoparasitic spiders to distinguish it from “specialist” kleptoparasites.

The Pleistocene Aggregate Island Complex (PAIC) model of speciation (Inger, 1954; Heaney, 1985, 1986; review: Brown & Diesmos, 2002, 2009) has been used as an operational hypothesis to generate testable predictions related to the analysis of diversification patterns among Philippine biota (Evans et al., 2003; Sánchez-González & Moyle, 2011; Su et al., 2014). The Pleistocene glacial cycles (between 2.5 MYA to 18 KYA) resulted in the repeated rising and lowering of sea levels (100–140 m). In the Philippines, this led to the repetitive isolation and formation of land bridges between neighboring islands separated by shallow seas (Figure 1a). With the tracing of bathymetric contours (100–140 m) within this period, Pleistocene islands can be estimated with the maximum extent of land bridges. This resulted in six major larger island-amalgamations known as PAICs: Luzon, Mindanao, Western Visayas, Mindoro, Sulu, and Palawan (Brown & Diesmos, 2002; Heaney, 1985, 1986). These paleoisland connections among islands in the Philippines served as a basis for predicting patterns of species diversity and distribution. To date, several vertebrate taxa like mammals, lizards, frogs, and birds showed nearly complete concordance to PAIC boundaries (Evans et al., 2003; Heaney, 1985, 1986; McGuire & Alcala, 2000; Sánchez-González & Moyle, 2011). However, applying the PAIC speciation model to highly dispersive arthropod species is sparse in literature, except for one pilot study (Su et al., 2014). Even though it has not been utilized more often to terrestrial invertebrate species due to the characteristic of flight and ballooning, it is also worth noting that this speciation model has been used to explain the diversification patterns of widely distributed volant mammals and birds (Heaney et al., 2005; Sánchez-González & Moyle, 2011).
The predictions derived from a strict interpretation of the PAIC Paradigm would include (1) a homogenized (or nearly so) gene pool of island populations within PAICs and (2) limited gene flow, leading to pronounced geographical structure, among and between PAICs. It follows, then, that if a particular taxon colonized the archipelago before or during the Pleistocene, the distribution of its species (or populations) would likely be found today in concordance with the PAIC model’s six major faunal regions. The Philippines Archipelago has a dynamic geologic history (Hall, 2002; Yumul et al., 2003, 2009), which likely influenced the diversification of its fauna and flora (Brown & Diesmos, 2009; Brown et al., 2013). Therefore, we assumed that heterogeneous, interrupted, and partitioned geographic template of land area throughout the archipelago might have led to distinct populations of Argyrodes lanyuensis across oceanic islands including the island banks stretching north toward Taiwan and Orchid Island (Figure 1c). However, if we consider the dispersal ability of spiders through long-distance ballooning (Bell et al., 2005; Bishop & Riechert, 1990), then we would expect to see little to no differentiation of Argyrodes lanyuensis populations, as it would greatly affect the gene flow of this species. Additionally, the behavior of this species, which is a generalist kleptoparasite, would also explain a little to no differentiation of taxa because generalists do not need to specifically adapt to a particular host (Su et al., 2018).

To ascertain how Argyrodes lanyuensis may have dispersed and colonized in the Philippine Archipelago and Orchid Island, we first update its geographical distribution and used time-calibrated phylogenetic analyses. We infer the ancestral area range evolution using biogeographical reconstruction models. Initially, we hypothesized that this species diverged from Sundaland and colonized Philippine islands via Eastern and Western arcs (Figure 1a; Route 1, 2), through the northern-most islands (Babuyan and Batanes island groups; Route 3), and eventually colonized Orchid Island, as suggested by the results of Su and Smith (2014). Alternatively, if the current distribution of the species came about by recent southward colonization (<1MYA), the species may have originated on Orchid Island (Route 4; Figure 1a) and subsequently colonized the Philippines via the Taiwan-Batanes-Babuyan island chain (Dickerson, 1928; Esselstyn & Oliveros, 2010; Oliveros et al., 2011). Thus, we undertook the current study to test the north-to-south versus south-to-north predictions derived from PAIC and analyze dispersal or vicariance events.
2 | MATERIALS AND METHODS

2.1 | Taxon sampling

We collected *A. lanyuensis* samples from the main islands in the Philippines and Taiwan between 2005 and 2007, and from July to August 2019. We found *A. lanyuensis* at only 13 collection sites on six islands: Orchid Island, Luzon, Palawan, Negros, Panay, and Mindanao (Figure 1a). Samples were collected from the webs of orb-spinning spiders of families Araneidae, Tetragnathidae, and Uloboridae. We preserved the specimens in 95% ethanol and stored at −30°C, for subsequent morphological examination and DNA extraction. All specimens were deposited in Evolution and Ecological Genomics (EEG) Laboratory, Kaohsiung Medical University, Kaohsiung, Taiwan. Specific collection information and sample accession number for each specimen are reported in Appendix 1.

2.2 | Morphological variation

To assess the geographic variation of *A. lanyuensis* populations from the Philippines and Orchid Island, we examined adult specimens for variation in continuous morphometric measurements. Male (n = 37) and female (n = 38) samples were observed under a Leica stereomicroscope. We embedded each specimen in a gel-loaded calibration slide (1 division = 0.1 mm; 1 division = 0.01) and used tethered Nikon camera D5600 to capture high-resolution images (Appendix 5). We utilized measure3 software (Tsai, 2021; https://github.com/yucenwan/Spider-measure) to generate calibrated measurements of specific body characteristics from captured high-resolution images. We normalized body morphometrics using carapace length, following character definitions of Yoshida et al. (1998). The measured body characteristics include total length (TL), carapace length (CL), carapace width (CW), total length of each leg (L1TOT; L2TOT; L3TOT, and L4TOT), and the length of each leg (I-IV) segment: femur (L1F-L4F); patella + patella (L1PT-L4PT); metatarsus (L1M-L4M), and tarsus (L1T-L4T). We additionally measured palp morphometrics from the male specimens, which include total palp length (PL), bulb length (BL), median apophysis (MA), accessorial apophysis (AP), and emboles length (EL). Bulb length was used to normalize all the palp morphometrics. All body and palp measurements used in this study were displayed in Appendix 5. Variation in morphometric dimensions (separately for males vs. females) was summarized in Principal Component Analysis (PCA) using the "prcomp" function in R 3.6.1 (R Core Team, 2017). Data visualization was carried out using the R package ggfortify (Hori koshi & Tang, 2018). We used nonlinear iterative partial least squares (NIPALS, followed Wold, 1973) in which the algorithm conducts local regressions using the latent components to predict and impute missing values caused by poor preservation conditions (Female, n = 9; 1.03% of the data matrix; Male, n = 50; 4.83% of the data matrix). To avoid multicollinearity problems among the measurements of our morphological data, we followed Vignon (2011) to adopt the Partial Least Square–Discriminant Analysis (PLS-DA), assessing if individuals clustered into geographical distributions based on morphology. We used the "plsda" function within the R package mixOmics (Rohart et al., 2017), where all measurements were included as response variables. Permutational test with 9,999 repetitions was performed based on cross-model validation procedures, where estimation of the classification error rate (CER) was used as the test statistics. Additionally, the function "pairwise. MVA.test" in the same R package was implemented for pairwise comparisons of clusters.

2.3 | DNA extraction, marker choice, and PCR amplification

We extracted the genomic DNA from legs and prosomal tissues of preadult and adult specimens following the Maxwell® RSC Blood DNA Kit AS1400 protocol. Tissues were homogenized in 300 μl Lysis Buffer and 30 μl Proteinase K (PK) Solution and incubated at 56°C for 2 hr. We purified the genomic DNA through the Maxwell® RSC Instrument following the manufacturer’s instructions. The extracted genomic DNA was stored at −30°C condition until used for polymerase chain reaction (PCR) amplification.

We sequenced the mitochondrial cytochrome oxidase I (CO1) partial gene region, which is an effective genetic marker in species identification and taxonomic delimitation (Hebert et al., 2004), especially for invertebrates (Cao et al., 2016; Carew et al., 2007; Gutiérrez et al., 2014). The CO1 fragment was targeted and amplified using primer pairs, CO1-F and CO1-r designed by Su and Smith (2014). PCR amplification was performed in a TurboCycler 2 thermal cycler (TCST-9622, Taiwan) with a total volume of 25 μl with 12 μl of premix, 10 μl of nuclelease-free water, and 0.5 μl to each of the primers. PCR products were visualized through 1.5% agarose gel electrophoresis to check amplified DNA fragments of the expected size and sequenced at the genetic sequencing facility of Genomics Co. Ltd., Taiwan.

2.4 | Sequence alignment and molecular data analysis

We filtered all the sequences according to the quality control reports and obtained a total of 95 CO1 sequences. Some samples used in morphological analyses have poor quality and thus were not included in the population genetic analyses. Contigs were generated from merged forward, and reverse, sequences and their consensus sequences were aligned using Genious Prime 2020.2. Alignment was refined manually to generate a complete alignment of 840 base pairs.

We reconstructed a time-calibrated phylogenetic tree using BEAST v1.10.4 (Drummond et al., 2012). We incorporated seven species (nine sequences in total) from GenBank as an outgroup (Appendix 1b). Species included in the outgroup are the closest relatives of *A. lanyuensis* according to the phylogenetic tree inferences of Su and Smith (2014). The program jModelTest2 v. 2.1.10 was used...
to calculate the best-fit nucleotide substitution model for the CO1 gene using the Akaike Information Criterion (AIC) (Posada, 2008). The GTR + I + G best-fit nucleotide substitution model, Yule process speciation tree model prior (Heled & Drummond, 2012), and the uncorrelated lognormal relaxed clock model (Drummond et al., 2006) were applied for node age time calibration. We used the uclsd.mean = 0.0112 site$^{-1}$ Myr$^{-1}$ based on the spider mitochondrial substitution rate estimates (Bidegaray-Batista & Arnedo, 2011; Kuntner et al., 2013) with an arbitrary standard deviation (uclsd.stdv = 0.01).

The MCMC parameters were fixed to $1 	imes 10^9$ generations with tree sampling every $1 	imes 10^4$ generations, after conducting preliminary runs (chain length $1 	imes 10^8$ and $5 	imes 10^8$). Tracer v.1.7.1 was used to determine burn-in (discarded the first $10\%$ of the trees) and to check the effective sample sizes (ESS $\geq$ 200; Rambaut et al., 2018). Maximum clade credibility (MCC) tree was then generated using the program TreeAnnotator v.1.8.4 (Rambaut & Drummond, 2010) and visualized using FigTree v.1.4.3 (Rambaut, 2014).

Additionally, nucleotide and haplotype diversity of the in-group sequences were calculated based on the PAIC boundaries and current island boundaries using DnaSp v.6.12.03 (Rozas et al., 2017). Haplotype networks were also created in DnaSP v.6 (Clement et al., 2000) and displayed as a final network using tcsBU v.1.0 (Múrias dos Santos et al., 2016). We conducted an isolation by distance (IBD) test among PAIC islands through Mantel’s test of correlation between Edward’s distances and Euclidian geographic distances. IBD test was implemented in R package “adegenet” (Jombart, 2008). Cline and distant patches of points were checked using the 2-dimensional kernel density estimation (kde2d) in R package “MASS.” Gene flow among current islands was further assessed by calculating pairwise fixation indices ($F_{ST}$) using the R package “StAMPP” (Pembleton & Pembleton, 2013).

### 2.5 | Biogeographical analyses

The ancestral geographic ranges were reconstructed by two programs: R package “BioGeoBEARS” (Matzke, 2014), and Reconstruct Ancestral State in Phylogenies (RASP) (Yu et al., 2015). The best-fit historical biogeographical model selection was conducted among six available models in “BioGeoBEARS”: DEC, DEC+I, DIVALIKE, DIVALIKE+I, BAYAREALIKE+I (Matzke, 2014). We applied the best-fit historical model (BAYAREALIKE+I) with the highest corrected Akaike information criterion (AICc) weights to the time-calibrated BEAST trees dataset and consensus tree dataset. Additionally, we applied the Bayesian Binary MCMC (BBM) and Statistical Dispersal-Vicariance models in RASP as alternative biogeographical reconstruction analyses.

We designated the geographical distributions of *Argyrodes lanyuensis* according to PAIC islands, while the known geographical distribution of the outgroup was based on the descriptions from World Spider Catalog (2020) and other published literature. There were five current distinct geographical areas included for the in-group: Orchid Island (A), Luzon PAIC (B), Palawan PAIC (C), West Visayas PAIC (D), and Mindanao PAIC (E). Five geographical areas were also included for the outgroup, namely Sundaland (F), Papua New Guinea (G), Japan (H), China (I), and Australia (J). The geographical range allowed at each node was set up to four geographical areas since no extant species occupied more than four geographical areas. Additionally, the density of evolutionary events such as dispersal and vicariance events was calculated and visualized along a time-calibrated tree.

### 3 | RESULTS

*Argyrodes lanyuensis* samples were collected from 13 sampling sites distributed across Orchid Island, Taiwan, the main (northern) component of Luzon Island, its southern Bicol Peninsula, Palawan, Negros, and Panay islands; plus, the northern, eastern, and southwestern (Zamboanga Peninsula) faunal subregions of Mindanao Island. Our sampling efforts have also reached the Ryukyu Islands (Japan) and Green Island (Taiwan). Additionally, we surveyed Cebu, Samar, Leyte, and Mindoro (Philippines), but did not find *A. lanyuensis* on these islands (2005 to 2019). At present, *A. lanyuensis* has a geographical distribution including the Philippine faunal regions of the Luzon, Palawan, West Visayas, and Mindanao PAICs, in addition to the original records (Yoshida et al., 1998) from Orchid Island, Taiwan.

We analyzed the measurements of morphological characters of *A. lanyuensis* males ($n = 37$) and females ($n = 38$) using PCA with 28 and 23 variables, respectively (Appendix 3). We then classified and sorted samples into Mindanao, the West Visayas, the Palawan, the Luzon PAIC, and the Orchid Island. Palawan female samples were not included because adult specimens were not available. The PCA showed limited clustering to both *A. lanyuensis* males and females across different geographic areas (Figure 2a,b; Appendix 4). Although, we observed three samples from Luzon PAIC that deviated from the main male clusters (Figure 2a). The first principle component (PC) accounted for 35.39% of the variance, and the second PC explained 17.15% of the variance for male morphometrics. The first PC explained 35.29% of the variance in females, and the second PC accounted for an additional 17.89%. Overall, we observed no PAIC-based clustering or divisions in males and females in the PCA results.

Alternatively, we used the PLS-DA, which emphasized a dimension reduction technique for handling multicollinearity data (Vignon, 2011), to detect the morphological clustering among samples. Because individual samples were assigned according to PAICs a priori, the PLS-DA score plot was able to discriminate PAIC clusters in both males and females (Figure 2c,d). For males, we identified one cluster (Orchid Island) that was clearly separated from the other samples, while Mindanao PAIC, Palawan PAIC, and West Visayas PAIC samples merge into a single overlapping cluster (Figure 2c). The Orchid Island cluster was significantly different with Mindanao PAIC cluster (Orchid Island vs. Mindanao PAIC: $CER = 0.23741$, $p$-value $< .05$; Table 1a) and Visayas PAIC cluster (Orchid Island vs. West Visayas PAIC cluster: $CER = 0.191$, $p$-value $< .05$; Table 1a). The Luzon PAIC samples were scattered with one sample overlapped with Orchid
FIGURE 2  Principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) score plots of male (a–c) and female (b–d) A. lanyuensis based on 28 and 23 morphometrics, respectively. Individuals are plotted against components 1 and 2 with 95% confidence ellipse for PLS-DA plot. The lists of the characters used in these analyses are in Appendices 3 and 5.
Table 1a. The overall discrimination method based on PLS-DA cluster (Luzon PAIC vs. Mindanao PAIC: CER samples. This cluster was significantly different with Mindanao PAIC Island cluster and two samples overlapped with the rest of the PAIC samples. This cluster was significantly different with Mindanao PAIC cluster (Luzon PAIC vs. Mindanao PAIC: CER = 0.111, p-value < .05; Table 1a). Thus, we inferred two morphologically discrete clusters for male data as Mindanao+Palawan+West Visayan populations and Luzon+Orchid Island populations were undifferentiated (Figure 2c). In contrast, we did not find obvious differentiation in PLS-DA plot with the female data; however, the Orchid Island and Mindanao PAIC clusters were significantly different from each other (Orchid Island vs. Mindanao PAIC: CER = 0.277; p-value < .05; Table 1b). Nonetheless, the overall discrimination method based on PLS-DA among the female samples was found to be nonsignificant (CER = 0.567, p-value > .05; Table 1b), which is consistent with our initial PCA results.

To visualize and explore the correlations among variables, we used the latent components in the PLS-DA to display a loading vector plot. The loading vector plot demonstrates the importance of each variable and its contribution to the overall variance in males and females. Figure 3 shows the results of the male and female loading vector plot obtained using two components from PLS-DA. For male data, the two most important variables showed for the first component (31.00% variance explained) L1F and L1TOT, while BL and TL are the most important variables for the 2nd component (14.00% variance). These variables have substantial contributions to the variations of Mindanao samples and Orchid Island samples, respectively (Figure 3a). For females, the two most important variables using the first component (30.00% variance explained) were CW and L2TOT, while L1PT and L2M are the two most important variables for the 2nd component (18.00% variance). These variables contribute to female variation in the Orchid Island and Mindanao samples, respectively.

We analyzed the genetic structure among all Taiwan and Philippine populations, using 840 bps of CO1 gene region. The aligned matrix showed a total nucleotide diversity (Pi) of 0.00015, and haplotype diversity (Hd) of 0.122 (Table 1). The TCS network indicated four major haplotypes (L1–L4) across our samples (Figure 4a). Geographically, the most distant population sampled is Orchid Island with four primary haplotypes. Based on haplotype diversity, Orchid Island has the highest haplotype diversity of any islands (Hd = 0.4100; Table 2). However, surprisingly, no geographic pattern in haplotype distribution can be discerned (Figure 4a). The IBD scatterplot shows a single consistent density of points suggesting a genetic homogenization (Appendix 7), which showed a weak and nonsignificant correlation between genetic and geographical distances across PAICs (FST values that ranged from −0.395 to 0.054). We also obtained low pairwise FST values that ranged from −0.395 to 0.054 with nonsignificant p-values (Appendix 8). The lack of IBD and low FST values suggested a limited population differentiation and high gene flow among PAIC populations and in present-day islands.

The same lack of pattern is also apparent in our BEAST maximum clade credibility (MCC) tree (Figure 4b), which shows two, strongly supported (Posterior Probability, or PP = 1.00) major clades, each of which exhibits no differentiation among PAIC or current island boundaries. Furthermore, all nodes within these two major clades have low posterior probability support (PP < 0.5), which is surprising given that CO1 is a rapidly evolving mitochondrial gene region. The divergence time of A. lanyuensis from the outgroup suggests that this species emerged in 3.1241 MYA (95% height posterior density: 0.2774–11.30 MYA), within the Neogene; specifically, Miocene–Pliocene epochs.
FIGURE 3  PLS-DA loading plots for the 1st and 2nd components where colors indicate the PAICs for which the selected variable has a maximal mean value.
The biogeographical analyses from the best-fit model in BioGeoBEARS (BAYAREALIKE +j) suggested that A. lanyuensis most likely originated from the Mindanao PAIC [node 198; area E; marginal probability (MP) = 55.33%; Figure 5a]. A similar ancestral area was also suggested by the S-DIVA analysis (node 198; area E; MP = 68.64%; Appendix 9), while the BBM analysis inferred both Mindanao and West Visayas PAIC as ancestral areas (node 198; area DE; MP = 48.14%; Appendix 10). Figure 5b shows the probability density of evolutionary events along the time-calibrated tree. We observed a consistent higher probability density of dispersal events than vicariance events that started from node 198, specifically at ~3 MYA (Miocene-Pliocene epochs) when A. lanyuensis diverged from the outgroup. Dispersal events continued toward later nodes wherein more dispersal events have occurred (Figure 5b). Therefore, based on our phylogenetic analyses and biogeographical reconstruction analyses, we reject the strict PAIC biogeographical patterns/predictions and the recent southward colonization (north-to-south prediction) and thus accept the south-to-north colonization as our best interpretation, but with little to no differentiation due to recent dispersal events and in response to a wide array of host species during range expansion.

4 | DISCUSSION

Our study demonstrated an updated geographic distribution of Argyrodes lanyuensis that covers almost the entire Philippine Archipelago, aside from Orchid Island, Taiwan, on which this species previously was thought to be endemic (Figure 1a). This species exhibits two phenotypically differentiated units in male morphology (Orchid Island Taiwan+Luzon, Philippines populations vs. Palawan+West Visayan+Mindanao populations; Figure 2c). Our
TABLE 2 Haplotype and nucleotide diversities of *A. lanyuensis* collected from Taiwan and Philippines according to PAIC (a) and current geographic boundaries (b)

|        | Orchid island | Luzon PAIC | West Visayas PAIC | Palawan PAIC | Mindanao PAIC | Total |
|--------|---------------|------------|-------------------|--------------|---------------|-------|
| (a)    |               |            |                   |              |               |       |
| Replicate | 13           | 17         | 20                | 3            | 42            | 95    |
| Haplotype | 3            | 3          | 1                 | 1            | 2             | 4     |
| Hd      | 0.410         | 0.228      | 0                 | 0            | 0.04762       | 0.122 |
| Pi      | 0.00052       | 0.00028    | 0                 | 0            | 0.00006       | 0.00015 |
| Theta   | 0.00077       | 0.00071    | 0                 | 0            | 0.00028       | 0.00070 |

|        | Orchid island | Luzon island | Negros Island | Panay island | Palawan | Mindanao PAIC | Total |
|--------|---------------|--------------|---------------|--------------|---------|---------------|-------|
| (b)    |               |              |               |              |         |               |       |
| Replicate | 13           | 17         | 10            | 12           | 3       | 42            | 95    |
| Haplotype | 3            | 3          | 1             | 1           | 1       | 2             | 4     |
| Hd      | 0.410         | 0.228      | 0             | 0           | 0       | 0.04762       | 0.122 |
| Pi      | 0.00052       | 0.00028    | 0             | 0           | 0       | 0.00006       | 0.00015 |
| Theta   | 0.00077       | 0.00071    | 0             | 0           | 0       | 0.00028       | 0.00070 |

The estimated divergence time suggests that this species originated ca. 3.1241 MYA, during the Pliocene epoch (Figure 4b). Thus, it may have already existed before Pleistocene glacial fission–fusion cycles or PAIC fragmentation. We identified no genetic structure across PAIC divisions or current island boundaries based on our time-calibrated tree and haplotype distribution (Figure 4). Additionally, the biogeographical reconstruction based on "BioGeoBEARS" and RASP suggested Mindanao as the most likely ancestral range (Figure 5; Appendix 9 and Appendix 10). Hence, our results favor south-to-north colonization over north-to-south colonization (Figure 1a) with no PAIC-genetic-structured variations.

The estimated divergence time of this species, which may have preceded Pleistocene glacial cycles, is inconsistent with the PAIC-based geographically structured genetic variation. The south-to-north colonization appears most plausible based on our results. This species may have diverged from an ancestral lineage in Sundaland and first colonized the southern Philippine islands via the eastern island arc or/and western island arc (Figure 1a; Route 1, 2 and Figure 5). The eastern island arc follows the colonization patterns from Borneo–Sulu archipelago–Mindanao–Leyte–Samar–Luzon (Huxley, 1868), while the western island arc follows the colonization route from Borneo–Palawan–Mindoro–Luzon (Dickerson, 1928). The south-to-north colonization inference was also consistent based on our MCC tree with strong nodal support (PP = 1.00) obtained for the *A. lanyuensis* clade, given that the outgroups are Australasian (e.g., *A. rainbowi*, *Faiditus xiphias*; Figure 4b) and Philippine (*A. tripunctatus*) species. Similar results were obtained by Su and Smith (2014) using different genetic markers. Thus, we suggest that this species invaded from the southern Philippines, with subsequent range expansion toward northern islands, eventually including Orchid Island of Taiwan via the Batanes-Babuyan island's route (Figure 1a; Route 3). However, further analyses of colonization patterns with higher genomic marker coverage should be explored in the Philippines, including the island chains to the south of Orchid Island to test the hypotheses of island, stepwise colonization (e.g., Su et al., 2016; Yang et al., 2018).

The lack of IBD (*R*² = 0.02313, *p*-value = 2.2e−16, Appendix 7) and low *F*<sub>ST</sub> values (−0.395 to 0.054; Appendix 8) imply high gene flow and limited population differentiation of *A. lanyuensis*. Based on the inference of evolutionary events using the best-fit model in "BioGeoBEARS," we observed a high density of recent dispersal events over vicariance (Figure 5b). These events enabled *A. lanyuensis* to disperse among islands most likely by "ballooning" with no signals of local adaptations. Even though spiders can disperse through long-distance "ballooning," evolutionary patterns are usually evident in these animals because of their unique ecological attributes that can be seen through their strong habitat affinities (Gillespie, 2016). For example, genetic structure was observed in excellent dispersalist, *Nephila pilipes* (Kuntner & Agranov, 2011; Su et al., 2007), and *Arpogoe brunnichi* (Krenwinkel et al., 2016). However, we could not observe local adaptations in the case of *A. lanyuensis*. The specific behavioral phenotype of this species, which is a "generalist" kleptoparasite, could explain the limited differentiation exhibited in this species and implies higher tolerance on different host webs (a case of ecological adaptation) without specialized functions in host-specific feeding strategies. Other spider kleptoparasites (e.g., *A. fissifrons* and *A. miniaceus*) utilize webs of specific host spiders to forage prey items (Tso & Severinghaus, 2000) and are thus called ecological "specialist" kleptoparasites (Su et al., 2018). These specialists demonstrate a strong association of these kleptoparasites to their specific host species which in turn may have caused genetic-structured populations across different islands in the Australasian region (Su & Smith, 2014). Thus, we assume that specialized kleptoparasitism could interrupt gene flow between different groups or regions.

![FIGURE 5](image-url) Ancestral area reconstruction from BioGeoBEARS derived from BEAST maximum clade credibility tree (a). The best-fit model was BAYEREALLIKE + I model with geologic time scale presented. Circles at each node show the most likely ancestral areas, while circles at the tips indicate the extant geographic distribution.
(a) BAYAREALIKE+j model

Legend
- **x**: Orchid island
- **A**: Luzon PAIC
- **B**: Palawan PAIC
- **C**: Mindanao PAIC
- **D**: West Visayas PAIC
- **E**: Sundaland
- **F**: Papua New Guinea
- **H**: Japan
- **I**: China
- **J**: Australia
- **ABF**: ABF
- **ABJ**: ABJ
- **AFJ**: AFJ
- **BFJ**: BFJ
- **ABFJ**: ABFJ
- **BCDE**: BCDE
- **FGHI**: FGHI

(b) Pliocene | Pleistocene

| Dispersal | Vicariance | Extinction | Standard |
|-----------|-----------|------------|----------|
geographic populations and might promote speciation, in contrast to generalist kleptoparasites (e.g., A. lanyuensis). The pilot study on terrestrial invertebrates, the Philippine endemic treehopper, Pyrgonota bifoliata (Membracidae), that applies a similar PAIC model of speciation shows more evident population subdivisions among PAIC islands (Su et al., 2014). Each subpopulation of P. bifoliata appears to specialize on a species-specific host plant, per PAIC island (Su et al., 2014). In contrast, the results presented here could be a special case for the Philippines archipelago in that we estimate a deeper, pre-Pleistocene temporal divergence time, and yet we did not detect any clear differentiation among PAICs or modern, current-day islands.

The phenotypic clustering evident in males from Orchid Island (southern Taiwan) and Luzon Island (northern Philippines) may suggest founder effects or could be related to sexual selection. The possible colonization of A. lanyuensis from the southern portions of the archipelago toward northern islands and eventually Orchid Island might have led to founder events. The most important variables contributing to clustering patterns of males are lengths of the first legs (L1F, L1TOT; Figure 3a) and palp bulb length (BL; Figure 3b). These variables contribute greatly to the samples from the inferred ancestral range (Mindanao island) and the recent population from Orchid Island, respectively. The phenotypic variations observed in these two populations, specifically in the leg I and palp bulb, could be attributed to sexual selection in males. Male A. lanyuensis typically have longer Leg I than females (Yoshida et al., 1998), in which similar observations were recorded in this study (Appendix 2). Leg 1 was usually used by both male and female A. lanyuensis, for moving around the web to locate the host’s silk and prey items for food consumption (Yoshida et al., 1998). For most of the Argyrodes spiders, leg I is very important in the male–male competition for female copulation, which results from highly modified intrasexual selection in males (Whitehouse, 1991, 2016). With these phenotypic variations in the leg I and palp bulb lengths between the ancestral (Mindanao) and recent populations (Orchid Island), we hypothesize that different mating strategies may evolve in recent populations given the selective pressures in the new environment. In argyrodiinae spiders, species-specific differences and intersexual differences in foraging strategies have been noted (Cangialosi, 1990; Kerr, 2005; Tso & Severinghaus, 2000). Female A. lanyuensis may be able to utilize the same foraging strategies across different spider hosts in which the functional genes for a specialized foraging behavior are not well expressed, even though a unique form of foraging strategy (host silk consumption) has been noted on this species in Orchid Island (Yoshida et al., 1998). Hence, our results on the population structure of females could be related to their foraging behavior. On the other hand, mating strategies in males could lead to the morphological differentiation of this spider kleptoparasite generalist (Whitehouse, 2016). However, these results should be further validated due to the limited sample size and genetic markers.

Our study demonstrates the possible exchange of taxa between two geographical entities. In this case, faunal transfers (dispersal) were possible between Taiwan and the Philippines through the Luzon-Taiwan strait, in which dispersal events originated from the Philippines. This study added to the cases of Philippine fauna that have been recorded to disperse from the Philippines to Orchid Island. These include Eutropis cumingi (skink; Ota & Huang, 2000), Polypedates leucomystax (frog; Kuraishi et al., 2009; Ota, 2004); five species of geckos (Ota, 1987; Siler et al., 2014; Wang, 1962), two species of butterflies, Macroglossum ungues cheni (Yen et al., 2003), and Catopyrops ancyra almana (Lu & Hsu, 2002). In contrast, other taxa (e.g., plants, shrews) have been recorded to disperse in the Philippines from Orchid Island (south-to-north colonization; Dickerson, 1928; Esselstyn & Oliveros, 2010; Oliveros & Moyle, 2010). Thus, careful analyses should be done for the diversification of taxa along the Philippine-Lanyu oceanic island chain.

5 | CONCLUSION

In conclusion, our results revealed the presence and widespread distribution of A. lanyuensis in the Philippines, far beyond its originally assumed microendemic distribution in Orchid Island, Taiwan. Our study also emphasized northward colonization of A. lanyuensis from the Philippines toward adjacent Orchid Island, Taiwan, through recent dispersal events. The molecular data highlight the importance of behavioral phenotype such as foraging behavior, rather than isolation by distance, sea-level vicariance, and climatic oscillations (e.g., PAICs Paradigm biogeographic isolation) as drivers of diversification of kleptoparasitic spiders. However, it is also important to note the structured variations we observed in males for the northern populations, which possibly contributed to mating strategies. Future work on this study system may be best informed by a higher coverage of genomic data, to get a more robust and finely differentiated characterization of its population structure across the Philippines, northwards directing to Orchid Island.

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CONFLICT OF INTERESTS
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
Mae Responde: Formal analysis (lead); Investigation (lead); Methodology (equal); Software (supporting); Visualization (equal); Writing- original draft (lead). Yi-Fan Chiu: Formal analysis (supporting); Methodology (equal); Software (supporting). Po Peng: Formal analysis (supporting); Methodology (equal); Software (supporting); Visualization (supporting). Rafe M. Brown: Methodology (equal); Validation (equal); Writing-review & editing (equal). Chia-Yen Dai: Supervision (equal); Validation (equal); Writing-review & editing (equal). Yong-Chao Su: Conceptualization (lead); Funding acquisition (lead); Methodology (equal); Software (supporting); Visualization (supporting); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT
DNA sequences: GenBank accessions MN881069- MN881072; KJ648441.1; KJ648369.1; KJ648430.1; KJ648426.1; KJ648436.1; MW549752; MW549751; KJ648385.1. Responde et al. (2021), Dryad, Dataset, https://doi.org/10.5061/dryad.1ns1r

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### APPENDIX 1

(a) Collection information of *Argyrodes lanyuensis* samples used for morphological and molecular analyses with GenBank accession number

| No. | Sample number | PAIC | Island boundary | Latitude/Longitude | Gender (M/F/subadult) | Molecular Analyses (DNA No.) | GenBank accession number | Morphological Analyses (Yes/No) |
|-----|---------------|------|-----------------|--------------------|------------------------|-------------------------------|--------------------------|------------------------------|
| 1   | SU56.57.58–1  | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 582                      | MN881070                 | Yes                          |
| 2   | SU56.57.58–2  | Luzon | Luzon           | 13.663188/123.3325 | M                      | EEG 583                      | MN881070                 | Yes                          |
| 3   | SU56.57.58–3  | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 584                      | MN881070                 | No                           |
| 4   | SU54.55A      | Luzon | Luzon           | 13.663188/123.3325 |                       | Subadult EEG 536             | MN881070                 | No                           |
| 5   | SU54.55B      | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 585                      | MN881070                 | Yes                          |
| 6   | Su40.41A      | Luzon | Luzon           | 13.663188/123.3325 | M                      | EEG 349                      | MN881071                 | Yes                          |
| 7   | SU40.41B      | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 350                      | MN881070                 | Yes                          |
| 8   | SU40–41C      | Luzon | Luzon           | 13.663188/123.3325 | Subadult              | EEG 519                      | MN881070                 | No                           |
| 9   | SU40–41D      | Luzon | Luzon           | 13.663188/123.3325 | Subadult              | EEG 520                      | MN881070                 | No                           |
| 10  | SU42–43       | Luzon | Luzon           | 13.663188/123.3325 | Subadult              | EEG 521                      | MN881070                 | No                           |
| 11  | SU46.47B      | Luzon | Luzon           | 13.663188/123.3325 | M                      | EEG 351                      | MN881070                 | Yes                          |
| 12  | Su52.53A      | Luzon | Luzon           | 13.663188/123.3325 | M                      | EEG 330                      | MN881070                 | No                           |
| 13  | Su52.53B      | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 331                      | MN881070                 | No                           |
| 14  | Su52.53C      | Luzon | Luzon           | 13.663188/123.3325 | F                      | EEG 332                      | MN881070                 | No                           |
| 15  | Su32.33       | Luzon | Luzon           | 14.121641/121.353872 | Subadult              | EEG 345                      | MN881070                 | No                           |
| 16  | Su34.35A      | Luzon | Luzon           | 14.121641/121.353872 | Subadult              | EEG 337                      | MN881070                 | No                           |
| 17  | SU60 61       | Luzon | Luzon           | 13.663188/123.3325 | M                      | EEG 538                      | MN881072                 | No                           |
| 18  | Su355B        | Palawan | Palawan     | 9.5627778/126.228372 | Subadult              | EEG 335                      | MN881070                 | No                           |
| 19  | Su355A        | Palawan | Palawan     | 9.5627778/126.228372 | Subadult              | EEG 334                      | MN881070                 | No                           |
| 20  | Su347A        | Palawan | Palawan     | 9.5627778/126.228372 | Subadult              | EEG 354                      | MN881070                 | No                           |
| 21  | SU141–142–143| West  | Panay          | 11.026671/122.658891 | F                      | EEG 529                      | MN881070                 | Yes                          |
| 22  | SU141–142–143| West  | Panay          | 11.026671/122.658891 | F                      | EEG 530                      | MN881070                 | Yes                          |
| 23  | SU141–142–143| West  | Panay          | 11.026671/122.658891 | M                      | EEG 531                      | MN881070                 | Yes                          |
| 24  | SU141–142–143| West  | Panay          | 11.026671/122.658891 | M                      | EEG 532                      | MN881070                 | Yes                          |
| 25  | SU147–148A    | West  | Panay          | 11.026671/122.658891 | Subadult              | EEG 511                      | MN881070                 | No                           |
| 26  | SU147–148B    | West  | Panay          | 11.026671/122.658891 | Subadult              | EEG 512                      | MN881070                 | No                           |
| 27  | SU107         | West  | Visayas        | 10.50888889/123.1052778 | F                      | EEG 319                      | MN881070                 | No                           |
| 28  | SU110.111–A   | West  | Visayas        | 10.50888889/123.1052778 | F                      | EEG 586                      | MN881070                 | Yes                          |
| 29  | SU110.111–B   | West  | Visayas        | 10.50888889/123.1052778 | F                      | EEG 587                      | MN881070                 | Yes                          |
| 30  | SU110–111–C   | West  | Visayas        | 10.50888889/123.1052778 | Subadult              | EEG 510                      | MN881070                 | No                           |
| 31  | SU134–A       | West  | Visayas        | 11.026671/122.658891 | F                      | EEG 580                      | MN881070                 | Yes                          |
| 32  | SU134–B       | West  | Visayas        | 11.026671/122.658891 | Subadult              | EEG 581                      | MN881070                 | No                           |
| 33  | SU118         | West  | Visayas        | 10.50888889/123.1052778 | F                      | EEG 333                      | MN881070                 | No                           |
| 34  | SU141.115.116B| West  | Visayas        | 10.50888889/123.1052778 | Subadult              | EEG 346                      | MN881070                 | No                           |
| No. | Sample number | PAIC | Island boundary | Latitude/Longitude | Gender (M/F/subadult) | Molecular Analyses (DNA No.) | GenBank accession number | Morphological Analyses (Yes/No) |
|-----|---------------|------|-----------------|--------------------|-----------------------|-----------------------------|-------------------------|-----------------------------|
| 35  | Su106–2       | West Visayas | Negros          | 10.5088889/123.1052778 | F                     | EEG 311                    | MN881070                | Yes                         |
| 36  | Su94.95.96    | West Visayas | Negros          | 10.5088889/123.1052778 | Subadult             | EEG 339                   | MN881070                | No                          |
| 37  | SU97–98       | West Visayas | Negros          | 10.5088889/123.1052778 | F                     | EEG 509                   | MN881070                | Yes                         |
| 38  | SU145–146     | West Visayas | Panay           | 11.026671/122.658891 | M                     | EEG 513                   | MN881070                | Yes                         |
| 39  | Su138.139B    | West Visayas | Panay           | 11.026671/122.658891 | M                     | EEG 323                   | MN881070                | Yes                         |
| 40  | Su108.109     | West Visayas | Negros          | 10.5088889/123.1052778 | Subadult             | EEG 343                   | MN881070                | No                          |
| 41  | SU2096        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 545               | MN881070                  | No                      |                             |
| 42  | SU2106        | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 525               | MN881070                  | No                      |                             |
| 43  | Su320.321A    | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 340               | MN881070                  | Yes                     |                             |
| 44  | Su320.321A    | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 347               | MN881070                  | No                      |                             |
| 45  | SU320–321 B   | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 527               | MN881070                  | No                      |                             |
| 46  | SU320–321 C   | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 528               | MN881070                  | No                      |                             |
| 47  | SU2102        | Mindanao     | Mindanao        | 9.052554/125.610306  | F EEG 499             | MN881070                  | Yes                     |                             |
| 48  | SU2103A       | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 500               | MN881070                  | No                      |                             |
| 49  | SU2103B       | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 501               | MN881070                  | No                      |                             |
| 50  | SU2126C       | Mindanao     | Mindanao        | 7.637164/124.045471  | EEG 504               | MN881070                  | No                      |                             |
| 51  | Su318.319A    | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 342               | MN881070                  | No                      |                             |
| 52  | SU318–319B    | Mindanao     | Mindanao        | 7.5855556/125.9865278| EEG 523               | MN881070                  | No                      |                             |
| 53  | SU2089        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 524               | MN881070                  | Yes                     |                             |
| 54  | SU118B        | Mindanao     | Mindanao        | 7.637164/124.045471  | EEG 507               | MN881070                  | No                      |                             |
| 55  | SU118C        | Mindanao     | Mindanao        | 7.637164/124.045471  | EEG 508               | MN881070                  | No                      |                             |
| 56  | SU2076A       | Mindanao     | Mindanao        | 8.172717/126.228372  | EEG 493               | MN881070                  | Yes                     |                             |
| 57  | SU2076B       | Mindanao     | Mindanao        | 8.172717/126.228372  | EEG 494               | MN881070                  | Yes                     |                             |
| 58  | SU2086A       | Mindanao     | Mindanao        | 8.172717/126.228372  | EEG 495               | MN881070                  | No                      |                             |
| 59  | SU2086 C      | Mindanao     | Mindanao        | 8.172717/126.228372  | EEG 497               | MN881070                  | No                      |                             |
| 60  | SU2109 B      | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 534               | MN881070                  | Yes                     |                             |
| 61  | SU2109 C      | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 535               | MN881070                  | No                      |                             |
| 62  | SU2097        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 541               | MN881070                  | No                      |                             |
| 63  | SU2095        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 542               | MN881070                  | Yes                     |                             |
| 64  | SU2098        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 544               | MN881070                  | No                      |                             |
| 65  | SU2101        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 498               | MN881070                  | No                      |                             |
| 66  | Su408A        | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 352               | MN881070                  | No                      |                             |
| 67  | Su408B        | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 353               | MN881070                  | No                      |                             |
| 68  | SU408C        | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 540               | MN881070                  | No                      |                             |
| 69  | SU408 D       | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 539               | MN881070                  | No                      |                             |
| 70  | SU2110 A      | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 515               | MN881069                  | Yes                     |                             |
| 71  | SU2110 B      | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 516               | MN881070                  | Yes                     |                             |
| 72  | SU2110 C      | Mindanao     | Mindanao        | 7.891919/123.77847   | EEG 517               | MN881070                  | Yes                     |                             |
| 73  | SU2099        | Mindanao     | Mindanao        | 9.052554/125.610306  | EEG 543               | MN881070                  | No                      |                             |
| 74  | SU367         | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 328               | MN881070                  | No                      |                             |
| 75  | Su405A        | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 338               | MN881070                  | No                      |                             |
| 76  | SU405B        | Mindanao     | Mindanao        | 7.0075/122.0230556   | EEG 536               | MN881070                  | No                      |                             |
| No. | Sample number | PAIC      | Island boundary | Latitude/Longitude      | Gender (M/F/subadult) | Molecular Analyses (DNA No.) | GenBank accession number | Morphological Analyses (Yes/No) |
|-----|---------------|-----------|-----------------|-------------------------|-----------------------|-------------------------------|--------------------------|-------------------------------|
| 77  | SU2091        | Mindanao  | Mindanao        | 9.052554/125.610306     | Subadult EEG 546      | MN881070                      | Yes                      |
| 78  | Su400A        | Mindanao  | Mindanao        | 7.0075/122.023055       | F EEG 341             | MN881070                      | Yes                      |
| 79  | SU2107        | Mindanao  | Mindanao        | 7.891919/123.77847      | M EEG 514             | MN881070                      | Yes                      |
| 80  | SU2126D       | Mindanao  | Mindanao        | 7.637164/124.045471     | Subadult EEG 505      | MN881070                      | No                       |
| 81  | SU2108        | Mindanao  | Mindanao        | 7.891919/123.77847      | F EEG 518             | MN881070                      | Yes                      |
| 82  | SU314–315     | Mindanao  | Mindanao        | 7.58555556/125.965278   | F EEG 522             | MN881070                      | Yes                      |
| 83  | SU511         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 306      | MN881069                      | No                       |
| 84  | SU489         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 310      | MN881069                      | No                       |
| 85  | Su114A        | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 336      | MN881070                      | No                       |
| 86  | Su503         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 329      | MN881070                      | No                       |
| 87  | Su181.182.M1  | –         | Orchid island   | 22.0097222/121.570865   | M EEG 305             | MN881070                      | Yes                      |
| 88  | Su284.285     | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 317      | MN881070                      | No                       |
| 89  | Su520         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 304      | MN881070                      | No                       |
| 90  | Su483         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 307      | MN881070                      | No                       |
| 91  | Su508         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 324      | MN881070                      | No                       |
| 92  | Su521         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 344      | MN881070                      | No                       |
| 93  | Su177.178     | –         | Orchid island   | 22.0097222/121.570865   | F EEG 303             | MN881070                      | Yes                      |
| 94  | Su499         | –         | Orchid island   | 22.0097222/121.570865   | Subadult EEG 318      | MN881070                      | No                       |
| 95  | SU175         | –         | Orchid island   | 22.0097222/121.570865   | F EEG 290             | MN881071                      | Yes                      |
| 96  | SU46.47A      | Luzon     | Luzon           | 13.663188/123.3325       | M – –                 | –                             | Yes                      |
| 97  | SU46.47C      | Luzon     | Luzon           | 13.663188/123.3325       | M – –                 | –                             | Yes                      |
| 98  | SU46.47D      | Luzon     | Luzon           | 13.663188/123.3325       | F – –                 | –                             | Yes                      |
| 99  | SU138_139 M   | West Visayas | Panay        | 11.026671/122.658891     | M – –                 | –                             | Yes                      |
| 100 | SU141_142_143E | West Visayas | Panay      | 11.026671/122.658891     | M – –                 | –                             | Yes                      |
| 101 | SU138_139F    | West Visayas | Panay      | 11.026671/122.658891     | F – –                 | –                             | Yes                      |
| 102 | SU134_135A    | West Visayas | Panay      | 11.026671/122.658891     | F – –                 | –                             | Yes                      |
| 103 | SU2115        | Mindanao  | Mindanao        | 7.637164/124.045471     | M – –                 | –                             | Yes                      |
| 104 | SU2116        | Mindanao  | Mindanao        | 7.637164/124.045471     | M – –                 | –                             | Yes                      |
| 105 | SU2117M1      | Mindanao  | Mindanao        | 7.637164/124.045471     | M – –                 | –                             | Yes                      |
| 106 | SU2117M2      | Mindanao  | Mindanao        | 7.637164/124.045471     | M – –                 | –                             | Yes                      |
| 107 | SU2118        | Mindanao  | Mindanao        | 7.637164/124.045471     | M – –                 | –                             | Yes                      |
| 108 | SU2090        | Mindanao  | Mindanao        | 9.052554/125.610306     | M – –                 | –                             | Yes                      |
| 109 | SU325M1       | Mindanao  | Mindanao        | 8.056852/126.219838     | M – –                 | –                             | Yes                      |
| 110 | SU325M2       | Mindanao  | Mindanao        | 8.056852/126.219838     | M – –                 | –                             | Yes                      |
| No. | Sample number | PAIC | Island boundary | Latitude/Longitude | Gender (M/F/subadult) | Molecular Analyses (DNA No.) | GenBank accession number | Morphological Analyses (Yes/No) |
|-----|---------------|------|-----------------|--------------------|-----------------------|-------------------------------|---------------------------|-------------------------------|
| 111 | SU235M3       | Mindanao | Mindanao | 8.056852/126.219838 | M | - | - | Yes |
| 112 | SU2111        | Mindanao | Mindanao | 7.891919/123.77847 | F | - | - | Yes |
| 113 | SU2114        | Mindanao | Mindanao | 7.637164/124.045471 | F | - | - | Yes |
| 114 | SU2117        | Mindanao | Mindanao | 7.637164/124.045471 | F | - | - | Yes |
| 115 | SU2118F1      | Mindanao | Mindanao | 7.637164/124.045471 | F | - | - | Yes |
| 116 | SU2118F2      | Mindanao | Mindanao | 7.637164/124.045471 | F | - | - | Yes |
| 117 | SU2092        | Mindanao | Mindanao | 9.052554/125.610306 | F | - | - | Yes |
| 118 | SU2102F2      | Mindanao | Mindanao | 9.052554/125.610306 | F | - | - | Yes |
| 119 | SU407         | - | Orchid island | 7.0075/122.0230556 | M | - | - | Yes |
| 120 | SU181_182M2   | - | Orchid island | 22.0096611/121.57086111111111 | M | - | - | Yes |
| 121 | SU484         | - | Orchid island | 22.0096611/121.57086111111111 | M | - | - | Yes |
| 122 | SU487         | - | Orchid island | 22.0096611/121.57086111111111 | M | - | - | Yes |
| 123 | SU491         | - | Orchid island | 22.0096611/121.57086111111111 | M | - | - | Yes |
| 124 | SU1351        | - | Orchid island | 22.0295240264385/121.57560297288 | M | - | - | Yes |
| 125 | SU1368        | - | Orchid island | 22.009242000582/121.572734015062 | M | - | - | Yes |
| 126 | SU1387        | - | Orchid island | 22.0097780227661/121.574569987133 | M | - | - | Yes |
| 127 | SU1697        | - | Orchid island | 22.6631840039044/121.501822024583 | M | - | - | Yes |
| 128 | SU1706M       | - | Orchid island | 22.0291449967771/121.576519031077 | M | - | - | Yes |
| 129 | SU181_182F1   | - | Orchid island | 22.0096611/121.57086111111111 | F | - | - | Yes |
| 130 | SU181_182F2   | - | Orchid island | 22.0096611/121.57086111111111 | F | - | - | Yes |
| 131 | SU484         | - | Orchid island | 22.0096611/121.57086111111111 | F | - | - | Yes |
| 132 | SU1383        | - | Orchid island | 22.0095020006946/121.573526021093 | F | - | - | Yes |
| 133 | SU1701        | - | Orchid island | 22.0281270146369/121.577498959377 | F | - | - | Yes |
| 134 | SU1706M       | - | Orchid island | 22.0291449967771/121.576519031077 | F | - | - | Yes |

(b) Collection information of outgroup samples used for molecular analyses with GenBank accession number

| Species                  | Sample number | Accession number |
|--------------------------|---------------|-----------------|
| *Faiditus xiphas*        | SU694         | KJ648441.1      |
| *Faiditus xiphas*        | SU629         | KJ648369.1      |
| *Argyrodes rainbowi*     | SU527         | KJ648430.1      |
| *Argyrodes nasutus*      | SU396         | KJ648426.1      |
| *Argyrodes tripunctatus* | SU334         | KJ648436.1      |
| *Argyrodes rainbowi*     | SU181_182M2   | KJ648385.1      |
| *Argyrodes kulczynski*   | SU181_182F1   | KJ648387.1      |
## APPENDIX 2

### Total number of individuals examined per PAIC island

| PAIC       | Number of individuals examined for morphological analyses | Number of individuals examined for molecular analyses |
|------------|----------------------------------------------------------|------------------------------------------------------|
|            | Male | Female | Male | Female |
| Orchid island | 10    | 8      | 13   |        |
| Luzon      | 5     | 4      | 13   |        |
| Palawan    | 3     | -      | 3    |        |
| West Visayas | 6    | 8      | 23   |        |
| Mindanao   | 13    | 18     | 43   |        |
| Total number of Individuals | 37    | 38     | 95   |        |

Abbreviation: PAIC, Pleistocene Aggregate Island Complexes.

## APPENDIX 3

### Morphometrics of A. lanyuensis male and female samples with 28 and 23 variables, respectively

| Characters code | Characters definition | Male (N = 37) | Female (N = 38) |
|-----------------|-----------------------|---------------|-----------------|
|                 | Mean ± SD (mm)        | Range (mm)    | Mean ± SD (mm)  | Range (mm)    |
| CL              | Carapace Length       | 1.396 ± 0.147 | 1.12 ± 1.804    | 1.158 ± 0.088 | 0.918 ± 1.326 |
| TL              | Total Length          | 2.198 ± 0.145 | 1.900 ± 2.631   | 0.644 ± 0.630 | 0.352 ± 2.947 |
| CW              | Carapace Width        | 0.453 ± 0.073 | 0.344 ± 0.604   | 2.440 ± 0.589 | 0.645 ± 3.173 |
| L1F             | Leg I Femur           | 2.474 ± 0.486 | 1.010 ± 3.297   | 2.621 ± 0.227 | 1.998 ± 3.128 |
| L1PT            | Leg I Patella & Tibia | 2.439 ± 0.243 | 1.945 ± 3.112   | 2.403 ± 0.239 | 1.738 ± 2.802 |
| L1M             | Leg I Metatarsus      | 2.294 ± 0.342 | 0.831 ± 3.068   | 2.231 ± 0.298 | 0.788 ± 2.683 |
| L1T             | Leg I Tarsus          | 0.930 ± 0.097 | 0.689 ± 1.108   | 0.935 ± 0.096 | 0.630 ± 1.119 |
| L1TOT           | Leg I Total Length    | 8.239 ± 0.875 | 6.368 ± 10.585  | 8.190 ± 0.690 | 6.696 ± 9.648 |
| L2F             | Leg II Femur          | 1.033 ± 0.159 | 0.303 ± 1.227   | 1.025 ± 0.108 | 0.815 ± 1.257 |
| L2PT            | Leg II Patella & Tibia| 0.922 ± 0.100 | 0.623 ± 1.099   | 0.845 ± 0.106 | 0.578 ± 1.033 |
| L2M             | Leg 2 Metatarsus      | 0.696 ± 0.071 | 0.445 ± 0.807   | 0.725 ± 0.102 | 0.439 ± 0.929 |
| L2T             | Leg II Tarsus         | 0.413 ± 0.058 | 0.314 ± 0.493   | 0.426 ± 0.056 | 0.303 ± 0.509 |
| L2TOT           | Leg II Total Length   | 3.064 ± 0.301 | 2.237 ± 3.508   | 0.678 ± 0.923 | 0.231 ± 3.569 |
| L3F             | Leg III Femur         | 0.550 ± 0.074 | 0.416 ± 0.770   | 0.555 ± 0.101 | 0.388 ± 0.921 |
| L3PT            | Leg III Patella & Tibia| 0.413 ± 0.058 | 0.295 ± 0.626   | 0.428 ± 0.076 | 0.323 ± 0.686 |
| L3M             | Leg III Metatarsus    | 0.306 ± 0.036 | 0.234 ± 0.418   | 0.319 ± 0.064 | 0.207 ± 0.532 |
| L3T             | Leg III Tarsus        | 0.243 ± 0.042 | 0.156 ± 0.339   | 0.268 ± 0.041 | 0.187 ± 0.347 |
| L3TOT           | Leg III Total Length  | 1.512 ± 0.167 | 1.196 ± 2.153   | 1.569 ± 0.247 | 1.224 ± 2.484 |
| L4F             | Leg IV Femur          | 0.879 ± 0.094 | 0.603 ± 1.010   | 0.879 ± 0.183 | 0.401 ± 1.153 |
| L4PT            | Leg IV Patella & Tibia| 0.648 ± 0.083 | 0.413 ± 0.852   | 0.636 ± 0.129 | 0.324 ± 0.867 |
| L4M             | Leg IV Metatarsus     | 0.467 ± 0.055 | 0.311 ± 0.654   | 0.481 ± 0.094 | 0.205 ± 0.693 |
| L4T             | Leg IV Tarsus         | 0.309 ± 0.036 | 0.223 ± 0.380   | 0.324 ± 0.064 | 0.174 ± 0.512 |
| L4TOT           | Leg IV Total Length   | 2.303 ± 0.223 | 1.582 ± 2.811   | 2.320 ± 0.405 | 1.186 ± 3.225 |
| PL              | Palpal Length        | 2.288 ± 0.208 | 1.983 ± 2.749   | -             | -             |
| BL              | Bulb length          | 0.547 ± 0.049 | 0.414 ± 0.627   | -             | -             |
| MA              | Median Apophysis     | 0.164 ± 0.024 | 0.112 ± 0.238   | -             | -             |
| AP              | Accessorial Apophysis| 0.167 ± 0.034 | 0.093 ± 0.264   | -             | -             |
| EL              | Embolus length       | 0.834 ± 0.091 | 0.557 ± 1.053   | -             | -             |
APPENDIX 4

PLS-DA loading weight values for the 28 variables of males and 23 variables for females listed from the most important to the least important variable

| Characters* | Component 1 | Component 2 | Characters* | Component 1 | Component 2 |
|-------------|-------------|-------------|-------------|-------------|-------------|
| Male (n = 37) |             |             | Female (n = 38) |             |             |
| L1F          | 0.38351198  | -0.45315538 | CW          | 0.43181394  | L1PT        | 0.45755393  |
| L1TOT        | 0.30376023  | -0.38248365 | L2TOT       | 0.33830543  | L2M         | 0.38784592  |
| L1PT         | 0.3027413   | -0.24934683 | L2PT        | 0.32477021  | L1F         | 0.35094616  |
| L2TOT        | 0.28166924  | 0.24539086  | L2F         | 0.30267685  | L1TOT       | 0.31340983  |
| EL           | -0.263136   | 0.23294068  | L3M         | 0.28311415  | CW          | -0.25730231 |
| L1M          | 0.24531684  | 0.22777035  | TL          | -0.26712752 | CL          | -0.23350157 |
| L2PT         | 0.23990613  | 0.22514304  | L3F         | 0.26598773  | L2TOT       | -0.22787851 |
| L2F          | 0.23806233  | -0.21431921 | L3TOT       | 0.2550646   | L2PT        | 0.22516641  |
| L1T          | 0.22988256  | -0.1927407  | L3PT        | 0.17869766  | L4F         | 0.18745189  |
| AP           | -0.22721389 | -0.18158622 | L4PT        | 0.17201315  | L2F         | 0.16876584  |
| L2M          | 0.21167709  | 0.17199691  | L2T         | 0.17095882  | L4TOT       | 0.16272747  |
| L2T          | 0.20418632  | 0.15818057  | L4TOT       | 0.1570943   | TL          | 0.14710686  |
| L4PT         | 0.17081701  | -0.15451271 | L4M         | 0.15582413  | L3PT        | 0.1333082   |
| L3F          | -0.16459783 | -0.15382933 | L4T         | 0.15414956  | L4PT        | 0.12769284  |
| L4F          | 0.13855817  | -0.14256483 | CL          | 0.1355591   | L4M         | 0.1216094   |
| CL           | -0.13085052 | -0.13641849 | L3T         | 0.11292653  | L2T         | 0.09883316  |
| L4TOT        | 0.12354748  | -0.13456348 | L3F         | 0.09088741  | L1T         | 0.09503677  |
| PL           | -0.11906856 | 0.13064304  | L4F         | -0.07524103 | L1PT        | 0.06081118  |
| L3TOT        | -0.11391175 | -0.1295035  | L2M         | 0.06857097  | L3F         | 0.05480712  |
| L3PT         | -0.09760581 | -0.12116636 | L1T         | -0.02694092 | L3TOT       | 0.05449961  |
| L3M          | -0.06407602 | -0.12047511 | L1TOT       | -0.02037519 | L3M         | -0.05382282 |
| L3T          | 0.05026468  | -0.10240951 | L1M         | 0.01787341  | L4T         | 0.05072841  |
| BL           | 0.04300805  | -0.09576307 | L1F.S       | 0.00526043  | L3T         | 0.03043119  |
| MA           | -0.0342114  | 0.0774619   |              |              |              |
| CW           | -0.01969183 | -0.04569395 |              |              |              |
| TL           | 0.01933653  | -0.04537034 |              |              |              |
| L4T          | 0.00938999  | 0.0314079   |              |              |              |
| L4M          | 0.00025733  | -0.00843207 |              |              |              |
APPENDIX 5
Body and male palp characters used for multivariate analyses (Please refer to Appendix 3 for the definition of each character.)
APPENDIX 6

PLS-DA score plots of *A. lanyuensis* males (a–b) and females (c–d) based on 28 and 265 morphometrics, respectively. Individuals are plotted against components 1 and 2, grouped according to current island boundary (a and c) and locality (b and d).
APPENDIX 7
Isolation by distance (IBD) analysis using mantel test between the Dgeo (spatial Euclidean distance) and Dgen (Edward's genetic distance). Color contours indicate kernel density estimation, where higher densities are shown by red color.

APPENDIX 8
Pairwise $F_{ST}$ estimates among island boundaries. Red colored cells show $F_{ST}$ values, while blue colored cells show the corresponding $p$-values.

| Current island boundary | Orchid | Panay | Mindanao | Negros | Luzon | Palawan |
|------------------------|--------|-------|----------|--------|-------|---------|
| Orchid                 | NA     | -0.030| 0.054    | -0.075| -0.080| -0.339  |
| Panay                  | 0.922  | NA    | 0.000    | 0.000  | -0.093| 0.000   |
| Mindanao               | 0.000  | 0.901 | NA       | -0.124| 0.001 | -0.355  |
| Negros                 | 0.998  | 0.000 | 0.901    | NA     | -0.132| 0.000   |
| Luzon                  | 0.994  | 0.998 | 0.294    | 0.998  | NA    | -0.395  |
| Palawan                | 0.998  | 0.000 | 0.901    | 0.000  | 0.998 | NA      |
APPENDIX 9
Statistical dispersal-vicariance S-DIVA biogeographical reconstruction analysis using RASP
APPENDIX 10
Bayesian binary MCMC biogeographical reconstruction analysis using RASP