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Research Article

Historical Collections Help Trace the Origin of Introduced and Native Populations of the Endangered Butterfly, *Troides magellanus sonani* (Lepidoptera: Papilionidae)

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Island endemics are highly vulnerable due to restricted ranges, small population size, and genetic erosion. The Magellan birdwing on Lanyu Island (*Troides magellanus sonani*) exemplifies this vulnerability. Despite decades of legal protection, whether it constitutes an independent evolutionarily significant unit (ESU) has remained uncertain. Recent reports of introductions into northern Taiwan highlight the need for a genetic assessment. Here, we applied genome skimming to analyze 46 specimens of the Magellan birdwing, mostly from museum collections. Conservation genetic and phylogenetic analyses revealed that the Lanyu population is genetically distinct, derived from a south-to-north expansion originating in the southern Philippines and colonizing Lanyu around 210,000 years ago. Haplotypes from Lanyu, even spanning a century, remained nearly identical, and those from northern Taiwan were identical to those from Lanyu, confirming human-mediated introductions. These findings establish the Lanyu population as an ESU and emphasize the urgency of targeted conservation actions. This study demonstrates how integrating next-generation sequencing (NGS) with museum specimens can provide critical insights into the evolution and conservation of endangered insular taxa while minimizing impacts on wild populations.

1. Introduction

Island species, restricted by small and isolated ranges, are highly vulnerable to natural disturbances and anthropogenic pressures [1–3]. Such pressures further intensify resource scarcity on islands, limiting the long-term viability of resident species. Some taxa persist solely on local resources, whereas others are maintained as sink populations through repeated influxes of immigrants from adjacent regions [4]. Evaluating the population structure and ecological constraints of island taxa, particularly those under legal protection, is therefore essential for developing evidence-based conservation and management strategies.

The Magellan birdwing (*Troides magellanus*), a typical insular species, has been listed as a protected species under Taiwan's Wildlife Conservation Act since 1989 [5]. This butterfly is renowned for its large size (Figure 1) and brilliantly iridescent wings [6]. It occurs widely across the Philippine archipelago and extends northward to Lanyu Island, off the southeastern coast of Taiwan [7]. The Lanyu population,

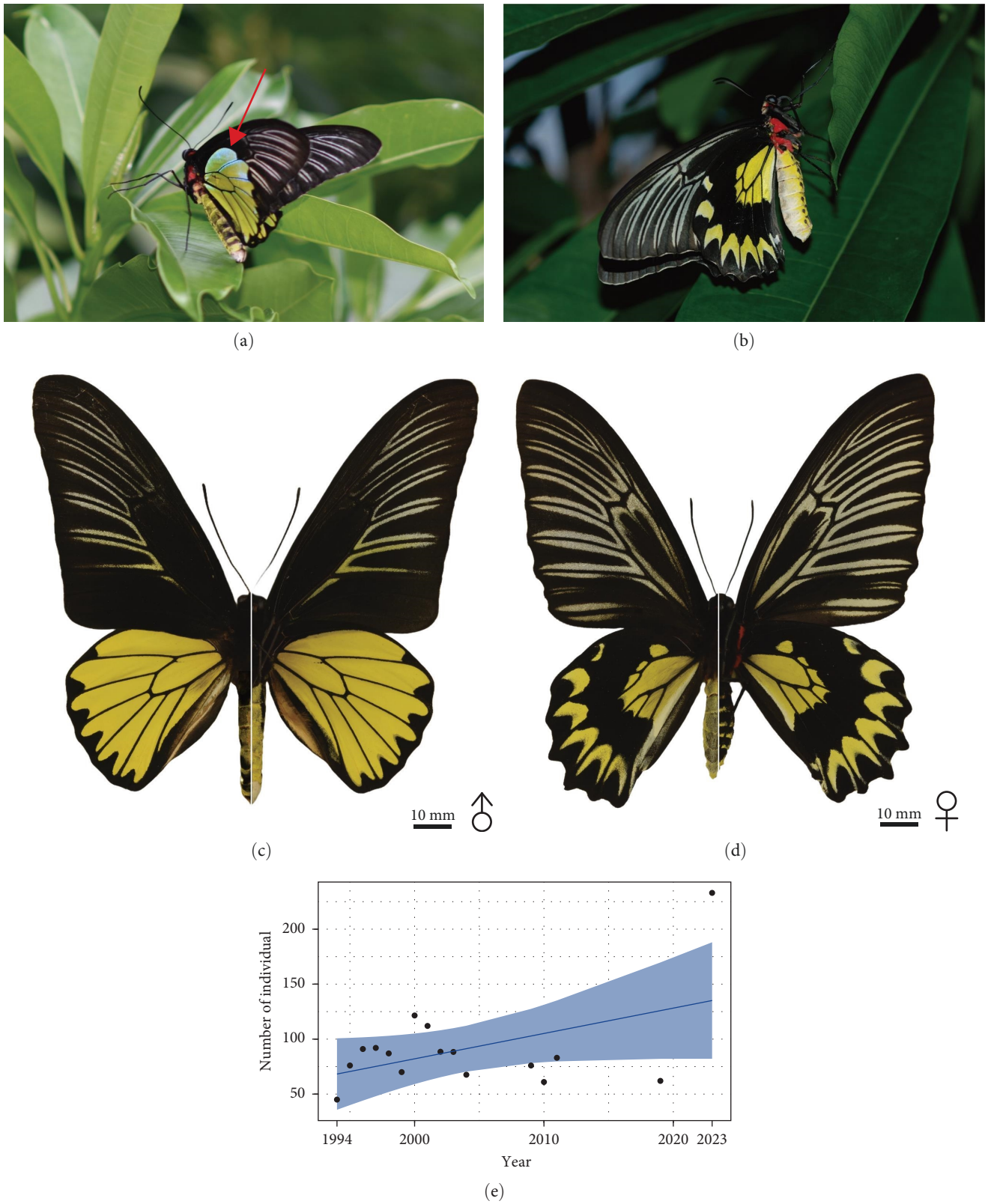

FIGURE 1: Wing patterns and population trend of the Magellan birdwing on Lanyu. (a) Male, with a red arrow indicating pearl-like coloration; (b) female; (c) male, showing dorsal (left) and ventral (right) views; (d) female; and (e) annual counts of observed adults from 1993 to 2023. The mean population abundance was 80 individuals, but the temporal trend was not statistically significant (slope = 2.3, $p = 0.07$).

situated at the northernmost limit of the species' distribution, has often been recognized as a distinct endemic subspecies, *Troides magellanus sonani* [8]. Historically, the butterfly was considered common on Lanyu until the 1960s, but populations declined sharply during the 1970s owing to habitat degradation and overcollection [9–11]. In response, conservation efforts focused on maintaining its larval hostplant, *Aristolochia zollingeriana*, to facilitate population recovery [9, 10, 12, 13]. Current monitoring indicates that the Lanyu population persists at low but stable numbers (Figure 1). Nevertheless, its long-term viability remains uncertain, underscoring the need for more targeted and sustained conservation actions.

Despite these conservation actions, an unexpected event occurred when the Magellan birdwing was discovered at multiple non-native sites in northern Taiwan between December 2021 and May 2022 (Figure 2). Although rare records exist from southern and eastern Taiwan [5, 14–16], these were regarded as occasional strays with no evidence of permanent establishment. In contrast, the individuals observed in northern Taiwan are generally considered to have resulted from intentional releases. However, their precise origin remains uncertain, reflecting the lack of diagnostic characters that distinguish regional populations.

Next-generation sequencing (NGS) technologies and associated bioinformatics tools [17] provide powerful means to characterize population relationships and trace the origins of individuals. Importantly, NGS also enables the use of museum specimens, whose DNA is typically highly fragmented [18]. This approach reduces the need for destructive sampling of extant populations and allows temporal assessments of genetic variation through historical collections.

Here, we applied genome skimming [19] to recover mitochondrial and nuclear sequences from historical specimens of the Magellan birdwing. This approach allows us to investigate the origin of the recently introduced individuals in northern Taiwan and to assess the molecular characteristics of the Lanyu population. We propose two possible scenarios (Figure 3): (i) the Lanyu population has experienced limited gene flow with other regions, and its small population size has promoted genetic drift [20], leading to the fixation of unique haplotypes (Figure 3A); or (ii) the Lanyu population is part of a larger metapopulation connected to the Philippines, functioning as a sink population that receives immigrants from Philippine sources (Figure 3B). Under the latter scenario, Lanyu haplotypes would not be unique but instead represent a subset of Philippine genetic diversity, consistent with higher levels of interisland movement. Finally, we aim to determine the origin of the introduced individuals in northern Taiwan and propose conservation strategies based on our findings.

2. Materials and Methods

2.1. Sampling. We analyzed mitogenomic and nuclear sequences from 46 individuals of the Magellan birdwing, including 39 obtained from museum collections (Supporting Information 1: Table S1). These specimens were sourced from the National Museum of Natural Science (NMNS, Taiwan), the National Taiwan Normal University (NTNU, Taiwan), the Taiwan Agricultural Research Institute (TARI, Taiwan), the Taiwan Biodiversity Research Institute (TBRI, Taiwan), and the University Museum, and the University of Tokyo (UMUT, Japan). Four specimens were collected from introduced sites in northern Taiwan, and three from Lanyu under official permits. In total, 27 Lanyu samples collected between 1920 and 2022 provided a historical perspective on temporal genetic changes. Fifteen museum specimens from the Philippines (Luzon, Bohol, Leyte, and Mindanao) were used for origin tracing. Additionally, three Philippine barcode sequences were retrieved from BOLD Systems (NIFB051-06, NIFB054-06) and GenBank (KT179917) to expand comparative coverage. Six outgroup species (three *Ornithoptera*, two *Troides*, and one *Trogonoptera* species) were used for phylogenetic analyses.

2.2. Ethics Statement. No vertebrates or cephalopods were involved in this study. The only protected taxon was *T. m. sonani* from Lanyu, listed as an endangered species in Taiwan. Fieldwork was conducted under permits issued by the Forestry and Nature Conservation Agency, Taiwan (Permit Numbers: 111YULI027 and D112002034, valid from 2022 to 2024). To minimize impacts on the wild population, three individuals were reared from eggs or larvae and used for molecular analysis. All other samples came from museum collections, and DNA sampling was performed with permission from each institution.

2.3. DNA Extraction and Sequence Assembly. Laboratory procedures followed previously published protocols [21, 22]. Genomic DNA was extracted from one leg per specimen, with an extended lysis step (2 h) for historical material. Libraries were constructed using the Celero DNA-Seq Library Preparation Kit (NuGEN, Redwood City, CA, USA) and sequenced on an Illumina NovaSeq 6000 platform. Fresh tissues were fragmented to 300–500 bp using a Covaris S220 ultrasonicator (Covaris, Inc., Woburn, MA), while DNA from historical specimens was already highly degraded (<100 bp) and required no further fragmentation.

Assembly targeted high-copy loci, including the mitochondrial genome and nuclear ribosomal DNA (18S–28S). Raw reads were trimmed for adaptors and bases with low-quality regions (<Q20), then mapped to the mitogenome and 18S–28S references [21, 22] in CLC Genomics Workbench 20 (CLC Bio, Aarhus, Denmark) with a 70% similarity threshold. Mapped reads were assembled de novo using the CLC assembler and MEGAHIT v1.2.9 [23] at 97% similarity. Consensus contigs were edited and validated in Sequencher 4.10 (Gene Codes, Ann Arbor, MI, USA). To further ensure sequence accuracy, independent de novo assemblies (97% similarity) were conducted for each specimen, filtered against reference sequences at 70% similarity, and cross-validated with mapped assemblies to confirm consistency.

2.4. Gene Annotation, Alignment, and Model Selection. Assembled contigs were verified using BLAST. Mitochondrial genes were annotated in MITOS2 [24], and gene boundaries

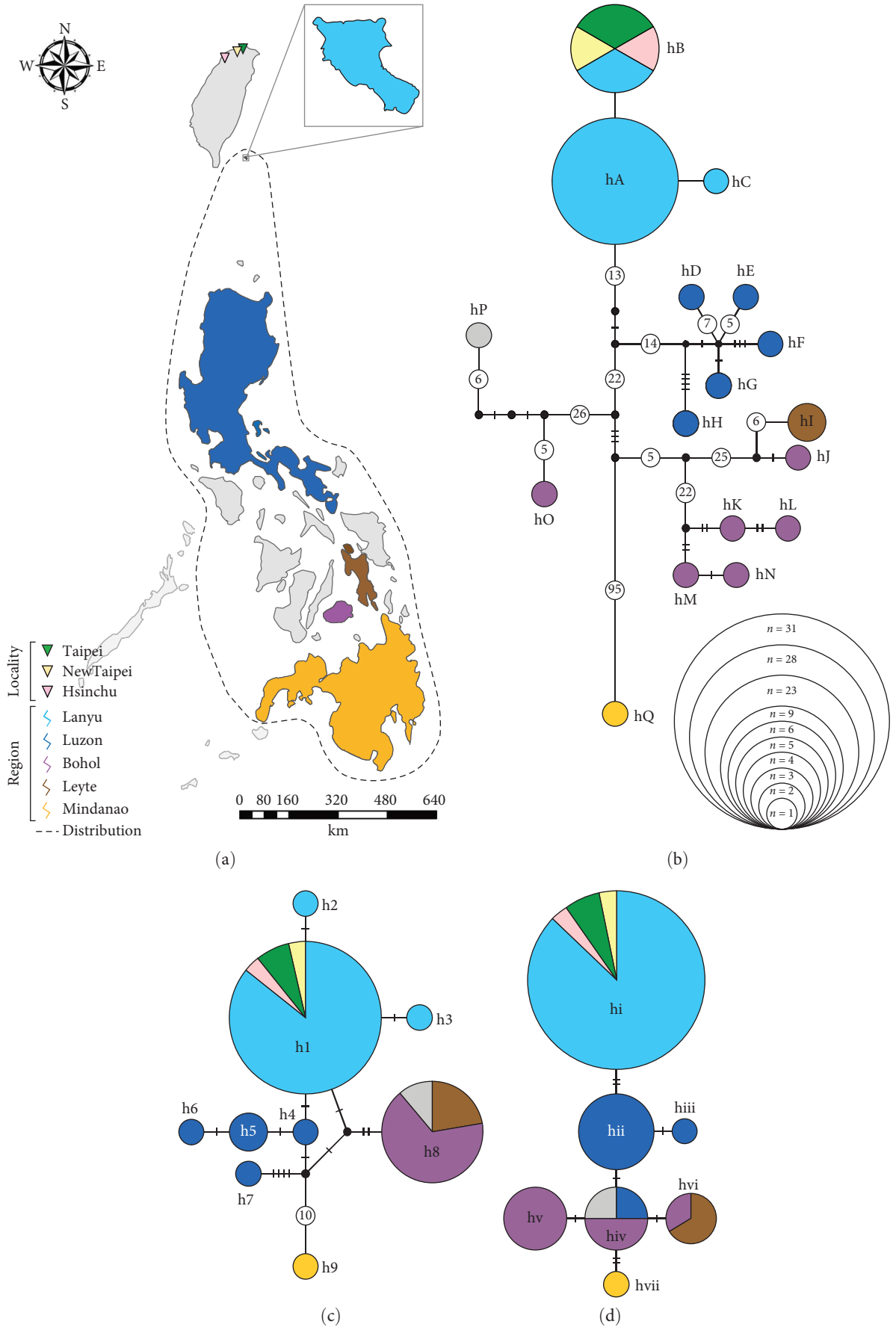

FIGURE 2: Distribution and haplotype networks of the Magellan birdwing. (a) The dashed line indicates the natural distribution, with sampling localities represented in different colors. Triangles mark the three introduced sites in northern Taiwan. Haplotype networks based on (b) 37 mitochondrial genes (dataset B), (c) nuclear 18S–28S sequences (dataset C), and (d) the barcode region (dataset D).

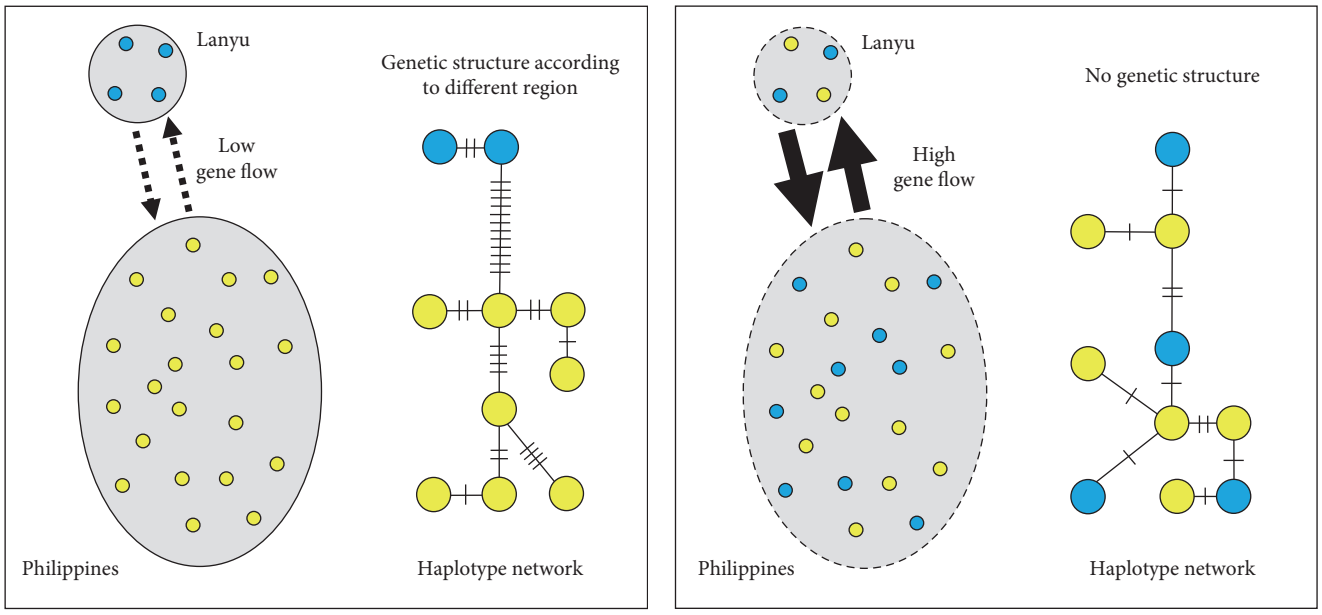

FIGURE 3: Two hypothesized scenarios for the genetic composition of Magellan birdwing populations between Lanyu and the Philippines. (a) Low gene flow, with distinct haplotypes restricted to each locality, and (b) high gene flow, resulting in shared haplotypes and weak differentiation.

were validated against the published mitogenome of *Papilio maraho* [51]. Protein-coding genes (PCGs) were checked to ensure the absence of internal stop codons. Nuclear loci (18S, 5.8S, 28S, ITS1, and ITS2) were annotated following Tseng et al. [22]. All newly obtained sequences were deposited in GenBank under accession numbers PP506337–PP506382 and PX123983–PX124028 (Supporting Information 1: Table S1).

Four datasets were generated: dataset A, comprising 46 Magellan birdwings plus six outgroups, for phylogenetic reconstruction and divergence dating; dataset B, containing only mitogenomic genes; dataset C, containing nuclear sequences only; and dataset D, consisting of barcode region plus three public sequences, used to validate dataset B. Alignments were performed in MEGA 11 [25] using MUSCLE [26]. PCGs were aligned according to amino acid translation, while tRNAs, rRNAs, and nuclear regions were aligned under default settings.

Partitioning schemes and substitution models were estimated in PartitionFinder 2.1 [27]. To find the best-fit models, 11 initial partitions were defined, including the three codon positions of PCGs, tRNAs, rrnL, rrnS, and nuclear regions (18S, 5.8S, 28S, ITS1, and ITS2).

2.5. Population Genetics. Summary statistics, including the number of haplotypes (h), polymorphic sites (s), nucleotide diversity (π), and haplotype diversity (h_d), were calculated in DnaSP 6 [28]. Genetic distances were estimated under the Kimura-2-parameter (K2P) model in MEGA. Analyses of molecular variance (AMOVA) were performed in Arlequin 3.1 [29] under three grouping strategies: (i) two divisions (Lanyu vs. Philippines) following the classification of two subspecies (*sensu* [30]); (ii) three divisions (Lanyu, Luzon, Southern Philippines) following three subspecies (*sensu* [8]); and (iii) five divisions (Lanyu, Luzon, Bohol, Leyte, and Mindanao) based on sampling localities.

2.6. Phylogenetic Analyses. Phylogenetic relationships were inferred from dataset A. Maximum likelihood (ML) analysis was conducted in IQ-TREE 2.1.2 [31] under the best-fit models and partitioning scheme (Table 1). Nodal supports were evaluated through ultrafast bootstrapping (UB) [32] and the SH-like approximate likelihood ratio test (SH-aLRT) [33], each with 1000 replicates. Bayesian inference (BI) was performed in BEAST 1.10.4 [34], with a birth-death process. Three independent Markov chain Monte Carlo (MCMC) runs were performed, each comprising 100 million generations, and sampled every 1000 generations. The first 10% of samples were discarded as burn-in, and the remaining trees were summarized to generate a maximum clade credibility (MCC) tree. Haplotype networks were inferred using the median-joining method implemented in PopArt 1.7 [35] with 1000 iterations.

2.7. Divergence-Time Estimation. Divergence times were estimated in BEAST using dataset A. Because no fossil records are known for the genus *Troides* [36], 3 secondary calibrations from Condamine et al. [37] were applied: (i) origin of birdwings at 25.9 million years ago (Ma) with a normal distribution (95% highest posterior density [HPD]: 22–29.8 Ma; mean = 25.9, SD = 2), (ii) divergence of *Ornithoptera* and *Troides* at 19.3 Ma (95% HPD: 15.8–22.8 Ma; mean = 19.3, SD = 1.8), and (iii) crown age of *Ornithoptera* and *Troides* at 11.5 Ma (95% HPD: 7.6 Ma–15.4 Ma; mean = 11, SD = 2). An uncorrelated lognormal relaxed clock was applied, and the impact of the

Table 1: The best-fit partition scheme inferred by PartitionFinder.

Subset	Partition names	Best model
1	PCGcodonpos1	GTR+G
2	PCGcodonpos2	K81UF+I
3	PCGcodonpos3	HKY+G
4	rrnS, rrnL	GTR+I+G
5	tRNAs	HKY+I+G
6	18S	JC
7	28S, 5.8S	F81
8	ITS1, ITS2	HKY

Yule process tree prior was also tested under the same calibration settings.

3. Results

3.1. General Information. After trimming low-quality regions ($Q < 20$), a total of 2,480,164,663 reads were obtained from 46 Magellan birdwing specimens (Supporting Information 1: Table S1). Most samples contained all targeted loci at depths over 100x. The only exception was the oldest museum specimen (22LW02007), which recovered 29 mitochondrial genes but lacked the genes *atp8*, *trnE*, *trnF*, *trnG*, *trnQ*, *trnS1*, *trnS2*, and *trnW*, along with partial regions of nuclear 18S (672 bp missing) and 28S (942 bp missing). This specimen was included in phylogenetic analyses but excluded from genetic diversity estimates.

Dataset A ($N = 52$) totaled 22,330 bp and contained 3325 polymorphic sites. Dataset B ($N = 45$) comprised 14,920 bp with 243 polymorphic sites, while dataset C ($N = 45$) covered 7410 bp with 22 polymorphic sites (Table 2). Dataset D ($N = 49$) was trimmed to 476 bp from the original 658 bp with eight polymorphic sites due to incomplete public sequences.

3.2. Haplotype Distribution. Across datasets, most haplotypes were island-specific, showing minimal overlap among regions (Figure 2). From dataset B, a total of 17 haplotypes were identified, including three from Lanyu (hA–hC), and 14 from the Philippines: Luzon (hD–hH), Bohol and Leyte (hI–hO), and Mindanao (hQ). All individuals from northern Taiwan carried haplotype hB, identical to one from Lanyu. From dataset C, nine haplotypes were identified. Lanyu and northern Taiwan shared haplotype h1, which was distinct from Philippine haplotypes (h4–h9). Dataset D recovered seven haplotypes, showing a simplified but geographically consistent pattern compared with dataset B.

3.3. Genetic Diversity. Genetic diversity is summarized in Table 2. Within-island haplotype differences were smaller than among islands. In Lanyu, haplotypes differed by only 1–2 mutational steps, despite representing a temporal span of more than a century (1920–2022). Both mitogenomic and nuclear datasets consistently showed lower nucleotide and haplotype diversity in Lanyu compared with the Philippine regions (particularly Luzon and Bohol). Notably, Bohol and Leyte exhibited the highest haplotype diversity in Dataset B (Table 2; Figure 2B), whereas dataset C revealed only a single nuclear haplotype for these islands. Neutrality tests were mostly nonsignificant across datasets B–D. An exception was observed in dataset C, which showed significantly negative values (Tajima's $D = -1.88$ *; Fu–Li's $F = -3.83$ *), suggesting a recent population expansion (Table 2).

Table 2: Summary of sample sizes and genetic diversity.

A							
Divisions	***n***	***S***	***h***	H_d	π	**Tajima's *D***	**Fu–Li's *F***
Taiwan	4	1	2	0.5	0.00003	−0.61	−0.48
Lanyu	26	3	4	0.29	0.00003	−1.29	−2.00
Philippines	15	230	14	0.99	0.00433	−0.43	−0.69
Luzon	5	21	5	1	0.00057	−1.23	−1.32
Bohol	6	92	6	1	0.00246	−0.65	−0.64
Lyete	2	0	1	—	—	—	—
Total	45	243	17	0.73	0.00231	−1.42	−2.00
B							
Divisions	***n***	***S***	***h***	H_d	π	**Tajima's *D***	**Fu–Li's *F***
Taiwan	4	0	1	—	—	—	—
Lanyu	26	2	3	0.15	0.00002	−1.51	−2.32
Philippines	15	20	6	0.65	0.00058	−1.23	−2.05
Luzon	5	8	4	0.9	0.00046	−0.81	−0.85
Bohol	6	0	1	—	—	—	—
Lyete	2	0	1	—	—	—	—
Total	45	22	9	0.58	0.00029	−1.88[a]	−3.83[a]
C							
Divisions	***n***	***S***	***h***	H_d	π	**Tajima's *D***	**Fu–Li's *F***
Taiwan	4	0	1	—	—	—	—
Lanyu	27	0	1	—	—	—	—
Philippines	18	7	7	0.86	0.00401	−0.11	−0.70
Luzon	7	2	3	0.52	0.00103	−0.11	−1.37
Bohol	7	4	4	0.71	0.00289	−0.11	−0.70
Lyete	2	0	1	—	—	—	—
Total	49	8	7	0.58	0.00361	−0.11	−0.66

Note: A, 37 mitochondrial genes; *B*, nuclear 18S–28S region; *C*, mitochondrial barcode.

[a]Indicates that neutrality tests (Tajima's *D* and Fu–Li's *F*) were statistically significant ($p < 0.05$).

3.4. Genetic Distance. Comparing K2P genetic distances among localities revealed clear geographic divergence (Table 3). In dataset B, Lanyu and Luzon were closest (K2P = 0.0023), while divergence was greatest between Lanyu and Mindanao (K2P = 0.0078). In dataset C, the Lanyu population differed substantially only from Mindanao, with smaller distances to other Philippine islands.

3.5. Population Structure and Gene Flow. AMOVA results revealed strong differentiation between the Lanyu and Philippine populations (Table 4). For mitochondrial data, variance among populations accounted for over 51.3% of total variation, with a high fixation index (F_{ST}). When subdivided into three or five groups, variance among populations increased to 89.2% ($F_{ST} = 0.89$, $p < 0.001$), indicating extremely limited maternal gene flow. Similarly, results for nuclear data were congruent, with 45.6% of the variance

Table 3: Pairwise genetic distances (K2P-distance) among Magellan birdwing populations from different localities.

Datasets	Regions	Lanyu	Luzon	Bohol	Leyte
Dataset B	Luzon	0.0023	—	—	—
	Bohol	0.0047	0.0050	—	—
	Leyte	0.0049	0.0054	0.0034	—
	Mindanao	0.0078	0.0080	0.0084	0.0088
Dataset C	Luzon	0.0004	—	—	—
	Bohol	0.0004	0.0007	—	—
	Leyte	0.0004	0.0007	0	—
	Mindanao	0.0016	0.0017	0.0018	0.0018

Table 4: Analysis of molecular variance (AMOVA) of the Magellan birdwing under different grouping strategies based on (*A*) 37 mitochondrial genes and (*B*) nuclear 18S–28S region.

A

Grouping	Source of variation	*df*	*SS*	Variance	Percentage of variance (%)	F_{ST}
Two divisions	Among population	1	241.7	12.3	51.3	0.51 ($p<0.0001$)
	Within population	38	442.2	11.6	48.7	
Three divisions	Among population	2	385.3	18.3	69.4	0.69 ($p<0.0001$)
	Within population	37	298.6	8.1	30.6	
Five divisions	Among population	4	573.6	26.2	89.2	0.89 ($p<0.0001$)
	Within populations	35	110.3	3.2	10.8	

B

Grouping	Source of variation	*df*	*SS*	Variance	Percentage of variance (%)	F_{ST}
Two divisions	Among population	1	13.4	0.7	45.6	0.46 ($p<0.0001$)
	Within population	38	31.4	0.8	54.4	
Three divisions	Among population	2	21.7	1.0	62.0	0.62 ($p<0.0001$)
	Within population	37	23.0	0.6	38.0	
Five divisions	Among population	4	36.8	1.7	88.1	0.88 ($p<0.0001$)
	Within populations	35	7.9	0.2	11.9	

Note: F_{ST} values are shown with significance levels estimated by 1000 permutations.

among populations explained by differences between the Lanyu and the Philippine populations, increasing to 88.1% ($F_{ST}=0.88$, $p<0.001$) when subdivided into five groups. Both markers consistently indicated strong population structure and negligible gene flow between the Lanyu and Philippine populations.

3.6. Phylogenetic Relationships and Dating. Haplotype networks showed that Lanyu was most closely related to Luzon (Figure 2), followed by central or southern Philippine populations according to both mitochondrial and nuclear datasets. Dataset C also suggested a potential connection between Lanyu and Bohol + Leyte. In all comparisons, the Mindanao haplotype was the most divergent from Lanyu.

Both BI and ML phylogenetic analyses produced congruent, well-supported topologies (Figure 4; Supporting Information 2: Figure S1). The Magellan birdwing formed a strongly supported monophyletic group, with relationships following a south-to-north gradient: the Mindanao lineage was sister to all other populations, Bohol and Leyte were sister to Lanyu plus Luzon, and Lanyu and Luzon were reciprocally monophyletic.

Molecular dating estimated the origin of the Magellan birdwing at 12.53 Ma, with diversification occurring within the last 1 Ma (Figure 4). The Mindanao lineage diverged at 0.86 Ma, the Bohol and Leyte clade at 0.42 Ma, and the Luzon and Lanyu clade most recently at 0.21 Ma, indicating a recent colonization event of Lanyu.

4. Discussion

4.1. The Origin of the Magellan Birdwing of Lanyu and Northern Taiwan. Our analyses show that the phylogeography of the Magellan birdwing is consistent with sequential founder events [38]. Divergence followed a south-to-north trajectory (Figure 4), beginning in the southern Philippines and extending through the central and northern Philippine islands to Lanyu. This pattern is consistent with the island-hopping and founder-speciation hypothesis proposed for *Troides* butterflies [37]. Haplotype diversity declined toward the northern distributional margin (Figure 2), and phylogenetic relationships reflected geographic proximity. However, as most Philippine samples in our study were derived from museum specimens, interpretations of genetic distance and

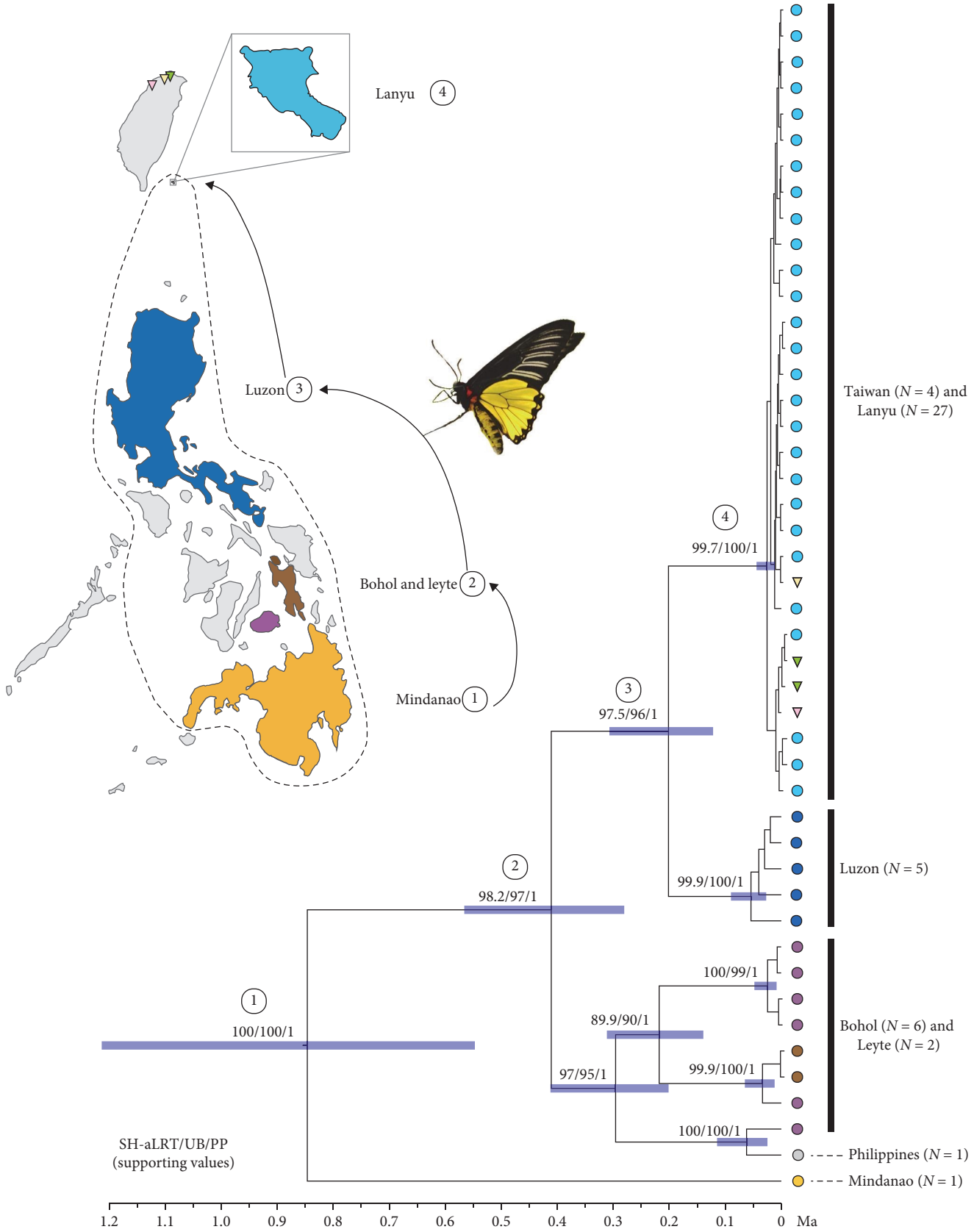

Figure 4: Molecular dating and phylogenetic relationships of Magellan birdwing inferred from a Bayesian analysis. Horizontal bars at nodes represent the 95% highest posterior density (HPD) intervals of estimated divergence times. An inset illustration shows the hypothesized northward dispersal of the species. PP, Bayesian posterior probability; SH-aLRT, the SH-like approximate likelihood ratio test; UB, ML bootstrap value.

diversity should be made with caution. Expanded sampling across the Philippines will be critical for clarifying the current demographic and evolutionary status of the Magellan birdwing.

Lanyu Island emerged around 2.61 Ma [39], and its biota has been strongly shaped by colonization. The Magellan birdwing conforms to this pattern: the Lanyu population is genetically distinct (Figure 3A), with divergence dating to 0.21 Ma (Figure 4). Comparisons with historical specimens revealed minimal haplotype turnover, and long-term monitoring showed persistently low but stable adult numbers (Figure 1E). Together, these findings indicate that the Lanyu lineage has persisted with low genetic variation for a long time. The presence of an identical haplotype in northern Taiwan strongly suggests that those individuals were introduced from Lanyu.

4.2. Subspecies Delimitation and Evolutionarily Significant Unit (ESU) of the Magellan Birdwing. The Lanyu population is currently classified as the subspecies *T. m. sonani*. However, subspecies delimitations within this species remain controversial, with authors recognizing one to four subspecies [8, 30, 40]. Some have even argued that the Lanyu population could have resulted from an artificial introduction for commercial purposes rather than representing a valid subspecies [41]. Although our sampling does not resolve subspecies boundaries across the entire range, our results demonstrate that the Lanyu population is genetically distinct and should be regarded as an ESU for conservation [42]. Moreover, the strong genetic structure revealed in this study supports the existence of at least three ESUs in the Philippines: Luzon, Bohol + Leyte, and Mindanao (Figures 2 and 4). Additional sampling is necessary to refine subspecies delimitations and to evaluate the number of ESUs, particularly given the pronounced variation in wing morphology among populations [16].

4.3. Conservation Implications. The Lanyu population is small, has low genetic diversity, and shows clear genetic distinctiveness (Figures 1, 2, and 4; Table 2). These characteristics highlight the urgent need for targeted conservation actions. Based on our findings, we propose three priorities for *T. m. sonani*, which may also serve as a framework for managing other endangered insular taxa, as follows:

1. Maintain larval hostplant resources: The Lanyu population depends exclusively on *A. zollingeriana* as its larval hostplant, making resource stability critical. Habitat loss from tourism and land conversion has historically reduced hostplant abundance [43]. Earlier planting efforts [13] and recent restoration programs since 2021 demonstrate the feasibility of sustaining hostplant resources. Continued planting, carrying capacity assessments, and routine habitat monitoring are essential to ensure hostplant persistence and butterfly survival.
2. Establish conservation refugia: Small insular populations are highly vulnerable to extreme climate events [44–46]. For example, Typhoon Koinu in 2023 sharply reduced butterfly numbers in Lanyu [47]. To minimize bottleneck risks, designated refugia should be established to safeguard subsets of the population against catastrophic events.
3. Apply conservation genomics: While our study clarifies the origin and genetic distinctiveness of the Lanyu population, the genetic markers used here provide limited resolution for assessing inbreeding or deleterious mutation accumulation. Conservation genomics now enables genome-wide assessments of effective population size, inbreeding coefficients, and mutation load [48–50]. Applying these approaches will generate baseline data on genetic health, guide adaptive management, and improve the long-term viability of this endangered lineage.

5. Conclusion

This study demonstrates that the Lanyu population of the Magellan birdwing is both small and genetically distinct, and that the introduced cases in northern Taiwan most likely originated from Lanyu. We further show that genome skimming is an effective method for obtaining mitochondrial and nuclear sequences from museum specimens, enabling origin tracing while minimizing impacts on extant populations. This method provides an effective framework for building conservation genetic datasets for rare or endangered species, extending across temporal and spatial scales, and thereby offering critical insights into their evolutionary and conservation dynamics.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Wen-Chen Chu and Masaya Yago contributed equally to this work. The study was conceptualized by Wen-Chen Chu, Masaya Yago, Pei-Tzu Hsu, Lan-Wei Yeh, Yen-Po Lin, Ren-Fang Chao, Yu-Feng Hsu, and Li-Wei Wu. Formal analyses were performed by Lan-Wei Yeh, Pei-Tzu Hsu, and Li-Wei Wu. Funding acquisition was acquired by Wen-Chen Chu, Masaya Yago, and Li-Wei Wu. The original draft was prepared by Wen-Chen Chu, Pei-Tzu Hsu, and Li-Wei Wu. All authors contributed to the review and editing.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information 1. Table S1: NGS read counts and general information for all analyzed specimens.

Supporting Information 2. Figure S1: Detailed Bayesian phylogeny of Troides butterflies based on 37 mitochondrial genes and the nuclear 18S-28S region. Support values are indicated as SH-aLRT: the SH-like approximate likelihood ratio test, UB: ML bootstrap value, and PP: Bayesian posterior probability. Node 1: origin of birdwings; node 2: divergence between *Ornithoptera* and *Troides*; node 3: crown age of *Ornithoptera* and *Troides*.

References

[1] C. Bellard, C. Leclerc, B. Leroy, et al., "Vulnerability of Biodiversity Hotspots to Global Change," *Global Ecology and Biogeography* 23, no. 12 (2014): 1376–1386.

[2] T. J. Matthews and K. Triantis, "Island Biogeography," *Current Biology* 31, no. 19 (2021): R1201–R1207.

[3] J. C. Russell and C. Kueffer, "Island Biodiversity in the Anthropocene," *Annual Review of Environment and Resources* 44, no. 1 (2019): 31–60.

[4] R. W. Howe, G. J. Davis, and V. Mosca, "The Demographic Significance of 'Sink' Populations," *Biological Conservation* 57, no. 3 (1991): 239–255.

[5] H.-C. Cheng, C.-T. Yao, F.-H. Hsu, et al., *Field Guide to Protected Wildlife of Taiwan (in Chinese)* (Endemic Species Research Institute, Council of Agriculture, 2004).

[6] E. Van Hooijdonk, S. Berthier, and J.-P. Vigneron, "Contribution of Both the Upperside and the Underside of the Wing on the Iridescence in the Male Butterfly *Troïdes Magellanus* (Papilionidae)," *Journal of Applied Physics* 112, no. 7 (2012): 1-2.

[7] S. Igarashi and H. Fukuda, *The Life Histories of Asian Butterflies*, 1 (Tokai University Press, 1997).

[8] E. Tsukada and Y. Nishiyama, *Butterflies of the South East Asian Islands. Vol. 1 Papilionidae* (Plapac Co, 1982).

[9] W.-S. Chen, *Study on the Troides magellanus (in Chinese)* (Forestry Bureau, Council of Agriculture, Executive Yuan, 1987).

[10] H.-S. Fang and Y.-L. Yang, *Conservation of the Magellanus Birdwing Butterfly, Troides magellanus in Lanyu (in Chinese)* (Endemic Species Research Institute, Council of Agriculture, Executive Yuan, 2003).

[11] E. Hamano, *Ecological Encyclopedia of Taiwanese Butterflies (in Japanese)* (Kodansha Ltd., Tokyo, Japan, 1986).

[12] H.-S. Fang, Y.-H. Tseng, C.-C. Huang, et al., *Recovery of Troides magellanus, Rhododendron Kanehirai, and Hygrophila pogonocalyx (in Chinese)* (Endemic Species Research Institute, Council of Agriculture, Executive Yuan, 2004).

[13] H.-S. Fang, Y.-L. Yang, and C.-T. Yao, *Ecology and Rehabilitation of the Birdwing Butterfly (Troides magellanus) in Lanyu (in Chinese)* (Endemic Species Research Institute, Council of Agriculture, Executive Yuan, Nantou, Taiwan, 1998).

[14] T. Shimonoya and C. K. Yu, "Remarks on Some Unrecorded Butterflies From Formosa (Taiwan), With Notes on the Butterflies from Is. Ching-Meng," *Transactions of the Lepidopterological Society of Japan* 22, no. 1 & 2 (1971): 57–61.

[15] M. Yamanaka, "Distribution of Formosan Butterflies (1)," *Special Bulletin of the Lepidopterological Society of Japan* 5 (1971): 115–191.

[16] S.-H. Yen and P.-S. Yang, *Illustrated Identification Guide to Insects Protected by the CITES and Wildlife Conservation Law of Taiwan, R.O.C* (Council of Agriculture, Executive Yuan, 2001).

[17] J. Kulski, *Next-Generation Sequencing—An Overview of the History, Tools, and "Omic" Applications* (IntechOpen, 2016).

[18] N. Nakahama, "Museum Specimens: An Overlooked and Valuable Material for Conservation Genetics," *Ecological Research* 36, no. 1 (2021): 13–23.

[19] S. C. Straub, M. Parks, K. Weitemier, M. Fishbein, R. C. Cronn, and A. Liston, "Navigating the Tip of the Genomic Iceberg: Next-Generation Sequencing for Plant Systematics," *American Journal of Botany* 99, no. 2 (2012): 349–364.

[20] C. D. Siler, J. R. Oaks, K. Cobb, H. Ota, and R. M. Brown, "Critically Endangered Island Endemic or Peripheral Population of a Widespread Species? Conservation Genetics of Kikuchi's Gecko and the Global Challenge of Protecting Peripheral Oceanic Island Endemic Vertebrates," *Diversity and Distributions* 20, no. 7 (2014): 756–772.

[21] Y.-C. Chen, C.-T. Wang, D. C. Lees, and L.-W. Wu, "Higher DNA Insert Fragment Sizes Improve Mitogenomic Assemblies From Metagenomic Pyrosequencing Datasets: An Example Using Limenitidinae Butterflies (Lepidoptera, Nymphalidae)," *Mitochondrial DNA Part A* 29, no. 6 (2018): 840–845.

[22] H.-Y. Tseng, H. Chiba, D. J. Lohman, et al., "Out of Asia: Intercontinental Dispersals After the Eocene-Oligocene Transition Shaped the Zoogeography of Limenitidinae Butterflies (Lepidoptera: Nymphalidae)," *Molecular Phylogenetics and Evolution* 170 (2022): 107444.

[23] D. Li, C. M. Liu, R. Luo, K. Sadakane, and T.-W. Lam, "MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph," *Bioinformatics* 31, no. 10 (2015): 1674–1676.

[24] M. Bernt, A. Donath, F. Jühling, et al., "MITOS: Improved De Novo Metazoan Mitochondrial Genome Annotation," *Molecular Phylogenetics and Evolution* 69, no. 2 (2013): 313–319.

[25] K. Tamura, G. Stecher, and S. Kumar, "MEGA11: Molecular Evolutionary Genetics Analysis Version 11," *Molecular Biology and Evolution* 38, no. 7 (2021): 3022–3027.

[26] R. C. Edgar, "MUSCLE: Multiple Sequence Alignment With High Accuracy and High Throughput," *Nucleic Acids Research* 32, no. 5 (2004): 1792–1797.

[27] R. Lanfear, P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott, "PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses," *Molecular Biology and Evolution* 34, no. 3 (2017): 772-773.

[28] J. Rozas, A. Ferrer-Mata, J. C. Sánchez-DelBarrio, et al., "DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets," *Molecular Biology and Evolution* 34, no. 12 (2017): 3299–3302.
[29] L. Excoffier, G. Laval, and S. Schneider, "Arlequin (version 3.0): An Integrated Software Package for Population Genetics Data Analysis," *Evolutionary Bioinformatics* 1 (2005): 1–47.
[30] J. Haugum and A. M. Low, *A Monograph of the Birdwing Butterflies, Vol. 2: The Genera Trogonoptera, Ripponia, and, Troides* (EJ Brill/Scandinavian Science Press, 1985).
[31] L.-T. Nguyen, H. A. Schmidt, A. Von Haeseler, and B. Q. Minh, "IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies," *Molecular Biology and Evolution* 32, no. 1 (2015): 268–274.
[32] B. Q. Minh, M. A. T. Nguyen, and A. Von Haeseler, "Ultrafast Approximation for Phylogenetic Bootstrap," *Molecular Biology and Evolution* 30, no. 5 (2013): 1188–1195.
[33] S. Guindon, J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel, "New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0," *Systematic Biology* 59, no. 3 (2010): 307–321.
[34] M. A. Suchard, P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut, "Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10," *Virus Evolution* 4, no. 1 (2018): vey016.
[35] J. W. Leigh, D. Bryant, and S. Nakagawa, "POPART: Full-Feature Software for Haplotype Network Construction," *Methods in Ecology and Evolution* 6, no. 9 (2015): 1110–1116.
[36] R. De Jong, "Fossil Butterflies, Calibration Points and the Molecular Clock (Lepidoptera: Papilionoidea)," *Zootaxa* 4270, no. 1 (2017): 1–63.
[37] F. L. Condamine, E. F. Toussaint, A.-L. Clamens, G. Genson, F. A. Sperling, and G. J. Kergoat, "Deciphering the Evolution of Birdwing Butterflies 150 Years after Alfred Russel Wallace," *Scientific Reports* 5, no. 1 (2015): 11860.
[38] S. M. Clegg, S. M. Degnan, J. Kikkawa, C. Moritz, A. Estoup, and I. P. Owens, "Genetic Consequences of Sequential Founder Events by an Island-Colonizing Bird," *Proceedings of the National Academy of Sciences of the United States of America* 99, no. 12 (2002): 8127–8132.
[39] W. Y. Shao, S. L. Chung, and W. S. Chen, "Zircon U-Pb Age Determination of Volcanic Eruptions in Lutao and Lanyu in the Northern Luzon Magmatic Arc," *Terrestrial, Atmospheric and Oceanic Sciences* 25, no. 2 (2014): 149.
[40] N. Kondo and M. Nishida, *The World of Birdwing Butterflies (in Japanese)* (Shinzansha, 1998).
[41] B. D'Abrera, *Butterflies of the Oriental Region, Part I: Papilionidae, Pieridae and Danaidae* (Hill House, 1982).
[42] C. Moritz, "Conservation Units and Translocations: Strategies for Conserving Evolutionary Processes," *Hereditas* 130, no. 3 (1999): 217–228.
[43] W.-C. Chu, *Conservation Action Plan for the Magellanus Birdwing (Troides magellanus) (in Chinese)* (Forestry and Nature Conservation Agency, Taiwan Biodiversity Research Institute, Ministry of Agriculture, 2023).
[44] D. E. Harter, S. D. Irl, B. Seo, et al., "Impacts of Global Climate Change on the Floras of Oceanic Islands–Projections, Implications and Current Knowledge," *Perspectives in Plant Ecology, Evolution and Systematics* 17, no. 2 (2015): 160–183.
[45] C. Leclerc, F. Courchamp, and C. Bellard, "Future Climate Change Vulnerability of Endemic Island Mammals," *Nature Communications* 11, no. 1 (2020): 43–49.
[46] J.-W. Lin, C.-P. Liao, C.-C. Chou, et al., "Loss of Sea Turtle Eggs Drives the Collapse of an Insular Reptile Community," *Science Advances* 9, no. 50 (2023): 7052.
[47] Y.-F. Hsu, Y.-M. Hsu, K.-C. Wang, et al., *Ecological Survey of the Magellan Birdwing (Troides magellanus) Population in Lanyu II (in Chinese)* (Taitung Branch, Forestry and Nature Conservation Agency, Ministry of Agriculture, 2023).
[48] G. Bertorelle, F. Raffini, M. Bosse, et al., "Genetic Load: Genomic Estimates and Applications in Non-Model Animals," *Nature Reviews Genetics* 23, no. 8 (2022): 492–503.
[49] M. Kardos, E. E. Armstrong, S. W. Fitzpatrick, et al., "The Crucial Role of Genome-Wide Genetic Variation in Conservation," *Proceedings of the National Academy of Sciences of the United States of America* 118, no. 48 (2021): e2104642118.
[50] N. J. Ouborg, C. Pertoldi, V. Loeschcke, R. K. Bijlsma, and P. W. Hedrick, "Conservation Genetics in Transition to Conservation Genomics," *Trends in Genetics* 26, no. 4 (2010): 177–187.
[51] L.-W. Wu, D. C. Lees, S.-H. Yen, and Y.-F. Hsu, "The Complete Mitochondrial Genome of the Near-Threatened Swallowtail, *Agehana maraho* (Lepidoptera: Papilionidae): Evaluating Sequence Variability and Suitable Markers for Conservation Genetic Studies," *Entomological News* 121, no. 3 (2010): 267–280.