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Distribution, Habitat Diversity, and Phylogeny of *Anochetus shohki* Terayama, 1996 (Hymenoptera: Formicidae) from Taiwan and the Ryukyu Archipelago of Southern Japan

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ABSTRACT. *Anochetus shohki* Terayama, 1996, has been considered endemic to Ishigaki Island in the Ryukyu Archipelago of southern Japan for decades. Although subsequent records have extended its known range to other islands within the archipelago, including Miyako, Iriomote, Yonaguni, and Okinawa Islands, its full geographic range and ecological requirements remain poorly understood; furthermore, the absence of molecular sequences has left its phylogenetic placement unresolved. In this study, we report the first record of *A. shohki* from central and southern Taiwan, complementing this with new distributional data from the Yaeyama Islands. To clarify phylogenetic relationships, four nuclear genes (*CAD*, *LW Rh*, *TOPI*, and *Wg*) and one mitochondrial gene (*COI*) from *A. shohki* were sequenced. Our phylogenetic results support the placement of *A. shohki* within the *A. graeffei* species group and reveal minimal genetic divergence between the Taiwan and Yaeyama populations at these markers. Our field observations indicate diverse habitat preferences in *A. shohki*, suggesting that its occurrence on beaches may reflect high dispersal ability and ecological flexibility. Finally, an updated taxonomic key to the workers of Taiwanese *Anochetus* species is provided.

Keywords	Ponerinae, distribution, phylogeny, species group, habitat
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INTRODUCTION

The ant genus *Anochetus* Mayr, 1861 is widely distributed across tropical and subtropical regions, such as South America, Africa, Australia, and Asia (Schmidt & Shattuck, 2014), and currently comprises 114 extant species and nine fossil species (AntCat, 2026). In contrast to its sister genus *Odontomachus, Anochetus* is relatively understudied, likely due to its small body size, modest colony populations, and cryptic foraging behaviors and habitats (Schmidt & Shattuck, 2014).

In Taiwan, three *Anochetus* species have been formally recorded: *Anochetus risii* Forel, 1900, *Anochetus subcoecus* Forel, 1912, and *Anochetus lanyuensis* Leong et al., 2018. While *A. risii* and *A. subcoecus* are also distributed in other neighboring regions such as Southeast Asia and China, *A. lanyuensis* has so far been documented exclusively on Lanyu (Orchid Island).

Recently, an examination of historical specimens from the Social Insect Laboratory at National Changhua University of Education revealed an unrecorded species collected from central and southern Taiwan. Based on morphological analysis, this unrecorded species was preliminarily identified as *Anochetus shohki*. Notably, *A. shohki* is the only *Anochetus* species recorded in Japan (Terayama, 1996; Yamane et al., 2024), where it was long thought to be endemic to Ishigaki Island in the Ryukyu Archipelago. Due to this restricted geographic range, Japan's Ministry of the Environment has listed *A. shohki* as threatened (Ministry of the Environment, 2015). Even though subsequent surveys have identified *A. shohki* on other islands within the archipelago (Miyako, Iriomote, Yonaguni, and Okinawa Islands) (Komatsu, 2009; Hisasue et al., 2019; Hisasue & Tokushige, 2021) and current records indicate that Okinawa Island is its northernmost limit (Hisasue & Tokushige, 2021), its full geographic range remains unclear.

Furthermore, no genetic sequences for *Anochetus shohki* are currently available in GenBank, and little is known about its phylogenetic relationships with other species. Doré et al. (2025) recently reconstructed a complete phylogenetic tree of Ponerinae, incorporating taxa lacking genetic data by grafting them onto the tree based on taxonomic and biogeographic information. Their analysis placed *A. shohki* within the *A. africanus* species group as a sister species to *Anochetus pupulatus* Brown, 1978. However, despite a similar absence of genetic data, Fernandes et al. (2021) assigned *A. shohki* to the *A. graeffei* species group based on morphological traits, highlighting an inconsistency with the placement by Doré et al. (2025). Obtaining molecular evidence for *A. shohki* is therefore crucial to empirically test these morphology- or biogeography-based hypotheses. Beyond resolving its phylogenetic position, reliable molecular data provide a baseline for species delimitation, helping to distinguish it from morphologically similar congeners.

In the present study, we integrated morphological and molecular approaches to confirm the first formal record of *Anochetus shohki* in Taiwan. To resolve the conflicting taxonomic placements proposed by recent large-scale studies, we reconstructed the phylogenetic relationships between *A. shohki* and other species within the *A. africanus* and *A. graeffei* species groups. Additionally, our field observations reveal the habitat preferences and ecological diversity of *A. shohki,* and we provide an updated identification key to the workers of *Anochetus* species found in Taiwan.

MATERIALS & METHODS

Ant collection and Taxonomic study

Historical ant specimens of *Anochetus* were hand-collected from under rocks or soil in Taiwan between 2012 and 2023. Species identification was based on the characteristic descriptions, taxonomic illustrations and images of Terayama (1996), Leong et al. (2018), and Yamane et al. (2024). Among the historical specimens examined, an unrecorded species was preliminarily identified as *Anochetus shohki*. To ensure taxonomic accuracy and facilitate subsequent molecular analyses, additional *Anochetus* ants were collected from the Yaeyama Islands, Japan in 2024. Detailed collection data, including specimen counts, specimen IDs, collection dates, and localities, are provided in Table 1.

Table 1. Collection data and specimen IDs for *Anochetus shohki* from Taiwan and the Yaeyama Islands (2012 – 2024)

Country	W / Q[a]	Date	Locality[b]	Latitude	Longitude	Collector	Specimen ID	SEM specimen ID
Taiwan	1 / 0	06.III.2012	Wuri, Taichung County	24.15885	120.54632	D.Y. Huang	NCUESIL001696	
	1 / 0	16.III.2012	PU, Taichung County			D.Y. Huang	NCUESIL001697	
	4 / 0	04.III.2016	NCUE, Changhua County	24.08001	120.55602	P.C. Hsu	NCUESIL001698	NCUESIL002627–NCUESIL002628
	1 / 0	08.X.2020	Wushantou Reservoir, Tainan County	23.20892	120.36892	P.C. Hsu		
	0 / 2	09.IX.2020	THU, Taichung City	24.17917	120.6025	Y.C. Hsiao	NCUESIL002623	NCUESIL002632
	4 / 1	09.VI.2021	Wuri, Taichung City	24.12656	120.58872	Y.C. Hsiao		
	2 / 0	26.V.2022	THU, Taichung City	24.17917	120.6025	Y.C. Hsiao	NMNS: NCUESIL002620–NCUESIL002621	
	2 / 0	31.V.2022	Shoushan, Kaohsiung City			S.H. Lai	NCUESIL002622	
	1 / 0	21.VII.2022	Zuocang Trai, Hualien County	24.02000	121.57444	Y.C. Hsiao		
	1 / 0	26.II.2023	Xiaoliuqiu Island, Pingtung County	22.35535	120.37773	Z.J. Xie		
	0 / 1	01.VI.2023	NCHU, Taichung city	24.1215	120.67949	J.T. Lin		
Yaeyama Islands, Japan	6 / 1	08.VII.2024	Inoda Beach, Ishigaki Island	24.46691	124.2523	F.Y. Chung	NCUESIL002633–NCUESIL002636	
	3 / 0	09.VII.2024	Ishigaki Island	24.42308	124.1998	F.Y. Chung	NCUESIL002637	
	0 / 2	13.VII.2024	Shirahama, Iriomote Island	24.36167	123.74973	F.Y. Chung	NCUESIL002624	
	8 / 2	14.VII.2024	Mandabaru Forest Park, Yonaguni Island	24.45646	122.97584	F.Y. Chung	NCUESIL002625–NCUESIL002626	NCUESIL002629–NCUESIL002631
	5 / 0	14.VII.2024	Mt. Kubura, Yonaguni Island	24.44814	122.96805	F.Y. Chung		

[a] W / Q, number of workers / queens.

[b] PU, Providence University; NCUE, National Changhua University of Education; THU, TungHai University; NCHU, National Chung Hsing University.

All specimens were examined and pinned specimens (Tab. 1) were photographed using a Leica M205C stereomicroscope (Wetzlar, Germany) mounted with a Nikon Z5 digital camera (Tokyo, Japan). The images were produced via focus stacking using Helicon Focus 6.8 software (Helicon Soft Ltd. 2012). For detailed morphological study, two workers and one queen each from Taiwan and the Yaeyama Islands (Tab. 1) were examined and photographed using a Hitachi TM3000 tabletop scanning electron microscope (SEM). SEM samples were attached to aluminum stubs and dried using an FD4.5-8P-D desktop vacuum freeze dryer (KINGMECH CO., LTD., Taiwan). After drying, the samples were coated with platinum for 90 seconds using Hitachi E-1010 Ion Sputter (Tokyo, Japan). All images were processed using Affinity Photo (v. 2.6.0).

Measurements

The following measurements were based on Terayama (2009) and Yoshimura & Fisher (2014). All measurements are recorded in millimeters.

HL, Head length, maximum length from anterior margin of clypeus to posterior margin of head in full face view. **HW**, Head width, maximum head width including compound eyes in full face view. **SL**, Scape length, maximum length of antennal scape excluding the basal constriction or neck close to condylar bulb. **EL**, Eye length, maximum diameter of compound eye in lateral view of head, where anterior and posterior compound eye margins are in the same plane. **ML**, Mandibular length, maximum length of dorsal surface of mandible. **WL**, Weber's length of alitrunk, maximum diagonal distance from the base of anterior slope of pronotum to metapleural lobe in profile view. **PnW**, Pronotal width, maximum width of the pronotum in dorsal view. **PtL**, Petiolar length, maximum length of petiole in profile view. **PtW**, Petiolar width, maximum width of petiolar node in dorsal view.

Specimen depositories

NCUE National Changhua University of Education, Changhua County, Taiwan.
NMNS National Museum of Natural Science, Taichung City, Taiwan.

DNA extraction and phylogenetic analysis

Total genomic DNA was extracted from seven *Anochetus shohki* samples (six workers and one queen) using the Qiagen Gentra Puregene Kit (Venlo, Netherlands). Samples were placed into 1.5 mL microcentrifuge tubes containing 150 µL of Cell Lysis buffer and 0.8 µL of Proteinase K, then incubated at 55°C overnight to maximize DNA yield. After incubation, 0.8 µL of RNase A were added, and the mixture was incubated at 37°C for 30 min. Subsequently, 50 µL of Protein Precipitation Solution was added, followed by vigorous vortexing at high speed for 20 s. The lysate was then centrifuged at 13,500 x *g* for 20 min. The resulting supernatant was carefully transferred into a new tube containing 150 µL of isopropanol and centrifuged at 13,500 x *g* for 10 min. The supernatant was discarded, and the DNA pellet was washed with 500 µL of 70% ethanol and centrifuged again at 13,500 x *g* for 10 min. After carefully removing the supernatant, the tubes were inverted on absorbent paper to drain. Finally, the DNA pellets were air-dried, rehydrated in 50 µL of DNA Hydration Solution, and stored at -20°C for subsequent PCR analysis.

Four nuclear genes, Rudimentary (*CAD*), Long-wavelength rhodopsin (*LW Rh*), Topoisomerase I (*TOPI*), and Wingless (*Wg*) and the mitochondrial cytochrome c oxidase subunit I (*COI*) gene were amplified via polymerase chain reaction (PCR). The primers used to produce sequences are listed in Table S1. PCR was performed in a total volume of 25 µL reagent mixtures, containing 12.5 µL 2 x Ampliqon Taq DNA polymerase Master Mix (Odense, Denmark), 0.5 µL each of the forward and reverse primer, 0.5 µL genomic DNA template, and 11 µL ddH$_2$O. PCR reaction conditions of *CAD*, *LW Rh*, *TOP I*, and *Wg* consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 50-58°C (depending on the primer set, Table S1) for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. Similarly, the amplification of *COI* was performed under the following conditions: 95°C for 5 min; 35 cycles of 95°C for 30 s, 45°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. PCR amplicons were sent to Tri-Biotech Inc. (Taipei, Taiwan) for purifying and Sanger sequencing. The *COI* gene was sequenced for all seven *A. shohki* samples, while four nuclear loci were sequenced for two of them.

The successfully amplified sequences were compared with those available in the GenBank (NCBI) nucleotide database using the BLASTn 2.17.0+ (with megablast algorithm) (Zhang et al., 2000) to verify both gene identity and taxonomic assignment. For phylogenetic inference, the sequences of four species from the *A. africanus* species group (*Anochetus africanus* (Mayr, 1865); *Anochetus bequaerti* Forel, 1913; *Anochetus madagascarensis* Forel, 1887*;* and *Anochetus obscuratus* Santschi, 1911) and five species from the *A. graeffei* species group (*Anochetus graeffei* Mayr, 1870; *Anochetus pattersoni* Fisher, 2008; *Anochetus pubescens* Brown, 1978; *Anochetus turneri* Forel, 1900; and *Anochetus yerburyi* Forel, 1900) were retrieved from GenBank and included in the analysis. A comprehensive list of the taxa used in this study, including GenBank accession numbers, sample IDs, localities, and original references, is provided in Table 2.

All *COI* and nuclear gene (*CAD*, *LW Rh*, *TOPI*, and *Wg)* sequences were aligned using the MUSCLE algorithm, and the nuclear loci were subsequently concatenated in Geneious Prime® 2025.2.1. The final alignment length for the *COI* dataset was 632 bp, while the concatenated nuclear dataset (comprising four nuclear loci) totaled 2,870 bp. Phylogenetic inference for both datasets were performed using Maximum Likelihood (ML) analyses via the IQ-TREE web server (Trifinopoulos et al., 2016). The best-fit model of nucleotide substitution was automatically selected by IQ-TREE, and the analysis was conducted with partitioning, based on 1,000 bootstrap (BS) replicates. The resulting phylogenetic trees were visualized in FigTree v1.3.1, and the graphical

Table 2. GenBank accession numbers and location data associated with the sequences used in this study

Species	Sample ID	COI	CAD	LW Rh	TOP I	Wg	Locality	Reference
Anochetus shohki	Ano160304	PX485161					Changhua County, Taiwan	This study
Anochetus shohki	Ano201008	PX485162	PX991853	PX991857	PX991855	PX991859	Tainan County, Taiwan	This study
Anochetus shohki	Ano220721	PX485163					Hualien County, Taiwan	This study
Anochetus shohki	Ano230226	PX485164					Pingtung County, Taiwan	This study
Anochetus shohki	Ano240708	PX485165	PX991854	PX991858	PX991856	PX991860	Ishigaki Island, Japan	This study
Anochetus shohki	Ano240713	PX485166					Iriomote Island, Japan	This study
Anochetus shohki	Ano240714	PX485167					Yonaguni Island, Japan	This study
Anochetus africanus I108		MT655632	MT872823	MT764348	MT679839	MT780367	Gabon	Fernandes et al., 2021
Anochetus bequaerti		PX697291					Kakamega, Kenya	Fisher et al., 2025
Anochetus cf. *madagascarensis* I80			MT828514	MT764347	MT679816	MT780337	Anjouan, Comoros	Fernandes et al., 2021
Anochetus madagascarensis		EU119728					Toliara, Madagascar	Fisher & Smith, 2008
Anochetus obscuratus I238		MT655627			MT679834	MT780361	Uganda	Fernandes et al., 2021
Anochetus graeffei I133			MT828510	MT763983	MT679813		Efate, Vanuatu	Fernandes et al., 2021
Anochetus graeffei I134			MT828511	MT763984	MT679814		Efate, Vanuatu	Fernandes et al., 2021
Anochetus graeffei I135		MT655603	MT828512	MT763985	MT679815	MT780336	New Caledonia	Fernandes et al., 2021
Anochetus pattersoni I82		MT655631	MT872822	MT764010		MT780366	Aldabra Island, Seychelles	Fernandes et al., 2021
Anochetus pubescens I76		MT655633	MT872826	MT764012	MT679841	MT780369	Grande Comore, Comoros	Fernandes et al., 2021
Anochetus pubescens I78		MT655613	MT828526	MT763995	MT679824	MT780346	Mohéli, Comoros	Fernandes et al., 2021
Anochetus pubescens I106			MT872825	MT764011	MT679840		Sazile, Mayotte	Fernandes et al., 2021
Anochetus turneri I254		MT655651	MT872848	MT764033	MT679859		Lockhart River, Australia	Fernandes et al., 2021
Anochetus yerburyi I55		MT655654	MT872850	MT764035	MT679861	MT780387	Karnataka, India	Fernandes et al., 2021

outputs were exported as EMF files for final figure preparation. Additionally, pairwise distances of the *COI* dataset were analyzed using Assemble Species by Automatic Partitioning (ASAP v 1.0) (Puillandre et al., 2021) under Kimura-2P (K80) model.

RESULTS

Morphological analysis

A total of 17 workers and four queens of historical specimens from Taiwan along with 22 workers and five queens of newly collected specimens from the Yaeyama Islands (Tab. 1), were identified as *Anochetus shohki* based on the morphology (for species diagnosis, see Terayama (1996) and Yamane et al. (2024)). Detailed SEM micrographs of the worker and queen, including the fine sculpture of the head and mesosoma, mouthparts, and petiole, are documented in Figures S1 and S2.

In *Anochetus shohki*, a reduced stridulitrumis present on the pretergite of abdominal segment IV (Fig. S1M–N) when abdominal segments III and IV are extended. However, the absence of a plectrum on the inner side of abdominal tergite III (Fig. S1O) indicates that its abdomen is incapable of sound production. Morphological comparisons revealed no significant differences in either worker or queen castes between the Taiwan and Yaeyama populations (Fig. 1). Observed morphological variations in workers were restricted to the overall size range (Tab. S2) and the sculpture patterns of the mesopleural anepisternum (Fig. S1J–K) within both populations.

Compared to members of the *A. africanus* species group, *Anochetus shohki* significantly differs in several characters. In contrast to *A. shohki,* species within the *A. africanus* species group (*A. africanus*, *A. bequaerti*, *A. madagascarensis*, and *A. obscuratus*) possess propodeal spines, a smooth and shining head in lateral view, and different mesosoma sculpture patterns; furthermore, their mesopleuron is not divided into an anepisternum and katepisternum.

Among the *A. graeffei* species group, *Anochetus shohki* resembles *Anochetus annettae* Sharaf, 2017 from Oman and *Anochetus validus* Bharti & Wachkoo, 2013 from India (Fig. 2). While the workers of these species share a similar morphology, they differ in body size: *A. validus* is the largest (TL 5.50-5.66 mm), followed by *A shohki* (TL ~4 mm), while *A. annettae* is the smallest (TL 3.35-3.57 mm) (Terayama, 1996; Bharti & Wachkoo, 2013; Sharaf et al., 2017). Moreover, *A. annettae* can be distinguished from *A. shohki* by smaller compound eyes, head striae extending only to mid-length, and the irregularly rugulose sculpture of the pronotum, mesonotum, and the abdominal segment III (Sharaf et al., 2017). *Anochetus validus* differs from *A. shohki* by having a conical petiolar node, and a smooth, shining lateral head, metapleuron, and abdominal segment III (Bharti & Wachkoo, 2013).

Molecular analysis and Phylogenetic relationships

BLASTn results from GenBank did not show any 100% matches with our sequences for either the *COI* or the four nuclear loci. The highest *COI* sequence identity was 95.89% (Accession No. KY835025, Formicidae sp. from Pakistan). The resulting genetic distance (4.11%) exceeds the commonly accepted 2–3% threshold for intraspecific variation in Formicidae (Hebert et al., 2003)*,* suggesting that this GenBank sample (labeled only as Formicidae sp.) represents a closely related species that remains unidentified at both the genus and species levels.

Phylogenetic analyses of the *COI* and nuclear datasets using the Maximum Likelihood (ML) method demonstrated that all *Anochetus shohki* sequences form a strongly supported monophyletic clade within the *A. graeffei* species group (Fig. 3; BS = 99 for *COI*, BS = 100 for nuclear). However, the topological structures of *COI* and nuclear trees exhibited marked incongruence. In the *COI* tree, *A. shohki* was recovered as sister to a clade comprising five other species of the *A. graeffei* species group (Fig. 3A), whereas in the nuclear tree, it was placed as the sister species to *A. yerburyi* (Fig. 3B).

Fig. 1. Morphological comparison between *Anochetus shohki* populations from Taiwan and the Yaeyama Islands (workers and queens). (**A–C**) worker specimen from Taiwan (NCUESIL001696); (**D–F**) worker specimen from Ishigaki Island (NCUESIL002636); (**G–I**) worker specimen from Yonaguni Island (NCUESIL002625); (**J–L**) queen specimen from Taiwan (NCUESIL002623); (**M–O**) queen specimen from Iriomote Island (NCUESIL002624). (**A, D, G, J, M**) full-face view; (**B, E, H, K, N**) body in profile view; (**C, F, I, L, O**) body in dorsal view.

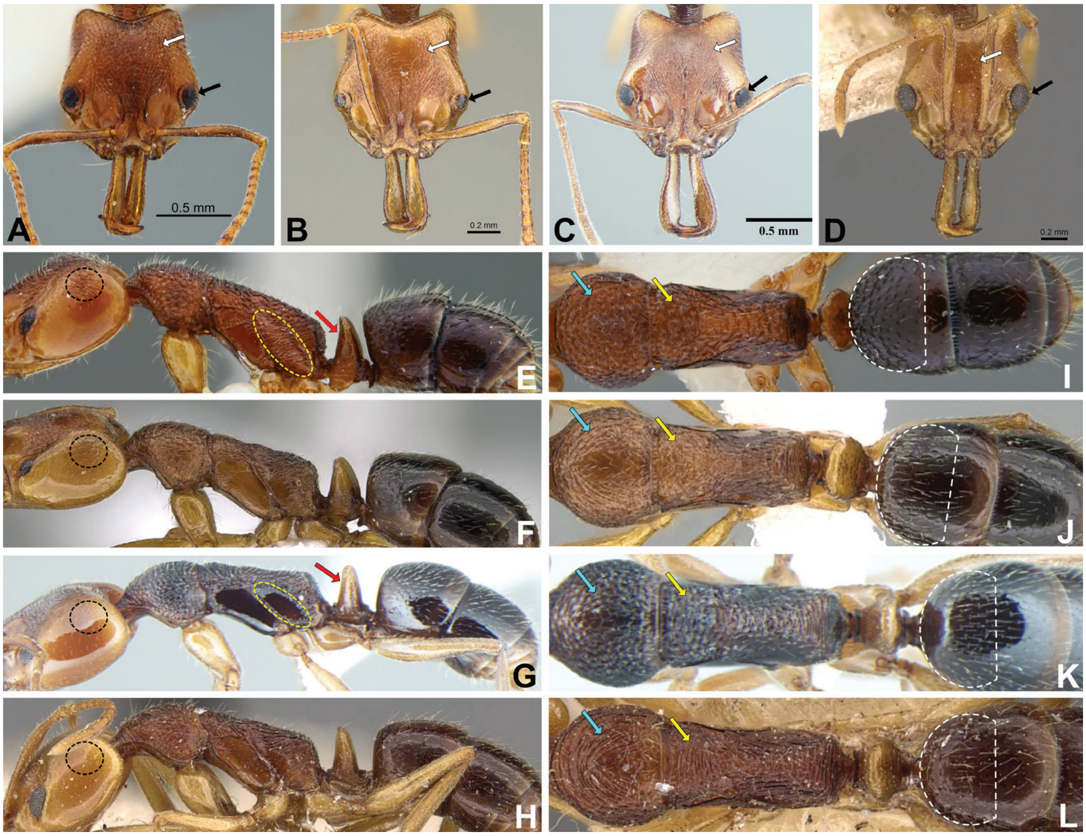

Fig. 2. Morphological comparison of *Anochetus shohki* and three other species within the *A. graeffei* species group (workers). (**A, E, I**) *A. shohki* (NCUESIL001696); (**B, F, J**) *Anochetus annettae* (CASENT0919949, Michele Esposito); (**C, G, K**) *Anochetus validus* (ANTWEB1008023, H. Bharti); (**D, H, L**) *Anochetus yerburyi* (CASENT0907416, Z. Lieberman). White arrows indicate cephalic striae; black arrows indicate the eye; red arrows indicate the petiole; blue arrows indicate pronotal sculpture; yellow arrows indicate mesonotal sculpture. Black dashed circles indicate lateral head sculpture; yellow dashed circles indicate metapleural sculpture; and white dashed circles indicate abdominal segment III.

The *COI* pairwise distance distribution (Fig. 4A) revealed a clear barcoding gap between maximum intraspecific distance (2%) and majority of interspecific distance (5%). The interspecific divergences within the species groups ranged from 3.0% to 11.0%. The minimum interspecific distance (3%) remained higher than the maximum intraspecific variation observed in *A. shohki* (< 1%) and *A. pubescens* (2%).

Importantly, no genetic variation was observed in either *COI* or nuclear markers between the Taiwan and Yaeyama populations, as all *A. shohki* sequences formed a single lineage with zero branch length (Fig. 3) and a high frequency of zero pairwise distance (Fig. 4B). This lack of divergence is noteworthy, given that a degree of genetic differentiation is often expected among populations separated by geographical distances, such as the marine gaps within the Taiwan-Ryukyu Archipelago.

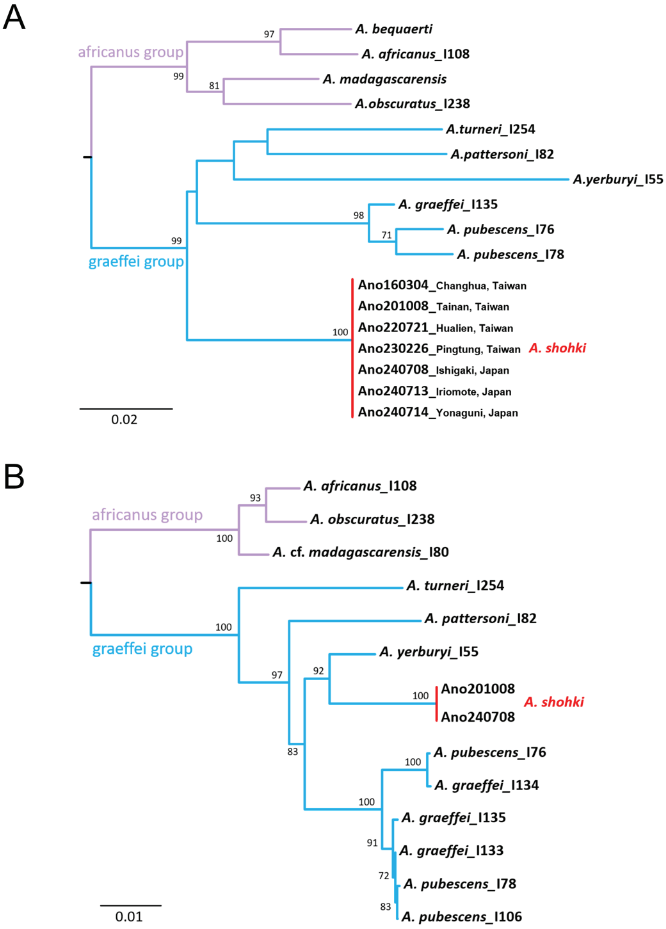

Fig. 3. Phylogenetic tree based on the (**A**) mitochondrial *COI* and (**B**) nuclear (*CAD*, *LW Rh*, *TOP1*, and *Wg*) datasets reconstructed using Maximum Likelihood (ML) with branch support values (Bootstrap, BS) via IQ-TREE. BS values > 70 % are shown at the nodes.

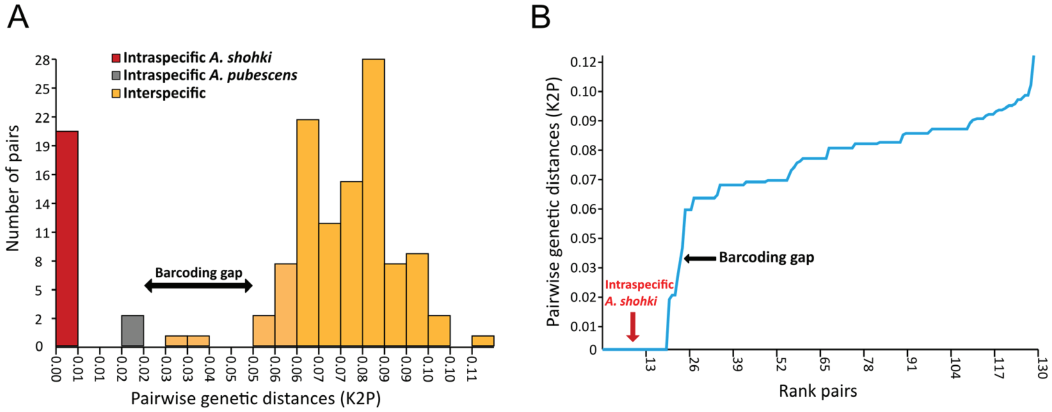

Fig. 4. Barcode divergence analysis of the 10 species included in Figure 2A, based on the *COI* dataset using Assemble Species by Automatic Partitioning (ASAP) under the Kimura-2P (K80) model. (**A**) distribution of pairwise distances; (**B**) ranked pairwise distances.

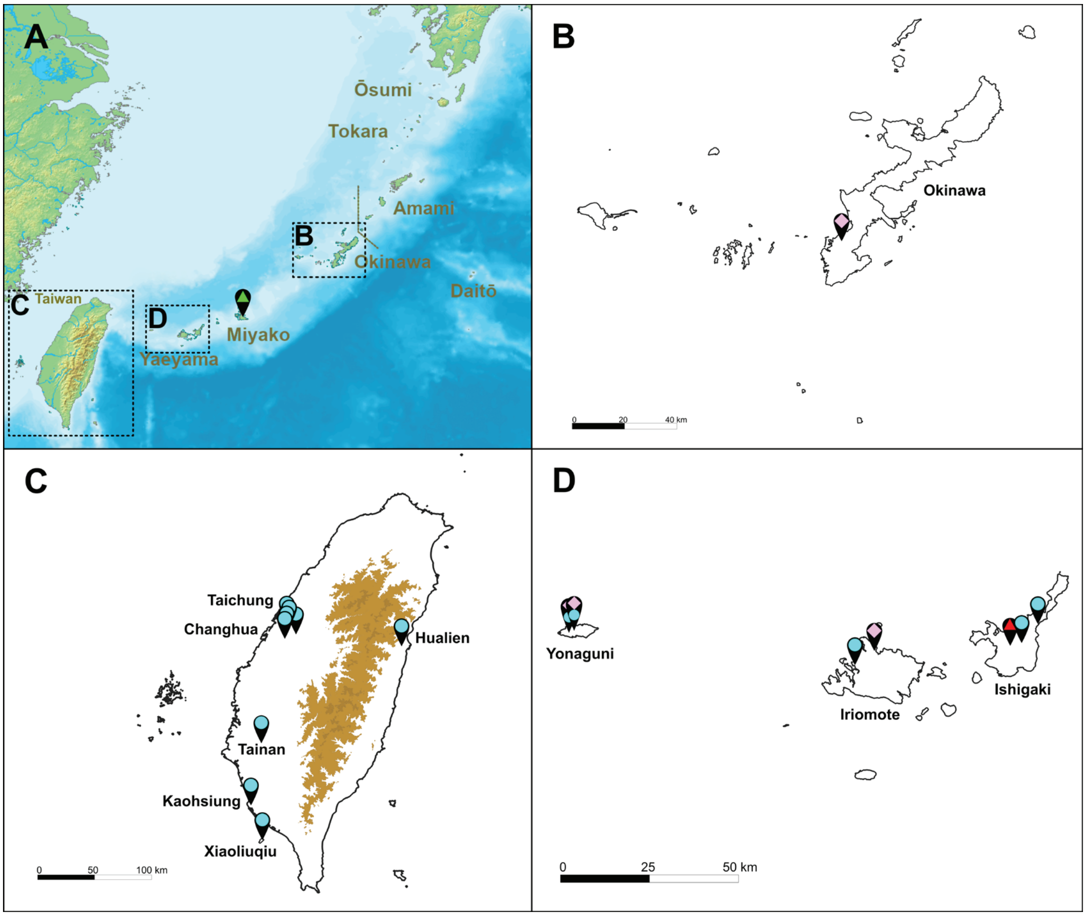

Fig. 5. Distribution of *Anochetus shohki* in Taiwan and the Ryukyu Archipelago, Japan. (**A**) map of surrounding area of Taiwan and the Ryukyu Islands; (**B**) Okinawa Islands; (**C**) Taiwan; (**D**) Yaeyama Islands. Red triangle indicates type locality, pink diamonds refer to Hisasue et al. (2019) and Hisasue & Tokushige (2021), green triangle refers to Komatsu (2009), and blue circles indicate records in this study.

Distribution records and habitat preferences

The distribution of *Anochetus shohki* is scattered across central and southern Taiwan (including Taichung, Changhua, Tainan, Kaohsiung, Pingtung, and Hualien) at low altitudes up to approximately 500 m ASL, yet it has not been recorded in northern Taiwan (Fig. 5). Our study also identified two new localities on the Yaeyama Islands: Inoda Beach on Ishigaki Island and Shirahama on Iriomote Island. Field observations revealed a diverse range of habitat preferences, ranging from leaf litter on roadsides and forest trails to compacted, dry soil near farmland and even sandy beaches (Fig. 6). These ants are typically found in moist microhabitats under the soil, though they also inhabit sun-exposed, disturbed areas. Notably, this species is recorded from sandy beaches on Xiaoliuqiu and Ishigaki Islands for the first time and is also commonly found in the university green spaces in central Taiwan.

Key to Taiwanese *Anochetus* species based on worker caste

1. Posterior half of head smooth and shining in full face view; antenna scape long, exceeding posterior corner of head; inner margin of mandible with several denticles .... *A. risii* Forel, 1900

- Posterior half of head with numerous longitudinal striate in full face view; antenna scape short, not exceeding posterolateral corner of head; inner margin of mandible without denticles 2

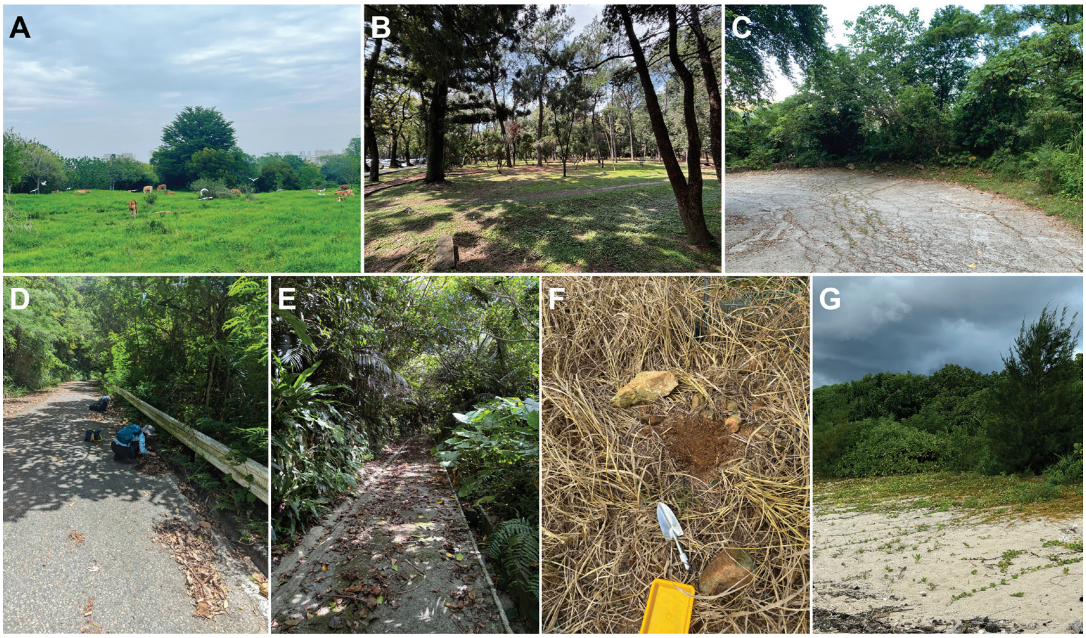

Fig. 6. Habitats of *Anochetus shohki* in Taiwan and the Yaeyama Islands, Japan. (**A**) grassland (Taichung City, Taiwan, Y.C. Hsiao); (**B**) university campus green spaces (Taichung City, Taiwan, F.Y. Chung); (**C**) nature trail (Hualien County, Taiwan, Y.C. Hsiao); (**D**) leaf litter on the roadside (Iriomote Island, Japan, J.R. Liao); (**E**) forest trail (Yonaguni Island, Japan, F.Y. Chung); (**F**) land of hard soil and dry grass (Ishigaki Island, Japan, F.Y. Chung); (**G**) beach (Ishigaki Island, Japan, F.Y. Chung).

2. Compound eye small with 6-7 ommatidia; propodeal spine present .... *A. subcoecus* Forel, 1912
- Compound eye large with over 30 ommatidia; propodeal spine absent 3
3. Dorsum of pronotum longitudinally striate; abdominal segment III smooth and shining .... *A. lanyuensis* Leong et al., 2018
- Dorsum of pronotum reticulate-rugose; abdominal segment III with weak transverse striae .... *A. shohki* Terayama, 1996

DISCUSSION

In this study, we integrated morphological examination and molecular analyses to report the first record of *Anochetus shohki* from Taiwan and to clarify its phylogenetic position within the *A. graeffei* species group. The BLASTn results further highlight the novelty of our molecular data, as none of the sequences available in GenBank matched our sequences with 100% identity. Although *A. shohki* can be identified through morphological characteristics, the previous deficiency of molecular sequences has hindered a robust assessment of its phylogenetic placement. Our study demonstrates the congruence between morphological characters and genetic data for *A. shohki* through a well-supported molecular evolutionary clade (Fig. 3). Furthermore, our findings substantiate its genetic uniformity across disjunct regions and provides a molecular baseline for future systematic research.

The phylogenetic placement of *Anochetus shohki* reveals a significant discordance between *COI* and nuclear datasets (Fig. 3). While the *COI* tree recovers *A. shohki* as a sister to the broader *A. graeffei* species group, the nuclear phylogeny strongly supports its sister-group relationship with *A. yerburyi*. This incongruence in our study potentially resulting from long-branch attraction (LBA) and incomplete lineage sorting (ILS), which are frequently observed in single-locus mitochondrial DNA (Funk & Omland, 2003; Bergsten, 2005; Ross & Shoemaker, 2005). Specifically, an excessive number of samples from the same species may lead to topological errors and instability in phylogenetic placement due to LBA.

Consequently, we favor the nuclear phylogenetic hypothesis regarding these relationships; the combined signal from multiple nuclear loci recovers a species-group-level topology, consistent with the phylogenetic framework proposed by Fernandes et al. (2021). This multi-locus approach is typically more robust against substitutional saturation when resolving deeper phylogenetic relationships and provides a more reliable reflection of the species' evolutionary history by mitigating the potential impacts of ILS (Funk & Omland, 2003; Bergsten, 2005). Nonetheless, mitochondrial *COI* gene remains a rapid and efficient tool for species-level delimitation and routine identification.

Our nuclear phylogenetic placement of *Anochetus shohki* as a sister species to *A. yerburyi* provides a framework for interpreting its zoogeographic history. Both *A. shohki* and *A. yerburyi* are recovered within the Indomalayan realm, whereas other clades within the group occupy the Australasian and Afrotropical realms (Tab. 1). Although the placement of *A. shohki* differs between Fernandes et al. (2021) and Doré et al. (2025), the phylogenetic hypotheses regarding the evolutionary origin of the *A. graeffei* species group remain consistent across both studies. The *A. graeffei* species group is regarded as one of the most ancient lineages within the genus, likely originating in the Neotropics and subsequently spreading to the Afrotropics, Australasia, and Southeast Asia (Fernandes et al., 2021; Doré et al., 2025). This broad biogeographic pattern, which is consistent with our nuclear phylogeny, suggests that *A. shohki* represents a specialized Indomalayan lineage derived from these ancestral dispersal events.

Notwithstanding their close phylogenetic relationship according to the nuclear data, *Anochetus shohki* and *A. yerburyi* exhibit significant differences in the extending position of the cephalic striae (Bharti & Wachkoo, 2013) and the sculpture type on the mesosomal dorsum (Fig. 2). Conversely, while *A. shohki* morphologically resembles *A. annettae* and *A. validus*, the phylogenetic relationships among these taxa remain unresolved due to the lack of available molecular sequences for the latter two species. In contrast to these interspecific differences, our visual comparison of key morphological characters reveals high intraspecific similarity between the Taiwan and Yaeyama populations (Fig. 1). This consistency in diagnostic features, despite noticeable variation in body size (Tab. S2), implies morphological uniformity and evolutionary conservatism across these regions.

Miyazaki (2023) reported thelytokous parthenogenesis in *Anochetus shohki* and confirmed the presence of spermatheca in queens. Although the retention of the spermatheca might suggest the occurrence of facultative thelytoky in this species, no males have been recorded to date in the Taiwan and Yaeyama colonies. Notably, approximately half of known thelytokous ant species (including lineages from Dorylinae, Ponerinae, Formicinae, and Myrmicinae) are categorized as tramp or invasive species, suggesting that thelytoky may offer reproductive and dispersal advantages to these ants (Rabeling & Kronauer, 2013; Wang et al., 2023). In addition to their specialized reproductive strategies, our field observations reveal a broad habitat preference in *A. shohki* (Fig. 6), a trait shared by several congeners. For instance, *Anochetus kempfi* Brown, 1978 has been recorded in diverse environments, ranging from rainforests and dry forests to sandy beaches (Torres et al., 2000). Such ecological flexibility suggests that members of this genus may possess high adaptability to both natural and urbanized environments. This adaptability, coupled with thelytokous reproduction, likely explains the extremely low genetic diversity observed in our study.

A striking finding of our study is the genetic homogeneity observed between the Taiwan and Yaeyama populations, as evidenced by the lack of genetic divergence across both *COI* and nuclear datasets (Fig. 3). This absence of genetic variation is particularly notable given the oceanic gaps separating these regions, which often promote a degree of genetic differentiation reflecting long-term isolation (Blatrix et al., 2020). Recent ant invasions in Taiwan, such as those of *Dolichoderus thoracicus* (Smith, 1860) (Hsu et al., 2022) and *Odontomachus troglodytes* Santschi, 1914 (Lin et al., 2023), further demonstrate how founder effects can lead to significant genetic uniformity in newly established populations. The genetic homogeneity observed across disjunct geographic regions suggests that our findings are not a localized sampling artifact but rather reflect a very recent evolutionary history, implying that the Taiwan and Yaeyama populations of *A. shohki* represent a single and homogeneous lineage.

These findings collectively suggest that *Anochetus shohki* might possess strong dispersal abilities, potentially via human-mediated transport or natural rafting, or has undergone a recent range expansion. The potential for oceanic dispersal by natural rafting among island-dwelling organisms is supported by studies on coastal termites and *Pachyrhynchus* weevils (Yeh et al., 2018; Chiu et al., 2021). Although *A. shohki* has been found on beaches on Ishigaki and Xiaoliuqiu Islands, whether it can disperse via oceanic rafting remains unknown. Given this uncertainty, the precise dispersal route and the full distribution range of *A. shohki* remain to be elucidated.

Future studies incorporating more extensive sampling across Southeast Asia will be crucial to determine whether the Taiwanese population represents a natural range expansion or a recent introduction. Effective monitoring and continued ant taxonomic efforts will not only clarify species distributions but also improve our understanding of their regional and global population trends.

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