



## Insights into social organization and population genetics of introduced *Odontomachus troglodytes* (Hymenoptera: Formicidae) in Taiwan

Hsiao-Yang CHU, Yi-Ting FANG, Tzong-Han LIN, Joanne Tzu-Chia CHEN & Shu-Ping TSENG

### Abstract

Supercoloniality and unique reproductive strategies are critical factors driving the ecological dominance of invasive ant species. However, their role in the post-introduction success of species belonging to the Ponerinae subfamily remains largely unexplored. *Odontomachus troglodytes*, a ponerine ant native to sub-Saharan Africa, has been recently reported to have established populations in southern Taiwan. In the present study, we conducted behavioral assays and developed 11 novel polymorphic microsatellite markers to investigate the social organization, breeding system, and population structure of *O. troglodytes* in its introduced range. Genotypic analyses indicated sexual reproduction with no evidence of clonal propagation. Both field observations and genetic data revealed high levels of polygyny and polyandry, accompanied by a notable frequency of diploid males, likely resulting from inbreeding. Our findings suggest that the Kaohsiung population exhibits some supercolonial traits, including polygyny, polydomy, and the absence of inter-nest aggression across distances of several kilometers. This marks the first reported case of such a social structure in the Odontomachini tribe. While workers from different nests displayed tolerance, genetic data suggest incomplete homogenization across nests. The present study offers new insights into the colony structure and reproductive biology of *O. troglodytes* in an introduced context and highlights the potential role of social structure in supporting population persistence and spread.

**Key words:** Invasive species, microsatellite, Ponerinae, supercolony.

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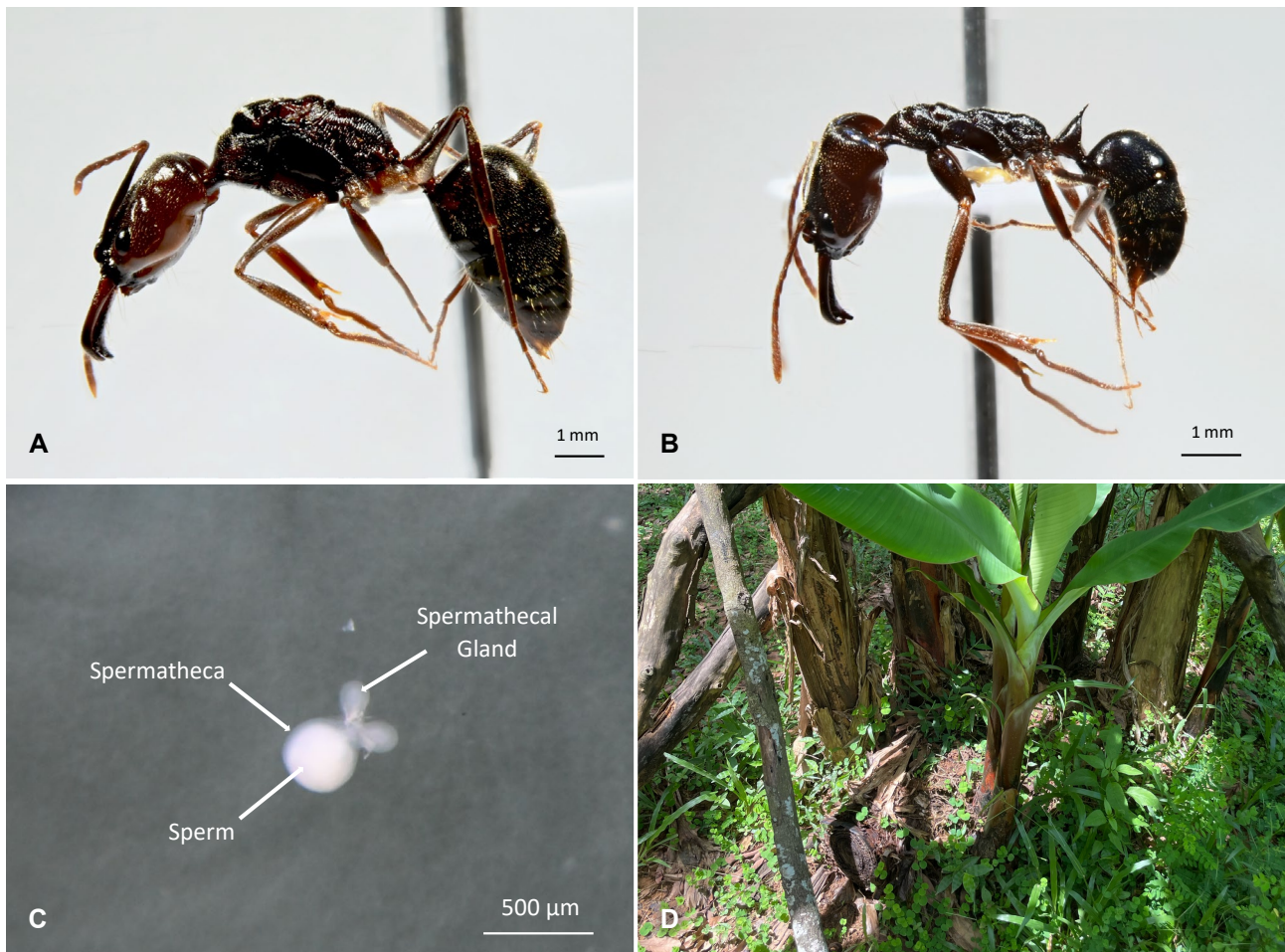
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### Introduction

Invasive species represent a significant threat to global biodiversity, with globalization and modern transportation accelerating their spread and causing substantial ecological and economic damage (SIMBERLOFF & al. 2013, EARLY & al. 2016, SEEBENS & al. 2017). Among invasive invertebrates, ants are particularly successful, often causing severe declines in native communities and disrupting ecosystems (HOLWAY & al. 2002, LACH & HOOPER-BÛI 2010, RABITSCH 2011, BERTELSMEIER & al. 2017). Successful invasive ant species often exhibit traits such as supercoloniality and polygyny, which enhance their ability to colonize new habitats (HELANTERÄ & al. 2009, BERTELSMEIER & al. 2017, EYER & VARGO 2021). Supercoloniality is characterized by extensive networks of interconnected nests with reduced aggression and territoriality, enabling invasive ants to achieve high population densities and ecological dominance (HELANTERÄ & al. 2009, HELANTERÄ

2022). Polygyny, in which colonies harbor multiple queens, promotes colony growth and survival, increasing the likelihood of successful establishment and spread in new environments (TSUTSUI & SUAREZ 2003, EYER & VARGO 2021). Asexual reproduction aids invasion by enabling a single foundress to establish colonies without requiring mates in introduced ranges (RABELING & KRONAUER 2013, EYER & VARGO 2021). Despite the extensive studies on these traits in several ant taxa (HELANTERÄ & al. 2009, EYER & VARGO 2021), their prevalence and role in post-introduction success remain poorly understood in certain groups, particularly in the primitively eusocial Ponerinae subfamily.

*Odontomachus troglodytes*, a ponerine ant native to sub-Saharan Africa, presents a unique opportunity to elucidate these dynamics. In its native range, *O. troglodytes* individuals form large colonies that often exceed



**Fig. 1:** Morphology and habitat of *Odontomachus troglodytes*. (A) Queen, (B) worker, (C) queen spermatheca, and (D) typical nesting site at the base of cash crops.

several thousand individuals (BROWN 1976, SCHMIDT & SHATTUCK 2014), contrasting with most Ponerinae species that typically maintain smaller colonies of only a few hundred workers (WHEELER 1900, PAIVA & BRANDÃO 1995, KELLER & PEETERS 2021). Field observations from Cameroon indicate that colony clusters may be separated by only a few meters and linked by underground galleries, with no aggression among workers (COLOMBEL 1970). This suggests the possibility of short-range polydomous or budding colony structures in its native range. Such attributes, including large population sizes and potentially low intraspecific aggression, imply that *O. troglodytes* could possess the traits necessary for successful colonization beyond its native range. Recently, *O. troglodytes* has been detected in southern Taiwan, where it was first reported in 2017 (LIN & al. 2023) (Fig. 1A & 1B). However, ecological studies of *O. troglodytes* in Taiwan are virtually absent, with no systematic investigation of its social organization, reproductive system, or behavioral ecology conducted yet. Understanding these attributes is crucial for assessing its potential as an introduced species and for informing future management.

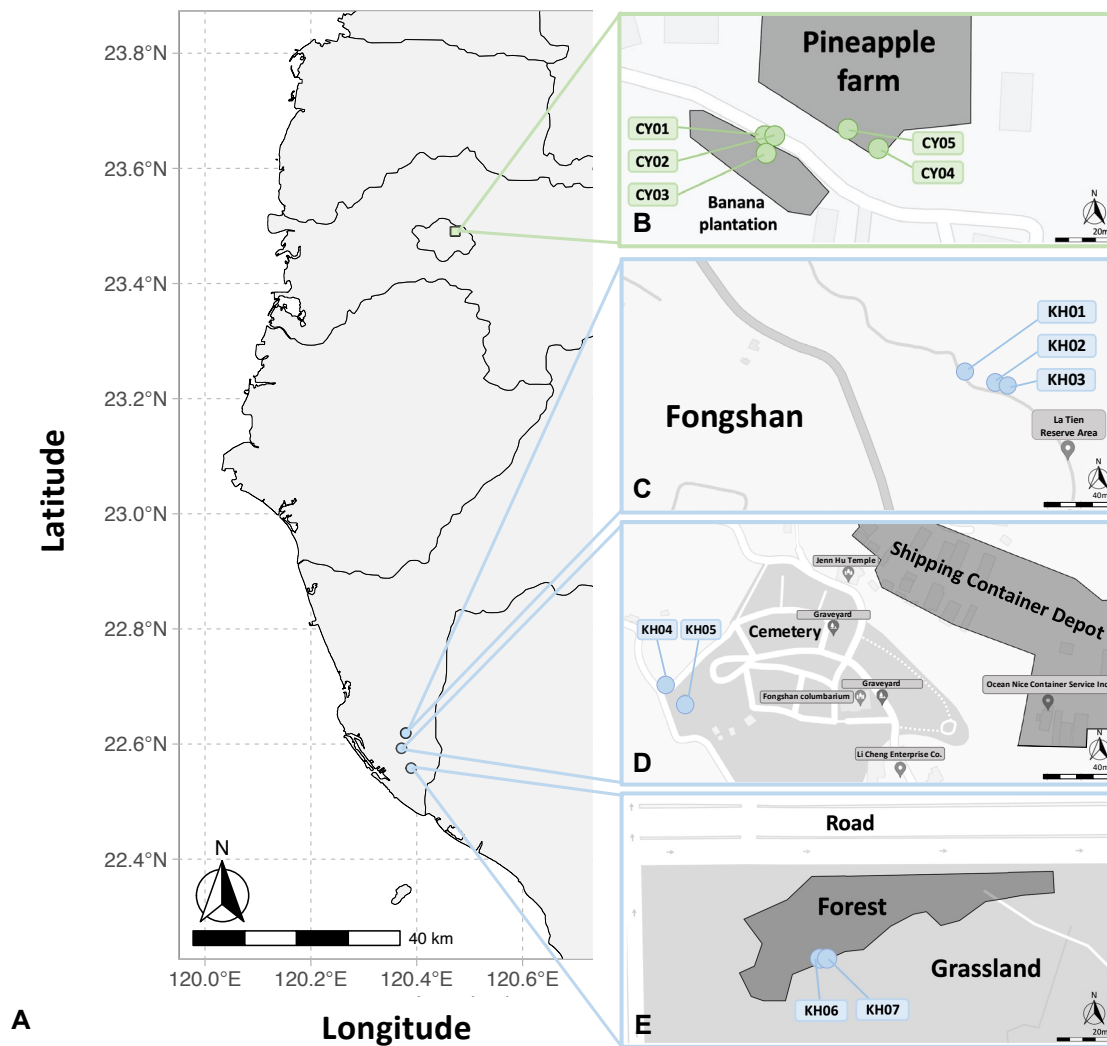
To address these knowledge gaps, we conducted behavioral assays and developed 11 novel polymorphic mi-

cro-satellite markers to evaluate the colony structure and breeding system of *O. troglodytes* in Taiwan. Specifically, we aimed to (1) investigate the presence of supercoloniality in *O. troglodytes* using behavioral assays and genetic analyses, (2) elucidate the breeding system of the species through molecular markers and field observations, and (3) assess the genetic diversity and differentiation of introduced populations to infer the invasion history within Taiwan.

## Material and methods

### Sampling

Twelve nests of *Odontomachus troglodytes* were collected from two distinct geographic regions in Taiwan: five nests from Chiayi and seven nests from Kaohsiung, between 14 and 15 August 2023 (Fig. 2A; Tab. S1, as digital supplementary material to this article, at the journal's web pages). Species identification was based on morphological diagnoses provided by BROWN (1976) and confirmed through cytochrome c oxidase I sequence analysis, following the protocols and comparative framework established by LIN & al. (2023). In Chiayi, nests were found in banana plantations (Fig. 1D) and near pineapple



**Fig. 2:** Collection sites in Taiwan. (A) Overview of sampling locations: five nests from Chiayi and seven nests from three Kaohsiung sites. Detailed collection sites in (B) East District, Chiayi: nests CY01 - 03 and CY05 from banana plants, CY04 from a fruit tree; (C) North Fongshan, Kaohsiung: three nests from tree bases; (D) South Fongshan, Kaohsiung: KH04 from banana rhizome, KH05 from tree base; and (E) Siaogang District, Kaohsiung: KH06 from dead trunk, KH07 from fallen wood.

farmlands (Fig. 2B). In Kaohsiung, nests were collected from the bases of bushes and tree trunks along hiking trails in northeastern Fongshan (Fig. 2C), on a hillside near Fongshan Cemetery (Fig. 2D), and from decomposing wood in the Dapingding Tropical Botanical Garden in Siaogang District (Fig. 2E). GPS coordinates of all sampled nests were recorded and are provided in the appendix (Tab. S1). Pairwise geographic distances between nests were calculated based on these coordinates to estimate the spatial extent of colony boundaries in each region. After collection, each nest was maintained in a pie-shaped plastic container with a moist plaster substrate at room temperature (25°C) in the laboratory and was provided with a water source prior to aggression assays or molecular experiments. Colonies were maintained with access to water only to avoid potential variability introduced by in-lab feeding. All assays were completed within one week of collection. No unusual mortality or signs of worker debilitation were observed during handling or after marking.

All colonies used in the assays were queen-right at the time of testing and contained comparable numbers of workers (estimated at 50 - 200 individuals).

### Aggression assays

To evaluate the levels of intraspecific aggression among ants from different nests and regions, aggression assays were conducted within a week of nest collection. Ten nests with sufficient worker numbers (four nests from Chiayi: CY02, CY03, CY04, CY05; and six from Kaohsiung: KH02, KH03, KH04, KH05, KH06, and KH07) were selected for the trials. Workers were placed in 7-mL plastic tubes and chilled in an ice bucket for 3 min to induce chill-coma and subsequently marked on the thorax and abdomen with SNOWMAN-CP paint markers (CP, Kurayoshi, Japan) for identification. Marked workers were returned to their respective nests for at least 24 h prior to the assays to allow marker odors to dissipate and ensure normal behavior.

Aggression trials consisted of one-on-one pairings, with a total of 107 pairings performed. These included (1) within-nest pairings (individuals from the same nest), (2) between-nest pairings within the same region (e.g., nest CY02 vs. CY04), and (3) between-nest pairings from different regions (e.g., CY02 vs. KH02). Each trial was conducted in a plastic arena (8 cm in diameter), with one worker ant initially confined under a transparent lid of a Petri dish. Workers and arena bases were not reused across trials to prevent any residual odors or interference. After a 10-min acclimatization period, the Petri dish lid was removed, and interactions were recorded for 10 min using an FHD Webcam (Keystone-AVA001, Taipei, Taiwan). Interactions were scored using a modified aggression scale (SUAREZ & al. 1999, GIRAUD & al. 2002, MURATA & al. 2017): (0) ignore; (1) mild antennation; (2) rapid antennation (RA), considered pre-aggressive behavior; (3) bite and pull; and (4) snap (a rapid mandible strike) and sting. Interactions scoring 3 or higher were classified as aggressive, whereas scores of 2 or lower were considered non-aggressive. For each trial, the interaction was scored based on the highest level of observed behavior. To assess temporal patterns, the RA duration, which is the total time two ants are engaged in RA (in seconds), was recorded for each interaction.

All trials, regardless of group pairing, exhibited the same aggression level (non-aggressive,  $\leq 2$ ; see Results section). Therefore, statistical tests on aggression levels were unnecessary. Instead, the analysis focused on the RA behavior within the 10-min recording period. Specifically, detailed RA duration patterns across the different group pairings and encounter sequences were analyzed using generalized estimating equations (GEE; LIANG & ZEGER 1986) with individual ID as a random effect. Moreover, differences in RA duration between the first and second encounters were examined using GEE, with model selection conducted using quasi-information criterion (QIC; PAN 2001). Differences in RA duration during the first encounter among different treatment groups were assessed using Kruskal-Wallis tests with post-hoc pairwise Wilcoxon rank-sum tests. Finally, the number of encounters was evaluated by counting the interactions between paired workers during the 10-min recording period. Differences among groups were first assessed using a Kruskal-Wallis test. When significant, post-hoc pairwise Wilcoxon rank-sum tests were applied, and P-values were adjusted using the Benjamini-Hochberg correction for multiple comparisons. All statistical analyses were conducted in R version 4.3.2 (R DEVELOPMENT CORE TEAM 2010), with GEE models implemented using the “geepack” package. Statistical significance was set at an alpha level of 0.05 for all analyses. Workers were preserved in 95% ethanol after the assay for subsequent molecular analysis.

#### **Additional behavioral observations**

In addition to the standardized aggression assays, further behaviors were observed during routine maintenance and colony monitoring. Worker carrying and queen-queen

dominance interactions were noted in laboratory colonies during both aggression trials and long-term observations. To assess the potential for worker reproduction, six queenless colonies were maintained under laboratory conditions and monitored for 13 months. Colonies were checked regularly for the presence of eggs and brood.

#### **Development of microsatellite markers**

Microsatellite markers were developed based on genetic data from two *Odontomachus troglodytes* samples collected in Kaohsiung and Chiayi. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA extraction, library preparation, and sequencing were performed by Tri-I Biotech Inc. (Taipei, Taiwan) using the Celero EZ DNA-Seq Library Preparation Kit (TECAN, Männedorf, Switzerland). Sequencing was performed on the NovaSeq 6000 Sequencing Illumina platform (Illumina Inc., San Diego, CA), and each sample was sequenced to generate 12 Gb of data. Sequence reads were trimmed for low-quality bases (quality value  $\geq 25$ ) using CLC Genomic Workbench v10. De novo assembly was performed using SPAdes v3.15.3 (<https://github.com/ablab/spades>) using the default settings. Draft contigs were screened for microsatellite loci, and dinucleotide repeats of 10 - 17 units were identified using Krait v1.4.0 (DU & al. 2018). From this analysis, 20 potential microsatellite loci were selected, with 10 derived from the Kaohsiung sample and 10 from the Chiayi sample. Primers were designed using Primer3 (ROZEN & SKALETSKY 2000) embedded in Krait v1.4.0, targeting amplicon sizes of 100 - 250 bp. The primer sequences are listed in the appendix (Tab. S2). Primer pairs were synthesized by Genomics BioSci and Tech Corp. (Taipei, Taiwan).

#### **DNA extraction and microsatellite genotyping**

Nine nests containing reproductive castes were genotyped: CY01, CY02, CY04, CY05, KH01, KH03, KH04, KH05, and KH06. Up to three queens, all of the collected males ( $N = 15$ ), and four workers from each colony were sampled for population genetics analysis. Additionally, sperm samples were genotyped from dissected spermathecae (Fig. 1C) of all the genotyped queens. Samples with possible contamination from queen tissues, identified using sperm genotype matching the alleles of the queen at each locus, were excluded from the analyses. Genomic DNA was extracted using the Gentra Puregene Tissue kit (Qiagen) according to the manufacturer's instructions. Eluted DNA samples were stored at  $-20^{\circ}\text{C}$  until further use. Microsatellite genotyping was conducted following the method of BLACKET & al. (2012), with slight modifications. An initial PCR was performed to screen 20 microsatellite loci. Each 20- $\mu\text{L}$  PCR reaction consisted of 10  $\mu\text{L}$  AMPLIQON 2  $\times$  Master Mix RED (Ampliqon, Odense, Denmark), 1  $\mu\text{L}$  of a 1  $\mu\text{M}$  primer pair, 8  $\mu\text{L}$  nuclease-free water, and 1  $\mu\text{L}$  genomic DNA from each ant sample (Kaohsiung). The PCR conditions were as follows:  $95^{\circ}\text{C}$  for 5 min, followed by 35 cycles of  $95^{\circ}\text{C}$  for 30 s,  $58^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 30 s, with a final extension at  $72^{\circ}\text{C}$  for

10 min and a hold at 4°C. PCR products exhibiting a single band of the expected size were selected for further PCR reactions using fluorescently labeled universal primers, following the modified method of BLACKET & al. (2012). Four fluorescent dyes – FAM, VIC, NED, and ROX – were used along with locus-specific primers containing 5' universal primer tails. Each microsatellite locus was amplified separately. PCR reactions were prepared similarly to the initial round, with diluted products (1:50) from the first PCR used as templates. Amplified products were analyzed on an ABI-3730XL DNA Analyzer (Genomics BioSci and Tech Co., Ltd, Taipei, Taiwan). The alleles were visualized and scored using GeneMarker v3.0.1 (SoftGenetics LLC, Pittsburgh, PA). A statistical summary of the novel microsatellite markers, including the number of alleles (Na), Shannon's information index (I), observed heterozygosity (Ho), and private allele summary, was calculated using GenAlEx 6.503 software (PEAKALL & SMOUSE 2006, 2012).

### Colony and breeding structure

Field observations and genetic data were integrated to understand the breeding system and reproductive mode of *Odontomachus troglodytes*. The number of queens was recorded during field collections and further estimated based on worker genotypes. Reproductive mode (i.e., sexual or clonal) was inferred by comparing genotypic data across all castes, including queens, alate queens, males, workers, and sperm from queen spermathecae. COLONY v.2.0 (JONES & WANG 2010) was used to estimate the number of reproductive individuals (queens and males) and assess mating patterns within the populations. Analyses were conducted separately for the Chiayi and Kaohsiung populations. Queens (maternity) and their mates (paternity) were provided as references based on available genotypic data. Queens were set as polygamous, reflecting the observation of multiple queens per colony (COLOMBEL 1970, 1978). Males were also set as polygamous based on sperm genotyping, which indicated possible polyandry. Inbreeding was included as a parameter in the COLONY software to account for the high proportion of diploid males observed in the population. The species setting in COLONY was set to "HaploDiploid," and the run length to "very long." Marker error rates were estimated using CREATE v.1.37 (COOMBS & al. 2008) based on genotypic data from all sampled colonies. Queen genotypes were included as maternal genotypes when available.

### Nest-level genetic differentiation within regions

To determine the colony affiliation of samples obtained from different nests within the same region (e.g., whether the four nests collected from Chiayi belong to the same colony), groups of ants were tested for significant genetic differentiation using permutation tests with standard Bonferroni corrections as implemented in FSTAT v.2.9.4 (<https://www2.unil.ch/popgen/software/fstat.htm>). Samples demonstrating significant genetic differentiation were considered to belong to separate colonies

(VARGO 2003, HUSSENER & al. 2005, 2007). Pairwise comparisons were conducted between the following nest groups: (1) CY01 / CY02 / CY04 / CY05 and (2) KH01 / KH03 / KH04 / KH05 / KH06.

### Relatedness analysis

Relatedness between queens and workers, as well as among workers, was estimated using the QUELLER & GOODNIGHT (1989) estimator implemented in GenAlEx 6.503 (PEAKALL & SMOUSE 2006, 2012). Analyses were conducted separately for the Kaohsiung and Chiayi populations. Pairwise relatedness values were calculated for all individual pairs and categorized as either within-nest or between-nest comparisons for both worker-worker and queen-worker pair types. All statistical analyses and visualizations were performed using the software R, version 4.3.2 (R DEVELOPMENT CORE TEAM 2010). The normality of relatedness distributions was assessed using Shapiro-Wilk tests. As some group distributions deviated from normality, non-parametric Wilcoxon rank-sum tests were used. The following analyses were performed: (1) comparisons of relatedness between individuals from the same nest versus those from different nests, conducted separately for each pair type and region; and (2) tests of whether within-nest and between-nest relatedness values significantly differed from zero. Statistical significance was set at an alpha level of 0.05. Boxplots were generated using the "ggplot2" package to visualize patterns of relatedness based on pair type and nest category.

### Population-level genetic structure

Analysis of molecular variance (AMOVA) was used to hierarchically analyze genetic diversity among regions, nests within regions, within nests, and individuals, using GenAlEx 6.503 (PEAKALL & SMOUSE 2006, 2012). The variance between regions indicated the genetic differentiation between the Chiayi and Kaohsiung populations. The significance of F-statistics was estimated based on 999 permutations (NEI 1973, LIU & al. 2022).

Regional genetic differentiation was assessed in GenAlEx 6.503 using Wright's  $F_{ST}$ , Jost's differentiation estimate ( $D_{est}$ ), and Hedrick's standardized  $G_{ST}$  for small populations ( $G'_{ST}$ ), using one worker per nest. To account for potential variability, estimates were obtained by selecting one worker from each nest at random, and the calculations were repeated four times. Genetic clustering patterns were explored using principal coordinate analysis (PCoA), based on pairwise genetic distances among individuals, calculated in GenAlEx 6.503 (PEAKALL & SMOUSE 2006, 2012) from all available worker genotypes. The results were visualized using the ggplot2 package in R (R DEVELOPMENT CORE TEAM 2010). Bayesian clustering analysis was conducted in STRUCTURE version 2.3.1 (PRITCHARD & al. 2000) to determine the number of genetic clusters, using genotypes from all available workers. Ten replicates were performed for each K value ranging from 1 to 9. Each run consisted of 500,000 Markov chain Monte Carlo iterations following a burn-in phase of 500,000 iterations.

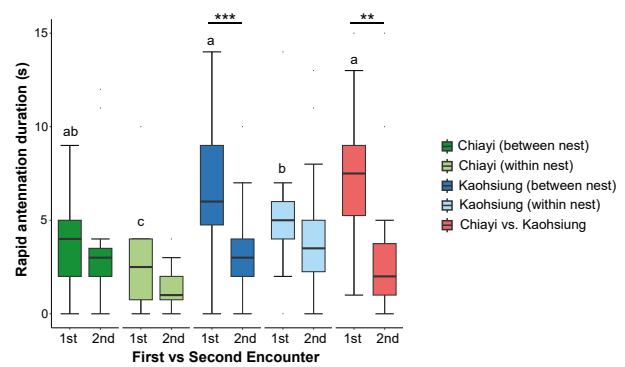
The most likely number of clusters was determined using the  $\Delta K$  method (EVANNO & al. 2005) as implemented in StructureSelector (LI & LIU 2018). STRUCTURE results were visualized in R with “ggplot2”.

## Results

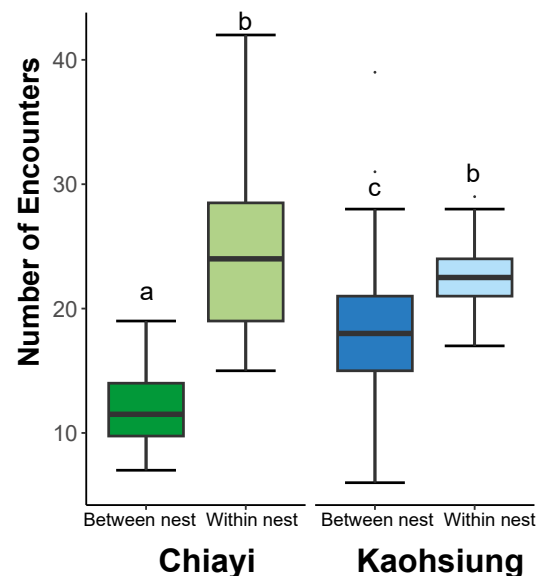
### Non-aggressive interactions among nests

All 107 aggression trials resulted in non-aggressive interactions, with the highest observed interaction levels classified as less than or equal to two on the aggression scale. No aggressive behaviors, such as biting, pulling, snapping, or stinging, were observed in the experimental (between nests from different regions) or control (within nests) groups. Additional observations indicated that *Odontomachus troglodytes* individuals are not inherently non-aggressive. Specifically, *O. troglodytes* workers exhibited aggressive behaviors toward heterospecific ants, including the red imported fire ant (*Solenopsis invicta*) and the black cocoa ant (*Dolichoderus thoracicus*), displaying biting, mandible snapping, and stinging responses in laboratory settings. Model selection using QIC values indicated that the interaction between the encounter count and group provided the best fit for analyzing RA duration patterns (Tab. S3). Moreover, GEE analysis revealed that after the first encounter, subsequent encounters exhibited no significant differences in RA duration ( $W = 2.15$ ,  $p = 0.142$ ; Fig. S1). The RA durations were significantly longer in the first encounter than in the second encounter ( $W = 10.93$ ,  $p < 0.001$ ; Fig. S2). Therefore, we focused on comparing initial and second encounters across groups. In Chiayi, the RA durations did not differ significantly between the initial and second encounters for both within-nest and between-nest groups (within-nest mean RA duration: initial = 2.67 s vs. second = 1.33 s,  $W = 2.19$ ,  $p_{adj} = 0.173$ ; between-nest mean RA duration: initial = 11.75 s vs. second = 5.33 s,  $W = 3.57$ ,  $p_{adj} = 0.098$ ; Fig. 3). Conversely, in Kaohsiung, the between-nest group displayed significantly longer RA durations during initial encounters than during second encounters (mean RA duration: initial = 10.87 s vs. second = 4.22 s,  $W = 27.5$ ,  $p_{adj} < 0.001$ ). However, the within-nest group exhibited no significant difference between initial and second encounters (mean RA duration: initial = 5.78 s vs. second = 4.44 s,  $W = 1.05$ ,  $p_{adj} = 0.304$ ). Similarly, the between-region group (Chiayi vs. Kaohsiung) displayed significantly longer RA durations during the initial encounter than during the second encounter (mean RA duration: initial = 13.07 s vs. second = 5.83 s,  $W = 10.9$ ,  $p_{adj} < 0.01$ ; Fig. 3).

The initial encounter durations significantly differed across groups (Kruskal-Wallis test, chi-square = 25,  $p < 0.001$ ). Post-hoc pairwise comparisons revealed that the RA duration in within-nest pairs was significantly shorter than that in between-nest pairs in both the Chiayi (pairwise Wilcoxon rank-sum test with Benjamini-Hochberg correction,  $p_{adj} = 0.048$ ; Fig. 3) and Kaohsiung regions (pairwise Wilcoxon rank-sum test with Benjamini-Hochberg correction,  $p_{adj} = 0.040$ ; Fig. 3).



**Fig. 3:** Rapid antennation (RA) analysis. Duration of RA during the initial and second encounter across five comparison groups: within-nest Chiayi (light green), within-nest Kaohsiung (light blue), between-nest Chiayi (dark green), between-nest Kaohsiung (dark blue), and Chiayi vs. Kaohsiung (red). Asterisks indicate significant differences between the initial and second encounter within each group, based on generalized estimating equations with Benjamini-Hochberg correction for multiple comparisons ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ). Letters above the first encounter boxplots indicate significant differences among the five groups during the first encounter, based on a Kruskal-Wallis test followed by pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction ( $p < 0.05$ ).



**Fig. 4:** Number of encounters per trial across four pair types: within-nest Chiayi (light green), between-nest Chiayi (dark green), within-nest Kaohsiung (light blue), and between-nest Kaohsiung (dark blue). Boxplots show the number of physical encounters recorded during 10-minute trials. Group differences were first assessed using a Kruskal-Wallis test, followed by post-hoc pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction for multiple comparisons. Letters above boxes indicate statistically significant differences; groups not sharing a letter differ significantly at  $p < 0.05$ .

**Tab. 1:** Genetic diversity across the 12 microsatellite loci in *Odontomachus troglodytes* workers. N: number of individuals genotyped; Na: number of alleles; I: Shannon's information index; Ho: observed heterozygosity; He: expected heterozygosity.

Locus	Chiayi population					Kaohsiung population				
	N	Na	I	Ho	He	N	Na	I	Ho	He
OT1	16	1.000	0.000	0.000	0.000	20	2.000	0.688	0.400	0.495
OT2	16	2.000	0.311	0.188	0.170	20	2.000	0.688	0.400	0.495
OT4	16	1.000	0.000	0.000	0.000	20	2.000	0.682	0.150	0.489
OT5	16	3.000	0.875	0.313	0.549	20	3.000	1.067	0.600	0.645
OT8	16	3.000	1.092	0.625	0.662	20	3.000	0.999	0.350	0.605
OT11	16	1.000	0.000	0.000	0.000	20	1.000	0.000	0.000	0.000
OT12	16	3.000	0.692	0.438	0.420	20	3.000	0.976	0.650	0.579
OT15	16	1.000	0.000	0.000	0.000	20	2.000	0.377	0.150	0.219
OT16	16	1.000	0.000	0.000	0.000	20	2.000	0.673	0.300	0.480
OT17	16	1.000	0.000	0.000	0.000	20	2.000	0.631	0.150	0.439
OT18	16	3.000	0.99	0.688	0.596	20	3.000	0.633	0.400	0.339
OT20	16	2.000	0.234	0.125	0.117	20	2.000	0.377	0.150	0.219
Mean (± SD)	16 (0)	1.833 (0.271)	0.349 (0.126)	0.198 (0.075)	0.209 (0.077)	20 (0)	2.250 (0.179)	0.649 (0.086)	0.308 (0.057)	0.417 (0.055)

The overall number of encounters differed significantly among groups (Kruskal-Wallis test, chi-square = 31.90,  $p < 0.001$ ). Post-hoc pairwise comparisons revealed that within-nest encounter counts were significantly higher than between-nest counts in both Chiayi (Wilcoxon rank-sum test,  $W = 4$ ,  $p_{adj} < 0.001$ ) and Kaohsiung (Wilcoxon rank-sum test,  $W = 195$ ,  $p_{adj} = 0.002$ ; Fig. 4). Pairwise comparisons across regions further revealed that between-nest encounter numbers were significantly lower in Chiayi than in Kaohsiung ( $W = 91$ ,  $p_{adj} < 0.001$ ), whereas within-nest encounter numbers did not differ significantly between the two regions ( $W = 128$ ,  $p_{adj} = 0.419$ ).

#### Characterization of microsatellite loci

Of the 20 primer sets tested, 12 loci were successfully amplified, with 11 displaying polymorphisms (Tab. S2). Across the 12 loci, 27 alleles were identified from 36 genotyped worker samples. The number of alleles per locus ranged from 1 to 3, with an average of 2.04 alleles per locus in the worker dataset (Tab. S2). The 12 loci were also successfully amplified in the queens, males, and sperm samples. Genetic diversity, indicated by average Na per

**Tab. 2:** Summary of the private alleles of the Kaohsiung population.

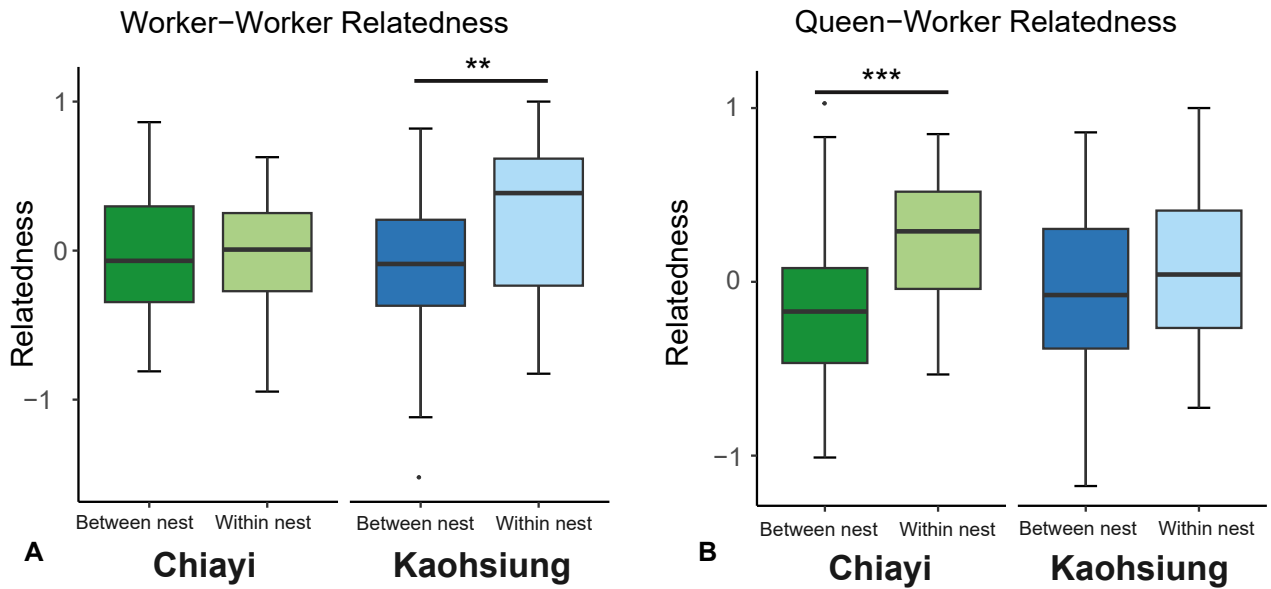
Locus	Allele	Frequency
OT1	114	0.406
OT4	115	0.311
OT15	221	0.123
OT16	212	0.387
OT17	219	0.302

locus and I, was higher in the *Odontomachus troglodytes* population from Kaohsiung (Tab. 1). Moreover, Ho was low in both regions, with average values of 0.198 in Chiayi and 0.308 in Kaohsiung. Among the 27 alleles observed, 5 private alleles (unique to a single population) were detected exclusively in the Kaohsiung populations at loci OT1, OT4, OT15, OT16, and OT17, with frequencies of 0.406, 0.311, 0.123, 0.387, and 0.302, respectively (Tab. 2).

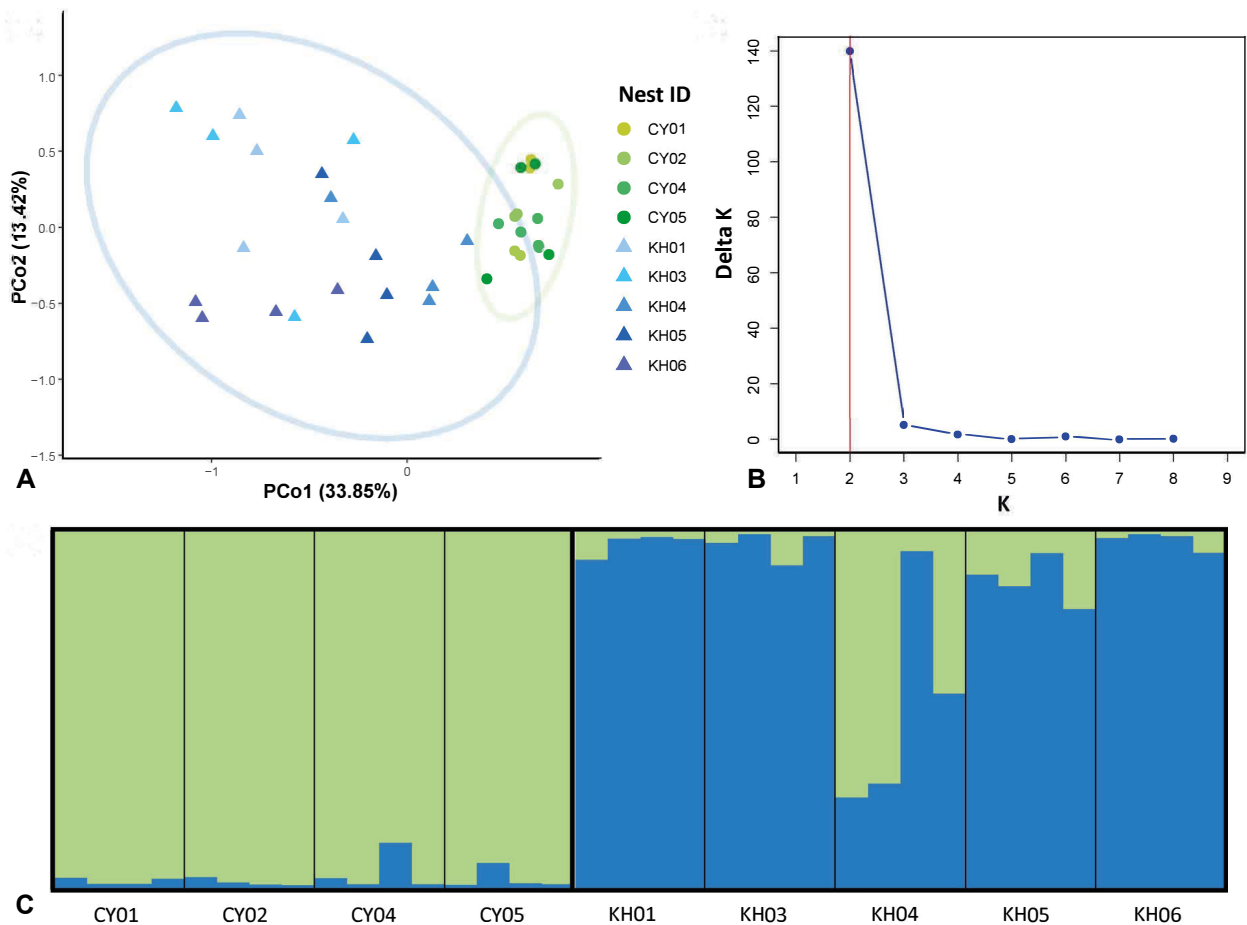
#### Polygyny and sexual reproduction as key features of introduced *Odontomachus troglodytes*

Genotypic analysis indicated that the introduced *O. troglodytes* population in Taiwan reproduced sexually, with no evidence of clonal reproduction. Male samples exhibited a high incidence of diploidy, with two out of six males from Chiayi and seven out of nine males from Kaohsiung exhibiting multiple alleles – defined as the presence of two alleles at one or more loci in a single individual – during allele screening. Sperm samples from the queen spermathecae also displayed multiple alleles in 2 out of 5 samples from Chiayi and 4 out of 12 samples from Kaohsiung, suggesting either diploid sperm or mating with multiple males. Six sperm samples (two from Chiayi and four from Kaohsiung) were excluded owing to potential contamination as their genotypes matched those of the queen at all loci. This exclusion may result in a slight underestimation of polyandry. In terms of social structure, 6 of the 12 nests were polygynous, with up to 9 queens per nest. The remaining nests comprised five monogynous colonies and one colony without a reproductive caste. No direct evidence of worker reproduction was observed over 13 months of monitoring 6 queenless lab colonies.

Parentage analysis inferred multiple maternal and paternal lines within colonies, indicating substantial



**Fig. 5:** Relatedness comparisons between within-nest and between-nest pairs in Chiayi and Kaohsiung populations. (A) Worker-worker relatedness. (B) Queen-worker relatedness. Relatedness was estimated using the QUELLER & GOODNIGHT (1989) estimator. Asterisks denote significant differences between within- and between-nest categories within each population based on Wilcoxon rank-sum tests (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).



**Fig. 6:** Population genetic structure analysis. (A) Principal coordinate analysis with 95% confidence ellipses, (B) Delta K plot from StructureSelector, and (C) STRUCTURE bar plot ( $K = 2$ ) showing nest assignments.

**Tab. 3:** Summary table of the analysis of molecular variance results. Df: Degree of freedom; MS: Mean square; \*:  $p < 0.05$ ; \*\*:  $p < 0.001$ .

Source	Df	MS	Percentage of variation (%)	F value
Among Regions	1	32.787	28	$F_{REGION-TOTAL} = 0.283^{**}$
Among Nests	7	3.913	8	$F_{NEST-REGION} = 0.110^{**}$
Among Individuals	27	2.106	10	$F_{IND-NEST} = 0.150^*$
Within Individuals	36	1.556	54	
Total	71		100	

genetic diversity among breeding individuals. The estimated number of maternal lines was consistent at three among the Chiayi nests and ranged from two to four among the Kaohsiung nests, with paternal lines estimated to range from three to four among the Chiayi nests and three consistently among the Kaohsiung nests. On average, 3.2 paternal lines and 3 maternal lines per nest were estimated from both regions.

#### Nest-level genetic differentiation within regions

Pairwise  $F_{ST}$  values indicated that the Chiayi nests belonged to the same colony ( $F_{ST}$  values ranged from -0.08 to 0.06, all adjusted  $p$ -values  $> 0.05$  after Bonferroni correction). Similarly, the nests in Kaohsiung appeared to belong to the same colony ( $F_{ST}$  values ranged from -0.01 to 0.25, all adjusted  $p$ -values  $> 0.05$  after Bonferroni correction). Pairwise geographic distances ranged from 0.004 to 0.05 km in Chiayi and from 0.003 to 6.863 km in Kaohsiung, indicating that colony ranges extend at least 0.05 km in Chiayi and 6.863 km in Kaohsiung.

#### Relatedness Analysis

In the Kaohsiung population, between-nest relatedness was negative for both pair types, with mean  $r = -0.081$  for worker-worker and  $-0.051$  for queen-worker comparisons. Within-nest relatedness was positive (mean  $r = 0.188$  for worker-worker and  $0.074$  for queen-worker), although neither value significantly deviated from zero (worker-worker:  $W = 325$ ,  $p = 0.060$ ; queen-worker:  $W = 695$ ,  $p = 0.275$ ; Tab. S4). A significant difference in relatedness was detected between within- and between-nest worker-worker comparisons ( $W = 3168$ ,  $p = 0.006$ ; Fig. 5), whereas queen-worker comparisons did not differ significantly between categories ( $W = 5323$ ,  $p = 0.097$ ; Fig. 5). In the Chiayi population, the mean relatedness was  $-0.013$  for both within- and between-nest worker-worker comparisons, and neither value significantly differed from zero (within-nest:  $W = 148$ ,  $p = 0.966$ ; between-nest:  $W = 2221$ ,  $p = 0.697$ ; Tab. S4). Queen-worker relatedness was significantly higher within nests (mean  $r = 0.221$ ) than between nests (mean  $r = -0.159$ ;  $W = 2797$ ,  $p = 0.0004$ ), and both values significantly deviated from zero (within-nest:  $W = 231$ ,  $p = 0.022$ ; between-nest:  $W = 770$ ,  $p = 0.002$ ; Fig. 5; Tab. S4).

#### Population-level genetic structure between regions

AMOVA revealed the following genetic variance distribution: 28% among regions, 8% among nests, 10% among individuals, and 54% within individuals (Tab. 3). Genetic differentiation was higher between regions ( $F_{REGION-TOTAL} = 0.283$ ,  $p < 0.001$ ) than among the nests within a sampling region ( $F_{NEST-REGION} = 0.110$ ,  $p < 0.001$ ).

All three genetic differentiation indices revealed significant differences between the Chiayi and Kaohsiung regions (mean  $F_{ST}$ :  $0.222 \pm 0.055$  standard deviation (SD), all  $p \leq 0.020$ ; mean  $D_{est}$ :  $0.213 \pm 0.017$  SD, all  $p < 0.001$ ; mean  $G''_{ST}$ :  $0.420 \pm 0.067$  SD, all  $p < 0.001$ ). PCoA revealed two partially overlapping clusters, suggesting genetic differentiation between the Chiayi and Kaohsiung populations, with the Kaohsiung population exhibiting greater genetic variation than the Chiayi population (Fig. 6A). Analysis using Evanno's method identified  $K = 2$  as the optimal number of genetic clusters (Fig. 6B). STRUCTURE analysis ( $K = 2$ ) revealed two distinct genetic clusters corresponding to the geographical regions of Chiayi and Kaohsiung (Fig. 6C). The Chiayi nests (CY01 - CY05) exhibited high proportions of ancestry from one genetic cluster (represented in green), whereas most Kaohsiung nests (KH01 - KH06) demonstrated high proportions of ancestry from the second genetic cluster (represented in blue). An exception was observed in nest KH04, which displayed substantial genetic contributions from both clusters, indicating possible gene flow between the two regions.

#### Discussion

##### Supercolonial traits in *Odontomachus troglodytes*

Supercoloniality is characterized by a set of traits, including the absence of intraspecific aggression, spatially expansive nest networks, polygyny, polydomy, and typically low genetic differentiation among nests (HELANTERÄ & al. 2009, HELANTERÄ 2022). This phenomenon has been broadly documented in formicoid clades, such as the Myrmicinae, Formicinae, and Dolichoderinae, and is often implicated in the ecological success of invasive ant species (reviewed in EYER & VARGO 2021 and HELANTERÄ 2022). Notable cases include *Wasmannia auropunctata*, *Pheidole megacephala*, *Anoplolepis gracilipes*, and *Linepithema*

*humile*, all of which can form supercolonies that facilitate rapid resource monopolization and range expansion (GIRAUD & al. 2002, LE BRETON & al. 2004, HOFFMANN & HAGEDORN 2014, EYER & VARGO 2021, HELANTERÄ 2022). In contrast, supercoloniality has not been convincingly demonstrated among poneroid ants, which generally have small colony sizes, typically non-claustral colony founding, and limited queen-worker dimorphism (KELLER & PEETERS 2021). While two poneroid species, *Brachyponera chinensis* and *Anochetus ghilianii*, have been reported to exhibit reduced aggression toward non-nestmates, they have not been formally validated as supercolonial (TAHERI & al. 2015, MURATA & al. 2017).

Our study identifies the Kaohsiung population of *Odontomachus troglodytes* as the first poneroid ant exhibiting multiple traits consistent with supercoloniality. In aggression assays, workers from nests located up to 6.9 km apart exhibited minimal antagonism. This broad tolerance resembles the behavioral cohesion commonly observed in invasive supercolonies of formicoid ants. However, our genetic analyses challenge the notion of a “fully integrated” supercolony. Although we detected no significant differentiation among Kaohsiung nests in pairwise  $F_{ST}$  comparisons, within-nest relatedness was significantly greater than between-nest relatedness. Moreover, AMOVA indicated weak but significant genetic structuring across nests, differing from the near-complete genetic homogenization observed in highly integrated supercolonies such as *Linepithema humile* (see TSUTSUI & al. 2000, PEDERSEN & al. 2006).

Given the varying definitions of “supercoloniality” in the literature, encompassing behavioral, genetic, and ecological dimensions, we compiled a comparative summary (Tab. 4) to assess how the Kaohsiung population

aligns with several commonly used definitions. Although minimal aggression between workers from the Chiayi and Kaohsiung populations was observed, clear genetic differentiation between the two regions suggests that these populations do not form a single supercolony. *Odontomachus troglodytes* in Kaohsiung exhibit several key traits of supercoloniality, including polygyny, polydomy, and minimal aggression among spatially distant nests. However, the relatively elevated within-nest relatedness suggests partial integration rather than the near-boundless homogeneity of fully unicolonial systems. These findings are consistent with the view that supercoloniality should be conceptualized along a continuum of social organization (HELANTERÄ 2022), with the Kaohsiung population occupying an intermediate position between classic multicolonial structures and fully integrated supercolonies.

The present observation raises intriguing questions about the evolutionary and ecological factors that may support supercolony-like organization in poneroid ants. Our results suggest that the ability to form supercolonial-like social structures may not be exclusive to formicoids. To interpret these findings, it is necessary to consider the ecology of the species in its native range. In its native range in Cameroon, *Odontomachus troglodytes* nests are relatively limited in physical size, with tunnels extending outward up to 3 m from the main nest chamber, and may form colonies of up to 1300 workers under optimal conditions (COLOMBEL 1970, 1972, BROWN 1976). Moreover, while limited data are available on native-range nestmate discrimination in this species, congeners such as *Odontomachus hastatus* and *Odontomachus mayi* exhibit strong aggression toward conspecific non-nestmates (ORIVEL & al. 1997, BERTHELOT & al. 2017). In this context, the widespread absence of aggression among

**Tab. 4:** Summary of supercoloniality definitions in ants and the level of support for each criterion in the Kaohsiung population of *Odontomachus troglodytes*. Definitions are drawn from key literature sources spanning behavioral, genetic, and ecological perspectives. Evidence from Chiayi is not included as the limited spatial scale and sampling in that region prevent assessment of supercolonial traits.

Source	Definition of supercoloniality	Key characteristics	Support in this study
WILSON (1971)	Introduced the term unicoloniality to describe ant populations lacking behavioral colony boundaries, allowing free movement among multiple nests within a large, dispersed colony.	- No intraspecific aggression - Free inter-nest movement	Partially supported: Absence of aggression in the Kaohsiung population supports behavioral unity. However, this social unity coexists with modest but statistically significant genetic structuring, suggesting partial alignment to the unicolonial model.
GIRAUD & al. (2002)	Described supercolonies as expansive cooperative networks of nests with no aggression among members, sometimes stretching over thousands of kilometers.	- Absence of inter-nest aggression - Wide spatial range (not explicitly definitional, but consistently observed) - Considerably low nestmate relatedness (observed, not part of formal definition)	Partially supported: No inter-nest aggression is observed in Kaohsiung; however, the spatial extent is moderate (6.9 km) and not comparable to the continental scale of Argentine ant supercolonies. Within-nest relatedness is positive but not significant; between-nest relatedness is negative and significantly low.

Source	Definition of supercoloniality	Key characteristics	Support in this study
PEDERSEN & al. (2006)	Defined supercolonies as large social networks of nests in which direct cooperative interactions between individuals from distant nests are not feasible. Behavioral boundaries are absent as aggression does not occur among members within the supercolony.	<ul style="list-style-type: none"> <li>- Lack of intraspecific aggression</li> <li>- No behavioral boundaries within the colony</li> <li>- Large spatial scale</li> </ul>	Supported: The Kaohsiung population does not exhibit aggression and spans a spatial extent (up to 6.9 km), making direct cooperation among all individuals unlikely. These observations are consistent with the absence of behavioral boundaries.
STEINER & al. (2007)	Proposed a behavioral transition model relevant to supercoloniality, suggesting that the loss of aggression while retaining nestmate recognition may represent an early stage of supercolony formation.	<ul style="list-style-type: none"> <li>- Lack of intraspecific aggression</li> <li>- Retained recognition ability</li> <li>- Food exchange between non-nestmates observed under laboratory conditions</li> </ul>	Partially supported: The Kaohsiung population shows no intraspecific aggression and maintains nestmate discrimination, inferred from antennation duration. Although food exchange was not tested, the observed behavior resembles early-stage patterns described by STEINER & al. (2007).
HELANTERÄ & al. (2009)	Defined supercolonies as extreme forms of polydomy, where the large spatial scale and nest number prevent direct interactions among workers from distant nests.	<ul style="list-style-type: none"> <li>- Aggression between nests is typically absent</li> <li>- Polydomy (definitional)</li> <li>- Low relatedness (not explicitly definitional, but often present)</li> </ul>	Supported: The Kaohsiung population shows no inter-nest aggression, displays polydomy, and spans a spatial extent of up to 6.9 km that likely prevents direct interactions among all individuals. While within-nest relatedness is positive, it is not significantly different from zero.
MOFFETT (2012)	Defined supercolonies as cooperative colonies capable of expanding without bounds under suitable conditions, maintaining colony identity and cohesion without internal aggression. The term is typically applied to colonies that have grown exceptionally large, with over 1 million individuals in the Argentine ant.	<ul style="list-style-type: none"> <li>- No internal aggression</li> <li>- Potential for large-scale expansion</li> <li>- Commonly applied to very large colonies</li> <li>- Shared colony identity</li> </ul>	Partially supported: Absence of internal aggression is evident. However, the colony size, identity retention, and growth dynamics were not evaluated. Moreover, the colony does not exceed population size thresholds typically associated with the term.
GORDON & HELLER (2012)	Defined supercolonies as lineages of polydomous colonies that share identity by descent but lack functional ecological coherence, such as resource sharing and reproductive integration.	<ul style="list-style-type: none"> <li>- Polydomy</li> <li>- Identity by descent</li> <li>- Absence of functional integration (e.g., no resource / reproductive exchange across distant nests)</li> </ul>	Partially supported: The Kaohsiung population exhibits polydomy. STRUCTURE and principal coordinate analyses suggest shared ancestry within the Kaohsiung population, consistent with identity by descent. Functional ecological integration, including resource sharing and reproductive connectivity, was not assessed.
PEDERSEN (2012)	Argued that supercolonies represent a distinct life-history strategy, not defined by size alone but rather by traits such as common origin, identity by descent across sites, budding-based nest networks, and self-contained reproduction. These traits form a unique social organization that fundamentally differs from other large ant colonies.	<ul style="list-style-type: none"> <li>- Budding-based colony expansion</li> <li>- Common origin within each site</li> <li>- Identity by descent across sites</li> <li>- Self-contained reproduction (within-supercolony mating)</li> <li>- Not defined by size but by life-history syndrome</li> </ul>	Partially supported: Budding was not tested. STRUCTURE and principal coordinate analyses support a common origin within Kaohsiung, and mating appears to occur within the same population based on sperm genotyping. However, the population remains spatially restricted and exhibits low but detectable genetic structuring.
HELANTERÄ (2022)	Suggested that supercoloniality represents a continuum, not a discrete category, defined by the co-occurrence of extensive polygyny, polydomy, budding dispersal, and reduced aggression across nests. These traits lead to spatially expansive colonies that often lack centralized functional organization.	<ul style="list-style-type: none"> <li>- Low or absent aggression</li> <li>- Polygyny</li> <li>- Polydomy</li> <li>- Budding dispersal</li> <li>- Degree of supercoloniality varies across species and between populations of the same species</li> </ul>	Supported: Budding was not tested. The Kaohsiung population shows no inter-nest aggression, displays polygyny and polydomy, occupying an intermediate position between classic multicolonial structures and fully integrated supercolonies.

*O. troglodytes* workers in Taiwan, both within and between regions, appears exceptional and invites further scrutiny.

Reduced aggression in the introduced range could reflect an invasion-associated shift in nestmate recognition, driven by either genetic bottlenecks or selection on recognition cues. One possibility is that genetic bottlenecks can lead to a loss of diversity in both neutral markers and nestmate recognition loci, weakening their discriminatory ability (SUAREZ & al. 1999, TSUTSUI & al. 2000). However, this explanation appears insufficient in our case as the genetically distinct Kaohsiung and Chiayi populations also exhibit mutual tolerance. Alternatively, the genetic cleansing hypothesis suggests that relaxed ecological constraints in invaded habitats may intensify competition, favoring colonies with common recognition alleles and leading to the elimination of those with rare cues (GIRAUD & al. 2002, TSUTSUI & SUAREZ 2003). This process could account for convergence in recognition profiles across genetically distinct populations.

An additional possibility is that behavioral tolerance in *Odontomachus troglodytes* is not invasion-induced but instead reflects a previously overlooked component of its natural social system. This pattern is not without precedent: Native *Formica paralugubris* colonies also exhibit low aggression despite genetic differentiation between spatially distant nests (HOLZER & al. 2009). Similarly, native *Tetramorium alpestre* populations can exhibit peaceful interactions among non-nestmates under natural conditions, with no significant correlation between aggression and genetic relatedness (KRAPF & al. 2017). The tolerance observed in *O. troglodytes* might represent the natural social organization of the species. Distinguishing between these possibilities will require direct behavioral and genetic studies of *O. troglodytes* in its native range. Such studies can help determine whether the observed social tolerance represents a derived invasion syndrome or a more widespread, ancestral feature of the social system of the species.

#### **Rapid antennation duration and frequency**

The RA behavior in *Odontomachus* ants serves diverse functions in social interactions within and between colonies. Our findings in *Odontomachus troglodytes* demonstrate that the RA duration was significantly longer during initial encounters between non-nestmates, potentially reflecting a process of familiarization between individuals from different nests. The reduction in RA duration in subsequent interactions indicates a gradual accommodation of unfamiliar recognition cues over time. Within nests, the higher frequency of RA may play a role in maintaining colony organization and task allocation through social feedback mechanisms, as observed in other *Odontomachus* species (OLIVEIRA & HÖLLDOBLER 1989). In *Odontomachus bauri* and *Odontomachus troglodytes*, RA facilitates cooperative behaviors such as stimulating foraging and food sharing (OLIVEIRA & HÖLLDOBLER 1989, LACHAUD & DEJEAN 1991). In *Odontomachus brunneus*, workers use

RA to establish task-specific roles and stimulate foraging activity (POWELL & TSCHINKEL 1999). Notably, the RA is not restricted to worker interactions; in *Odontomachus chelifer*, it also facilitates reproductive hierarchies among queens (MEDEIROS & al. 1992). Within nests, the high RA frequency may play a role in maintaining colony organization and task allocation through social feedback mechanisms, as observed in other *Odontomachus* species (OLIVEIRA & HÖLLDOBLER 1989, POWELL & TSCHINKEL 1999). Investigating the specific contexts of nestmate RA in *O. troglodytes*, such as its involvement in task allocation or food sharing, could provide deep insights into the functional importance of this behavioral discrepancy.

#### **Additional behavioral observations in *Odontomachus troglodytes* – worker carrying and queen domination**

In laboratory nests, *Odontomachus troglodytes* workers exhibited a unique carrying behavior during both preliminary bioassay testing and aggression assays in our study. In this behavior, one worker frequently initiated RA toward another worker, which responded with a submissive posture and minimal RA. The dominant worker then gently grasped the thorax of the submissive individual with its mandibles, lifting it in a manner reminiscent of particle handling. Unlike higher-level aggressive interactions (levels 3 and 4), this carrying behavior was notably gentle. Previous studies have documented similar carrying behavior within *O. troglodytes* colonies, with mature foragers transporting premature workers that wandered into foraging areas back to the nest (DEJEAN & LACHAUD 1991).

Additionally, queen-to-queen dominance behavior was observed in multiple laboratory nests. Alpha queens engaged in agonistic interactions, including pulling the antennae and appendages of beta queens and performing RA, until the beta queens either adopted a submissive pupal-like posture or the alpha queens disengaged. This behavior aligns with previous reports of dominance hierarchies among *Odontomachus* queens to establish reproductive rights, which can influence colony reproductive rates (MEDEIROS & al. 1992). If similar dominance interactions influence the reproductive dynamics of *Odontomachus troglodytes*, they may serve as a regulatory mechanism of colony growth rates in introduced populations. However, such behavior may have been amplified in the confined laboratory setting, where limited space restricts queen separation. In natural environments, multiple queens might establish separate chambers, reducing reproductive inhibition through spatial segregation. Further studies investigating natural nest structures and queen distribution could clarify how queen-to-queen aggression impacts colony growth under field conditions.

#### **Diploidy and breeding structure of *Odontomachus troglodytes***

Our study revealed a high frequency of diploid males in the *O. troglodytes* population in Taiwan, with 60% of

the sampled males displaying two or more alleles at microsatellite loci. In newly introduced populations, elevated inbreeding rates often lead to an increased production of diploid males, which are typically inviable or sterile (ROSS & FLETCHER 1985a, b, 1986, ROSS & al. 1993, LENANCKER & al. 2019). To mitigate this risk, queens might adopt strategies such as increased mating frequency, which reduces the likelihood of mating with a male carrying a matching sex allele and minimizes the impact of diploid male production (FERNÁNDEZ-ESCUADERO & al. 2002, HAGAN & GLOAG 2021). Although multiple matings are uncommon in many social Hymenoptera, they can enhance reproductive success by increasing genetic diversity within the colony (STRASSMANN 2001, DING & al. 2017). In the Taiwanese *O. troglodytes* population, 35% of sampled queens carried multiple types of sperm, suggesting that mating with multiple males is possible. Moreover, genetic data and field observations indicate that all sampled *O. troglodytes* nests were polygynous, with an estimated average of three queens per nest. Notably, field observations suggest that queen numbers often exceed this genetic estimate. This reproductive strategy, combining polygyny with potentially increased mating frequency, likely supports the establishment and expansion of the species in Taiwan by maintaining colony resilience and mitigating inbreeding effects in the introduced population.

#### **Genetic differentiation and invasion pathways of *Odontomachus troglodytes* in Taiwan**

Our genetic differentiation analysis revealed significant divergence between the *O. troglodytes* populations in Chiayi and Kaohsiung, which may suggest independent introduction events. However, the presence of private alleles exclusive to Kaohsiung, along with higher overall allele diversity in this region, supports a possible alternative scenario: A single invasion event initially established the Kaohsiung population. From Kaohsiung, the population may have expanded inland to Chiayi, resulting in a derivative population with decreased genetic diversity and no unique alleles. This genetic differentiation between regions can be attributed to isolation and reduced gene flow following the initial invasion. The second interpretation aligns with the findings of LIN & al. (2023), who proposed that the proximity of Kaohsiung to port facilities makes it a likely entry point for the alien species. Specifically, our sampling sites in central Kaohsiung (KH04 and KH05) are located near the shipping container depot of Ocean Nice Container Service Inc. (Fig. 2D), further supporting Kaohsiung as the probable introduction site. Whereas this bridgehead scenario is consistent with our observations, it does not entirely exclude the possibility of multiple introduction events over time. Additional research incorporating broader sampling across Taiwan and long-term genetic monitoring is needed to clarify the invasion history of *O. troglodytes*. Such studies could also provide valuable insights into the frequency and scale of introduction events and the dynamics of population establishment and expansion.

#### **Conclusion**

Our study provides important insights into the ecological and evolutionary factors underlying the successful establishment of *Odontomachus troglodytes*, a recently introduced ant species in Taiwan. Behavioral assays and genetic analyses revealed a polygynous, polydomous social structure exhibiting traits associated with supercoloniality, including low aggression between geographically distant nests. This represents the first report of supercolonial traits in the Ponerinae subfamily. High-density, polygynous colonies with reduced nestmate aggression may facilitate local spread and resource dominance. However, further research is needed to determine their role in establishment success. The findings underscore the need for monitoring and further research to inform pest management strategies and preserve native biodiversity.

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#### **Declaration on the use of generative artificial intelligence tools**

The authors declare that no AI tool was utilized for the generation of content, scientific ideas, data analysis, or any other part of the composition. All content, data analyses, and conclusions presented in this work are entirely the result of the authors' independent efforts. ChatGPT was used for initial linguistic support, and the manuscript was subsequently reviewed and edited by the professional English editing service *Editage* to ensure clarity and conformity with academic standards. The authors take full responsibility of the content of the manuscript in every detail.

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