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Research Article

Population Genetic Structure in the Invasive Ant *Tapinoma indicum* (Forel) (Hymenoptera: Formicidae) in Penang Island, Malaysia

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ABSTRACT

Tapinoma indicum (Forel, 1895) (Hymenoptera: Formicidae) is a common household ant in Southeast Asia and has become increasingly dominant in urban environments on Penang Island, Malaysia. Its ability to establish persistent infestations indoors presents a growing concern. As its presence expands, understanding its genetic structure and dispersal patterns becomes increasingly important to uncover how it colonizes new areas and maintains high population densities. This study presents the first population genetic analysis of *T. indicum* using seven polymorphic microsatellite markers. A total of 300 individuals from 30 residential sites across three districts were analyzed to assess genetic diversity and population structure. All loci exhibited high polymorphism, with an average of 31 alleles per locus and high expected heterozygosity (mean H_E = 0.926), indicating substantial genetic variation. However, observed heterozygosity was lower (mean H_O = 0.437), and some loci showed signs of null alleles and inbreeding. F-statistics revealed moderate inbreeding within populations (mean F_{IS} = 0.490) and low genetic differentiation between districts (F_{ST} = 0.075). Principal Coordinates Analysis and STRUCTURE results showed weak spatial genetic structuring and evidence of admixture, suggesting widespread gene flow. These findings indicate that *T. indicum* populations on Penang Island are genetically diverse but not strongly differentiated, forming a largely interconnected population across the island.

Key words: Ants, nuisance pest, microsatellite marker, population genetic

INTRODUCTION

Ants are among the most diverse insect groups in the world, with over 2,693 identified species and 7,953 morphospecies recorded globally (Gibb *et al*., 2017). Despite only around 20 species being classified as pests, ants are consistently ranked as the top nuisance pest in the United States (NPMA, 2012). In 2019, ant control services accounted for an average of 22.2% of pest management firm income, more than any other service (Syngenta, 2020).

In Malaysia, a 2016 survey recorded 13 ant species from eight genera and three subfamilies in residential areas on Penang Island. Among them, *Tapinoma indicum* (Forel, 1895), a species widespread across southern Asia, was one of the most abundant (Ab Majid *et al*., 2016). Known for nesting and foraging indoors, *T. indicum* frequently invades kitchens, bathrooms, and food storage areas, making it a persistent nuisance in households and commercial settings.

Surveys over the past few decades show a clear shift in the composition of household ant communities in Penang. In the mid-1990s, *Monomorium pharaonis* (Pharaoh ant) was the most dominant, with no record of *T. indicum* (Yap & Lee, 1994). By 2001, *T. indicum* appeared at low frequency (4.2%) (Lee *et al*., 2001), but by 2016, it had become the second most common species (17.74%) in areas including Balik Pulau, Relau, Sungai Ara, and George Town (Ab Majid *et al*., 2016). This sharp rise in abundance signals a growing problem that *T. indicum* is not only expanding its range but also increasingly displacing other species and establishing itself as a major indoor pest.

In response, various control strategies have been tested in Malaysia. Studies have focused on understanding *T. indicum*'s feeding and foraging behavior (Chong & Lee, 2006), evaluating plant-based insecticides (Lim & Ab Majid, 2019), and developing effective bait formulations (Lee, 2008; Chong & Lee, 2009; Ab Majid *et al*., 2018). While these efforts have provided practical tools for management, they have yet to address deeper questions about the species' adaptability, colony structure, and possible re-invasion dynamics. This is where genetic research becomes essential.

Microsatellite markers, known for their high degree of polymorphism, are widely used in population genetics (Seri Masran & Ab Majid, 2018; Hicks & Marshall, 2018), phylogeography (Goropashnaya *et al*., 2007), and phylogenetic studies (Trible *et al*., 2020). These markers have been especially useful in revealing colony organization, reproductive strategies, and dispersal patterns of invasive ants (Zheng *et al*., 2018; Blumenfeld *et al*., 2021). For *T. indicum*, genetic data could uncover whether infestations originate from a single supercolony, multiple introductions, or local re-invasions. This information is critical to long-term control success.

This research aims to investigate the genetic diversity and population structure of *T. indicum* on Penang Island. The research will provide valuable insights to support more effective, targeted, and sustainable pest management strategies by identifying genetic patterns and likely sources of introduction or spread.

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MATERIALS AND METHODS

Samples collection

Tapinoma indicum was collected from 30 residential areas across three districts on Penang Island (Table 1) using a baiting method described by Ab Majid *et al*. (2016). Ten residential dwellings were randomly selected in each area, and bait traps were set using 15 mL centrifuge tubes filled with a 50:50 mixture of peanut butter and honey based on the known food preferences of *T. indicum* (Lee, 2002). Five baited tubes were placed along *T. indicum* trails at each sampling site and left for three hr, from 4:00 to 7:00 p.m. Collected ants were stored in glass vials containing 95% ethanol and kept at –20°C for preservation.

Table 1. Population and location of sample collection

Collection site	Area	Code for each population	Latitude	Longitude
1	Balik Pulau	R01	N 5°22'22.60"	E 100°13'05.88"
2	Balik Pulau	R02	N 5°21'07.20"	E 100°14'18.84"
3	Balik Pulau	R03	N 5°21'02.10"	E 100°13'51.56"
4	Balik Pulau	R04	N 5°20'42.22"	E 100°13'47.67"
5	Balik Pulau	R05	N 5°20'33.77"	E 100°13'41.02"
6	Balik Pulau	R06	N 5°20'02.22"	E 100°12'59.39"
7	Balik Pulau	R07	N 5°20'40.07"	E 100°13'26.74"
8	Balik Pulau	R08	N 5°20'05.71"	E 100°13'39.84"
9	Balik Pulau	R09	N 5°21'20.07"	E 100°13'47.53"
10	Balik Pulau	R10	N 5°22'02.93"	E 100°12'56.45"
11	Gelugor	S01	N 5°21'00.649"	E 100°18'26.68"
12	Gelugor	S02	N 5°20'59.21"	E 100°18'02.37"
13	Gelugor	S03	N 5°20'58.00"	E 100°17'50.05"
14	Gelugor	S04	N 5°21'07.85"	E 100°18'26.12"
15	Gelugor	S05	N 5°20'41.96"	E 100°17'41.08"
16	Gelugor	S06	N 5°21'03.22"	E 100°18'18.46"
17	Gelugor	S07	N 5°21'08.39"	E 100°18'10.54"
18	Gelugor	S08	N 5°20'57.90"	E 100°17'39.40"
19	Gelugor	S09	N 5°21'20.29"	E 100°17'32.91"
20	Gelugor	S10	N 5°21'10.27"	E 100°17'58.28"
21	George Town	U01	N 5°25'04.52"	E 100°19'34.66"
22	George Town	U02	N 5°25'08.12"	E 100°19'37.49"
23	George Town	U03	N 5°25'43.45"	E 100°19'05.87"
24	George Town	U04	N 5°25'42.61"	E 100°19'02.56"
25	George Town	U05	N 5°24'36.31"	E 100°19'11.42"
26	George Town	U06	N 5°24'41.33"	E 100°19'06.76"
27	George Town	U07	N 5°26'23.01"	E 100°18'18.76"
28	George Town	U08	N 5°26'05.98"	E 100°17'45.96"
29	George Town	U09	N 5°24'13.35"	E 100°20'01.58"
30	George Town	U10	N 5°25'52.78"	E 100°18'38.76"

Morphological identification

A total of 30 *T. indicum* workers from each sampling location were examined under a compound microscope (model: BS-2020BD) (BestScope, China). Morphological measurements included head width (HW), defined as the maximum width of the head excluding the eyes; head length (HL), measured from the anterior to the posterior clypeal margin; eye width (EW), the maximum width of the eye; eye length (EL), the maximum length of the eye; scape length (SL), the maximum length of the scape; and mesosoma length (ML), the maximum length of the mesosoma from the midpoint of the anterior pronotal declivity to the posterior basal angle of the metapleuron (Branstetter, 2013).

The cephalic index (CI) was calculated as HW/HL × 100, the eye index (EI) as EL/HL × 100, and the scape index (SI) as SL/HW × 100. Species identification followed the identification keys for household ants provided by Mallis and Moreland (2011). Morphological and molecular identification had previously been confirmed in another study (Lim & Ab Majid, 2022).

DNA extraction

From each location, 10 samples were randomly selected to represent a population for DNA extraction. Genomic DNA was extracted from the heads of the ants using the HiYield Plus Genomic DNA Mini Kit (Blood/Tissue/Cultured Cells; Real Biotech Corp., Taipei, Taiwan), following the manufacturer's instructions. The obtained DNA was quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, MA).

Microsatellite marker amplification

Seven microsatellite markers were used to analyze genetic polymorphism, following the protocol by Lim and Ab Majid (2021). These markers were used to amplify DNA from 300 individual *T. indicum* ants sampled from 30 locations (one location represents one population), with 10 individuals representing each population. PCR amplification was carried out using a Thermal

Cycler (TaKaRa, Japan).

Each 25 µL PCR reaction contained 12.5 µL of Master Mix with green buffer (NX, Nuclix Biosolution, Malaysia), 1.0 µL of 0.4 µM of each primer, 5.0 µL of DNA template, and sterile, cold distilled water to make up the final volume. The PCR conditions consisted of an initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 75°C for 1 min, with a final extension step at 75°C for 10 min.

Fragment analysis was conducted using the Fragment Analyzer Automated CE System (Agilent Technologies, CA), and allele scoring was performed using ProSize 3.0 software (Agilent Technologies, CA).

Data analysis

To check for potential genotyping errors such as null alleles, stuttering, or large allele dropout, Micro-Checker v2.2.0.3 (Van Oosterhout *et al*., 2004) was used. Allele frequency metrics, including observed and expected heterozygosity, the number of alleles, and the polymorphic information content (PIC), were calculated using Cervus v3.0.7 (Kalinowski *et al*., 2007).

Wright's F-statistics (F_{IT}, F_{ST}, and F_{IS}) (Weir & Cockerham, 1984), along with the relatedness coefficient r (Hamilton, 1971), were computed using FSTAT v2.9.4 (Goudet, 2003), considering hierarchical levels of individuals (I), sampling sites (S), and district areas (T).

Principal coordinates analysis (PCoA) based on the F_{ST} distance matrix was performed using GenAlEx v6.5 (Peakall & Smouse, 2012). The genetic structure was further examined using STRUCTURE v2.3.4 (Hubisz *et al*., 2009), with the number of genetic clusters (K) ranging from 1 to 30. Each run consisted of 100,000 iterations after a burn-in period of 100,000. The optimal number of clusters was determined using Evanno's Delta K method.

RESULTS

Table 2 presents the genetic diversity statistics for seven microsatellite loci used in the analysis of *T. indicum* populations. The number of alleles per locus ranged from 21 (Ti-Pe05) to 46 (Ti-Te04), with an average of 31 alleles, indicating a high level of polymorphism. Expected heterozygosity (H_E) values were consistently high across all loci, ranging from 0.884 to 0.949, with a mean of 0.926, reflecting a high level of genetic diversity. Observed heterozygosity (H_O), however, showed greater variability, ranging from 0 (Ti-Tr04 and Ti-Pe05) to 1 (Ti-Di02), with a mean of 0.437. Some loci, such as Ti-Tr04 and Ti-Pe05, showed no observed heterozygosity. Null allele frequency estimates (F_{null}) ranged from 0 (no null alleles detected) to 1 (complete presence of null alleles), with a mean value of 0.476. Notably, Ti-Tr04 and Ti-Pe05 had an estimated F_{null} of 1, suggesting a high probability of null alleles at those loci.

Table 2. Number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), and null allele frequency estimate (F_{null}) of the seven microsatellite loci

Locus	N_A	H_E	H_O	F_{null}
Ti-Di02	33	0.943	1	0
Ti-Di06	25	0.919	0.94	0
Ti-Tr04	23	0.894	0	1
Ti-Tr09	39	0.945	0.41	0.396
Ti-Te04	46	0.949	0.237	0.602
Ti-Te13	30	0.948	0.47	0.335
Ti-Pe05	21	0.884	0	1
Mean	31	0.926	0.437	0.476

Table 3 shows the number of alleles detected at each of the seven microsatellite loci across 30 sampling sites of *T. indicum*. For primer, Ti-Te04 displayed the highest total number of alleles (46), indicating it is the most polymorphic marker, while Ti-Pe05 had the lowest total number of alleles (21), suggesting it is the least polymorphic among the loci. Mean allele numbers ranged from 4.90 (Ti-Tr04) to 9.67 (Ti-Di02), showing variation in diversity across loci. The standard deviation was highest in Ti-Tr09 (S.D. = 3.35), reflecting substantial variation in allele numbers across sites for that locus. Allelic richness varied across sampling sites, with some locations like U06 and R10 showing high allele counts across multiple loci, while others, such as R07 and S03, showed relatively lower counts.

Table 4 presents the results of F-statistics and the relatedness coefficient (*r*) for *T. indicum* populations across three districts on Penang Island. The F_{IS} values ranged from 0.442 (Gelugor) to 0.536 (Balik Pulau), indicating moderate to high levels of inbreeding within subpopulations. The F_{IT} values, which reflect total inbreeding, were highest in Balik Pulau (0.572) and lowest in Gelugor (0.492). The F_{ST} values were relatively low overall (0.075), with the highest in Gelugor (0.086) and the lowest in George Town (0.052), suggesting low to moderate genetic differentiation between districts. Relatedness (*r*) was highest in Gelugor (0.116) and lowest in George Town (0.068), implying that individuals in Gelugor are more genetically related to each other compared to those in George Town.

Table 3. Number of alleles per sampling site and locus

Sampling site	Locus						
	Ti-Di02	Ti-Di06	Ti-Tr04	Ti-Tr09	Ti-Te04	Ti-Te13	Ti-Pe05
S01	9	6	5	4	5	13	6
S02	12	7	4	6	9	5	6
S03	8	7	4	3	5	4	3
S04	13	9	8	7	7	7	6
S05	10	7	5	6	6	11	5
S06	11	8	5	12	8	9	4
S07	9	7	4	13	8	6	6
S08	8	7	4	7	11	7	4
S09	13	8	4	10	10	10	3
S10	7	11	4	11	8	10	4
R01	9	9	4	4	7	7	4
R02	7	7	3	6	7	7	6
R03	9	9	4	6	3	8	8
R04	10	7	6	7	7	10	6
R05	10	8	5	5	5	9	6
R06	7	6	4	7	5	5	5
R07	5	8	4	7	6	6	5
R08	9	7	4	10	7	9	7
R09	12	11	5	12	6	7	4
R10	12	10	5	13	9	7	7
U01	11	8	7	3	5	10	5
U02	8	6	5	5	7	8	8
U03	11	12	6	9	4	8	6
U04	9	6	5	3	5	11	5
U05	9	7	3	7	8	6	5
U06	13	10	6	15	11	7	7
U07	11	9	6	12	5	4	3
U08	8	10	4	10	8	8	6
U09	10	7	7	12	6	10	5
U10	10	8	7	10	8	10	7
Total	33	25	23	39	46	30	21
Mean	9.67	8.07	4.90	8.07	6.87	7.97	5.40
Standard deviation	1.96	1.59	1.22	3.35	1.93	2.15	1.36

Table 4. F-statistics (F_{IS}, F_{IT}, and F_{ST}) and relatedness coefficient (*r*) with 95% CI of *T. indicum* from Gelugor, Balik Pulau, and George Town

District	F_{IS} (CI)	F_{IT} (CI)	F_{ST} (CI)	r (CI)
Gelugor	0.442 (0.160-0.749)	0.492 (0.215-0.775)	0.086 (0.064-0.110)	0.116 (0.102-0.129)
Balik Pulau	0.536 (0.187-0.864)	0.572 (0.244-0.875)	0.074 (0.051-0.103)	0.094 (0.063-0.127)
George Town	0.492 (0.161-0.818)	0.519 (0.208-0.827)	0.052 (0.032-0.075)	0.068 (0.040-0.098)
Overall Penang Island	0.490 (0.177-0.800)	0.529 (0.232-0.817)	0.075 (0.060-0.092)	0.098 (0.079-0.112)

Principal Coordinates Analysis (PCoA) based on pairwise F_{ST} values among all 30 *T. indicum* populations on Penang Island revealed no distinct clustering pattern (Figure 2) except populations S06, U06, U08, and U09 formed a close cluster. The first and second principal coordinates accounted for 15.15% and 9.24% of the total genetic variation, respectively.

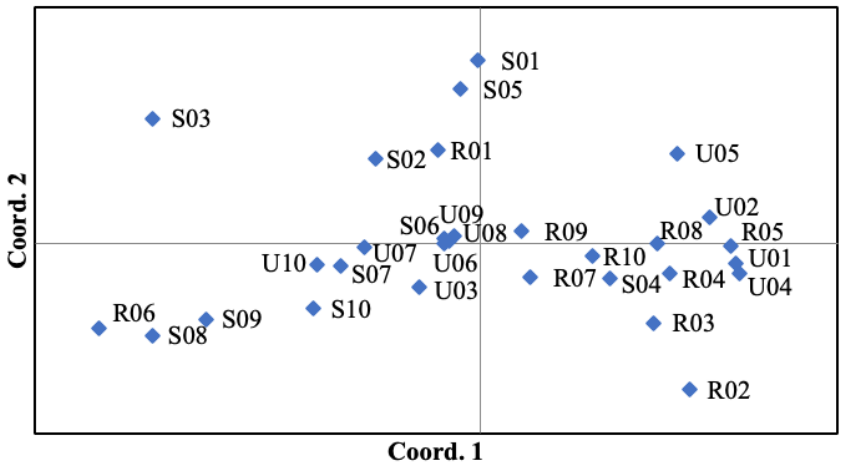

Fig. 2. Principal coordinates analysis with pairwise F_{ST} of all 30 *T. indicum* sampling sites on Penang Island. Abbreviations refer to supplement data 1.

As shown in Figure 3, Evanno's ΔK method identified *K*=2 as the optimal number of genetic clusters across the *T. indicum* sampling sites. This finding suggests that the populations are best represented as two genetically distinct subgroups.

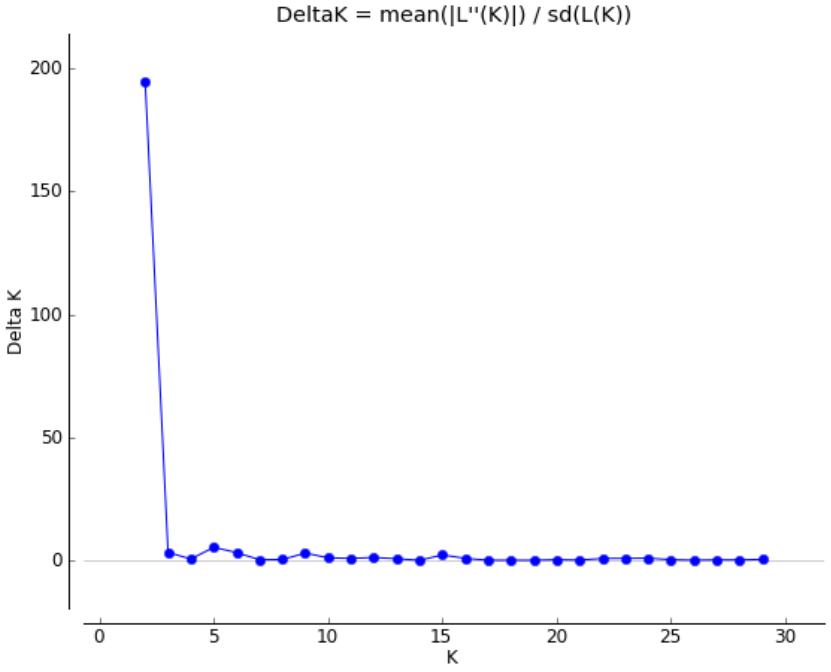

Fig. 3. Optimal Delta K values identified by the Evanno method from *K*=2 to *K*=30.

Table 5 provides the estimated proportion of the membership of each sampling site in the two clusters inferred by STRUCTURE. These proportions form the basis of the genetic structure visualization in Figure 4, where sites are grouped according to their dominant cluster assignment (red or green).

Table 5. Proportion of membership of each pre-defined population in each of the 2 clusters

Sampling sites	Inferred clusters	
	1	2
S01	0.654	0.346
S02	0.904	0.096
S03	0.987	0.013
S04	0.118	0.882
S05	0.407	0.593
S06	0.634	0.366
S07	0.825	0.175
S08	0.976	0.024
S09	0.944	0.056
S10	0.950	0.050
R01	0.297	0.703
R02	0.042	0.958
R03	0.037	0.963
R04	0.067	0.933
R05	0.061	0.939
R06	0.979	0.021
R07	0.171	0.829
R08	0.097	0.903
R09	0.464	0.536
R10	0.065	0.935
U01	0.149	0.851
U02	0.121	0.879
U03	0.552	0.448
U04	0.113	0.887
U05	0.18	0.82
U06	0.612	0.388
U07	0.691	0.309
U08	0.61	0.39
U09	0.394	0.606
U10	0.676	0.324

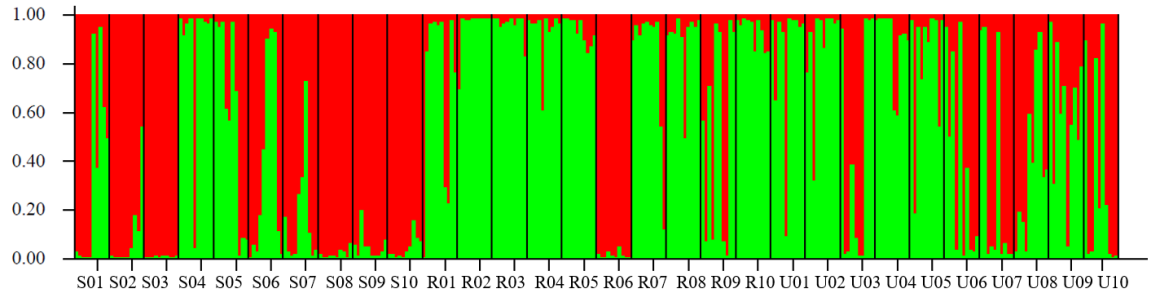

Fig. 4. STRUCTURE plot of *T. indicum* from Penang Island for *K*=2. Sampling sites are delimited by black lines with corresponding abbreviations from supplement data 1.

DISCUSSION

This study represents a comprehensive genetic assessment of *T. indicum* populations across Penang Island using seven polymorphic microsatellite markers. The findings provide critical insights into genetic diversity, spatial structure, and gene flow within and between urban populations in a rapidly developing tropical environment.

The genetic analysis (Table 2) revealed high levels of polymorphism among the seven microsatellite loci tested, with a total number of alleles per locus ranging from 21 (Ti-Pe05) to 46 (Ti-Te04) and an average of 31 alleles. This level of allelic richness indicates that the selected loci are highly informative for assessing genetic variation in *T. indicum*. The observed expected heterozygosity (H_E) values, which ranged from 0.884 to 0.949 (mean H_E = 0.926), further confirmed the markers' effectiveness in detecting variation, suggesting a genetically diverse gene pool at the marker level.

However, observed heterozygosity (H_O) was markedly lower than expected, with a mean H_O of 0.437 and certain loci (e.g., Ti-Tr04 and Ti-Pe05) showing complete homozygosity (H_O = 0) (Table 2). These discrepancies likely reflect the presence of null alleles, as estimated F_{null} values for those loci reached 1. The presence of null alleles can arise from mutations at primer binding sites, leading to allele dropout and underestimation of heterozygosity (Inokuchi *et al*., 2017). It may also indicate underlying biological processes such as inbreeding or clonal reproduction in parts of the population (Zhivotovsky *et al*., 2015).

Allelic variation across the 30 sampling sites showed modest variation, with the number of alleles per locus per site ranging from 3 to 15. Sites such as U06 (George Town) and R10 (Balik Pulau) displayed consistently high allele counts across multiple loci, suggesting that these locations may harbor larger or more genetically mixed populations. In contrast, sites like R07 and S03 exhibited lower allelic richness, potentially reflecting founder effects, localized bottlenecks, or smaller colony sizes (Nei *et al*., 1975; Zheng *et al*., 2018).

Despite some variability among sites, the mean number of alleles per locus remained within a relatively narrow range (4.90 to 9.67) (Table 3), indicating consistent levels of genetic variation across populations. This range is considered relatively consistent because the difference between the highest and lowest mean values across loci is modest (less than five alleles). In population genetics, such a narrow spread suggests that no particular population is dramatically more or less diverse than others. The absence of extreme outliers and the generally uniform distribution of allele counts across multiple sites imply a balanced contribution to genetic diversity island-wide (Leberg, 2002; Frankham, 2018).

Furthermore, this range (4.90 to 9.67) reflects moderate genetic diversity. Typically, populations with low diversity exhibit fewer than four alleles per locus. In contrast, in highly diverse populations, such as those from native or long-established, the ranges can exceed 10 or more alleles per locus on average. With mean allele counts falling between these two thresholds, *T. indicum* in Penang Island appears to harbor an intermediate level of diversity. This suggests that while the species may not be native to the area, it has either undergone multiple introductions or persisted long enough to accumulate moderate levels of variation. Such a pattern is consistent with invasive species that experience recurrent introductions or limited but ongoing gene flow, maintaining diversity without allowing it to escalate dramatically (Roderick & Navajas, 2003; Dlugosch & Parker, 2008).

The F-statistics (Table 4) provided further insights into the population structure of *T. indicum* on Penang Island. The inbreeding coefficient within subpopulations (F_{IS}) was relatively high across all districts, with values ranging from 0.442 (Gelugor) to 0.536 (Balik Pulau), indicating a substantial excess of homozygosity. The overall F_{IT} value of 0.529 also supports the presence of inbreeding at the metapopulation level. Such patterns could reflect reproductive strategies such as intranidal mating, polygyny with closely related queens, or reduced gene flow from external populations (Ross, 1993; Pamilo *et al*., 1997).

As measured by F_{ST} (Table 4), genetic differentiation between populations was relatively low (overall F_{ST} = 0.075), suggesting weak population subdivision and considerable gene flow between districts. The highest genetic differentiation (F_{ST} = 0.086) was observed in Gelugor, suggesting that populations in this district are more genetically distinct from those in George Town and Balik Pulau. At the same time, Gelugor also exhibited the highest within-district relatedness (r = 0.116), indicating that individuals in this area are more genetically similar to one another compared to those in other regions. These two findings are consistent: the elevated relatedness within Gelugor may reflect localized inbreeding or a smaller gene pool, which in turn contributes to its greater differentiation from the other districts. In contrast, George Town showed both the lowest F_{ST} (0.052) and lowest r (0.068), suggesting higher gene flow and more genetic mixing in this urbanized area.

Principal Coordinates Analysis (PCoA) of pairwise F_{ST} values (Figure 2) supported the above findings. The first two axes explained only 15.15% and 9.24% of total genetic variance, respectively, and only a few sites, such as S06, U06, U08, and U09, clustered closely. The lack of clear regional groupings in the remaining sites suggests a weak spatial genetic structure and implies widespread gene flow across the island (Jombart *et al*., 2010; Iacchei *et al*., 2013).

STRUCTURE analysis (Figures 3, 4; Table 5) revealed two major genetic clusters (K=2), but these clusters were not spatially confined. Several sampling sites, including S01, S05, R01, R09, U03, U08, and U09, exhibited no clear dominance of either genetic cluster, with individual membership coefficients below 0.75 for both clusters (Table 5). This intermediate assignment indicates potential admixture and suggests ongoing gene flow or recent secondary contact between populations. This admixture pattern reinforces the conclusion of ongoing gene flow and suggests that ants move between regions, either through natural dispersal or, more likely, via anthropogenic means.

The weak genetic structuring, high admixture, and moderate allelic richness across all sites strongly suggest that *T. indicum* dispersal is influenced by human activity. Urban landscapes provide abundant resources, nesting opportunities, and continuous movement of materials and people, all of which can facilitate passive transport. This is particularly relevant in a densely populated and highly urbanized area like Penang Island, where commercial, residential, and industrial zones are closely interlinked (Ward *et al*, 2006; Gippet *et al*., 2019).

Similar patterns of weak genetic structuring and high gene flow have been documented in other urban-dwelling invasive ants, such as *T. melanocephalum* (Zheng *et al*., 2018) and *Solenopsis invicta* (Yang *et al*., 2008), where human-mediated movement played a significant role in shaping population structure. In the case of *T. indicum*, frequent re-introductions through goods transport, building renovations, and waste management could contribute to genetic homogenization across districts.

The lack of strong spatial genetic structuring and evidence of gene flow suggest that control strategies targeting *T. indicum* should be implemented at a broader, island-wide scale. Localized treatments may be ineffective in the long term if reinvasion occurs from neighboring populations. Genetic monitoring can aid in identifying reinvasion sources, tracking spread, and evaluating the efficacy of control interventions (Rollins *et al*., 2009; Destour *et al*., 2024).

CONCLUSION

This study provides the first detailed population genetic analysis of *T. indicum* on Penang Island using microsatellite markers. The high level of polymorphism observed across all loci, coupled with moderate allelic richness and low observed heterozygosity, indicates that *T. indicum* populations possess substantial genetic variation, although likely shaped by inbreeding and the presence of null alleles. F-statistics revealed moderate inbreeding within districts and low genetic differentiation between them, while PCoA and STRUCTURE analyses showed weak spatial genetic structuring and clear signs of admixture. These results suggest high levels of gene flow across the island, likely facilitated by human activity and urban connectivity.

Importantly, the findings highlight that *T. indicum* infestations are not genetically isolated but interconnected across Penang Island. This underscores the need for coordinated, island-wide management strategies rather than localized control efforts. Ongoing genetic monitoring will be essential for tracking reinvasion, understanding dispersal dynamics, and informing sustainable

pest control strategies tailored to the genetic landscape of this emerging urban pest.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

ETHICS STATEMENT
Not applicable.

DATA AVAILABILITY STATEMENT
The datasets generated and /or analyzed during the current study are available in the NCBI repository, (https://www.ncbi.nlm.nih.gov/bioproject/598521) BioProject: PRJNA598521.

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