Wing geometric morphometric analysis of dwarf honeybee (*Apis florea*) populations in Thailand

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Abstract. *Soipijit S, Sopaladawan PN. 2024. Wing geometric morphometric analysis of dwarf honeybee (*Apis florea*) populations in Thailand. Biodiversitas 25: 3568-3575.* The dwarf honeybee, *Apis florea* Fabricius, 1787, is native to Thailand and distributed throughout the country. It plays a vital role in agriculture and the environment as an excellent pollinator of crops and economic plants in Thailand. Deforestation and insecticide usage have decreased populations of *A. florea*. There is a need for more information about the diversity of this species in Thailand, particularly its morphological diversity. This study aimed to determine variation among *A. florea* populations in Thailand based on geometric morphometrics of right forewings. Samples of adult *A. florea* worker were collected from 81 colonies across Thailand. Forewing shape variation was examined based on 19 landmark coordinates. The results revealed that landmark number 15 had the highest variance, followed by landmark numbers 14 and 16 with the $S^2=0.00004646$, 0.00004436, and 0.00004024, respectively. Principal component analysis (PCA) showed that *A. florea* populations were distributed as one group, consistent with discriminant and cluster analyses. In addition, multivariate analysis of variance (MANOVA, $\alpha=0.05$) was not significantly different among populations. In summary, *A. florea* in Thailand had less morphological diversity than *Apis cerana* Fabricius, 1793 and *Apis mellifera* Linnaeus, 1758. This exciting information provides valuable insight for conserving *A. florea* in Thailand.

Keywords: Discriminant analysis, landmark, principal component analysis, right forewing

INTRODUCTION

Apis florea Fabricius, 1787, commonly known as the dwarf honeybee, is a species native to South and Southeast Asia. It belongs to the genus *Apis,* which encompasses several important honeybee species vital to global agriculture and ecosystems (Wongsiri et al. 2000). Previous studies of insect pollinators have reported that 90% of them are primarily honeybees (Mashilingi et al. 2022). Among these bee species, *A. florea* is regarded as the most crucial pollinator for wild and economic crops due to its non-selective foraging behavior on flowers (Oldroyd 2021). Additionally, it exhibits specialized behavior for collecting pollen and nectar (Layek et al. 2022), contributing to pollination and enhancing genetic diversity in wild and economically important plants, thereby increasing agricultural productivity (Fitch et al. 2019).

Apis florea is distinct for its small size and is considered a wild bee species (Hepburn and Radloff 2011). Initially found on the eastern fringe of the Arabian Peninsula in Oman, *A. florea* has since expanded its range across various regions from Iraq, Iran, India, Pakistan, Bangladesh, Saudi Arabia, Oman, Vietnam, Sumatra, Java, Borneo, Thailand, the Philippines, and China (Al-Katahni and Taha 2014; Ghassemi-Khademi 2014; Rajak and Basavarajappa 2016; Radjabi et al. 2018; Parichehreh et al. 2020; Al-Etby et al. 2022). *Apis florea* frequently build solitary nests on shrubs, trees, buildings, or caves (Shwetha et al. 2020) and are commonly found in urban and rural areas. These honeybees have smaller body sizes and are not aggressive when compared to other bee species. They exhibit adaptability and can tolerate hot climates (Cui and Corlett 2016). Moreover, they compete effectively for foraging resources with other bee species (El-Niweiri et al. 2019).

The identification of honeybee species can be comprehensively studied using several methodological approaches. The morphological study focuses on physical characteristics such as body size, coloration patterns, wing venation, and other morphological traits (Aluri and Naidu 2016; Koca et al. 2018). Geometric morphometrics (GM) has been developed based on the coordinates of landmarks on wing venation (Sappasat and Wongsiri 2017; Nogueira et al. 2019; Zahara et al. 2022). This method is informative, easy to perform, less time-consuming, cost-effective, and more accessible to beekeepers. GM is a powerful method for studying shape and size variations in organisms, including honeybees. It focuses on the shape of structures rather than their absolute size. It can be used to rapidly identify *A. mellifera* (Combey et al. 2018; Buala and Sopaladawan 2022). GM uses geometric and statistical methods to analyze landmark points on organisms (Theska et al. 2020). Statistical analysis such as principal component analysis (PCA), Procrustes analysis, and multivariate regression are used to analyze the shape variations. These methods are used to simplify a large data set into a smaller set and to analyze the distribution of a set of shapes, which help in identifying patterns of variation

among different populations, subspecies, or species of honeybees (Ghassemi-Khademi et al. 2024). It offers insights into evolutionary relationships, adaptation to various environments, and occasionally behavioral traits linked to morphology. For instance, changes in wing size and morphology under environmental stress can improve our understanding of the adaptive evolution of *A. cerana* in Changbai Mountain (Nannan et al. 2022). Compared to traditional morphometrics, geometric morphometrics offers more precise quantification of shape differences and can handle complex shape variations more effectively.

In addition, genetic studies utilize techniques such as quantitative Real-Time PCR (qRT-PCR) (Jeon et al. 2020), DNA sequencing (Bhatta et al. 2024), mitochondrial DNA (Porrini et al. 2021), and microsatellite DNA analysis (Rahimi et al. 2023), which enable the assessment of genetic variability among populations. This genetic data offers insights into evolutionary relationships, population structure, and patterns of gene flow among different populations. The biogeographical study examines the distribution patterns and historical biogeography across its geographic range, providing insights into how populations have dispersed over time, adapted to local environmental conditions, and potentially evolved into distinct populations or subspecies. Each of these methodological approaches contributes unique perspectives to understanding the diversity, evolutionary history, and ecological adaptations, thereby aiding in their conservation and management strategies.

Today, honeybees are experiencing a worldwide decline due to factors closely tied to human activities (Oldroyd 2021). "Bee hunting" is a well-known profession, and hundreds of *A. florea* nests may be "harvested" per week by one person and sold intact at the market. As honey is harvested by destroying the brood, *A. florea* populations in Thailand have been rapidly reduced (Wongsiri et al. 2000). This decline varies depending on species and geographic regions. Major anthropogenic factors contributing to the decline include habitat destruction and fragmentation, pollution and pesticide toxicity, and diseases. Therefore, this study aimed to investigate if *A. florea* in Thailand is composed of distinct geographical populations using a wing geometric morphometric-based analysis. The results can be applied for more effective natural resource management and conservation of *A. florea* in Thailand.

MATERIALS AND METHODS

Sample collections and measurements

We collected *A. florea* workers randomly from 81 natural colonies at 26 locations across Thailand during March 2017-April 2018 (Figure 1, Table 1). Bee specimens were captured from each colony using a sweep net and preserved in 80% ethanol at -20°C. Ten worker bees per colony were selected randomly, dissected, and mounted on glass slides before taking photographs of the right forewing under a SZX7 stereo microscope and images files were transferred to a TPS file using Util 1.63 software (Rohlf 2004a). Nineteen homologous landmarks on the right forewing were plotted at the forewing vein intersections using tpsDig2 software version 2.21 (Rohlf 2004b) (Figure 2). The Cartesian coordinates of these vein junctions were aligned, and partial warp analysis was conducted using tpsRelw software version 1.61 (Rohlf 2004c). The research project had been reviewed and approved by the Institutional Animal Care and Use Committee, Mahasarakham University (IACUC-MSU). The approval number is IACUC-MSU-0019/2017.
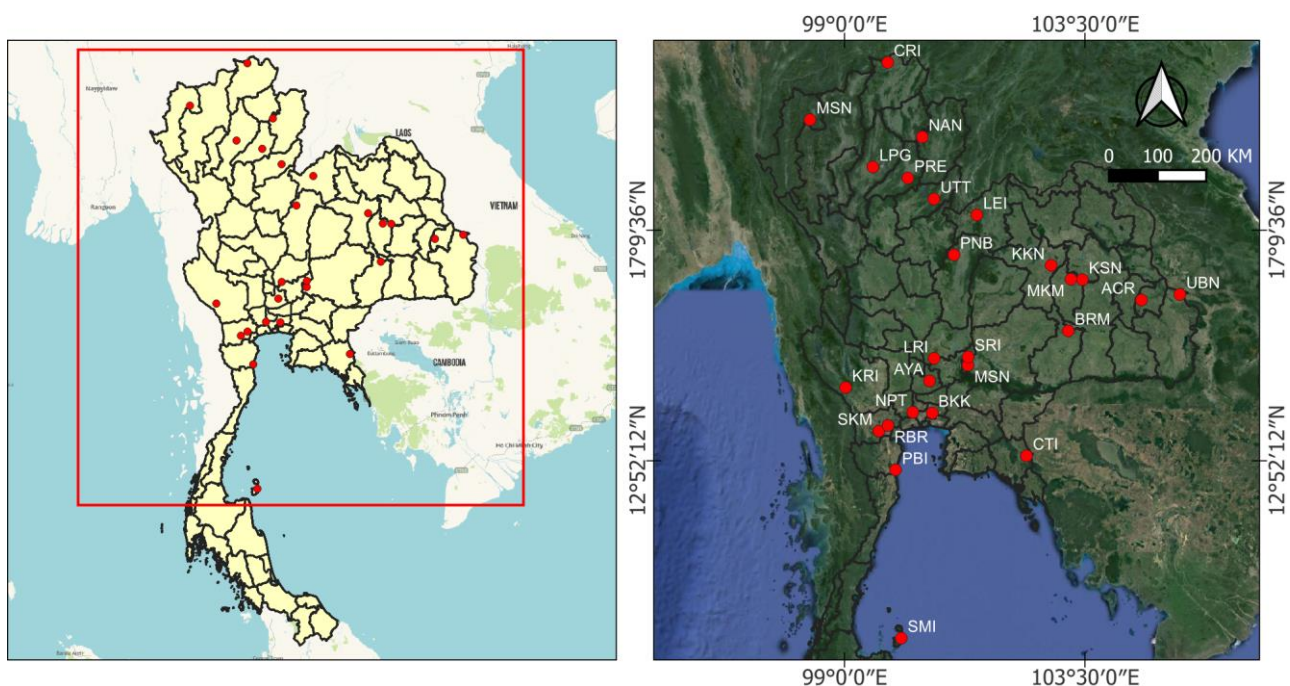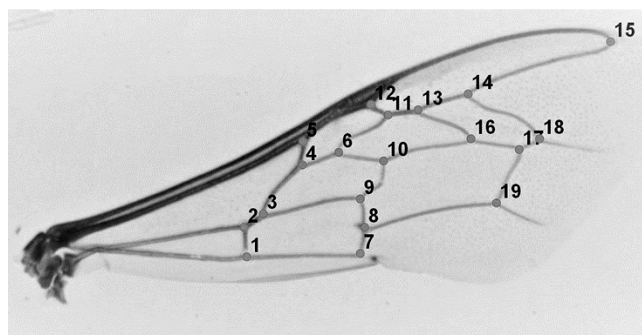

Figure 1. Sampling locations of *Apis florea* across Thailand

Table 1. Sampling locations of *Apis florea* in Thailand

Regions	Locations	GPS	No. of colonies	Sample code
North	1. Chiang Rai	20°17′20″N, 99°48′35″E	2	N-CRI1-2
	2. Nan	18°53′58″N, 100°27′23″E	1	N-NAN1
	3. Phetchabun	16°42′21″N, 101°03′03″E	1	N-PNB1
	4. Phrae	18°08′03″N, 100°11′08″E	4	N-PRE1-4
	5. Mae Hong Son	19°13′16″N, 98°21′17″E	3	N-MSN1-3
	6. Lampang	18°20′29″N, 99°32′00″E	1	N-LPG1
	7. Uttaradit	17°44′43″N, 100°40′32″E	5	N-UTT1-5
Northeast	8. Kalasin	16°14′37″N, 103°27′13″E	4	NE-KSN1-4
	9. Khon Kaen	16°30′38″N, 102°52′05″E	4	NE-KKN1-4
	10. Buri Ram	15°17′24″N, 103°11′24″E	1	NE-BRM1
	11. Nakhon Ratchasima	14°39′07″N, 101°18′41″E	1	N-MSN3
	12. Maha Sarakham	16°15′07″N, 103°14′21″E	4	NE-MKM1-4
	13. Loei	17°26′55″N, 101°28′41″E	1	NE-LEI1
	14. Amnat Charoen	15°51′55″N, 104°33′38″E	2	NE-ACR1-2
	15. Ubon Ratchathani	15°58′06″N, 105°16′38″E	7	NE-UBN1-7
Central	16. Bangkok	13°45′42″N, 100°38′41″E	2	C-BKK1-2
	17. Kanchanaburi	14°14′07″N, 99°01′23″E	3	C-KRI1-3
	18. Chanthaburi	12°57′51″N, 102°24′24″E	3	C-CTI1-3
	19. Nakhon Pathom	13°46′32″N, 100°16′51″E	4	C-NPT1-4
	20. Phra Nakhon Si Ayutthaya	14°21′34″N, 100°35′34″E	2	C-AYA1-2
	21. Phetchaburi	12°42′15″N, 99°57′28″E	2	C-PBI1-2
	22. Lop Buri	14°46′47″N, 100°40′42″E	11	C-LRI1-11
	23. Saraburi	14°48′19″N, 101°18′56″E	1	C-SRI1
	24. Samut Songkhram	13°25′32″N, 99°38′41″E	1	C-SKM1
	25. Ratchaburi	13°31′47″N, 99°48′51″E	1	C-RBR1
South	26. Ko Samui, Surat Thani	9°34′12″N, 100°03′49″E	10	S-SMI1-10
	Total		81	

Figure 2. Position of 19 landmarks on the right forewing of *Apis florea* worker

Data analysis

Apis florea populations were assigned according to the four major geographic regions of Thailand (Northern, Northeastern, Central, and Southern). Multivariate statistical analyses using canonical analysis, principal component analysis (PCA), discriminant analysis, and cluster analysis were employed to assess the variation between the populations. PCA was carried out using the relative Cartesian coordinates of each landmark after alignment. A forward stepwise analysis (tolerance 0.01; F to enter 1.00) using the same measures was conducted to determine discriminant functions. A canonical analysis and a cross-validation test were carried out to check the accuracy of the equations in identifying the samples. The square Mahalanobis distances between the centroids of the

groups were correlated to the geographical distances between the populations as calculated using a Mantel test (Buala and Sopaladawan 2022). Then, cluster analysis was performed on Mahalanobis distances of the morphometric data set to investigate the relationships between groups of samples in Thailand. All statistical analyses were performed using SPSS version 21.0 software.

RESULTS AND DISCUSSION

Geometric morphometric analysis of right forewings

The relative warp values of the right forewing of the specimens were calculated using the orthogonal alignment projection method. The Singular Values (SV) were presented along with an explanation of the percentage of relative values, where factor 1 and factor 2 were 0.207 and 0.193, respectively, and can explain 20.42% of the variance. Analysis of the relative contribution values of each landmark on the right forewing, consisting of 19 landmarks, found that landmarks no. 11 and 7 had the highest values (0.212 and 0.094, respectively). Analyzing the variances at landmarks for aligned specimens (S^2), it was found that landmarks 15, 14, and 16 had the highest variance values at 0.00004646, 0.00004436, and 0.00004024, respectively. However, landmark no. 8 had the lowest variance value of 0.00001762 (Table 2). The examination of relative warp values revealed a total of 34 values. The first three factors can be explained by 28.89% of the variance within the groups, with contributions of 10.94, 9.48, and 8.47%, respectively.

Principal component analysis

We found that there were 5 factors with eigen values greater than 1, which can explain 73.33% of the total variance. Specifically, factor 1 (20.03%) and 2 (16.50%) together could explain 36.53% of the total variance. Additionally, the landmark influences two factors: 6y and 8y. A scatter plot of *A. florea* populations in Thailand resolved into 4 regions: northern, northeastern, central, and southern. The study found that the populations in Thailand exhibited overlapping distributions (Figure 3).

Discriminant analysis

We analyzed the cross-validation test, which showed a low level of correct classification. Our assessment employed the equations generated in the discriminant analysis (DA) (Figure 4). The discriminant analysis results demonstrated that *A. florea* populations in Thailand are panmictic, a population in which the individuals are not structured by any network of acquaintances and are free to mate with any other individual (Fernandes et al. 2020), supporting the PCA and cluster analysis results.

Cluster analysis

The hierarchical cluster analysis divided the retrieved cluster into regions according to geographic characteristics. We found that the dendrograms of *A. florea* populations from different regions in Thailand were very close, and so they were unable to clearly classify the clusters (Figure 5).

Multivariate analysis of variance

No significant differences were found among the honeybee populations in Thailand (Wilk's λ=0.581; P=0.099).

Discussion

Geometric morphometry is a method that quantifies the shapes and sizes of organisms' features, allowing researchers to analyze variation within and between populations in a detailed manner (Mitteroecker and Schaefer 2022). Geometric morphometric analyses have been confirmed as an effective method in the microtaxonomy of *A. florea* populations among different countries, ranging from China to Egypt (about 7000 km) (Ghassemi-Khademi et al. 2024). The differentiation of both morphology and genetics in the species with a wide distribution range may caused by distance (geographic isolation), barrier (natural or anthropogenic barriers), landscape resistance, and/or adaptation to the environment (Lomolino et al. 2016).

Table 2. Variance values of the 19 landmark coordinates

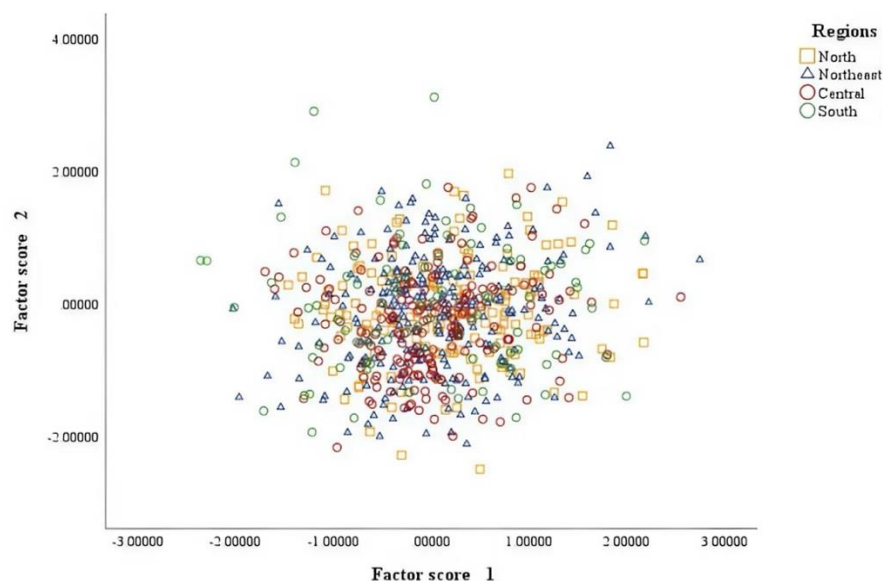| Landmarks	S^2X	S^2Y	S^2
1	0.00002031	0.00000985	0.00003016
2	0.00001307	0.00000640	0.00001947
3	0.00001521	0.00000672	0.00002193
4	0.00001325	0.00000773	0.00002098
5	0.00001537	0.00001507	0.00003044
6	0.00001856	0.00001166	0.00003023
7	0.00001384	0.00000869	0.00002253
8	0.00001033	0.00000729	0.00001762
9	0.00001768	0.00000618	0.00002386
10	0.00003176	0.00000560	0.00003736
11	0.00002596	0.00000681	0.00003277
12	0.00001925	0.00001194	0.00003119
13	0.00002087	0.00000626	0.00002712
14	0.00003655	0.00000781	0.00004436
15	0.00003279	0.00001367	0.00004646
16	0.00003275	0.00000749	0.00004024
17	0.00001930	0.00000593	0.00002523
18	0.00001983	0.00000760	0.00002743
19	0.00002266	0.00001115	0.00003381

Figure 3. The scatterplot illustrates the distribution of workers of *Apis florea* Fabricius, 1787 based on the two most influential factors identified through PCA of 19 landmarks. Factor 1 and factor 2 were determined to be the most significant in explaining variation among the measured landmarks. Each point on the scatterplot represents an individual worker specimen, positioned according to its scores on factor 1 (x-axis) and factor 2 (y-axis)
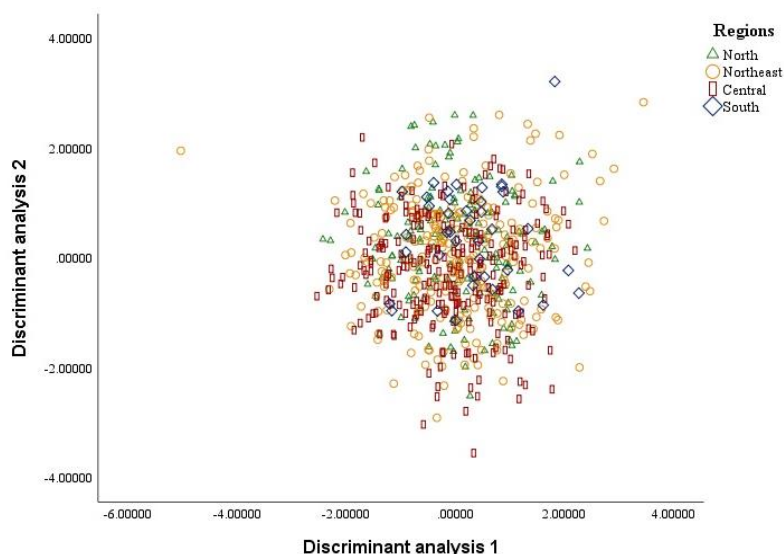
Figure 4. Discriminant analysis of the partial warps extracted from the right forewing of *Apis florea* Fabricius, 1787 workers
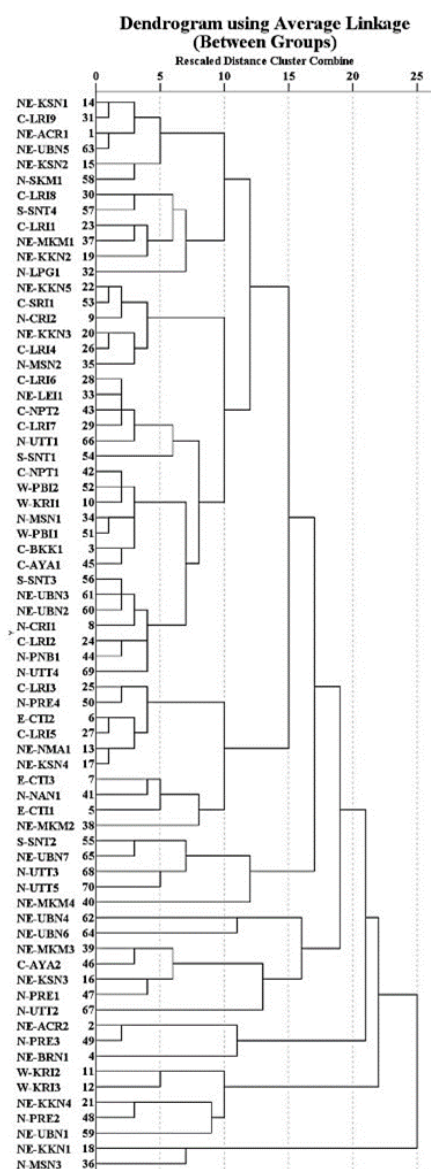

Figure 5. The dendrogram of *Apis florea* Fabricius, 1787 populations in Thailand based on geographical characteristics

In this study, PCA analysis of 19 landmarks on the right forewings of *A. florea* in Thailand was performed. The results indicated overlapping distribution among *A. florea* populations across 4 regions: northern, northeastern, central, and southern Thailand (Figure 3). The discriminant analysis results demonstrated that *A. florea* populations in Thailand are panmictic, the random mating and genetic exchange occurring across the entire population without any significant geographical or genetic barriers, supporting the PCA and cluster analysis results. This finding aligns with previous studies using traditional morphometric methods (Chaiyawong et al. 2004; Hepburn and Radoff 2011) and molecular genetic markers (Nanork 2001). Chaiyawong et al. (2004) used more than 20 informative morphological characters to examine the diversity of *A. florea* populations in Thailand. Despite using these characters, the populations of *A. florea* across Thailand did not show any geographical separation. Instead, they appeared as a single group, indicating a panmictic population. This consistency across different analytical approaches underscores the robustness of the findings, suggesting that the wing morphology of *A. florea* individuals does not vary significantly across the studied populations.

A molecular study on intergenic COI-COII mtDNA variability among *A. florea* populations in Thailand was reported by Nanork (2001). The Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) technique detected no difference on mtDNA among Thai *A. florea* populations. Results from previous and recent studies are crucial for understanding the population structure and genetic diversity of *A. florea*, contributing to broader studies on bee ecology and evolution. In the context of *A. florea* in Thailand, this suggests that the bees are freely interbreeding and exchanging genetic material across different regions of Thailand, leading to a lack of distinct population subdivisions based on the morphological characters studied. This finding could have implications for understanding the genetic structure, behavior, and

evolutionary dynamics of *A. florea* in Thailand, highlighting the importance of gene exchange and connectivity among different geographical regions.

Our data demonstrates the potential for a high degree of gene flow between populations. Likewise, previously reported by Rattanawannee et al. (2012), using geometric morphometrics to analyze giant honeybee (*Apis dorsata* Fabricius, 1793) populations in Thailand, indicated similar results to the study on *A. florea* in Thailand. It was found that there were no significant barriers to gene flow among *A. dorsata* populations in mainland Thailand.

Apis florea colonies are known to move relatively short distances, typically from a few meters up to several hundred meters (Nanork 2001). This movement is part of their swarming behavior, where a new colony is established at a new location (Nagaraja 2020). *Apis florea* colonies can swarm frequently, reportedly up to 8 times a year (Deowanish et al. 2001). Swarming is a natural reproductive behavior of honeybee colonies, where a new queen and a portion of the worker bees leave the original colony to form a new colony (Vijayan and Somanathan 2023). After swarming and establishing a new colony site, honeybees from *A. florea* colonies may revisit the old site (Deowanish et al. 2001). They do this to collect resources such as honey and wax from the old comb. These resources are important for building new comb and sustaining the new colony. This behavior ensures efficient resource utilization and continuity in colony development. It also underscores the adaptability of *A. florea* to maintain productivity and survival during swarming events (Ali et al. 2023). When *A. florea* colonies abscond, it refers to a specific behavior where the entire colony relocates over a longer distance than when routine swarming occurs (Deowanish et al. 2001). Absconding in *A. florea* colonies occurs when the colony leaves its current nest site and relocates to a new location. This behavior is distinct from routine swarming because it involves moving over longer distances, and colonies may abscond two to three times a year (Srinivasan et al. 2020). Absconding is often triggered by overcrowding, predation pressure, or environmental stressors that make the current nest site less favorable. During absconding, *A. florea* colonies can move significant distances compared to the shorter moves associated with routine swarming (Ibrahim 2020). The seasonal migration behavior of *A. florea* in Thailand, as observed by Deowanish et al. (2001), is influenced primarily by periods of resource scarcity. *Apis florea* colonies in Thailand exhibit seasonal migration in response to scarcity of resources such as nectar and pollen. When these resources become limited, colonies may relocate to areas where food availability is better, ensuring their survival and sustainability. Temperature changes play a significant role in inducing changes in nesting sites for *A. florea*. Additionally, human activities, including disturbances or habitat changes, can influence nesting site selection by *A. florea*.

These factors collectively influence the migratory and nesting behaviors of *A. florea* in Thailand. It demonstrates adaptive responses to environmental changes and threats, ensuring their ability to thrive and maintain healthy populations in varying conditions. Understanding these behaviors is significant for conservation efforts and the sustainable management of these important pollinators.

According to Charistos et al. (2014), a geometric morphometric approach is a powerful tool for studying the systematics and evolutionary dynamics of bees, particularly in differentiating species, subspecies, populations, and even castes within a species. This approach uses geometric coordinates to analyze and quantify subtle differences in the shape and size of morphological features, such as wings. Moreover, it provides a more detailed and nuanced view of morphological variation than traditional linear measurements. Each species exhibits specific patterns of variation in wing morphology, which can be quantitatively assessed and compared. Investigation is sensitive enough to detect differences between species, subspecies, and populations within a species. In social insects like honeybees, different castes (e.g., queens, workers, and drones) can exhibit distinct morphological differences, including wing shape and size. Wing characteristics that change over time can reflect adaptations to different environmental conditions, ecological niches, or behavioral traits, providing clues about the evolutionary history of Apoidea. It can be applied to both males and females (Oliveira and Gonçalves 2023). In addition, its ability to analyze damaged wings is advantageous for studies where fossilized wings may be incomplete or damaged, such as in fossil Darwin wasps (Viertler et al. 2022). It is reliable and low-cost alternative for the identification, discrimination, and preliminary estimation of the population structure (Ndungu et al. 2023).

In conclusion, analysis of wing geometric morphometrics from this study and a previous molecular study indicated that dwarf honeybees (*A. florea*) in Thailand are panmictic. Seasonal migration, hive separation, swarming, and their adeptness at adapting to the environment enable these honeybees to thrive in nature and disperse rapidly, which may have resulted in a high level of gene flow among populations. Our finding provides valuable insight for natural resource management and conservation of *A. florea* in Thailand.

ACKNOWLEDGEMENTS

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