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Landmark-Based Geometric Morphometric of *Apis dorsata* and *A. d. binghami* Wing Venation in Indonesian Archipelagos

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ABSTRACT

The giant honey bee *Apis dorsata* has a vast distribution in Asia, including in the Indonesian archipelagos, whereas *A. d. binghami* is endemic to Sulawesi Island. This research aimed to analyze the variations of landmarks in wing venation of *A. dorsata* from Sumatra, Belitung, Java, Sumbawa, Moluccas islands, and *A. d. binghami* in Sulawesi, based on geometric morphometric analysis. Nineteen landmarks from 200 wing venations were analyzed using Thin Plate Spline software. Our study found landmarks 16 and 17, known as Cubital Index in traditional morphometrics, contributed to the wing shape of *A. dorsata* and *A. d. binghami* Among all samples, these two landmarks show high displacement in the wing shape of *A. d. binghami* in Sulawesi and *A. dorsata* in the Moluccas. On the contrary, we found that the Sumatra, Belitung, and Java *Apis dorsata* revealed low displacement in the deformation grid; resulting in their shapes being more similar to the reference landmark. The variations of wing shape separate *A. d. binghami* from Sulawesi and Sumbawa in the Principle Component Analysis and agreement with the Neighbor-joining tree. Therefore, the geometric morphometric based on landmarks of wing venation is a powerful tool to discriminate the subspecies level of *A. dorsata*.

1. Introduction

The giant honey bee *Apis dorsata* migrates every year for foraging the nectar, pollen, and water resources (Ruttner 1988). The distributions of *A. dorsata* encompass a vast area from South Asia to Eastern Indonesian and Philippines archipelagos (Ruttner 1988). This giant honey bee comprises three subspecies, i.e. *A. d. breviligula* (Sakagami *et al.* 1980), *A. d. binghami*, and *A. d. dorsata* (Ruttner 1988). The latter (hereafter referred to as *A. dorsata*) has the most widely distributed, i.e. in India, Bhutan, Nepal, Sri Lanka, Pakistan, Thailand, and Indonesia archipelagos (Ruttner 1988; Sakagami *et al.* 1980). However, *A. d. breviligula* is only distributed in the Philippines, whereas *A. d. binghami* is endemic in Sulawesi, Indonesia (Maa 1953; Nagir *et al.* 2016). In general, the morphologically *A. d. binghami* has white stripes abdomen whereas a black abdomen with yellow stripes occurred in *A. dorsata* (Maa 1953).

Wings are part of insect body structure that provide genetic data based on the landmarks interconnection of the venation (Ruttner 1988; Mondal *et al.* 2015). By using the traditional morphometric analysis, the angle and ratio of the wing venations of *A. koschevnikovi* and *A. cerana* in Borneo showed the geographical area (Hadisoesilo *et al.* 2008) as well as between *A. andreniformis* and *A. florea* in Thailand (Wongsiri 1990).

Geometric morphometrics that studied the landmark configuration based on coordinates points has a strong analytical measurement (Cassanova *et al.* 2020; Webster and Sheets 2010; Mondal *et al.* 2015). This analysis can differentiate *A. mellifera* into subspecies levels such as *A. m. intermissa*, *A.m. sahariensis*, and *A. m. capensis* from Algeria that

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showed a differential wing deformation (Barour and Baylac 2016). Moreover, this morphometric approach even has a powerful statistical analysis to differentiate within the subspecies of honey bee *A. m. adansonii* as seen in the three agro-landscape in Ghana (Combey *et al.* 2018). This approach was also used to study a panmictic population of giant honey bee *A. dorsata* in five geographic regions in Thailand, however, no significant difference among the regions based on the wing variations was revealed in this study (Rattanawannee *et al.* 2012).

The honey bee wing venations possess 19 anatomically homolog landmarks (Michener 2007). Geometric morphometric analysis among five honeybee species of *A. cerana, A, koschevnikovi, A. mellifera, A. andreniformis*, and *A. dorsata* has generated landmark data variation and defined their relationship in a neighbor-joining tree. *Apis mellifera, A. cerana*, and *A. koschevnikovi* are clustered in one clade, whereas *A. dorsata* and *A. andreniformis* are clustered in another clade differentiated by the landmarks 1, 2, 11, 14, and 17 (Santoso *et al.* 2018). However, currently a lack of landmark variations data between *A. dorsata* and *A. d. binghami* in the Indonesian archipelagos. Our research, therefore, aimed to analyze landmark variations based on geometric morphometrics among *A. dorsata* from Sumatra, Belitung, Java, Sumbawa, Moluccas, and *A. d. binghami* from Sulawesi and to analyze the relationship of those giant honey bees.

2. Materials and Methods

2.1. Dissection and Wing Digitation

Eighteen colonies of *A. dorsata* from five islands and two colonies of *A. d. binghami* from Sulawesi were used for geometric morphometric analysis with ten individuals of honey bees from each colony as also applied in the previous studies (Abou-Shara 2013; Raffiudin *et al.* 1999; Santoso *et al.* 2018). A total of 180 and 20 samples from the right anterior wing of *A. dorsata* and *A. d. binghami* respectively (Table 1, Figure 1) were dissected and photographed by using an Olympus SZ61 stereomicroscope equipped with an Optilabviewer2.2 under the standard position. Nineteen anatomical landmarks on wing venation (Michener 2007) were digitized (Figure 1, Table 2) for five replicates (configurations) using tpsDig232 software (Rohlf and Slice 1990).

2.2. Geometric Morphometric Analysis

We aligned five landmark configurations on the (x, y) coordinates to obtain the mean landmark as the reference (Rohlf *et al.* 1996) by using tpsRelw32 software. The reference configurations were visualized as deformation grids using tpsSplin software. All Thin Plate Spline (TPS) software used in the current study is available at https://life.bio.sunysb.edu/morph/. Deformation grids were generated based on the island population of *A. dorsata* as well as *A. d. binghami* and aligned to the deformation grid from the mean landmarks of the total 200 wing venation. The mean landmarks for all bee samples generated the values of relative contribution and variance. We also constructed deformation grids of wing venation shapes based on the mean landmark of *A. dorsata* and *A. d. binghami* from each island. We measured the Procrustes distance and angles during the alignment of landmark coordinates (Rohlf and Slice 1990).

Subsequently, the bending energy values were analyzed based on landmark displacements in the deformation grid of the bee wing venations. The bending energy represents several non-affine differences between landmarks. Bending energy describes the Thin Plate Spline (TPS) process to the shape transformations and landmark displacement in the x and y (landmark coordinates) (Mitteroecker and Gunz 2009). Thus, it centered, scaled, and rotated the landmarks and consequently showed the bending energy value between landmarks configuration (Cooke and Terhune 2015).

2.3. Principal Component Analysis and Tree Construction

The mean landmark (refer as the reference) from the wing deformations were clustered in the plot ordination of Principal Component Analysis (PCA). PCA was constructed based on the two largest relative warps (RW) cumulative values in landmark configurations generated from tpsRelw32 software (Rohlf 2016). This process resulted in matrix data in R software that were used to generate the unrooted phylogeny tree using a package of Analyses of Phylogenetics and Evolution (APE) implemented in R software (https://cran.r-project.org/web/packages/ape/index.html) (R Development Team 2016).

Table 1. Location and samples ID of *A. dorsata* and *A. d. binghami*

Species/Islands	Locations	GPS	ID colony	Collector
A. dorsata/ Sumatra	Gunung Sahilan Village, Riau	N 00°00'38.7" E 101°19'50.7"	AdRiau1	RIR, MD, RN
	Gunung Sahilan Forest, Riau	N 00°00'04.7" E 101°19'01.2"	AdRiau2	RIR, MD, RN
	Gunung Sahilan Forest, Riau	N 00°00'04.7" E 101°19'01.2"	AdRiau3	RIR, MD, RN
	Mount Kerinci, Jambi	S 01°44'14.3" E 101°15'58.6"	AdJambi1	RIR, MD, RN
	Mount Kerinci, Jambi	S 01°44'14.3" E 101°15'58.6"	AdJambi2	RIR, MD, RN
A. dorsata/ Belitung	Keciput Village, Sijuk Regency	S 02°34'32.2" E 107°42'05.4''	AdBelitung1	RIR, NIS, MRD, DD
	Prepat Village, Membalong Regency	S 03°02'56.8" E 107°40'40.1''	AdBelitung2	RIR, NIS, MRD, DD
	Prepat Village, Membalong Regency	S 03°02'56.8" E 107°40'40.1''	AdBelitung3	RIR, NIS, MRD, DD
A. dorsata/Java	Bogor	S 06°40'08.9" E 106°44'46.8"	AdJava1	NA
	Mount Sampai, Bojong Kalong Village, Sukabumi	S 6°57'50.6" E 106°57'36.4"	AdJava2	CF, DKH, MEU
	Mount Sampai, Bojong Kalong Village, Sukabumi	S 6°57'50.6" E 106°57'36.4"	AdJava3	CF, DKH, MEU
A. d. binghami/ Sulawesi	Sausu Village, Sausu Regency	S 01°06'18.8" E 120°28'37.2"	AdbSulawesi1	RIR, FR, YSF, NHR
	Tolai Village, Torue Regency	S 01°02'56.1" E 120°19'26.8"	AdbSulawesi2	RIR, FR, YSF, NHR
A. dorsata/ Sumbawa	Ai Mena Batu Dulang Village, Batu Lanteh Regency	S 08°35' 13.7" E 117°15'37.5"	AdSumbawa1	QRT, SVF, YLI, FJR
	Keongkong Batu Dulang Village, Batu Lanteh Regency	S 08°36' 32.9" E 117°17'27.4"	AdSumbawa2	QRT, SVF, YLI, FJR
	Kayu Batu Lantung Village, Ropang-lantung Regency	S 08°46'05.2" E 118°00'30.1"	AdSumbawa3	QRT, SVF, YLI, FJR
	Kayu Batu Lantung Village, Ropang-lantung Regency	S 08°44'50.9" E 117°33'30.8"	AdSumbawa4	QRT, SVF, YLI, FJR
	Sawah Baru Omal Tingi Boal Village, Empang Regency	S 08°48'02.3" E 117°57'21.4"	AdSumbawa5	QRT, SVF, YLI, FJR
	Orong Motong Boal Village, Empang Regency	S 08°47'56.2" E 117°57'55.8"	AdSumbawa6	QRT, SVF, YLI, FJR
A. dorsata/ Moluccas	Sermata Island, Mahaleta Village, Mollucas	S 08°11'33.0" E 128°55'21.9"	AdMoluccas1	JLK
			Total colonies = 20	

[a] Collectors: CF: Cut Ferawati, DKH: Desmina Kristiani Hutabarat, DD: Diardi, FR: Fahri, FJR: M. Fajri Al Fateeh, JLK: Jacobus SA Lamerkabel, MR: Meggi Romadhona, MD: Meis Dyahastuti, MEU: Meutya, NHR: Nuhra, NIS: Nurul Insani Shullia, QRT: Qashiratuttarafi, RIR: Rika Raffiudin, RN: Rahmia Nugraha, SVF: Sevi Fortunika, TN: Teguh Nagir, YLI: Yulianti, YSF: Yusuf

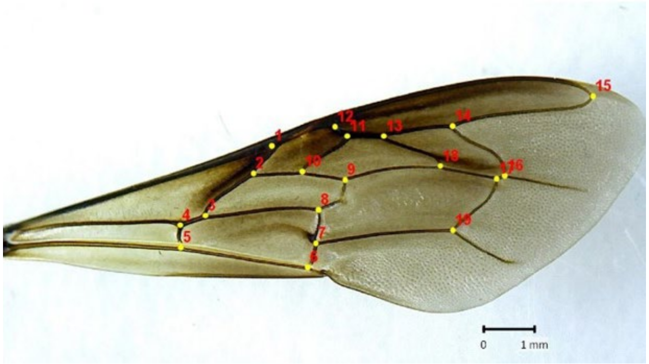


Figure 1. Digitation of nineteen anatomical landmarks of *Apis dorsata* worker forewing venation

Table 2. Description of 19 anatomical landmarks of *A. dorsata* forewing venation (Michener 2007)

Description
Rs and prestigma junctions
The point Rs + M
Point M + Cu 1
Point M + Cu 2
Cu-v point
V & Cu2 junctions
The vein junctions of Cu1 & Cu2
Point 1m-cu 1
Point 1m-cu 2
The second vein junctions of Rs + m & arbiscissa is Rs
Arbiscissa junctions both Rs and Rs
The junctions of r & stigma
Rs & 1r-m junctions
Rs & 2r-m junctions
The tip point of Rs
2r-m point
Endpoint 2m-cu 1
1r-m point
2m-cu and Cu junction

[b]Description: 1m-cu, medio-cubital opposite the first vein; 2m-cu, medio-cubital across the second vein; 1r-m, radio-medial cross-first vein (cross-second submarginal vein); 2r-m, radio-medial opposite-second vein (third submarginal vein); C, costa; Cu, cubitus; Cu1, the first branch of the cubitus; Cu2, second branch cubitus; M, medium (basal vein); r, radial cross-vein; r, radius; R1, the first branch of the radius; Rs, radial sector (Michener 2007)

3. Results

3.1. The Deformation Grids Representing the Variation of Landmarks of *A. dorsata* and *A. d. binghami* in Indonesia

Nineteen anatomical landmarks on wing venation were digitized for five replications. The deformations grid from island populations was aligned to the deformation grid based on the mean of all individual landmarks (Figure 2). Most deformation grids were influenced by bending energies (Figure 2A, B, C, E).

The highest contribution landmark variations influenced the bending energies was found in landmarks 14, 16, 17, and 18 (Table 3) on the deformation grid of the *A. d. binghami* wings (Figure 2D). Thus, these four landmarks are able to differentiate in the subspecies level between *A. dorsata* and *A. d. binghami*, which is endemic in Sulawesi. Landmark 14 showed the third-highest variance among all landmark variances in *A. dorsata* wing venations. Moreover, we also found the second-highest landmark variations in the deformation grid of the *A. dorsata* from the Moluccas with landmarks 16 and 17 that have the highest displacement (Figure 2F). These two landmarks also show the highest relative contribution value that plays a significant role in the formation of venation. We found interesting phenomena, the results of *A. dorsata* from Sumatra, Belitung, and Java revealed low displacement in the deformation grid, thus showing the most similar shapes to the reference landmark compared to the others (Figure 2A-C).

We further analyzed the deformation grid of the wing shape of the *A. dorsata* population on each island, which was compared to the mean population landmark of each island as a reference (Figure 3). This landmark produces a deformation grid to examine the shape differences of landmark configurations on each island. The wing shape of *A. d. binghami* from Sulawesi and *A. dorsata* from Sumbawa has a significantly different form (Figure 3D, E). These shape differences were contributed by the highest contribution values of 0.37278 and 0.42903, respectively, of the landmarks 16 and 17 (Table 3). Therefore, our study found that besides determining the landmark of wing venations, the geometric morphometric analysis was also able to differentiate the wing shape of *A. dorsata.*

The wing shape of *A. dorsata* is represented by the bending energy value derived from grid deformation (Figure 3, Table 4). The high value of bending energies and wing shape were found in Sumbawa and Moluccas (Figure 3, Table 4). However, although the wing shape of *A. d. binghami* is highly different, we found a low (0.08150) bending energy value in this Sulawesi giant honey bee compared to *A. dorsata* from other islands (Figure 3, Table 4).

3.2. Principle Component Analysis and Phylogeny Tree

The first and the second principal component (RW1 and RW2) plotted the clustering of 200 *A. dorsata* wing venations landmark samples in an

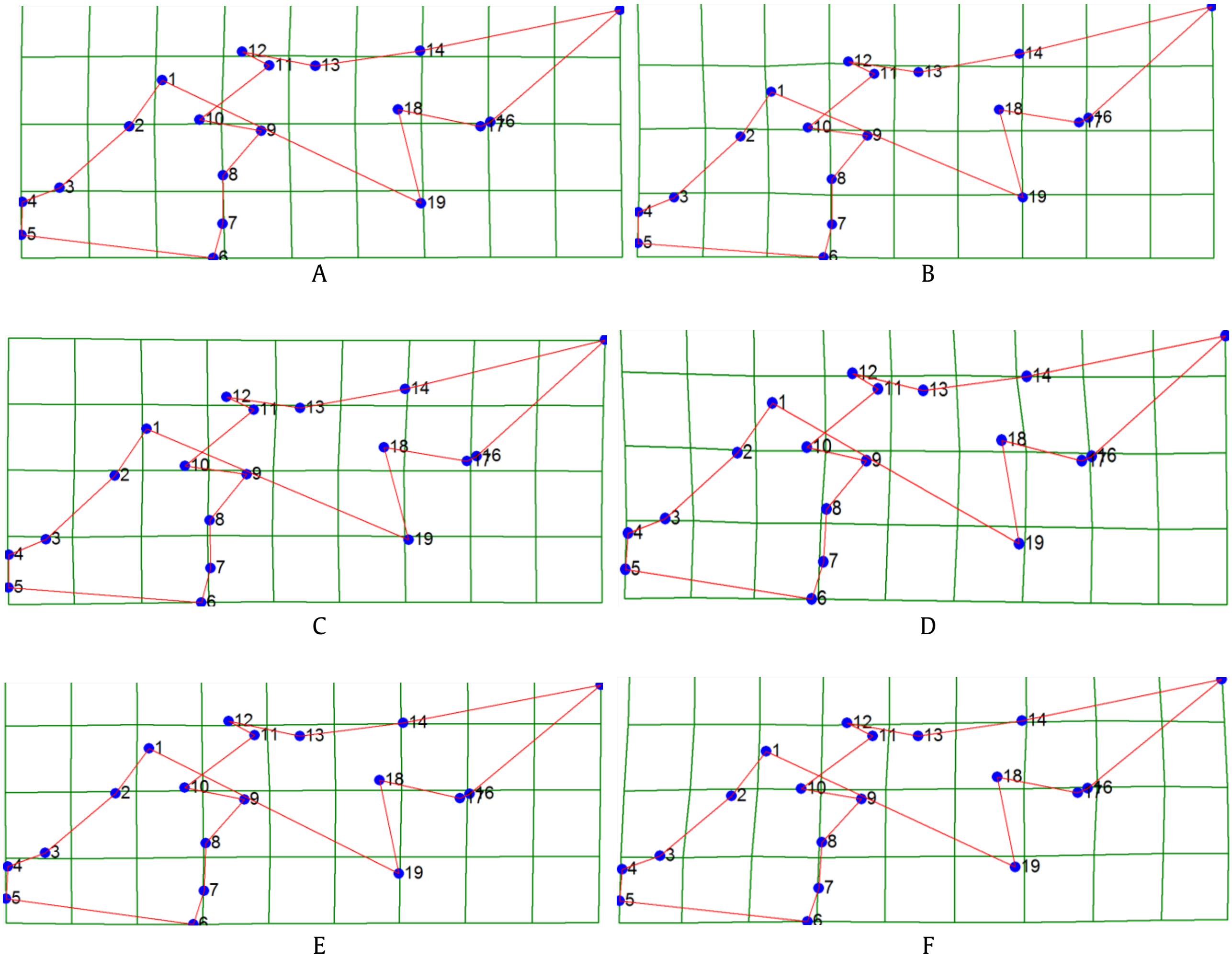
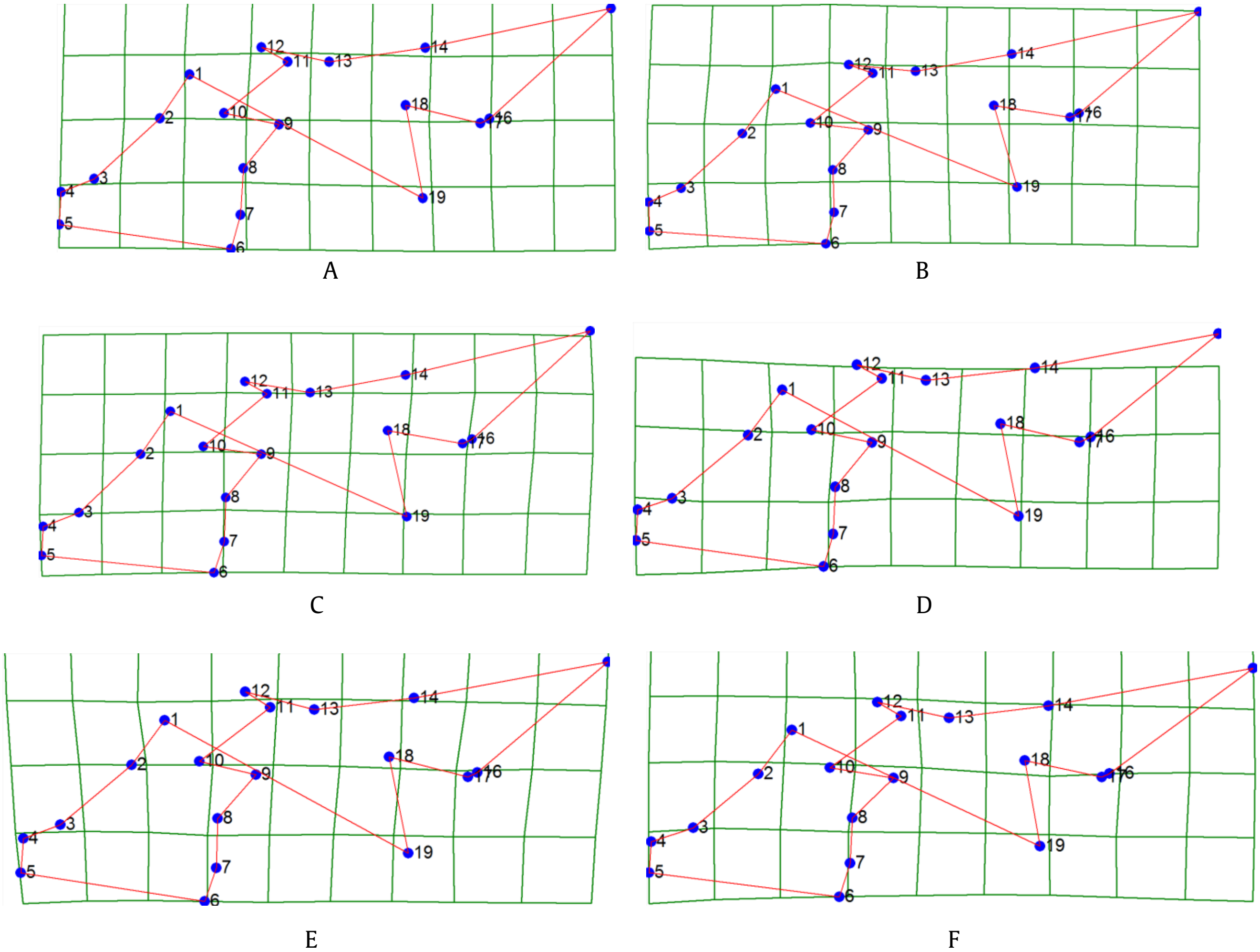


Figure 2. Deformation grid of wing venation *A. dorsata* (Ad) and *A d. binghami* (Adb) population from each island that aligned to the total samples; Ad Sumatra (A), Ad Belitung (B), Ad Java (C), Adb Sulawesi (D), Ad Sumbawa (E), Ad Moluccas (F)

Table 3. Relative contribution and variance of wing venation landmarks across all bee samples

Landmark	Contribution value	S^2
1	0.00629	0.00004421^{c}
2	0.00505	0.00002655
3	0.00747	0.00002984
4	0.01467	0.00002910
5	0.00556	0.00003975
6	0.00891	0.00003314
7	0.02718	0.00003067
8	0.01903	0.00001921
9	0.00689	0.00002444
10	0.00958	0.00003106
11	0.04733^{c}	0.00002658
12	0.02281	0.00003842
13	0.00910	0.00003041
14	0.00142	0.00004033^{c}
15	0.00026	0.00005812^{c}
16	0.37278^{c}	0.00002136
17	0.42903^{c}	0.00002252
18	0.00567	0.00002220
19	0.00098	0.00003912

^{c}the most contribution and variation of landmarks values that have high variations



A B C D E F

Figure 3. Deformation grid of wing venation of *A. dorsata* (Ad) and *A d. binghami* (Adb) population from each island; Ad Sumatra (A), Ad Belitung (B), Ad Java (C), Adb Sulawesi (D), Ad Sumbawa (E), Ad Moluccas (F)

Table 4. Bending energies, Procrustes distance (d), and Angles population of wing venation of *A. dorsata* and *A. d. binghami* in each island

Apis dorsata	Bending energies	Procrustes distance (d)	Angles
Sumatra	0.01775	0.01699	0.01699
Belitung	0.01295	0.02054	0.02054
Java	0.03302[d]	0.01877	0.01877
Sulawesi	0.01850	0.01874	0.01875
Sumbawa	0.04949[d]	0.02972	0.02973
Moluccas	0.03322[d]	0.02309	0.02309

[d] the high values of bending energy

ordination plot (Figure 4). The data resulted in a total cumulative value of RW1 and RW2, about 31.98% reflecting the variation of the wing venation in *A. dorsata*. The PCA shows that individuals of *A. dorsata* from Sumbawa and *A. d. binghami* from Sulawesi were distributed scattered from the center (0.0) in quadrants 3 and 4 (Figure 4).

The clustering of RW data was used to reconstruct the unrooted Neighbor-Joining phylogeny tree (Figure 5). In agreement with the PCA, the phylogenetic trees also revealed the separate clades of *A. d. binghami* from Sulawesi and *A. dorsata* from Sumbawa.

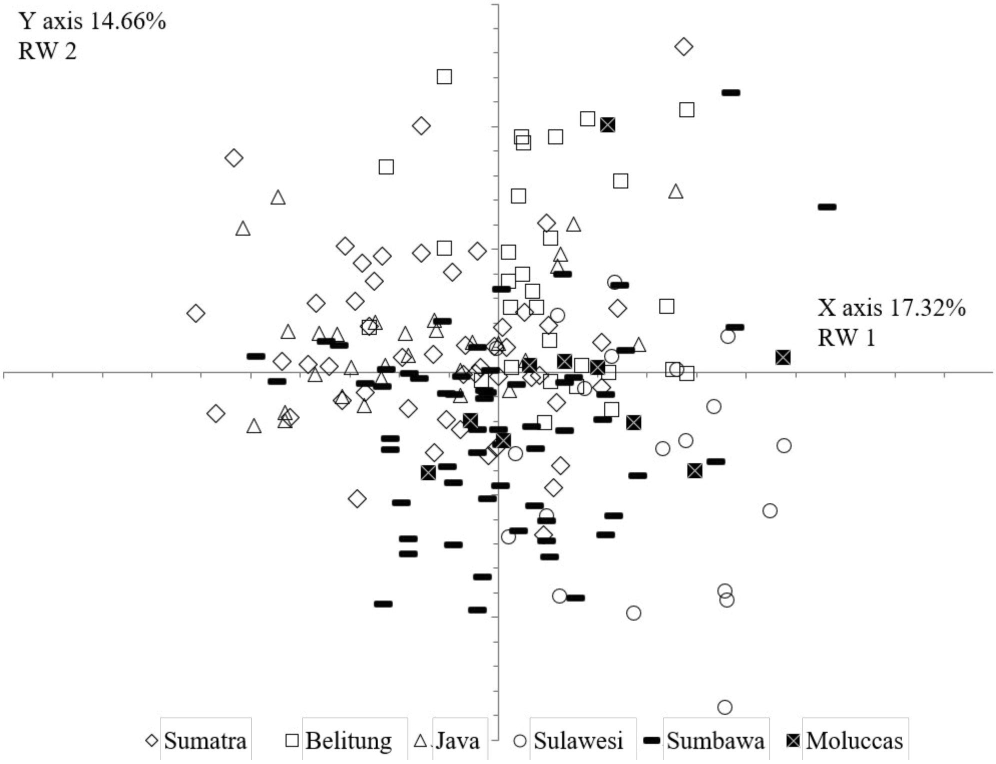


Figure 4. Principle Component Analysis of *A. dorsata* and *A. d. binghami* worker honeybees measured for 19 anatomical landmarks from six islands in Indonesia

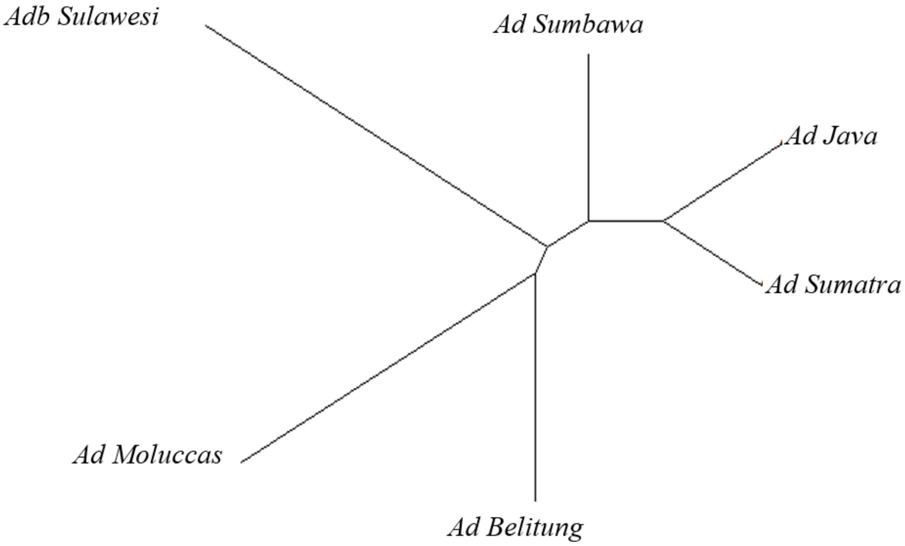


Figure 5. Neighbor-joining unrooted phylogenetic tree of *A. dorsata* and *A. d. binghami*, Abbreviation of the OTUs refer to Table 1

4. Discussion

Our research explored the landmark variations using geometric morphometrics among *A. dorsata* from six islands in the Indonesian archipelagos, i.e., Sumatra, Belitung, Java, Sumbawa, Moluccas, and seek the difference in the subspecies of *A. d. binghami*, the endemic subspecies of the honey bee in from Sulawesi. We then further confirmed the relationships of those giant honey bees.

4.1. The Highest Bending Energy on Wing Venation of *A. dorsata* from Moluccas and Sumbawa

The deformation grid depicted the location of landmark variations (Rohlf 1999) that presented different shapes from random sizes of samples in geometric morphometrics. The different shape was analyzed based on the connecting pair lines of the landmark from the effects of translation, rotation, and scale superimposed to Euclidean distance (Rohlf 2000). The shape of the different deformation grids aligned from anatomical landmarks was processed into a general coordinate system (Slice 2007). In this study, the deformation grid was visualized from the connecting pair lines of 19 landmarks from *A. dorsata* populations on each island and aligned with the mean landmarks. This approach was also used for *A. mellifera* populations in Algeria from different geographic locations or altitudes and revealed no effect between size and wing shape (Barour *et al.* 2011). Their result agrees with our study; that is the variability of different wing shapes is not related to the size contribution in all colonies.

The bending energy of the deformation grid has derived from the location of landmarks that have the most contribution variations of the shape of wing venation (Aytekin *et al.* 2007; Baracchi *et al.* 2011). The high value of bending energies and wing shape was found in Sumbawa and Moluccas (Figure 3, Table 4), as mentioned by Baracchi *et al.* (2011) that the deformation grid is able to differentiate the wing shape from landmarks. On the other hand, the Sumatra *Apis dorsata* revealed very low displacement in the deformation grid; which has bending energy similar to the reference landmark. The low bending energy values indicate low landmark variations for each individual in the group from each population. This is due to bending energy being processed from the average landmark reference when aligned with other landmark configurations (Rohlf 1993).

4.2. Landmark of Wing Venations Differentiate the Subspecies of *A. d. binghami* from *A. dorsata*

Almost all *A. dorsata* from 18 populations comprised of 180 individuals from different islands are clustered in the PCA plot to the centroid of cartesian coordinate based on wing landmark venations. The variation of landmarks of *A. dorsata* wing venation has a discriminant function of 31.98% relative warps score. The discriminant percentage was accurate if the PCA analysis could show variance above 75% cumulative for the RW1 and RW2 (Bro and Smilde 2014). It means that all giant honey bees sampled in Indonesia have a close relationship based on the geometric morphometric of wing venations. This close relationship of *A. dorsata* was also found among the 700 individuals of 70 colonies across five geographic groups of North, West, Central, NE, and Southern in Thailand (Rattanawannee *et al.* 2012), showing a single group for all populations. They hypothesized that the phenomenon occurred panmictic population of random mating in the population of *A. dorsata*. It is plausible due to *A. dorsata* performing a long migration for nectar source and water (Sihag 2014). Our study revealed that the two populations of 20 individuals of *A. d. binghami* were more discrete from the centroid of the cartesian coordinate. The *A. mellifera* in Pantanal Brazil also showed more discrete from the centroid due to the temperature influence in the different niches; thus, slight variations were seen in this population (Peiel and Arranda 2021).

The Sulawesi Island had been uniquely formed by the collision of the three different plates of the Eurasian, Philippine, and Australian in the Oligocene-Miocene period (Hall and Wilson 2000; Hall 2012). This collision played a role in forming endemic and native species of flora and fauna in Sulawesi (Michaux 2010; Stelbrink *et al.* 2012; Hall 2013). The emergence of endemic honey bee *A. d. binghami* revealed a high displacement of the wing venations. The other current study of the significant different morphological of six populations of *A. cerana* derived with the increasing elevation of land in Southwestern China that the body color of *A. cerana* enhances darker and more giant size wings (Zhu *et al.* 2017). This finding also affirmed that *A. cerana* morpho-cluster study based on 12 characters of worker honey bees showed related distributions to ecoregion in climatic zones and affected honey bee taxonomy (Combey *et al.* 2018; Radloff *et al.* 2010).

The presence of a subspecies of giant honey bee *A. d. binghami* endemic to Sulawesi (Maa 1953) indicates the high genetic resources resulting from evolution shaped by the environment and biotics (Alsina *et al.* 2021; Michaux 2010). The wing venation of *A. d. binghami* and *A. dorsata* from the Wallacean towards Eastern Indonesia are more discrete in grid deformation compared to the Sundaland region Sumatra, Belitung, and Java giant honey bee.

Our second finding is that the landmark coordinates 16 and 17 can identify the subspecies level in the population of *A. dorsata* in Indonesia and *A. d. binghami* from Sulawesi generated from the deformation grid. This area landmarks 16 and 17 is known as a cubital index (CI). CI also differentiates the three honey bee subspecies of *A. m. mellifera*, *A. m. ligustica*, and *A. m carnica* in North Wales, England (Keller *et al.* 2014). In Poland, three honeybee subspecies *A. m. mellifera*, *A. m. carnica*, and *A. m. caucasica*, also can discriminate and the distribution of CI of the *A. mellifera* subspecies is suspected influenced by bending energy (Tofilski 2008). The cubital index on bee wing morphology has an a/b ratio that significantly discriminated the subspecies of *A. cerana* from different zones in India (Ibrahim *et al.* 2019). These area landmarks also have relatively high contribution values by geometric morphometrics that were found as the most contribution variation of landmarks in wing venation of wasp in the subfamily of Stenogastrinae (Baracchi *et al.* 2011).

Nevertheless, the CI has long been known to discriminate at the species level, such as *A. andreniformis* with a very high a/b ratio on the wings compared to *A. florea* (Wongsiri *et al.* 1990). The high a/b ratio of *A. koschevnikovi* wing venations compare to the sympatric *A. cerana* in Kalimantan (Hadisoesilo *et al.* 2008). *Apis dorsata* and *A. andreniformis* have displacement in landmarks 14 and 17 (Bustamante *et al.* 2021), which agrees with this current study.

Our geometric morphometric results supported by the molecular phylogeny based on mtDNA showed the clade of *A. d. binghami* close to *A. dorsata* from Asia and *A. breviligula* from the Philippines (Cao *et al.* 2012; Raffiudin and Crozier 2007). The study of wing venation based on geometric morphometrics combined with DNA microsatellite revealed that there is an agreement in clustering the honey bee *A. m. iberiensis, A. m. mellifera, A. m. intermissa*, and *A. m. major, A. m. ligustica*, and *A. m. macedonica* (Miguel *et al.* 2011). It is tempting to explore the relationship of the *A. dorsata* population in this study using the molecular approach, thus can explain the correlation of the geometric morphometric in the relationship of *A. dorsata* in the Indonesia archipelago.

In conclusion, the *A. dorsata* population from Sumatra, Belitung, Java, Sumbawa, the Moluccas and *A. d binghami* from Sulawesi can be distinguished from the displacement of landmarks of wing venation in the deformation grid. The clustering of *A. d. binghami* in an unrooted phylogeny tree has affirmed the subspecies. However, all shape variances clustered in a single group in the cartesian coordinate. The giant honey bee *A. d. binghami* from Sulawesi was discrete in the landmark's variation for 14, 16, 17, and 18 in the deformation grid, and *A. dorsata* landmarks from the Moluccas for 16, 17. Overall, landmarks 16 and 17 differentiate the subspecies level of this giant honey bee *A. d. binghami*, and these landmarks are an important area of the wing venation known as the cubital index.

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