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To cite this article: M A D Santoso *et al* 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **197** 012015

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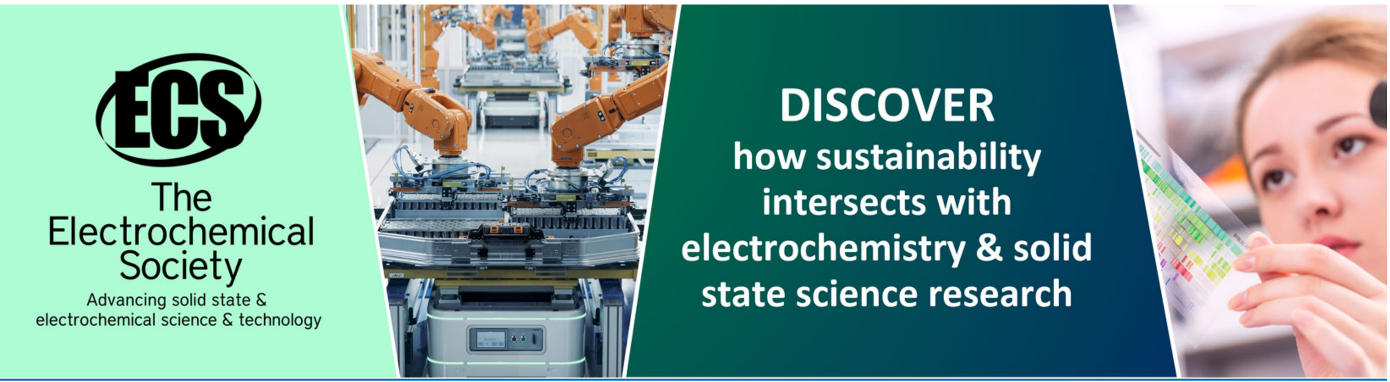

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Honey Bees Species Differentiation using Geometric Morphometric on Wing Venations

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Abstract. Wings are the most interesting part for morphometric study in any species of insect, including honey bees. The geometric morphometric is a strong analytical method to identify the species of insect, therefore we used wing venations to explore the wing variations among five honey bee species in Indonesia, i.e. *A. mellifera, A. cerana, A. dorsata, A. andreniformis,* and *A. koschevnikovi*. We used Thin Plate Splin (TPS) software to conduct the landmark digitation on wing venation and geometric morphometric analysis based on the 19 anatomically points of venation through *tpsdig2* and *tpsRelw* software to describe the variations of wing venations. Our result of Relative Warp ordination plot showed variations of wing venation of five honey bee species, particularly on the five points. Grid deformations show the difference patterns among the five species of honey bees as well. Moreover, based on phylogenetic tree by using Neighbour Joining approach, the morphometric geometric wings venation is also able to separate the species of honey bees; thus, a possibility method to differentiate the honey bee species in Indonesia.

Keywords: Geometric morphometric, wing venations, *Apis andreniformis, Apis dorsata, Apis koschevnikovi, Apis mellifera*

1. Introduction

Honey bees (Hymenoptera: Apidae) is a social insect producing honey that have role as the main pollinators of plants [1]. In the size of a morphology, honey bee can be divided into three types : dwarf honeybees, medium sized honeybees, and giant honeybees. Based on the type of their hive, honey bee can be divided into two groups, open nesting honeybees and cavity nesting honeybees. Indonesia has a lot of diversity of honey bee species. There are seven species of honey bee in Indonesia such as *Apis cerana*, *A. koschevnikovi*, *A. nigrocincta*, *A. mellifera*, *A. dorsata*, *A. andreniformis* and *A. florea* [2]. The four species that mentioned first are a cavity nesting honey bee, while *A. dorsata, A. andreniformis* and *A. florea* are an open nesting honey bee [3]. *A. andreniformis, A. dorsata, A. cerana, A. koschevnikovi,* and *A. nigrocincta* are native honey bees native from Indonesia [4].

Various methods have been developed to facilitate the identification process of the honey bees species on the field which is important to see their diversity. Methods that have been developed include

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morphometric [5], biochemistry [6] and molecular methods, including mitochondrial DNA [7] and microsatellite [8]. Morphological characters have been long used to study the diversity and kinship in the honey bee species. Wing morphology venation is often used as the distinctive characteristics on each species of insects, including honey bees. Variations on honey bee's wings can be used to distinguish between species or subspecies as well to see their close relations. One of the studies to see variations of the wing venation of the honey bee species is through the morphometric method.

Morphometric is a method describing morphological characters through measurement, calculation and granting score [9]. The morphometric method is a pretty powerful biology research device because it can be applied to deduce numerical data and graph the morphology of a species, knowing a certain kinship of a species, morphological variation of a species as well as species identification [10-11]. Geometric morphometric is known better as compared with the traditional morphometric, because geometric morphometric is based on the analysis representations of the form in cartesian coordinate. In addition to using distance and angle, geometric morphometric using dots coordinates called landmark [12].

Morphometric geometric method is able to mapping a deformation process of a shape to other shapes with the movement of the anatomical dots one example is the Thin Plate Splin (TPS). The change form on TPS device can be differentiated into affine component and non-affine component. Both component will show the shape of grid deformation [9]. The use of geometric morphometric method are using TPS software. The geometric morphometric study of honey bee wings is a new method and are already used to see wings variations of four species of honey bee wings in Thailand as a database for studies of bees biodiversity or to the fossil record [13]. However, wing venation variation research of honey bee species in Indonesia has not been done yet.

2. Materials and methods

2.1 Taking a photo of a bee wing

This research uses right front wing from ten specimens of *A. andreniformis, A. dorsata, A. cerana, A. koschevnikovi* and *A. mellifera* worker that obtained from various locations in Indonesia (table 1). Right front wings used in this study for consistency because many research generally use the right front wing for morphometric analysis [13-16], while the left front wing used by Bouga and Hajitna [17] and Tolfiski [18].

Ten samples of bees can represent the population data for the purposes of statistical analysis [14]. The right wing cut out at the base of the wings attached to the mesoscutum using scissors and placed on top of a glass object using tweezers. The photos of bee wing are taking using microscope with camera (Optilab). The photos of bee wings are further used for digitations analysis.

Table 1. Honeybee sampling location

Species	Location	Collector[a]	Year	Amount
A. andreniformis	Barangan, West Sumatra	CF, RR	2014	10
A. dorsata	Gunung Sampai, Sukabumi	DH, CF	2016	10
A. mellifera	Boyolali	YN, DH	2016	10
A. cerana	West Java	RR	1998	10
A. koschevnikovi	South Kalimantan	AR	2004	10
			Total	50

[a] Collector: CF = Cut Ferawati, RR = Rika Raffiudin, DH = Desmina Hutabarat, YN = Yuan Natalia, AR = Arif Rohmatullah.

2.2 Data Analysis
The picture of honey bee wings are analyzed by using multiple software series of TPS. TPS decomposed changes the form of affine or linear components and non-affine component, which can be displayed into the shape of the grid deformation. Affine component maintains parallel lines on the grid deformation, while non affine component reflect the specific changes on the specific anatomical points so it can be known the change from it grid deformation [9,19].

This studies are used non affine changes in form, because this forms can reflect variation changes. Every picture of honey bee wings are digitized using the *tpsDig2* programme [20] to get the coordinates of an anatomical point on the wing venation. Description of wing is done by using 19 anatomical point of wing venation (table 2). Samples of wing digitations can be seen in figure 1. Pictures of each individual honey bee wings are digitized 5 times to reduce errors on digitation. Furthermore, the average value of the coordinates of each repeat is calculated by the *tpsRelw* software [21]. The value of the coordinate average is then stored as a database of wings venation coordinates to honey bee species that concerned. The average coordinate species calculated from the coordinates of each individual member of the species by using *tpsRelw* software [21]. The visualization from the result of digitation species average are analyzed with *tpsSuper* software. Wing shape deformation from every species of honey bees are visualize using the software *tpsSplin* to see the variations of each point in the not affine form [22].

Each individual from the calculation result of *tpsRelw* occupies a point in the space that formed by Relative Warp (RW) based on their deformation changes. The distance value between species is calculated based on the euclideus distance of each species on the wing venation space shapes. Each species grouped by similarity with the Neighbour Joining method [23] in the packet of Analysis Phylogenics And Evolutions (APE) [24]. The tree of kinship between species is then created with the *R* software [25].

3. Results

3.1 Relative warps
Honey bee wing variation in non affine component been summarized with the principal component analysis on the cuvation partial value that produces relative warp (RW) value. The RW value obtained according to the average form of each individual. Bee wing venation RW obtained 34 RW values. Variation in the RW value are described in an ordination plot form (figure 2) Ordination plot singular values that explained by the first two relative warps for the front wing consensus were 0.044 and 0.030 respectively. Wing venation of *A. cerana, A. koschevnikovi, A. mellifera* has close relation.

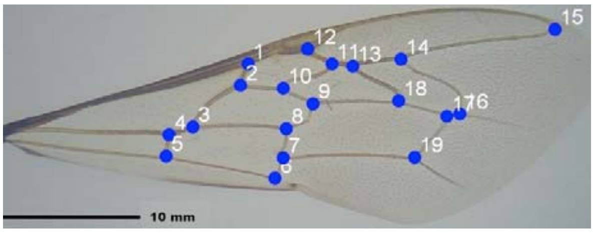

Figure 1. Digitation consensus of honey bee wing

Honey bee wing visualitation are described by grid deformation venation. Grid deformation TPS showed direction of anatomical point movement on *Apis* wing (figure 3). Generally, landmark 15, 14, 10, and 1 has highest variances amongs every species with values of S^2 = 0.0003630, 0.0002261, 0.0001857, and 0.0001607. While landmark 12, and 13 has lowest variances with values of S^2 = 0.0000208 and 0.0000203.

Table 2. Honey bee anatomical venation point [1]

Landmark	Description
1	Meeting point of Rs & prestigma
2	Rs+M Point
3	M+Cu 1 Point
4	M+Cu 2 Point
5	cu-v Point
6	Meeting point of V & Cu2
7	Meeting point of Cu1 & Cu2
8	1m-cu 1 point
9	1m-cu 2 point
10	Meeting point of Rs+m & 2nd arbiscissa of Rs
11	Meeting point of 2nd arbiscissa of Rs & Rs
12	Meeting point of r & stigma
13	Meeting point of Rs & 1r-m
14	Meeting point of Rs & 2r-m
15	Rs end point
16	2r-m point
17	2m-cu 1 end point
18	1r-m point
19	Meeting point of 2m-cu & Cu

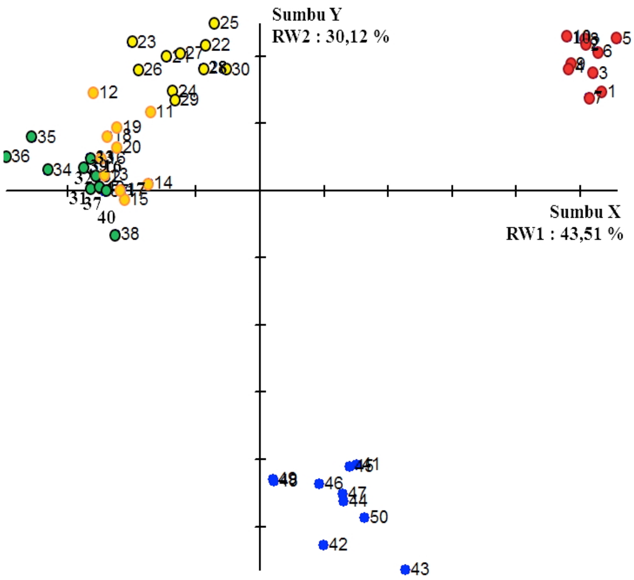

Figure 2. Ordination plot on RW1 and RW2. *A. andreniformis* (1-10; red), *A. cerana* (11-20; orange), *A. koschevnikovi* (21-30; yellow), *A. mellifera* (31-40; green), *A. dorsata* (41-50; blue).

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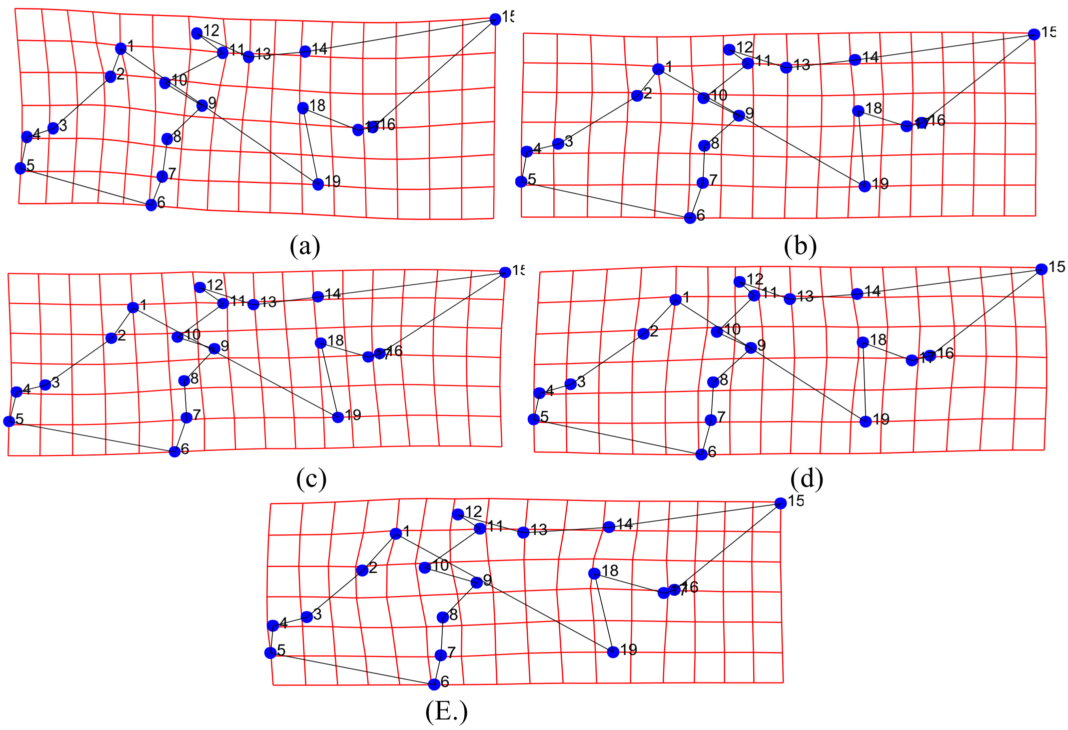
A. andreniformis is a species that have highest difference deformation form than the other species. Generally, the position of anatomical landmark venation on the grid deformation of *A. andreniformis* are more moving toward the inferior when compared with the cavity nesting honey bee group, i.e. at the landmark 4, 5, 6, 7, 8, 9, 10, 14, 15 and 18. *A. andreniformis* and *A. dorsata* is different at the landmark 1, 2, 11, 14, and 17. Landmark 1 on *A. andreniformis* are more moving toward the lateral left. Landmark 2 is moving toward landmark 1, contrary on *A. dorsata*. Landmark 11 on *A. andreniformis* are moving toward inferior than *A. dorsataa*. Landmark 14 and 17 on *A .dorsata* is more moving toward right.

A. mellifera and *A. cerana* just have different on landmark 1 and 15. Landmark 1 and 15 on *A. cerana* is moving toward the inferior, while landmark 1 and 15 of *A. mellifera* is moving toward the superior. Although in a morphological manner *A.cerana* and *A. koschevnikovi* are close, both spesies have different on landmark 1, 4, 15, and, 18, *A. koschevnikovi* and *A. mellifera* is different on landmark 1, 4, 7, 12, 15, and 18. The difference between open nesting and cavity nesting are in the area landmark 1 to 10, where on the open nesting honeybee those points more moving towards in.



(a) (b) (c) (d) (E.)

Figure 3. Wing venation of honey bee with it deformation grid.*Apis andreniformis* (a), *Apis cerana* (b), *Apis koschevnikovi* (c), *Apis mellifera* (d), *Apis dorsata* (e).

3.2 Phylogenetic tree

Relative warp value then used for the next following process that would see phylogenetic relation between using R software. Euclideus distance matrix used as data for grouping forms honey bee wing on similarity. The result showed *A .andreniformis* are in one clade *A. dorsata* (figure 4), and *A. koschevnikovi, A. cerana, A. mellifera* are in different clade but still in one group. Figure 4b showed the average result from each *Apis* because there is an overlap on individu result number 14 and 11.

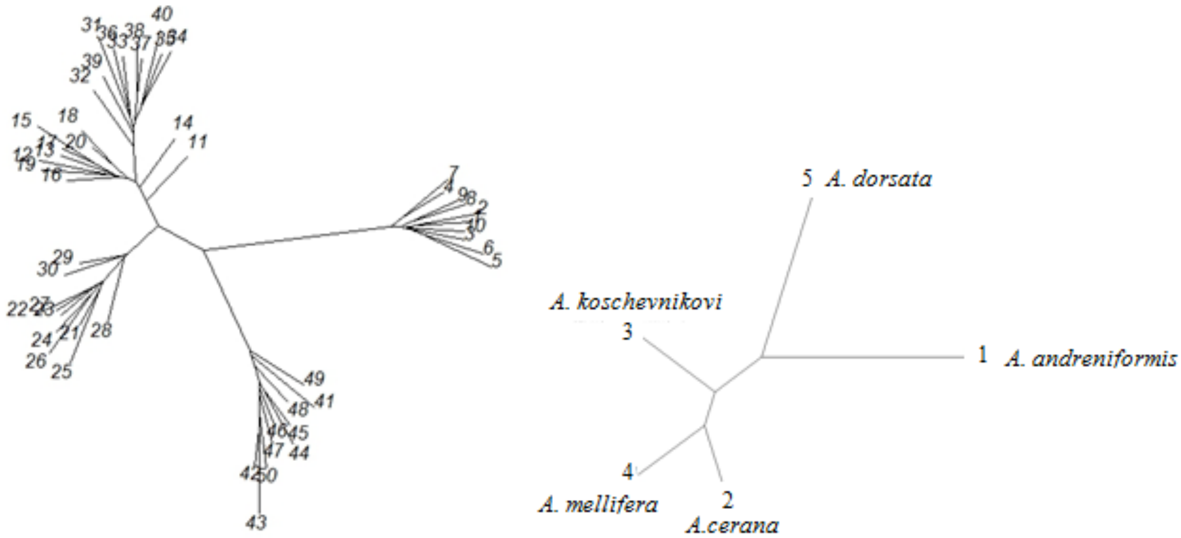


Figure 4. *Neighbour Joining* phylogenetic tree. The tree between each individual of *Apis, A. andreniformis* (1-10), *A. cerana* (11-20), *A. koschevnikovi* (21-30), *A. mellifera* (31-40), dan *A. dorsata* (41-50) (A). The tree between average species of *Apis* (B).

4. Discussion
Insects have high variation of wing venation patern and complexity, so wing venation are often used as peculiar characteristic on each spesies of insect. Several study have explained that wing morphometric can be used for identify several species of bees and diverging venation variation between the species such as *stingless bees* [26], *bumble bees* [27], and on the subspecies of *Apis mellifera* [28]. The study based on average digitation of all individual species can showed the anatomical points picture on the right wing of five species honey bees. The anatomical landmark are obtained from the result of consensus or average from 19 point of landmark digitation. The wing form and variation explained with homologous anatomical point. Insect wings have two dimentional structure so it easy to give labeling number on, and provide specific information on different taxa using geometric morphometric [29].

The geometric morphometric analysis of *Apis* wings anatomical point are served on relative warp ordination plot form. The plot result showed that honey bees wing analysis generates 34 relative warp (RW). Variation between species can be clearly seen in the ordination plot. The distribution from five species of honey bee showed that there is a clustering from *A.cerana, A.koschevnikovi and A.mellifera* group. It mean there are a morphological resemblance of wing venation between these three species that belongs to a group of cavity nesting honeybees or medium size honeybees. Meanwhile, *A.andreniformis* and *A. dorsata* form a different cluster from theese three species.

The result of relative warp is consistent with topology phylogenetic tree which construct with Neighbour Joining method. *A. andreniformis* and *A.dorsata* are in different clade. Another conformity is proximity clade on three species cavity nesting : *A. mellifera, A. cerana, A. koschevnikovi*. Amongs theese three species, *A.cerana* is more cluster with *A.mellifera* compared with *A. koschevnikovi*. The closeness between *A. cerana* and *A. mellifera* are supported by only two points venation variation between theese two cavity nesting species. This result are different from the bee phylogeny analysis based on morphology [30] and molecular [2], that say *A. cerana* and *A. koschevnikovi* are in the nearby clade. Thus, geometric morphometric can be used for identifying species of honey bee.

Acknowledgements
We thank to Andi Trisnadi, Achmad Alfiyan, Nurul Insani, and Tiara Sayusti for the asistance.

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