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Morphometric diversity of *Apis cerana* Fabr. within the Philippines

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(Invited paper)

Abstract – The diversity of *Apis cerana* Fabr. in the Philippines was studied using morphometric methods. A total of 101 samples of *A. cerana* from feral and hived colonies, and foragers were collected throughout the Philippine archipelago. The 39 morphometric characters recommended by Ruttner and Ruttner et al. were measured. The data were statistically analyzed by means of factor analysis, discriminant analysis, and cluster analysis. The bees from Palawan were unequivocally distinct and separate from the other Philippine samples. The bees from the other Philippine islands still showed a high degree of variation. The bees of Luzon differed clearly from those of Visayas and Mindanao. Within Luzon, the bees from the highland differed clearly from those in the lowland and were regarded as separate groups. Bees from Visayas and Mindanao were still very variable and showed potential for further sub-structuring. The present analysis could not distinguish whether the difference between Luzon and Visayas-Mindanao was based on a north-south clinal structure, or on distinct groups.

***Apis cerana* / Philippines / morphometry / biogeography**

1. INTRODUCTION

Apis cerana Fabr., also referred to as the Eastern honeybee, exhibits a wide variation in body size, productivity and behavior in different parts of Asia. Unlike its western counterpart, *A. mellifera* L., the understanding of its taxonomy and biology is only in the beginning. Early taxonomists like Maa [11] had split up the eastern cavity-nesting bees into 11 species and several subspecies. Based on groups generated by

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multivariate analysis of morphometric data, Ruttner [13] proposed a subdivision of the species into only four subspecies, namely: *A. c. cerana, A. c. indica, A. c. himalaya, and A. c. japonica*. The recent upsurge of interest in Asiatic bees has led to the rediscovery of Maa's species *A. koschevnikovi* in Sabah, Borneo [17] and to the discovery of the new species *A. nuluensis* in Sabah [18] and *A. nigrocincta* in Sulawesi, Indonesia [7].

In the Philippines *A. cerana* is found to be geographically distributed and abundant throughout the archipelago. In his book 'Biogeography and Taxonomy of Honeybees', Ruttner [13] did not give a clear and definite position for these bees, due to the paucity of samples from the region. He grouped the Philippine bees with the 'plains variety' of *A. c. indica*, although he noted the need for a more thorough knowledge of their variation before deciding whether they could be considered as a separate subspecies or not. Even from these earlier studies it was evident that within the archipelago *A. cerana* varies widely, and Maa [11] had mentioned two groups he regarded as different species: *A.* (*sigmatapis*) *samarensis* from the island of Samar and *A. (sigmatapis) philippina* from Luzon.

This study aims at investigating the variation of *A. cerana* in the Philippines through sampling from different regions and morphometric analysis. It sets out to describe their morphometric properties, to determine whether distinct populations of *A. cerana* exist in this area and to establish the relationships among them.

2. MATERIALS AND METHODS

2.1. Collection of bee samples

Honey bee samples were collected from different localities and islands throughout the Philippine archipelago. Selection of the areas of collection was made in consideration of established Philippine faunal regions; the routes of entry or origin of Philippine floral and faunal composition; the presence of natural barriers such as bodies of water and mountain ranges. Traditional beekeeping with *A. cerana* is not popularly practiced in most of the areas of collection. As feral colonies abound in most of the areas, the few *A. cerana* beekeepers do not practice import of colonies. Hence, human transfer of colonies from one island to another or from one locality to another can effectively be ruled out.

A total of 101 samples was collected. Mostly worker bees were sampled from feral colonies (90), and a few from managed hives (3). In some cases (8) foragers were collected from flowers, especially in areas where it was very hard to locate the bees' nests. A total of 22 samples was collected from mainland Luzon, 19 from Mindanao, 14 from 4 different Visayas islands, and 46 from the Palawan island. Figure 1 presents a map of the sampling locations.

At least 30 worker bees were collected from each colony. Traps were fitted at the hive entrance catching the bees flying out to forage. The trapped bees were immediately killed by immersion in 70% ethanol. Final preservation of the samples was carried out by replacing the ethanol after two days. Complete sets of voucher material were deposited at the Bee Bank of the Institut für Bienenkunde in Oberursel, Germany and at the UPLB Bee Program, Los Baños, Philippines.

2.2. Preparation and measurement techniques

Fifteen bees from each colony were prepared for morphometric analysis using the techniques and characters described in Ruttner [13] and Ruttner et al. [14]. A total of 39 characters was measured: 18 size characters, 11 wing venation angles, and 7 color characters, hairlength, proboscis, and hooks (Tab. II). Quantitative and qualitative measurements were carried out using a

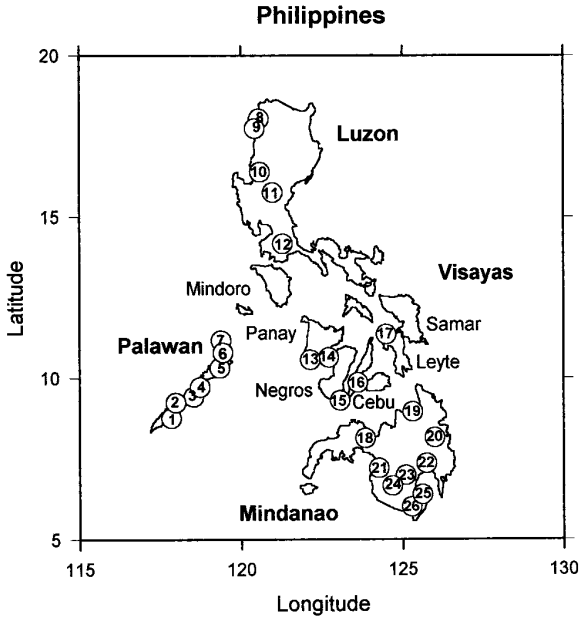

Figure 1. Map of *A. cerana* sampling locations. Number of samples is given in parentheses. Palawan: 1 Brooke's Point (7); 2 Quezon (8); 3 Aborlan (7); 4 Puerto Princesa (11); 5 Roxas (6); 6 Tatay (3); 7 El Nido (4). Luzon northern lowland: 8 Batac (5); 9 San Vincente (1). Luzon highland: 10 Baguio City and region (8). Luzon southern lowland: 11 San Jose City (1); 12 Laguna City and region (7). Visayas: 13 San Joaquin (5): 14 Valencia (4); 15 Negros Occidental (1) 16 Argao (2); 17 Isabel (2). Mindanao: 18 Ozamis City (1); 19 Carmen City (2) 20 Bunawan (1); 21 Polomolok (1); 22 Panabo (3); 23 Kitapawan (3); 24 Takurong (1); 25 Davao del Sur region (6); 26 General Santos City (1).

stereomicroscope and a computer-aided measuring system based on a video system and a measuring program developed by Meixner [12].

2.3. Statistical analysis of the data

Means, standard deviations and standard errors of the samples were automatically computed for each character by the morphometric program. Sample means were analyzed using factor analysis and discriminant analysis. Relations between groups were investigated by cluster analysis using the Euclidian distances between the group centroids calculated from the discriminant analysis (SPSS for Windows 7.0).

To test for the presence of a gradient or cline, the three factor scores generated from the factor analysis were plotted against latitude and longitude, and the respective linear regressions were calculated. To test for deviations from the clinal pattern, multiple regression analysis of the sample factor scores on both longitude and latitude was calculated and a one-way analysis of variance (ANOVA) of the resulting residuals was performed on the respective groups.

3. RESULTS

Principal component analysis (PCA) of the 39 morphometric characters performed on the 101 sample means yielded 3 factors with high eigenvalues (> 4). The first factor accounted for 21.7% of the total variation in the data and was mainly associated with wing venation angles (E9, J10, J16, and L13), variables of size pertaining to the wax mirror, tergites 3 and 4, sternite 3, tomentum, cubital 2, and pigmentation of tergite 4. The second factor was mainly positively associated with size variables pertaining to the leg, forewing, sternites 3 and 6, proboscis, and cubital 1. This factor accounted for 20.5% of the total variation in the data. The third factor was mainly associated with the characters pertaining to pigmentation pattern (tergites 2, 3, 4 and scutellum 1), length of hair on tergite 5, and the angle A4,

and accounted for 11.3% of the total variation in the data. All three factors accounted for 53.6% of the total variation in the data.

Figures 2a and 2b present the plots of the three factor scores generated from the principal component analysis (PCA). The bee samples were coded based on nine major collection localities. Two clusters can very clearly be distinguished from the graphs. One cluster contains the Palawan bees, and a second cluster contains the bees from the rest of the Philippines (Luzon mainland, the Visayas islands, and Mindanao).

The cluster of bees from the rest of the Philippines exhibits a wide range of variability. The PCA graphs suggest two possibilities: 1) the group may be substructured into a Luzon cluster and a Visayas-Mindanao

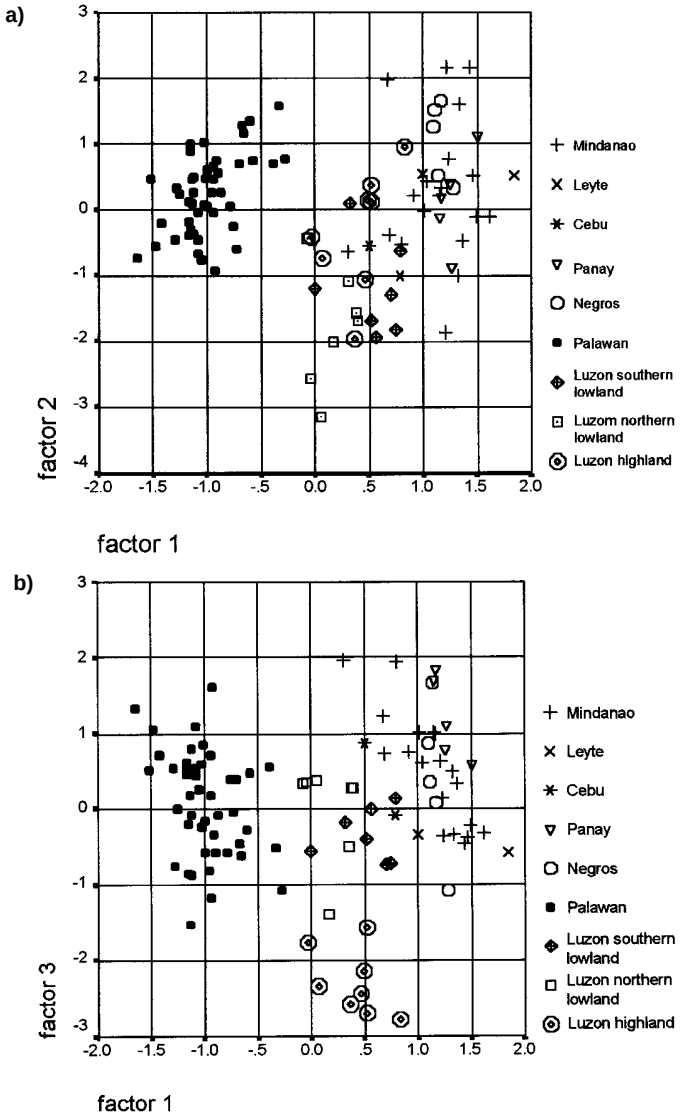

Figure 2. Position of Philippine *A. cerana* samples on the factor axes derived from the PCA of morphometric characters. **a:** Ordinate: factor 1; abscissa: factor 2. **b:** Ordinate: factor 1; abscissa: factor 3.

cluster; or 2) the presence of a north-south (Luzon-Mindanao) clinal component, strongly indicated from factors 1 and 3. In the first case, the distinction of a Luzon cluster is supported by on average lower values on all three factor axes, compared to the Visayas-Mindanao samples. However, there is some overlap on each axis, which is highest in factor 2 and only slight in factor 3. Nevertheless, a line without overlap can be drawn in the factor 1 versus factor 3 plot (Fig. 2b). A further delineation of the Luzon component into the highland bees (Luzon HL) and the lowland bees (Luzon northern lowland NL and Luzon southern lowland LL: Luzon LL) is indicated along factor 3 (Fig. 2b).

The above-mentioned patterns and trends were consistently obtained even with the varied PCA analyses. If only the more conservative characters of wing venation angles were considered, an even clearer resolution of the Philippines (without Palawan) into a Luzon and Visayas-Mindanao cluster was obtained along the factors 1 and 3, and of the Luzon HL bees along factors 1 and 2. The same trends were obtained by including only the size-independent variables and computed indices. An analysis excluding the characters of pigmentation, on the other hand, supported only the Luzon component and a Visayas-Mindanao component along factors 1 and 3. In all these analyses, the distinctness of the Palawan group was clearly expressed.

Discriminant analysis with the bees assigned to four a priori groups, Palawan, Luzon HL, Luzon LL, and Visayas-Mindanao, yielded a 100% classification of the samples to their preassigned groups with post-hoc probabilities of $P > 0.999$. When forced to be classified into the other major collection sites, the Luzon bees were assigned only to the other Luzon groups; 89% of the Palawan bees were assigned to the Luzon bees and 11% to the Cebu bees; all the Visayas and Mindanao bees, except the Cebu bees, were assigned only to the other Visayas and Mindanao bees; and the Cebu bees were assigned to either the Southern Luzon LL bees (50%) or to the Leyte bees (50%).

Figure 3 shows the dendrogram constructed from a cluster analysis of the squared Euclidian distances between centroids of the factor scores for the samples grouped by the major collection localities as previously defined (c.f. Fig. 2). Two main branches are shown, one that combines the Luzon bees with the Palawan bees, and the other that combines all the Visayas and Mindanao bees, except the Cebu bees, which grouped with the Luzon-Palawan cluster. The dendogram supports the distinctness of the Palawan group and the separation of Luzon into Luzon HL and Luzon LL, and the presence of a variable Visayas-Mindanao component.

To explore clinal patterns in the characteristics of the Luzon-Visayas-Mindanao bees, factor scores of the samples were plotted against latitude and longitude (Figs. 4a–4f). Gradual transition of characters from north to south and from west to east are

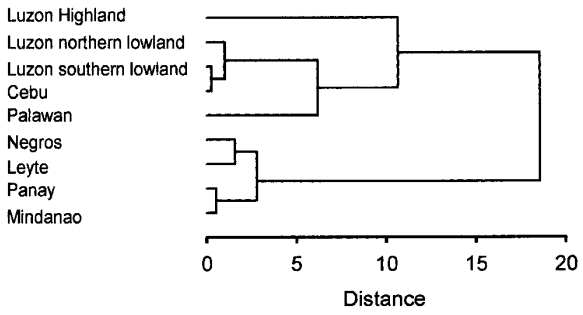

Figure 3. Relations of morphometric similarity between Philippine *A. cerana* from major sampling locations. Similarities are based on squared distances between centroids from prinzipal component analysis; the tree was constructed from clustering by Ward's method.

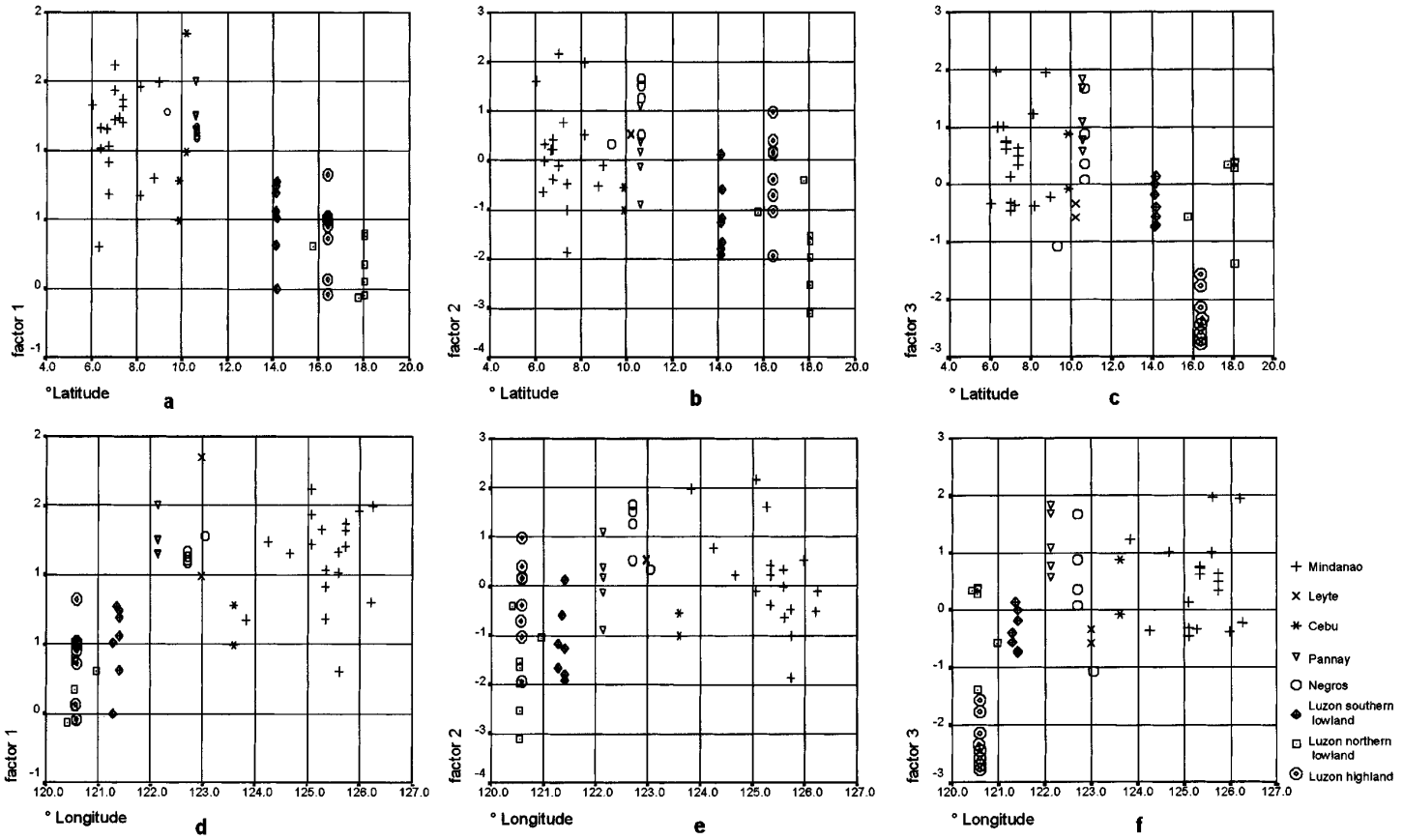

Figure 4. Geographic trends in morphometric characters in Philippine *A. cerana*. Abscissa: latitude (**a, b, c**) or longitude (**d, e, f**). Ordinate: factor scores 1 (**a, d**), 2 (**b, e**), 3 (**c, f**), as derived from PCA. Value labels refer to major sampling locations.

indicated in most of the graphs. In particular, Luzon HL bees seem to deviate from the overall trend (cf. Figs. 4c, 4f). Results of linear regression analyses of the factor scores against latitude and longitude (taken one at a time and with or without the Luzon HL samples) are summarized in Table I. A distinct and highly significant ($P < 0.0005$) slope is observed in latitude for both factors 1 and 2 (with or without Luzon HL), whereas for factor 3 the slope is highly significant ($P < 0.0005$) only in the presence of Luzon HL bees, and less obvious ($P < 0.013$) if they are excluded. A significant slope ($P < 0.0005$) is also indicated in longitude for factor 1 both with or without Luzon HL, it is less pronounced for factor 2 (with Luzon HL, $P < 0.008$; without Luzon HL, $P < 0.003$), and significant for factor 3 only in the presence of Luzon HL. Multiple regression analysis of the factor scores with both longitude and latitude taken simultaneously for all samples grouped according to major collection localities and one-way ANOVA of the resulting residuals for the localities showed that all localities were homogenous for factor 1 and factor 2, but not for factor 3. Multiple comparison showed that this was caused by Luzon HL bees, which was the only locality which showed significant differences from part of the others (NL: $P < 0.0005$; SL: $P < 0.024$; Leyte 0.0005; Mindanao: $P < 0.01$).

The major collection sites were divided into four main groups: Palawan, Luzon LL, Luzon HL, and Visayas-Mindanao. For these main groups, Table II gives the group means and standard deviations of the measured morphometric characters and indices. One-way ANOVA performed on colony means grouped by sampling sites was performed within the Luzon HL, Luzon LL, and Palawan main groups and yielded non significant variability for most of the characters, implying homogeneity of the characters within these three groups. In the Visayas-Mindanao main group, samples grouped by major collection locality as shown in Table II showed significant differences in 20 out of the 48 characters, reflecting the still wide variability within this large group.

For the single characters, the Palawan group had the highest values in the length and width of the wings, width of sternite 6, distance of wax mirrors, tomentum 2, tibia, in the pigmentation on tergite 2, cubital vein 1, and the wing angles J10 and L13. They scored lowest in the length of tergites 3 and 4, and thus in the body size, the width of the wax mirror, and tomentum 1 (and thus in

Table I. Linear regression of geographic trends in morphometric characters in Philippine *A. cerana* derived from prinzipal component analysis.

Predictor	Dependent variable	*R* value		Significance P	
		With Luzon HL	Without Luzon HL	With Luzon HL	Without Luzon HL
Latitude	Factor 1	0.768	0.729	0.0005	0.0005
	Factor 2	0.505	0.614	0.0005	0.0005
	Factor 3	0.575	0.358	0.0005	0.013
Longitude					
	Factor 1	0.670	0.606	0.0005	0.0005
	Factor 2	0.352	0.424	0.008	0.003
	Factor 3	0.519	0.287	0.0005	0.051

HL: highland; LL: lowland.

tomentum index), in the total length of the legs, the relation of length and width of the metartarsus, and the relation of body size to leg size, further in the pigmentation on labrum 2 and the wing venation angles J16 and K19. Only in two of these measurements, i.e., the distance of the wax mirrors and the wing angle L 13, differences to the other groups were found to be significant by multiple comparison (ANOVA, LSD post-hoc, $P < 0.05$).

The Visayas-Mindanao main group had the highest values for most of the size-related variables, particularly for the characters that pertained to the abdomen and legs (length of tergites 3 and 4, length of sternites 3 and 6, and the relation of length to width of sternite 6 = slenderness, proboscis, length of femur, length and width of metatarsus, length of complete leg, the relation of body size to leg size, tomentum 1 and tomentum index, pigmentation on tergites 3 and 4, as well as the wing venation angles E9, G18, J16, N23 together with a high cubital index. The group scored lowest in the distance of wax mirrors and some wing venation angles (A4, D7, L13 and particularly O26). Of these measurements, 10 were significantly lower or higher than the other groups in a multiple comparison (length of tergites 3 and 4, length of sternite 3, length of tarsus, body size, slenderness, pigmentation on tergite 4, cubital index and wing venation angles G18 and N23; ANOVA, LSD post-hoc, $P < 0.05$).

The Luzon HL main group was highest in most of the pigmentation scores (pigmentation on tergites 2, 3 and 4, and scutella 1 and 2); they possessed the biggest wax mirrors in length and width together with the highest length to width relation of wax mirrors, and the longest hair. Two of the wing venation angles were the highest (A4 and K19), and two others (B4 and G18) the lowest. The difference to the other groups was significant in the length of the wax mirror, the hairlength and the wing venation angle A4. The Luzon LL bees showed the lowest values in more than half of the size measurements (length of sternites 3 and 6, width of sternite 6, length of wax mirror, length and width of forewing, length of femur, tibia and metatarsus, and tomentum 2, which led to a low tomentum index. They further showed low values in the wing venation angles J10 and N23. They showed the highest values only in the pigmentation of scutella 1 and 2 and the wing venation angle O26. However, none of these differences were statistically significant.

4. DISCUSSION

Ruttner [13] was the first to initiate morphometric studies of Philippine bees. In his analyses, he placed the Philippine bees among the smallest *A. cerana* bees, and mentioned the possibility of these bees belonging to a separate subspecies, *A. cerana philippina*. The separateness of these bees from other *A. cerana* groups has since been confirmed in a number of morphometric and molecular studies [2–5, 15, 16]. However, even the limited samples of Philippine bees up to now included in morphometric analyses made on *A. cerana* have suggested a great deal of morphometric variability among these bee populations [3, 13].

Our morphometric analysis of 101 samples of *A. cerana* from collection sites covering the main parts of the Philippines revealed a pronounced variation within the archipelago. The most apparent feature is the position of the Palawan bees as separate from the rest of the Philippine *A. cerana* samples, as confirmed by factor and discriminant analysis. These bees showed some characteristic morphological features; the most noteworthy of these being the comparatively long wings and legs, long distance between the wax mirrors, and a low tomentum index. Also, two of the wing venation angles, J10 and L13, were distinctly higher than in the other groups. The apparent homogeneity of the different characters in the sample site ANOVA suggested no further subdivision on this extremely long

Table II. Mean values and standard deviations of the different morphometric characters among the four main bee groups, and for the major collection localities within the Visayas-Mindanao main group.

	Luzon HL *N* = 8	Luzon LL *N* = 14	Palawan *N* = 46	Visayas-Mindanao *N* = 33	Negros	Iloilo	Cebu	Leyte	Mindanao
Size-related characters									
Cubital vein1	0.43 ± 0.01	0.43 ± 0.02	0.47 ± .02	0.48 ± .03***	0.50 ± 0.01	0.50 ± 0.004	0.44 ± 0.01	0.44 ± 0.006	0.47 ± 0.02
Cubital vein 2	0.13 ± 0.007	0.13 ± 0.006	0.13 ± 0.01	0.11 ± 0.01*	0.12 ± 0.004	0.11 ± 0.004	0.12 ± 0.01	0.13 ± 0.02	0.11 ± 0.01
W sternite 6	2.46 ± 0.06	2.38 ± 0.08	2.48 ± 0.05	2.43 ± 0.08	2.43 ± 0.07	2.45 ± 0.06	2.39 ± 0.02	2.49 ± 0.02	2.42 ± 0.09
L sternite 6	1.98 ± 0.04	1.94 ± 0.04	2.03 ± 0.03	2.03 ± 0.06**	2.07 ± 0.03	2.03 ± 0.03	1.91 ± 0.003	2.05 ± 0.01	2.03 ± 0.06
L sternite 3	2.19 ± 0.04	2.12 ± 0.06*	2.16 ± 0.04	2.24 ± 0.05	2.27 ± 0.04	2.23 ± 0.04	2.18 ± 0.03	2.29 ± 0.03	2.23 ± 0.06
L tergite 3	1.75 ± 0.04	1.71 ± 0.04	1.69 ± 0.03	1.81 ± 0.04*	1.82 ± 0.03	1.79 ± 0.03	1.72 ± 0.02	1.85 ± 0.06	1.81 ± 0.04
L tergite 4	1.70 ± 0.03	1.66 ± 0.04	1.64 ± 0.04	1.76 ± 0.05	1.77 ± 0.03	1.75 ± 0.04	1.68 ± 0.01	1.80 ± 0.07	1.76 ± 0.05
L femur	2.19 ± 0.07	2.16 ± 0.05	2.22 ± 0.04	2.24 ± 0.07	2.28 ± 0.08	2.20 ± 0.07	2.20 ± 0.03	2.28 ± 0.03	2.23 ± 0.07
L tibia	2.73 ± 0.09	2.71 ± 0.07	2.81 ± 0.07	2.80 ± 0.09	2.84 ± 0.08	2.77 ± 0.10	2.75 ± 0.05	2.87 ± 0.004	2.79 ± 0.10
L metatarsus	1.73 ± 0.07	1.71 ± 0.04	1.76 ± 0.03	1.80 ± 0.06	1.83 ± 0.06	1.79 ± 0.06	1.75 ± 0.01	1.82 ± 0.02	1.80 ± 0.06
W metatarsus	1.00 ± 0.04	0.99 ± 0.04	0.99 ± 0.02*	1.02 ± 0.03	1.05 ± 0.04	1.02 ± 0.03	0.99 ± 0.01	1.05 ± 0.01	1.02 ± 0.03
L forewing	7.58 ± 0.15	7.36 ± 0.32	7.69 ± 0.16	7.61 ± 0.24	7.76 ± 0.09	7.50 ± 0.04	7.42 ± 0.03	7.51 ± 0.09	7.63 ± 0.29
W forewing	2.62 ± 0.05	2.50 ± 0.04	2.66 ± 0.05	2.60 ± 0.07	2.66 ± 0.04	2.57 ± 0.02	2.85 ± 0.02	2.57 ± 0.03	2.60 ± 0.08
D wax mirror	0.31 ± 0.02	0.31 ± 0.03*	0.35 ± 0.03	0.26 ± 0.03*	0.25 ± 0.03	0.23 ± 0.02	0.23 ± 0.01	0.30 ± 0.01	0.27 ± 0.03
W wax mirror	1.88 ± 0.03	1.81 ± 0.06	1.79 ± 0.04	1.86 ± 0.04	1.87 ± 0.02	1.88 ± 0.03	1.87 ± 0.007	1.85 ± 0.01	1.86 ± 0.05
L wax mirror	0.94 ± 0.03	0.87 ± 0.03	0.88 ± 0.03	0.90 ± 0.03	0.93 ± 0.03	0.91 ± 0.01	0.89 ± 0.01	0.92 ± 0.006	0.89 ± 0.03
W tomentum 1	0.35 ± 0.02	0.34 ± 0.02	0.31 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.40 ± 0.03	0.35 ± 0	0.40 ± 0.01	0.36 ± 0.03
W tomentum 2	0.82 ± 0.03	0.79 ± 0.04	0.83 ± 0.04	0.80 ± 0.04	0.83 ± 0.04	0.77 ± 0.03	0.76 ± 0.02	0.80 ± 0.003	0.81 ± 0.04
Color characters									
P tergite 2	5.73 ± 1.09	7.20 ± 0.81	7.31 ± 1.05	7.01 ± 0.84	7.13 ± 0.76	7.36 ± 1.15	6.63 ± 0.61	6.63 ± 1.18	6.96 ± 0.81
P tergite 3	6.78 ± 0.39	7.36 ± 0.35	7.18 ± 0.40	7.48 ± 0.30	7.44 ± 0.36	7.69 ± 0.26	7.2 ± 0.28	7.43 ± 0.24	7.46 ± 0.29
P tergite 4	5.87 ± 0.58	6.76 ± 0.68	5.79 ± 0.59	7.10 ± 0.30	7.04 ± 0.37	7.43 ± 0.42	7.07 ± 0.09	6.93 ± 0.09	7.05 ± 0.23
P scutellum 1	3.38 ± 0.73	6.92 ± 1.33	5.16 ± 1.21	6.86 ± 1.18	6.97 ± 0.77	7.25 ± 1.02	7.13 ± 0.57	7.8 ± 0	6.60 ± 1.37
P scutellum 2	2.74 ± 1.60	4.71 ± 1.44*	3.83 ± 0.69	4.64 ± 0.87	4.21 ± 0.52	4.67 ± 1.31	5.07 ± 0.85	5.23 ± 0.71	4.64 ± 0.85
P labrum 1	7.00 ± 0.0	7.00 ± 0.0	6.99 ± 0.03	6.99 ± 0.05**	7.00 ± 0.0	7.00 ± 0	6.87± 0.19	6.97 ± 0.05	7.00 ± 0.0
P labrum 2	7.28 ± 0.53	7.92 ± 0.20	7.24 ± 0.43	8.10 ± 0.26**	8.15 ± 0.21	8.48 ± 0.34	7.83 ± 0.14	7.9 ± 0.33	8.04 ± 0.15

Table II. (Continued).

Wing venation angles									
Angle A4	36.63 ± 0.53	34.68 ± 1.24	34.13 ± 1.12	33.37 ± 1.63*	33.32 ± 1.10	32.12 ± 0.69	35.37 ± 0.87	35.98 ± 0.42	33.23 ± 1.63
Angle B4	100.44 ±1.88	101.91 ± 1.67	103.59 ± 2.53	102.65 ± 2.58	101.80 ± 1.85	104.38 ± 1.41	101.56 ± 0.31	99.48 ± 1.61	102.87 ± 2.86
Angle D7	98.30 ± 1.30	98.03 ± 2.00	97.13 ± 1.62	96.49 ± 1.56*	97.66 ± 1.41	95.71 ± 0.94	97.72 ± 1.84	98.26 ± 0.77	96.08 ± 1.50
Angle E9	18.61 ± 0.40	18.94 ± 0.63	18.16 ± 0.80	19.17 ± 0.91***	19.15 ± 0.94	18.90 ± 0.44	17.10 ± 0.28	18.25 ± 1.05	19.56 ± 0.65
Angle G18	87.13 ± 1.31	87.58 ± 1.23	90.06 ± 1.57	91.72 ± 1.70	91.46 ± 1.36	91.71 ± 1.18	92.13 ± 1.15	88.56 ± 0. 02	92.08 ± 1.75
Angle J10	48.95 ± 0.88*	47.74 ± 2.27	54.90 ± 1.88	48.28 ± 2.09*	50.15 ± 1.00	48.84 ± 0.88	50.60 ± 0.05	47.74 ± 0.19	47.45 ± 2.23
Angle J16	106.96 ±1.62	108.1 ± 1.73*	105.03 ± 1.84	108.97 ± 2.12	109.68 ± 0.76	108.05 ± 1.27	108.42 ± 1.30	107.40 ± 1.72	109.24 ± 2.54
Angle K19	77.38 ± 0.88	76.76 ± 1.56	76.53 ± 1.60*	76.57± 1.60***	75.46 ± 1.44	74.34 ± 0.62	78.56 ± 0.10	75.94 ± 1.47	77.31 ± 1.04
Angle L13	13.48 ± 0.67	13.51 ± 0.93	15.28 ± 0.73*	13.42 ± 0.75*	12.68 ± 0.36	13.75 ± 0.37	14.01 ± 0.15	12.43 ± 0.22	13.56 ± 0.77
Angle N23	84.38 ± 1.05	83.84 ± 1.57	85.52 ± 1.55	88.01 ± 1.74	88.17 ± 1.15	88.56 ± 1.24	87.06 ± 0.14	89.29 ± 1.83	87.79 ± 2.04
Angle 026	34.13 ± 2.37	34.66 ± 2.22	33.71 ± 1.46	32.21 ± 2.51*	33.61 ± 0.79	35.22 ± 1.54	32.57 ± 1.51	31.32 ± 2.33	31.11 ± 2.38
Others									
L hair	0.17 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.13 ± 0.01	0.13 ± 0.02
Hooks	0.16 ± 0.006	0 .17 ± 0.01*	0.17 ± 0.006	0.17 ± 0.006**	0.17 ± 0.01	0.17 ± 0.001	0.18 ± 0.003	0 .18 ± 0.001	0.17 ± 0.006
L proboscis	4.53 ± 0.15	4.46 ± 0.12	4.66 ± 0.10	4.70 ± 0.18	4.79 ± 0.17	4.61 ± 0.21	4.8 ± 0. 0	4.72 ± 0.06	4.68 ± 0.18
Computed indices									
Cubital index	3.44 ± 0.17	3.28 ± 0.18	3.58 ± 0.30	4.26 ± 0.44**	4.19 ± 0.23	4.77 ± 0.21	3.68 ± 0.20	3.57 ± 0.70	4.28 ± 0.36
Body size									
(L tergite 3+4)	3.45 ± 0.07	3.36 ± 0.08	3.33 ± 0.07	3.57 ± 0.09*	3.60 ± 0.05	3.54 ± 0.06	3.40 ± 0.005	3.65 ± 0.14	3.57 ± 0.09
L complete leg	6.52 ± 0.02	6.58 ± 0.16	6.49 ± 0.01	6.83 ± 0.21	6.96 ± 0.22	6.76 ± 0.22	6.71 ± 0.09	6.97 ± 0.05	6.82 ±.23
Body size / leg	0.52 ± 0.02	0.51 ± 0.02	0.49 ± 1.14	0.52 ± 0.02	0.52 ± 0.02	0.52 ± 0.01	0.51 ± 0.006	0.52 ± 0.02	0.52 ± 0.02
L/W metatarsus	0.58 ± 0.005	0.58 ± 0.01	0.56 ± 0.01	0.57 ± 0.01*	0.57 ± 0.01	0.57 ± 0.006	0.57 ± 0.001	0.58 ± 0.001	0.57 ± 0.007
L/W sternite 6	0.81 ± 0.02*	0.81 ± 0.02	0.82 ± 0.01	0.84 ± 0.02*	0.85 ± 0.03	0.83 ± 0.01	0.80 ± 0.01	0.82 ± 0.0	0.84 ± 0.02
L/W wax mirror	0.50 ± 0.01	0.48 ± 0.01	0.49 ± 0.01*	0.48 ± 0.01*	0.49 ± 0.01	0.49 ± 0.01	0.47 ± 0.01	0.50 ± 0.001	0.48 ± 0.001
L/W forewing	0.35 ± 0.002	0.34 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.002	0.34 ± 0.002	0.35 ± 0.001	0.34 ± 0.0	0.34 ± 0.01
Tomentum index	0.43 ± 0.04	0.44 ± 0.05	0.37 ± 0.05	0.47 ± 0.06	0.45 ± 0.001	0.53 ± 0.06	0.47 ± 0.01	0.51 ± 0.01	0.46 ± 0.07

Size measurements are given in mm, angles in degrees; L: length; W: width; D: distance. Significance for inhomogeneity between sampling sites within Luzon HL, Luzon LL or Palawan) or between major collection localities in Visayas-Mindanao: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.00005$.

island with a total length of more than 400 km, although molecular evidence suggests that the group may not be completely homogeneous [4].

The bees from the other Philippine islands do not present groupings that are as distinct and separate as the Palawan group. It is, however, very apparent that the bees from the northern part, namely Luzon, differ from the bees in southern parts, i.e., Visayas and Mindanao. As factor analysis would not clearly indicate whether morphometric characters support separate groups in these areas rather than a gradual north-south cline, both possibilities were explored.

In an approach that contrasts the bees of Luzon to those of Visayas and Mindanao, both groups showed consistency in a discriminant analysis, but with some ambiguity in respect to the island of Cebu which tended to be grouped with Luzon. In a clinal approach, significant north-south and east-west trends were apparent in most of the factor scores. While the clinal pattern was also apparent within the Luzon samples alone, this was not clearly expressed within the Visayas-Mindanao samples. Owing to the paucity or lack of samples in intermediate areas it cannot be decided at this stage which model would be more adequate; this question might be resolved in the future with more samples, especially from the important islands such as Mindoro, Samar, Panay, Bohol, and from other parts of Mindanao.

The Luzon bees showed a distinct contrast between the bees from the highland (> 1 000 m) area of Baguio, and those from the lowland, south and north, which was likewise apparent from factor analysis and discriminant analysis. Again, the distinctness of the Luzon HL group was also apparent in a clinal approach, as it did not yield to this overall trend, particularly on factor 3. This was further ascertained in an ANOVA on the residuals of multiple regression analysis (on longitude and latitude), in which this was the only group found to differ significantly from other groups. After defining these two groups as separate populations, variation between sampling sites was no longer statistically different, indicating that within-Luzon variation was mainly expressed by this classification.

The Luzon HL bees were set apart predominantly by their dark color and longer hair, but also by their larger wax mirrors. While they were still smaller than the bees from Visayas-Mindanao, they were distinctly larger than the LL bees of Luzon, except for the length of the legs. The Luzon LL bees, on the other hand, were the smallest bees and occupied extreme positions in 10 out of the 19 size measurements. The elevated position of the mountain province in Luzon permits the prevalence of a temperate climate, which allows a notable amount of temperate-zone vegetation [6], making the area unique and with isolated conditions. The elevated regions in Luzon, especially in northern Luzon, are traceable to the mid-Miocene period [6]. The main characters, which are greater size compared to the neighboring low-elevation population, hairiness, shorter legs, dark color, mostly agree with the general pattern found in mountain-dwelling bees [10, 12, 13]. Without any evidence for a strong genetic contrast [4] to the surrounding population, these may be so far be considered as a typical mountain variety, which evolved due to differences in ecological conditions.

The Visayas-Mindanao group also showed distinctive morphometric features that separated them from the other groups. In particular, they were the largest of the bees, and showed the highest values in most of the size characters (10 out of 19). However, in two size measurements, cubital vein 1 and the distance between wax mirrors, they showed distinctly lower measurements. The high length-width ratio of sternite 6 characterized them as slender bees. They were further set apart by a distinctly higher cubital index, a higher wing venation angle N23 and a lower angle 026. Although this group

was fully separated from the others in the discriminant analysis, it still contained a wide range of variability and thus a potential for further substructuring. This variability was apparent from the comparatively spread-out cluster in the factor analysis, and was also reflected in the cluster tree. In particular, an ANOVA performed according to collection sites showed that 14 out of the 40 single characters were inhomogeneous, thus strongly indicating a need for more detailed studies. However, none of the main sampling sites showed a convincing distinctness, such that further identification of subpopulations was not attempted. In particular, the island structure of Visayas certainly provides an appropriate environment for the evolution of distinct local types. Among the bees in this group, the Cebu samples tended to deviate most from the general pattern and may be a likely candidate for a separate group. However, with only a few samples from only part of the islands a clarification of the relations of bee populations between the islands needs to be postponed until a more adequate database is available.

The general pattern of the biogeography of morphological differentiation of honey bees in the Philippines provided in this study coincides with and parallels the biogeographic history of some well-studied Philippine fauna [1, 6, 9]. The distinctness of the Palawan bees is an additional support to the well-documented uniqueness of the flora and fauna of the region from the rest of the Philippines, which are basically of strong Bornean influence [6, 9]. This is attributable to the fact that Palawan is part of the Asian continental shelf and, from geological evidence, was probably connected to Borneo by way of a land-bridge during the Pleistocene (Everett 1889; cited in [9]). A close relationship between *A. cerana* from Palawan and the bees of Borneo, *A. cerana indica*, has been confirmed in a recent study [2, 3], and fits well into this picture.

The rest of the Philippines was probably never a part of the Asian continental shelf. Heaney's [9] documentation of Philippine geology as determined from the works of various authors points to a tectonic origin for the Philippine mainland. Luzon is postulated to be the oldest island. Its beginnings date as far back as the late Eocene or early Oligocene as a series of small islands, achieving its present one large landmass status by the late Pliocene. The other major islands in existence at the present time achieved initial subaerial status during the lower Miocene (Mindanao), Pliocene (Leyte), early Pliocene (Mindoro), late Pliocene (Negros and Panay), and Miocene-Pliocene (Samar). The present large landmass status of these islands was mostly achieved during the Plio-Pleistocene. Based on the present 120 m bathymetric line [9], there is a strong indication for the existence of a continuous connection among the Philippine islands from Luzon to Mindanao.

The question of whether the *A. cerana* bees inhabiting the Luzon-Visayas-Mindanao islands are essentially one population which has differentiated over this 1 500 km geographical range, or whether the differences between northern and southern bees are relicts from two or even more immigration events, with possible zones of admixture, cannot be resolved at this stage. Molecular evidence points to some degree of genetic distance [4, 16], which might support such a view. This would, however, not exclude a gradual spread of *A. cerana* from Luzon over the archipelago. The history of the colonization of the Philippines (except Palawan) by *A. cerana* is still obscure, and an issue of debate. More recent morphometric studies [3] on Philippine bees collected particularly in Mindanao have indicated a close relationship between the bees of Mindanao and those described from Sulawesi as *A. nigrocinta* [8], which was also present in a mtDNA study [16]. However, evidence is not yet sufficient to reconstruct the pathways and their direction with any degree of certainty as to how *A. cerana* may have spread throughout the Malaysian-Indonesian-Philippine archipelago.

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Résumé – Variabilité morphométrique d'*Apis cerana* Fabr. aux Philippines. La diversité d'*Apis cerana* Fabr. aux Philippines a été étudiée par les méthodes morphométriques. Au total 101 échantillons d'*A. cerana* provenant de colonies sauvages et de ruches ont été prélevés dans l'ensemble de l'archipel des Philippines (Fig. 1). Les 39 caractères morphométriques recommandés par Ruttner [13] et Ruttner et al. [14] ont été mesurés : 18 caractères de taille, 11 angles de la vénation alaire, 7 caractères de pigmentation, longueur des poils, longueur du proboscis et crochets. L'analyse factorielle a montré que les abeilles de Palawan se distinguaient nettement des abeilles des autres îles de l'archipel (Figs. 2a et 2b). Les abeilles du reste de l'archipel présentaient des différences manifestes entre la région sud (Mindanao et Visayas) et la région nord (Luzon). La régression selon la latitude géographique n'a pas pu trancher définitivement s'il s'agissait là de deux groupes différents ou d'un cline géographique (Fig. 3). Dans Luzon un groupe (massif montagneux de Luzon) se distingue nettement des abeilles environnantes de moindre altitude ; c'est ce qui ressort de façon frappante de l'évolution des caractères selon un cline nord-sud. L'analyse discriminante a séparé clairement les quatre groupes ainsi obtenus. Les abeilles de Palawan se caractérisent par des ailes et des pattes relativement grandes pour une taille corporelle moyenne. Les abeilles des régions basses de Luzon sont nettement les plus petites, celles du massif montagneux de Luzon nettement plus grandes et plus foncées. Les abeilles de Visayas-Mindanao sont les plus grosses et possèdent un indice cubital nettement plus élevé (Tab. II). L'étude de la variabilité entre les lieux de prélèvement au sein de ces groupes principaux a montré que dans trois de ces groupes (régions basses de Luzon, massif montagneux de Luzon et Palawan) seuls des caractères isolés présentaient des différences significatives. Chacun de ces groupes pouvait donc être considéré comme homogène. Le troisième groupe le plus étendu (Visayas et Mindanao) a par contre présenté des différences significatives pour de nombreux caractères. Ce groupe n'est donc pas homogène et peut potentiellement être sous-divisé, mais les données issues de l'échantillonnage ne permettent pas d'aller plus loin. Les résultats de cette étude mettent en évidence de grosses différences morphologiques nettement marquées entre les populations d'*A. cerana* des différentes contrées des Philippines. Ces différences sont en accord avec l'histoire géologique de l'archipel.

***Apis cerana* / Philippines / morphométrie / biogéographie**

Zusammenfassung – Morphometrische Variabilität von *Apis cerana* Fabr. innerhalb der Philippinen. Die Diversität von *A. cerana* Fabr. in den Philippinen wurde mit morphometrischen Methoden untersucht. Insgesamt wurden über das ganze Inselreich 101 Proben von wildlebenden oder in Beuten gehaltenen *A. cerana* gesammelt (Abb. 1). Die von Ruttner [13] und Ruttner et al. [14] empfohlenen 39 morphometrischen Charaktere (18 Größenmessungen, 11 Flügeladerungswinkel, 7 Farbcharaktere, Haarlänge, Rüssellänge und Häkchen) wurden vermessen. Die Faktorenanalyse zeigte, dass die Bienen von Palawan sich deutlich von den übrigen Bienen des philippinischen Archipels unterschieden (Abb. 2a und 2b). Die Bienen des übrigen Archipels zeigten klare Unterschiede zwischen dem südlichen (Mindanao und Visayas) und nördlichen Bereich (Luzon).

Die Regression auf die geographische Breite konnte allerdings nicht endgültig klären, ob es sich hierbei um zwei unterschiedliche Gruppen oder eine geographische Kline handelt (Abb. 3). Innerhalb Luzon unterschied sich eine Gruppe (Luzon Hochland) deutlich von den umliegenden Bienen aus geringerer Höhe, diese stach insbesondere aus dem Nord-Südklinalen Merkmalsverlauf hervor. Die hieraus ermittelten vier Hauptgruppen waren anhand einer Diskriminanzanalyse klar gegeneinander abgegrenzt. Die Bienen von Palawan waren durch verhältnismäβig lange Flügel und Beine bei sonst mittlerer Größe gekennzeichnet. Die Bienen von Luzon Tiefland waren deutlich am kleinsten, die des Hochlandes von Luzon deutlich größer und von dunklerer Farbe. Die Bienen von Visayas-Mindanao waren die größten und hatten einen deutlich höheren Cubitalindex (Tab. II). Die Untersuchung der Variabilität zwischen Sammelorten innerhalb dieser Hauptgruppen zeigte, dass drei von diesen (Luzon Tiefland, Luzon Hochland und Palawan) nur in vereinzelten Merkmalen signifikante Unterschiede zeigten und diese Gruppen daher als in sich homogen angesehen werden können. Die dritte ausgedehnteste Gruppe (Visayas und Mindanao) zeigte dagegen in vielen der Merkmale signifikante Unterschiede. Diese Gruppe ist daher in sich inhomogen, dies legt eine weitere Unterstrukturierung nahe. Für eine verbindliche Festlegung reichen allerdings die vorliegenden Daten mit oft nur einzelnen Proben insbesondere von den verschiedenen Inseln von Visayas nicht aus. Das Ergebnis der Untersuchung belegte damit deutliche großräumige morphologische Unterschiede zwischen *A. cerana* aus verschiedenen Gebieten der Philippinen. Diese stehen mit der geologischen Geschichte des Inselreiches in Übereintimmung.

***Apis cerana* / Philippinen / Morphometrie / Biogeographie**

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