Apidologie (1995) 26, 387-394

Original article

A morphological comparison of the dwarf honey bees of southeastern Thailand and Palawan, Philippines

TE Rinderer [1], BP Oldroyd [1], S Wongsiri [2], HA Sylvester [1], LI de Guzman [3], JA Stelzer [1], RM Riggio [3]

[1] *USDA, Agricultural Research Service, Honey-Bee Breeding, Genetics and Physiology Research Laboratory, 1157, Ben Hur Rd, Baton Rouge, LA 70820 USA;*
[2] *Bee Biology Research Unit, Department of Biology, Chulalongkorn University, Bangkok 10330, Thailand;*
[3] *Dept of Entomology, Louisiana State University, Baton Rouge, LA 70893, USA*

(Received 20 February 1995; accepted 11 April 1995)

Summary — Morphological descriptions using measurements common to honey bee taxonomy are provided for *Apis andreniformis* Smith (1858) from southeastern Thailand and Palawan, Philippines and *Apis florea* Fabricius (1787) from southeastern Thailand. Overall, *A andreniformis* has a very different morphology from the sympatric *A florea* and from all other well-described species of honey bees. One difference, the color of the scutellum, although not species specific, is sufficient in many cases to facilitate field identifications. Very few morphological differences occurred between the Thai and the Philippine populations of *A andreniformis.*

Asian bees / *Apis andreniformis* / *Apis florea* / morphometry / population genetics / Thailand / Philippines

INTRODUCTION

The small dwarf honey bee, *Apis andreniformis* Smith (1858) has recently been re-evaluated and recognized as a valid biological species based on morphological evidence of a unique endophallus, characteristic worker bee wing venation, and a distinctive furcation of the male basitarsus (Wongsiri *et al,* 1990). The Wongsiri *et al* study supported the conclusions of Wu and Kuang (1986, 1987) who studied furcated basitarsus differences between drones of *A florea* Fabricius (1787) and *A andreniformis.* A species-specific characteristic of *A andreniformis* identified by Smith (1858) of worker bees having black hairs on the hind tibia and dorsolateral surface of the hind basitarsus as opposed to the white hairs of *A florea* was confirmed by these studies.

Further confirmation of the valid biological species status of *A andreniformis* was provided by Rinderer *et al* (1993a) who reported that the mating flights of drones from sympatric *A andreniformis* and *A florea* were temporally separate. While these reports clearly show *A andreniformis* to be a separate species, they do not describe all the morphological measurements commonly used in modern honey bee taxonomy (Ruttner, 1987). This paper provides these morphometric details for 2 populations of *A andreniformis* occurring in southeastern Thailand and Palawan, Philippines (de Guzman *et al,* 1992).

Since *A andreniformis* is most similar to *A florea* and since little is known of the interspecies morphological variation of either species, comparative data for the sympatric population of *A florea* in Thailand are also provided.

MATERIALS AND METHODS

Twenty-seven colonies of *A florea* and 18 colonies *A andreniformis* were sampled in Thailand where the 2 species are sympatric. Four colonies of *A andreniformis* were sampled from Palawan, Philippines, an island which is outside the distribution of *A florea.* Ten workers from each colony were dissected and 44 morphometric measurements were made using computer-assisted techniques (Daly *et al,* 1982; Rinderer *et al,* 1993b). These measurements and their alphabetical designations are given in table I and follow the system of morphometric analysis described by Ruttner *et al* (1978), Ruttner (1987) and Rinderer *et al* (1989). Numerical designations for wing venation angles (fig 1) are a combination of the angles studied by Ruttner *et al* (1978) and Daly and Balling (1978) and follow the designations of Rinderer *et al* (1989). For the most part, numbers represent lengths or widths of various structures and are reported in millimeters. Interior angles of vein intersections in wing venation patterns are reported in degrees. Pigmentation characteristics of the second, third and fourth tergites, the scutellum, the metanotum and the mesonotum are scored according to the procedures of Ruttner *et al* (1978) and Ruttner (1987). Scores for tergite color intensity range from 0 to 10 with 0 being completely black and 10 being completely yellow. Scutellum scores range from 0 to 9 with 0 being completely black and 9 being completely yellow. Scores for the color intensity of the metanotum (B) and the mesonotum (K) range from 0 to 5 with completely black being 0 and completely yellow being 5.

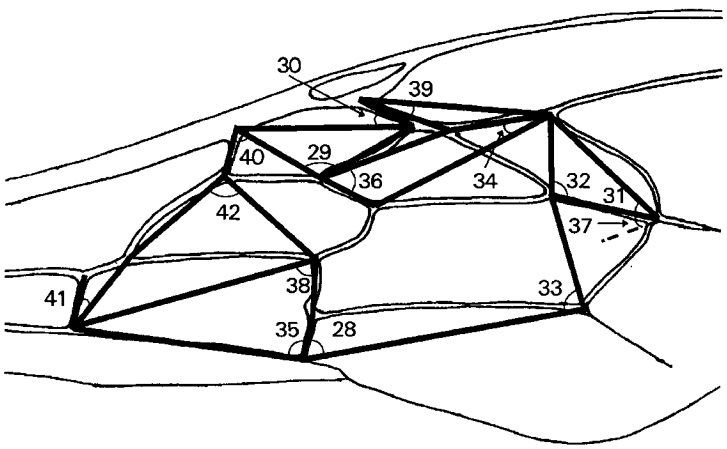

Fig 1. Wing venation measured for *A andreniformis* and *A florea.* The 15 angles are indicated on a representation of the wing of *A andreniformis* and follow a unique numbering system combining the measurements of Ruttner *et al* (1978), Daly and Balling (1978) and Rinderer *et al* (1989).

Table I. Descriptive statistics (± *STD*) and *t*-test probabilities for equality of means of 44 morphometric characteristics of 10 worker bees each from 42 colonies of *A florea (Af)* and 36 colonies of *A andreniformis* from Thailand (*Aa*T) and 4 colonies of *A andreniformis* from Palawan, Philippines (*Aa*P).

Character	A andreniformis		A florea	*Probability of*	
	Philippines	*Thailand*		Aa = Aa*T*	Aa*T*= Af
Proboscis (P)	2.797 ± 0.09	2.798 ± 0.12	3.273 ± 0.18	0.9220	0.0000
Femur length (Fe)	1.703 ± 0.04	1.704 ± 0.035	1.757 ± 0.05	0.9388	0.0000
Tibia length (Ti)	2.120 ± 0.05	2.121 ± 0.05	2.218 ± 0.07	0.9035	0.0000
Basitarsus length (M_L)	1.285 ± 0.03	1.276 ± 0.03	1.338 ± 0.05	0.5052	0.0000
Basitarsus width (M_T)	0.630 ± 0.02	0.610 ± 0.02	0.634 ± 0.03	0.0022	0.0000
Tergite 3 length (T_3)	1.327 ± 0.04	1.329 ± 0.05	1.406 ± 0.05	0.9082	0.0000
Tergite 4 length (T_4)	1.284 ± 0.06	1.281 ± 0.05	1.359 ± 0.06	0.8478	0.0000
Sternite 3 length (S_3)	1.689 ± 0.06	1.696 ± 0.05	1.817 ± 0.06	0.7179	0.0000
Wax mirror length (W_L)	0.655 ± 0.03	0.649 ± 0.04	0.776 ± 0.04	0.6667	0.0000
Wax mirror width A (W_T)	1.318 ± 0.03	1.330 ± 0.04	1.417 ± 0.05	0.4259	0.0000
Wax mirror width B (W_D)	0.054 ± 0.02	0.045 ± 0.02	0.065 ± 0.03	0.2526	0.0001
Sternite 6 length (L_6)	1.339 ± 0.04	1.352 ± 0.04	1.452 ± 0.04	0.3290	0.0000
Sternite 6 width (T_6)	1.721 ± 0.04	1.702 ± 0.06	1.754 ± 0.06	0.3731	0.0002
Forewing length (F_L)	6.435 ± 0.12	6.493 ± 0.05	6.454 ± 0.16	0.3165	0.2186
Forewing width (F_B)	2.167 ± 0.05	2.213 ± 0.06	2.254 ± 0.06	0.0332	0.0018
Angle 20	62.95 ± 3.16	61.25 ± 3.27	57.096 ± 3.57	0.0639	0.0000
Angle 29	121.79 ± 2.38	121.78 ± 3.42	119.591 ± 5.23	0.9631	0.0008
Angle 30	34.46 ± 2.48	34.35 ± 3.29	42.205 ± 5.02	0.8347	0.0000
Angle 31	31.38 ± 2.40	31.79 ± 2.05	32.929 ± 2.42	0.4028	0.0012
Angle 32	104.57 ± 5.33	101.64 ± 5.06	94.35 ± 5.83	0.0290	0.0000
Angle 33	83.02 ± 3.19	81.581 ± 3.07	87.36 ± 3.54	0.1509	0.0000
Angle 34	19.921 ± 1.52	20.565 ± 1.47	19.67 ± 2.04	0.1639	0.0037
Angle 35	98.51 ± 3.33	100.07 ± 3.63	102.16 ± 4.18	0.1741	0.0005
Angle 36	42.07 ± 2.54	42.40 ± 2.50	40.94 ± 2.87	0.6503	0.0004
Angle 37	30.40 ± 4.04	31.48 ± 4.03	28.03 ± 3.66	0.2341	0.0000
Angle 38	76.24 ± 2.41	72.49 ± 2.67	70.50 ± 3.06	0.0000	0.0000
Angle 39	13.81 ± 1.20	14.27 ± 1.32	15.85 ± 1.99	0.2081	0.0000
Angle 40	72.97 ± 3.18	75.25 ± 3.55	76.98 ± 4.78	0.117	0.0010
Angle 41	26.69 ± 2.95	26.25 ± 2.63	26.38 ± 3.42	0.4169	0.8038
Angle 42	99.84 ± 1.96	97.68 ± 2.55	102.05 ± 3.40	0.0123	0.0000
Cubital A (a)	0.506 ± 0.036	0.525 ± 0.033	0.493 ± 0.04	0.0901	0.0000
Cubital B (b)	0.084 ± 0.02	0.090 ± 0.02	0.176 ± 0.03	0.3073	0.0000
Cubital index (a/b)	6.378 ± 1.672	6.282 ± 1.793	2.864 ± 0.552	0.7830	0.0000
Hamuli number	10.88 ± 1.07	10.38 ± 0.92	11.10 ± 0.93	0.0372	0.0000
Hindwing length (H_L)	3.22 ± 0.07	3.23 ± 0.09	3.17 ± 0.08	0.7522	0.0011
Hindwing width (H_W)	1.25 ± 0.04	1.28 ± 0.04	1.36 ± 0.04	0.0440	0.0000
Tomentum A	0.387 ± 0.044	0.392 ± 0.064	0.399 ± 0.159	0.8592	0.8155
Tomentum B	0.667 ± 0.054	0.660 ± 0.062	0.723 ± 0.166	0.7986	0.0990
Pigmentation tergite 2	6.60 ± 1.65	5.85 ± 1.37	9.99 ± 0.12	0.1959	0.0000
Pigmentation tergite 3	7.75 ± 0.776	7.234 ± 0.546	8.774 ± 0.419	0.0295	0.0000
Pigmentation tergite 4	7.650 ± 0.770	7.154 ± 0.407	5.111 ± 1.402	0.0158	0.0000
Scutellum pigmentation (Sc)	5.375 ± 2.789	3.406 ± 3.137	0.196 ± 0.398	0.1154	0.0000
Metanotum pigmentation (B)	1.525 ± 2.050	0.491 ± 1.304	0.256 ± 0.437	0.0176	0.0879
Mesonotum pigmentation (K)	0.575 ± 1.196	0.046 ± 0.209	0.063 ± 0.243	0.0001	0.6985

After measurements were made on individual bees, colony averages for each characteristic were calculated. These averages were used to calculate means, variances and ranges for each measurement within each species (table I). The colony averages were also used to calculate *t*-test evaluations of differences between *A florea* and *A andreniformis* from Thailand and between *A andreniformis* from Thailand and the Philippines. A multivariate discriminant analysis was also conducted to estimate Mahalanobis distances between population centroids.

RESULTS

In general, *A florea* from Thailand are larger than sympatric *A andreniformis*. Of the 17 measurements of length or width involving wings, legs, mouthparts and sternites, in only 2 cases was *A andreniformis* equal to or greater than *A florea*. Interestingly, both these cases involved wing length: forewing lengths were about equal and hindwing lengths of *A andreniformis* were larger.

The 2 species differed in all the angles of wing vein intersection save number 40. Cubital segment A was larger in *A andreniformis* and cubital segment B was larger in *A florea*. Consequently, the cubital index of *A andreniformis* (6.37) was much larger than that of *A florea* (2.86).

In general, *A florea* had less black pigment on the various structures measured in congruence with the general visual impression that *A florea* are mostly yellow bees and *A andreniformis* are mostly black bees. A notable exception to this rule is the pigmentation of the scutellum. Scutellum color for *A andreniformis* tends toward yellow while the scutellum color for *A florea* tends toward black. The range of *A andreniformis* scutellum coloration does extend into the black range. However, the range of scutellum coloration for *A florea* of our sample does not include yellow. Thus, this highly visible character may be useful for field identifications of *A andreniformis*.

The 2 populations of *A andreniformis* differed for only a few characters. The Thai *A andreniformis* had longer forewings and longer hindwings, but fewer hamuli. Wing length differences with similar wing widths probably led to the 3 observed significant differences in wing angles. Thai *A andreniformis* also had significantly narrower metatarsi and a scutellum which was, on average, lighter than that of both *A florea* and *A andreniformis* from the Philippines.

A multivariate discriminant analysis of data summarizes the population differences. A Mahalanobis difference of 110 252 separates Thai *A andreniformis* from Thai *A florea* while a Mahalanobis difference of 2 891 separates Thai *A andreniformis* from Philippine *A andreniformis* (fig 2).

DISCUSSION

Overall, *A andreniformis* has a very different morphology from the sympatric *A florea*. The color of the scutellum appears to be a useful field character to identifying *A andreniformis*. Additionally, the remainder of the measurements provide a picture of *A andreniformis* as a remarkably different honey bee, unique in its morphological organization.

The differences between the Thai and the Philippine populations of *A andreniformis* are rather small considering their geographical separation. Similar distances with geographical isolation result in strong subspecific variation in *A mellifera* (Ruttner, 1987). However, the differences between the Thai and Philippine populations of *A andreniformis* are less than differences typically used to support subspecific designations. But the 2 groups show no overlap in the multivariate discriminant analysis procedures with each group forming a tight grouping about its centroid. If more samples confirmed these observations, the lack

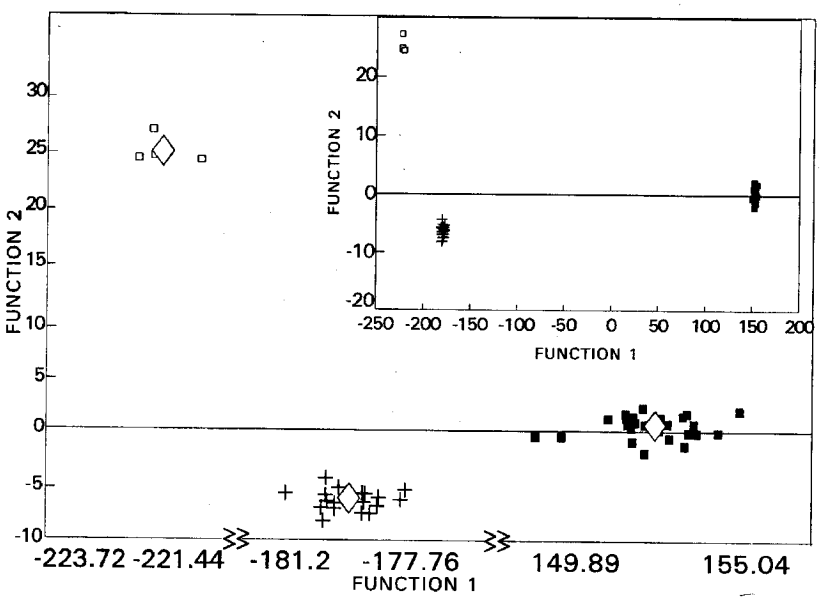

Fig 2. Scatterplot of the results of the multivariate discriminant function analyses of *A andreniformis* and *A florea:* each + represents an *A andreniformis* colony from Thailand (*Aa*T), each □ represents an *A andreniformis* colony from Palawan, Philippines (*Aa*P) and each ■ represents an *A florea* colony from Thailand *(Af).* Due to a singular matrix, scutellum color was not used in the analysis. The centroid for each group is marked by a ◊. Using a pooled covariance matrix, the Mahalanobis distances between centroids are: *Aa*T to *Af* = 110 252, *Aa*P to *Af* = 141 585 and *Aa*T to *Aa*P = 2 891. The larger figure shows a plot with an interrupted scale to display intra-group variation along the first discriminant function. The uninterrupted scale in the insert displays the large inter-group variation.

of overlap might be interpreted as indicating subspecific differences (Ruttner, 1987).

Much remains to be learned about *A andreniformis.* Its historical confusion with *A florea* has probably resulted in many published studies on what was actually *A andreniformis* being reported as information on *A florea.* Clear information on the ranges of these 2 species will provide a guide as to which literature resulted from studies of bees in areas of sympatry and needs to be considered suspect. The dwarf honey bees represent great potential for comparative studies in honey bee biology and, indeed, have already provided some interesting insight into the evolutionary development of honey bee characteristics (Rinderer *et al,* 1992, 1993a).

ACKNOWLEDGMENT

This study was made in cooperation with Louisiana Agricultural Experiment Station.

Résumé — Comparaison morphologique des abeilles naines du sud-est de la Thaïlande et de l'île de Palaouan (Philippines). La petite abeille naine, *Apis andreniformis* Smith (1858), a été récemment confirmée comme étant une véritable espèce biolo-

gique (Wongsiri *et al,* 1990). Bien que cette publication, ainsi que d'autres (Wu et Kuang, 1986, 1987; Rinderer *et al,* 1993a), le démontrent clairement, ces travaux étaient incomplets puisqu'ils n'incluaient pas toutes les mesures morphologiques habituellement utilisées en taxonomie moderne (Ruttner, 1987). Ces mesures sont présentées dans le présent travail, elles concernent 2 populations d'*A andreniformis* du sud-est de la Thailande et de l'ile de Palaouan (Philippines) (de Guzman *et al,* 1992).

Vingt-sept colonies d'*A florea* et 18 colonies d'*A andreniformis* ont été échantillonnées en Thaïlande où les 2 colonies sont sympatriques. Quatre colonies d'*A andreniformis* ont été échantillonnées à Palaouan, une île qui est allopatrique pour *A florea.* Dix ouvrières de chaque colonie ont été disséquées et 44 mesures morphométriques ont été réalisées en utilisant une méthode de mesures assistée par ordinateur (Daly *et al,* 1982 ; Rinderer *et al,* 1993b). Ces mesures et leurs désignations alphabétiques présentées dans le tableau I suivent la méthode d'analyses morphométriques décrite par Ruttner *et al* (1978), Ruttner (1987) et Rinderer *et al* (1989). Après que les mesures ont été réalisées sur les abeilles individuelles, les moyennes des colonies pour chaque caractéristique ont été calculées, ainsi que les moyennes, les variances et les écarts pour chaque espèce (tableau I). Les moyennes par colonie ont également été utilisées pour réaliser le test t sur les différences entre les colonies d'*A florea* et d'*A andreniformis* de Thaïlande et entre les colonies d'*A andreniformis* des Philippines.

En général, *A florea* est, en Thaïlande, plus grande qu'*A andreniformis.* Ces 2 espèces diffèrent dans tous les angles d'intersection des veines des ailes, sauf une. Le segment cubital A est plus grand chez *A andreniformis* et le segment cubital B chez *A florea.* Par conséquent, l'index cubital d'*A andreniformis* (6,37) est beaucoup plus grand que celui d'*A florea* (2,86). *A florea* a moins de pigment noir, ce qui est conforme à l'impression générale qu'*A florea* est principalement une abeille jaune alors qu'*A andreniformis* est principalement une abeille noire.

Les différences entre les populations d'*A andreniformis* de Thaïlande et des Philippines sont plutôt petites malgré leur séparation géographique. Cependant, les 2 groupes ne présentent pas de recouvrement dans l'analyse discriminante multivariée, et chaque groupe forme un groupement serré autour de son centroïde. Le manque de recouvrement peut être interprété comme indiquant des différences sous-spécifiques (Ruttner, 1987).

Par dessus tout, *A andreniformis* présente une morphologie très différente d'*A florea* sympatrique. La couleur du scutellum apparaît être un caractère très utile dans la nature pour identifier cette espèce. De plus, l'ensemble des mesures montre qu'*A andreniformis* est une espèce d'abeille remarquablement différente des autres, et unique dans son organisation morphologique.

abeille asiatique / *Apis andreniformis* / *Apis florea* / morphométrie / génétique des populations / Thaïlande / Philippines

Zusammenfassung — Ein morphologischer Vergleich der Zwerghonigbienen von Südost-Thailand und Palawan, Phillipinen. Die Zwerghonigbiene, *Apis andreniformis* Smith (1858) wurde erst kürzlich erneut kritisch untersucht und als eigene biologische Art anerkannt (Wongsiri *et al,* 1990). Diese und andere Arbeiten über *A andreniformis* (Wu and Kuang, 1986, 1987; Rinderer *et al,* 1993a) zeigen deutlich, daß es sich wirklich um eine Art handelt. Sie sind dennoch unvollständig, da sie nicht alle morphologischen Eigenschaften beschreiben, die in der modernen Taxonomie der Honigbienen im allgemeinen angegeben werden (Ruttner, 1987). Die jetzige Veröffentlichung

beschreibt diese morphologischen Einzelheiten für 2 Populationen von *A andreniformis* aus Südost-Thailand und Palawan von den Philippinen (de Guzman *et al,* 1993).

Siebenundzwanzig Völker von *Apis florea* Fabricius (1787) und 18 *A andreniformis* Völker wurden in Thailand gesammelt, wo beide Arten sympatrisch vorkommen. Vier *A andreniformis* Völker stammen von Palawan, einer Insel auf der es keine *A florea* gibt. Von jedem Volk wurden 10 Arbeiterinnen präpariert und 44 morphometrische Daten mit einer Computer unterstützten Technik (Daly *et al,* 1982; Rinderer *et al,* 1993b) erhoben. Diese Meßdaten und ihre alphabetische Einordnung sind in Tabelle I wiedergegeben, die auf dem System der morphometrischen Analyse von Ruttner *et al* (1978), Ruttner (1987) und Rinderer *et al* (1989) beruhen.

Nach Messungen der Einzelbienen wurde der Volksdurchschnitt für jede Eigenschaft berechnet. Diese Durchschnittswerte wurden benutzt, um Mittelwerte, Varianzen und Bereich für jeden Meßwert innerhalb jeder Art zu berechnen (Tabelle I). Die Unterschiede der Volksmittelwerte zwischen *A florea* und *A andreniformis* in Thailand und zwischen *A andreniformis* von Thailand und von den Philippinen wurden mit dem *t*-Test bestimmt.

A florea in Thailand ist insgesamt größer als die sympatrische *A andreniformis*. Die beiden Arten unterschieden sich in allen Winkeln des Flügelgeäders bis auf einen. Das Cubitalsegment A war größer in *A andreniformis* und das Cubitalsegment B war größer in *A florea*. Entsprechend war der Cubitalindex von *A andreniformis* mit 6,37 viel größer als der von *A florea* mit 2,86.

A florea hat weniger schwarzes Pigment. Das stimmt mit dem Eindruck überein, daß es sich bei *A florea* um überwiegend gelbliche und bei *A andreniformis* um überwiegend dunkle Bienen handelt. Eine auffällige Ausnahme zu dieser Regel ist die Färbung des Scutellums. Die Farbe des Scuttellums tendiert bei *A andreniformis* mit einigen Ausnahmen zum gelblichen, während es bei *A florea* zu dunkler Farbe tendiert.

Die Unterschiede zwischen der Thai- und der philippinischen Population sind recht gering, wenn man die geographische Entfernung bedenkt. Beide Gruppen zeigen jedoch keine Überlappung bei einer multivariaten Diskriminanzanalyse. Jede Gruppe bildet eine enge Gruppierung um ein Centroid. Das Fehlen einer Überlappung kann als rassenspezifische Unterschiede gedeutet werden (Ruttner, 1987).

Insgesamt sind *A andreniformis* und die sympatrische *A florea* morphologisch sehr unterschiedlich. Die Farbe des Scutellums scheint eine nützliche Eigenschaft für eine Feldbestimmung zu sein. Die übrigen Messungen zeichnen das Bild einer erstaunlich unterschiedlichen Honigbienenart, die in ihrer morphologischen Organisation einzigartig ist.

Asiatische Bienen / *Apis andreniformis* / *Apis florea* / Morphometrie / Populationsgenetik / Thailand / Philippinen

REFERENCES

Daly HV, Balling SS (1978) Identification of Africanized honey bees in the western hemisphere by discriminant analysis. *J Kans Entomol Soc* 51, 857-869

Daly HV, Hoelmer K, Norman P, Allen T (1982) Computer-assisted measurement and identification of honey bees (Hymenoptera: Apidae). *Ann Entomol Soc* 75, 591-594

Fabricius JC (1787) *Mantissa Insectorum.* Vol I Proft, Hafniae

Guzman de LI, Forbes M, Cervancia C, Rinderer TE, Somera S Jr (1992) *Apis andreniformis* Smith in Palawan, Philippines. *J Apic Res* 31, 111

Rinderer TE, Koeniger N, Tingek S, Mardan M, Koeniger G (1989) A morphological comparison of the cavity dwelling honeybees of Borneo *Apis koschevnikovi* (Buttel-Reepen, 1906) and *Apis cerana* (Fabricius, 1793). *Apidologie* 20, 405-411

Rinderer TE, Oldroyd BP, Wongsiri S, Guzman de LI, Sylvester HA (1992) Evolution of bee dances. *Nature (Lond)* 306, 305

Rinderer TE, Oldroyd BP, Wongsiri S *et al* (1993a) Time of drone flight in 4 honey bee species in southeastern Thailand. *J Apic Res* 32, 27-33

Rinderer TE, Buco SM, Rubink WL *et al* (1993b) Morphometric identification of Africanized and European honey bees using large reference populations. *Apidologie* 24, 569-585

Ruttner F (1987) *Biogeography and Taxonomy of the Honeybees.* Springer Verlag, Berlin, 284 p

Ruttner F, Tassencourt L, Louveaux J (1978) Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. *Apidologie* 9, 363-381

Smith F (1858) Catalogue of the Hymenopterous insects collected at Sarawak, Borneo, Mount Ophir, Malacca, and at Singapore, by AR Wallace. *J Prov Linn Soc Lond* 2, 42-130

Wongsiri S, Limbipichai K, Tangkanasing P *et al* (1990) Evidence of reproductive isolation confirms that *Apis andreniformis* Smith (1858) is a separate species from sympatric *Apis florea* (Fabricius, 1787). *Apidologie* 21, 47-52

Wu Y, Kuang B (1986) A study of the genus *Micrapis* (Apidae). *Zool Res* 7, 99-102; *In: Chinese Bee World* 68, 153-155 (a translation of *Zool Res* 7, 99-10)