

## Evidence of reproductive isolation confirms that *Apis andreniformis* (Smith, 1858) is a separate species from sympatric *Apis florea* (Fabricius, 1787)

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**Summary** — The species *Apis andreniformis* (Smith, 1858), the small dwarf honey bee of South-east Asia, is recognized as a valid biological species. This recognition is based on distinctive endophallus characteristics in comparison with sympatric *Apis florea* (Fabricius, 1787). Additionally, scanning electron microscope images of drone basitarsi are presented, as are preliminary comparisons of wing venation.

*Apis florea* / *Apis andreniformis* / taxonomy / reproductive isolation

### INTRODUCTION

In 1984, our group collected dwarf honey bees in Thailand in the province of Chantaburi near the border with Kampuchea. Laboratory examinations of worker bees from these collections revealed that some bees had the species specific characteristics of *Apis andreniformis* (Smith, 1858) and that others had the species specific

characteristics of *Apis florea* (Fabricius, 1787) that are reported for worker bees (Maa, 1953).

Wu and Kuang (1986, 1987) reported that secondary sex characteristics differed between drones of *A. florea* and *A. andreniformis*. Specifically, both have a furcated basitarsus, presumably modified to grasp queens during mating (see Ruttner, 1988). The furcated basitarsus is quite different in

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the 2 groups. Following the report of Wu and Kuang, we re-examined the sympatric populations in 4 Thai provinces, as well as bees from East and West Malaysia. Drones and workers were collected in 1988 from nests of both *A florea* and *A andreniformis* in the Thai provinces of Chantaburi (Southeastern), Uthaitanee (Western), Chiang Rai (Northern) and Chumporn (Southern). *A andreniformis* was collected in 1989 in Malaysia.

Comparative studies of endophalli revealed evidence of reproductive isolation between the 2 groups, and hence confirmed the biological validity of naming 2 species of dwarf bees. In addition, drone hind legs were examined using electron microscopy, and worker bee wing venation was studied.

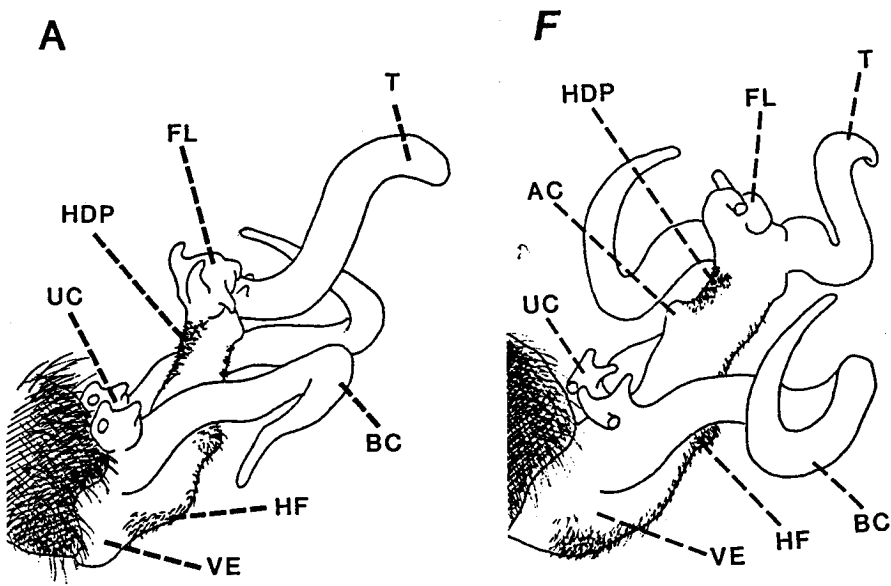
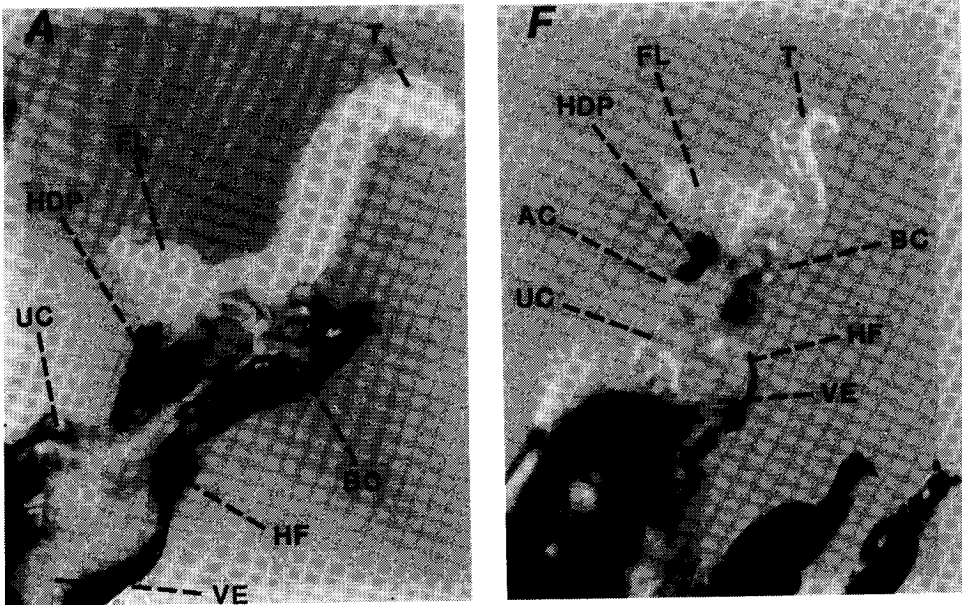
### ***Species-specific characteristics***

Comparisons of the endophalli of *A andreniformis* and *A florea* reveal numerous structural characteristics which assure complete reproductive isolation between these species (fig 1). A full or stage 9 eversion (Woyke and Ruttner, 1958; Ruttner *et al*, 1973) of an *A andreniformis* endophallus has a pair of bursal cornua that fold forward once near their end; a pair of upper cornua at the sides of the vestibulum, each of which has small protrusions; a hairy field on the ventral surface that has 4 separate hairy patches distal to the primary hairy area near the vestibulum; an indented triangular hairy patch on the dorsal surface proximal to a fimbriate lobe; a fimbriate lobe having 6 small protrusions; and a comparatively thick and straight terminal portion extending distal to the fimbriate lobe.

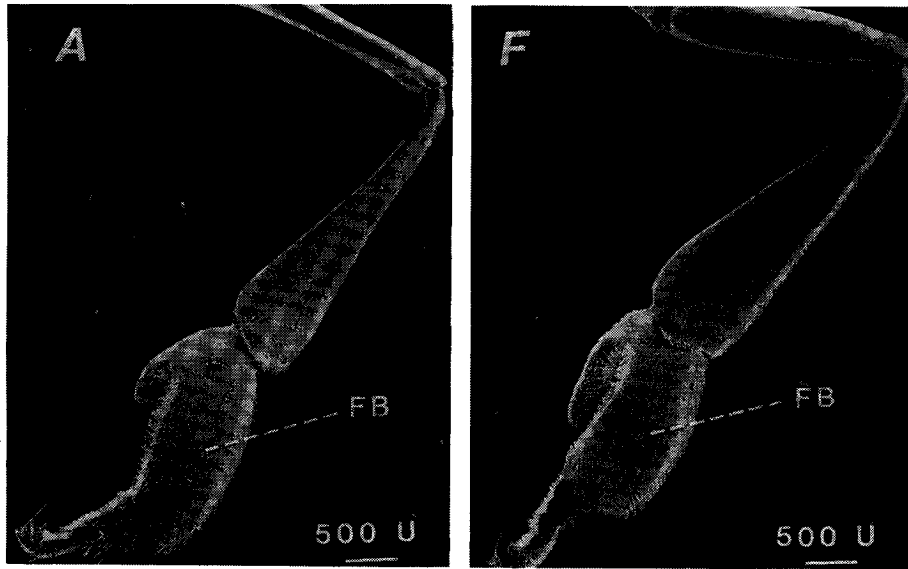
In contrast, a full stage 9 eversion of an *A florea* endophallus has a pair of bursal cornua that coil or generally fold twice, a pair of upper cornua at the sides of the vestibulum, each of which is composed of 3 joined protrusions; a continuous, non-indented hairy field on the ventral surface extending from the vestibulum; a small protrusion proximal to a small dorsal hairy patch; a fimbriate lobe having 3 protrusions and a comparatively thin and strongly curved terminal portion extending distal to the fimbriate lobe.

Figure 1 illustrates these characters. The drawings are based on direct observations of several specimens as well as the accompanying photographs.

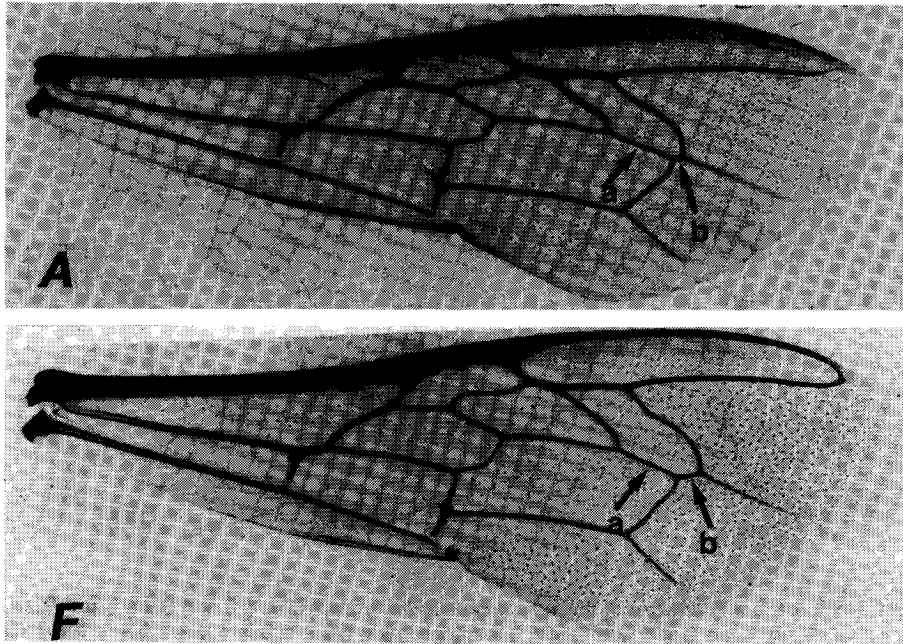
Figure 2 shows photographs of the basitarsal extensions described by Wu and Kuang (1986, 1987). Quite clearly, the thumb-like extension is comparatively short in *A andreniformis*, and was absent from the only drone of *A andreniformis* examined from Sabah, Malaysia. This extension presumably provides a "clasper organ" that fits on the queen's hind tibia during mating (Ruttner, 1975). However, whether or not this description of function is accurate awaits confirmation from observations of matings. The wing venation of *Apis andreniformis* workers is more similar to that of *Apis dorsata* and *Apis koschevnikovi* than it is to *Apis cerana* or *Apis florea*. The cubital index of *Apis andreniformis* is large ( $\bar{x}$  for colonies = 6.07,  $N = 11$  colonies, 10 worker bees each; ranges = 5.03 to 9.21 for colony averages and 3.5 to 13.8 for individual bees) while the cubital index of *A florea* is comparatively small ( $\bar{x}$  for colonies = 2.78,  $N = 6$  colonies, 10 worker bees each; ranges = 2.53 to 3.14 for colony averages and 1.93 to 5.27 for individual bees).



**Fig 1.** Photograph and drawing of the *Apis andreniformis* endophallus (A) and of the *Apis florea* endophallus (F). AC = additional cornua, BC = bursal cornua; FL = fimbriate lobe, HDP = hairy dorsal plate, HF = hairy field, T = terminal portion of endophallus, UC = upper cornua, VE = vestibulum of endophallus.



**Fig 2.** The hind legs of an *Apis andreniformis* drone (A) and an *Apis florea* drone (F). These electron micrographs show the short and long basitarsal extensions of males of the two species of dwarf bees.



**Fig 3.** The fore-wings of workers of *Apis andreniformis* (A) and *Apis florea* (F) showing marked differences in cubital index, the ratio of the length of vein segments a/b.

## CONCLUSION

*Apis andreniformis* (Smith, 1858) is a valid biological species which is found in south-east Asia and is sympatric with *Apis florea* in much of Thailand. It is also found in the Southern China peninsula, Malaysia and Borneo but its exact distribution and sympatry with *Apis florea* are unknown. Equally unknown are most comparative aspects of *A andreniformis* biology. Much that was published concerning *A florea* may be *A andreniformis* biology. Extensive comparative studies are required to describe and properly attribute known dwarf honey bee biology to the correct species and discover what is unique to each species.

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**Résumé — La preuve de l'isolement sexuel confirme qu'*Apis andreniformis* (Smith, 1858) et *Apis florea* (Fabricius, 1787) sont deux espèces sympatriques séparées.** *Apis andreniformis* (Smith, 1858) et *Apis florea* (Fabricius, 1787) ont été trouvées en position de sympatrie dans les régions thaïlandaises suivantes : Chantaburi, Uthaitanee, Chiang Rai et Chumporn. L'examen de l'endophallus des mâles de ces abeilles naines a révélé des différences dans presque tous les détails de structure (fig 1) : dans les cornules ven-

trales (BC) et dorsales (UC), dans la surface pileuse ventrale (HF) et dorsale (HDP), dans le lobe feuilleté (FL) et dans la longueur et la forme de la partie terminale qui s'étend après le lobe feuilleté (T). Ces différences sont la preuve de l'isolement reproductif des deux espèces sympatriques et donc, la preuve qu'il s'agit bien dans le cas présent de véritables espèces. Les endophallus d'*A andreniformis* de Thaïlande et de Malaisie sont semblables.

Sont jointes en outre des photos au microscope électronique de l'extension basitarsale caractéristique des pattes antérieures des mâles des deux espèces (fig 2). Les photographies de l'aile antérieure des 2 espèces montrent clairement l'index cubital élevé, caractéristique d'*A andreniformis* ( $x = 6,07$ ) et celui petit, caractéristique d'*A florea* ( $x = 2,78$ ) (fig 3).

La répartition précise d'*A andreniformis* aussi bien que sa biologie sont encore inconnues. Des études comparatives sont nécessaires, car dans le passé certains caractères d'*A andreniformis* ont certainement été attribués à tort à *A florea*.

## ***Apis florea* / *Apis andreniformis* / systématique / isolement sexuel**

**Zusammenfassung — Der Beweis für die reproduktive Isolierung bestätigt, dass *Apis andreniformis* (Smith, 1858) eine von der sympatrischen *Apis florea* (Fabricius, 1787) getrennte Art ist.** *Apis andreniformis* (Smith, 1858) und *Apis florea* (Fabricius, 1787) wurden in folgenden Provinzen von Thailand sympatrisch nachgewiesen : Chantaburi, Uthaitanee, Chiang Rai und Chumporn. Untersuchungen des Endophallus der Drohnen beider Zwergbienen ergaben bei folgenden strukturellen Einzelheiten Unterschiede (Abb 1) : bei den ventralen (BC) und dorsalen (UC) Hörnchen

(Cornua), beim ventralen Haarfeld (HF), beim dorsalen Haarfeld (HDP), beim Federanhang (FL) und in der Länge und Form des Endteils nach dem Federanhang (T). Diese Unterschiede sind Beweise für eine reproduktive Isolation zweier sympatrischer Formen, also Beweise, dass es sich hierbei um echte Arten handelt. Die Endophalli von *A. andreniformis* von Thailand und Malaysia sind ähnlich.

Zusätzlich wurden elektronenmikroskopische Fotos über den basitarsalen Fortsatz (Klammerorgan) des Hinterbeins der Drohnen von beiden Arten beigefügt (Abb 2). Fotografien der Vorderflügel beider Arten zeigen deutlich den charakteristisch grossen Kubital-Index von *A. andreniformis* ( $x = 6,07$ ) und den charakteristisch kleinen Kubital-Index von *A. florea* ( $x = 2,78$ ) (Abb 3).

Sowohl die genaue Verbreitung von *A. andreniformis* als auch ihre Biologie sind noch unbekannt. Vergleichende Untersuchungen sind nötig, da in der Vergangenheit sicher einige Eigenschaften der *A. andreniformis* fälschlich *A. florea* zugeordnet wurden.

***Apis florea* / *Apis adreniformis* /  
Taxonomie / reproduktive Isolation**

## REFERENCES

- Fabricius JC (1787) *Mantissa Insectorum* Vol I Prof. Hafniae
- Maa T (1953) An inquiry into the systematics of the tribus *Apidini* or honey bees Hym. *Treubia* 21, 525-640
- Ruttner F (1975) Ein metatarsaler Haftapparat bei den Drohnen der Gattung *Apis* (Hymenoptera: Apidae). *Entomol Germanica* 2, 22-29
- Ruttner F (1988) *Biogeography and taxonomy of honey bees*. Springer Verlag, Berlin NY
- Ruttner F, Woyke J, Koeniger N (1973) Reproduction in *Apis cerana* 2. Reproductive organs and natural insemination. *J Apic Res* 12, 21-34
- Smith F (1858) Catalogue of the Hymenopterous insects collected at Sarawak, Borneo; Mount Ophir, Malakka; and at Singapore, Wallace AR. *J Proc Linn Soc London Zool* 2, 42-130
- Woyke J, Ruttner H (1958) An anatomical study on the mating process in the honeybee. *Bee World* 39, 3-18
- Wu Y, Kuang B (1986) A study of the genus *Micrapis* (Apidae). *Zoological Research* 7, 99-102, In Chinese
- Wu Y, Kuang B (1986) Two species of small honeybee — A study of the genus *Micrapis*. *Bee World* 68, 153-155. A translation of *Zoological Research* 7, 99-102