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**Original article**

# Drone flight times confirm the species status of *Apis nigrocincta* Smith, 1861 to be a species distinct from *Apis cerana* F, 1793, in Sulawesi, Indonesia

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**Summary** — The drone flights of two distinct morphs of cavity-nesting honey bees were compared in Sulawesi. Drones of the 'black' morph (*Apis cerana)* flew in the early afternoon and had nearly completed their flights before the main flight of drones of the 'yellow' morph. This pattern was consistent in all three study sites but there were significant differences in timing of drone flights between the sites. The differences in timing of mating flights provide the biological justification to recognize the 'yellow' morph as a distinct species, *Apis nigrocincta* Smith, 1861.

***Apis nigrocincta* / *Apis cerana* / drone flight / reproductive isolation / taxonomic status / Sulawesi / Indonesia**

## INTRODUCTION

Recently two morphs of cavity-nesting honey bees, were discovered on Sulawesi (Otis and Hadisoesilo, 1990; Otis, 1991). Morphometric and DNA analyses of specimens (Damus, 1995; Hadisoesilo et al, 1995; Smith and Hagen, 1996) indicated that the smaller, darker bees are *Apis cerana*, the broadly distributed Asian hive bee. In Sulawesi, this species has been collected only from the extreme southern part of south Sulawesi and from a small region of central Sulawesi. In contrast, the larger, yellower morph is widely distributed on Sulawesi as well as the islands of Sangihe and Mindanao to the north (Damus, 1995; Otis, 1996). It is easily separated from *A cerana* of Sulawesi on the basis of size, color, and detailed morphometric analyses.

The obvious question is whether the yellow bee morph of Sulawesi constitutes a subspecies of *A cerana* or a distinct species, *Apis nigrocincta*, as previously discussed

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by Hadisoesilo et al (1995). For convenience it will be tentatively referred to as '*A nigrocincta*' throughout this paper. One relevant observation is that all specimens of cavity-nesting honey bees from Sulawesi have been clearly assignable to one morph or the other; no intermediate forms have been observed (Damus, 1995; Hadisoesilo et al, 1995; Hadisoesilo, unpublished data), including those sampled from two different areas of sympatry (Bontobulaeng area in south Sulawesi and Kamarora village in central Sulawesi). Contrasting with this is the preliminary observation that "there are no substantial differences in the male genitalia of the two morphotypes" (Otis, 1991). The objective of this study was to assess the species status of '*A nigrocincta*' by comparing the timing of its drone flights with those of *A cerana*.

## MATERIALS AND METHODS

Drone flights were quantified in Palangisang and Bontobulaeng (south Sulawesi) and Kamarora/ Bobo (central Sulawesi; fig 1). Palangisang (PAL) is located 20 km NE of Bulukumba at elevation 200 m. Drone flights were monitored on one *A cerana* colony and one '*A nigrocincta*' colony for 7 days, 17–23 August 1995 (table I). The black colony originated in Palangisang. Because '*A nigrocincta*' does not exist in PAL, one colony was brought from Manipi (800 m elevation, 90 km NW of Palangisang) in January 1995. Bontobulaeng (BBL) is 30 km NW of Bulukumba at 420 m elevation and is within a zone of sympatry that extends over approximately 20 km (Hadisoesilo, unpublished data). One *cerana* colony (B-1) was moved from Palangisang in March 1995; the other *cerana* colony and three '*nigrocincta*' colonies were endemic feral colonies transferred to hives in March and April 1995 (table I). Drone flights were observed for 15 days during the period of 5–27 September 1995. Kamarora (KR), at 650 m, is located 55 km SE of Palu in central Sulawesi; both morphs of bee occur in the village, but *cerana* is more common (Hadisoesilo, unpublished data). One *cerana* colony was observed. Because the only two known '*nigrocincta*' colonies in KR lacked adult drones, a '*nigrocincta*' colony was observed in Bobo (BO), a village at 730 m situated 16 km NW of KR. KR and BO were considered a single study site. Observations were made from 17–20 November 1995 (table I).

Colonies were placed 150–500 m apart to reduce drifting of drones between them. One *cerana* and one or two '*nigrocincta*' colonies were watched simultaneously by two or three people on observation days. Prior to data collection, we observed colonies of both morphs from 1200 to 1800 hours for 5 days to obtain preliminary information on timing of drone flights. The earliest observed departures of *cerana* and '*nigrocincta*' drones occurred at 1245 and 1445 hours respectively. Consequently we initiated data collection at 1200 hours for *cerana* and 1400 hours for '*nigrocincta*' to record all drone flights; on all days of data collection a minimum of 15 min passed before any drones took flight. After recording the time at which the first drones departed, we recorded the number of drones that entered each nest during 5 min intervals. Observations were terminated when 15 min had passed with no drones returning to colonies. Observers were 0.5–0.8 m from the hive entrances to enable drones to be readily counted without disturbing the colonies. Times were recorded in Central Indonesian Standard Time (GMT + 8 h). Because all locations were very close to 120° east latitude, data required no adjustment to solar zenith time prior to statistical comparisons.

Mean values are followed by standard errors of the means. Data on first drone departure, last drone entrance, and mean drone entrance times (after grouping data into 15 min intervals) of the two bee morphs were analyzed using oneway analyses of variance (Statistical Analysis System©, SAS Institute Inc). Location effects on these three parameters were analyzed with a two-way analysis of variance. Durations of the drone flight periods for the two morphs in the three locations were compared with a two-way analysis of variance after log-transformation of the data. The percentages of drones of both morphs that returned during the period of overlap (the period during which bees of both morphs were away from colonies on flights) were calculated. Proc GLM was used for all statistical analyses.

## RESULTS

At each of the three sites, the times of the first departures of drones, the last returns,

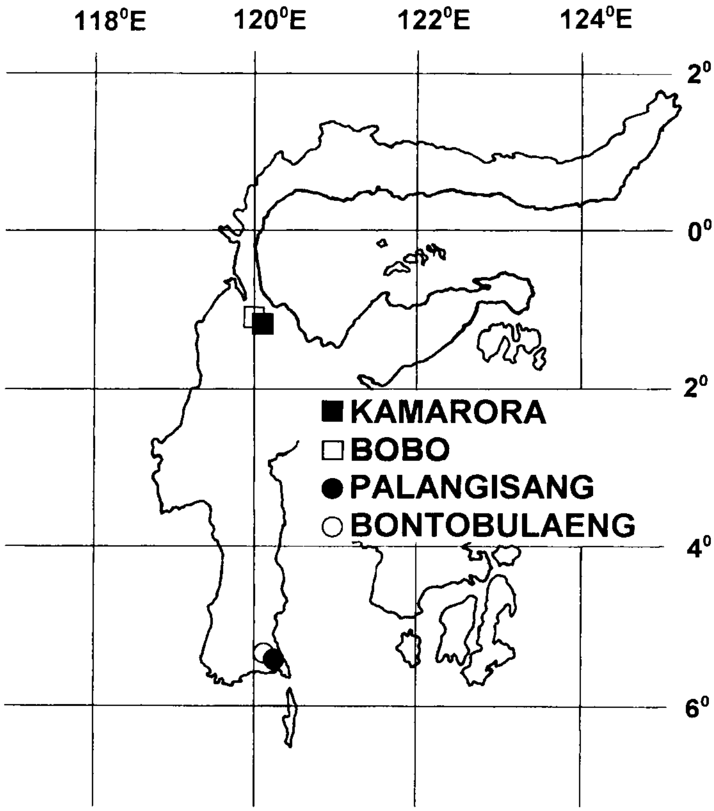


**Fig 1.** Map of Sulawesi indicating locations of study sites.

and mean times of returns differed significantly ($P < 0.0001$; table II; fig 2), with *cerana* drones nearly completing their flights before the first '*nigrocincta*' drones initiated their mating flights. At PAL there was considerable overlap in the distributions of the drone flight times of the two morphs (18.9–8.99% (range: 0–49.8%) of *cerana* drones flew in the period of overlap; for '*nigrocincta*' drones: 2.3–1.2% (0–8.2%). In the other two sites there was extremely little overlap in the distributions of flight times of the *cerana* and '*nigrocincta*' drones (BBL: *cerana* drones: 0.41–0.27% (0–4.5%); '*nigrocincta*' drones: 0.33–0.27% (0–1.4%); KR/BO: *cerana* drones: 0.3–0.20% (0–0.7%); '*nigrocincta*' drones: 0.5–0.31% (0–1.3%)).

For both morphs, the mean times of first departure, mean drone entrance times, and mean times of last returning drones differed significantly between the three sites ($P < 0.05$; table II). The mean times of first departure of drones in KR/BO occurred earlier than in PAL. Drones of both morphs stopped flying later in PAL than in BBL and KR/BO. The earliest mean entrance times of *cerana* and '*nigrocincta*' drones were recorded in KR and BBL, respectively.

**Table I.** Locations, dates and colonies observed for drone flights.

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *17/8* | *18/8* | *19/8* | *20/8* | *21/8* | *22/8* | *23/8* | | | | | | | | |
| Palangisang | B | B | B | B | B | B | B | | | | | | | | |
| | Y | Y | Y | Y | Y | Y | Y | | | | | | | | |
| | *5/9* | *12/9* | *13/9* | *14/9* | *15/9* | *16/9* | *17/9* | *18/9* | *19/9* | *20/9* | *21/9* | *24/9* | *25/9* | *26/9* | *27/9* |
| Bontobulaeng | B-1 | B-1 | B-1 | B-1 | B-1 | B-1 | B-1 | – | – | – | – | – | B-1 | B-1 | B-1 |
| | – | – | – | – | – | – | – | B-2 | B-2 | B-2 | B-2 | B-2 | B-2 | – | – |
| | Y-1 | Y-1 | Y-1 | Y-1 | – | – | – | – | – | – | – | – | – | – | – |
| | – | Y-2 | – | – | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 | Y-2 |
| | – | – | – | Y-3 | Y-3 | Y-3 | Y-3 | – | Y-3 | Y-3 | Y-3 | Y-3 | Y-3 | – | – |
| *Kamarora and Bobo* | *17/11* | *18/11* | *19/11* | *20/11* | | | | | | | | | | | |
| | B | B | B | B | | | | | | | | | | | |
| | Y | Y | Y | Y | | | | | | | | | | | |

**Table II.** Means of time of first departure mean entrance time, and last entrance time of drone flights of *A cerana* (B) and '*A nigrocincta*' (Y) at Palangisang, Bontobulaeng (south Sulawesi), Kamarora and Bobo (central Sulawesi).

| *Location* | *No obs (days)* | *Mean time of first departure ± SE (hours ± min)* | | *Mean drone entrance times ± SE (hours ± min)* | | *Mean time of last drone entrance ± SE (hours ± min)* | |
|---|---|---|---|---|---|---|---|
| | | *B* | *Y* | *B* | *Y* | *B* | *Y* |
| Palangisang | 7 | 1336 ± 9[a] | 1510 ± 9[a] | 1432 ± 2[a] | 1647 ± 2[a] | 1514 ± 3[a] | 1735 ± 3[a] |
| Bontobulaeng | | | | | | | |
| B-1 and Y-1 | 4 | 1315 ± 9 | 1528 ± 9 | 1346 ± 9 | 1621 ± 9 | 1450 ± 5 | 1715 ± 5 |
| B-1 and Y-2 | 7 | 1315 ± 6 | 1507 ± 6 | 1356 ± 2 | 1612 ± 2 | 1453 ± 4 | 1713 ± 4 |
| B-2 and Y-2 | 6 | 1316 ± 6 | 1514 ± 6 | 1357 ± 3 | 1615 ± 3 | 1439 ± 3 | 1713 ± 3 |
| B-2 and Y-3 | 5 | 1318 ± 10 | 1509 ± 10 | 1357 ± 4 | 1628 ± 4 | 1437 ± 4 | 1728 ± 4 |
| B-1 and Y-3 | 5 | 1310 ± 7 | 1520 ± 7 | 1354 ± 2 | 1622 ± 2 | 1457 ± 5 | 1728 ± 5 |
| All colonies combined | 15 | 1312 ± 5[b] | 1517 ± 4[a] | 1356 ± 1[b] | 1618 ± 2[b] | 1447 ± 3[b] | 1717 ± 3[b] |
| Kamarora and Bobo | 4 | 1246 ± 7[c] | 1456 ± 7[b] | 1344 ± 5[c] | 1635 ± 5[c] | 1450 ± 3[b] | 1725 ± 3[b] |

Significant differences ($P < 0.05$) between values within a column are indicated by different letters. All pairwise comparisons between *cerana* and '*nigrocincta*' are highly significant ($P < 0.0001$).

There was variation in the mean duration of the drone flight periods of the two morphs in the three locations. Comparing the two morphs, the durations of the flight period were significantly different in PAL ($P = 0.0006$) and BBL ($P = 0.0001$), but did not differ in KM/BO ($P = 0.1654$). Considering only the *cerana* drones, the drone flight period was longer in KR (129–14.5 min) than at PAL (103–10.9 min; $P = 0.049$) or BBL (100–7.2 min; $P = 0.010$); the flight periods at the latter two sites were not significantly different ($P = 0.62$). For the '*nigrocincta*' drones, the mating flight period was shorter in Bontobulaeng (127–5.8 min) than in either PAL (149–10.9 min; $P = 0.048$) or BO (155–14.5 min; $P = 0.035$); duration of the flight periods in the latter two sites did not differ ($P = 0.64$).

## DISCUSSION

This study demonstrates that there is a large difference in the timing of flights of *A cerana* and '*A nigrocincta*' drones in Sulawesi. Because queen mating flights largely coincide with the flight times of drones within a population (reviewed by Koeniger, 1991; see also Verma et al, 1990; Yoshida et al, 1994; Yoshida , 1995), quantification of the timing of drone flights is an easy means to determine whether two populations differ with respect to this component of their mate recognition systems. Although the timing varied between sites, the almost complete separation in mating flight times results in virtually no opportunity for hybridization between the two morphs. Morphometric and genetic analyses (Damus, 1995; Hadisoesilo et al, 1995; Smith and Hagen, 1996) have

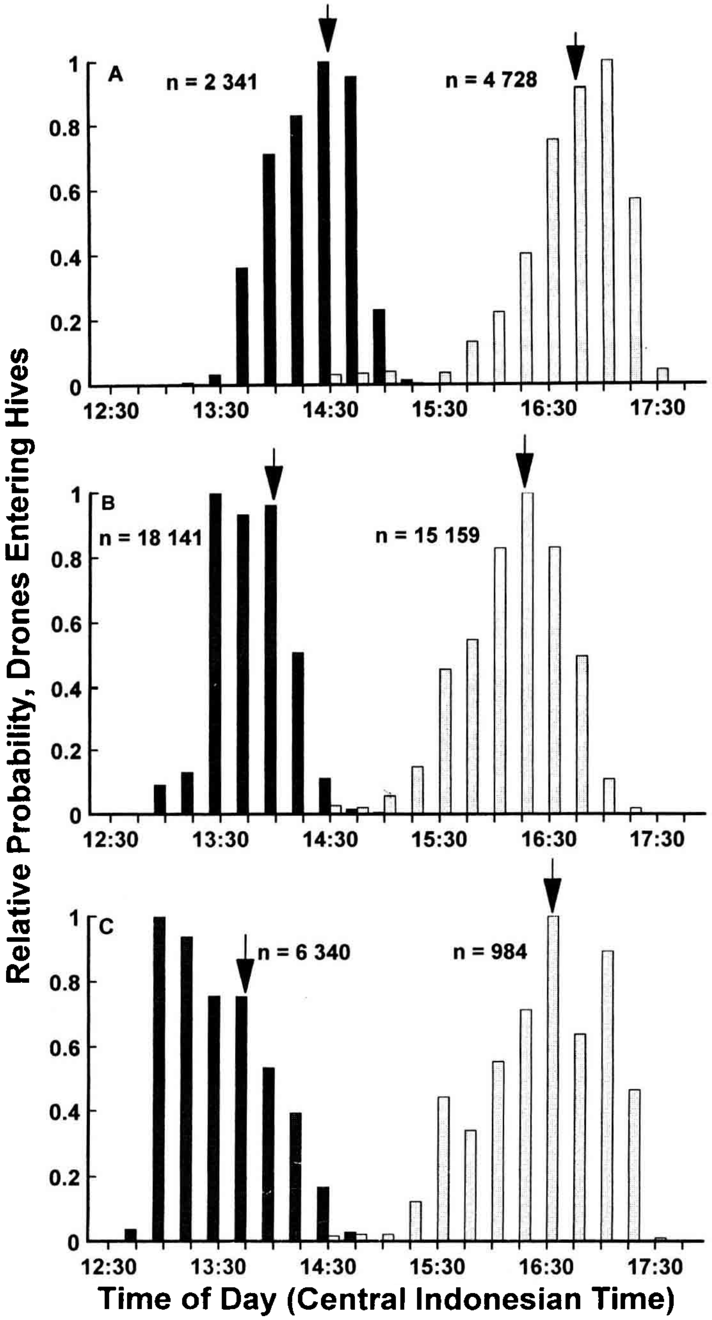


**Fig 2.** Timing of flights of drones in three sites in Sulawesi. Numbers of drones are represented as values relative to the maximal number ($P = 1.0$) recorded returning to hives during 15 min periods. Dark and light bars represent the *cerana* and '*nigrocincta*', respectively. Arrows indicate mean drone entrance times. Study sites: A = Palangisang; B = Bontobulaeng; C = Kamarora/Bobo.

demonstrated that '*A nigrocincta*' is clearly distinct from *A cerana* from all the islands surrounding Sulawesi (Damus, 1995) and from *Apis koschevnikovi* of Borneo (Damus, 1995; Hadisoesilo et al, 1995). Consequently, on the basis of drone flight data we concluded that it represents a currently unrecognized species of *Apis*. Specimens we have examined are similar to a specimen collected by AR Wallace near "Makassar" (=Ujung Pandang) in 1856 that was later described by Frederick Smith (1861) as *A nigrocincta*. The type specimen is housed in the Oxford Museum Collection. Subsequent reports will consider its geographic distribution in more detail, male genitalia of *A nigrocincta* and *A cerana,* and other aspects of the biology of *A nigrocincta*.

There were significant differences between sites in the timing of drone flights and the duration of the drone flight periods of *A cerana* and *A nigrocincta*. The reasons for these differences are not clear, but may be related in part to afternoon temperatures (Taber, 1964; Rowell et al, 1986): at the higher elevation sites (BBL and KR/BO), recorded temperatures were cooler (maximum 29 °C) and drone flights generally occurred earlier than at Palangisang (maximum 34 °C). Unfortunately, detailed weather data were not taken, thereby eliminating the possibility of conducting more detailed analyses. Because day length, latitude, and longitude were almost identical at the times of observation in Palangisang and Bontobulaeng, they do not help in understanding the differences between the sites.

A comment is warranted concerning the specific timing of *A cerana* drone flights in Sulawesi relative to other sites were data have been obtained. In Sulawesi, the mean flight time (returning drones) was 1344 to 1432 hours. This is somewhat earlier than observed in relatively close proximity in Sabah, Borneo (Koeniger et al, 1988), where the mean flight time (exiting and returning drones combined) occurred at approximately 1420 hours (data corrected to solar zenith time). In contrast, *A cerana* drones in Thailand have a mean flight time of approximately 1540 hours (corrected to solar zenith time by subtracting 24 min; Rinderer et al, 1993) which is much later than in the previous two sites. The mean flight (combined exiting and entering bees) occurs even later in the day in Japan (exiting bees only), at approximately 1515 hours (Yoshida *et al*, 1994), and in Sri Lanka (combined exiting and entering bees), at approximately 1655 hours (corrected to solar time; Koeniger and Wijayagunasekera, 1976). The variability between sites is remarkable. It is undoubtedly influenced by the other species of honey bees that co-exist with *A cerana* at the above sites, as pointed out by most of these authors but that alone does not explain all the differences. In Sulawesi, in addition to *A nigrocincta* and *A cerana*, the only other species present is *Apis dorsata binghami* whose drones fly at dusk.

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This paper is dedicated to CD Michener, who in 1990 bet GWO that '*A nigrocincta*' would eventually be recognized as a distinct species. We particularly recognize the Indonesian Agency for Forest Research and Development for providing a scholarship for graduate studies of SH; the Balai Penelitian Kehutanan Ujung Pandang for providing funding for bee hives, some transportation, and logistical support; M Sila for advice; A Rahim for beekeeping assistance; research assistance of S Halim and F Halim; M Nasr for statistical advice; and C O'Toole for access to the type collection at Oxford University Museum. Partial support has been provided by the Biodiversity Support Program, a consortium of World Wildlife Fund, The Nature Conservance and the World Resources Institute, with funding by the United States Agency for International Development. The opinions expressed herein are those of the authors and do not necessarily reflect the views of the US Agency for International Development or the Nature Conservancy.

**Résumé — Les périodes de vol des mâles confirment la statut d'*Apis nigrocincta* Smith, 1861 comme espèce distincte d'*Apis cerana* F, 1793, à Sulawesi, Indonésie.** La découverte récente de deux formes distinctes d'abeilles mellifères nidifiant dans des cavités à Sulawesi (fig 1) nous a incités à étudier la période de vols des mâles afin de déterminer le statut taxonomique d'*A nigrocincta*. On a relevé les vols des mâles de quatre colonies d'*A cerana* et de cinq d'*A nigrocincta* en trois sites différents comprenant deux zones de sympatrie (tableau I). Dans tous les sites les mâles du morphe *cerana* volaient significativement plus tôt ($p < 0{,}0001$), l'heure moyenne se situant environ 2,4 heures plus tôt pour les mâles *cerana* que pour les mâles *nigrocincta*. Quelques mâles *nigrocincta* volant de bonne heure ont causé un petit chevauchement dans la distribution des périodes de vol des deux morphes (fig 2). La période de vol et la durée des vol de mâles différaient significativement d'un site à l'autre (tableau II et fig 2), les vols ayant lieu généralement plus tôt dans les sites plus élevés (plus froids). Ces données soutiennent fortement la conclusion selon laquelle les morphes représentent deux espèces distinctes, puisque la répartition temporelle des vols de mâles est séparée. D'après les études morphométriques et génétiques il est évident que le morphe le plus gros et le plus jaune correspond à une espèce non encore reconnue, qui fut autrefois décrite sous le nom d'*A nigrocincta* par F Smith en 1861.

***Apis cerana* / *Apis nigrocincta* / isolement reproductif / statut taxonomique / vol des mâles / Indonésie**

**Zusammenfassung — Beobachtungen der Drohnenflugzeiten in Sulawesi, Indonesien, bestätigen den Status von *Apis nigrocincta* Smith, 1861, als von *A cerana* F, 1793, verschiedener Art.** An den kürzlich entdeckten zwei verschiedenen Morphen der höhlenbrütenden Honigbienen von Sulawesi (Abb 1) wurden zur Bestimmung des Status von A nigrocincta als möglicherweise eigene Art Untersuchungen der Drohnenflugzeiten durchgeführt. Hierbei wurden die Drohnenflugzeiten von vier *A cerana*-Völkern und fünf A nigrocincta-Völkern quantifiziert. Die Völker befanden sich an drei verschiedenen Orten, an zwei dieser Orte kommen beide Morphen sympatrisch vor (Tabelle I). An allen Orten flogen die Drohnen des *Cerana*-Morphs signifikant früher ($P < 0.0001$), wobei der Mittelwert für *Cerana*-Drohnen etwa 2,4 Stunden früher lag als der der *Nigrocincta*-Drohnen (Tabelle II). Einige wenige sehr früh ausfliegende *Nigrocincta*-Drohnen führten zu einem geringen Ausmaß an zeitlicher Überlappung der Flugzeiten beider Morphen (Abb 2). Zwischen den Standorten gab es signifikante Unterschiede der Flugzeiten (Tabelle II; Abb 2) und der Dauer der Ausflugzeiten, wobei die Flüge generell an den höhergelegenen und kühleren Orten früher stattfanden. Das Ergebnis zeitlich getrennter Drohnenausflugzeiten unterstützt die Ansicht, daß die beiden Morphen zwei unterschiedlichen Arten zuzuordnen sind. Aus morphometrischen und genetischen Untersuchungen ist andererseits deutlich, daß es sich bei dem größeren und gelblicher gefärbten Morph um eine zur Zeit nicht anerkannte Art handelt, die bereits 1861 von F Smith als *A nigrocincta* beschrieben worden war.

***Apis nigrocincta* / *Apis cerana* / reproduktive Isolation / taxonomische Status / Drohnenflug / Sulawesi / Indonesia**

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