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# Drone cell cappings of Asian cavity-nesting honey bees (*Apis* spp.)

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**Abstract**– Drone pupae of *Apis cerana* are sealed in their cells under a hard silken cap with a central pore, a feature notably lacking in the closely related species, *Apis nigrocincta*. In this paper, the hard cap with a pore is confirmed for drone cells of four additional species or putative species of cavity-nesting honey bees of Asia. Inaccuracies in the literature related to cell cappings of Asian bees are clarified. The character state of the drone cell cap for each taxon was added to a recent phylogeny for the genus *Apis*. Parsimony analysis indicates that the hard cap evolved once in the common ancestor to all Asian cavity-nesting honey bees and was subsequently lost in *Apis nigrocincta*. Possible functions of the hard drone cell cap are reviewed, but the true functions of the cap and the reasons underlying its loss in *A. nigrocincta* remain unresolved.

*Apis nigrocincta* / *Apis koschevnikovi* / *Apis nuluensis* / *Apis cerana philippina* / *Apis cerana indica* / drone cell

## 1. INTRODUCTION

A notable feature of *Apis cerana* F. is the hard inner capping of drone cells (Hänel and Ruttner 1985; fig. 9.13 in Ruttner 1988). In all species of honey bees, worker bees seal mature larvae into their cells with a layer of beeswax, following which the larvae inside spin a cocoon. In sealed drone cells of *Apis cerana*, the outer beeswax covering is removed by worker bees a few days after they have sealed the cells, exposing a hard, conical silk cap with a central pore that is created by the drone larva before it pupates (Hänel and Ruttner 1985; Boecking et al. 1999). After an adult drone has eclosed from its pupal cuticle, it cuts through the edge of the cell capping and exits its cell. In their article published more than three decades ago, Hänel and Ruttner (1985) referred to

this hard drone capping with central pore of *Apis cerana* as “unique in the genus *Apis*.”

Because of advances in our understanding of honey-bee diversity, particularly of the cavity-nesting taxa, that statement is no longer true. In 1985, when Hänel and Ruttner described the cappings of *Apis cerana* drone cells, only four species of *Apis* were universally recognized, including only one Asian cavity-nesting species, *A. cerana* (Ruttner 1988). However, the rediscovery of *A. koschevnikovi* (Mathew and Mathew 1988; Tingek et al. 1988) and Maa’s (1953) intriguing treatise on *Apis* taxonomy led to a surge in exploration within Asia for additional taxa. Since 1990, additional collections of honey bees throughout Asia and increasingly sophisticated genetic analyses have provided evidence of greater species diversity within *Apis*: 7 species (Arias and Sheppard 2005), 10 species (Raffiudin and Crozier 2007), 11 species (Lo et al. 2010), and 13+ taxa that deserve species status (Smith 2020; Su and Smith, unpubl. data). Six Asian taxa nest

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in cavities, have multiple combs, and are members of the same phylogenetic clade (Lo et al. 2010, Smith 2020; Su and Smith, unpubl. data).

Interestingly, the hard cell capping with central pore described for *Apis cerana* is absent in drone cells of *Apis nigrocincta*, which are sealed with only a thin layer of beeswax (Hadisoesilo and Otis 1998). This character state of capped drone cells is the sole apomorphic character that distinguishes *Apis nigrocincta* from *Apis cerana sensu lato* (Engel 1999). Descriptions of the drone cell cappings of the other four Asian cavity-nesting taxa are currently obscure, confounded, or nonexistent. In this paper, we clarify the presence/absence of the hard drone cell cap in *A. c. indica*, *A. c. philippina*, *Apis nigrocincta*, *A. koschevnikovi*, and *A. nuluensis*. (As *A. c. indica* and *A. c. philippina* require further research before recognition as species, we refer to them by their subspecific names throughout this paper.) This information clarifies the distribution of *A. nigrocincta* that has been erroneously reported from the southern island of Mindanao by numerous authors. Finally, by mapping the character state of the drone cell cap onto a recent phylogeny of the genus *Apis*, the evolutionary transitions of the drone capping are revealed.

## 2. METHODS

We have personally determined the presence or absence of the hard drone cell cap with central pore in five cavity-nesting taxa. Our observations are supplemented by those of other researchers who have regularly inspected colonies of honey bees in Asia (Table I).

Additionally, we have reviewed the published literature related to all taxa of Asian cavity-nesting bees for relevant information. We assume that the character state of the capped drone cells of a bee taxon in a few localities in a region (e.g., on islands such as Mindanao, Sulawesi, Borneo, and Sri Lanka) is a consistent qualitative character for that bee taxon.

To infer the evolution of the hard drone cell cap, we added the character state for each taxon on a recent phylogenetic tree for the genus *Apis* (Smith 2020; Su et al., unpubl. data). The tree was rooted with the

giant (*Megapis*) and dwarf (*Micrapis*) honey bee clades, both of which lack the hard drone cell cap. The cavity-nesting nesting taxa *A. mellifera*, *A. koschevnikovi*, *A. nigrocincta*, *A. cerana indica*, *A. cerana philippina*, and *A. cerana sensu stricto* were included in the analysis; *A. nuluensis* could not be included because it was not included in the analysis of Su and Smith. Ancestral states were reconstructed in Mesquite version 3.61 (Maddison and Maddison 2019) using parsimony, with evolution from pore present → pore absent and from pore absent → pore present equally likely.

## 3. RESULTS AND DISCUSSION

### 3.1. *Apis cerana* Fabricius, 1793

The central holes, or pores, in the cappings of cells of *Apis cerana* were first observed by Jacobson on Java and Buttell-Reepen on Sumatra (Buttell-Reepen 1907, Buttell-Reepen 1918; as reviewed by Hänel and Ruttner 1985). In photos published by Hänel and Ruttner (1985), Ruttner (1988), and Hadisoesilo and Otis (1998), they appear very similar to those presented here of *A. c. indica*, *A. c. philippina*, and *A. koschevnikovi*.

### 3.2. *Apis cerana indica* Fabricius, 1798

In southern India, people familiar with honey bees refer to the “yellow plains bees” and the “black hills bees” (summarized by Oldroyd et al. 2006). The “yellow” bees also occur in Sri Lanka (refer to figs. 1.9, 1.12, 7.18, and 10.4 in Punchihewa 1994). With the advent of DNA analyses, it has become evident that the “yellow” and “black” bee morphs differ genetically in profound ways (Smith and Hagen 1996; Smith et al. 2000; Oldroyd et al. 2006; Lo et al. 2010; Smith 2020; Su and Smith, unpubl. data), to the extent that they deserve to be considered different species. By the rules of nomenclatural priority in taxonomy, the yellow morph of India (and of Sri Lanka based on genetic similarities; c.f., Smith and Hagen 1996), if elevated to species status, would be referred to by the name *Apis indica* assigned by Fabricius in 1798 (Engel 1999).

**Table I.** Locations in Asia in which researchers have made observations of drone cells and confirmed the type cell capping

| Honey bee taxon         | Observer                 | Geographic region   |
|-------------------------|--------------------------|---|
| <i>Apis cerana</i>      | G.W. Otis                | Malaysia (Borneo [Sabah] and Peninsular Malaysia); Indonesia (Sulawesi—South and Central); Vietnam                              |
|                         | D.R. Smith               | Malaysia (Borneo [Sabah]); Thailand   |
|                         | D. Anderson              | Indonesia (throughout Sundaland and major islands of the Lesser Sunda Islands; Bangka and Belitung; Sulawesi—South and Central) |
|                         | N. & G. Koeniger         | China; Indonesia (Java and Sumatera); (Borneo [Sabah] and Peninsular); Malaysia; Thailand; Pakistan                             |
|                         | Hänel & Ruttner (1985)   | China; Malaysia (Peninsular); Pakistan;   |
| <i>A. c. indica</i>     | R.W.K. Punchihewa        | Sri Lanka   |
| <i>A. c. philippina</i> | G.W. Otis                | Philippines (Luzon, Mindanao)   |
|                         | D.R. Smith               | Philippines (Luzon)   |
|                         | D. Anderson              | Philippines (Bohol, Cebu, Leyte, Luzon [N and S], Mindanao, Panay, Samar)   |
| <i>A. nigrocincta</i>   | D. Anderson              | Indonesia (Sulawesi—South)  |
|                         | S. Hadisoesilo           | Indonesia (Sulawesi—South and Central)  |
|                         | G.W. Otis                | Indonesia (Sulawesi—South, Central, and North)  |
| <i>A. koschevnikovi</i> | K.P. & S. Mathew (1990), | Malaysia (Borneo—Sabah)   |
|                         | N. & G. Koeniger,        |   |
|                         | G.W. Otis, D.R. Smith    |   |
|                         | N. & G. Koeniger         | Malaysia (Borneo—Sabah)   |

**Figure 1.** *Apis cerana indica* comb, Mawathagama, Kurunegala District, Sri Lanka. Visible on the far left are sealed worker cells (red arrow). To the right of them, with darker wax cappings, are sealed drone cells (blue arrow). Those on the left have the outer wax capping intact, while cells at the far right have had the outer caps removed, exposing the inner capping with pore (Photo courtesy of R.W.K. Punchihewa.).

Punchihewa (1994, fig. 6.4) depicted drone brood of *A. c. indica* in Sri Lanka. In the accompanying figure legend, he stated that a distinct hole appears in the middle of the capping as the drone brood ages. Figure 1 shows that the drone cells of the yellow Indian bee, *A. c. indica*, have a hard silk cap with a pore.

### 3.3. *Apis cerana philippina* Skorikov, 1929

Cavity-nesting honey bees occur throughout the Philippines where they are referred to as simply *A. cerana* (Tilde et al. 2000; Radloff et al. 2005), although Engel (1999) suggested that they may warrant subspecies status (i.e., *A. c. philippina*). The most recent genetic analyses suggest that the bees of the Philippines, excluding Palawan, are genetically quite distinct from *A. cerana* of mainland Asia, comprise the sister taxon to *A. nigrocincta* (section 3.4 below), and may warrant being elevated to species status (Smith 2020; Su and Smith, unpubl. data).

From 1998 to 2003, Denis Anderson opened sealed drone cells in honey bee colonies to collect reproducing *Varroa* mites on several major islands of the Philippines (Table 1). He stated: “I never saw the drone cell caps without the pore in the Philippines, even though I was quite aware of [the pore] and always looked for it. All *A. cerana* colonies I looked at in the Philippines always had the pore in the drone cap” (D. Anderson, pers. comm.). His observations are supported by those of others (Table 1) and verify that drone cells of *A. c. philippina* are capped in a manner similar to those of *A. cerana* elsewhere in Asia. Figure 2 shows drone cells of *A. c. philippina* with a hard silk cap with a pore.

Both Anderson and GWO observed the hard cap with pore in colonies on Mindanao Island (Anderson: 30 km NE of Davao City, Davao del Norte Province, and in Manolo Fortich, Bukidnon Province; Otis: in Catalunan Pequeño, 10 km SSE of Davao City, Davao del Sur Province). This information is relevant to the discussion of *A. nigrocincta* that follows.

### 3.4. *Apis nigrocincta* F. Smith, 1861

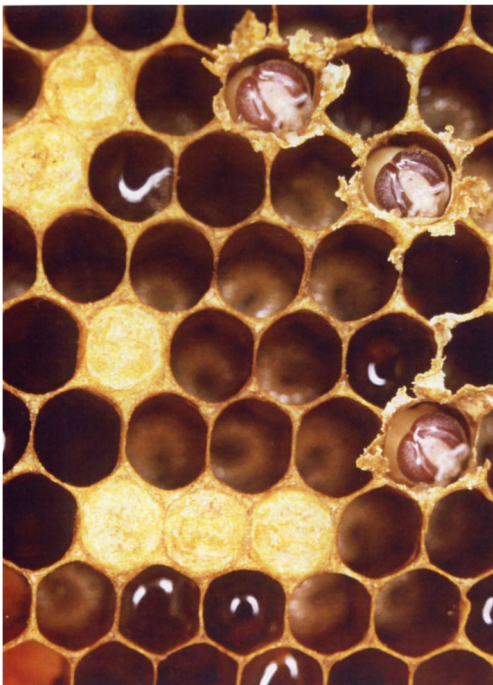
*A. nigrocincta* has been documented in South, West, Central, and North Sulawesi and on the island of Sangihe approximately 200 km north of the northeastern tip of Sulawesi, based on genetic analyses (e.g., Smith et al. 2000) and personal observations by S. Hadisoesilo and GWO (Otis 1996; Hadisoesilo 1997). It likely occurs in forest habitats throughout Sulawesi and on nearby islands. Over a portion of its range, it is sympatric with *A. cerana* (Hadisoesilo et al. 1995; Damus and Otis 1997; Otis and Hadisoesilo 1999; Su and Smith, unpubl. data). Drone cells of bees that were morphometrically (Hadisoesilo et al. 1995) and genetically (Smith et al. 2000) identified as *A. nigrocincta* are sealed with only a thin layer of beeswax (Figure 3); they lack the inner hard cell cap with pore (first observed on Sulawesi by Denis Anderson, unpubl. obs.; Hadisoesilo and Otis 1998). In contrast, drone cells in sympatric *Apis cerana* colonies exhibit the hard drone cap with central pore (Hadisoesilo and Otis 1998).

There is considerable confusion in the literature concerning the presence of *Apis nigrocincta* in the Philippines. Based on the morphological similarity (color and size) between bee specimens collected on Mindanao and *Apis nigrocincta* of Sulawesi, Otis (1996) initially reported: “It was surprising to find that bees from the north coast of Mindanao also belong to this species [*nigrocincta*].” The morphometric analyses of Damus and Otis (1997) supported that statement: their principal components analysis of bee populations resulted in overlapping polygons for bees from the islands of Sulawesi, Sangihe, and Mindanao, demonstrating the similarity in their morphology. Those two assessments, made without supporting genetic information or knowledge of the cappings of drone cells in the Philippines, have proven to be incorrect.

Because drone cell cappings are the single taxonomic character that differentiates *A. nigrocincta* from *A. cerana* (Engel 1999), we can confirm that *A. nigrocincta* does not occur on Mindanao or elsewhere in the Philippines (refer to section 3.3). Although Engel (1999) made this correction, the initial assessment that *A. nigrocincta* occurs on Mindanao



**Figure 2.** *Apis cerana philippina* comb, Don Mariano Marcos Memorial State University, Bacnotan campus, La Union Province, Luzon Island, the Philippines. Drone cells with silk capping and pore, one drone cell with outer wax capping still in place (blue arrow), and several sealed worker cells (red arrow). One drone has chewed its brood cell open, revealing the inner surface of the silk cap (Photo by DRS).



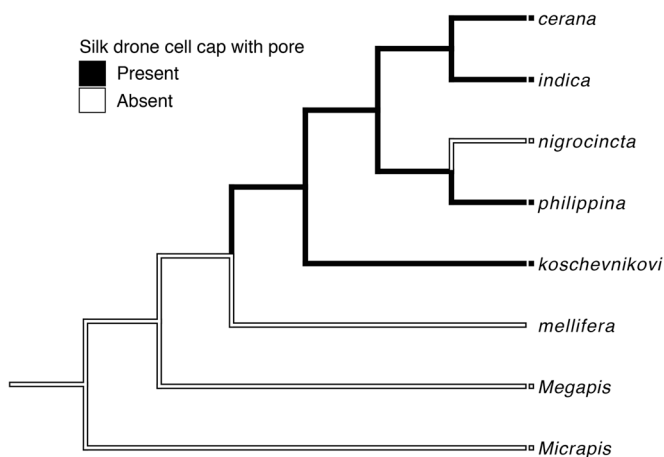
**Figure 3.** Drone cells of *Apis nigrocincta* are sealed with only a thin layer of beeswax, as is evident in the cells that have been opened to expose the heads of purple-eyed drones (Photo taken in Kamarora, Central Sulawesi, Indonesia, by GWO).

(Otis 1996) unfortunately resulted in subsequent erroneous statements (c.f., Damus and Otis 1997; Palmer et al. 2001; Takahashi et al. 2002; Eimanifar et al. 2017; Takahashi et al. 2018). The most serious error occurs in the entry for *Apis nigrocincta* in Wikipedia (2021) that not only states that this taxon occurs on Mindanao but provides a range map suggesting it inhabits most of the Philippine archipelago.

Initial evidence from mitochondrial DNA and AFLP markers indicated *A. nigrocincta* bees from Sulawesi and Sangihe comprise a group distinct from several other geographic groupings of *A. cerana* (Smith and Hagen 1996; Smith et al. 2000, 2003). The recent phylogenetic analysis of honey bee taxa places *A. nigrocincta* as the sister taxon to *A. cerana philippina* of the Philippines (Smith 2020; Su and Smith, unpub. data; see Figure 4).

### 3.5. *Apis koschevnikovi* Enderlein, 1906

K.P. and Susan Mathew (1988) were the first to report that the “red bee of Sabah” was unique and different from *Apis cerana*. It was formally “rediscovered” as a distinct species by Tingek et al.



**Figure 4.** Recent phylogeny for the genus *Apis* (Smith 2020; Su and Smith, unpubl. data), showing character states of drone cell cappings. Ancestral character states were reconstructed in Mesquite (Maddison and Maddison 2019) using parsimony and equal likelihood of change from “pore absent” to “pore present” and vice versa. The most parsimonious explanation for the evolution of the hard drone cell cap with pore shows it was absent in the common ancestor of the extant species of *Apis*, evolved once in the Asian cavity-nesting clade, and was subsequently lost in *A. nigrocincta*.

(1988) under the name *Apis vechti* Maa 1953, which was corrected to *A. koschevnikovi* (Ruttner et al. 1989) and correctly attributed to Enderlein, 1906, by Engel (1999). The species occurs in forested areas of Sundaland (Otis 1996; Hadisoesilo et al. 2008). Mathew and Mathew (1990) included this entry in their set of traits of *A. koschevnikovi*: “9. Drone cells of *A. koschevnikovi* are sealed with two layers of raised cappings. As in *A. cerana*, the inner one develops a pore in the centre which is exposed as the pupa matures.”

That description seems to have been the basis of Alexander’s (1991a, 1991b) use of this character in his phylogenetic analysis of the genus *Apis*, although he incorrectly attributed it to Mathew and Mathew (1988). Engel (1999) stated that “the apomorphic presence of such a pore [taxonomically] unites *Apis cerana* and *Apis koschevnikovi*.” He seems to have based this statement on Alexander (1991a, 1991b) because he did not cite Mathew and Mathew (1990).

Numerous people have inspected hives of *A. koschevnikovi* in Tenom, Sabah, Malaysia (Table I). They confirm that the hard cap with central pore is present on its sealed drone cells, as shown in Figure 5.



**Figure 5.** *Apis koschevnikovi* comb, Agricultural Research Center, Tenom, Sabah (Borneo), Malaysia. The drone cells in the upper portion of the comb show the central pore in their cappings. The outer wax cell cappings are still intact on the drone cells along the lower periphery of the comb. Several smaller empty worker cells are visible at the top of the photo. At the bottom edge of the comb a queen cell can be seen (Photo by DRS.).

### 3.6. *Apis nuluensis* Tingek et al. 1996

The structure of the drone cell capping in *A. nuluensis* has not been reported previously. N. and G. Koeniger (unpubl. data) inspected sealed drone cells in two colonies, in Mesilau and Gunung Alab, Sabah, Malaysia (Borneo). In both cases, drone cells had two cappings; the inner one was conical with a central pore, similar to the inner cappings of *A. cerana* (N. Koeniger, pers. com.). Unfortunately, there are no photos depicting the drone brood of this species.

### 3.7. Evolutionary history of drone cell cappings in the genus *Apis*

Figure 4 summarizes the most recent phylogeny for the genus *Apis*, based on phylogenetic analysis of genome-wide single nucleotide polymorphisms (SNPs) carried out in BEAST v1.8.4 (Drummond and Rambaut 2007; Smith 2020; Su and Smith, unpubl. data). This phylogeny of cavity-nesting bees is compatible with those constructed independently by Raffiudin and Crozier (2007) and Lo et al. (2010). Onto the new phylogeny, we have added the character state of the drone cell capping for each species. The absence of the hard silk drone cell cap with central pore is almost certainly a plesiomorphic character in *Apis*: the hard cap is lacking in the dwarf honey bee (*Micrapis*) and giant honey bee (*Megapis*) clades as well as in *A. mellifera* (Engel 1999). It is present in all of the currently recognized and putative Asian cavity-nesting species (including *A. nuluensis*) except *A. nigrocincta*. The most parsimonious explanation for the evolution of the hard drone cap, given the phylogenetic relationships depicted in Figure 4, is that it evolved once in the most recent common ancestor of Asian cavity-nesting honey bees following its split from *A. mellifera*, and was subsequently lost in *A. nigrocincta*.

### 3.8. Function of the hard drone cell cap

The hard drone pupal cap is difficult or impossible for worker bees to cut off and remove

(Boecking et al. 1999; Davis and Prabuswamy 2000), which suggests that it functions to prevent worker bees from contacting the contents of drone cells. Boecking et al. (1999) reviewed the functions that have been proposed for it: (a) protection of drone pupae from cannibalism/destruction by worker bees (Moritz and Southwick 1992) and (b) prevention of the removal of mite-infested or diseased/dead drone brood (Boecking 1998). Relevant to Boecking's second proposed function (b), one regularly sees old, dark brown sealed drone cells among empty drone cells in colonies of *Apis cerana* that, when opened, contain dead brood that appears to have been killed by a pathogen. It is plausible that as the hard cap evolved, either to protect live drones from being removed or to entomb mite-infested or diseased pupae, the pore simultaneously evolved to enable exchange of gases by live drone pre-pupae and pupae (Rath 1992). The pore may have a secondary function: it also would enable worker bees to detect chemical cues emitted from dead or heavily mite-infested drone brood that could stimulate the workers to seal the central pore with wax, thereby limiting pathogens or mites from affecting additional brood.

These explanations fail to explain the loss of the hard cell cap in *Apis nigrocincta* unless the pathogens or parasitic mites that negatively affect drone brood elsewhere did not exist on Sulawesi during most of *A. nigrocincta*'s evolutionary history, relaxing selection pressures favoring the hard cap found in other Asian cavity-nesting bees and resulting in the loss of that trait. Currently, *Apis nigrocincta* shares two parasitic mite species, *Varroa jacobsoni* and *V. underwoodi*, with *A. cerana* on Sulawesi (Anderson et al. 1997). These mites may have been introduced relatively recently to Sulawesi along with *A. cerana* colonies moved from Java, an explanation consistent with genetic analyses of bees and mites conducted by D. Anderson (unpubl. data). Unfortunately, because we lack a direct window into evolutionary processes, the true function of the hard inner drone cell capping and the reasons for its loss in *Apis nigrocincta* remain mysteries.

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**Availability of data and material** The data consist of the photos and the table with observations by scientists in various geographic regions that are provided in the manuscript. There are no other data.

**Code availability** Not applicable

## AUTHOR CONTRIBUTION

GWO conceived the research, reviewed the literature, contacted people for unpubl. obs., and wrote the 1st version. DRS created the phylogeny, performed parsimony analysis, provided photos, and edited. Funding

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## DECLARATIONS

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Statement for use of photo from RWK Punchihewa is in the Acknowledgements section.

**Conflict of interest** The authors declare no competing interests.

**Les operculations des cellules du couvain des mâles des abeilles asiatiques nichant dans des cavités (*Apis* spp.).**

*Apis nigrocincta* / *Apis koschevnikovi* / *Apis nuluensis* / *Apis cerana philippina* / *Apis cerana indica* / couvain mâle

**Die Zelleckel der Drohnenbrut der höhlennistenden asiatischen Honigbienen (*Apis* spp.).**

*Apis nigrocincta* / *Apis koschevnikovi* / *Apis nuluensis* / *Apis cerana philippina* / *Apis cerana indica* / Drohnenbrutzelle

## REFERENCES

- Alexander, B.A. (1991a) Phylogenetic analysis of the genus *Apis* (Hymenoptera: Apidae). *Ann Entomol Soc Am* 84:137-149
- Alexander, B.A. (1991b) A cladistics analysis of the genus *Apis*. In: Smith DR (Ed.) Diversity in the genus *Apis*. Westview Press, Boulder, Colorado, pp. 1-28
- Anderson, D.L., Halliday, R.B., Otis, G.W. (1997) The occurrence of *Varroa underwoodi* (Acarina: Varroidae) in Papua New Guinea and Indonesia. *Apidologie* 28:143-147
- Arias, M.C., Sheppard, W.S. (2005) Phylogenetic relationships of honey bees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data. *Mol Phylogen Evol* 37:25-35
- Boecking, O. (1998) Sealing up and non-removal of diseased and *Varroa jacobsoni* infested drone brood cells is part of the hygienic behaviour in *Apis cerana*. *J Apic Res* 38:159-168
- Boecking, O., Rosenkranz, P., Sasaki, M. (1999) The pore in the hard conical *Apis cerana* drone capping results from a spinning process. *Apidologie* 30:513-519
- Buttel-Reepen, H.v. (1907) Psychobiologische und biologische Beobachtungen an Ameisen, Bienen, und Wespen. *Naturwiss Wochenschr Neue Folge* 6/30:465-487
- Buttel-Reepen, H.v. (1918) Seltsame Milbewohner der Bienenzellen. *Bienenwirtsch Centralbl* 54:78-80
- Damus, M.S., Otis, G.W. (1997) A morphometric analysis of *Apis cerana* F. and *Apis nigrocincta* Smith populations from Southeast Asia. *Apidologie* 28:309-323
- Davis, A.R., Prabuswamy, H.P. (2000) Influence of cell type and mandibular dentition on the ability of captive honey bee workers and drones (*Apis cerana indica* Fabr. and *Apis mellifera* L.) to uncapp brood cells:

- biological implications of parasitic mites (*Varroa* spp.). *Can J Zool* 78:666-679
- Drummond, A.J., Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: article 214. doi:<https://doi.org/10.1186/1471-2148-7-214>
- Eimanifar, A., Kimball, R.T., Braun, E.L., Fuchs, S., Grünewald, B., Ellis, J.D. (2017) The complete mitochondrial genome and phylogenetic placement of *Apis nigrocincta* Smith (Insecta: Hymenoptera: Apidae), an Asian, cavity-nesting honey bee. *Mitochondrial DNA B Resour* 2: 249-250
- Engel, M.S. (1999) The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae; *Apis*). *J Hymenopt Res* 82:165-196
- Hadisoesilo, S. 1997. A comparative study of two species of cavity-nesting honey bees of Sulawesi, Indonesia. PhD Thesis, University of Guelph, xi and 199 pp
- Hadisoesilo, S., Otis, G.W. (1998) Differences in drone cappings of *Apis cerana* and *Apis nigrocincta*. *J Apic Res* 37:11-15.
- Hadisoesilo, S., Otis, G.W., Meixner, M. (1995) Two distinct populations of cavity-nesting honey bees (Hymenoptera: Apidae) in South Sulawesi, Indonesia. *J Kans Entomol Soc* 68:399-407
- Hadisoesilo, S., Raffiudin, R., Susanti, W., Atmowidi, T., Hepburn, C., Radloff, S.E., Fuchs, S., Hepburn, H.R. (2008) Morphometric analysis and biogeography of *Apis koschevnikovi* Enderlein (1906). *Apidologie* 39:495-503
- Hänel, H., Ruttner, F. (1985) The origin of the pore in the drone cell capping of *Apis cerana* Fabr. *Apidologie* 16:157-164
- Lo, N., Gloag, R.S., Anderson, D.L., Oldroyd, B.P. (2010) A molecular phylogeny of the genus *Apis* suggests that the giant honey bee of the Philippines, *A. breviligula* Maa, and the plains honey bee of southern India, *A. indica* Fabricius, are valid species. *Syst Entomol* 35:226-233
- Maa, T.C. (1953) An inquiry into the systematics of the tribus Apidini or honeybees (Hym.). *Treubia* 21:525-640
- Maddison, W.P., Maddison, D.R. (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61 <http://www.mesquiteproject.org>
- Mathew, K., Mathew, S. (1988) The red bees of Sabah, *Newsl Beekeep Trop and Sub-trop Countries* 12:10
- Mathew, K.P., Mathew, S. (1990) Further observations on characters and behaviour of *Apis koschevnikovi*, the 'red bee' of Sabah. *Bee World* 71:61-66
- Moritz, R.F.A., Southwick, E.E. (1992) Bees as superorganisms. Springer-Verlag, Berlin.
- Oldroyd, B.P., Reddy, M.S., Chapman, N.C., Thompson GJ, Beekman M (2006) Evidence for reproductive isolation between two colour morphs of cavity nesting honey bees (*Apis*) in south India. *Insectes Soc* 53:428-434
- Otis, G.W. (1996) Distribution of recently recognized species of honey bees (Hymenoptera: Apidae, *Apis*) in Asia. *J Kans Entomol Soc* 69:311-333
- Otis, G.W., Hadisoesilo, S. (1999) Insights into honey bee biology from *Apis nigrocincta* of Indonesia. In: Hoopingarner R, Connor L (Eds.). *Beekeeping in the 21st Century*, Wicwas Press, Cheshire, Connecticut, pp 69-79
- Palmer, K., Oldroyd, B., Franck, P., Hadisoesilo, S. (2001) Very high paternity frequency in *Apis nigrocincta*. *Insectes Soc* 48:327-332
- Punchihewa, R.W.K. (1994) Beekeeping for honey production in Sri Lanka. *Can. Internat. Dev. Agency and Sri Lanka Depart. Agric.* ISBN: 9559282018
- Radloff, S.E., Hepburn, R.H., Fuchs, S., Otis, G.W., Hadisoesilo, S., Hepburn, C., Ken, T. (2005) Morphometric multivariate analysis of the *Apis cerana* populations from oceanic Asia. *Apidologie* 36:475-492
- Raffiudin, R., Crozier, R.H. (2007) Phylogenetic analysis of honey bee behavioral evolution. *Mol Phylogen Evol* 43:543-552
- Rath, W. (1992) The key to *Varroa*: the drones of *Apis cerana* and their cell cap. *Am Bee J* 132:329-331
- Ruttner, F. (1988) Biogeography and taxonomy of honeybees. Springer Verlag, Berlin
- Ruttner, F., Kauhausen, D., Koeniger, N. (1989) Position of the red honey bee, *Apis koschevnikovi* (Buttel-Reepen 1906), within the genus *Apis*. *Apidologie* 20:395-404
- Smith, D.R. (2020). Biogeography of honey bees. In: Starr C.K. (Ed.) *Encyclopedia of social insects*. Springer, Cham. doi:[https://doi.org/10.1007/978-3-319-90306-4\\_60-1](https://doi.org/10.1007/978-3-319-90306-4_60-1)
- Smith, D.R., Hagen, R.H. (1996) The biogeography of *Apis cerana* as revealed by mitochondrial DNA sequence data. *J Kans Entomol Soc* 69:294-310
- Smith, D.R., Villafuerte, L., Otis, G., Palmer, M.R. (2000) Biogeography of *Apis cerana* F. and *Apis nigrocincta* Smith: insights from mtDNA studies. *Apidologie* 31:265-279
- Smith, D.R., Palmer, M.R., Otis, G., Damus, M. (2003) Mitochondrial DNA and AFLP markers support species status of *Apis nigrocincta*. *Insectes Sociaux* 50:185-190
- Takahashi, J.-i., Nakamura, J., Sasaki, M., Tingek, S., Akimoto, S.-i. (2002) New haplotypes for the non-coding region of mitochondrial DNA in cavity-nesting honey bees *Apis koschevnikovi* and *Apis nuluensis*. *Apidologie* 33:25-31
- Takahashi J.-i., Hadisoesilo, S., Okuyama, H., Hepburn, H.R. (2018) Analysis of the complete mitochondrial genome of *Apis nigrocincta* (Insecta: Hymenoptera: Apidae) on Sangihe Island in Indonesia. *Conserv Genet Resour* 10:755-760

- Tilde, A.C., Fuchs, S., Koeniger, N., Cervancia, C.R. (2000) Morphometric diversity of *Apis cerana* Fabr. within the Philippines. *Apidologie* 31:249-263
- Tingek, S., Mardan, M., Rinderer, T.E., Koeniger, N., Koeniger, G. (1988) Rediscovery of *Apis vechti* (Maa 1953)—the Saban honeybee. *Apidologie* 19:97-102
- Tingek, S., Koeniger, G., Koeniger, N. (1996) Description of a new cavity nesting honey bee of *Apis* (*Apis nuluensis* n. sp.) from Sabah, Borneo, with notes on its occurrence and reproductive biology (Insecta: Hymenoptera: Apoidea: Apini). *Senckenb Biol* 76:115-119
- Wikipedia (2021) *Apis nigrocincta*. [https://en.wikipedia.org/wiki/Apis\\_nigrocincta](https://en.wikipedia.org/wiki/Apis_nigrocincta). (Accessed 15 Mar 2020)

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