

Nyaton Kitnya¹, Axel Brockmann², Gard W. Otis³

¹Trivedi School of Biosciences, Ashoka University, Sonapat, Haryana, 131029, India;

²National Centre for Biological Sciences - Tata Institute of Fundamental Research, Bengaluru, 560065, India;

³Institute of Bee Health, University of Bern, CH-3097 Bern, Switzerland

BACKGROUND: The giant honeybees (subgenus *Megapis*) of Asia are important pollinators that build open nests on branches of tall trees and rocky cliffs. Two species are now generally recognised: *Apis dorsata* and *Apis laboriosa*. However, some researchers have argued that morphological variation between *dorsata* and *laboriosa* is due to their occurrence at different elevations: *dorsata* is widely distributed in lowlands whereas *laboriosa* is endemic to the higher elevations in Himalaya and neighbouring mountain ranges. Additionally, based on genetic analyses, some researchers have suggested that the three additional populations deserve species status: Sulawesi, Indonesia (*A. binghami*), the Philippines (*A. breviligula*), and South-India *A. dorsata*.

PROBLEM DESCRIPTION: The taxonomy and phylogeny of *Megapis* are controversial and unresolved. The species boundaries within this subgenus are unclear and species recognized on basis of genetic differences lack supporting morphological characters. Our goal was to (1) compare sympatric *dorsata* and *laboriosa* specimens morphologically and genetically; (2) uncover morphological differences between the populations, and (3) create a taxonomic key for identification of the species we could distinguish.

METHODOLOGY: We examined morphological characters of both drones and worker bees of all five populations. Thorax muscles from specimens from sites of sympatry were used for DNA extraction. Phylogeny reconstruction was performed using ~600bp of mtDNA COI. The specimens were deposited at the Research Collection Facility, NCBS-TIFR, Bengaluru, India.

RESULTS AND CONCLUSION: Based on numerous morphological characters, *laboriosa* is very distinctive from the other taxa. Mainland *dorsata* differed in several characters from the Philippines + Sulawesi populations based on colouration, ocellus size, and other characters. Despite its name “*breviligula*”, the tongue length of Philippine specimens did not differ from *binghami* of Sulawesi. We provided keys for identification of *A. dorsata*, *A. laboriosa*, and *A. binghami*.

The phylogenetic analysis of mtDNA COI from specimens from sites of sympatry perfectly matched morphological differences between *A. dorsata* and *A. laboriosa*, further confirming the species status of *laboriosa*.

Additional morphological and genetic analyses are required to resolve the status of *A. breviligula* and South-India *A. dorsata*.

Giant Honeybees of Asia: Based on Morphological and Genetic Study

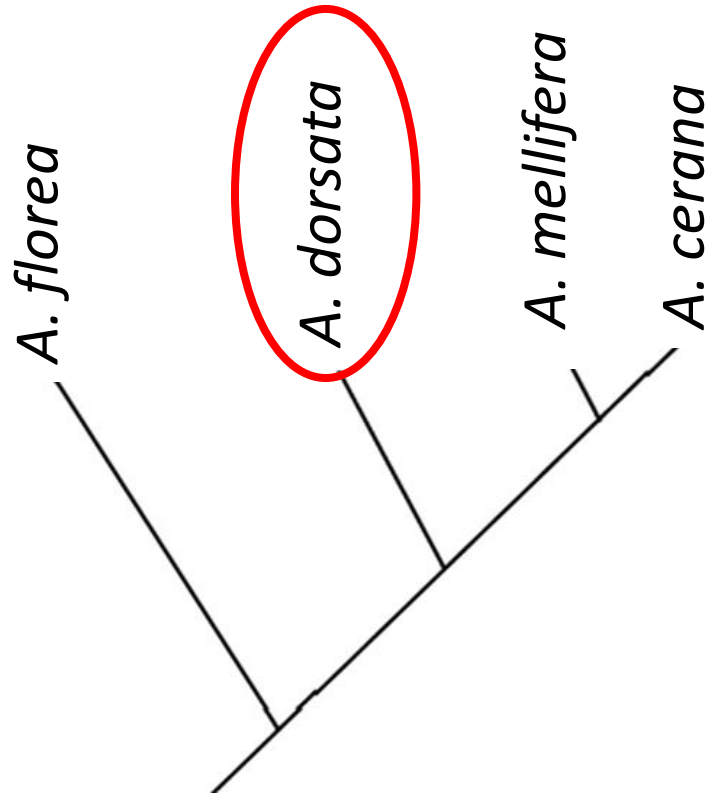
Nyaton Kitnya, PhD

¹Trivedi School of Biosciences,
Ashoka University, Sonipat, India

²Indira Gandhi Government
College, Tezu, India



The “traditional” 4 honeybee species



Apis florea



Apis dorsata

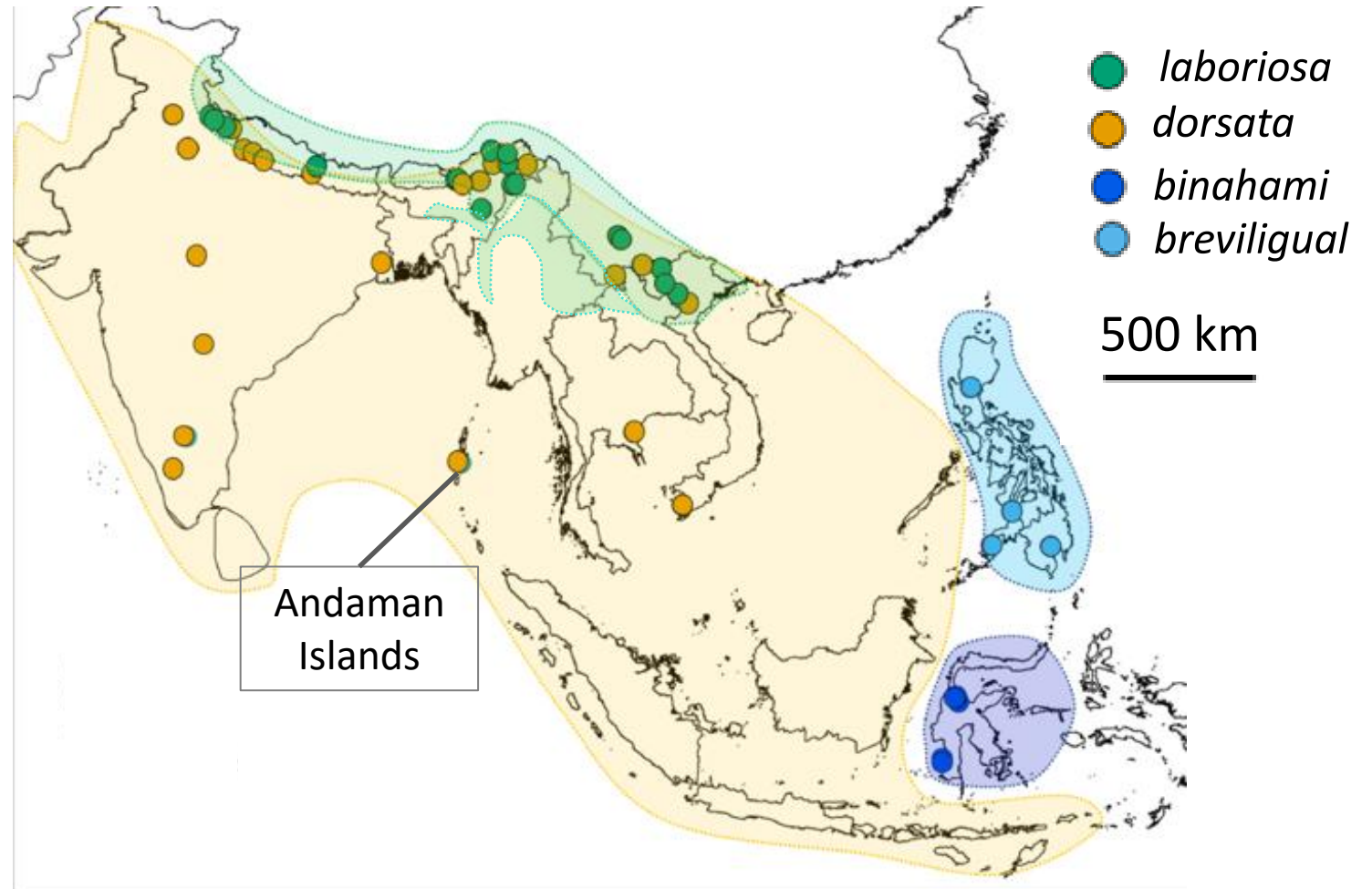
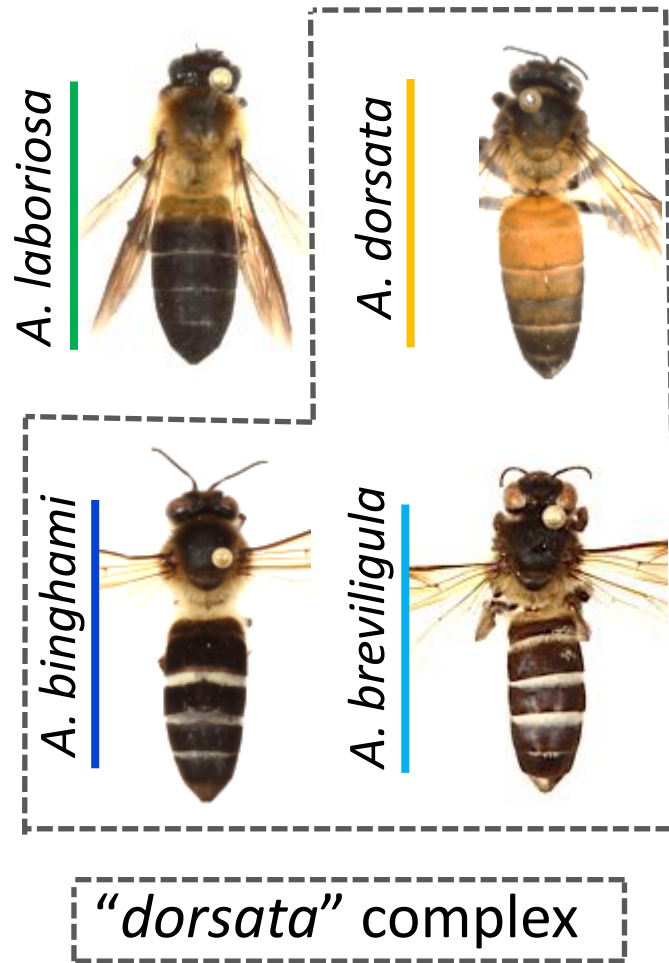


Apis mellifera



Apis cerana

The giant honeybees: Maa (1953) recognized 4 species



Kitnya et al. (2020), Smith (2021), and Otis et al 2024

The Controversy about *Apis laboriosa*

- First described from Yunnan, China, by Frederick Smith.
- Maa (1953) split *Apis dorsata* into 4 species of the subgenus *Megapis* & provided a key for their identification.
- Sakagami et al. (1980) studied morphology in detail and concluded that *Apis laboriosa* deserved species status. No detailed morphological study since.
- In contrast, *laboriosa* was proposed to be a high-elevation form of *dorsata* by Ruttner (1988 & 1992) and Engel (1999).



Photo: Shantam Ojha, inaturalist.org/observations/178510648

My talk today:

1) Is *Apis laboriosa* a valid species?

- a) Geographic distribution (partial sympatry - NE India, Vietnam, s. China)
- b) Behavioural evidence (mating flight times differ)
- c) Morphological evidence
- d) Morphometric and genetic evidence from sites
where *laboriosa* is partially sympatric with *dorsata*

2) Within the *Apis dorsata* complex:

Are there 1, 2 or 3 species? Morphology

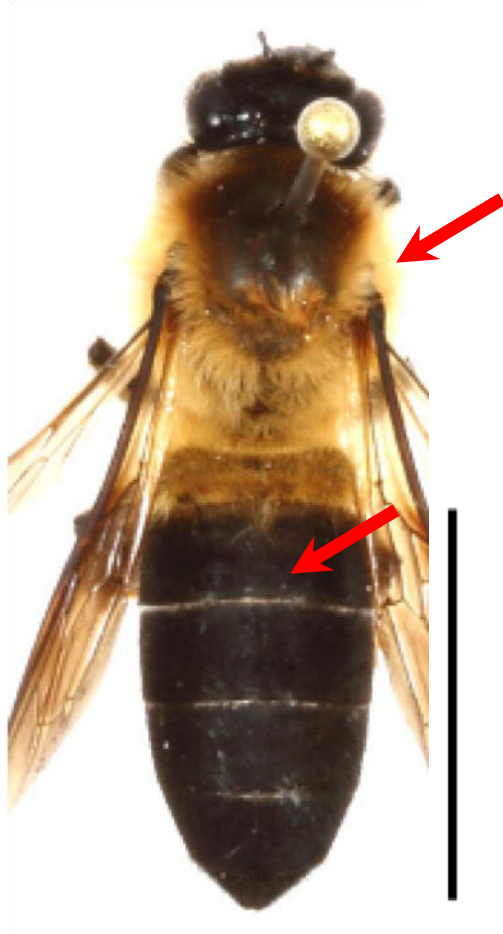
Apis laboriosa: Largest bees within *Apis*

- Worker bee: 1.75 cm long.
- Nest is a single large comb built from a cliff; often in aggregations.



A. laboriosa easily distinguished from mainland Asian *A. dorsata*:

Apis laboriosa (India)



Apis dorsata (China)

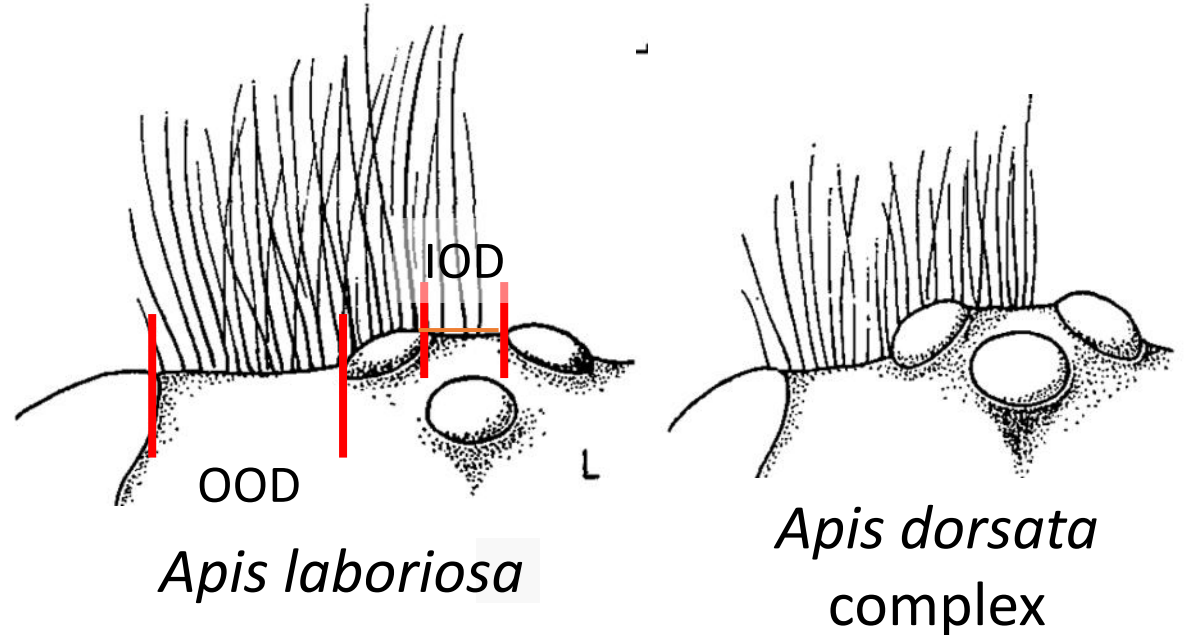


- colour of hairs on thorax;
- colour of abdomen

Morphological evidence for *Apis laboriosa*

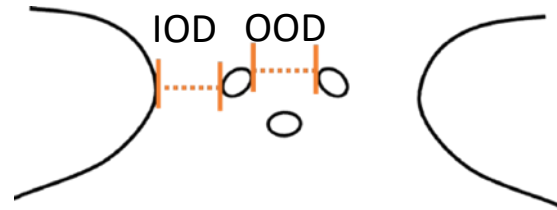


- Sakagami, Matsumura, and Ito (1980) provided several diagnostic characters; concluded that *A. laboriosa* is distinct from *A. dorsata*.
- Differences in ocelli (relatively flat in *laboriosa*, domed upward in *dorsata*)
- Engel (1999) claimed that the ocellar structures are variable within *laboriosa*.

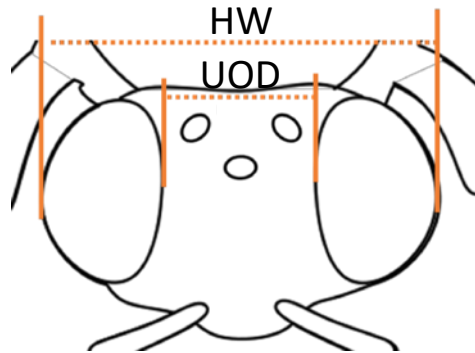


From Sakagami et al., 1980

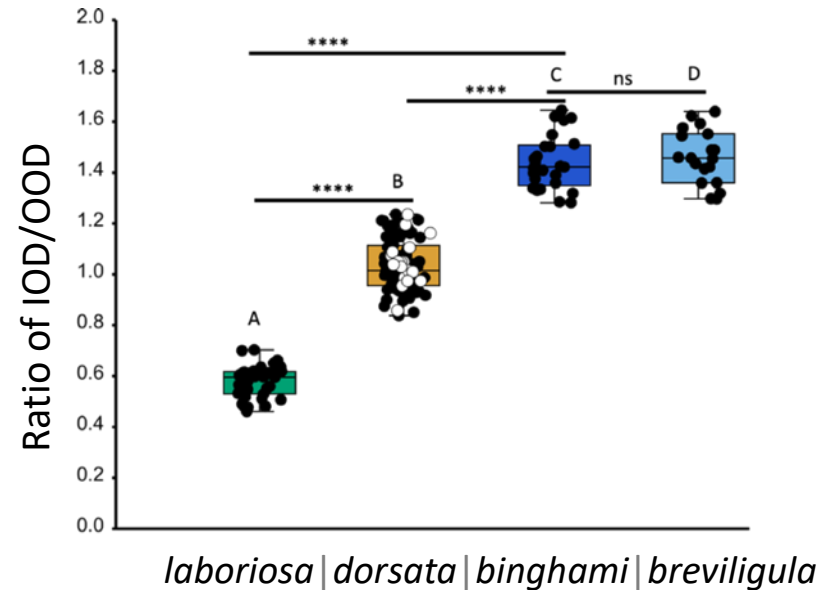
Worker: Head morphology



OOD: Ocellocular distance
IOD: Inter-ocular distance

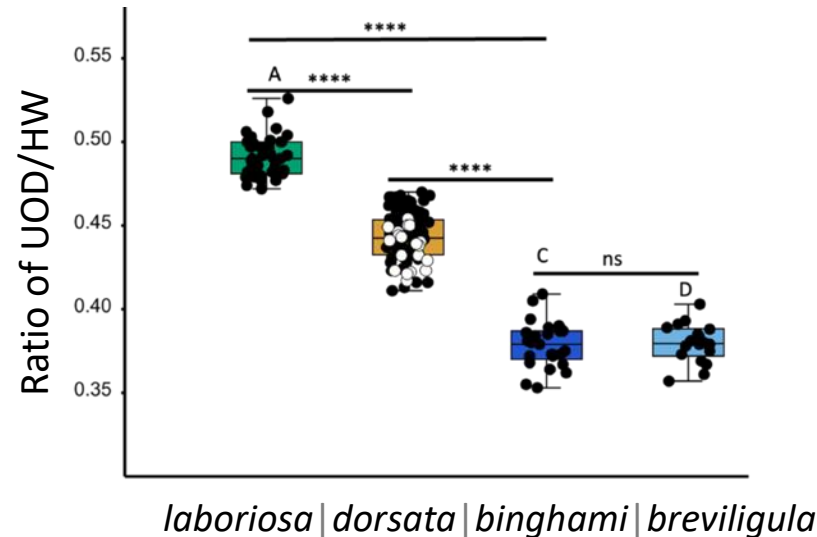


HW: Head width
IOD: Upper-ocular distance



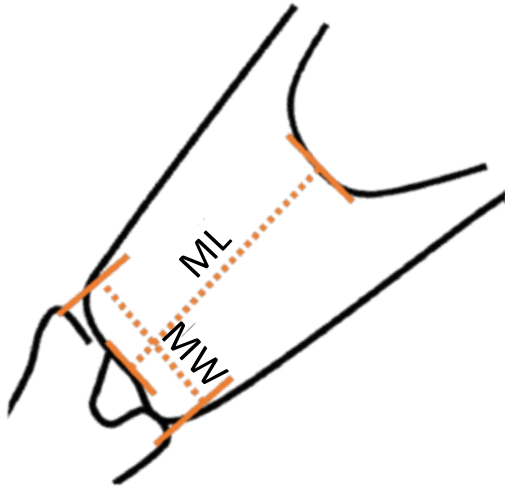
Ratios of IOD to OOD (top) and of UOD to HW (bottom) separate *laboriosa* from other 3 taxa.

Also, they separate *dorsata* from the island giant honey bees, *binghami* & *beviligula*.



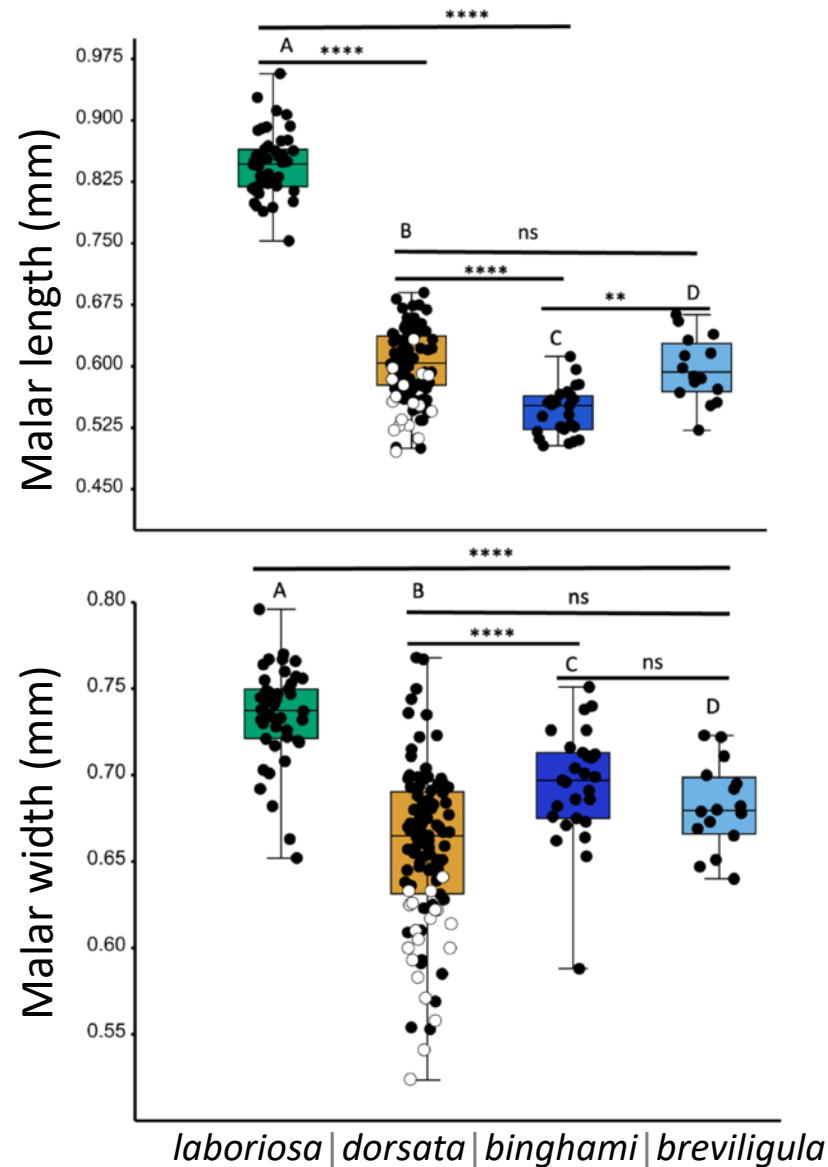
White circles indicate *dorsata* from the Andaman Islands; they are similar morphologically to *dorsata* from the mainland.

Worker: Head morphology



Malar length distinctly separates *laboriosa* from other 3 taxa.

Malar width is significantly larger in *laboriosa*, but values overlap broadly with other 3 taxa.



laboriosa

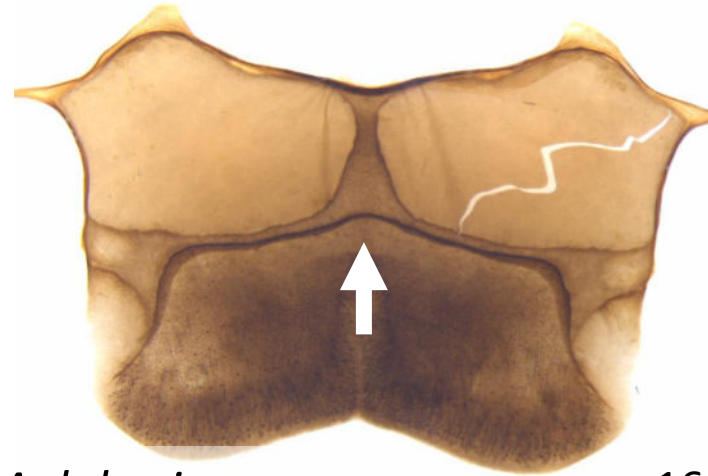


dorsata



Worker: Abdomen Morphology

Sternum 3



A. laboriosa

n=16



A. dorsata

n=22



A. binghami

n=16



A. breviligula

n=16

Drone: Head morphology



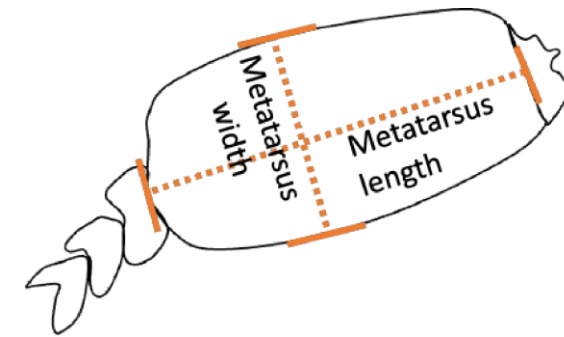
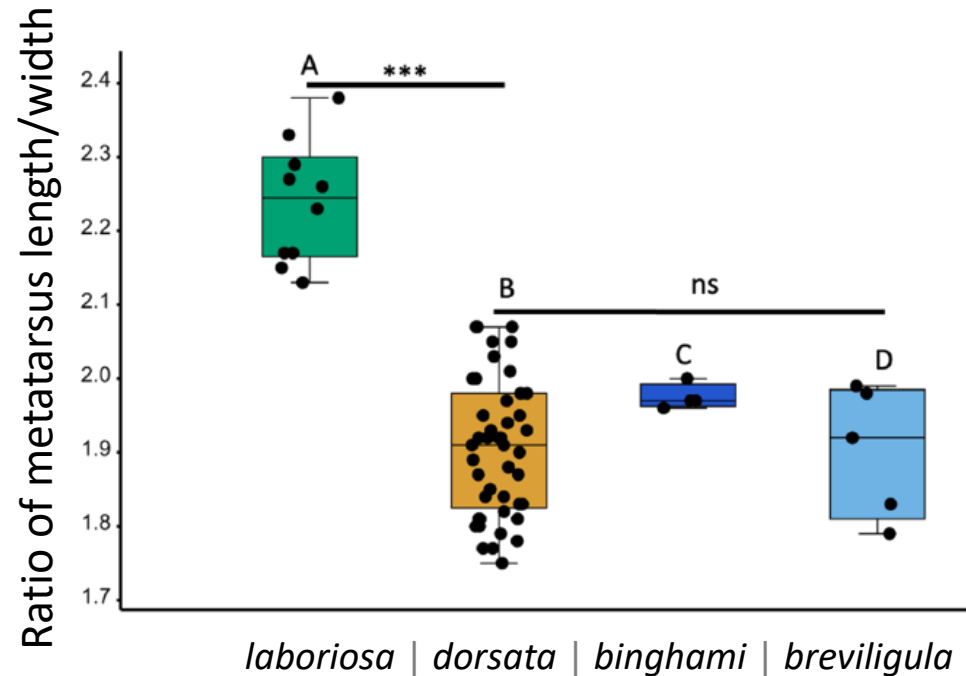
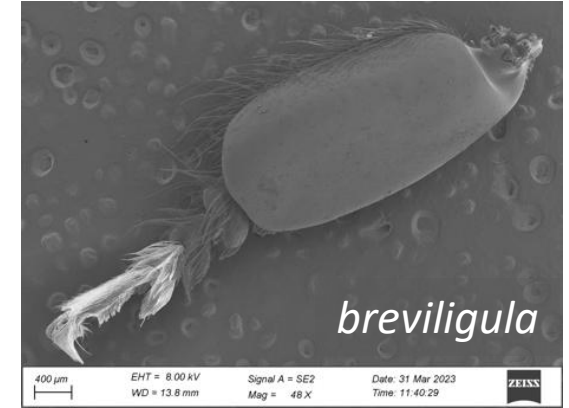
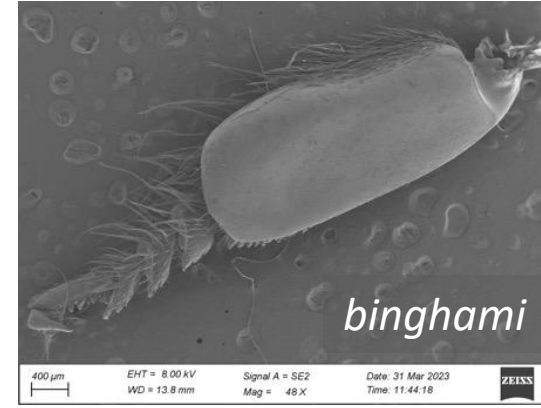
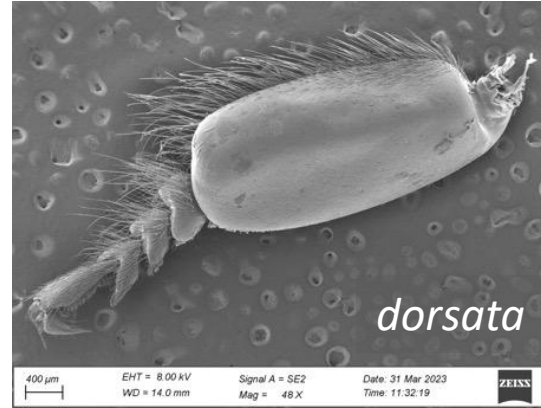
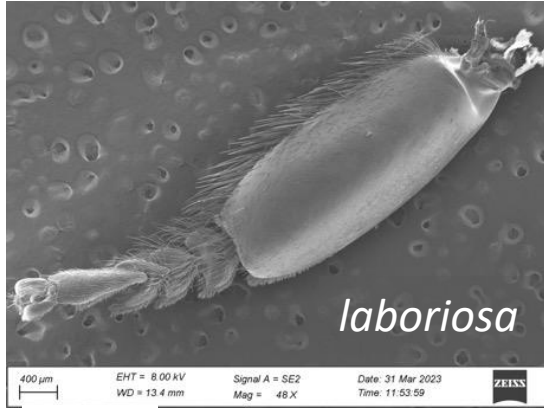
laboriosa



dorsata

- *laboriosa*: Ocelli relatively flat, on plane of head, & widely spaced.
- *dorsata* complex: ocelli domed, on strongly raised platform, & close together.

Drone hindleg morphology: Metatarsus length/width

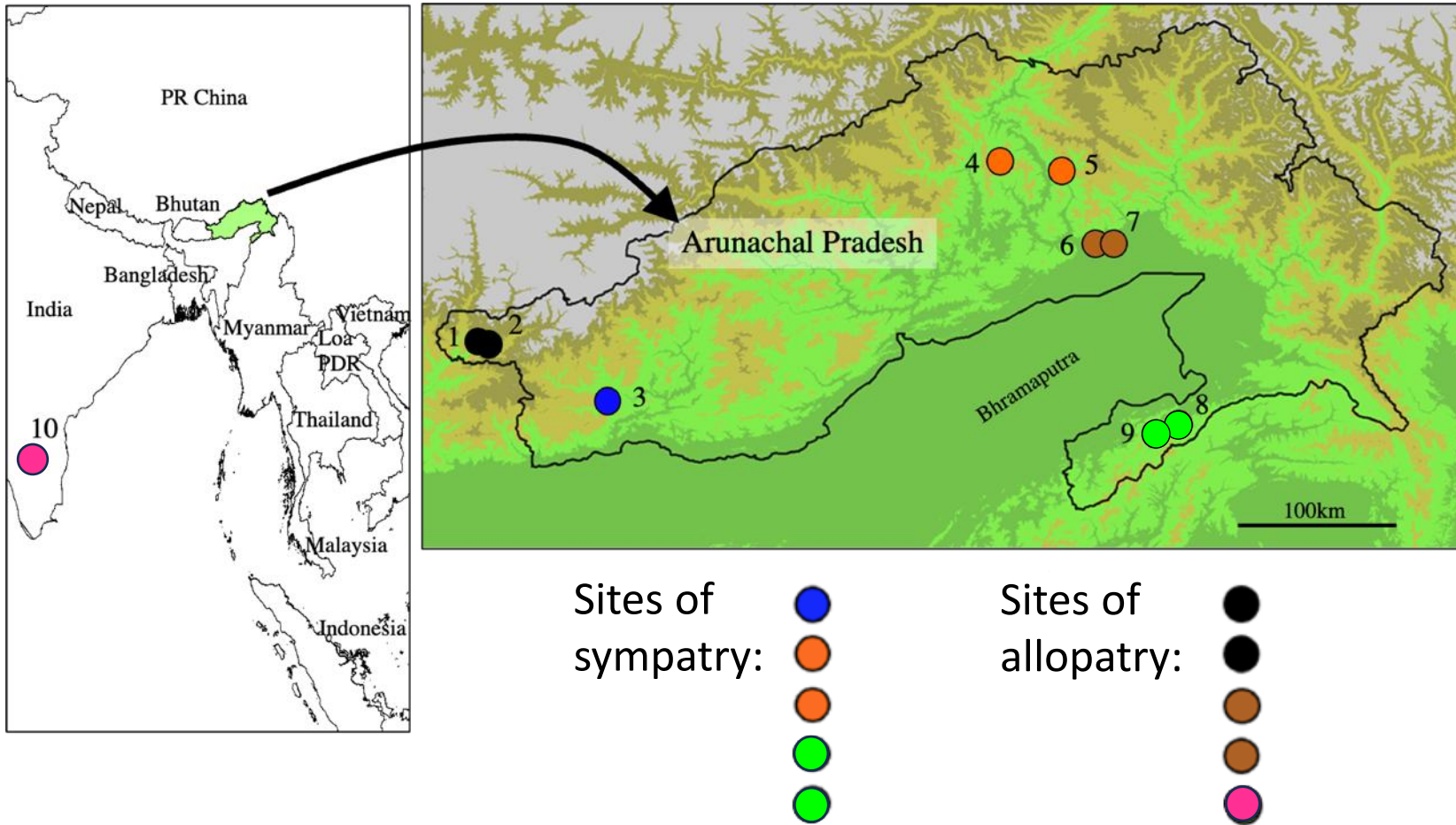


- Ratio of metatarsus length to width separates *laboriosa* from other three taxa
- This character is drone specific

Based on morphological data:
laboriosa meets criteria for valid species.

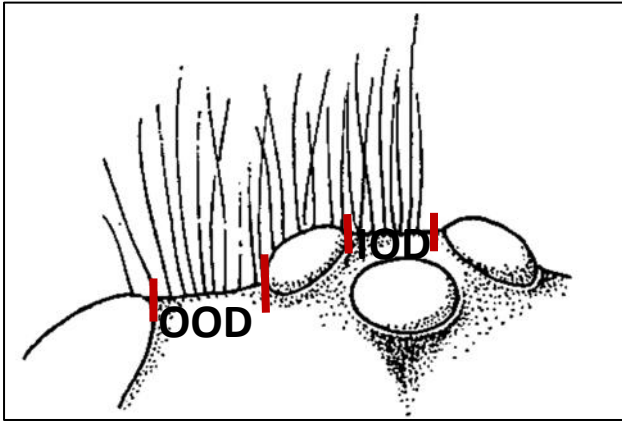


Sympatry of *A. dorsata* and *A. laboriosa*

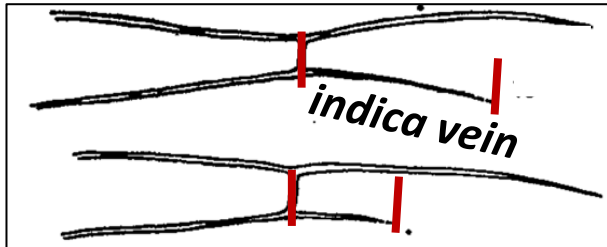


Morphometric analysis of bees: 17 Characters

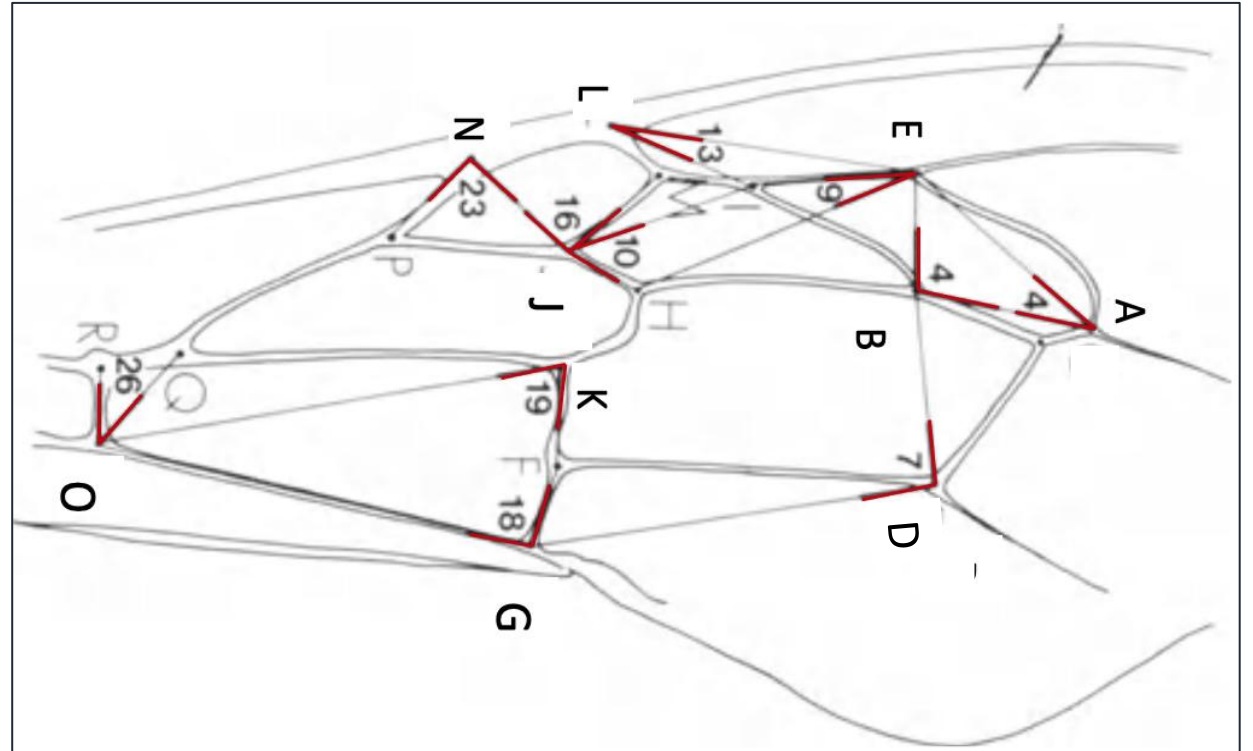
Malar length and width



Distances between ocelli and eyes

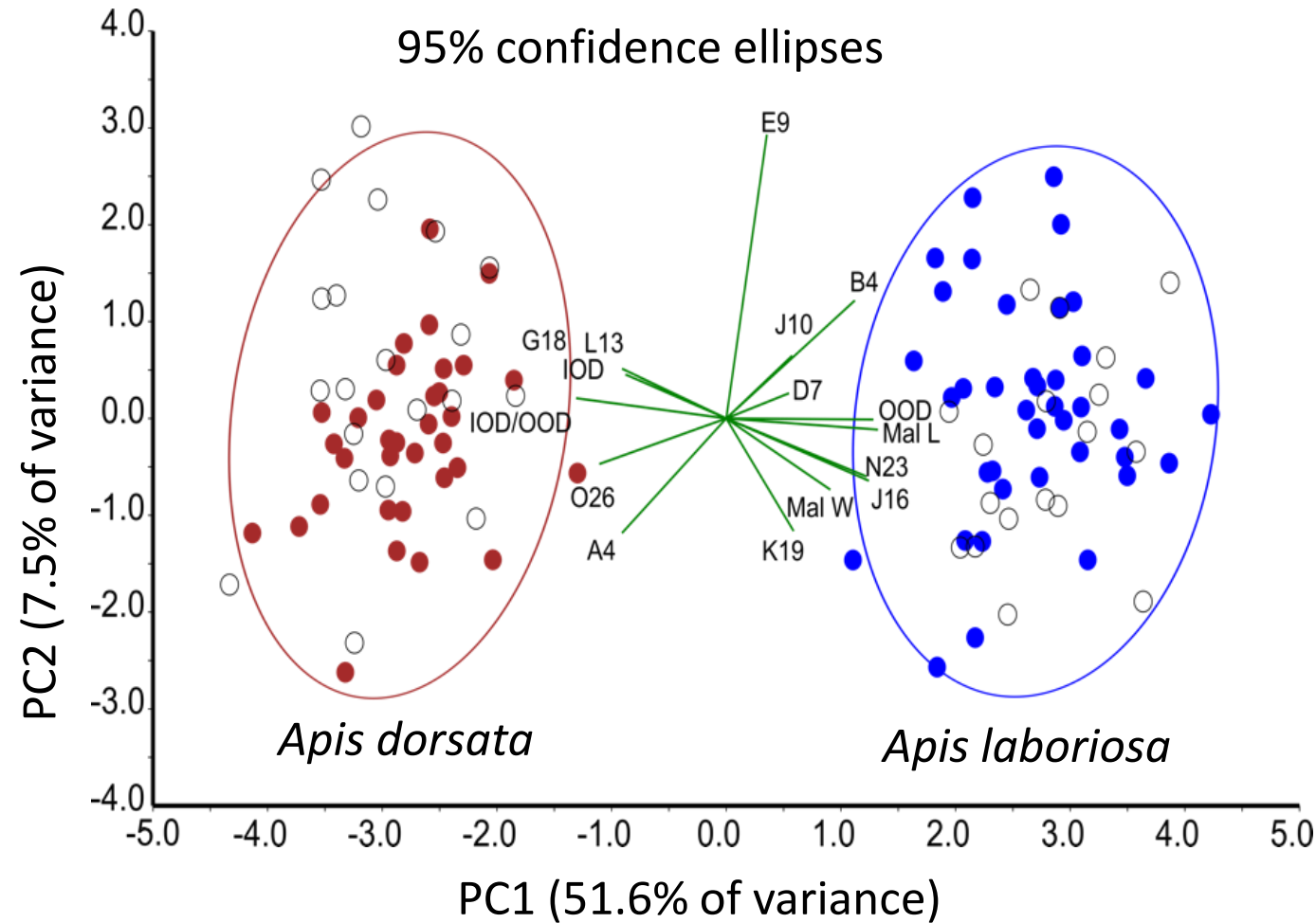


Length of indica vein (HW)



Wing vein angles (Forewing)

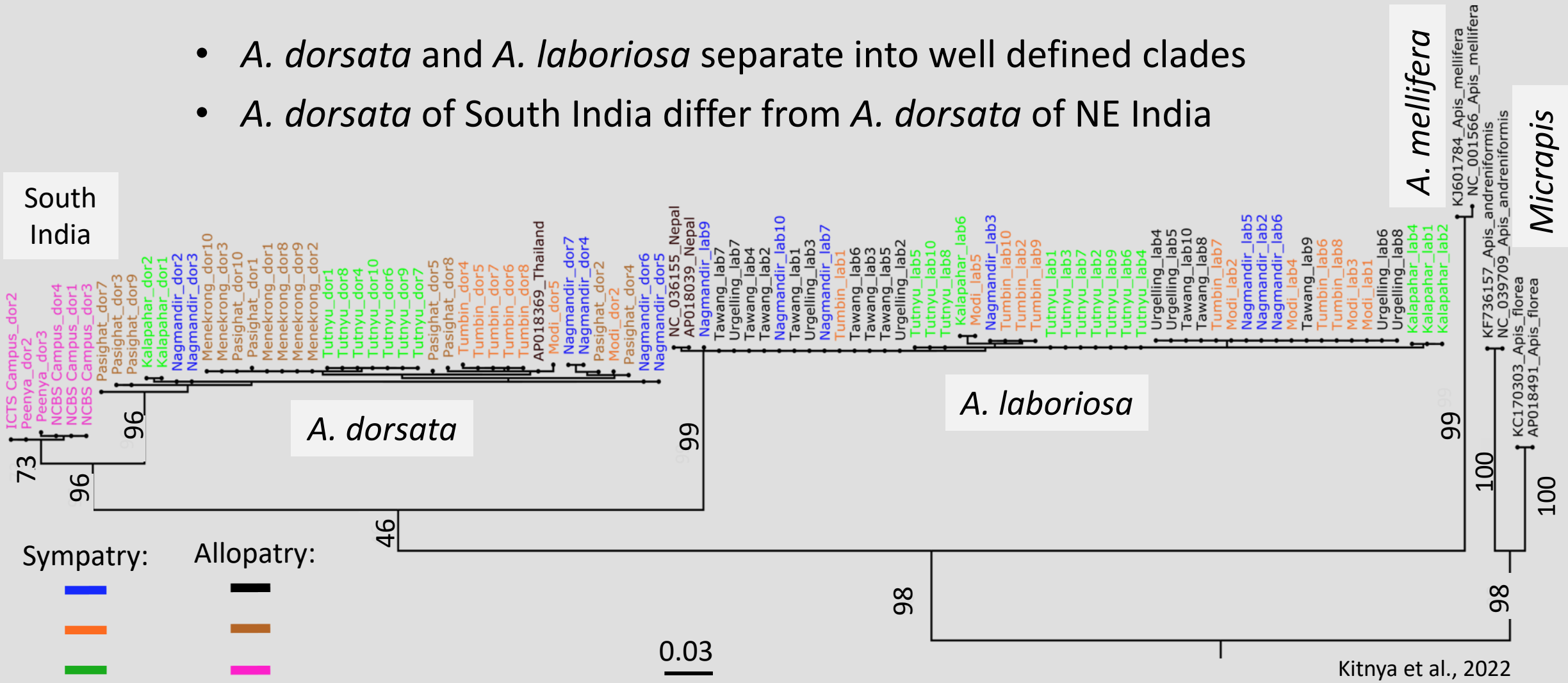
Principal component analysis of 16 size independent traits



**Solid dots =
samples from sites
of sympatry**

Cladogram based on 500 bp DNA sequence: mtDNA cytochrome oxidase subunit I

- *A. dorsata* and *A. laboriosa* separate into well defined clades
- *A. dorsata* of South India differ from *A. dorsata* of NE India



Next: the “*dorsata* complex: 1, 2, or 3 species?

dorsata: anterior of abdomen
always orange or yellow

Island taxa: abdomens lack
yellow/orange; white abdominal bands



dorsata



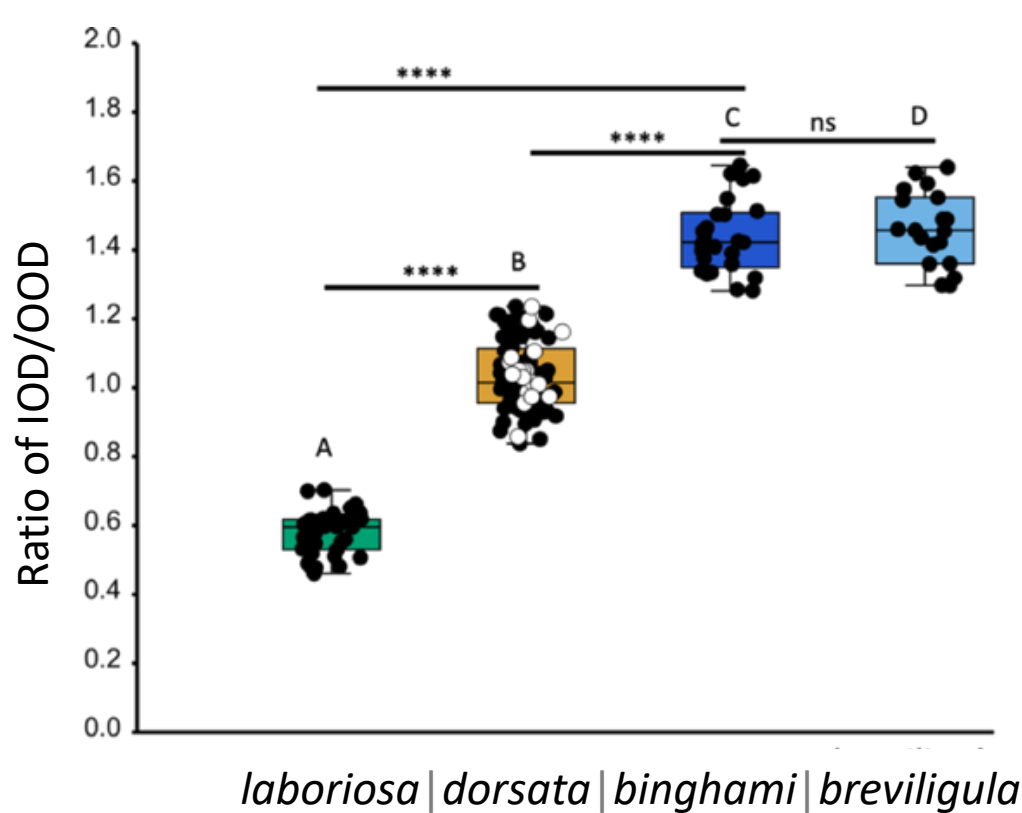
binghami



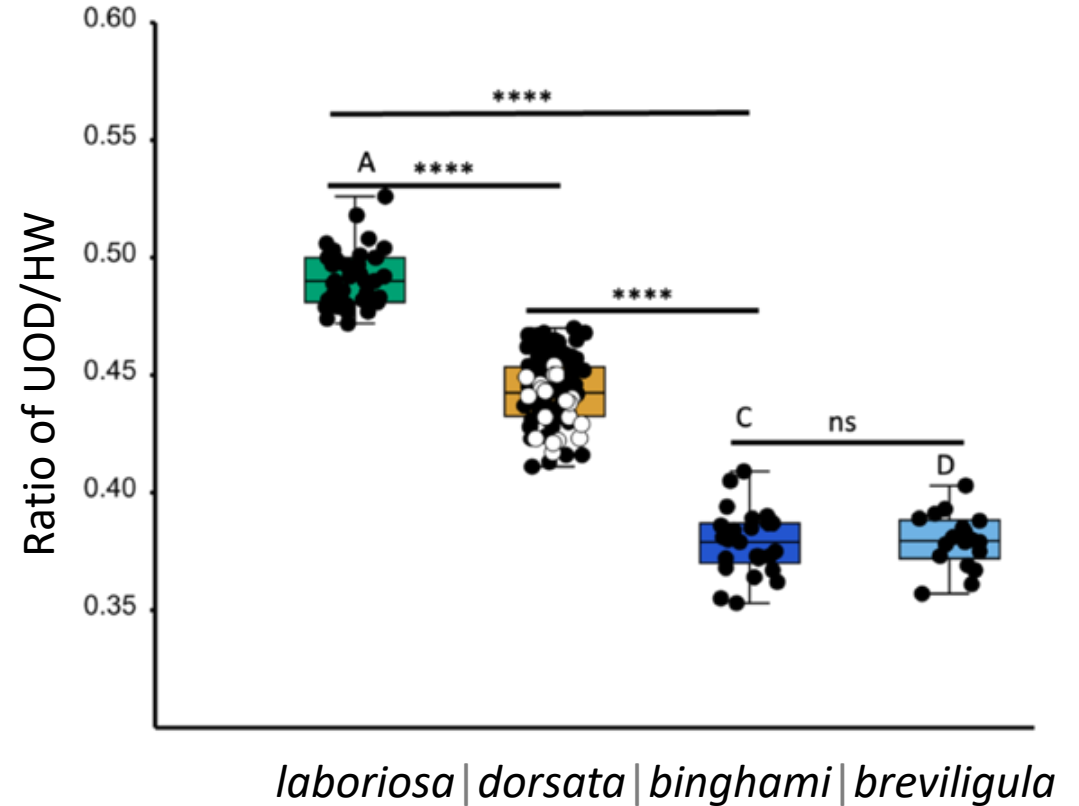
breviligula

dorsata vs. *binghami* + *breviligula*:

Worker: Head morphology



Spacing of ocelli: IOD/OOD

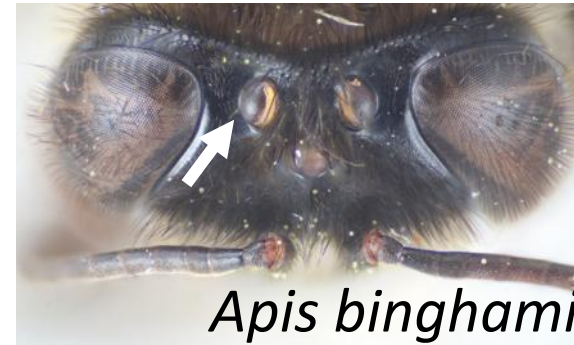


Upper ocular distance/head width

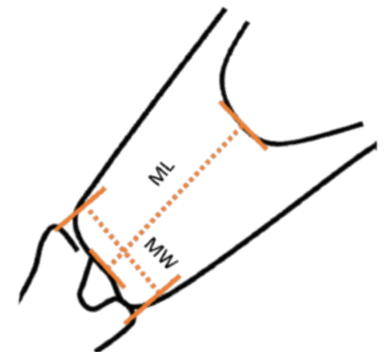
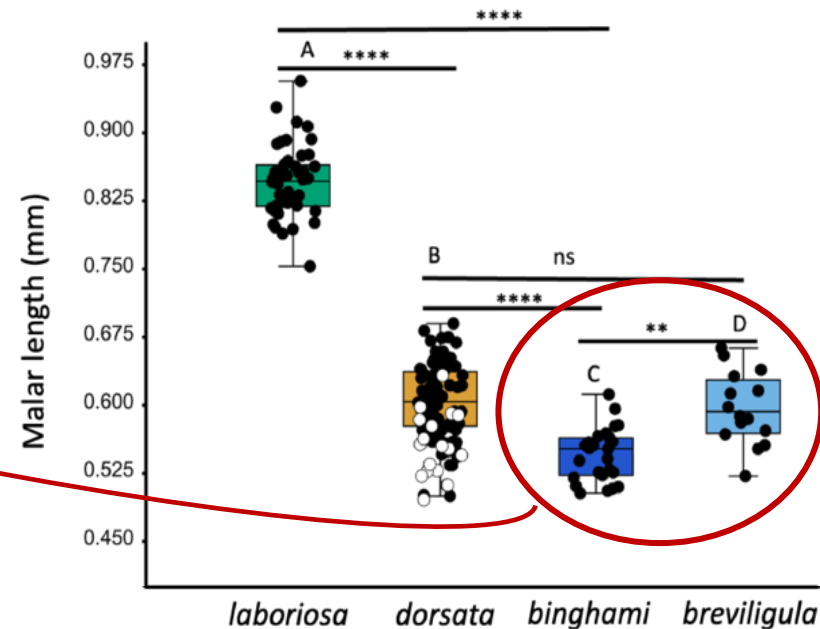
binghami vs. *breviligula*: Worker: Head morphology

These taxa were classified together as *A. binghami* until Maa (1953) named *Megapis breviligula* on the basis of:

- i) **Less strongly raised ocelli** ✓
- ii) **Longer malar area** ✓
- iii) **Sternite 5**
- iv) **Short tongue in *breviligula***



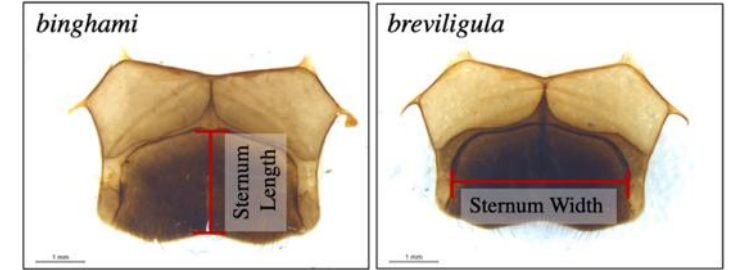
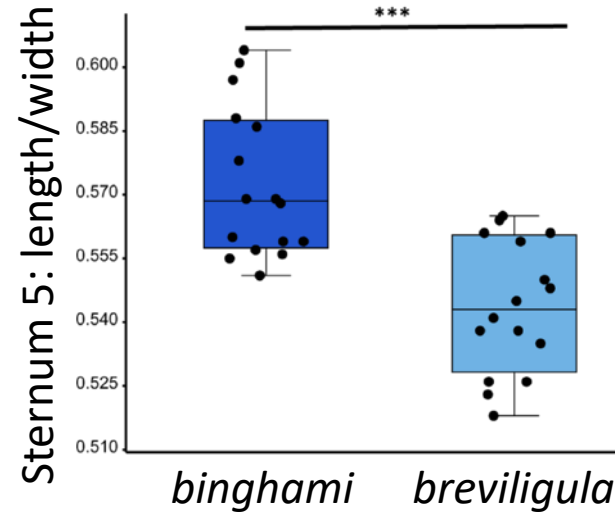
The malar area is statistically longer in *breviligula* than in *binghami*.



binghami vs. *breviligula*: Worker: Sternum 5 & Tongue length

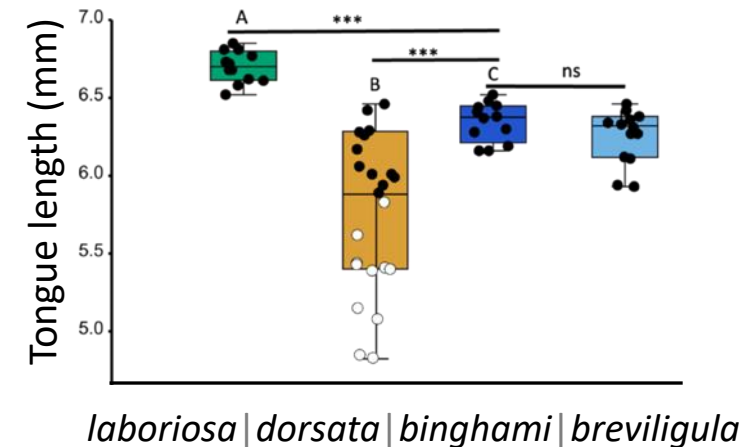
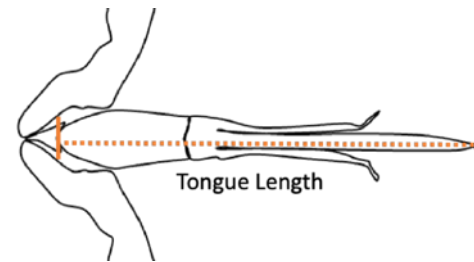
iii) Shorter & broader abdominal sternite 5 ✓

The ratio of length to width of sternum 5 partially separates *binghami* from *breviligula*



iv) Short glossa X

- Tongue of *breviligula* is not shorter than *binghami*, unlike previous report (Maa 1953)
- Tongue length is NOT a good character!



Summary / Conclusions: *Apis laboriosa*

***Apis laboriosa* and *A. dorsata*:**

Species status is supported by:

- Several autapomorphies of *laboriosa* workers: ocellar characters, UOD/HW, malar length, sternites 2 & 3, spiracular plate.
- Drones: ocellar shape, ocelli raised / not raised, shape of metatarsus.
- Partial sympatry, with no evidence of hybrids (morphometrically or genetically).

Apis laboriosa is
a valid species



Photo: Nyaton Kitnya, NE India

Summary / Conclusions: the *dorsata* complex

Apis dorsata “complex”:

- Allopatric distributions; no opportunity to interact.
- *dorsata* differs in colour, IOD/OOD, and head proportions (UOD/HW) from *breviligula* & *binghami*.
- Ocelli of *binghami* are slightly more raised than *breviligula*; sternum 5 differs slightly in length vs. width.
- Tongue length do not seem to differ between *binghami* and *breviligula*.
- Morphological evidence confirms that *dorsata* is distinct from [*binghami* + *breviligula*]; species name is ***Apis binghami***.
- Failed to differentiate *binghami* from *breviligula* as 2 species.



dorsata



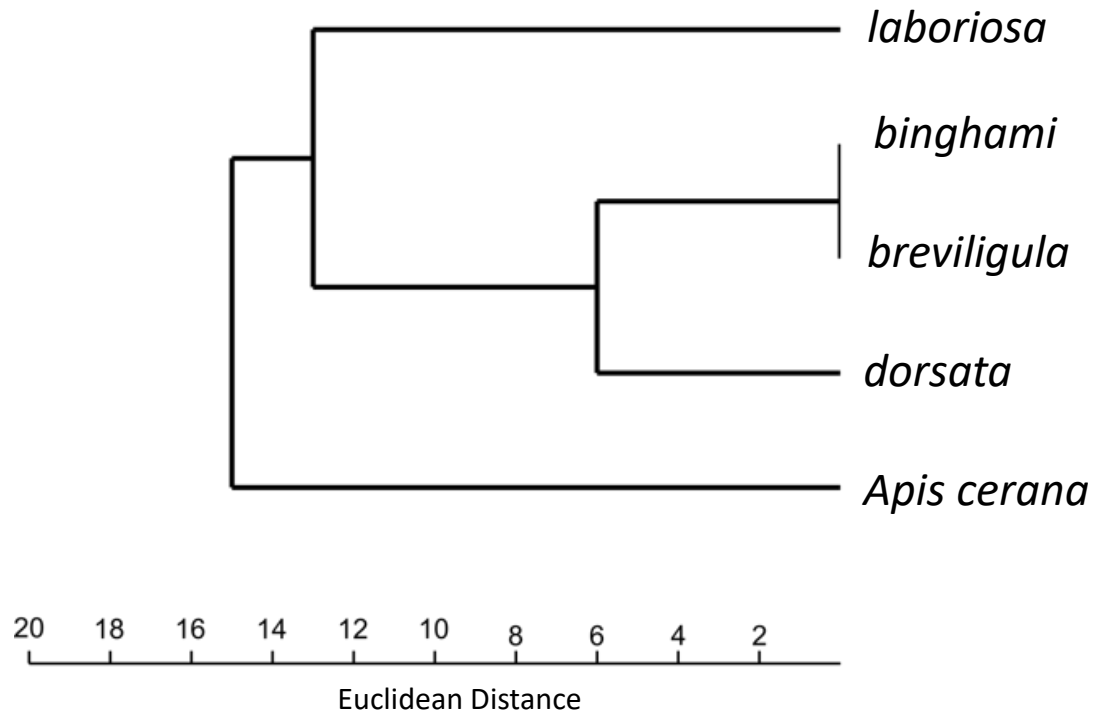
binghami



breviligula

Summary / Conclusions

We hypothesize the current evidence supports 3 *Megapis* species:



Cladogram based on 16 morphological characters

Future work: Genomic Wide Analysis



laboriosa



dorsata



A. binghami binghami
and
A. binghami breviligula

Acknowledgements

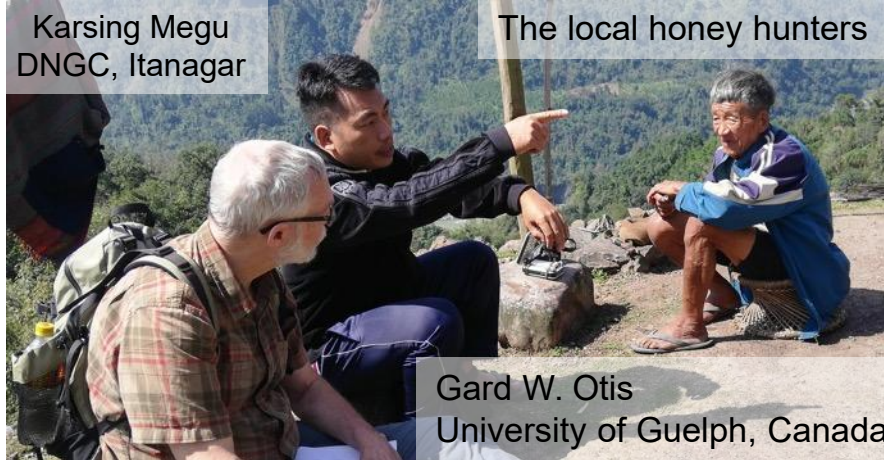
Co-authors



Dr. Axel Brockmann



Prof. Gard W. Otis



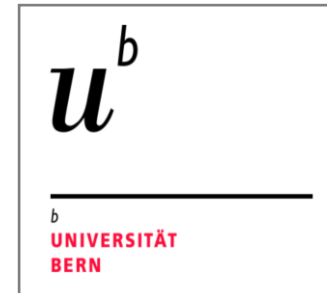
Prabhudev MV, Deborah Smith, Qiu Lifei, Chet Prasad Bhatta, Thai Hong Pham, Savita Chib, Tarun Karmakar, Yeshwant HM, Joby Joseph, Prabhudev MV, Vinay Hedge, Bharat, and Jaya Narah.



NGS Facility and
Research Collection
Facility, NCBS-
TIFR, Bangalore



Indira Gandhi Government
College (IGGC)



Trivedi School of
Biosciences



UNIVERSITY OF
GUELPH