

Phylogenetic and Morphological Characteristics Reveal Cryptic Speciation in Stingless Bee, *Tetragonula laeviceps* s.l. Smith 1857 (Hymenoptera; Meliponinae)

ABSTRACT

Tetragonula laeviceps sensu lato (s.l.) Smith 1857 has the most complicated nomenclatural history among the *Tetragonula* genera. The objective of this study was to investigate whether *T. laeviceps* s.l. individuals with worker bees are grouped in the same or nearly the same morphological characteristics and have similar COI haplotype cluster groups. A total of 147 worker bees of *T. laeviceps* s.l. were collected from six sampling sites in Sabah (RDC, Tuaran, Kota Marudu, Putatan, Kinarut and Faculty of Sustainable Agriculture (FSA)), but only 36 were selected for further studies. These specimens were first classified according to the most obvious morphological characteristics, i.e., hind tibia color, hind basitarsus color and body size. Group identification was based on morphological characteristics important for distinguishing the four groups within *T. laeviceps* s.l. The four groups of *T. laeviceps* s.l. had significantly different body trait measurements for the TL (total length), HW (head width), HL (head length), CEL (compound eye length), CEW (compound eye width), FWLT (forewing length, including tegula), FWW (forewing width), FWL (forewing length), ML (mesoscutum length), MW (mesoscutum width), SW (mesoscutellum width), SL (mesoscutellum length), HTL = (hind tibia length), HTW (hind tibia width), HBL (hind basitarsus length) and HBW (hind basitarsus width) ($p < 0.001$). Body color included HC (head color), CC (clypeus color), ASC (antennae scape color), CFPP (Clypeus and frons plumose pubescence), HTC (hind tibia color), BSC (basitarsus color), SP (leg setae pubescence), SP (Thorax mesoscutellum pubescence), SPL (thorax mesoscutellum pubescence length) and TC (thorax color) ($p < 0.05$). The most distinctive features of the morphological and morphometric characteristics measured by PCA and LDA biplot that distinguish Group 1 (TL6-1, TL6-2 and TL6-3) from the other groups were the yellowish-brown ASC and the dark brown TC. Group 2 (haplotypes TL2-1, TL2-2 and TL2-3 and TL4-1, TL4-2 and TL4-3) had a dark brown ASC and a black TC, while Group 3 (haplotypes TL11-1, TL11-2 and TL11-3) had a blackish-brown ASC, a black TC and the largest TL, FWW and FWL. As for phylogenetic relationships, 12 out of 36 haplotypes showed clear separation with good bootstrap values (97–100%). The rest of the haplotypes did not show clear differentiation between subclades that belonged together, regardless of their morphology and morphometric characteristics. This suggests that the combination of DNA barcoding for species identification and phylogenetic analysis, as well as traditional methods based on morphological grouping by body size and body color, can be reliably used to determine intraspecific variations within *T. laeviceps* s.l.