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NOTES AND COMMENTS

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A novel parasitic beetle, *Procoryphaeus violaceus*, in stingless bees, *Tetragonula pagdeni*

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ABSTRACT

Two beetle parasites of social bees are known to cause colony collapse: small hive beetles, *Aethina tumida,* in honey bees and *Haptoncus luteolus* in stingless bees. Here, we report for the first time parasitic beetles, *Procoryphaeus violaceus,* causing colony collapse in stingless bees, *Teteragonula pagdeni*.

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In March 2023, beekeepers detected that seven out of 54 *Tetragonula pagdeni* colonies in Songkhla province (Thailand) had collapsed due to *P. violaceus* infestations (Figure 1(a)). Careful visual inspections of the collapsed colonies revealed that none of them had brood, but all had damaged honey and pollen pots as well as cerumen present. Three colonies also still had a small number of adult bees (<100; Figure 1(b)). All collapsed colonies were highly infested with *P. violaceus* larvae ($N > 100$) throughout the entire hives. We further examined the collapsed colonies in the field (Neumann et al., 2013) and found three adult beetles of *P. violaceus*, but neither eggs nor pupae. Three of the seven infested colonies were randomly selected and brought to the laboratory for thorough dissections. In addition, three non-collapsed colonies were screened in the field as described above and no larvae, pupae or adult *P. violaceus* were detected and the colonies were considered healthy (Grüter, 2021).

The laboratory colony dissections included carefully combing through the entire hive boxes and content (i.e., cerumen, honey, and pollen pots as well as slimy degraded hive material) and recording the total number of *P. violaceus* eggs, larvae, pupae, as well as adults present (Figure 2). No living adult bees or brood patches were found indicating prior prepared absconding similar to heavily small hive beetle infested *A. mellifera* colonies (Neumann & Elzen, 2004). Across all colonies, a total of 853 larvae ($N = 398$, 148, and 307 per colony), eight pupae ($N = 1$, 0, and 7 per colony), and 5 adult *P. violaceus* ($N = 1$, 1, and 3 per colony) were found, but no eggs. Most larvae (~70%) were found within the pollen pots apparently feeding on pollen (Figure 1(c)), whereas the remaining larvae were found along the bottom of the hive within wax and cerumen patches. All three colonies had slimy and/or partially destroyed wax, cerumen, honey pots, and pollen pots, thereby resembling clinical symptoms of small hive beetle infestations (Neumann & Elzen, 2004). Interestingly, all beetle pupae were exclusively found in distinct bulges ($N = 8$) in the cerumen and wax along the bottom surface of the three nest boxes (Figure 1(d)). A subsample of beetle larvae, pupae as well as adult samples were taken from all colonies and stored in RNAlater until further use.

Three beetle specimens were compared morphologically with specimens conserved in the Georg Frey collection at Naturhistorisches Museum Basel

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D.W.C. and L.S. were responsible for conception and design. Material preparation and data collection were performed by D.W.C., L.S., & W.C. DNA barcoding was conducted by O.Y. and morphological species identification by C.G. J.M. and W.C., were responsible for research organization as well as providing laboratory material and infrastructure in Thailand. P.N. provided laboratory material and facilities in Switzerland. The first draft of the manuscript was written by D.W.C and L.S. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

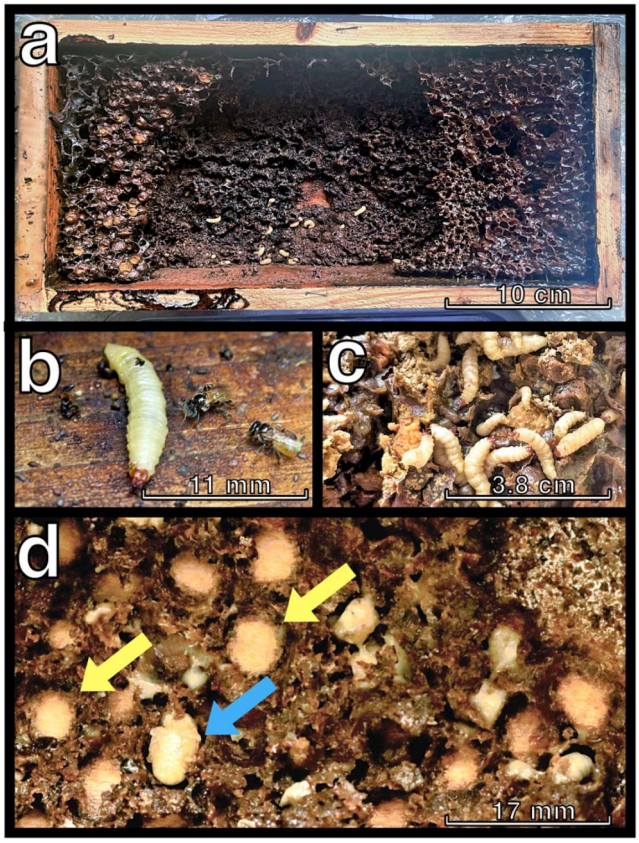

Figure 1. *Tetragonula pagdeni* colonies infested with *Procoryphaeus violaceus*: (a) A collapsed parasitized colony; (b) *T. pagdeni* workers with *P. violaceus* larvae; (c) *P. violaceus* larvae feeding in pollen pots; (d) *P. violaceus* pupation sites at bottom of the hive boxes. The blue arrow indicates a pupa, whereas yellow arrows indicate empty pupation sites from which adults most likely emerged.

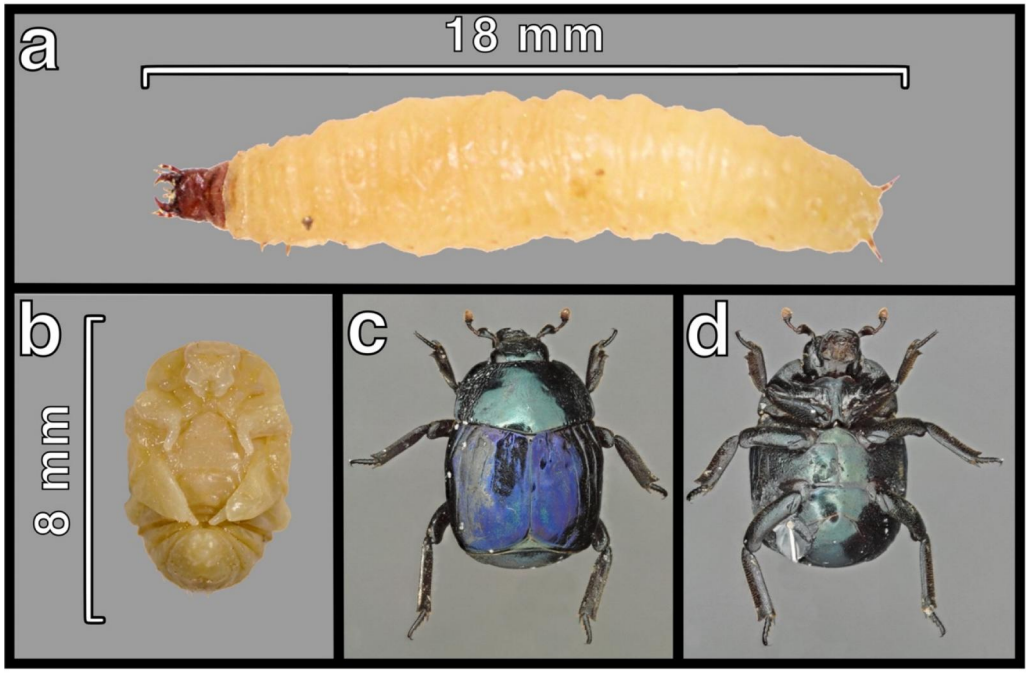

Figure 2. Developmental stages of the beetle *Procoryphaeus violaceus*: (a) Larva (lateral), (b) pupa (ventral), as well (c) adult dorsal and (d) adult ventral.

and the recent revision by Lackner (2015) was used for a detailed check. Furthermore, a high resolved stacking photo was used for a critical review of one of the specimens by Thomas Lackner (Bavarian State Collection of Zoology) (Figure 2(c,d)). Following Folmer et al. (1994), DNA barcoding on a subsample

of larvae, pupae, and adults was conducted to confirm that larvae and pupae were of the same species as the adults. DNA was extracted from the specimen using a Nucleospin® Tissue kit (Macherey–Nagel, Germany) and following the suppliers' recommended protocol. The PCR products were cleaned using a NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Germany) and commercially sequenced by Fasteris SA™ (Geneva, Switzerland). The DNA barcoding revealed that all samples were of the same species, yet no matches were found on either GenBank or Barcode of Life database (BOLD). The COI sequences were submitted to both databases. BOLD accessions include full collection details along with diagnostic images (Figure 2) of the specimens produced by using a Zeiss Stemi 508 stereomicroscope and a Canon D600 camera (Figure 2(a,b)).

To our knowledge, this is the first report of *P. violaceus* in association with social bees and infestations leading to *T. pagdeni* colony collapse. The genus *Procoryphaeus*, ($N = 3$ species) belongs to the family Histeridae and is known to occur in Malaysia, Indonesia as well as Thailand (Lackner, 2015). Other Histeridae beetles are known as scavengers, often feeding on decaying organic matter (e.g., Hawkeswood, 2011) and can be associated with social bees where they predate on larvae and pupae as well as feed of dead adult stingless bees yet these attacks usually do not lead to colony collapse (Carvalho et al., 2021). Interestingly, adult *Procoryphaeus wallacei* were observed attacking *Tetragonula minangkabau*, colonies in Indonesia, but colony collapse was not reported (Inoue et al., 1993). The presence of pupae suggests that *P. violaceus* can complete its entire life cycle within colonies of *T. pagdeni* similar to *Haptoncus luteolus* in *Tetragonula laeviceps* (Krishnan et al., 2015). Possibly, the seven *T. pagdeni* collapsing colonies were weak or stressed, analogous to *A. tumida* (Neumann et al., 2016), but mostly established healthy *T. minangkabau* colonies were attacked by adult *P. wallacei* (Inoue et al., 1993). Understanding why colony collapse and whether *Procoryphaeus* species can exploit different social bee species remains to be investigated. We thus raise caution that *P. violaceus* may cause damage to the Meliponini industry in Southeast-Asia as well as colonies of other wild and managed social bees. Subsequently, there is an urgent need to improve our understanding of the beetle's biology and behavior as well as how it interacts with its host. Such knowledge will enable the implementation of potential control measures to protect colonies as well as the spread of the parasite.

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Disclosure statement

The authors declare no competing interests.

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