Journal of Apicultural Research

ISSN: 0021-8839 (Print) 2078-6913 (Online) Journal homepage: www.tandfonline.com/journals/tjar20

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To cite this article: Alexis L. Beaurepaire, Lars Straub, Sanchai Naree, Jakkrawut Maitip, Steven Cook, Yanping Chen, Peter Neumann, Gareth S. Powell, James D. Ellis, Jay D. Evans & Guntima Suwannapong (15 Apr 2026): First report of small hive beetles infesting eastern and western honey bee, colonies in Thailand, Journal of Apicultural Research, DOI: 10.1080/00218839.2026.2649510

To link to this article: https://doi.org/10.1080/00218839.2026.2649510

Published online: 15 Apr 2026.

JOURNAL OF APICULTURAL RESEARCH
https://doi.org/10.1080/00218839.2026.2649510

NOTES & COMMENTS

First report of small hive beetles infesting eastern and western honey bee, colonies in Thailand

Alexis L. Beaurepaire[a,b], Lars Straub[b,d], Sanchai Naree[c], Jakkrawut Maitip[d], Steven Cook[e], Yanping Chen[e], Peter Neumann[b], Gareth S. Powell[f], James D. Ellis[g], Jay D. Evans[e] and Guntima Suwannapong[h]

[a]Swiss Bee Research Centre, Agroscope Liebefeld, Switzerland; [b]Institute of Bee Health, University of Bern, Switzerland; [c]Pharmacology and Biopharmaceutical Department, Faculty of Pharmaceutical Sciences, Burapha University, Thailand; [d]Faculty of Science, Energy and Environment, King Mongkut's University of Technology North Bangkok, Thailand, Rayong; [e]United States Department of Agriculture, Agricultural Research Services, Bee Research Laboratory, Beltsville, Maryland, USA; [f]North Carolina State University Insect Museum, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina, USA; [g]Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA; [h]Department of Biology, Faculty of Science, Burapha University, Thailand

ABSTRACT

Small hive beetles (SHBs), *Aethina tumida*, are parasites of social bee colonies endemic to sub-Saharan Africa and have become a widespread invasive species. SHBs have recently established populations in different regions of Asia, infesting both western (*Apis mellifera*) and eastern honey bees (*Apis cerana*) colonies. Here, we report for the first time on SHBs in Thailand. Suspicious adult beetles were collected for morphological and genetic analyses to confirm taxonomy and track the origin of this potential new invasion. Both morphometrics and genetics confirmed that the collected adults were *A. tumida*. The sequenced mtDNA haplotypes match those of SHB specimens from Hawaii and South Africa, but not those from neighbouring Asian countries that have previously been invaded. These results suggest that the invasion originated from a single source and that the pest was transported over long distances, rather than coming from neighbouring countries.

ARTICLE HISTORY
Received 16 September 2025
Accepted 17 February 2026

KEYWORDS
Barcoding; invasive species; morphometrics; phylogeography; small hive beetle

Small hive beetles (SHBs), *Aethina tumida*, are pests of social bee colonies (Neumann & Elzen, 2004). They originate from sub-Saharan Africa but have become a worldwide invasive species (Idrissou et al., 2019). Over the past decade, SHBs have established populations in Asia, including the Philippines (Cervancia et al., 2016), South Korea (Namin et al., 2019), and China (Liu et al., 2021), where this pest was also found in *Apis cerana* colonies. The beetle's ability to invade Asian honey bee colonies is of great concern (Chantawannakul et al., 2016).

In October 2023, beetles resembling *A. tumida* were observed by beekeepers in Eastern Thailand, in the provinces Chantaburi and Chon Buri. Mass reproduction in absconded *Apis cerana* colonies was observed in the infested apiaries. Twenty samples of beetles were collected from each two colonies of *A. mellifera* located in Bang Saen, Chon Buri Province (13°16′47.9″N, 100°55′29.0″ E) and Rayong Province (13°01′34.0″N, 101°21′49.6″E), and 30 beetles from one colony of *A. cerana* located in Bang Saen (13°16′47.9″N, 100°55′29.0″ E) and taken to the laboratory for species identification using morphological and genetic analyses (barcoding) (R Core Team, 2016). The general morphology and size of the collected specimens were analyzed. To do so, the ultrastructure of an SHB male from an *A. mellifera* colony located in Bang Saen was studied using a scanning electron microscope (LEO, LEO 1450 VP) following the procedure described by Suwannapong et al. (2011). Genetic analyses, including DNA extraction and sequencing of a portion of the mitochondrial DNA (mtDNA) Cytochrome Oxidase I (COI) gene of 14 adult specimens collected in Thailand (i.e., 12 in the *A. mellifera* colony of Bang Saen and two in Chantaburi) were conducted following standard methods as described in Liu et al. (2021). In brief, DNA was extracted using a NucleoSpin® Tissue Kit (Macherey-Nagel) and PCR was performed using the AT1904S and AT2953A primers from Evans et al. (2000). The PCR products were sent to Microsynth (Switzerland) for sequencing using the AT1904S primer. Sequences were trimmed using the software Chromas v. 2.6.6 (Technelysium, 2000n.d.). Sequences were then aligned using the ClustalW method, and a Maximum Likelihood phylogenetic tree was constructed using MEGA v. 12 (Kumar et al., 2024). The SHB sequences were then BLASTed against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.

CONTACT Guntima Suwannapong guntima@go.buu.ac.th; guntima@buu.ac.th; beefambuu@gmail.com

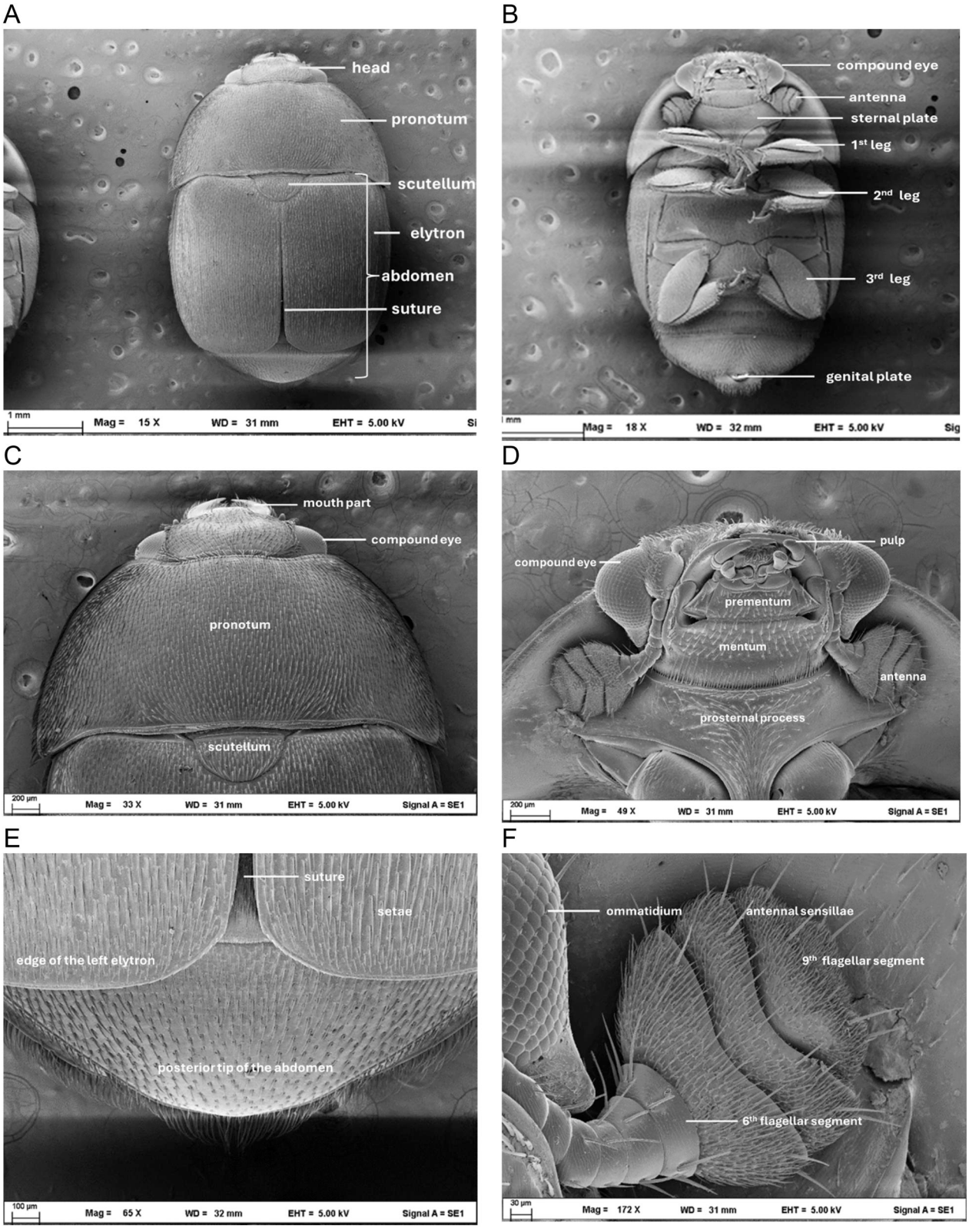

Figure 1. Scanning electron micrographs of *Aethina tumida*.
Adult male SHB collected in a *Apis mellifera* colony in Bang Saen. Dorsal view of (A); ventral view (B); dorsal view of the head and upper thoracic region (C); ventral view of the head and upper thoracic region (D); dorsal view of the posterior tip of the abdomen which covered partially by elytra (E); clubbed antenna of *A. tumida* showing three enlarged distal segments (F).

cgi) to identify the closest known haplotypes. Additional published sequences from previous studies (Duquesne et al., 2017; Granato et al., 2017; Idrissou et al., 2019), including specimens collected in the native and invasive ranges of SHBs, were obtained from the NCBI database and added to the analysis. Notably, sequences from China (Liu et al., 2021) were also included in order to test whether the geographical proximity between the two countries had resulted in the invasion. The aligned sequence file was then used to build a haplotype network using the software PopART (Bandelt et al., 1999).

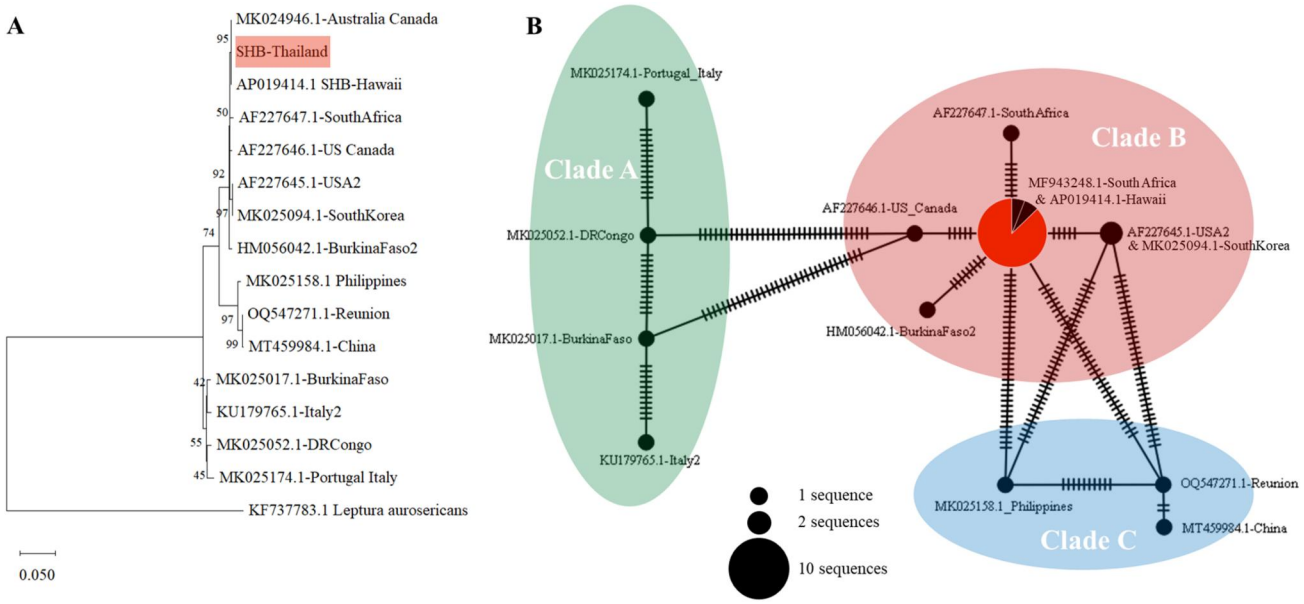

Figure 2. Comparison of small hive beetles (SHBs), *Aethina tumida*, mtDNA sequences from Thailand and other invasive and native populations.

Phylogenetic analysis of *A. tumida* from Thailand. (A) Maximum likelihood tree including sequences generated in this study (highlighted in red) as well as previously described SHB haplotypes with NCBI accession numbers and an outgroup (*Leptura aurosericans*). Numbers next to the nodes represent the statistical confidence associated with the branches (500 bootstraps). (B) COI haplotype network based on SHB samples collected in two apiaries in Thailand (N = 14, in red), as well as previously described haplotypes with NCBI accession numbers (in black). The size of the back and red circles reflects the number of individual sequences within the haplotypes. The hatch marks represent the number of mutations between sequences and nodes. Figure drawn with PopART (Bandelt et al., 1999).

Characteristic features of SHBs were detected through scanning electron microscopy, including the specific antenna structure and distinctive elytral and pronotal patterns of *A. tumida* adults (Figure 1). In parallel, the genetic analysis revealed a single COI haplotype (***Accession number PZ159312***) in the 14 Thai samples analyzed, identical to previously sequenced SHB specimens from South Africa and Hawaii (Figure 2a). Notably, the SHB sample with the closest Asian origin was from South Korea, with five nucleotide substitutions out of 1,022 bp in the sequences compared (Figure 2b).

In summary, the results from the morphological and genetic analyses confirmed that the specimens collected in Thailand are *A. tumida*. Moreover, the genetic analyses suggest that a single introduction from a distant source led to the invasion of the two provinces studied, and not from China as initially suspected. Interestingly, the beekeeper from whom the infested *A. mellifera* colonies were purchased buys their stocks in Taiwan, a country that is not known to be infected to date. This suggests that the infected colonies may have only transited through Taiwan, and that complex invasion routes may be at play. Alternatively, bee products and, in particular, wax trade - a known driver of SHB invasions (Idrissou et al., 2019) - may have played a role in this new introduction. These results highlight the need to regulate bee and bee product trade further to limit the spread of SHBs, and to understand its host range and impacts on native Asian bees better, as evidence shows that the pest can readily infest and mass-reproduce in *A. cerana* colonies.

Acknowledgments

Swiss Bee Research Centre, Agroscope Liebefeld and Institute of Bee Health, University of Bern, Switzerland; Agricultural Research Service, Bee Research Laboratory, USA, for research facilities and additional support.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was funded by the Bee Health and Their Hive Products for Supporting SDGs Research Unit, Department of Biology, Faculty of Science, Burapha University (GS), Thailand, Research Partnership Grant RPG_ASEAN_072022_03 of the Swiss Leading House Asia (AB, GS). Funding was provided by the Vinetum Foundation to PN and LS, and The Swiss National Science Foundation to PN (310030_204479).

ORCID

Alexis L. Beaurepaire http://orcid.org/0000-0002-9832-5609
Lars Straub http://orcid.org/0000-0002-2091-1499
Sanchai Naree http://orcid.org/0000-0001-7177-1863
Jakkrawut Maitip http://orcid.org/0000-0001-6265-1421
Steven Cook http://orcid.org/0000-0003-4927-1402
Yanping Chen http://orcid.org/0000-0002-5224-1100

Peter Neumann http://orcid.org/0000-0001-5163-5215
Gareth S. Powell http://orcid.org/0000-0003-0847-2718
James D. Ellis http://orcid.org/0000-0003-0318-8646
Jay D. Evans http://orcid.org/0000-0002-0036-4651
Guntima Suwannapong http://orcid.org/0000-0002-3180-4103

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