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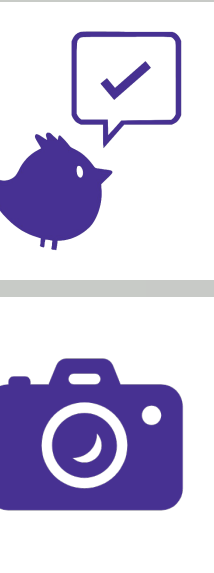
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Data Availability

The data associated with this publication are available upon request.



Applying Geometric Morphometrics to Identify Bee Species in the Genus *Halictus* (Hymenoptera: Halictidae) and to Quantify Island-Mainland Variation Within Species



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Introduction

Native bees play essential roles in their ecosystems, and accurately tracking bee diversity is important for understanding ecological change. Yet, identifying bees for research projects and recognizing variation in populations is difficult for non-experts. The objectives are:

- Find an accurate and inexpensive method of bee identification and detection of population variation
- Test morphometrics and landmark homologous wing venation patterns for identification and variation detection
- Analyze variation in landmarks between species within one genus
- Analyze variation between island and mainland specimens within one species

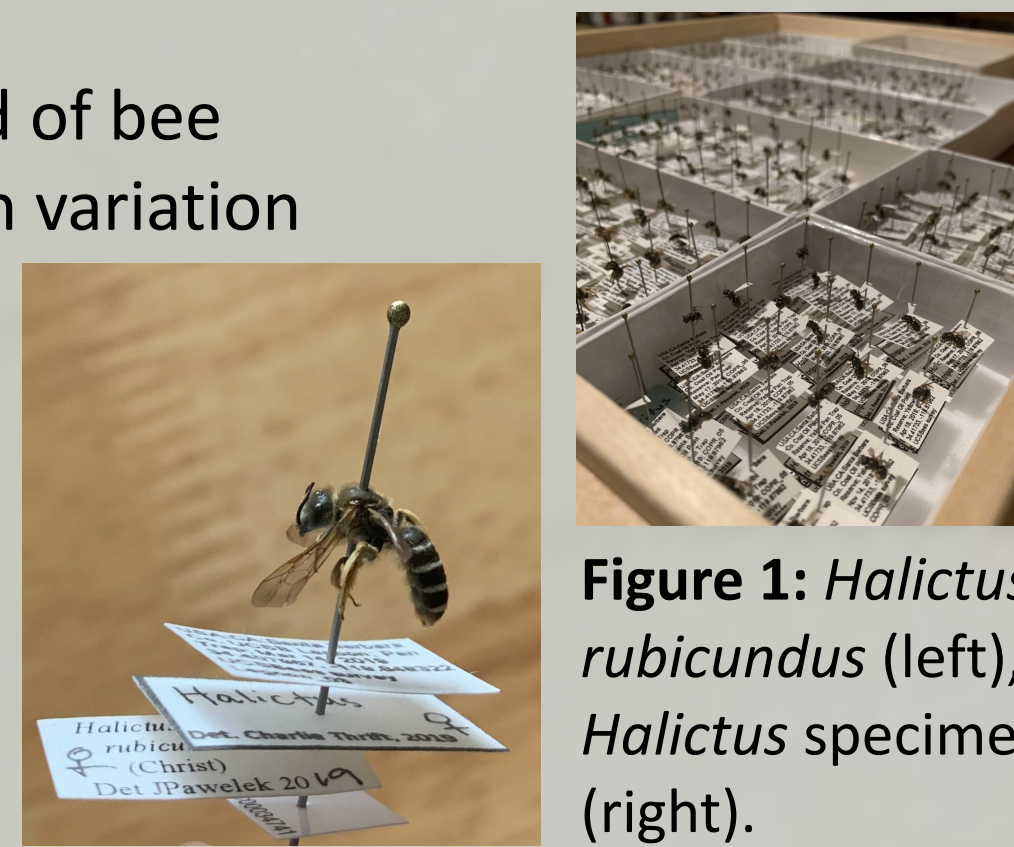


Figure 1: *Halictus rubicundus* (left), *Halictus* specimens (right).

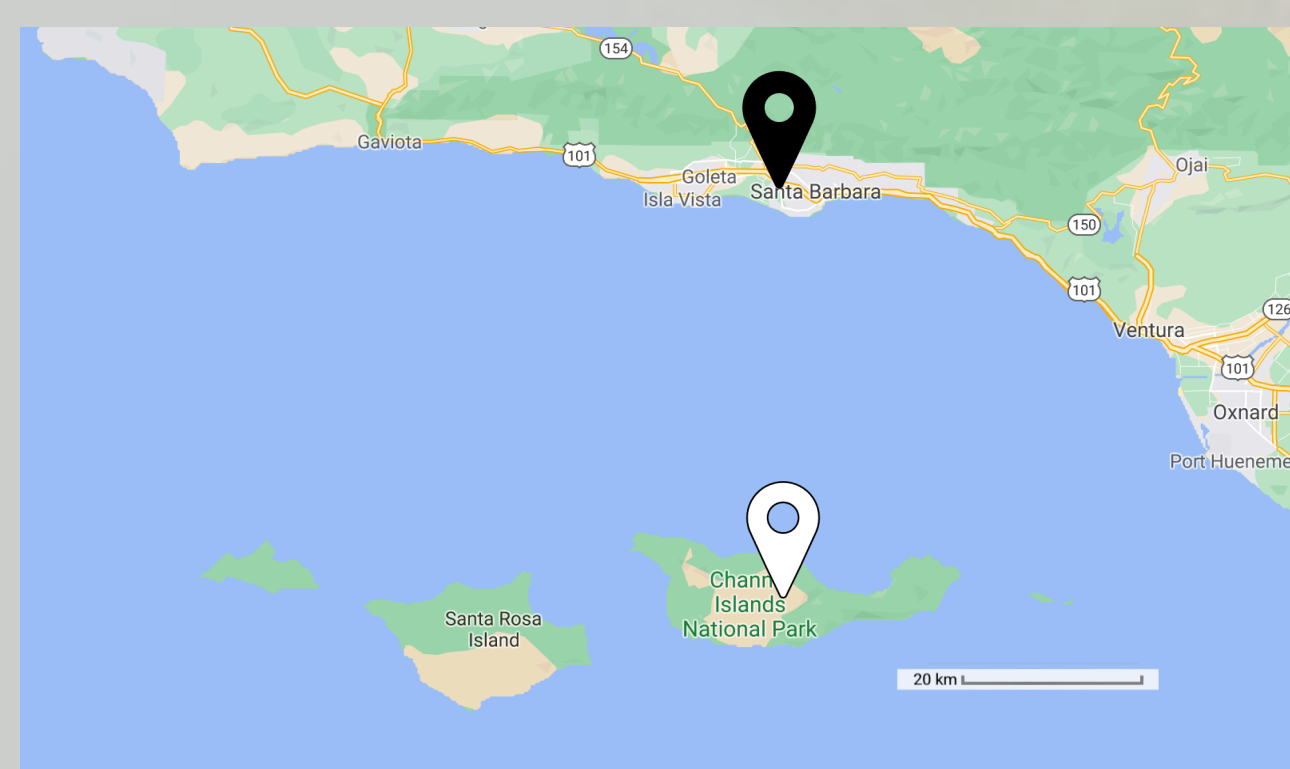
Methods

The Cheadle Center for Biodiversity and Ecological Restoration (CCBER) houses the University of California Santa Barbara Invertebrate Zoology Collection. The bees used in this study came from that collection, including four species of

Species	Total Specimens	Mainland Specimens	Island Specimens
<i>Halictus tripartitus</i>	253	171	82
<i>Halictus ligatus</i>	37	Table 1: abundance of <i>Halictus</i> specimens analyzed.	
<i>Halictus farinosus</i>	5		
<i>Halictus rubicundus</i>	4		

genus *Halictus* (*Halictidae*): *H. tripartitus*, *H. ligatus*, *H. farinosus*, and *H. rubicundus*. Bees were collected between 1954 and 2019 (mean 2018, median 2019). *H. tripartitus* was most abundant, as shown in Table 1.

H. tripartitus was collected from both island and mainland areas. Mainland specimens were collected in and around Santa Barbara, a coastal mainland town; island specimens were collected on Santa Cruz Island, a 35-kilometer long island about 32 kilometers off the coast of Santa Barbara in the Pacific Ocean, which creates a physical barrier between mainland and island populations, shown in Map 1.



Map 1: Santa Barbara mainland (black) and Santa Cruz Island (white).

Further Methods

Left forewings were removed from each specimen, slide-mounted, imaged with a DinoXcope stereomicroscope digital camera, then edited to the resulting standard picture shown in Figure 2.

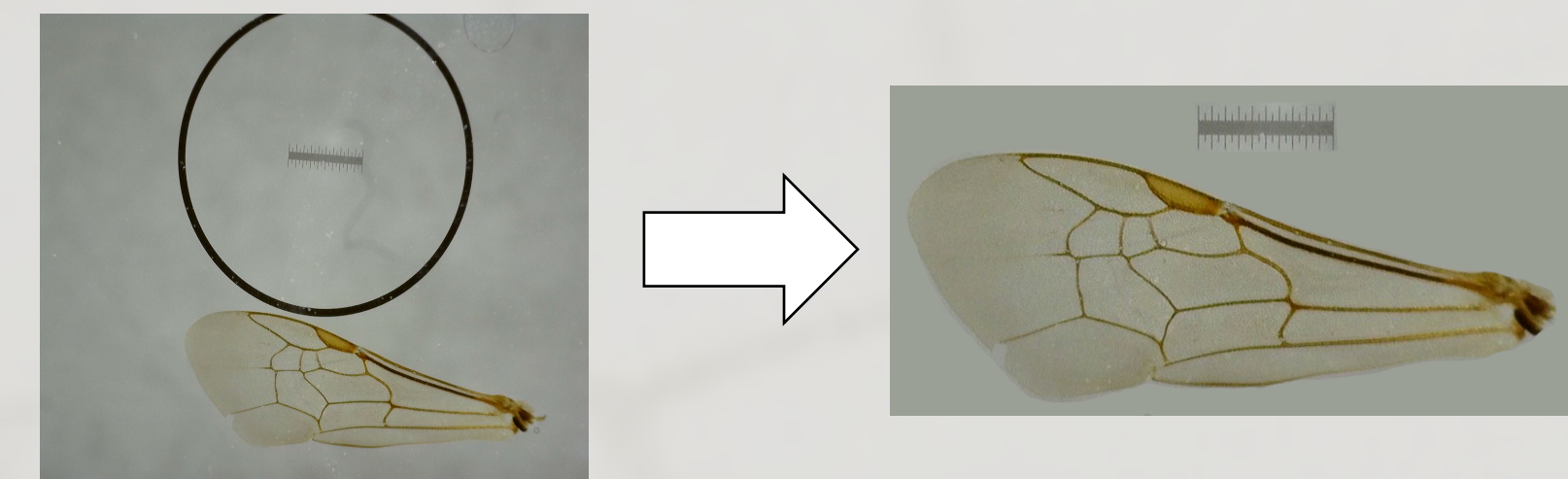


Figure 2: Original photograph (left) and edited image (right).

Homologous wing venation landmarks were plotted using tpsDig software, version 2.31 (Rohlf 2005).

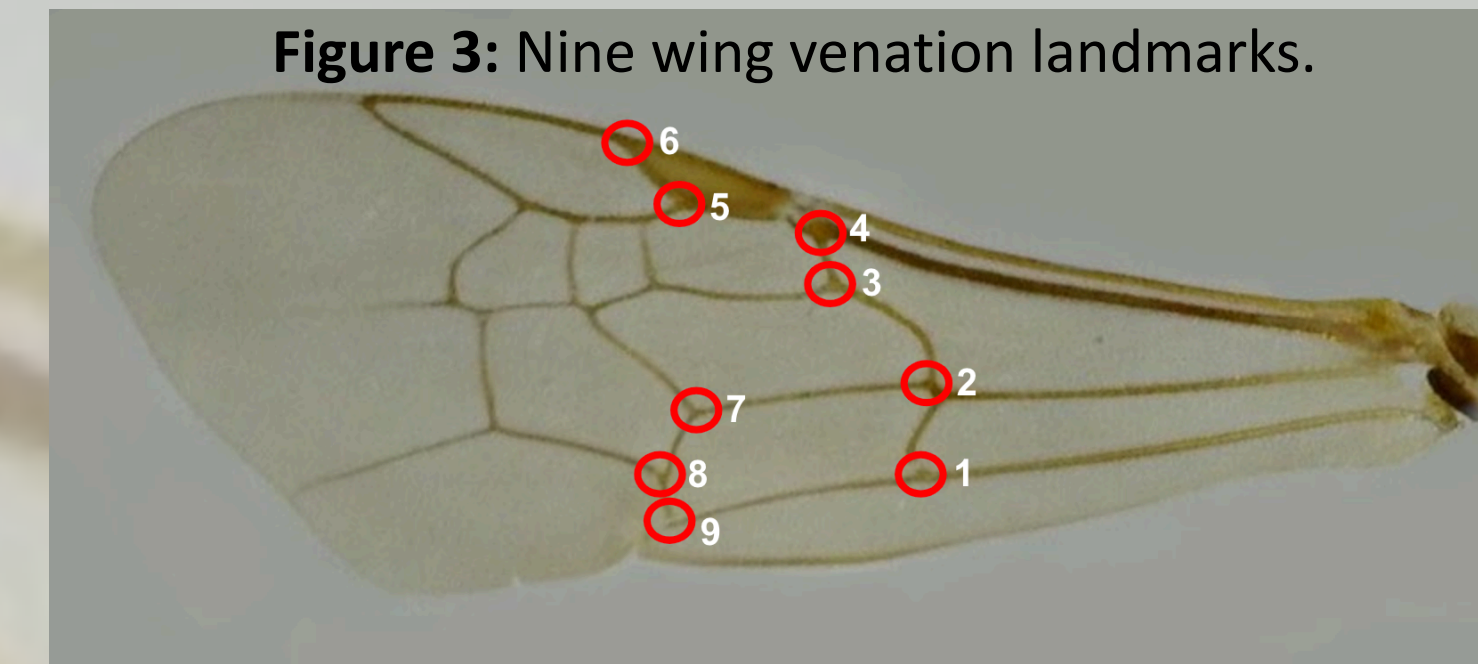


Figure 3: Nine wing venation landmarks.

The nine landmarks used (Fig. 3) are easily observed and common in insect identification. These landmarks were chosen following the study by Rattanawanee *et al.* in 2015.

R package geomorph was used to perform a Generalized Procrustes Analysis of the landmarks. A principal component analysis (PCA) was then run on the resulting coordinates. Eigenvalues and principal factors were extracted, and finally, the resulting PCAs were plotted using R package ggbiplot.

Results

Plotting by species, five principal factors with eigenvalues greater than one were extracted from the PCA analyses. Together, the five components were able to explain 78.00% of the data set variability. The first principle component explained 32.2% of the variation. *Halictus tripartitus* is distinguished from the other species (Fig. 4) and placed in the upper right-hand side. *H. farinosus* has minimal overlap with *H. ligatus* and *H. rubicundus*.

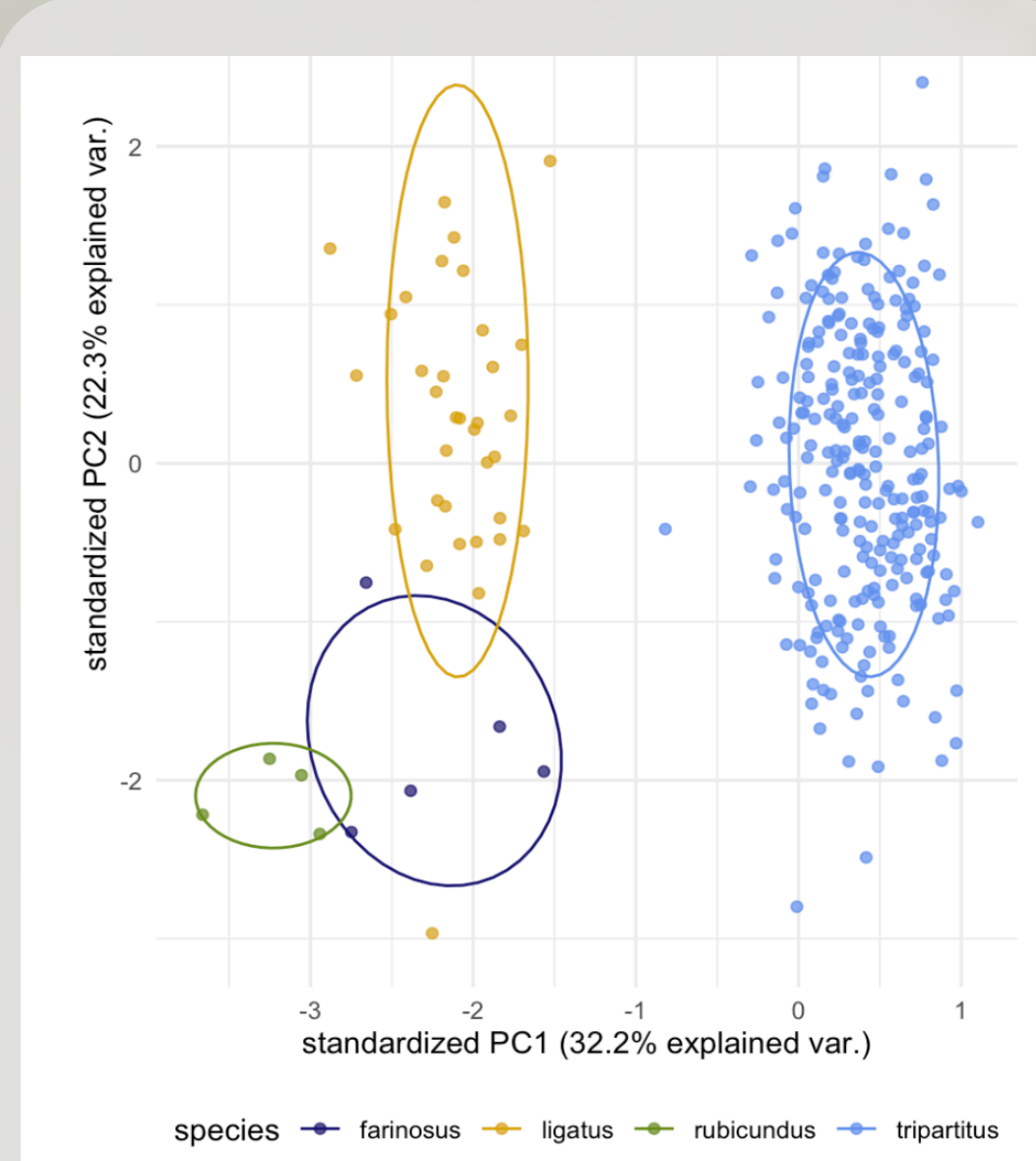


Figure 4: Scatterplot of two most influential factors from principal component analysis of four *Halictus* species, from nine measured landmarks.

The average time to process ten bees was 80 minutes, with 60 minutes for removing wings and imaging, 10 minutes for photo editing, and 10 minutes for digital landmarking, however, all steps took more time at the start of the process.

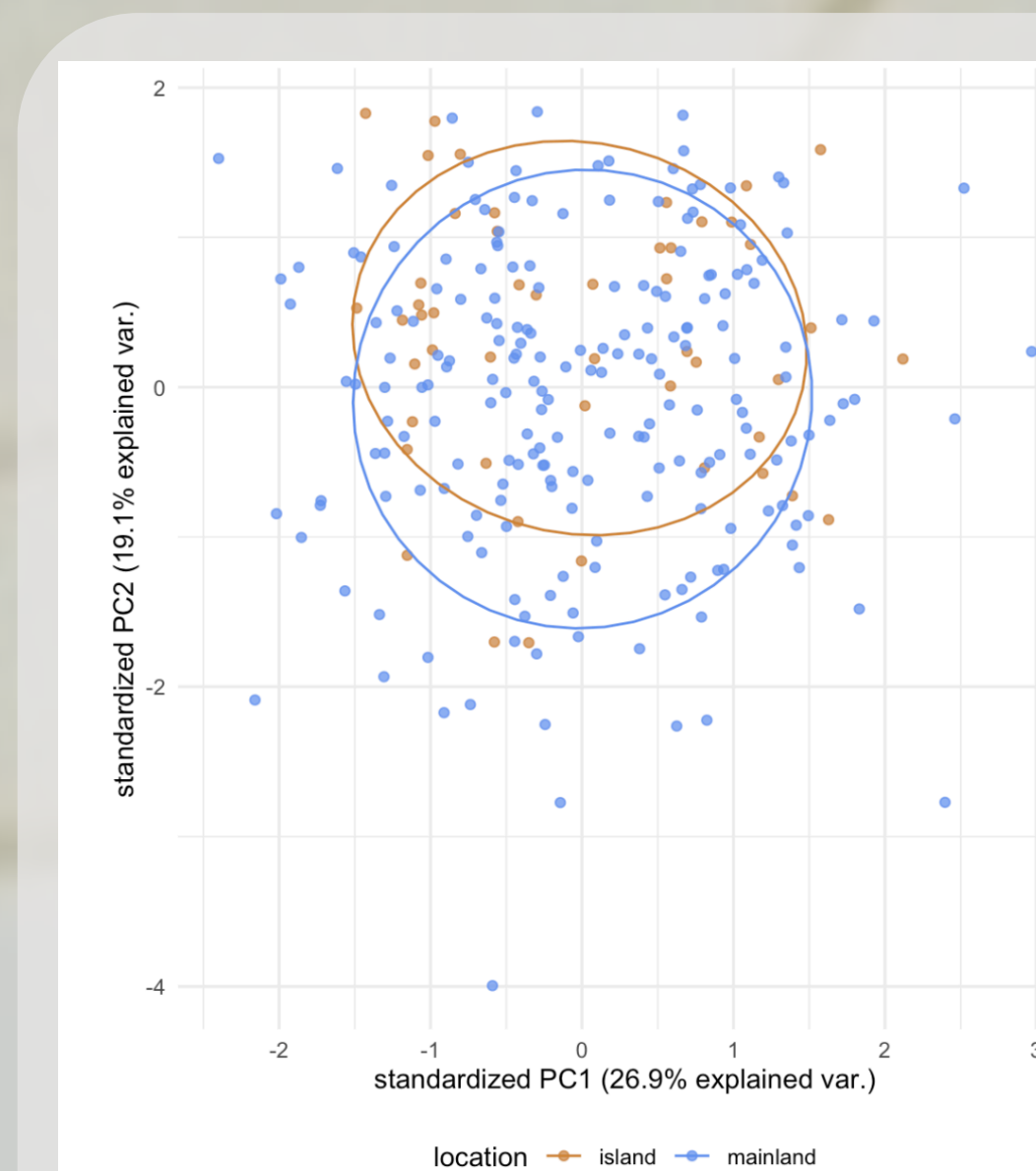


Figure 5: Scatterplot of two most influential factors from principal component analysis of island and mainland *Halictus tripartitus*, from nine measured landmarks.

Plotting by location for *H. tripartitus*, five principal factors with eigenvalues greater than one were extracted from the PCA analyses. Together, the five components were able to explain 72.06% of the data set variability. The first principle component explained 26.9% of the variation. As shown, island and mainland *H. tripartitus* specimens have considerable overlap (Fig. 5).

Discussion

The results show that applying geometric morphometrics to wing venation landmarks can be an effective source of identification for species within the genus *Halictus*, and those with more data have more resolution. However, variation of island and mainland populations within a single species is less clear.

The landmarking procedure, including photographing bee wings, was accomplished remotely and with relatively inexpensive equipment during the COVID-19 pandemic (Figure 6). This procedure is viable for future exploration of the diversity and variation in bees. In addition, it may be useful in supporting identifications in localized areas.



Figure 6: Remote experimental setup

This study would benefit from a larger sample size, particularly of the three species most underrepresented (*H. ligatus*, *H. farinosus*, and *H. rubicundus*).

Future directions of study will include adding specimens to the analysis and testing the accuracy of the plots by comparing undetermined *Halictus* specimens' assigned species to a taxonomist-obtained identification.



Figure 7: *Halictus* specimens

Acknowledgements

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References

- Rattanawanee, A., Duangpakdee, O., Rod-im, P., Hepburn, R. (2015). Discrimination of Two *Tetragonula* (Apidae: Meliponini) Species in Thailand using Geometric Morphometric Analysis of Wing Venation. *Agriculture and Natural Resources*, 49(5), 700-710.
- Rohlf, F.J. (2005). tpsDig, 2.31 ed. Department of Ecology and Evolution, State University of New York. Stony Brook, NY, USA.