Applying geometric morphometrics to assess phenotypic variation in bees

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Introduction

Species-level identification of insects is often challenging and can limit ecological studies, particularly those assessing insect biodiversity. Wing venation characteristics are fundamental in classifying insects, but traditional methods of species identification based on structures with complex geometries is difficult and time consuming. Bees have relatively conserved wing characteristics, but the patterns between groups remain poorly understood. We employed geometric morphometrics to assess variation in wing venation across bee taxa. Geometric morphometrics allows for detailed shape analysis of wing structures, which may provide insights into evolutionary relationships. By digitally landmarking nine homologous wing vein characters in a diverse sample of bees, we quantified and compared phenotypic variation to assess whether the resulting morphological clusters reflect evolutionary divergence and align with established phylogeny. This study examines over fifty primarily local bee species housed at the Cheadle Center's Invertebrate Zoology Collection, representing a broad coverage of the currently recognized bee families.

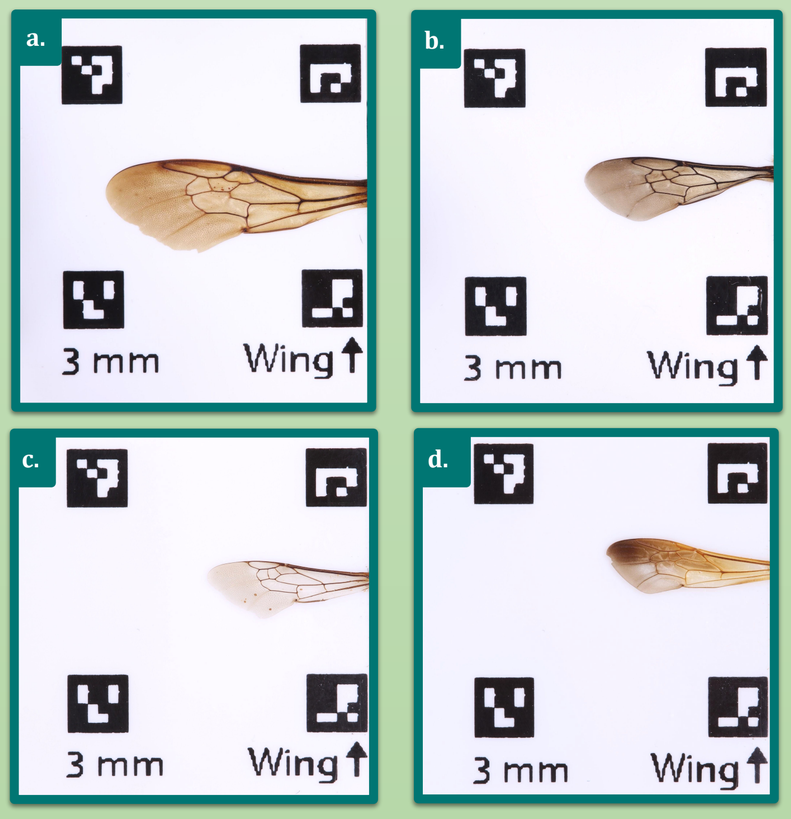

Fig 1. Wing images of different bee species (a. *Bombus vagans,* b. *Eucera frater albopilosa,* c. *Anthophora urbana,* d. *Andrena prunorum*)

Methodology

Imaging

A sample of >20 all-female bee wings across >50 recognized species spanning 4 families were imaged in high resolution using a Canon EOS 80D digital camera (Fig. 1). Non-destructive imaging techniques were developed as a part of this study. Prior to imaging, each specimen is left in a humid "relaxing chamber" for >24 hours in order to restore flexibility and reduce the chance of breakage. This relaxing chamber consists of an air-tight container with paper towels dampened with an even solution of water and ethanol. Each pinned bee specimen is positioned adjacent to a stage with an imaging card with a printed standardized scale bar. The wing is positioned on top of the card and flattened with a coverslip in order to photograph the wing in a planar view (Fig. 2)

Landmarking

Nine wing venation characters corresponding to homologous structures present across all bee species were selected for this study (Fig. 3). These venation "landmarks" were digitally plotted on the high-resolution wing images using tpsDig2 ver. 2.31 and tpsUtil ver. 1.83 software.

Analysis

Analyses were performed in R ver. 4.3.1. A Generalized Procrustes Analysis (GPA) test was performed using the R package "geomorph" and a Multivariate Analysis Of Variance (MANOVA) test was performed using the R package "RRPP." Discriminant Analysis of Principal Components (DAPC) tests were performed using the R package "adegenet" to produce Fig. 4 & 5.

Results

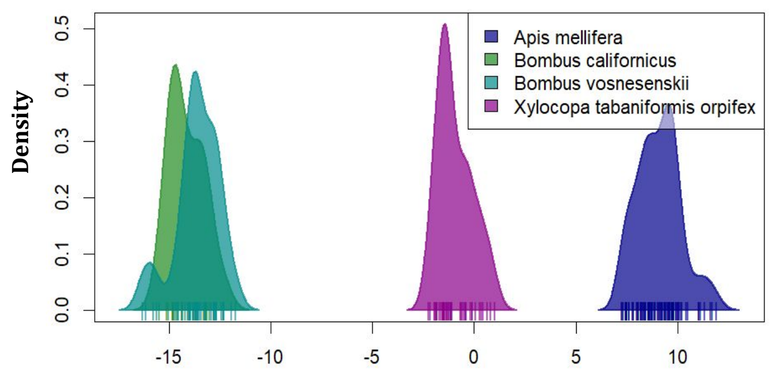

Fig 4. Species level DAPC Density Plot with single most influential discriminant function

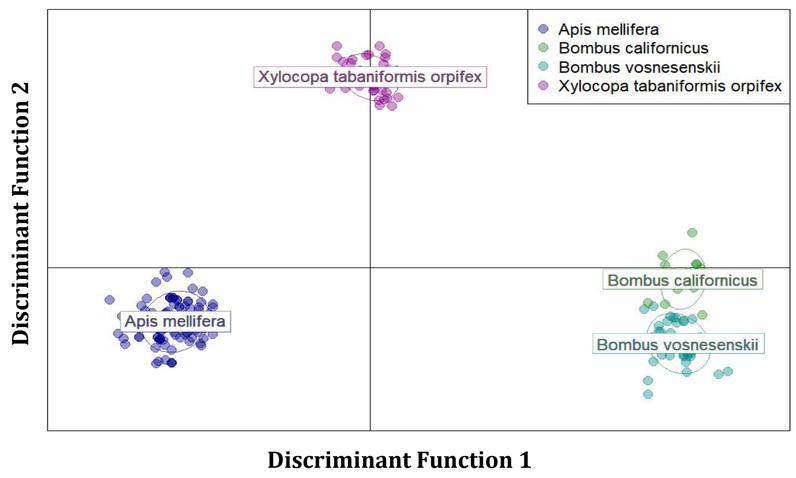

Fig 5. Species level DAPC Scatter Plot with two most influential discriminant functions

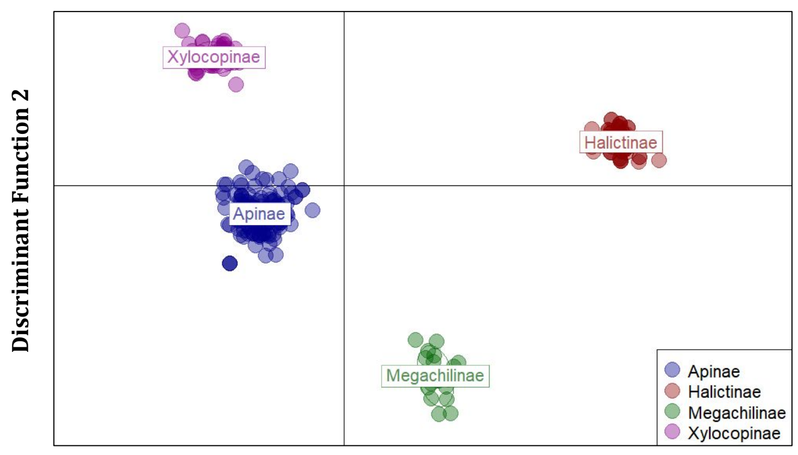

Fig 6. Subfamily level DAPC Scatter Plot with two most influential discriminant functions

Fig 2. Bee imaging setup (with prototype imaging card) with *Apis mellifera*

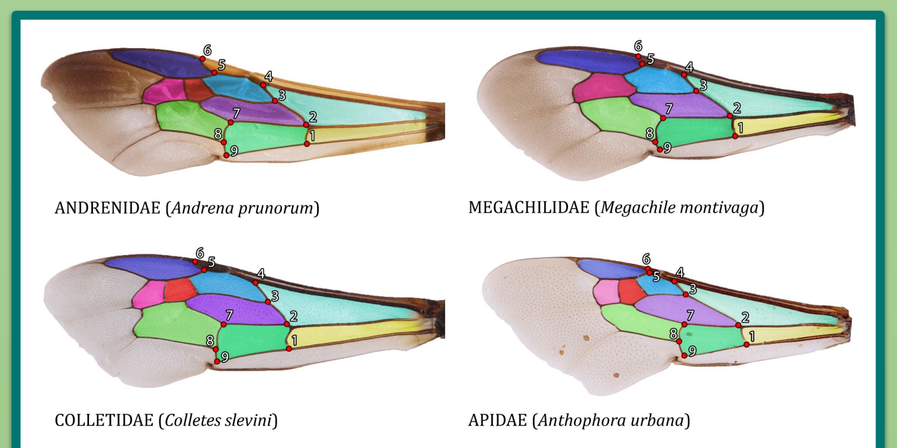

Fig 3. Nine wing venation landmarks chosen for analysis on various species

Discussion & Conclusion

While our results are preliminary, they consistently show that species can be distinguished based on wing venation characters alone. The MANOVA test on our data indicates that variance between predefined species groupings exceeds that within the groups. **Our DAPC density plot (Fig. 4) clearly separates taxa using the most influential principal component of the nine wing venation landmarks.** Notably, *B. vosnesenskii* and *B. californicus* exhibit the least variation and overlap, consistent with their classification in the genus *Bombus*. In our DAPC scatter plot (Fig. 5), which utilizes the two most influential principal components, the two species of *Bombus* show clearer separation. It also alludes to more similarities between the two *Bombus* species and *Apis mellifera*, which both belong to the subfamily Apinae.

Our DAPC plots are more difficult to interpret with the addition of more species due to the limitations of our DAPC analysis which only looks at the two most influential discriminant functions, without considering that species can vary through different characteristics. However, each individual species can be grouped into higher ranks, such as genus, subfamily, and family and analyzed on these levels. In our subfamily-level DAPC (Fig. 6) *Agapostemon texanus* and *Osmia nemoris* are introduced, belonging to the subfamilies Halictinae and Megachilinae. Of the groups sampled, Halictinae is the most phylogenetically distant which is reflected in our analysis. As *A. mellifera* and both species of *Bombus* belong to the subfamily Apinae, the variation between these species is now minimized, the DAPC only focusing on the characters that differ between Apinae and other subfamilies. Thus, it is important to note that the discriminant functions of this subfamily-level DAPC analysis are not the same as the previous species-level analyses.

This is important because it allows for a "tiered" classification system using geometric morphometrics to infer the phylogenetic placement of indeterminate bee species based solely on wing vein patterns. As the MANOVA and DAPC analyses test for statistically significant variation equating to discrimination between pre-defined groups, then the null hypothesis of a lack of discriminating variation could act as a positive identification. To identify an unknown bee, the group it cannot be distinguished from can be successively tested on a family level, then a subfamily level, and so on, until it reaches the most precise taxonomic rank possible. We are currently working to include more species within our analysis, especially those representing additional families, in order to further assess how morphological clusters can reflect evolutionary divergence and relations.

Additionally, novel imaging protocols were developed as a part of this study which could allow researchers to take standardized images in the field, allowing for the in situ identification of live bee specimens. Geometric morphometrics could provide an effective pathway for species identification which could accelerate research in bee conservation and ecology by offering an accessible alternative means for identification and ecological morphotype hypotheses.

References

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