Applying geometric morphometrics to assess phenotypic variation in bees

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Introduction

Bees have relatively conserved wing morphology, but the variation between groups remains poorly understood and has not been thoroughly quantified. Wing venation characteristics are fundamental for defining and classifying insects, but traditional methods of phenotypic identification for structures with complex geometries are challenging and time consuming. This poses a challenge to the ease and accessibility of biodiversity research and studies examining ecological morphotypes. In this study, we employed geometric morphometrics (GM) to assess variation in wing venation across bee taxa. GM allows for detailed shape analysis of wing structure, which may provide insights into evolutionary relationships. By digitally landmarking nine homologous wing vein characters of a diverse sample of bees, we quantified and compared phenotypic variation in order to assess whether the resulting morphological clusters reflect evolutionary divergence and align with established phylogeny—potentially serving as a pathway for the classification of indeterminate bee species.

How can we distinguish bee species through their wings?

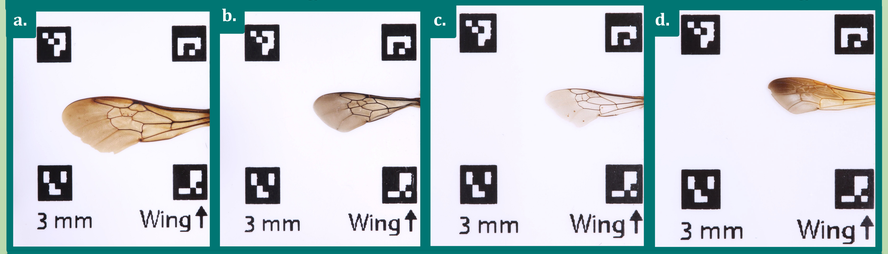


Figure 1. Wing images of different bee species (a. *Bombus vagans*, b. *Eucera frater albopilosa*, c. *Anthophora urbana*, d. *Andrena prunorum*)

Methodology

Imaging

A sample of ~20 all-female bee wings across 56 recognized species spanning 4 families were imaged in high resolution using a Canon EOS 80D digital camera (Fig. 1 & 2).

Landmarking

Nine homologous wing venation intersections 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

R (version 4.3.1) was used for statistical analyses, with the "geomorph" package utilized for a Generalized Procrustes Analysis (GPA) and to calculate Blomberg's K statistic. A Multivariate Analysis of Variance (MANOVA) test was performed using the "RRPP" package, and Discriminant Analysis of Principal Components (DAPC) was conducted using the "adegenet" package, producing Figures 4 and 5. The "ggtree" package was used to visualize the bee phylogenetic tree (Fig 6.)

Figure 3. Nine homologous wing venation intersections

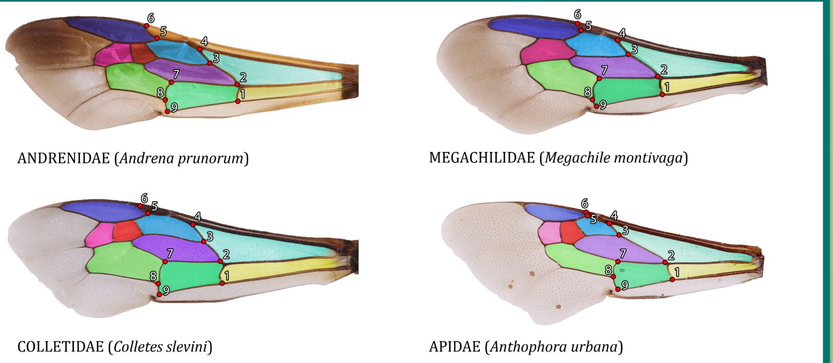


Figure 2. Bee imaging setup (with prototype imaging card)

Apis mellifera

Results

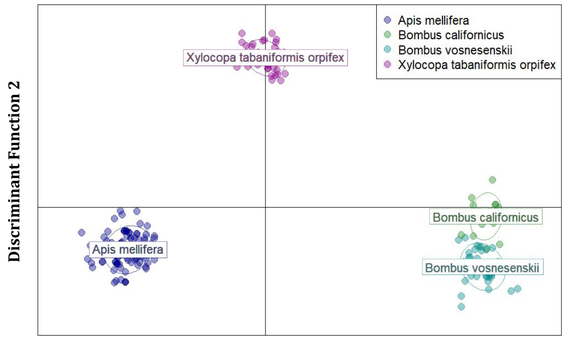


Figure 4. Species level DAPC Scatter Plot with two most influential discriminant functions

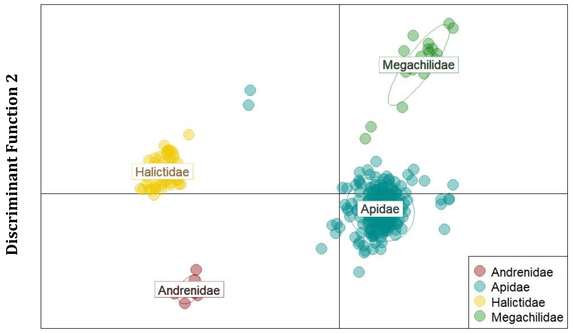
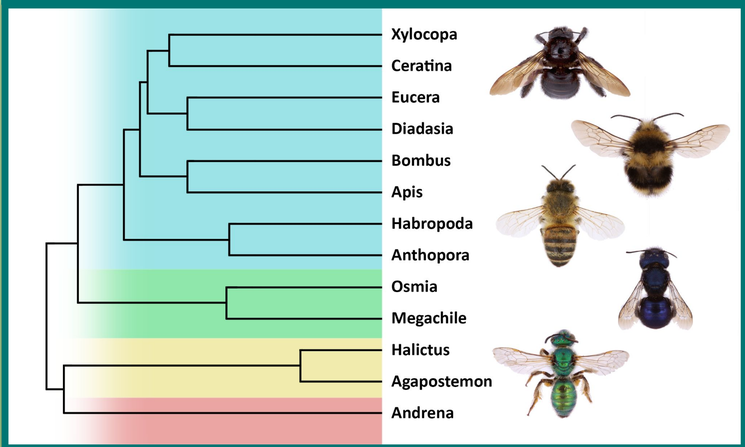


Figure 5. Family level DAPC Scatter Plot with two most influential discriminant functions

Figure 6. Bee Phylogenetic Tree (Hedkte et al., 2013)

Blue = Apidae Green = Megachilidae Yellow = Halictidae Red = Andrenidae



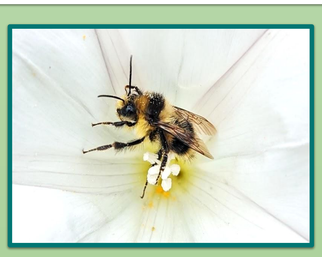
Discussion & Conclusion

Our results consistently demonstrate that species can be distinguished based on wing venation characters alone. The MANOVA test indicates that variance between species exceeds that within species. **Our DAPC plot (Fig. 4) clearly clusters four species from the Apidae family using the two most influential principal components derived from the nine wing venation landmarks.** This is consistent with prior research such as Speisman et al., 2024 and Ostwald et al., 2023.

The spatial distances between the morphological clusters is consistent with the relative phylogenetic distances between species. The alignment of the GM clusters with the accepted bee phylogenetic tree (Hedtke et al., 2013) was assessed by calculating the phylogenetic signal. The phylogenetic signal, quantified by Blomberg's K, measures the tendency of closely related species to resemble each other more than species chosen at random from a given phylogenetic tree. K was calculated from the GM shape data, permuted to the tree branch length distances under the assumption of a Brownian Motion (BM) model of trait evolution. Our results ($K = 1.062$, $p = 0.001$) indicate a stronger relationship between our morphological data (9 landmarks, 13 species) and the phylogeny (Fig. 6) than expected under BM. **Thus, the calculated phylogenetic signal strongly supports the ability of GM to accurately predict the evolutionary relationships between bee taxa according to established phylogeny.**

Additionally, each species can be grouped into higher ranks, such as genus, subfamily, and family and discriminated at these levels. In our family-level DAPC (Fig. 5) thirteen total species are sorted into their respective families. It is important to note that the discriminant functions of this family-level DAPC analysis are fundamentally different from the previous species-level analyses. Variance between individual species is disregarded, the new DAPC focusing only on character differences between families. **This is crucial as it allows GM to be a useful tool for the classification of indeterminate bee species based solely on wing venation at multiple taxonomic levels.** As the MANOVA and DAPC analyses test for statistically significant variation that indicates discrimination between taxonomic groups, the null hypothesis—indicating no discriminating variation—could serve as a positive identification tool. To identify an unknown bee, one can successively test the taxonomic groups it cannot be distinguished from, starting at the family level, then proceeding to the subfamily level, and so forth.

Novel imaging techniques 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. **GM could provide an effective pathway for species identification and quantitatively driven morphotype hypotheses, accelerating research in bee conservation and ecology by offering an accessible alternative means for identification and ecological morphotype hypotheses**.



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