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Title

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.

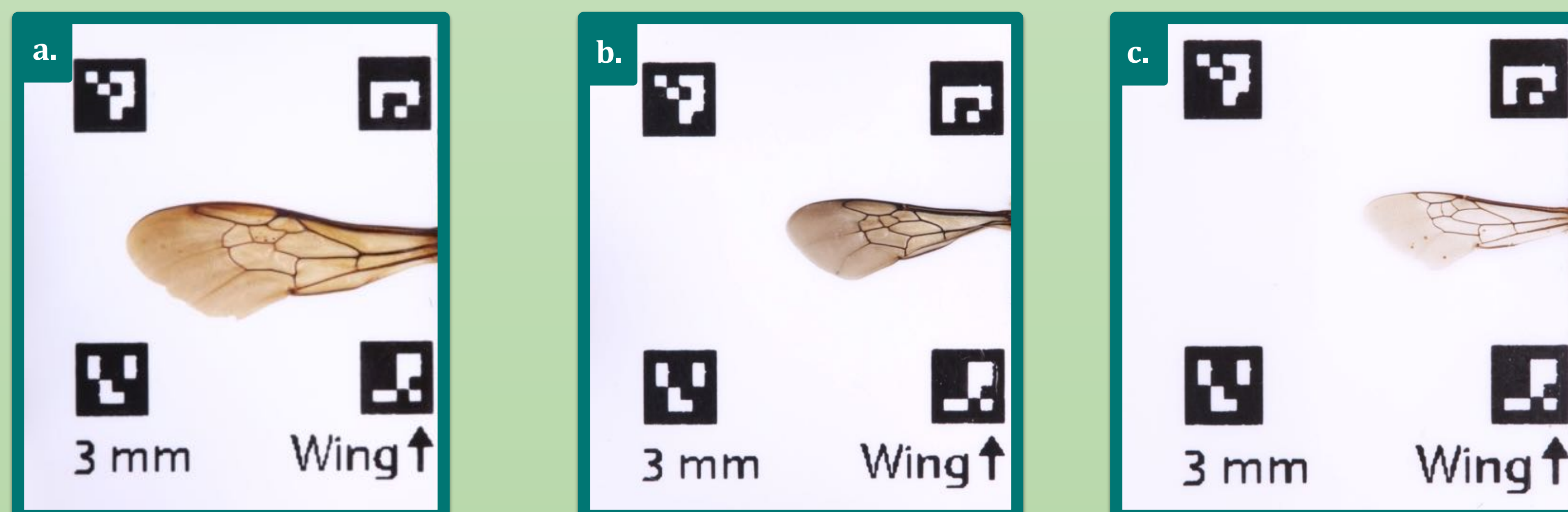


Fig 1. Wing images of bees within the family Apidae (a. *Bombus vagans*, b. *Eucera frater albopilosa*, & c. *Anthophora urbana*)

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.

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



Results

PRELIMINARY RESULTS

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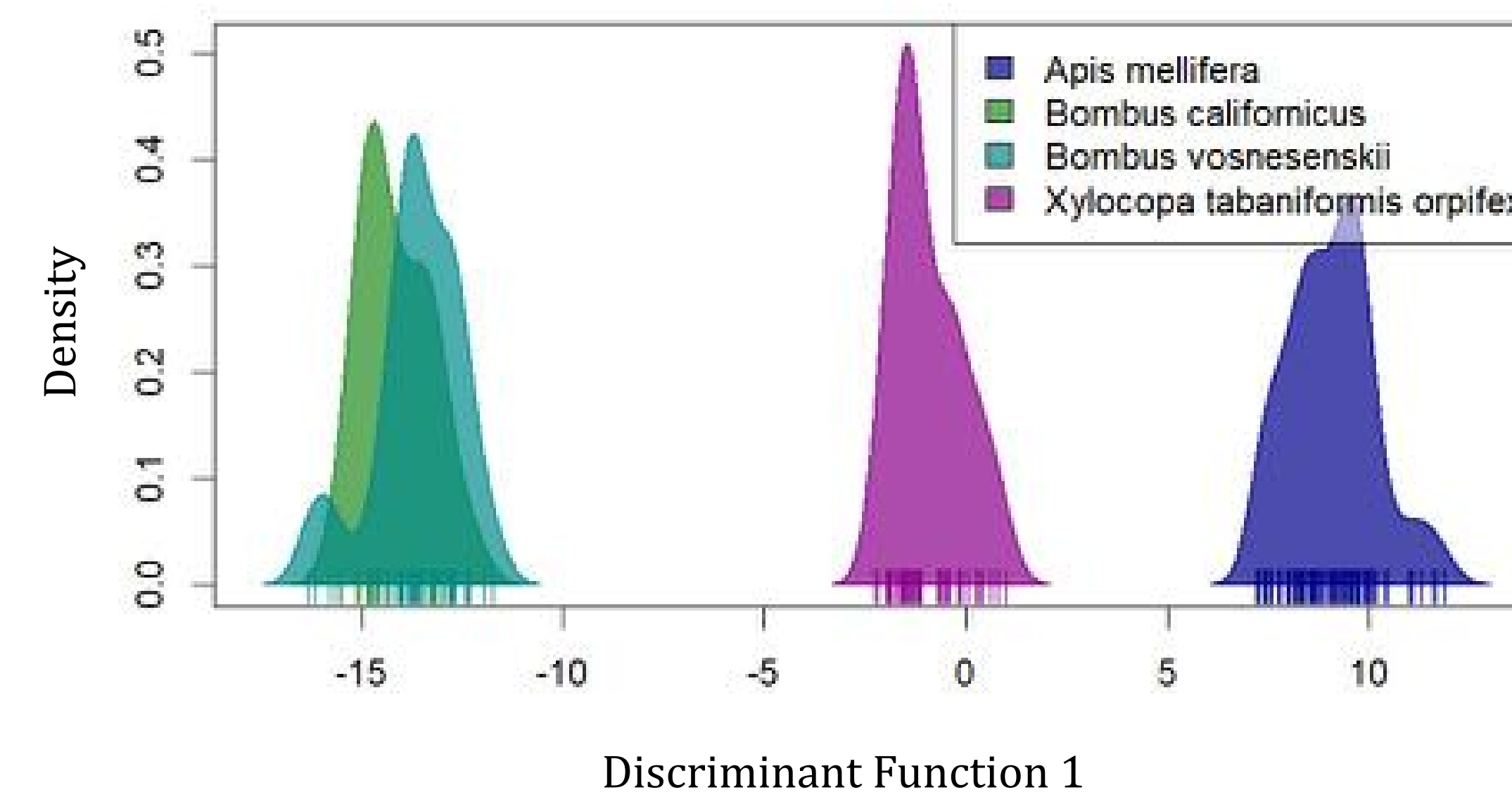


Fig 4. DAPC Density Plot with single most influential discriminant function

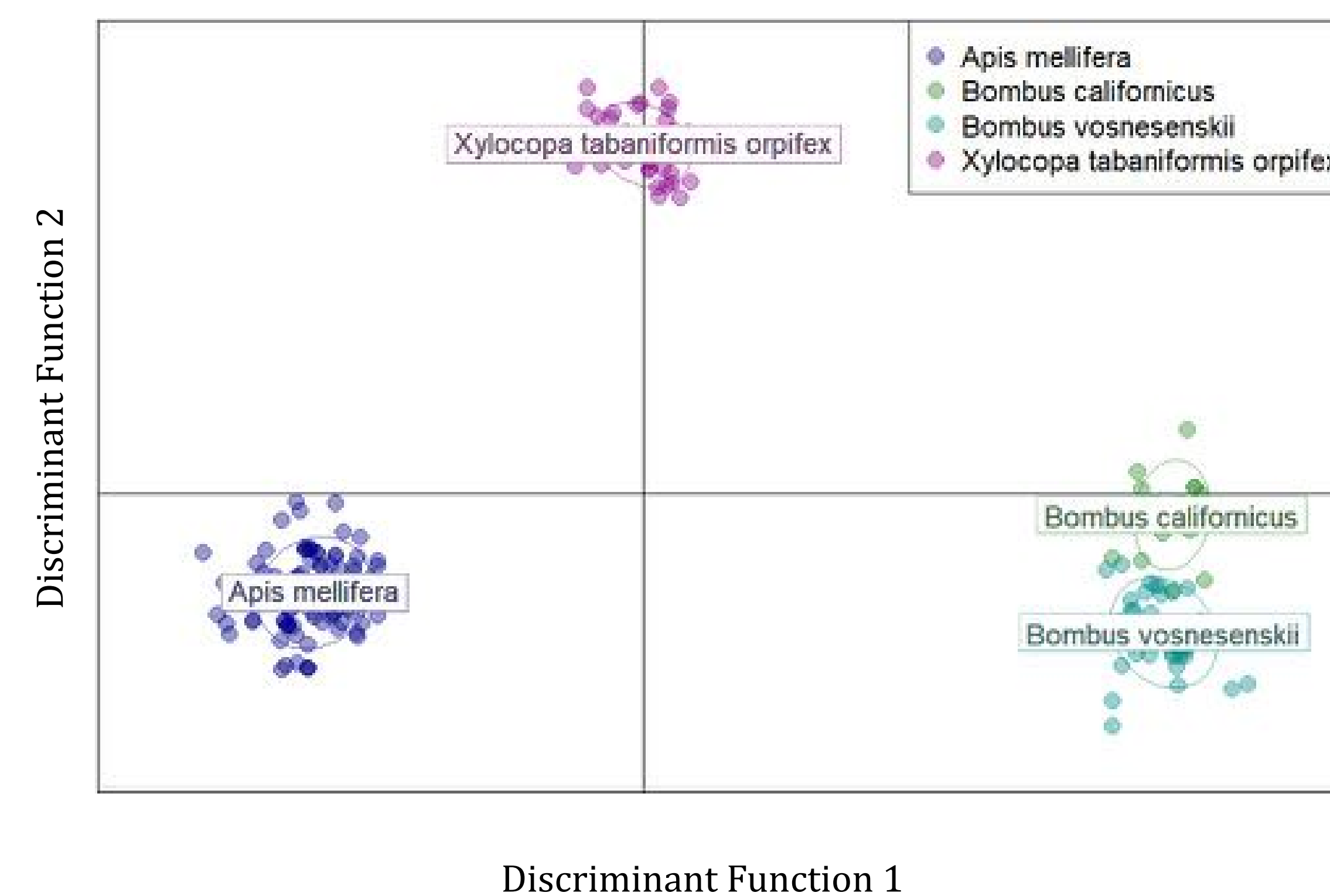


Fig 5. DAPC Scatter Plot with two most influential discriminant functions

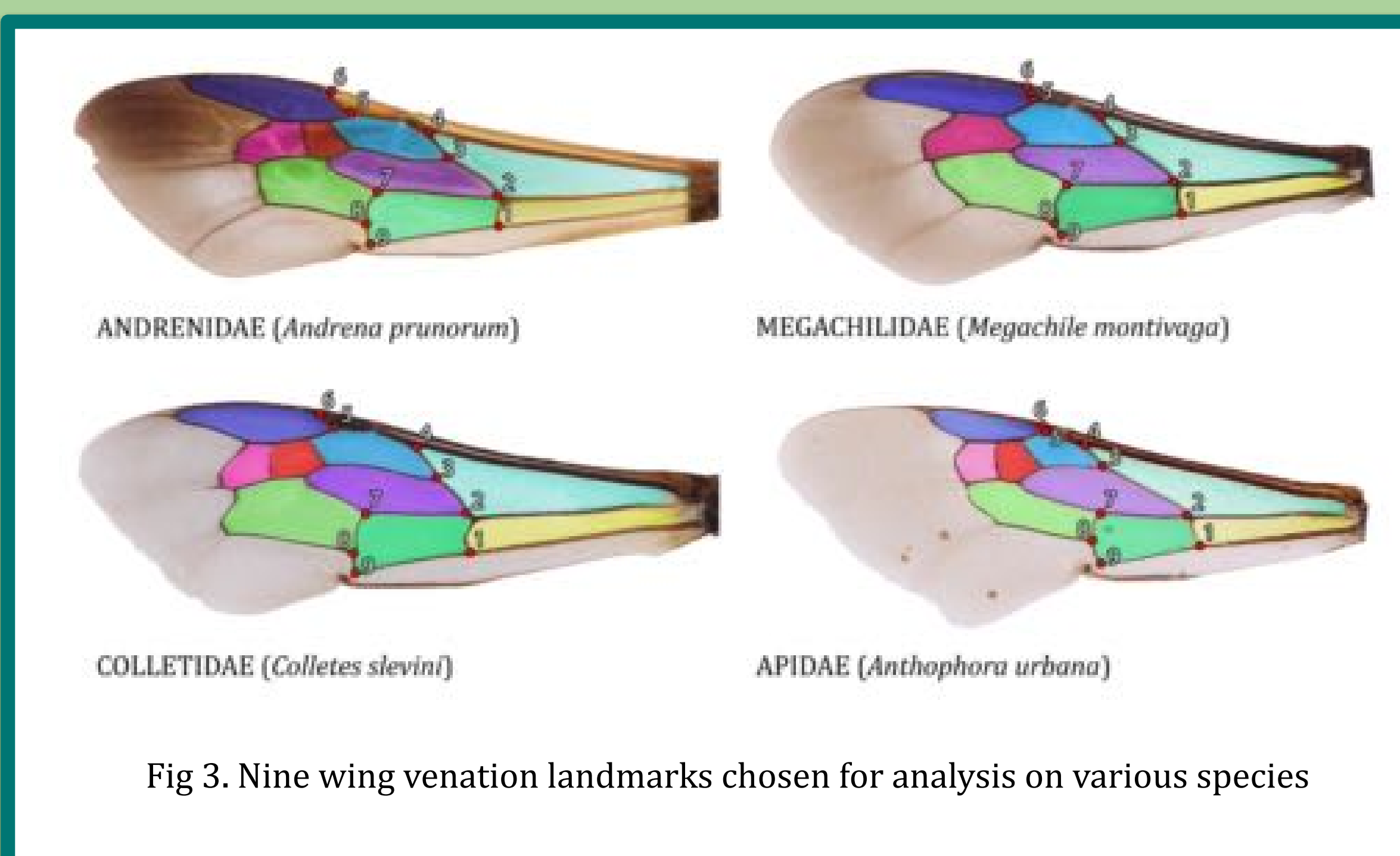


Fig 3. Nine wing venation landmarks chosen for analysis on various species

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 eludes to more similarities between the two *Bombus* species and *Apis mellifera*, which both belong to the subfamily Apinae. We are currently working to include more species within our analysis, especially those belonging to other families outside of Apidae to further assess how morphological clusters reflect evolutionary divergence.

The potential of geometric morphometrics to infer the phylogenetic similarity of indeterminate bee species based solely on wing vein patterns is being investigated. Additionally, 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. 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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