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Methods in Ecology and Evolution

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Attached is our standard article submission “*Protocol to increase accuracy and fidelity of pollen meta-genomic barcoding using Angiosperms353: a case study using pollen loads from wild bumble bees*”.

In this paper, we combine Hyb-Seq to generate *de novo* sequence databases, informed by museum records, and species distribution modelling. Further we use the results of phenological modelling to interpret our sequence alignment results to increase the likelihood of accurate results.

Given the rising popularity of metabarcoding and metagenomics, especially for projects over large spatial domains and with multiple seasonal time points, we believe that our method provides a framework for meaningfully assessing and contextualizing results. We have observed that many studies utilizing metabarcoding generate spurious results, which are precluded due to spatial or temporal incongruities with the focal animal species and plants. However, a simple and intuitive framework for programmatically assessing and rectifying these issues are lacking.

We believe that the use of the Angiosperms353 probes was novel at the inception of the study, and that several manuscripts utilizing them will be forthcoming to a variety of journals.

Our submission contains X supplementary materials, X figures and X tables. It unfortunately is currently at roughly 9,300 words. However *circa* 900 words are dedicated to a plain text overview of the methods, a section which we believe elucidates the integration of the various approaches, and clarifies adoption by subsequent users. Considerable length is dedicated to the methods, which contain five years of field work, and similarly thorough microscopy, and molecular work; but which we have cut by nearly 500 words.

We have no conflicts of interest to disclose. Additionally, this work - nor any part of it - has been submitted to any other journal.

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Thank you for considering this work.

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