

A targeted capture approach to generating reference sequence databases for chloroplast gene regions

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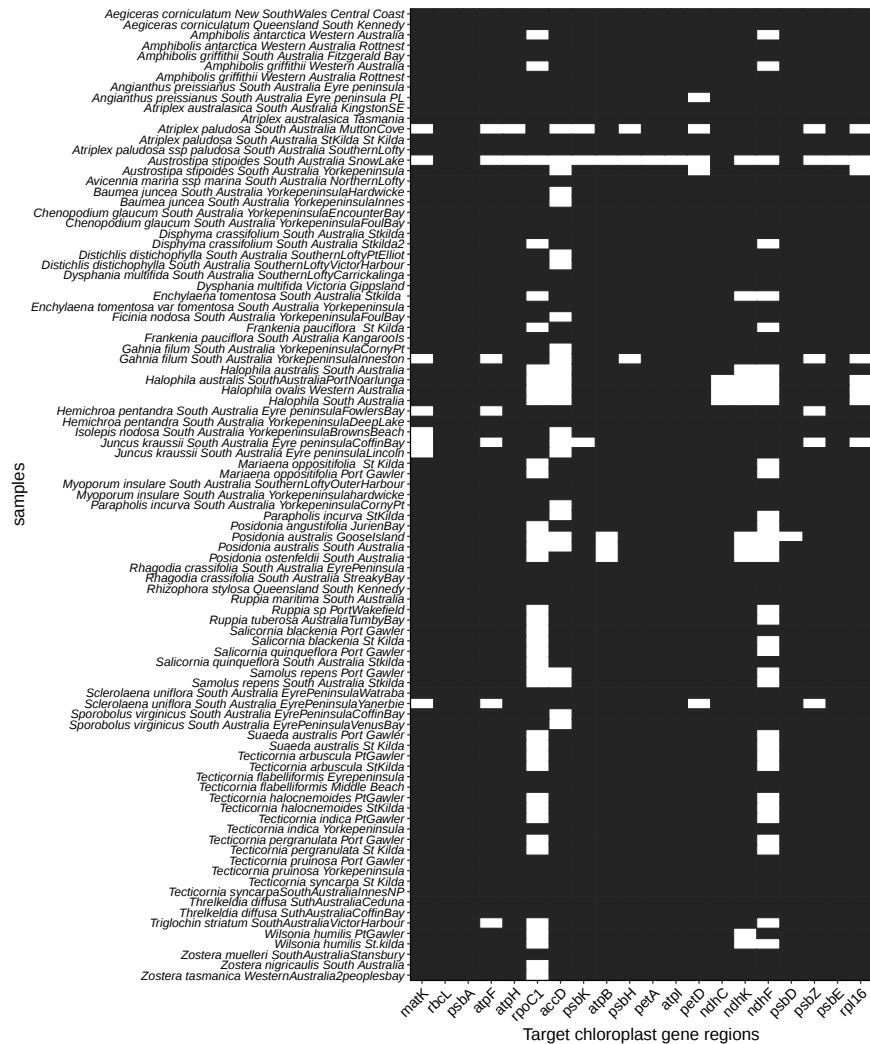
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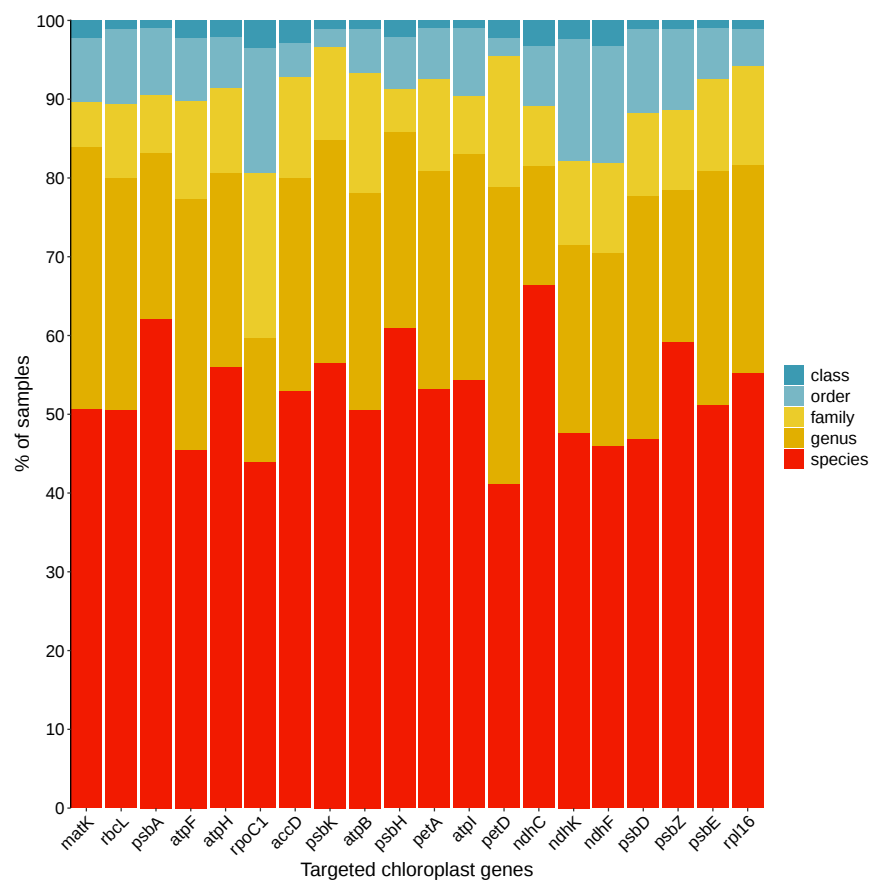
Abstract

Metabarcoding has improved the way we understand plants within our environment, from their ecology and conservation to invasive species management. The notion of identifying plant taxa within environmental samples relies on the ability to match unknown sequences to known reference libraries. Without comprehensive reference databases, species can go undetected or be incorrectly assigned, leading to false positive and negative detections. To improve our ability to generate reference sequence databases we developed a targeted capture approach using the OZBaits_CP V1.0 set, designed to capture chloroplast gene regions across the entirety of flowering plant diversity. We focused on generating a reference database for coastal temperate plant species given the lack of reference sequences for these taxa. Our approach was successful across all specimens with a target gene recovery rate of 92% which was achieved in a single assay (i.e., samples were pooled), thus making this approach much faster and more efficient than standard barcoding. Further testing of this database highlighted 80% of all samples could be discriminated to family level across all gene regions with some genes achieving greater resolution than others – which was also dependant on the taxon of interest. Thus, we demonstrate the importance of generating reference sequences across multiple chloroplast gene regions as no single loci is sufficient to discriminate across all plant groups. The targeted capture approach outlined in this study provides a way forward to achieve this.

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