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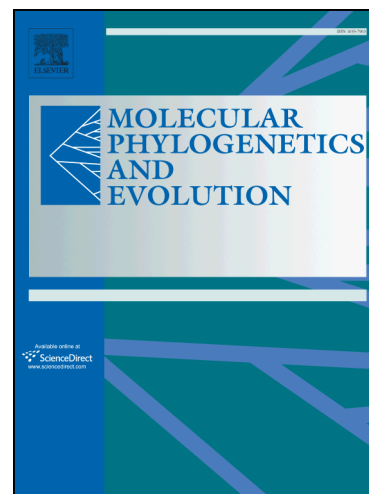
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Unpacking boxes: integration of molecular, morphological and ecological approaches reveals extensive patterns of reticulate evolution in the box eucalypts

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## Abstract

Reticulate evolution by hybridization is considered a common process shaping the evolution of many plant species, however, reticulation could also be due to incomplete lineage sorting in biodiverse systems. For our study we selected a group of closely related plant taxa with contrasting yet partially overlapping geographic distributions and different population sizes, to distinguish between reticulated patterns due to hybridization and incomplete lineage sorting. We predicted that sympatric or proximal populations of different species are more likely to have gene flow than geographically distant populations of the same widespread species. Furthermore, for species with restricted distributions, and therefore, small effective population sizes, we predicted complete lineage sorting. Eastern grey box eucalypt species (*Eucalyptus* supraspecies *Moluccanae*) provide an ideal system to explore patterns of reticulate evolution. They form a diverse, recently evolved and phylogenetically undefined group within *Eucalyptus*, with overlapping morphological features and hybridization in nature. We used a multi-faceted approach, combining analyses of chloroplast and nuclear DNA, as well as seedling morphology, flowering time and ecological spatial differentiation in order to test for species delimitation and reticulate evolution in this group. The multiple layers of results were consistent and suggested a lack of monophyly at different hierarchical levels due to multidirectional gene flow among several species, challenging species delimitation. Chloroplast and nuclear haplotypes were shared among different species in geographic proximity, consistent with hybridization zones. Furthermore, species with restricted distributions appeared better resolved due to lineage sorting in the absence of hybridization. We conclude that a combination of molecular, morphological and ecological approaches is required to disentangle patterns of reticulate evolution in the box eucalypts.

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