

Investigating underlying discordance and hybridization among nuclear phylogenies of *Packera* (Asteraceae)



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INTRODUCTION & METHODS

Packera Á. Löve & D. Löve (Asteraceae) is a genus containing ~88 species and varieties, and is known to be complicated by hybridization, polyploidy, and reticulation^{1,2,3}. An estimated 40% of *Packera* taxa present polyploidy, aneuploidy, and other cytological disturbances^{1,2}, complicating phylogenetic reconstruction of this group. Previous phylogenomic work showed high levels of underlying discordance, likely resulting from extensive paralogy and hybridization³. Thus, we wanted to investigate the causes and consequences of nuclear discordance to understand their influence on phylogenetic patterns in *Packera*.

To do this, we compared phylogenies resulting from normal paralog selection processes defined by HybPiper⁴ (ASTRAL-III^{5,6}), along with other paralog selection (ASTRAL-Pro⁷) or pruning (TEO^{8,9}) methods. We then determined whether pruning or selecting paralogs affected the topology and support of our phylogeny. We then investigated hybridization and its effect on the species relationships in our tree to find any evidence of gene flow between species lineages.

In doing so, we hoped to address:

- 1) Does utilizing different paralog selection/pruning methods generate different results and provide higher resolution than typical methods?
- 2) Is there evidence of ancient hybridization within *Packera*?
- 3) Can previous incongruences be explained by hybridization, introgression, ILS, or paralog duplication/loss?

Table 1. Pairwise adjusted Robinson-Foulds (RF_{adj}) distance values between the three different tree topologies: ASTRAL-III, ASTRAL-Pro, and TEO.

	ASTRAL-III	ASTRAL-Pro	TEO
ASTRAL-III	0	---	---
ASTRAL-Pro	0.3663366	0	---
TEO	0.3663366	0.3465347	0

Packera is a great system to address questions relating to polyploidy, hybridization, and introgression in complex genera

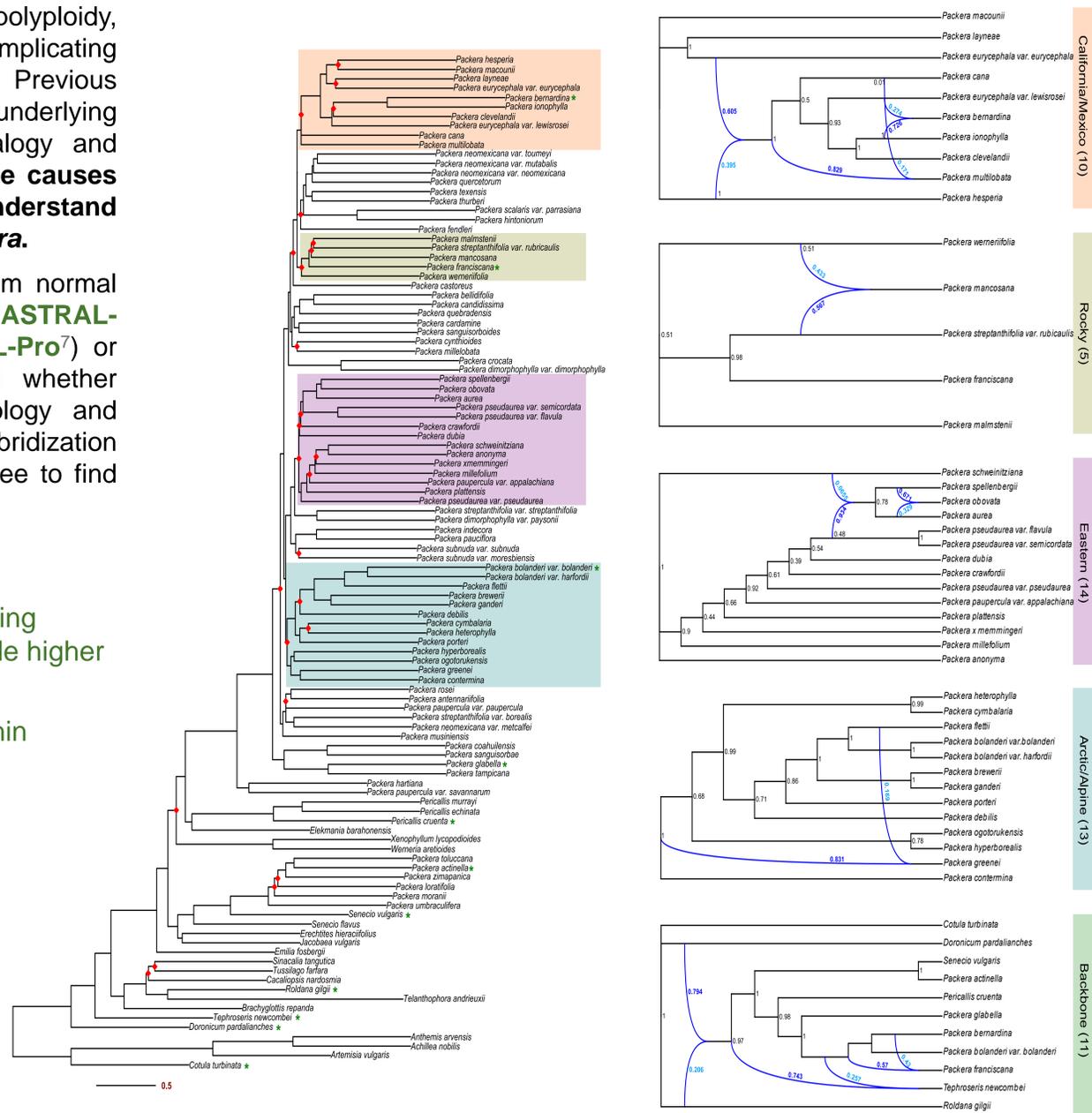


Figure 1. ASTRAL-III phylogeny of 108 taxa. Red diamonds at the node indicate discordance (QC < 0) given results from Quartet Sampling¹⁰. Clades are highlighted according to majorly discordant clades and follow the coloring scheme of the PhyloNetwork¹¹ networks to the right. Green stars to the right of some taxon names in the phylogeny relate to species included in the Backbone PhyloNetwork analysis. Black numbers in the PhyloNetwork networks indicate bootstrap support values, while light blue and dark blue numbering shows values of the hybrid edges.

RESULTS & DISCUSSION

We found that performing different paralog selection or pruning methods does impact our understanding of the evolutionary relationships within *Packera*. Overall, ASTRAL-Pro and TEO shared the most similar topologies when compared to ASTRAL-III (Table 1). ASTRAL-III was the most concordant and highly supported, with ASTRAL-Pro as next most supported (Table 2). ASTRAL-Pro had the most concordant nodes within *Packera*, meaning it provided higher resolution within our focal group than ASTRAL-III or TEO.

Table 2. General statistics between the different paralog selection and pruning processes.

	ASTRAL-III	ASTRAL-Pro	TEO
Paralog modification	Selection	Selection	Pruning
Final Paralog #	809	809	692
Final Locus #	1,049	1,049	932
# Concordant nodes	9	9	5
# Discordant nodes	25	28	29

Even so, there was still an overwhelming amount of discordance present within *Packera*, indicating that other biological processes may be influencing our results. In response, we investigated hybridization networks at highly discordant nodes within *Packera*, revealing a large amount of ancient hybridization and reticulation events (Figure 1), potentially explaining some of the discordance still seen within this group.

We hope that this approach to investigate the influence of paralogy and hybridization on underlying discordance in *Packera* can serve as a model in other complex plant groups.

CITATIONS

¹Barkley (1988), *Bot Rev*, 54(1), 82-106; ²Trock (2006), *FNA*, Vol. 20, 570-602; ³Moore-Pollard & Mandel, unpublished; ⁴Johnson *et al.* (2016), *Appl PI Sci*, 4(7), 1600016.; ⁵Zhang *et al.* (2018), *BMC Bioinformatics*, 19(Suppl 6), 15-30.; ⁶Zhang *et al.* (2020), *Mol Biol Evol*, 37, 3292-3307; ⁷Zhang & Mirarab (2022), *Bioinformatics*, 38, 4949-4950; ⁸Yang & Smith (2014), *Mol Biol Evol*, 31, 3081-3092; ⁹Morales-Briones *et al.* (2022), *Sys Bio*, 71, 190-207; ¹⁰Pease *et al.* (2018), *AJB*, 105, 385-403; ¹¹Solis-Lemus *et al.* (2016), *PLoS Gen*, 12, 1-21.