

Disentangling evolutionary histories: Target enrichment (HybSeq) as a tool for understanding population and species relationships

Juan Angulo¹, Matt Wang², Jeremie Fant^{2,3}, Norman Wickett^{2,3}
¹University of Georgia, ²Northwestern University, ³Chicago Botanic Garden

Introduction

The relationships between evolutionary lineages, either species or populations, are difficult to discern without comprehensive analysis of genes that represent a broad sampling of the genome. Target enrichment is a next-generation sequencing technique that uses short RNA probes to “capture” and sequence complementary DNA fragments in a highly efficient manner. In contrast to other sequencing methods, this method can be:

- Universal – diverse species can be targeted using the same set of RNA probes
- Cost effective – many species can be sequenced in a single reaction
- Data rich – using hundreds of coding regions leads to more accurate results

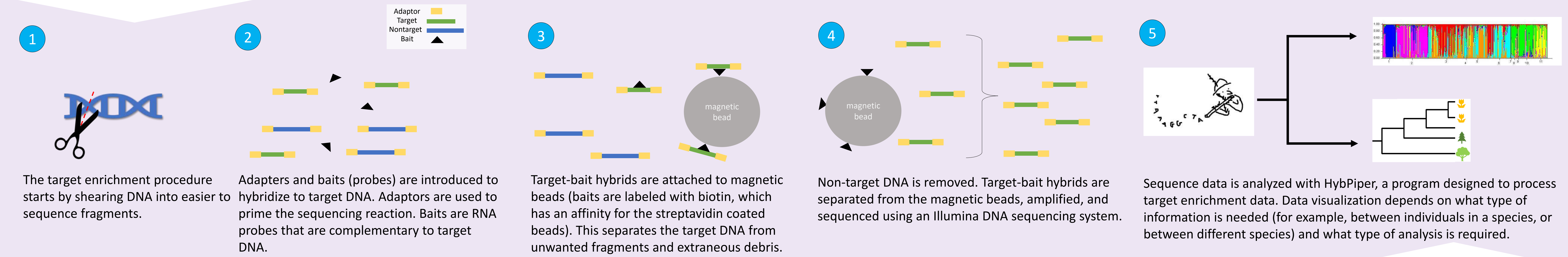
The effectiveness and universality of targeted sequencing is showcased here with two studies at different stages in the workflow.



The moss group Hypnanae, which accounts for 50% of all moss species on earth, is the subject of a phylogenetic investigation aiming to delineate longstanding, poorly resolved taxonomic groupings between its families.



A population genetic analysis using target enrichment on populations of the endangered cactus species *Sclerocactus wrightiae* has not been done before. Preliminary results using this procedure suggests it will be effective.



Results

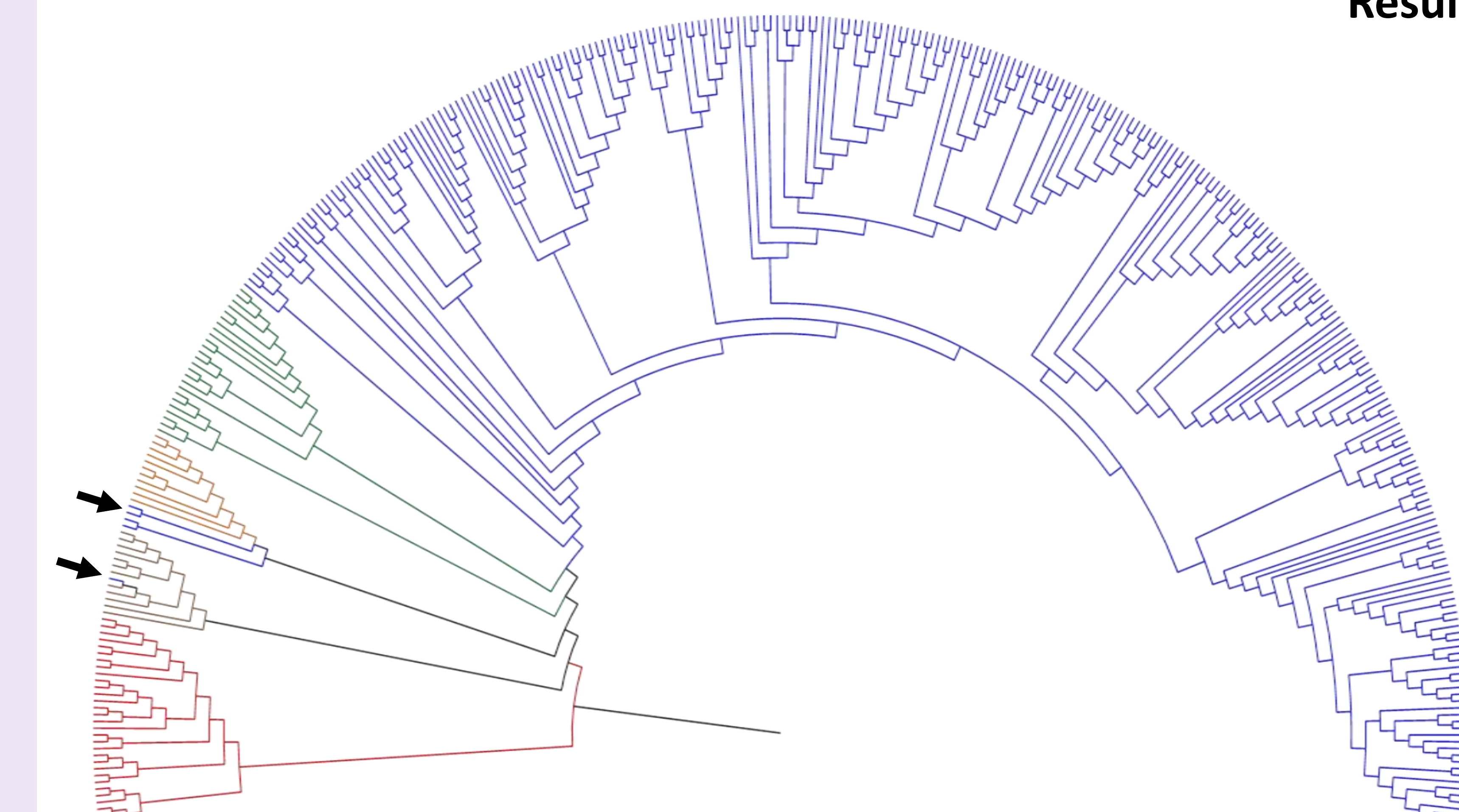


Fig. 1 Phylogenetic tree of Hypnanae organized by orders (Hypnales-blue, Hookeriales-dark green, Ptychomniales-orange, Hypnodendrales-brown. Outgroup taxa are in red. Paraphyletic groups in Hypnales are indicated by arrows. Constructed from gene trees acquired through Hyb-Seq.

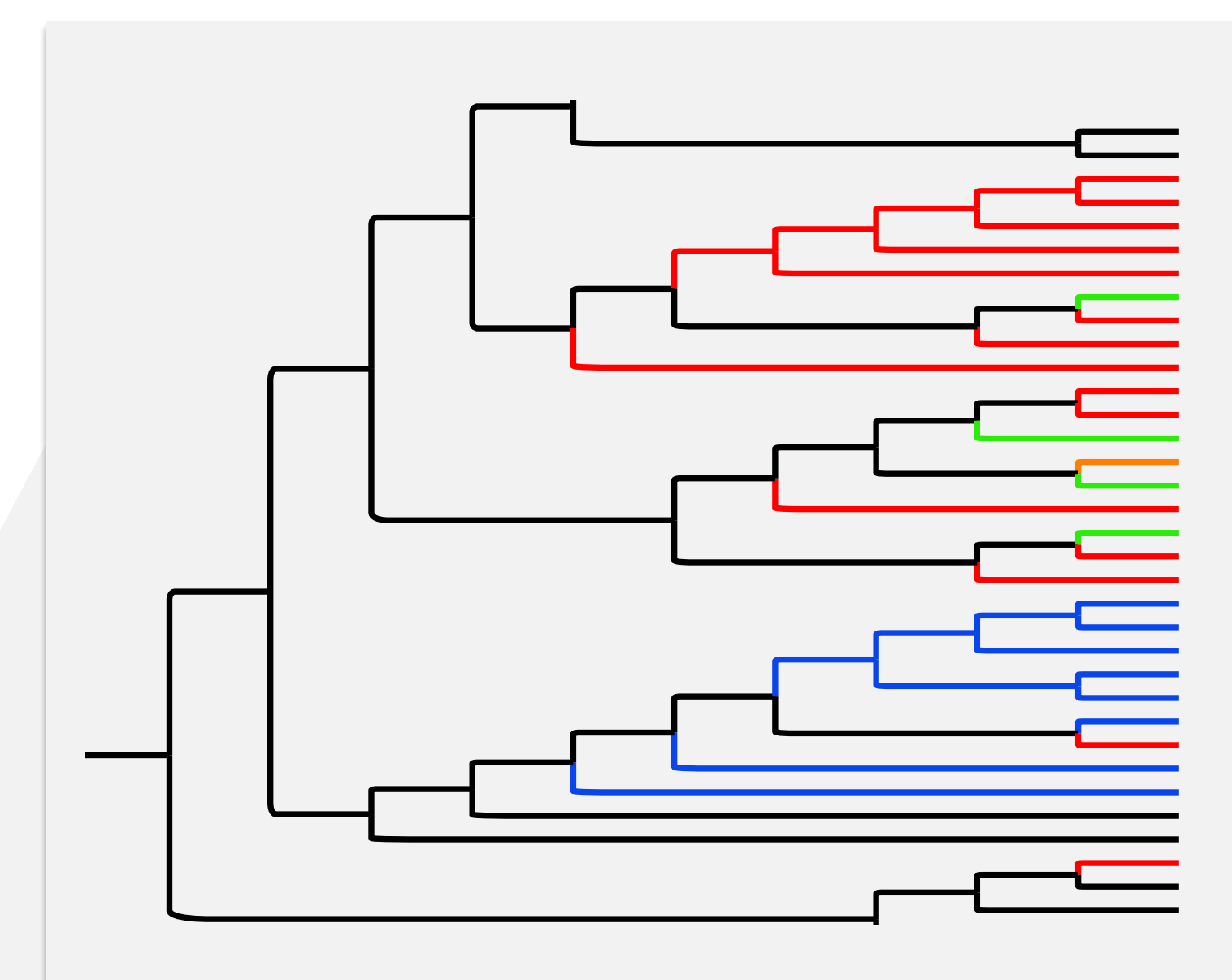


Fig. 2. Magnification of a branch in Hypnales organized by family showing paraphyletic families with high levels of taxonomic inconsistency. Neckeraceae (red), Lembophyllaceae (blue), and Amblystegiaceae (green)

Discussion

The potential for evolutionary analysis using Hyb-Seq is reflected in the preliminary results of two studies. The prepared *Sclerocactus* samples yielded much more than the minimum 2 ng/ul needed for proper sequencing, and are ready to be hybridized and sequenced.

As for *Hypnanae*, the analysis showed:

- A phylogenetic tree (Fig. 1) outlines the mostly monophyletic orders within the group, with Hypnales showing paraphyletic taxa that are more closely related to Ptychomniales
- On the family level, there are many classification errors that depict the importance of Hyb-Seq’s use for uncovering unresolved lineages within diverse groups
- Current taxonomic groupings do not reflect natural evolutionary lineages
- A strong need for **major taxonomic revisions** within families in Hypnanae