The Phylogenetic Utility of EPIC DNA Sequences in the Tribe Cecropieae

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ABSTRACT

Mutualisms, interactions between species that are beneficial to both partners, are useful systems for investigating how interactions may affect the evolution and diversification of lineages. Interactions between ants and plant species of the genus Cecropia are a classic example of mutualism but the origin and evolution of the mutualism is poorly understood. We examined whether the inclusion of an exon-primed intron-crossing (EPIC) marker could improve our understanding of Cecropieae phylogeny. We performed a Bayesian phylogenetic analysis of 15 species from which EPIC sequences were obtained (Fig. 2). By comparing clade support from analyses with and without EPIC, we concluded that the addition of a third gene region strongly supports the hypothesis that the Afrotropical and antless genus Musanga was derived from a Cecropia ancestor and is most closely related to the antless neotropical species C. sciadophylla (Fig. 1).

INTRODUCTION

The study of mutualism involving neotropical trees of the genus Cecropia and their associated ants could provide insights into their coevolutionary relationship. Distinctive features shared by Cecropia species—glycogen-rich Mullerian food bodies (Fig. 3 left panel) and internal prosomata enabling ant queens to nest in the hollow stems of the plant—suggest that the mutualism evolved in the common ancestor of members of the genus. We aimed to investigate phylogenetic relationships within the tribe Cecropieae of the plant family Urticaceae in order to identify the closest relatives of Cecropia. Previous work estimated the phylogeny based on 26S ribosomal DNA and ndhF chloroplast DNA sequences but results were not robust. In order to improve our understanding of the origin of the ant-Cecropia mutualism, we investigated the utility of EPIC DNA sequences had on resolving and supporting phylogenetic relationships within the tribe. The five genera of Cecropieae are distributed in either the neotropics of Central and South America (Cecropia, Coussapoa, Porouma) or the afrotropics (Musanga, Myrianthus). Objective: The study examined the effect of including an exon-primed intron-crossing (EPIC) marker on resolution and support for Cecropiaceae phylogeny.

METHODS

We sequenced 18 samples representing 15 species of Cecropieae and one outgroup taxon. Previously extracted DNA was amplified using a polymerase chain reaction mix and A1671T0b primers as outlined in Yao et al. (2013). PCR products were run on a 0.8% agarose gel to confirm amplification and products were cleaned using ethanol precipitation. The cleaned PCR products were quantified to determine DNA concentration and were then used as template for Big Dye cycle sequencing reactions. Cleaned products were sequenced by the BioMedical Genomics Center core facility at the University of Minnesota. The data were analyzed and edited using Geneious with additional alignment done using Se-Al v2.0a1. The analysis was run on the CIPRES portal using MrBayes v3.2.2. The analysis was run with four chains for 10,000,000 generations with a GTR model.

RESULTS

Fig. 2a: Bayesian phylogeny based on ndhF & 26S

- > 0.95 PP
- 0.75-0.95 PP

By comparing clade support from analyses with and without EPIC, we concluded that the addition of a third gene region strongly supports the hypothesis that the Afrotropical and antless genus Musanga was derived from a Cecropia ancestor and is most closely related to the antless neotropical species C. sciadophylla (Fig. 1).

Fig 2b. Phylogeny based on EPIC, ndhF & 26S

- > 0.95 PP
- 0.75-0.95 PP

CONCLUSIONS

With the inclusion of the EPIC markers, we observed augmented levels of support for the:
- Musanga/Cecropia clade—suggesting that Musanga diverged from the Cecropia genus before adopting mutualistic associations with ants.
- C. membranacea/C. putamayonensis clade
- C. pelitata clade
- C. multisecta/C. telealba clade

Conversely, decreased resolution in clades was associated with the EPIC marker addition (for currently unknown reasons), including the underlying Pourouma, Myrianthus, Cecropia, and Musanga node.

REFERENCES

6) Yao, Xiaobong, Chenhong Li, and Christopher W. Dick. "Exon-Primed Intron-Crossing (EPIC) Markers for Evolutionary Studies of Ficus and Other Taxa in the Fig Family (Moraceae)." Applications in Plant Sciences 1.10 (2013).

ACKNOWLEDGEMENTS

Many thanks to the LSSURP staff for their life changing commitment to the next generation of scientists. Jennifer McCurtain deserves special recognition for her exemplary leadership. I remain indebted to Aaron Shatzer and Matthew Sandler for providing the crucial support needed to begin this project. Howard D. Duke remains my wonderful friend and a prodigious colleague. Mountains of Thanks to my family. Our work would not be possible without the generosity of the University of Minnesota and the NSF.

Figure Legend

Figure 1: Geographic Range of the Tribe Cecropieae and Proposed Divergence

Figure 2a and Figure 2b: Cecropiaeae majority rule Bayesian consensus trees. Grey and white circles are associated, respectively, with ≥0.95 and 0.75-0.95 Bayesian posterior probability. Trees were rooted with Parietaria debilis, a member of the tribe Parietarieae (Urticaceae).