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 Cecropiaeae 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[1, 2, 3]. Distinctive features shared by *Cecropia* species—glycogen-rich Müllerian food bodies **(Fig. 3 left panel)** and internodal prostomata 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[3]. 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[5] 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[6] had on for resolving and supporting phylogenetic relationships within the tribe[2]. 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 Cecropieae 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 A167170b 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. Tree analysis was done on the CIPRES portal using Mr.Bayes v3.2.2. The analysis was run with four chains for 10,000,000 generations with a GTR model.
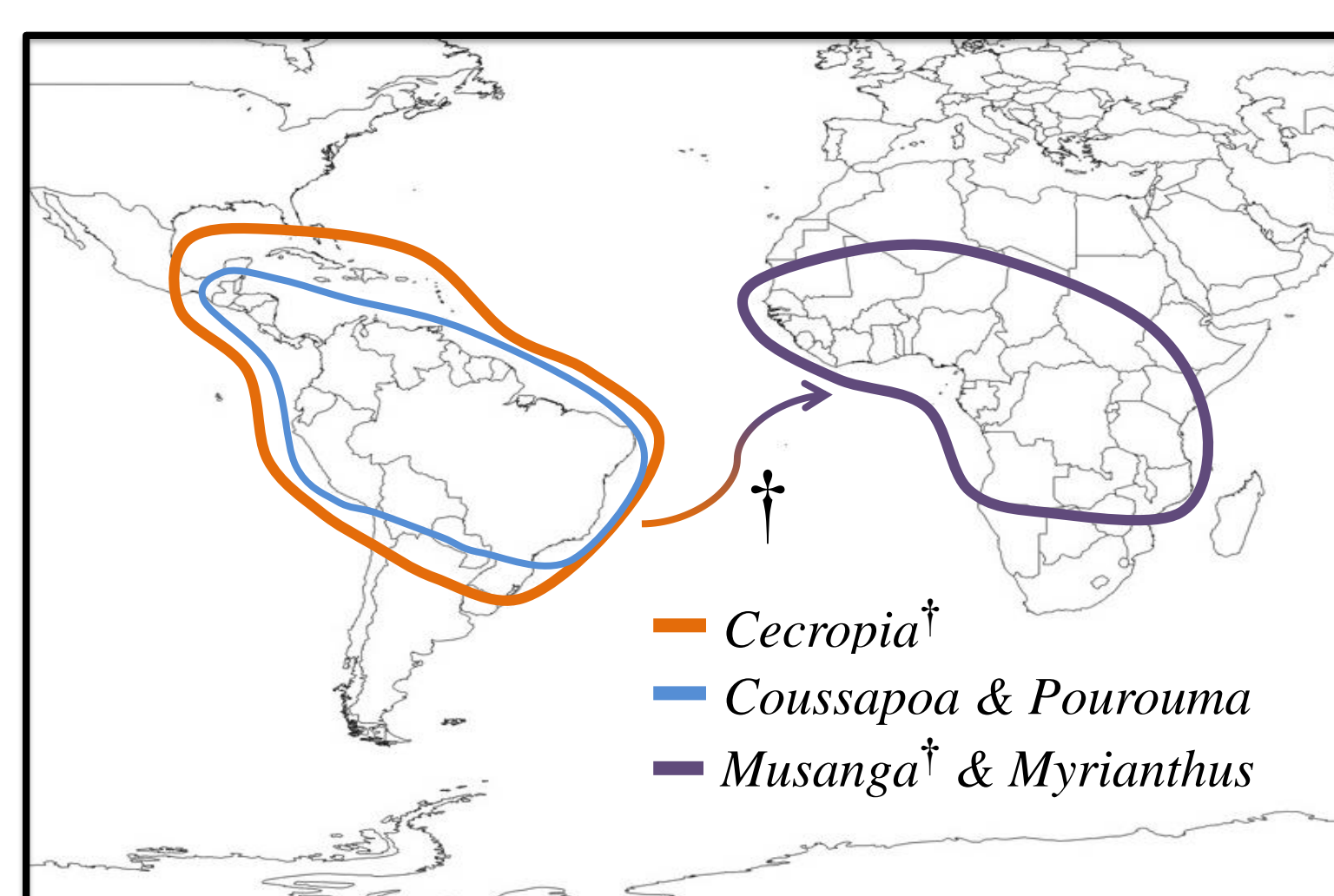
Figure 1: Geographic Range of the Tribe Cecropieae and Proposed Divergence

- *Cecropia*†
- *Coussapoa & Pourouma*
- *Musanga*† & *Myrianthus*

RESULTS

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

- ● > 0.95 PP
- ○ 0.75-0.95 PP

Taxa: *Coussapoa villosa*, *Pourouma myrmecophilia*, *Myrianthus serratus*, *Cecropia sciadophylla*, *Musanga cecropiodes*, *Musanga cecropioides*, *Cecropia marginalis*, *Cecropia membranacea*, *Cecropia peltata*, *Cecropia putamayonis*, *Cecropia multisecta*, *Cecropia obtusifolia*, *Cecropia telealba*, *Cecropia ficifolia*, *Cecropia herthae*, *Cecropia obtusifolia*, *Cecropia tacuna*, *Parietaria debilis*

0.0080

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

- ● > 0.95 PP
- ○ 0.75-0.95 PP

Taxa: *Coussapoa villosa*, *Pourouma myrmecophilia*, *Myrianthus serratus*, *Cecropia sciadophylla* *, *Musanga cecropioides*, *Musanga cecropioides*, *Cecopia marginalis*, *Cecropia membranacea* *, *Cecropia putamayonis* *, *Cecopria peltata* *, *Cecropia multisecta* *, *Cecropia telealba* *, *Cecropia obtusifolia*, *Cecropia obtusifolia*, *Cecropia tacuna*, *Cecropia ficifolia*, *Cecropia herthae*, *Parietaria debilis*

0.0070

*Greater support with addition of EPIC markers
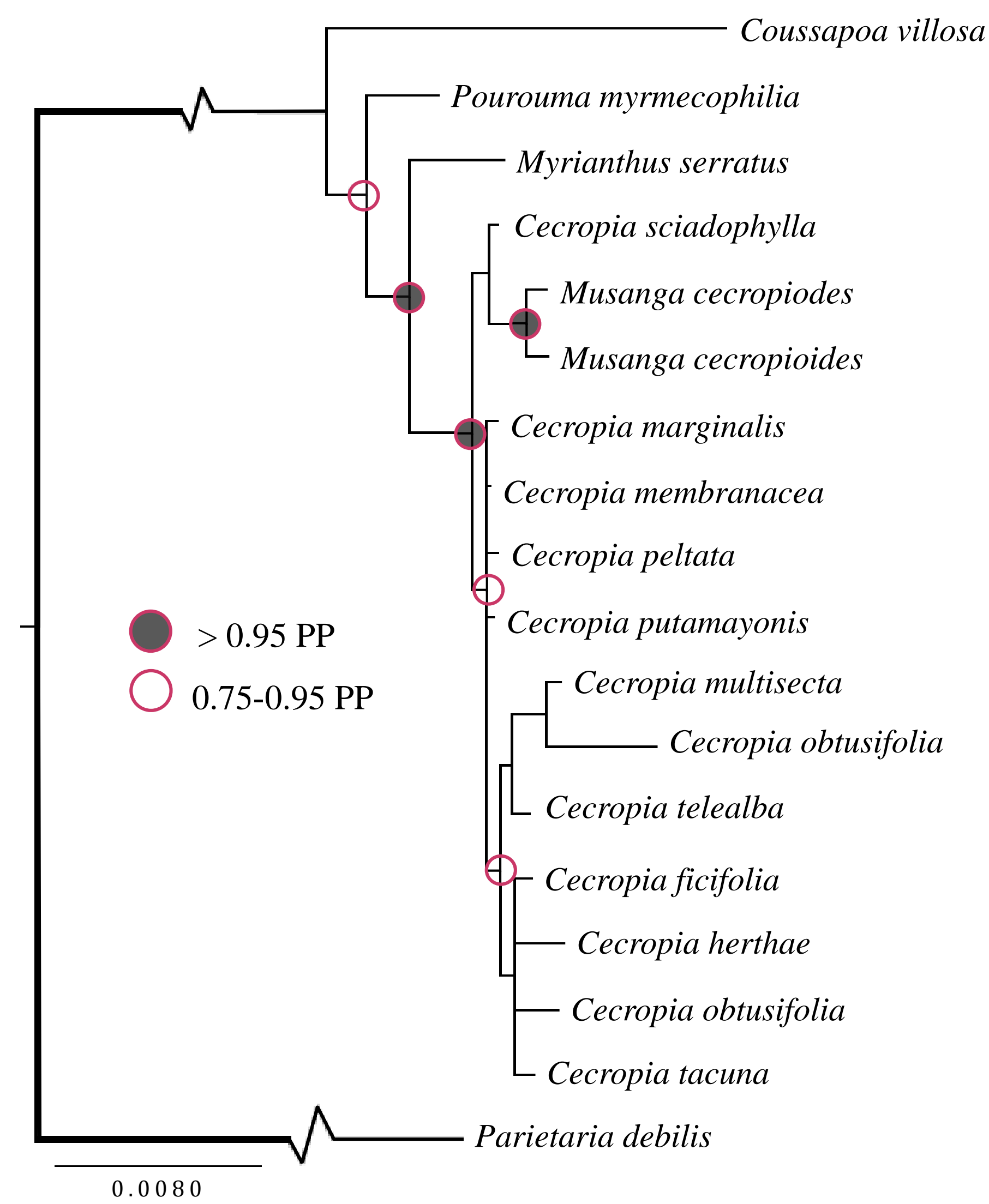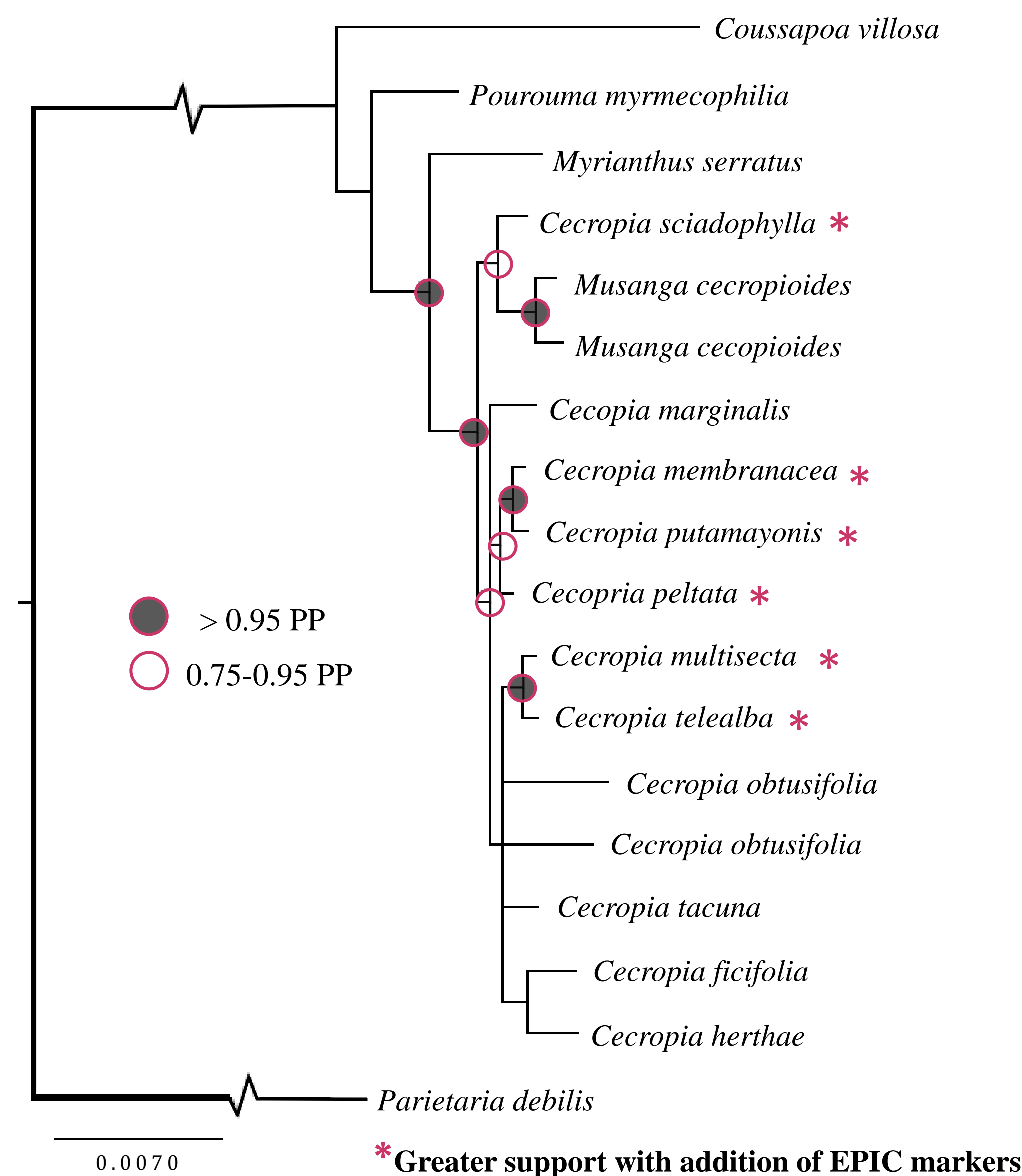
Figure Legend

Figure 2a and Figure 2b: Cecropieae 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 dibilis*, a member of the tribe Parietarieae (Urticaceae).

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. putamayonis* clade
- *C. peltata* 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.

Figure 3: Tree-produced Food Bodies (left) and *Cecropia* Leaf (right).

REFERENCES

1) Ayala, Francisco José, et al. "Molecular Phylogeny of *Azteca* Ants (Hymenoptera: Formicidae) and the Colonization of *Cecropia* Trees." *Molecular Phylogenetics and Evolution* 5.2 (1996): 423-428.

2) Bronstein, Judith L. "The Contribution of Ant-Plant Protection Studies to Our Understanding of Mutualism1." *Biotropica* 30.2 (1998):150-161.

3) Davidson, D. W. and D. McKey. 1993. Ant-plant symbioses: Stalking the chuyachaqui. Trends in Ecology & Evolution 8:326-332.

4) Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 - 8.

5) Strand, A. E., J. Leebens-Mack, and B. G. Milligan. 1997. Nuclear DNA-based markers for plant evolutionary biology. Molecular Ecology 6:113-118.

6) Yao, Xiaohong, 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).

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