



## Using multiple analytical methods to improve phylogenetic hypotheses in *Minaria* (Apocynaceae)

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### ABSTRACT

Metastelmatinae is a neotropical subtribe of Asclepiadoideae (Apocynaceae), comprising 13 genera and around 260 species whose phylogenetic relationships are often unresolved or incongruent between plastid and nuclear datasets. The genus *Minaria* is one of the first lineages to emerge in the Metastelmatinae and is highly supported based on plastid markers. It comprises 21 species, most of which are endemic to small areas with open vegetation in the Espinhaço Range, Brazil. In the work presented here, we use plastid (*rps16*, *trnH-psbA*, *trnS-trnG*, and *trnD-trnT*) and nuclear (ITS and ETS) datasets to investigate the relationships within *Minaria*. We show that the three methods mostly used in phylogenetic studies, namely, maximum parsimony, maximum likelihood, and Bayesian Inference, have different performances and that a pluralistic analytical approach combining results from them can increase tree resolution and clade confidence, providing valuable phylogenetic information.

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### 1. Introduction

Metastelmatinae is a neotropical subtribe, comprising 13 genera and nearly 260 species. The monophyly of most genera of Metastelmatinae has been questioned by phylogenetic studies, but a generic re-circumscription has not been possible because the relationships within the subtribe are often unresolved, phylogenetic signals are incongruent between plastid and nuclear datasets, and clades usually lack diagnostic morphological features (Silva et al., 2012). In spite of this difficulty, *Minaria* has been identified as one of the first lineages to diverge in the Metastelmatinae (e.g., Rapini et al., 2006). The genus was not recognized based on morphology, but is highly supported in phylogenetic studies based on the plastid *trnL-F* intergenic spacer and *trnL* intron (Rapini et al., 2003, 2007) and was confirmed by subsequent analyses with the additional plastid markers *trnT-L* and *trnS-G* intergenic spacers, *rps16* intron, and part of *matK* gene (Liede-Schumann et al., 2005; Rapini et al., 2006).

Originally, *Minaria* comprised 19 species that are mostly endemic to small areas with open vegetation in the Espinhaço Range, in the states of Minas Gerais, in eastern Brazil (Konno et al., 2006). Nevertheless, a study comprising 25% of the Metastelmatinae (Silva et al., 2012) suggested that two other species also endemic to the Espinhaço Range, but in the state of Bahia, should also be included in *Minaria* and contradicted the position of *Minaria*

*polygaloides* in the genus. These relationships were not resolved with ITS, but were strongly supported based on plastid dataset and plastid and ITS combined analyses.

Due to the distribution of *Minaria* spp., most of which are rare and/or narrowly distributed in the Espinhaço range, the phylogenetics of the genus may reveal biogeographic patterns and help to assess priority areas for conservation in the region (Ribeiro et al., 2012), besides questions about the origin and maintenance of endemism. Therefore, the present work has three primary objectives: (1) to evaluate the circumscription of *Minaria*, (2) to position the genus in the Metastelmatinae, and (3) to establish the relationships among its species. We present the results produced by the three most popular analytical methods in phylogenetic studies – maximum parsimony, maximum likelihood, and Bayesian Inference – and compare their performances. Finally, we show that combining results obtained by different methods can increase clade confidence and the resolution of our phylogenetic hypotheses.

### 2. Materials and methods

We sampled 41 species of Metastelmatinae, concentrating the sampling in *Minaria*, with 18 species (Appendix). Three specimens of *M. cordata* were sampled to include the whole variation of the species, which is divided into varieties (Fontella-Pereira, 1989). The tree was rooted in *Blepharodon ampliflorum* because it is closely related to *B. lineare* (and eventually considered a synonym of this species; e.g., Morillo, 1976; Fontella-Pereira et al., 1984),

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