

“Systematics, biogeography and anatomy of *Oocephalus* (Hyptidinae, Lamiaceae)”

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Introduction

The Hyptidinae subtribe, Ocimeae tribe and subfamily Nepetoideae (Lamiaceae), has its predominance occurrence in the Neotropical region, with only two species extending its range to Africa (Harley *et al.* 2004). The first phylogeny of Hyptidinae (Pastore *et al.* 2011) proved its monophyly but also has shown that the genus *Hyptis* Jacq. was seriously paraphyletic. Based on the molecular information and morphological evidence, Harley & Pastore (2012) proposed a new generic delimitation in the subtribe, elevating section of the genus *Hyptis* to generic level. Currently, the subtribe has 19 genera and approximately 400 species.

Currently, the newly dismembered genus *Oocephalus* (Benth.) Harley & J.F.B. Pastore, previously subsect. *Glomeratae* Benth. and subsect. *Oocephalus* (Benth.) Epling, (both located in *Hyptis* sect. *Polydesmia* Benth.), Has 18 species that occur mainly in the mountainous regions of central Brazil, such as the *campos rupestres* of Espinhaço range, Chapada Diamantina and the Planalto of the state of Goiás (Harley 2014). Although most species are restricted to these environments, *O. oppositiflorus* (Schrank) Harley & J.F.B. Pastore has a wider distribution, and can be found south of the state of Piauí, east of São Paulo state and eastern Bolivia (Harley 2014). Morphologically, *Oocephalus* is classified by the characters of its inflorescence, as the entire subtribe where it belongs (Harley & Pastore 2012). On the other hand, the previous classification (Epling 1949) usually divided the species in two distinct groups (subsect. *Glomeratae* and subsect. *Oocephalus*), based on the calyx morphology, making the present classification quite heterogeneous, with two subgroups.

Anatomy has shown to be quite efficient in elucidating taxonomic problems in several families, especially with leaf characters (Solleder 1908; Metcalf & Chalk 1950; Rudall 1993; Diane *et al.* 2003; Coutinho *et al.* 2013). Studies with this focus on Hyptidinae are scarce, highlighting the contributions of Rudall (1980a, 1980b 1981), where the leaf, wood and floral anatomy of the subtribe were studied. However, only the first showed relevant results on the taxonomy of the group due to the great taxonomic importance and ecological significance. Despite the great contribution that this study brought to the subtribe, only two species of *Oocephalus* were analyzed by the author, leaving the genus subsampled and lacking in anatomical information.

Since it was proposed, *Oocephalus* does not have a specific study to delimit its species, which was suggested by Harley & Pastore (2012), who recognized the need for more deeply morphological studies in the genus. A study of the anatomical and morphological characters of the two remaining species subgroups to investigate if there are, between them, any cohesion in these characteristics and to find synapomorphisms, will help to better understand the taxonomy of the group and resolve the unsatisfactory classification. Furthermore, a phylogenetic analysis with all species been sampled, will show the relationship between the species and, combined with the morphoanatomical data, it will be possible to know which characters are directly linked with the genus diversification.

Objectives

We will conduct a systematic study of the genus *Oocephalus*, presenting a phylogeny associated to morphoanatomic data to characterize the genus, as well as identify the characters which contributed to the diversification of it.

Material and methods

Sampling:

To obtain species samples, extensive field work will be conducted in two Brazilian states: Goiás and Minas Gerais. In the state of Goiás, the collections will be carried out in the municipalities of Alto Paraíso de Goiás and surrounding areas. In Minas Gerais, the destination will be the municipalities of Diamantina, Joaquim Felício and Itacambira. The predominant vegetation type of the localities cited is the *Campos rupestres*, as stated earlier is the main formation where the genus occurs. Besides, a number of other *Oocephalus* collections were made, including morphotypes which have not yet been identified at the species level, which with the fresh material from the original locality, can be identified. The two states are located in the central region of Brazil, making necessary a plane trip from the state of Rio Grande do Norte (Northeast region Brazil), where the applicant lives.

Phylogenetic analysis:

For the phylogenetic approach, a NGS genomic sequencing (nuclear and plastidial DNA simultaneously) will be realized using the Hyb-Seq protocol. Thus 50 µl aliquot of genomic DNA (total) will be fragmented by the sonication process, with subsequent construction of a library using the *NEBNext DNA Sample Prep Master Set 1* (New England Biolabs). The fragments obtained for each species in the initial phase of library construction of DNA will be amplified (by PCR) individually with addition, for further identification, of barcodes for each access. The ready-made DNA libraries will be quantified by quantifying PCR (qPCR) in a PCR thermocycler. Genomes of all species will be sequenced in a *paired-end* run using the Illumina Genome Analyzer. With this, it will be possible to understand the phylogenetic relation between the species. The resulted phylogeny will be submitted to **Taxon** journal.

Morphoanatomic revision:

The taxonomic revision will be made using specialized bibliographies to describe specified morphological terms (*e.g.* Epling 1949; Harley 1985, 1986, 1992, 2014). Morphological leaf terminology will follow Hickey (1973) and general morphological Harris & Harris (2001) and Radford *et al.* (1986). Additionally, visits to herbaria are being made to check collections of *Oocephalus* and type material of the genus species to observe the morphological variation. The results of this study will be submitted to **Systematic Botany**.

Species cauline leaves will be fixed in FAA₇₀ in the field, being stored on it for 48 hours and then transferred to alcohol 70% to be stored until be analyzed in laboratory. After being settled in paraffin, the samples will be cut using a rotary microtome and stained to observation of anatomical characters, such as mesophyll cells and epidermis, organizing this data in a matrix of characters, seeking to find and interpret the synapomorphies presented by each of the phylogenetic tree groups. Anatomical results combined with morphological and phylogenetic evidence will be submitted to **Botanical Journal of the Linnean Society**.

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