

Project proposal

Title: Evolution of Amazonian and Atlantic Forests: Historical Biogeography and Evolution of *Stiffitia* J.C. Mikan (Asteraceae)

Description of overall research project:

Amazon and Atlantic Forest are the two largest moist forests of South America. Estimates of diversity for Amazon found 14,003 plant species (Cardoso et al. 2017), with 16.1% of endemics (BFG 2015). However, 17% of the area of Amazon Forest (AM) have already been devastated until 2008, and in the last ten years, 61,600km² and 650,000km² have been devastated in Brazil by deforestation and fire, respectively. Furthermore, only in 2019, the fires increased 43% compared to 2018 in Brazil (Inpe 2019a, b). In turn, Atlantic Forest (AT) is the most diverse Brazilian biome, with 15,001 species of Angiosperms, of which almost half are endemics (BFG 2015). AT is very threatened due to its highly fragmentation, remaining less than 28% of natural cover (Rezende et al. 2018).

Scrutinize the evolutionary processes that shaped and maintain the current diversity are central questions in contemporary biology (Antonelli et al. 2018, Brown & Lomolino et al. 2006), also is fundamental to understand current world and can also be used for conservation purposes. Considering the current threats to these environments, it is imperative to understand the macroevolutionary processes AM and AF.

One of the most intriguing groups of the Asteraceae Family is the tribe Stifftieae, one of the basal tribes of Asteraceae (Mandel et al. 2019). Stifftieae is a morphologically and biogeographically complex group that present exclusively South American distribution (AM, AT, Andes, Pantepui) and no synapomorphy is found for the tribe yet (Roque & Funk 2013). The genus *Stiffitia* is the sister group of the remaining tribe Stifftieae (Mandel et al. 2019) and have six species, of which two occur in the east portion of the AM, *Stiffitia cayennensis* H. Rob. & B. Kahn and *S. uniflora* Ducke. The remaining species of the genus occur in different vegetation types of AT, *S. chrysantha* J.C. Mikan, *S. fruticosa* (Vell.) D.J.N. Hind & Semir, *S. hatschbachii* H. Rob. and *S. parviflora* (Leandro) D. Don (Katinas et al. 2008, Monge 2011). The remaining species of Stifftieae occur mainly in Guyana Shield and Andes (Katinas et al. 2008). In addition, there are polymorphisms on the morphology of the genus and in the tribe there are related to the habit (treelets vs. vines); color of the flower and pappus (both pink vs. pink and yellow vs. both orange vs. both beige); length of corolla (short vs. long); pappus bristles (capillari vs. scale); pappus fusion (free vs. fused).

Even though Stifftieae is monophyletic, the previous phylogenetic studies are based only on 15% of its diversity, and this can be a problem in groups with homoplasies. Furthermore, the current knowledge on the patterns of diversification, morphological evolution and biogeography are poorly known for *Stiffitia* and also for Stifftieae phylogenetic hypothesis is biased by low sampling (Monge 2011, Katinas et al. 2008, Mandel et al. 2019). Therefore, due to geographical distribution on AF and AT, complex morphological background, and basal phylogenetic position, *Stiffitia* is a good model for studies of diversification, biogeography, and evolution of the Biota of Amazon and Atlantic Forests. Furthermore, it may contribute substantially to the knowledge of the diversification of the Asteraceae family.

Objectives: So, the objectives of the study are: (1) Propose a well supported phylogenetic hypothesis for *Stiffitia* based on NGS data, (2) infer ancestral areas and its divergence times based on fossil calibrations; (3) infer morphological evolution searching for synapomorphies of the tribe; (4) increase data sampling of Stifftieae by studying its specimens housed at MO and US herbaria.

Material & Methods

To infer the phylogeny of *Stiffitia* we will use modern phylogenomic sequencing (NGS), a target enrichment approach developed for Asteraceae (Mandel et al. 2014), which have proved to be successful

(Mandel et al. 2019). This method will maximize the phylogenetic information available due to the amount of data collected, increasing the possibility of full resolution at the species level, avoiding the occurrence of paralogs or pseudogenes, which can potentially mislead the phylogenetic inference. For further details in data preparation are available at Mandel et al. (2014, 2019). We will infer the phylogeny under Maximum Likelihood on Raxml (Stamatakis 2014) with concatenated data. In addition, we will estimate a consensus coalescent species on tree based on individual gene trees under a Bayesian analyses on Astral II (Mirarab & Warnow 2015) and StarBEAST 2 (Heled & Drummond 2010), as well as estimate its divergence times with fossils based on the later.

Additionally, we will perform comparative phylogenetic analysis of discrete and continuous data. To infer the ancestral traits of morphological data we will perform stochastic character mapping with SIMMAP (Bollback 2006) on the package Phytools (Revell 2012) for discrete data and with ace function in APE package (Paradis & Schliep 2018) for continuous data. To infer the historical biogeography of the group on BioGeoBEARS (Matkze 2013) performing a model selection of the best approach using AIC. All comparative phylogenetic analyses will be implemented on R (R Core Team 2019).

We plan to sample more than 50% of the tribe, sequencing 40 samples in total. Regarding *Stiffitia*, we will have more than one sample per species. Currently, we have successfully extracted 28 samples with the amount of DNA suitable for NGS sequencing. The distribution range and the morphological characters of the studied species have been gathered from herbaria (CEPEC, HUFU, RB, S, SP, SPF, SPFR, UEC) and field work.

To complement our morphological and geographical dataset we need to study the specimens of Stifftieae housed at US and MO. If authorized by the curators, we will obtain leaf fragments for DNA extractions will allow to fill the sampling gaps in Stifftieae phylogeny, in order to better investigate the evolutionary history of the tribe. Furthermore, we plan to discuss my research project with Asteraceae NGS expert, Jennifer Mandel, at University of Memphis.

Additional Relevant information

This project has been well evaluated four times, but only funded twice by Appleyard Fund of the Linnean Society of London and CAPES. These awarded grants will be used to hire NGS library preparation and sequencing services with Rapid Genomics (California, US) for 30 samples. So, to develop this project we need a complementary founding to sequence the remainder 10 samples and to cover the costs of the flight tickets to USA.

The analysis of the material stored at MO and US are crucial to the development of this study, because those herbaria has the largest collections of plants from the Guiana Shield and Andes, where most of the remainder genera of Stifftieae occurs. Scrutinize the morphological traits of the members of Stifftieae is fundamental for the search of morphological synapomorphies for the group. Additionally, it will also help me to delimit more precisely the geographic distribution of the members of the tribe to better investigate the biogeography of Stifftieae and, as a consequence, the biogeography of the Amazon and Atlantic Forests. In addition, this travel to US will be a good opportunity to talk to the taxonomists from MO and US and to the Asteraceae NGS expert. This travel will contribute to stablish strong collaborations between my research projects with foreign researchers.

I have plans to publish the results of this project in high quality journals, such as Taxon (I.F. 3.8), Journal of Biogeography (I.F. 3.8), Molecular Phylogenetics and Evolution (I.F. 3.9). IAPT Research Grants was selected because it is one of the distinguished systematic grants available worldwide for early career researchers. In addition, Brazilian research funding have almost none grants available since 2018, making almost impossible to produce high quality research funded locally.

In conclusion, the IAPT Research Grants Program in Plant Systematics certainly will contribute to achieve the goals of my research, and the award will be used to complement the sequencing costs, supporting the travel to USA herbaria and strengthen the collaborations with US researchers.

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