

IAPT RESEARCH GRANT APPLICATION FORM

Systematic studies in *Agarista* (Ericaceae): phylogeny and taxonomic revision of the Neotropical species

Introduction

Ericaceae is a cosmopolitan family comprising 124 genera and 4.100 species, occurring in acid soils of temperate and subtropical regions in both hemispheres, and in mountain chains in the tropics (Kron et al. 1995). The major diversity of the family occurs in the Neotropics concentrated in five centres of diversity. These centres includes the highlands of Mexico and northern Central America, the Tropical Andes from Costa Rica to northern Bolivia and eastward to Trinidad and Tobago, the West Indies mountains, eastern Brazilian mountains and the Guayana highlands (Luteyn, 2002).

The family comprises nine subfamilies and 20 tribes (Kron et al., 2002; Freudenstein et al., 2016). Subfamily Vaccinioideae contains one of the larger clades in the family, composed by Andromedae Klotzsch + Gaultheriae Nied.+ Lyonieae Kron & Judd + Vaccineae Rchb. (Kron et al., 2002). *Agarista* D.Don ex G.Don. (Vaccinioideae, Lyonieae) comprises 31 species classified into two morphologically distinct sections (Judd, 1984). *Agarista* sect. *Agauria* (DC.) Judd includes a single, variable and widely distributed species (*Agarista salicifolia* G.Don) which is found in the mountainous areas of central Africa, Réunion and Mauritius islands, reaching its highest morphological diversity in Madagascar. *Agarista* sect. *Agarista* includes 30 species endemic to South America. The higher diversity of the later section occurs in Brazil, especially in the eastern mountains, and comprises 21 species (19 endemic).

Despite the high degree of endemism in Brazil, the absence of recent works and the difficulty to delimit and identify species makes *Agarista* a neglected genus. Besides the taxonomic and cladistic work from Judd (1984), there is no other taxonomic treatment neither comprehensive recent phylogenetic hypothesis. The only phylogenetic hypothesis based on molecular data sampling the genus comprises only two species: *Agarista salicifolia* (African specie) and *Agarista populifolia* (Lam.) Judd (North American specie). Thus, the monophyly of the group is unconfirmed and the relationships of *Agarista* with the other genera within Lyoneae are unknown. Therefore, we aim to provide a phylogenetic hypothesis to the Neotropical species of *Agarista*, clarifying inter and infragenic relationships, and a taxonomic revision to *Agarista* sect. *Agarista*.

Objectives

- Reconstruct a dated phylogenetic hypothesis of *Agarista* sect. *Agarista* testing the group's monophyly and determining its phylogenetic positioning within Lyoneae;
- Prepare a taxonomic treatment to the Neotropical species of *Agarista*, reevaluating the morphological characters of diagnostic value for the taxa that compose the genus, recognizing the ones that are efficient for section and species circumscription;
- Investigate the possible existence of species not yet described in the genus.

Material and methods

Fieldwork and herbaria revision: Fieldworks will cover the species distribution to obtain samples for morphological descriptions, DNA extraction and enable to analyse the species in their habitat, compile ecological information, as well as habit and characters better visualized in the field, such as colour of flowers, leaves and fruits. Vouchers will be deposited in ICN, with duplicates sent to the herbaria nearby the collection sites and international herbaria. I will revise most of the herbaria (ALCB, BHCB, BHZB, CESJ, CEPEC, ECT, EFC, ESA, ESAL, FLOR, FUEL, FURB, HAS, HBR, HUCS, HUEFS, HUFU, ICN, MBM, MPUC, OUPR, PACA, SP, SPF, SPSF, UB, UEC, UPC, VIES) in the geographic range of Neotropical *Agarista* to describe the species, compile distributional data and obtain samples for DNA extraction (fide herbaria guidelines and curator's authorization) from specimens of species that could not be collected in the field. Additionally, herbaria outside the range of distribution (BM, E, F, G, GH, K, M, MO, NY, P, S, US) will be visited depending on funding or loans of critical specimens will be requested to them.

DNA extraction, amplification and sequencing: DNA will be obtained using the Stratec® DNA Extraction Kit (Birkenfeld, Germany) from silica-gel dried samples or from recent herbarium specimens. I will amplify markers used in related taxa to test if they are informative for phylogenetic reconstruction (Kron *et al.*, 2002; Bush *et al.*, 2010; Fritsch *et al.*, 2011). Sequencing will be performed in Ludwig Biotec® (Porto Alegre, Brazil).

Phylogenetic analysis: The resulting DNA sequences will be evaluated and edited in Geneious 2020.0.3 Prime (Kearse *et al.*, 2012; Geneious Prime, 2019) and aligned with MAFFT 7 (Katoh and Standley, 2013) implemented in Geneious 2020.0.3. The evolutionary substitution models for separate plastid and nuclear data sets will be estimated under the Akaike Information Criterion implemented in jModelTest v.2.1.3. (Darriba *et al.*, 2012) and the two sets will be analyzed in a partitioned way. The generated matrices will be analyzed by Bayesian Inference (Huelsenbeck & Ronquist, 2001) and the consistency of the branches will be evaluated by the posterior probability test (PP). The phylogenetic inference to the complete data set will be reconstructed under a multispecies coalescence model (Mendes *et al.*, 2018). For molecular dating, the inference tree will be generated using the package *BEAST, employing the MCMC algorithm (Heled & Drummond, 2009; Bouckaert, 2019) and implemented in BEAST v1.10.4 (Drummond *et al.*, 2012; Suchard *et al.*, 2018).

Itemized budget

The proposed budget are destined to finance part of the work to be started in April 2020. At this moment this resource will be destined to herbaria revisions in Minas Gerais state, as well to pay for fieldwork expenses in areas that include high diversity of *Agarista* in that state. Additionally, part of this resource aims to acquire material and reagents for material collect in the field and subsequent DNA extraction.

Literature citations

- BUSH, C. M.; JUDD, W. S.; FRAZIER, FRAZIER, L. & KRON, K. A. 2010. The Phylogeny of *Leucothoë* s. l. (Ericaceae Vaccinioideae) Based on Morphological and Molecular (*ndhF*, *matK*, and *nrITS*) Data. *Systematic Botany* 35: 201-206.

- BOUCKAERT, R.; HELED, J., KÜHNERT, D.; VAUGHAN, T.; WU, C. H.; XIE, D. & DRUMMOND, A. J. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.*, v. 10, e1003537.
- DRUMMOND, A. J.; SUCHARD, M. A.; XIE, D. & RAMBAUT, A. 2012. Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969-1973.
- FREUDENSTEIN, J. V.; BROE, M. B. & FELDENKRIS, E. R. 2016. Phylogenetic relationships at the base of Ericaceae: Implications for vegetative and mycorrhizal evolution. *Taxon* 65 (4): 794-804.
- FRITSCH, P. W.; LU, L.; BUSH, C. M.; CRUZ, B. C.; KRON, K. A. & LI, DE-ZHU. 2011. Phylogenetic Analysis of the Wintergreen Group (Ericaceae) based on Six Genic Regions. *Systematic Botany* 36: 990-1003.
- HELED, J. & DRUMMOND, A. J. 2009. Bayesian Inference of Species Trees from Multilocus Data. *Mol. Biol. Evol.*, v. 27, p. 570-80.
- HUELSENBECK, J.P. & RONQUIST, F. 2001. MrBayes: bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755;
- JUDD, W. S. 1984. A taxonomic revision of the American species of *Agarista* (ERICACEAE). *Journal of the Arnold Arboretum* 65: 255-342.
- KATOH, T. & STANDLEY, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772-780.
- KEARSE, M.; MOIR, R.; WILSON, A.; STONES-HAVAS, S.; CHEUNG, M.; STURROCK, S.; BUXTON, S.; COOPER, A.; MARKOWITZ, S.; DURAN, C.; THIERER, T.; ASHTON, B.; MENTJIES, P. & DRUMMOND, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.
- KRON, K. A.; JUDD, W. S.; STEVENS. P. F.; CRAYN, D. M.; ANDERBERG, A. A.; GADEK, P. A.; QUINN, C. J. & LUTEYN, J. L. 2002. Phylogenetic classification of Ericaceae: molecular and morphological evidence. *The Botanical Review* 68: 335-424.
- KRON, K. A. & LUTEYN, J. L. 2005. Origins and biogeography patterns in Ericaceae: New insights from recent phylogeny analyses. *Biol. Skr.* 55: 479-500.
- MENDES, F. K.; FUENTES-GONZÁLEZ, J. A.; SCHRAIBER, J. G. & HAHN, M. W. 2018. A multispecies coalescent model for quantitative traits. *eLife* 7:e36482.
- SUCHARD M. A; LEMEY P., BAELE, G.; AYRES, D. L.; DRUMMOND, A. J. & RAMBAUT, A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution* 4: vey016.