

## Discovering new ‘superfruits’: from taxonomy to phytochemistry of Brazilian *Eugenia* (Myrtaceae) fruits

*Eugenia* L. (c. 1100 species) is the largest genus of neotropical Myrtaceae and the second most diverse tree genus worldwide (Beech et al. 2017). In Brazil, the genus is one of the largest in the country’s flora (c. 400 species) and its diversity is remarkably high in threatened environments such as the Atlantic forest and Cerrado biomes (Flora do Brasil 2020). Several studies have pointed out the ecological importance of *Eugenia* in these habitats, not just in its high number of species (e.g. Oliveira-Filho & Fontes 2000; BFG 2015; Lucas & Bunger 2015) but also as a source of food for frugivores as it bears fresh-fruits throughout the year (Gressler et al. 2006; Staggemeier et al. 2010).

In parallel, *Eugenia* fruits could also provide an opportunity to discover new sources of human food and pharmacological compounds. In fact, most Brazilian native species cultivated in backyards for fruit production are species of *Eugenia*, such as the Surinam cherry (*E. uniflora*), uvaia (*E. pyriformis*) and grumixama or Brazilian cherry (*E. brasiliensis*). There are also several recent studies reporting *Eugenia* species as potential sources of ‘superfruits’ (or functional fruits), fruits rich in vitamins, minerals and numerous other classes of biologically active compounds with beneficial characteristics such as antioxidant, anti-inflammatory, anti-obesity (e.g. Infante et al. 2016; Araújo et al. 2019).

Despite this potential, when searching for studies regarding *Eugenia* fruit chemical composition, available studies encompass just ten species of the Brazilian flora. This number represents about 2.5% of the *Eugenia* species diversity in Brazil and suggests that the bioavailability of phytochemicals from the genus is under-estimated. Another problem is that most studies don’t have vouchers in biological collections and chemical protocols are not standardized, meaning that even available data is unreliable.

Regarding the morphology, taxonomy and phylogenetic knowledge of the group, a notable improvement had been achieved in the last decade, especially in the neotropics. Several works providing more cohesive classification and evolutionary hypotheses have been published (Mazine et al. 2014; Faria 2014; Bünger 2015; Bünger et al. 2016; Mazine et al. 2018; Giaretta 2018). However, modern taxonomic treatment of *Eugenia* is at an early stage and very few descriptions of fruits are available. In addition, the fruits of most species are unknown (pers. comm. Mazine).

Molecular data can be used to improve the search for patterns traits of ecological, evolutionary and economic importance. In this context, modern botanical data are increasingly applied to questions of sustainable crops and natural capital. **As such, the use of current taxonomic and phylogenetic knowledge of *Eugenia* to promote better understanding of morphological and chemical composition of its fruits could not be more opportune.** Such data will further be used to test whether these characters are associated with the infrageneric groups of *Eugenia*.

This project aims to carry out a multidisciplinary study of *Eugenia* to: (1) undertake the first evolutionary study of its fruits, (2) increase knowledge of morphological and chemical diversity of Brazilian native fruits, (3) elucidate patterns of fruits traits among infrageneric groups of the genus and (4) promote discovery of potential new ‘superfruits’.

### Materials and Methods

**Taxonomic sampling.** A minimum of 10% of *Eugenia* diversity in Brazil will be sampled (c. 40 species), ensuring all infrageneric groups are proportionally represented. Well known fruit species will be included with care taken to include better representation from different Brazilian biomes. Other Myrtaceae fleshy-fruited genera will be included as outgroups, such as *Myrcianthes* and *Pimenta*. For chemical analysis, three individuals of

each species will be included to provide representative results. Currently, fruits of 31 species have been collected and collection efforts in the most taxonomically complex and species-rich section, *Eugenia* sect. *Umbellatae*, are ongoing (almost half of the target of 22 species have been collected from this section).

**Data Collection.** (1) *Field activities*: botanical collections have been made in natural and cultivated areas to obtain samples for taxonomic, chemical and phylogenetic analyses. Notes on fruit size, colour, form, number and seed/fruit ratio have been taken. Freshy-fruits are washed, non-edible parts are manually removed (sepals and seeds) and an aliquot of 30 g is freeze-dried, ground and stored at -80 °C for chemical analysis; (2) *Molecular activities*: sequences of two nuclear (ETS and ITS) and four plastid regions (rpL16, psbA-trnH, rpl32-trnL e trnQ-5-rps16) are being used in this work. *Eugenia* sequences available on GenBank from previous studies (Faria 2014; Bünger et al. 2016; Mazine et al. 2018) are being used to increase the scope of the phylogeny in which fruit characters will be examined; (3) *Chemical activities*: moisture, sugar (Cordenunsi et al. 2008), ascorbic acid (Pasternak et al. 2005), carotenoids (Pacheco et al. 2014), antioxidant capacity (Genovese et al. 2008), total phenolics (Watermar & Mole 1994) and tannin content (Watermar & Mole 1994) are being measured using appropriate protocols. Means are calculated from the three samples of a single species to minimize the phenotypic plasticity.

**Analysis.** (1) *Phylogenetic reconstruction*: Maximum Likelihood and Bayesian Inference will be carried out using RaxML 8.2 (Stamatakis 2006) and MrBayes 3.2 (Ronquist & Huelsenbeck 2003), respectively. The best evolutionary models for BI will be estimated using jModelTest 2; (2) *Ancestral reconstruction of characters*: phylogenetic signal will be estimated using the phytools package (Revell 2012) implemented using R software to examine dependent or independent occurrence of morphological and chemical patterns in *Eugenia* fruits. The use of discrete vs. continuous characters will be explored.

### Prospective budget and other financial support

This project is a crucial component of my PhD and will cost a total of \$14,356. \$11,426 of this has been secured from other sources to complete the molecular, chemical activities and part of the fieldwork (FUNBIO, The Emily Holmes Memorial Scholarship, The Explorers Club – Mamont Scholar Grant, The Mohamed bin Zayed Species Conservation Fund). The collection of fruits is a major component of this project and the most challenging one, as samples must be ripe at of collection, transported in a refrigerated condition and freeze-dried in 24 hours. The budget requested here will be used to complete three field expeditions, to Caatinga and Amazon biomes (Pernambuco, Rio Grande do Norte and Amazonas states) in order to better represent their *Eugenia* fruit diversity.

Total budget project	\$14,356.00
Financial support secured or in progress	
Institutional stipend (CAPES Scholarship not included)	\$1,000.00
In-kind contributions	\$10,426.00
Other proposals submitted	\$965.00
Outstanding field work activities	
Travel costs (airline tickets, car rent, fuel, daily expenses)	\$1,725.00
Labor costs	\$240.00
<b>Total request</b>	<b>\$1,965.00</b>

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