Proposal title: Towards an integrative revision of the overlooked genus *Ecclinusa* (Sapotaceae): taxonomy, molecular phylogeny, and diversification patterns

Introduction. The Neotropical genus *Ecclinusa* Mart. (Sapotaceae, Chrysophylloideae) includes 12 species distributed from Panama to tropical South America (Pennington 1990; Terra-Araujo et al. 2015a). Although *Ecclinusa* is a small genus, some species such as *Ecclinusa guianensis* Eyma and *E. lanceolata* (Mart. & Eichler ex Miq.) Pierre are among the most abundant species inside permanent plots in terra-firme (non-flooded) forests of Central and Peruvian Amazonia, respectively (Oliveira and Mori 1999; Waltke 2008). The majority of species are distributed generally of the Guyana Shield occurring in savannah (being the only genus of Neotropical Sapotaceae that is well represented in this biome), lowland rainforest and cloud forest (e.g. Peru) to 1300 m altitude, and they vary in habit from small understory treelets to large canopy emergents (Pennington 2004).

The classification history of the genus was controversial, which is reflected by the poorly understood taxonomy of Sapotaceae that has concealed definition by botanists for over 100 years. *Ecclinusa* was included in *Chrysophyllum* L. by Baehni (1965), later Aubréville (1961), which recognizing it as a genus distinct from *Chrysophyllum*, included in it species of both *Pradosia* Liais and *Chrysophyllum*. Of the 18 species treated in this study by Aubréville, five belong in *Ecclinusa*, seven belong in *Chrysophyllum* (sects. Ragala and Prieurella), and six in *Pradosia*. In the Flora Neotropica account of Sapotaceae, which consists in the latest taxonomic revision for the genus, Pennington (1990) recognized eleven species in *Ecclinusa*. However, over the past two decades it has become outdated, with newly described species and an accumulation of new material that has extended the geographical distribution of many species.

Ecclinusa and *Chromolocuma* Ducke are the only genera in Neotropical Chrysophylloideae showing stipules (*sensu* Faria et al. 2017). However, *Ecclinusa* is easily distinguished from *Chromolucuma* by the presence of white latex in bark, twigs and petioles (vs. yellow latex), sessile flowers (vs. pedicellate) and lack of staminodes (vs. presence of staminodes) (Pennington 1990, 1991; Swenson et al. 2008). Currently, the available molecular phylogenetic reconstructions for Sapotaceae-Chrysophylloideae point to a close relationship of *Ecclinusa* to *Chrysophyllum*, *Elaeoluma* Baill. and possibly *Pouteria* Aubl., and it is a strongly supported monophyletic group (Swenson and Anderberg 2005; Swenson et al. 2008; Terra-Araujo et al. 2015a; Faria et al. 2017), but internal resolution in *Ecclinusa* clade remains poorly resolved.

Given the ecological and evolutionary importance of *Ecclinusa* in the context of the Chrysophylloideae diversification across South America, it is critical to establish an even more robust phylogenetic hypothesis to include, for example, nuclear DNA markers and a complete sampling of the species diversity in the genus. A sound phylogenetic analysis of *Ecclinusa* will potentially help to understand how ecology and biogeography interact to shape its phylogeny. Furthermore, this will permit accurate species delimitation in a solid taxonomic monograph of the genus. Understanding the taxonomic and evolutionary history of *Ecclinusa* is particularly interesting because the genus is widely distributed with different ecological preferences, morphologically variable but easily recognizable, which allows exploring different issues on species diversification, such as transitions between Amazonia and Atlantic Forest, Amazonia and dry areas of Venezuela, and the occupation of sandy environments. A recent ecological estimate listed *Ecclinusa guianensis* in an oligarchy of 227 dominant species from an assemblage of ca. 16,000 woody species that make up the Amazonian forest (ter Steege et al. 2013). Thus, *Ecclinusa* can serve as key lineage to foster our understanding on ecological and diversification processes across the extremely diverse environments. We believe that only an integrative taxonomic approach on *Ecclinusa* will allow us to understand its evolutionary history and contribute to consistent species identifications without overestimating its diversity.

This proposal requests funds to enable visits to North American herbaria towards phylogenetic sampling and morphological study across the Neotropical distribution of the genus *Ecclinusa*. A travel to the USA would facilitate my visit to three important institutions such as MO, NY and US herbaria, which housing a representative collection of *Ecclinusa* (including type collections) as well related genera from Brazil and bordering countries, such as Venezuela, Colombia, Peru and also Guiana Shield. The herbarium visits will be of much use to consolidate accurate species descriptions and to consult important historical taxonomic publications, all of which shall be included in a forthcoming monograph of the genus.

Proposal aim. The first task of my thesis project is to review type collections, update the nomenclature and obtain comprehensive records on geographic distribution and ecological preferences of species, and DNA samples of preserved specimens (when possible). Thus, the specific goals to be reached at herbarium collections are the following: **1.** Analyze the *Ecclinusa* collection, including type specimens; **2.** Review taxonomic studies of *Ecclinusa* stored at the libraries of the interest institutions; **3.** Update the unidentified collections of *Ecclinusa* and related genera; **4.** Gather morphological data for studies on morphological evolution.

Method. The taxonomic treatment will be performed based on a bibliographic survey, consultation to herbarium collections and field expeditions throughout the Neotropics. In the case of herbarium studies, the flowers and fruits (if available) will be temporarily removed from herbarium specimens and boiled in Copenhagen mixture (70 mL of ethanol, 29 mL of distilled water, 1 mL of glycerol) in a microwave oven until the parts were soft enough to permit examination under a stereo-microscope. Terminology will follow Harris and Harris (2001) and Hickey and King (2000). DNA samples will be requested (when available) according to the herbarium use policies. Molecular sequence data will be extracted from three different nuclear regions: ETS, ITS and RPB2 (Oxelman and Bremer 2000). Extraction, amplification and primers of ETS, ITS and RPB2 will follow the protocol proposed by Swenson et al. (2013), and Swenson et al. (2008) for ETS and Bartish et al. (2005) for ITS. Phylogenetic analysis and reconstruction of the biogeographic pattern, ancestral states of habitat and morphology will be performed according to Terra-Araujo et al. (2015b).

Here, we will adopt the general lineage-based concept (GLC) (De Queiroz 1998) in delimiting species. Under GLC, a species is formed by separately evolving metapopulation lineage, where reproductive isolation, reciprocal monophyly or ecological divergence is reached independently at different times along the evolutionary history (De Queiroz 2007). Thus, a lineage does not have to be necessarily monophyletic, morphologically distinct, or reproductively isolated to be recognized as a species. In practice, any property that provides evidence of lineage separation is relevant to infer the boundaries and number of species. For this taxonomic treatment, the ecology, geographic distribution, phylogenetic relationships and morphological and spectral data of species will be considered as sources of evidence.

Background information. This proposal is part of my Ph.D. thesis research and will investigate how many species of *Ecclinusa* there are in Neotropics, how they can be identified using DNA sequences and morphological characters, and how the different species are related to one another in an evolutionary context. The project has four major goals: **1.** Reconstruct the evolutionary history based on a solid phylogenetic framework and test whether the previously described taxa are monophyletic; **2.** Obtain morphological, ecological and near-infrared spectroscopy data to delimit species based on molecular phylogeny, and test whether the species are supported by this integrated dataset; **3.** Estimate the divergence time between clades and discuss the historical biogeography (considering habitat transitions, climate changes and landscape evolution over time); **4.** Update the taxonomy of the genus (including possible new species), and make free online tools available, such as interactive keys and an illustrated identification guide for the genus.

Broader impacts. In addition to publications that will document the results, the project will benefit scientists and the general public with a source of reliable Sapotaceae taxonomy and diversity for the Flora of Brazil (BFG 2018). It will also produce online databases that will be a significant resource of biological data (morphological, near-infrared spectroscopy, ecological, geographical, and genetic data) for Neotropical Sapotaceae in the precursor (see duckewiki) of the ODB platform, which will be part of a dynamic taxonomy system using OpenDataBio, a web platform based on specimen-oriented approach. Here, all data obtained are integrated into this platform for building dynamic and updated taxonomic monograph. Biological data will be obtained from historical and recent collections housed at US, MO, NY and Brazilian herbaria and from field expeditions. Additional contributions are: (i) voucher specimens from field collections will be deposited in Brazilian herbaria and be available through Flora of Brazil project and SpeciesLink's Virtual Herbarium; (ii) geographic data will be available through GBIF; (iii) DNA sequence data will be made publicly available through GenBank.

Literature cited

Aubréville A (1961) Notes sur des Pouteriées américaines. Adansonia 1:171–173.

Baehni C (1965) Mémoires sur les sapotacées. 3. Inventaire de genres. Boissiera 11:1–262.
Bartish I V, Swenson U, Munzinger J, Anderberg AA (2005) Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): molecular evidence for generic polyphyly and repeated dispersal. Am J Bot 92:667–673.
BFG-Brazil Flora Group (2018) Brazilian Flora 2020: innovation and collaboration to meet Target 1 of the Global Strategy for Plant Conservation (GSPC). Rodriguésia 69:1513–1527.
De Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation. In: Howard DJB, Berlocher SH (eds) Endless Forms: species and speciation. Oxford, New York, USA, pp 57–75.
De Queiroz K (2007) Species Concepts and Species Delimitation. Syst Biol 56:879–886.
Faria AD, Pirani JR, Ribeiro JELS, et al (2017) Towards a natural classification of subfamily Chrysophylloideae (Sapotaceae) in the Neotropics. Bot J Linn Soc 185:27–55.
Harris JG, Harris MW (2001) Plant identification terminology: an illustrated glossary, 2nd edn. Spring Lake Publishing, Payson, USA.
Hickey M, King C (2000) The Cambridge Illustrated Glossary of Botanical Terms. Cambridge University Press, Cambrigde, UK.
Oliveira AA, Mori SA (1999) A central Amazonian terra firme forest. I. High tree species richness on poor soils. Biodivers Conserv 8:1219–1244.
Oxelman B, Bremer B (2000) Discovery of paralogous nuclear gene sequences coding for the second-largest subunit of RNA polymeras II (RPB2) and their phylogenetic utility in Gentianales of the asteroids. Mol Biol Evol 17:1131–1145.
Pennington TD (1990) Flora Neotropica Monograph 52: Sapotaceae. New York Botanical Garden, New York.
Pennington TD (2004) Sapotaceae. In: Kubitzki K (ed) The Families and Genera of Vascular Plants. Flowering Plants. Dicotyledons, Celastrales, Oxalidales, Cornales, Ericales. Springer-Verlag, Berlin, pp 390–401.
Pennington TD (1991) The genera of Sapotaceae. Royal Botanic Gardens, Kew.
Swenson U, Anderberg AA (2005) Phylogeny, character evolution, and classification of Sapotaceae (Ericales). Cladistics 21:101–130.
Swenson U, Nylinder S, Munzinger J (2013) Towards a natural classification of Sapotaceae subfamily Chrysophylloideae in Oceania and Southeast Asia based on nuclear sequence data. Taxon 62:746–770.
Swenson U, Richardson JE, Bartish IV (2008) Multi-gene phylogeny of the pantropical subfamily Chrysophylloideae (Sapotaceae): evidence of generic polyphyly and extensive morphological homoplasy. Cladistics 24:1006–1031.
ter Steege H, Pitman NCA, Sabatier D, et al (2013) Hyperdominance in the Amazonian tree flora. Science (80-) 342:1243092.
Terra-Araujo MH, Costa FM, Carvalho RB, Vicentini A (2015a) *Ecclinusa campinae* (Sapotaceae), a new species from the Middle Rio Negro region, Amazonas, Brazil. Brittonia 67:180–184.
Terra-Araujo MH, Faria AD, Vicentini A, et al (2015b) Species tree phylogeny and biogeography of the Neotropical genus *Pradosia* (Sapotaceae, Chrysophylloideae). Mol Phylogenet Evol 87:1–13.
Waltke AJ (2008) The Sapotaceae of a lowland rainforest: diversity and distribution in the Los Amigos Watershed, Madre de Dios, Peru. Master thesis, Texas Christian University, Fort Worth, Texas.

Financial support. National Council for Scientific and Technological Development (CNPq, in Portuguese) full-time fellowship number 142214/2018-3 for Caroline C. Vasconcelos.

Prospective itemized budget

Description	Amount ($)
Herbarium visits/ airline tickets (round trip)	950
Accommodation	720
Molecular genetics/consumables	170
Exchange Visitor US Visa (J1)	160
Total request	2000