DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	The Nature of Species project -
Travel Dates:	11 June 2024 – 14 July 2024
Location:	Campos rupestres (rupestrian grasslands), Cerrado and Caatinga regions in Brazil.
Group Members:	Flávia Fonseca Pezzini (RBGE), Thales de Lima (RBGE), Laís Zeferino (Unesp - Brazil), Livia Echternacht (UFOP – Brazil)
Aims:	(i) increase RBGE's herbarium collection of campos rupestres plant representatives; (ii) collect leaf material for high molecular weight DNA extraction to produce a reference genome using long-reads for *Comanthera* (sequencing funds already available), (iii) collect multiple individuals of species of *Comanthera* to be sequenced using high-throughput deep resequencing technique (sequencing funds already available), (iv) consolidate the collaboration with Dr. Echternacht (Universidade Federal de Ouro Preto - Minas Gerais, Brazil), specialist in the taxonomy of *Comanthera* and allied genera within Eriocaulaceae.

Photography consent form attached: ☒ Yes
(*please refer to your award letter)* ☐ No

Outcome (a minimum of 500 words):-

The expedition was part of the project of RBGE's PhD student Thales de Lima supervised by me, Professor Pete Hollingsworth and Dr. Alex Twyford, but also part of a broader project, The nature of species, aiming at several Cerrado and campos rupestres plant groups to establish a conceptual framework for investigating and comparing the nature of plant species across multiple clades occurring on the main biomes of the Neotropics, Rain forest (Amazon), Savanna (Cerrado, including campos rupestres) and Seasonally Dry Tropical Forests (Caatinga). We aimed to collect multiple individuals per species across the distribution of the genus *Comanthera* (Eriocaulaceae) to investigate plant species boundaries in the campos rupestres (or rupestrian grasslands), a threatened and megadiverse montane grassland within the Cerrado. I joined the expedition for four weeks, travelling over 3,000km across the Caatinga, Cerrado and campos rupestres. Overall, we collected 152 specimens as herbaria vouchers and silica samples under the collection numbers of Thales de Lima (107) and my own (45) and eight samples in cold-chain for long-read, whole genome sequencing. We collected 36 samples of *Comanthera* representing at least 15 species and species of four other Eriocaulaceae genera (Table 1). We collected samples not only of Eriocaulaceae (mostly under de Lima's numbers), but also of other plant families that are focus of research at RBGE (Leguminosae, Solanaceae, Ericaceae, Malvaceae) (Figures 1 and 2).

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Table 1. List of genera and species of Eriocaulaceae collected overall during the 2024 expedition under collection numbers of de Lima.

Genus	species	number of samples
Comanthera	*aciphylla*	1
	aurifibrata	2
	bisulcata	6
	borbae	1
	centauroides	6
	curralensis	2
	dealbata	3
	euschemus	2
	giuliettiae	1
	harleyi	3
	lanosa	1
	mucugensis	1
	nivea	1
	paepalophylla	2
	pignalii	1
	sp.	3
Comanthera Total		36
Eriocaulon	sp.	1
Eriocaulon Total		1
Leiothrix	*flagellaris*	2
	flavescens	2
	fluitans	2
	prolifera	1
	sp.	2
Leiothrix Total		9
Paepalanthus	*denudatus*	1
	elongatus	2
	sessiliflorus	1
	tortilis	4
	trichophyllus	1
	sp.	6
Paepalanthus Total		15
Syngonanthus	*caulescens*	1
	gracilis	1
	humboldtii	1
	polyaxis	1
	widgrenianus	1
	sp.	2
Syngonanthus Total		7

According to Brazilian legislation, all specimens collected in Brazil need to have one duplicate deposited in a national herbarium and duplicates can be sent elsewhere. We deposited our samples in the herbarium OUPR (Universidade Federal de Ouro Preto - Minas Gerais, Brazil, institution of Dr. Echternacht) and the final documentation is on its way to send duplicates and silica samples to E. During the expedition, we also had the opportunity to meet with de Lima's co-supervisor based in Brazil, Dr. Livia Echternacht, both in the field and in the herbarium. The days we spent together were important to talk about the species taxonomy and ecology, to discuss the details of the PhD project but also important to consolidate my collaboration with Dr. Echternacht for future grant proposals investigating the nature of plant species for other groups in campos rupestres. We dedicated some days of the expedition to develop the logistics of sampling non-model plant species in cold-chain (dry ice or liquid nitrogen) for whole genome, long-read sequencing. Long-read sequencing technologies require high molecular weight DNA, i.e., DNA that is not degraded (broken) and available in long fragments (at least longer than 50,000 base pairs or more). DNA starts to degrade as soon as plants are collected and to prevent that samples need to be stored in dry ice or liquid nitrogen as soon as collected until the DNA sequencing starts. We collected specimens of *Comanthera* in the field and kept them alive in pots until we reached a town where it was possible to hire a company to provide dry ice and transport the samples. During this time, we secured all the relevant paperwork to go along the samples in a cold-chain international shipment. It was very unfortunate that at the very last step – transportation from Rio de Janeiro/Brazil to Edinburgh – things did not go according to plans and the box arrived in Edinburgh cracked and a couple of days late. We are unsure if enough non-degraded DNA survived, but an extraction test will be done soon. We are organising a plan to re-collect representatives of such species. This was the first field expedition of Thales de Lima to collect herbarium, silica and cold-chain leaf samples. I am grateful to the Davis Expedition Fund for funding this expedition which allowed me to collect important samples for my research at RBGE to help deliver RBGE's Science and Biodiversity Strategy 2021-2030, for Research Theme 1 - Biodiversity Genomics, straighten local collaborations and to accompany a PhD student along a successful field expedition that also included some complicated bureaucratic and logistics steps. Samples collected during the expedition will allow me to continue the project The Nature of Species, complement the data from previous field work, expand the sampling to the often neglected campos rupestres and its flora and help setting up protocols to deal with the challenges of collection of plant leaf tissue in cold-chain to produce reference genomes particularly from tropical areas.

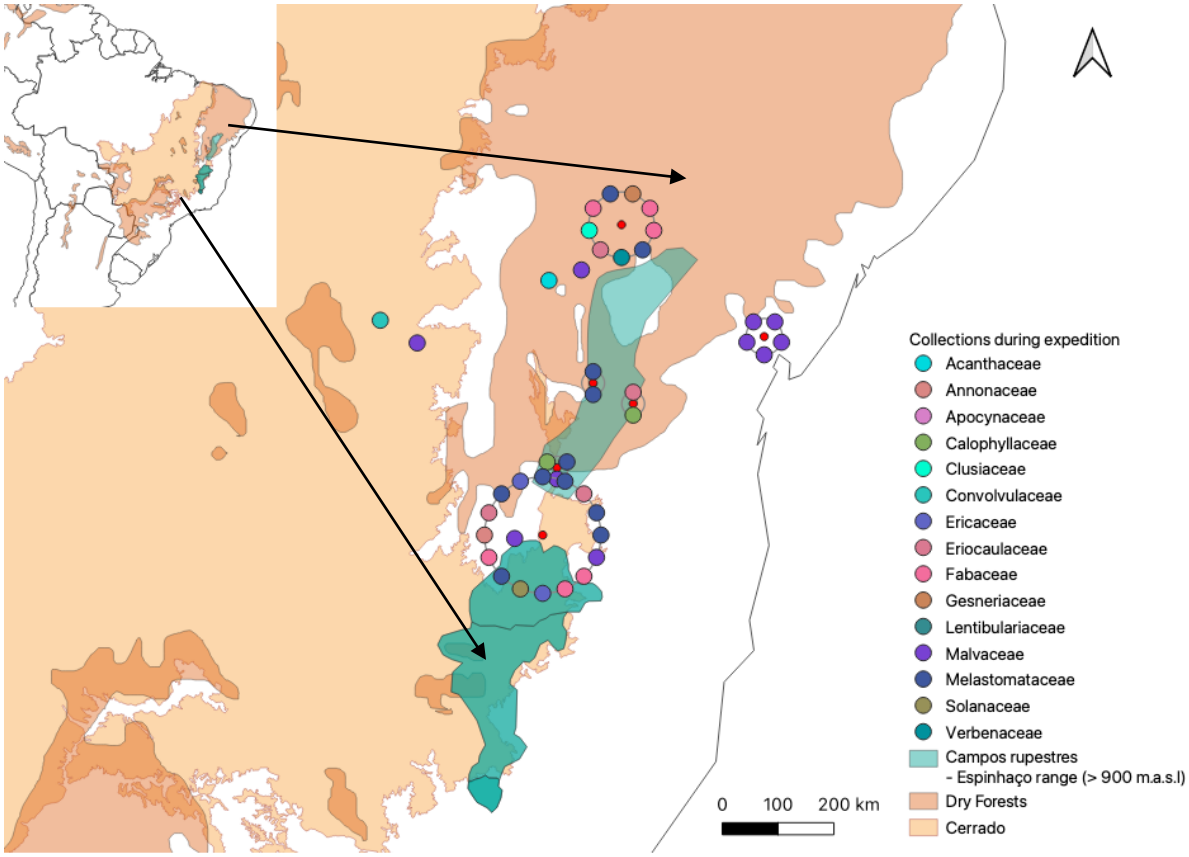

Figure 1. Map representing the sampling locations in NE Brazil and representative families collected during the field expedition.

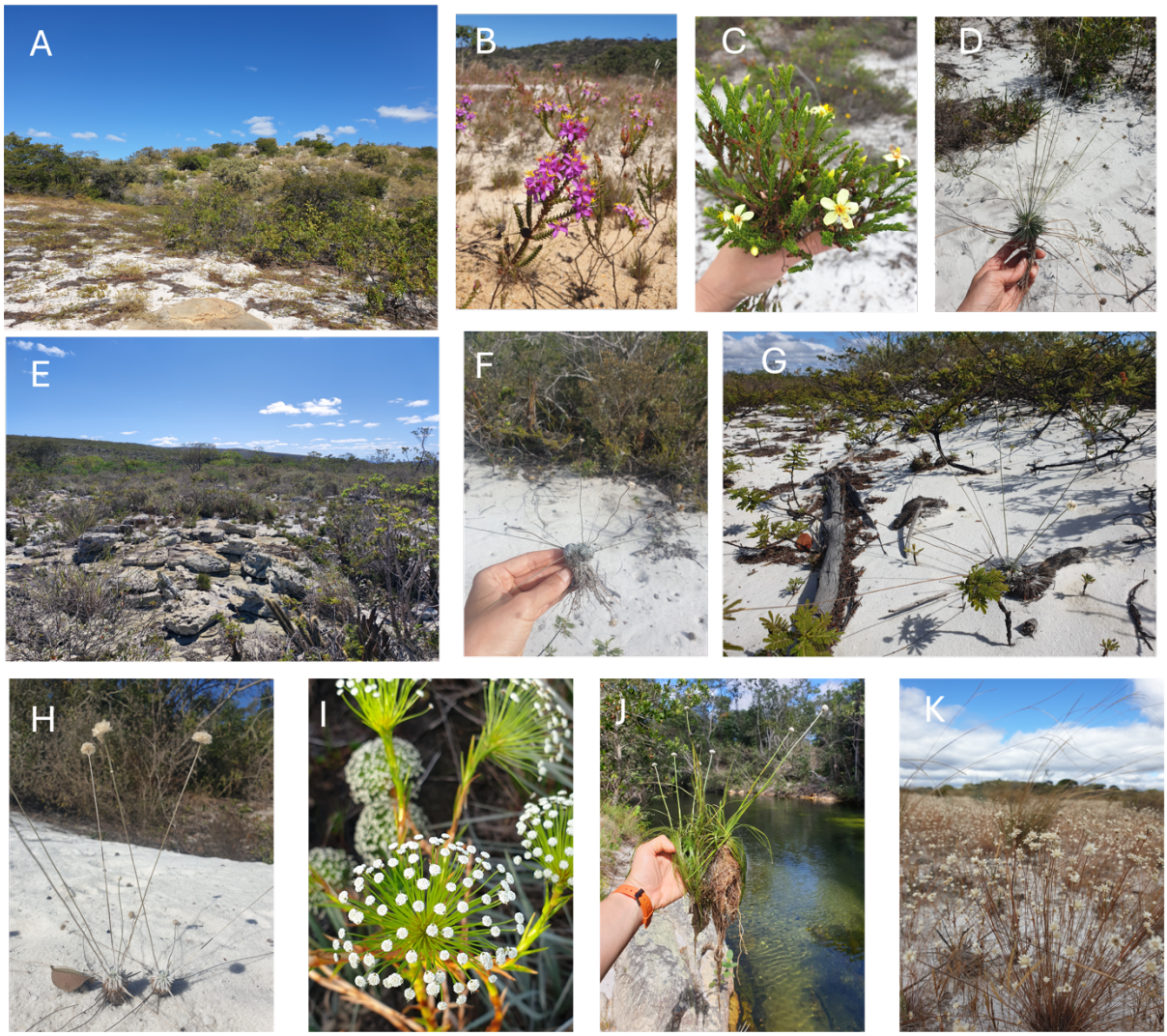

Figure 2. Photo plate of campos rupestres (A and B) and samples of *Comanthera* (D, F, G, H, K), other Eriocaulaceae (I, J) and Ericaceae (B, C) collected during the 2024 expedition.