DAVIS EXPEDITION FUND

REPORT ON EXPEDITION / PROJECT

Expedition/Project Title:	Exploring the roles of geographic barriers in determining mutualistic associations between the Neotropical plant *Tococa* and its *Azteca* ants.
Travel Dates:	March 9th 2016 to June 20th 2016
Location:	Antioquia, Santander, Tolima, Meta, Casanare, (Colombia)
Group Members:	María Fernanda Torres Jimenez
Aims:	To collect *Tococa* plants and its ant symbionts at different populations settled at both sides of the Andean cordillera.

Introduction

Myrmecophyte plants associate with ant colonies nesting the plant's hollow cavities (or ant domatia) and provide them with food resources (Davidson and McKey 1993; Rosumek et al. 2009). The ants act as the biotic defence of the plant host against herbivores and plant competitors (Bronstein et al. 2006). As components of tropical communities, ant-plant interactions are important for structuring food webs and promoting diversity (Heil and McKey 2003).

Tococa guianensis is one of the most widely distributed species of myrmecophyte Melastomataceae, commonly found from Central to South America (Michelangeli 2005). The Melastomataceae family includes 68 myrmecophyte plant species mostly belonging to *Tococa* and *Clidemia*, and representing 20% of myrmecophyte species globally (Michelangeli 2010). Ants nest in domatia placed at the petiole, the blade of the leaf, or the hollow stems (Michelangeli 2010). *Tococa* is mainly associated with *Pheidole* and *Azteca* ants, and at a less extent with *Crematogaster*, *Myrmelachista* and *Camponotus* (Alvarez et al. 2001; Dejean et al 2009). *Azteca* (Dolichoderinae) is a large Neotropical ant genus encompassing about 84 species exhibiting different nesting behaviours, including myrmecophytism, (Longino 1989). High morphological differences among individuals in different localities suggest species diversification throughout the South American tropics (Longino 1991). *Azteca* is commonly associated with *Tococa*, but also nests in *Cordia*, *Tachigali*, *Cecropia*, and *Triplaris* (Longino 1991).

The Neotropical region holds a high proportion of the world's species diversity and it is characterized by recent geographical changes and highly diversified taxa (Antonelli et al. 2010). Many studies have proposed the Andean mountain chain as the driver of such speciation via allopatric speciation and ecological displacement, and by changing the hydrology and climate of the region (Antonelli et al. 2009; Hoorn et al. 2010). But despite the implications that landscape changes might have on ecologically restricted taxa and its interspecific interactions, only few studies address the effect of geographic barriers in mutualisms, and even less include more than one of the mutualists.

This project aims to explore the role of Andean geography as barrier to gene flow on the population history of Neotropical plant-ant mutualisms, with Melastomataceae myrmecophyte plants (with emphasis in *Tococa*) and *Azteca* ants as a model. Using molecular data obtained from plant and ant genomes I will 1) barcode the ants and plants to identify Molecular Taxonomic Units and geographic structure; 2) assembly genomes of individuals from each population; 3) model plausible population history hypothesis based on genetic differentiation among populations across the Andes; and 4) detect possible population vicariance by comparing the timing of coalescence events and geographic events.

The main purpose of this expedition to Colombia was to collect material from the populations of ant and plant symbionts located in the areas not sampled in the first fieldwork season to complete the survey at both sides of the cordillera. In addition to the genetic data obtained from the samples, useful ecological information was recorded during the expedition I sampled as many domatia per plant as possible to capture allate ants (key for morphological identification) and the record of possible species cohabiting the plant.

The material collected during this fieldwork season is representative of the inter cordillera valleys (the Magdalena drainage system) and the easternmost lowland areas of Colombia.

Expedition itinerary

A total of ten locations for population sampling and diversity surveys were selected throughout the plant's distribution ranges in such way the distance among sites will be equidistant on both sides and across the Andes range. At each location, areas nearby were visited to try to sample as much diversity as possible.

Only one area that was planned for this fieldwork season could not been visited. The Catatumbo region (1 in Fig. 1) is of high interest and relevance for its biodiversity from which only a small proportion of it has been recorded. But unfortunately, and despite of the country's opening thanks to the recent social improvements, the area is of no access due to other groups taking over the previously opening territories. As security could not be granted, this location was removed from the plan. Unlike Catatumbo, the areas of Tauramena, Amalfi and Cimitarra (Fig. 1) were formerly restricted areas that now can be securely accessed, and collecting samples there contributes to the knowledge on Colombia's fauna and flora and its distribution.

At other areas selected for sampling the plant was not found (2-4 in fig. 1). Those areas were chosen for the purpose of my project to provide an equidistant comparison of populations. These regions (along with Antioquia and Santander), have been notoriously transformed by deforestation, cattle and agriculture. *Tococa* can grow in disturbed and secondary forest; nonetheless farmers cut off the plants because of the undesired presence of the ants (as local people told me during fieldwork). In the case of La Victoria-Caldas, *Tococa* records exist at the Universidad

Nacional de Colombia Herbarium, however the area has been flooded to build a dam and I did not find the plant in the surrounding areas. All this highlights the relevance of taking conservation and educative actions in rural areas of Colombia.

Figure 1. Map of Colombia showing the collecting locations sampled from December 2014 to June 2016. White circles represent areas sampled during the first fielwork season in 2014-2015. Orange circles represent areas successfully sampled during 2016. Black circles represent areas where the plant could not be collected: 1. Catatumbo in Norte de Santander; 2. La Victoria-La Miel in Caldas; 3. Honda-La Dorada in Tolima; 4. Armero in Tolima.

Methods

Plant and ant collection:

Each trip was planned to last for three to five working days excluding the two days to arrive and leave from the area. At each main location, a local assistant and I collected plants through large areas or sub-locations location at least 1km distant to increase the probability of sampling different populations. Each collection consisted on silica-dried tissue for DNA extraction, 10-30 ant workers and fertile individuals (when present) from 3-10 domatia, and all other insect found inside the domatia. Fertile material was collected, dried and deposited at the Universidad Distrital herbarium. Ant material was collected in 96% ethanol for identification and molecular purposes. A material transfer agreement between RBGE and Universidad Distrital has been sign and the exchange of material can be organised. All plant and ant collections were made under the Macro permit granted from the National Authority for Ambient Licences-Colombia to the Universidad Distrital. Other Melastomataceae plants were collected at areas where no myrmecophyte *Tococa* plants were found.

Plant and ant barcoding and phylogenetic analyses:
Plant samples were DNA barcoded using the nuclear Internal Transcribed Space 1 and 2 (ITS1 and ITS2), and the Yeast Cadmiun Factor 1 (ycf1) (Michelangeli 2004, Dong et al. 2015). Ant samples were DNA barcoded using the nuclear Internal Transcribed Space (ITS2) and Cytochrome Oxidase I (COI) regions (Pringle et al. 2012). A phylogeny was subsequently estimated under Bayesian methods. *Cecropia*-associated *Azteca* sequences from an older project but collected in the same areas were included in the phylogenetic analyses to evaluate species overlapping among those two common myrmechopyte plants.

Preliminary results

Myrmecophyte plants
Intraspecific morphological variation for *Tococa guianensis* is wide, especially in the Amalfi region where two different morphotypes, *Tococa guianensis* and *Tococa guianensis var. acuminata,* are found. Fertile samples of both plants were collected and the tissue will be used in future projects.

Table 1. Melastomataceae plants collected from 2013 to 2016 by location and sublocation (for each plant sample there is a corresponding ant collection).

Area	Department	Location	*Tococa guanensis*	Other Melastomataceae	Total samples
West north	Choco	Arusi_1	9	7	16
	Choco	Arusi_2	27	13	40
	Choco	Arusi_3	15	3	18
	Choco	Arusi_4	10	5	15
West south	Valle del Cauca	San Cipriano	12	0	12
	Valle del Cauca	Buenaventura	6	0	6
	Valle del Cauca	Bajo Calima	23	8	31
	Valle del Cauca	Bajo Calima	35	8	43
	Valle del Cauca	Bajo Calima	10	6	16
Central west north	Antioquia	San Luis	16	0	16
	Antioquia	San Carlos	10	2	12
	Antioquia	Amalfi_1*	24	0	24
	Antioquia	Amalfi_2*	19	0	19
Central east north	Santander	La India*	8	0	8
	Santander	Barrancabermeja*	12	0	12

East north	Casanare	Villanueva*	9	0	9
	Casanare	Tauramena_1*	8	0	8
	Casanare	Tauramena_2*	8	0	0
East south	Meta	San Juan de Arama_1	15	1	16
	Meta	San Juan de Arama_2	12	0	12
	Meta	San Juan de Arama_2	12	0	12
	Meta	Villavicencio_1*	5	0	5
	Meta	Acacias*	7	0	7
	Meta	Villavicencio_2*	9	0	9
Amazon west	Putumayo	Villagarzon_1	20	24	44
	Putumayo	Villagarzon_2	13	13	26
	Putumayo	Villagarzon_3	17	10	27
Amazon east	Amazonas	Kilometros	19	12	31
	Amazonas	Tarapaca	20	0	20
	Amazonas	Puerto Nariño	10	0	10
Other	Tolima and Cundinamarca*		0	7	7
		Total	420	119	531

* Samples collected during the last field season

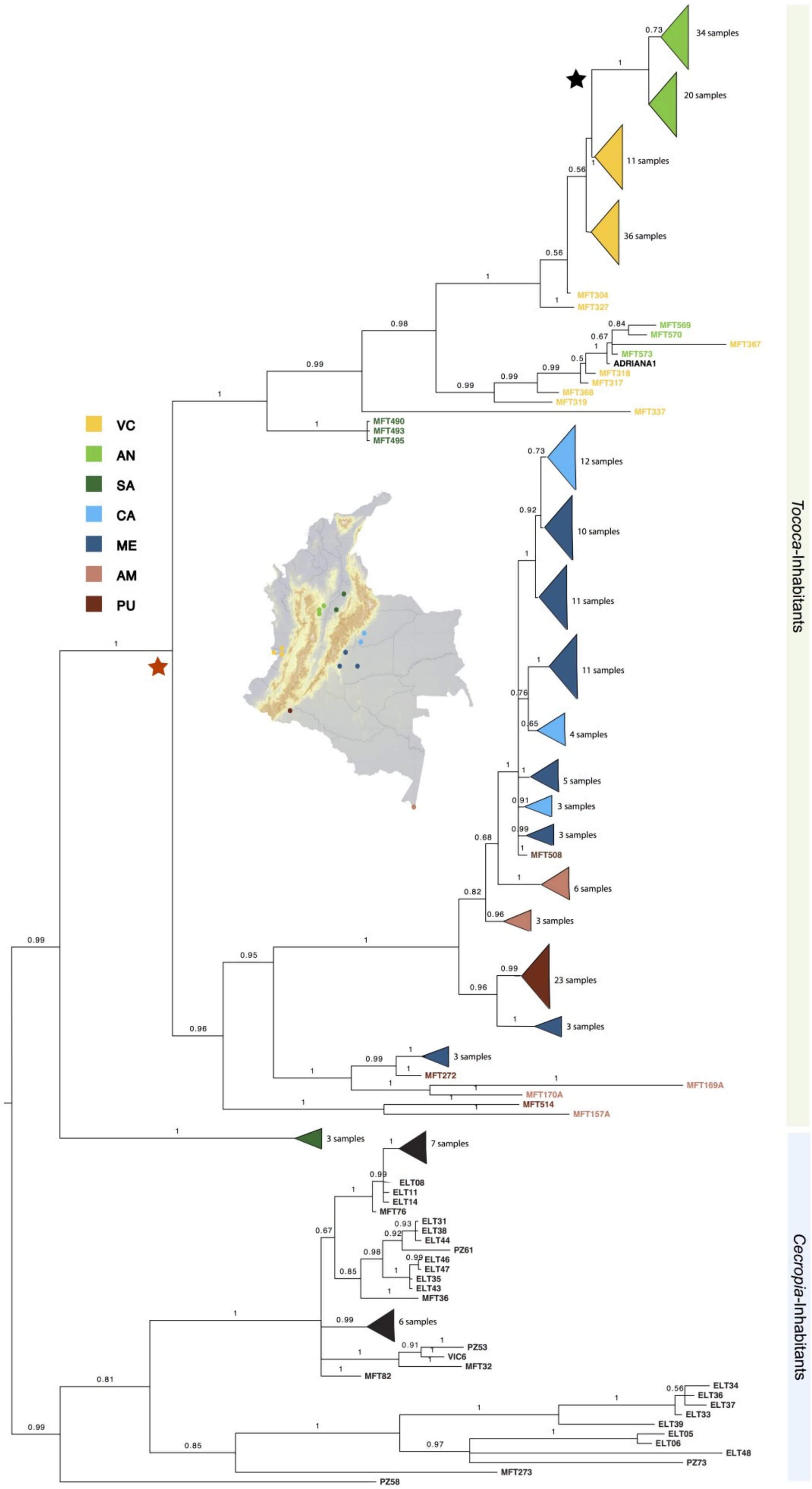
Plant-inhabiting ants
Two ant genera are the most common *Tococa* associated inhabitants (Table 2). *Pheidole* ants tend to inhabit plants growing inside conserved secondary and primary forest, while *Azteca* is more common in patched and disturbed areas. *Azteca* ants are bigger and have a higher muscular capacity that allows the queens to cover large areas in search of distant hosts to reduce competence with other ants. Contrary to the small *Pheidole* queens that tend to colonize areas densely populated by the host. This explains why in the area of Chocó, a preserved and not human-populated area, *Pheidole* was the only inhabitant of *Tococa*, despite of *Azteca* presence in bigger, more scattered myrmecophyte species such as *Cecropia.* *Azteca* was more common at other sampling locations were the presence of farmers has affected the forest, making *Tococa* individuals more scattered.

The best tree suggests geographical structure caused by both Western and Eastern Cordillera (Fig. 2). The role of Central cordillera as barrier to gene flow between populations in points 2-4 (Fig. 1) and the rest of populations cannot be tested since the plant could not been found.

Table 2. Ant colonies inhabiting *Tococa guianensis* and other Melastomataceae myrmecophytes. Ant species identification corresponds to the best BLAST hit and not necessarily correspond to a taxonomic revised identification.

Species	*Tococa guianensis*	Other Melastomataceae	N total
Allomerus octoarticulatus	2	12	14
Azteca beltii	8	1	9
Azteca nigricans	33	1	34

Azteca ovaticeps	4	0	4
Azteca quadraticeps	6	0	6
Azteca (unidentified)	167	34	201
Camponotus planatus	2	0	2
Crematogaster	2	1	3
Myrmelachista	17	5	22
Nylanderia	2	0	2
Pheidole ampla	1	0	1
Pheidole cramptoni	1	0	1
Pheidole erratilis	1	6	7
Pheidole gatesi	6	2	8
Pheidole jonas	1	0	1
Pheidole lacornis	1	0	1
Pheidole minutula	10	1	11
Pheidole monteverdensis	0	1	1
Pheidole oswaldi	33	31	64
Pheidole simonsi	1	0	1
Pheidole sospes	0	1	1
Pheidole (unidentified)	1	6	7
Solenopsis saevissima	2	0	2
Solenopsis (unidentified)	2	0	2
Tapinoma sessile	12	2	14
Unidentified ants	-	-	79
		Total colonies collected	419



VC
AN
SA
CA
ME
AM
PU
34 samples
20 samples
11 samples
36 samples
MFT304
MFT327
MFT569
MFT570
MFT367
MFT573
ADRIANA1
MFT318
MFT317
MFT368
MFT319
MFT337
MFT490
MFT493
MFT495
12 samples
10 samples
11 samples
11 samples
4 samples
5 samples
3 samples
3 samples
MFT508
6 samples
3 samples
23 samples
3 samples
3 samples
MFT272
MFT169A
MFT170A
MFT514
MFT157A
3 samples
7 samples
ELT08
ELT11
ELT14
MFT76
ELT31
ELT38
ELT44
PZ61
ELT46
ELT47
ELT35
ELT43
MFT36
6 samples
PZ53
VIC6
MFT32
MFT82
ELT34
ELT36
ELT37
ELT33
ELT39
ELT05
ELT06
ELT48
PZ73
MFT273
PZ58
Tococa-Inhabitants
Cecropia-Inhabitants

Figure 3. Bayesian phylogeny of COI for all *Azteca* samples collected from 2014-2016. The black star represents the split between the Valle del Cauca and the Antioquia clades by the Western Cordillera. The red star represents the split between the western clades (Valle del Cauca-Antioquia) and the eastern clades (remaining areas) by the Eastern cordillera.

Future work

The next step of the project involves exploring further the geographic structure and the whole genome sequencing of two ant individuals per population of the same species. With the help of bioinformatic tools and population analyses based on maximum likelihood approaches for model testing I will estimate the genetic divergence across the whole genome and will compare it by triplets of populations across and in the same side of the cordilleras (Fig. 3). Finally, I will test models of possible population histories and evaluate which of those fit best the data.

Three genomes of *Tococa guianensis* sampled during this project were sequenced and assembled. Though the quality of the assemblies is poor due to chromosome duplication and deletion processes, the aim is to sequence more *Tococa guianensis* samples at a deeper coverage to help with the assembly and annotation of the first whole genome of a plant from the Melastomataceae family.

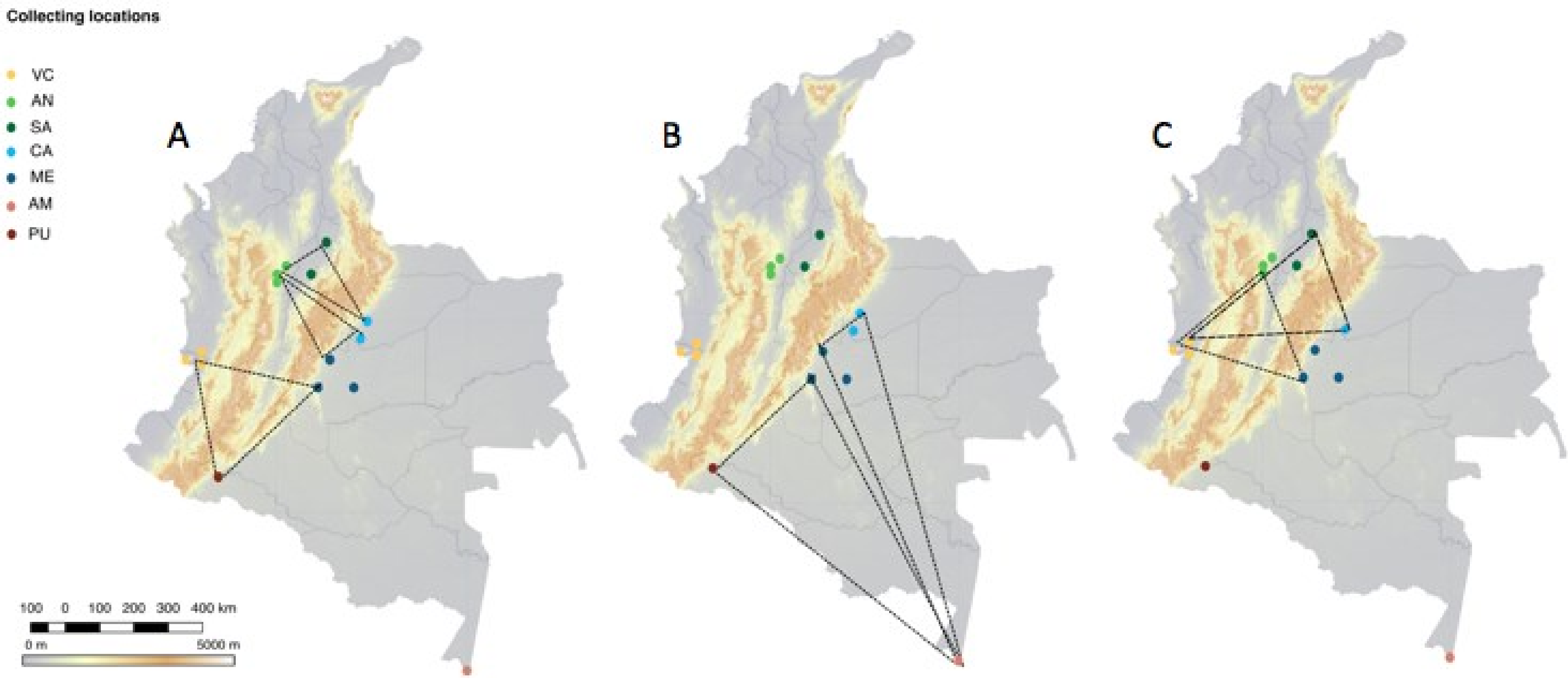


Figure 3. Map of Colombia showing the triplets of populations and the genetic divergence comparisons among them. A. Triplets of two populations in the same side and one across the cordillera; B. Triplets of three populations in the same side; C. Triplet of three populations across cordilleras.

References:

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Expenses

Location	Expense description	Number of days	Number of people	Amount in GBP
Bogotá	Tickets Edinburgh-Bogotá	NA	1	£1,300.00
	Taxi from house to the airport (round trip)	2	1	£10.00
	Transportation inside Bogotá	3 months	1	£50.00
	Silica, alcohol, paper and label printing for plant and ant collection	NA	NA	£17.50
	Subtotal			£277.50
Cimitarra (Santander)	Taxi from house to the bus station (round trip)	NA	1	£10.00
	Tickets Bogotá-Cimitarra (round trip)	NA	1	£40.00
	Tickets Cimitarra-La India (round trip)	NA	2	£17.50
	Stay in La India	5	1	£75.00

	Guide salary	5	1	£75.00
	Food (inluding the guide)	5	3	£70.00
	Moto La India - La herradura (round trip and waiting)	1	2	£32.50
	Boat pass La India-parcelas (roundtrip)	5	2	£50.00
	Subtotal			£370.00
Barrancabermeja (Santander)	Taxi from house to the bus station (round trip)	NA	1	£10.00
	Bus guide's home to bus station (round trip)	NA	1	£7.50
	Tickets Bogotá-Barrancabermeja (round trip)	NA	2	£90.00
	Stay in Barrancabermeja	1	2	£25.00
	Guide salary	1	1	£12.50
	Bus Barrancabermeja-Unipaz (round trip)		2	£15.00
	Food (inluding the guide)	2	2	£17.50
	Subtotal			£177.50
San Vicente de Chucurí (Santander)	Bus Barrancabermeja-San Vicente	NA	2	£15.00
	Stay in San Vicente	4	2	£92.50
	Guide salary	5	1	£62.50
	Food (inluding the guide)	5	2	£98.75
	Subtotal			£268.75
Tauramena, Villanueva (Casanare) Acacías, Villavicencio (Meta)	Total fuel expenses	NA	NA	£62.50
	Checkpoint payments (peajes) roundtrip	NA	NA	£33.50
	Stay in Tauramena	2	2	£62.50
	Stay in Acacias	3	2	£94.75
	Food (including driver)	6	2	£100.00
	Subtotal			£353.25
La Victoria-La Miel (Caldas), Honda, La	Total fuel expenses	NA	NA	£90.00
	Checkpoint payments (peajes) roundtrip	NA	NA	£37.50

Dorada, Armero (Tolima)	Stay in Honda (as a trip hub)	3	1	£72.50
	Food (including driver)	4	2	£75.00
	Subtotal			£275.00
Amalfi (Antioquia)	Taxi from house to the bus station (round trip)	NA	1	£10.00
	Tickets Bogotá-Medellín (roundtrip)	NA	1	£30.00
	Tickets Medellín-Amalfi	NA	1	£25.00
	Bus Amalfi-Arenas Blancas	NA	1	£10.00
	Stay in Arenas Blancas	4	1	£50.00
	Food (including guide)	5	2	£75.00
	Guide salary	5	1	£50.00
	Subtotal			£250.00
	TOTAL			£3,272.00
	Granted by Davis Expedition fund			£3,250.00
	For Davis Expedition Fund			(£-22.00)