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Para Clara y Ronaldo,
En memoria de Pepe Barletti

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ABSTRACT

Tropical areas represent the region with the highest diversity in the world. In plants, one major radiation having this high diversity is ferns, the sister lineage of seed plants, which, together with lycophytes, another group of free-sporing vascular plants, comprise around 10,500 species in the world. Previous studies in the Neotropics have shown that edaphic conditions, such as nutrient concentration and soil texture, influence fern and lycophyte species diversity, local species composition and geographical distribution patterns. As a consequence, fern and lycophyte species are effective indicators of environmental characteristics of tropical forests. In this thesis, I studied three different aspects of ferns and lycophytes. First, I used ferns and lycophytes as an indicator group for animal behaviour. I explored the relationship between the home range size of three groups of tamarins (*Leontocebus nigrifrons* I. Geoffroy Saint-Hilaire) and forest productivity at Estación Biológica Quebrada Blanco. For this purpose, I identified the fern and lycophyte species present in the area, and using previous knowledge of their soil fertility preferences, I estimated the soil's fertility within the tamarin home ranges. Contrary to my expectations, the home range size was not always negatively related to soil fertility. The group of tamarins inhabiting the poorest soils always had the biggest home range, but the group inhabiting the richest soils did not consistetly have the smallest home range, as it would be expected. Second, in order to improve the efficiency of ferns as indicator group, I revised the number of taxa present in the Neotropical fern genera *Metaxya* and *Salpichlaena*. I did this by combining morphological, molecular, phylogenetic and biogeographical studies. As a result, I delineated the boundaries of the taxa in these genera and described five new species and two subspecies, adding new records to Neotropical biodiversity. Third, I inferred fern phylogenetic relationships based on plastid genomes. Compared to more than 6,000 published plastomes of flowering plants, little is known about fern plastomes. Only around 130 fern plastomes have been published to date, with many important fern lineages completely unsampled. By applying Next-Generation Sequencing techniques, I generated eight new complete fern plastomes and built a phylogenetic hypothesis based on the protein coding regions of the newly produced and previously published plastomes. I was able to identify novel rearreagements in the genome structure revealing a contrasting evolutionary pattern between Polypodiineae and the other fern clades.

TIIVISTELMÄ

Trooppiset alueet edustavat maailman lajirikkaimpia seutuja. Siemenkasvien sisarryhmä saniaiset on yksi esimerkki merkittävästä kasvien lajiutumisesta tropiikissa. Saniaisiin ja liekomaisiin kasveihin, toiseen vapaasti itiöivien putkilokasvien ryhmään, kuuluu maailmanlaajuisesti yhteensä noin 10 500 lajia. Aiemmat tutkimukset uuden maailman tropiikissa ovat paljastaneet maaperätekijöiden, kuten ravinteiden määrän ja maannoksen rakenteen, vaikuttavan näiden kasvien monimuotoisuuteen, maantieteelliseen levinneisyyteen ja paikalliseen lajikoostumukseen. Tämän vuoksi saniaiset ja liekomaisten kasvit ovat tehokkaita trooppisten metsien ympäristöolosuhteiden ilmentäjiä. Väitöskirjassani tutkin saniaisia ja liekomaisia kasveja kolmesta eri näkökulmasta. Ensimmäisenä tutkin näiden kasvien ilmentämien ympäristötekijöiden vaikutusta apinoihin kuuluvien tamariinien käyttäytymiseen Perun Amazoniassa. Määritin Quebrada Blancon biologisen aseman alueella esiintyvät saniaiset ja liekomaiset kasvit. Hyödyntäen aiempaa tietoa löytämieni lajien suosimista ravinteisuustasoista arvioin metsän tuottavuutta kolmen tamariiniryhmän elinpiirien alueella. Odotusteni vastaisesti elinpiirien koko ei ollut käänteisessä suhteessa maaperän ravinteisuuteen: kaikkein vähäravinteisimmalla alueella elävän tamariiniryhmän elinpiiri oli aina laajin, mutta ravinteisimmalla alueella elävän ryhmän elinpiiri ei ollut säännönmukaisesti suppein. Seuraavaksi pyrin parantamaan saniaisten ja liekomaisten kasvien käyttökelpoisuutta ilmentäjäkasveina varmentamalla taksonien määrän uuden maailman saniaissuvuissa *Metaxya* ja *Salpichlaena* morfologisia, molekulaarisia, fylogeneettisiä ja eliömaantieteellisiä tutkimuksia yhdistämällä. Tuloksena sain selvennettyä näihin sukuihin kuuluvien taksonien väliset rajat ja kuvasin tieteelle uutena viisi lajia ja kaksi alalajia, kasvattaen näin luetteloa uuden maailman biologisesta monimuotoisuudesta. Lopuksi selvitin saniaisten fylogeneettisiä sukulaisuussuhteita plastidigenomien perusteella. Kukkakasveista on julkaistu 6 000 plastidigenomia, kun taas saniaisten plastidigenomit tunnetaan varsin huonosti. Niitä on julkaistu vain noin 130 lajilta, ja tämän otoksen ulkopuolelle jää monta tärkeää saniaisten kehityslinjaa. Uuden sukupolven sekvensointimenetelmää käyttäen tuotin kahdeksan uutta saniaisten täydellistä plastidigenomia ja rakensin fylogeneettisen hypoteesin sekä uusien että aiemmin julkaistujen plastidigenomien proteiinia koodaavien alueiden perusteella. Kykenin tunnistamaan genomeissa aiemmin havaitsemattomia rakenteellisia uudelleenjärjestäytymisiä, jotka paljastivat Polypodiineae-alalahkon genomirakenteen evoluution poikkeavan muista saniaisten kehityslinjoista.

ABBREVIATIONS AND CODES USED IN THE THESIS

AAU	Herbarium Aarhus University, Aarhus, Denmark
ABGD	Automatic Barcode Gap Discovery
ALCB	Herbário Alexandre Leal Costa, Bahia, Brazil
AMAZ	Herbarium Amazonense, Iquitos, Peru
B	Herbarium Botanischer Garten und Botanisches Museum Berlin-Dahlem, Berlin, Germany
BHCB	Herbário do Instituto de Ciências Biológicas, Minas Gerais, Brazil
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
BM	Herbarium The Natural History Museum, London, U.K.
bp	Base pair
bPTP	Bayesian implementation of the Poisson Tree Processes
CAY	Herbier de Guyane, Cayenne, French Guiana
COL	Herbario Nacional Colombiano, Bogotá, Colombia
CR	Herbario Nacional, Museo Nacional de Costa Rica, San José, Costa Rica
CVRD/VALE	Herbário Reserva Natural da Vale, Meio Ambiente, Espírito Santo, Brazil
DNA	Deoxyribonucleic acid
DOGMA	Dual Organellar GenoMe Annotator
EBQB	Estación Biológica Quebrada Blanco
F	Herbarium Field Museum of Natural History, Chicago, U.S.A.
FLOR	Herbário FLOR, Santa Catarina, Brazil
FURB	Herbário Dr. Roberto Miguel Klein, Santa Catarina, Brazil
GH	Herbaria Harvard University, Massachusetts, U.S.A.
HUEFS	Herbário Universidade Estadual de Feira de Santana, Bahia, Brazil
INPA	Herbário Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil
IR	Inverted Repeat
K	Herbarium Royal Botanic Gardens, London, U.K.
LE	Vascular Plants Herbarium of the Komarov Botanical Institute, Saint Petesburg, Russia
LSC	Large Single Copy
MAFFT	Multiple Alignment using Fast Fourier Transform
matK	Maturase K gene
MCP	Minimum Convex Polygon
ML	Maximum Likelihood
MO	Herbarium Missouri Botanical Garden, Missouri, U.S.A.
MORFFO	Mobile Open Reading Frames in Fern Organelles
NGS	Next Generation Sequencing
NY	William and Lynda Steere Herbarium, New York, U.S.A.
P	Herbier National, Paris, France
pgiC	Glucose-6-phosphate isomerase, cytosolic gene
RAxML	Randomized Axelerated Maximum Likelihood
rbcL	RuBisCO large subunit gene
RON	Herbário Rondoniensis - João Geraldo Kuhlmann, Rondônia, Brazil
rpoC1	RNA polymerase beta' subunit gene
rps4	Ribosomal protein S4 gene
rps4-trnS	Intergenic spacer between ribosomal protein S4 and tRNA-Ser
SP	Herbário Instituto de Botânica, São Paulo, Brazil

SPF	Herbário Universidade de São Paulo, São Paulo, Brazil
SSC	Small Single Copy
TIGER	Tree-Independent Generation of Evolutionary Rates
trnG-trnR	Intergenic spacer between mitochondrial transfer RNA:Glycine and mitochondrial transfer RNA:Arginine
trnH-psbA	Intergenic spacer between mitochondrial transfer RNA:Histidine and Photosystem II protein D1
TUR	Herbarium University of Turku, Turku, Finland
UC	University Herbarium, University of California, California, U.S.A.
UEC	Herbário Universidade Estadual de Campinas, São Paulo, Brazil
US	United States National Herbarium, Washington, U.S.A.
USM	Herbario Universidad Nacional Mayor de San Marcos, Lima, Peru
UTU-ART	Amazon Research Team of the University of Turku
Z	Herbarium Institut für Systematische Botanik, Zurich, Germany

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications and manuscripts, which are referred to by their Roman numerals in the text:

I. Cárdenas, G.G., Tuomisto, H., Jones, M. & Heymann, E. Characterising monkey habitats in Peruvian Amazonia using ferns and lycophytes as indicators. Manuscript.

II. Cárdenas, G.G., Tuomisto, H. & Lehtonen, S. (2016). Newly discovered diversity in the tropical fern genus Metaxya based on morphology and molecular phylogenetic analyses. Kew Bulletin 71:5.

III. Cárdenas, G.G., Lehtonen, S. & Tuomisto, H. (2019). Taxonomy and evolutionary history of the neotropical fern genus Salpichlaena (Blechnaceae). Blumea 64: 1-22 doi.org/10.3767/blumea.2018.64.01.01

IV. Lehtonen, S. & Cárdenas G.G. Dynamism in the fern plastome structure observed through the phylogeny. Manuscript submitted to Botanical Journal of the Linnean Society and accepted pending minor revision.

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Author contribution to the publications

Activities	I	II	III	IV
Research design	HT, GC	HT, SL, GC	HT, SL, GC	SL, GC
Field work	GC, EH	HT, GC, SL	HT, GC, SL	-
Herbarium work	GC, HT	GC, HT, SL	GC, SL, HT	-
Laboratory work	-	GC, SL	GC, SL	GC, SL
Ecological analysis	MJ	HT	-	-
Molecular data analysis	-	GC, SL	GC, SL	GC, SL
Writing the manuscripts	GC, MJ, HT, EH	GC, SL, HT	GC, SL, HT	GC, SL

GC = Glenda Cárdenas, EH = Eckhard Heymann, MJ = Mirkka Jones, SL = Samuli Lehtonen, HT = Hanna Tuomisto

1 INTRODUCTION

1.1 Biodiversity in the Neotropics

Tropical areas are considered to hold the highest biodiversity in the world (Gaston 2000), with the Neotropics being the richest region in some groups, such as plants (Corlett 2016, Antonelli & Sanmartín 2011) and birds (Jetz et al. 2012). In the Neotropics, the Western Arc forests of the Amazon Basin, the Atlantic forest ecoregion of Brazil, and the Chocó-Daríen ecoregion of north-western South America are among the most species-rich tropical moist forests on Earth (Olson & Dinerstein 1998). Several record numbers of biodiversity have been reported for this region, for example the presence of 420 species of birds around the Yasuní Scientific Station in Ecuador (Piedrahita et al. 2012), or the presence of around 300 species of trees in a single hectare plot (Gentry 1988a). Although it has been stated that high Neotropical biodiversity is a product of various processes (Lawton 1996, Antonelli & Sanmartin 2011, Rull 2011), numerous studies have focused on specific factors and processes in their attempt to explain this extraordinary diversity. Such specific factors include the forest fragmentation during the Pleistocene, which supposedly created islands of forest surrounded by drier habitats (Haffer 1969), the population fragmentation induced by cooling, reduced atmospheric CO_2 concentration and decreased precipitation (Bush 1994), or river dynamics that create heterogeneity in forest structure and in consequence high diversity (Salo et al. 1986). Environmental and geographical factors have been shown to have an important role in the high diversity of species and the habitats in which the species occur (Gentry 1988b).

Despite our awareness of its high biodiversity, a large part of the Neotropical species richness still remains unrevealed. Evidence of this are the continuous findings of new species. In mammals, for instance, in 2018, three new species of silky anteater were described (Miranda et al. 2018); in 2014, a new species of river dolphin was discovered (Hrbek et al. 2014); in 2013, a new species of tapir (Cozzuol et al. 2013) and a new species of rodent (Jiménez et al. 2013) were found; and in 2012, a new species of marsupial (Solari et al. 2012) was reported. Findings in smaller and lesser known animals are more common, such as 177 new species of ichneumonid parasitoid wasps found in just one study (Veijalainen et al. 2012). Findings of new plant species are also continuosly happening, for instance in the following families: Plantaginaceae (Hassemer & Rønsted 2016), Apocynaceae (Morales 2017), Polypodiaceae (Sundue 2017), Annonaceae (Pirie et al. 2018), and Orchidaceae (Díaz Hernández et al. 2018).

It is important to realise, that the Neotropics are invaluable not only for the high species diversity they hold, but also because of the high number of endemic species, for instance in Amazonia (Álvarez & Whitney 2003), in the Guayana Highlands (Rull & Vegas-Vilarrúbia 2006), and in the Atlantic forest (Ribeiro et al. 2009). But, paradoxically to the extraordinarily high species diversity and to the fragility of habitats where the endemic species inhabit, a great number of species and ecosystems in the Neotropics, many of them still unknown to science, are threatened as a result of habitat degradation and habitat loss caused

by deforestation, logging and oil and gas extraction (Brooks et al. 2002, Canaday & Rivadeneira 2001, Siqueira & Peterson 2003, Finer et al. 2008, Álvarez et al. 2013). This is alarming considering that losses of biodiversity and ecosystems may continue at least two more decades after the fragmentation (Haddad et al. 2015). The implementation of conservation planning and management is still a priority in the Neotropics.

1.2 Background of the use of ferns and lycophytes as an indicator group

Since its origin in the early 1980s, the Amazon Research Team of the University of Turku (UTU-ART) has been studying the relationship between the biodiversity and the environmental factors in the Amazon region. Early studies of UTU-ART reported that erosional disturbances of the rivers in the lowland forests have created a mosaic of different soils and forests of different ages, and are thus maintaining the high diversity (Salo et al. 1986, Salo & Kalliola 1991). Other studies showed that the tectonical activity on the Andes is causing long-term fluvial perturbances (Räsänen et al. 1987), and the age heterogeneity of the exposed sediments has implications on current biogeography of the Amazon forest (Räsänen et al. 1990). Kalliola et al. (1998) mapped the environmental variation in the Iquitos area (in western Amazonia) with sediments of different ages. In their map, they recognised 11 different geoecological units in an area of 990,000 ha and pointed out the presence of still more units to be recognised. A study in a larger area, also in western Amazonia, depicted the presence of around 100 biotopes (Tuomisto et al. 1995). These UTU-ART pioneer studies provided not only a broad overview on the possible factors responsible for the origination and maintenance of Amazonian biodiversity, but also deepened our understanding on how the diversity, mainly of plants, is distributed in Amazonia.

One of the current main goals of the UTU-ART is to classify and map the Amazonian vegetation; but mapping the complete Amazonia is a big challenge due to its vast area, its high species number and the time and cost that complete floristic inventories would demand. As an alternative, few groups of plants were tested to be used as indicator groups, taking into account that by studying a small part of the flora, it is possible to obtain a high proportion of information of all the study area (Kessler & Bach 1999). As a result, species of ferns and lycophytes were found to perform well as an indicator group (Tuomisto & Ruokolainen 1994, Tuomisto & Poulsen 1996). The features that make the species in this group suitable indicators include their morphological distinctness, making it easy to spot them in the forest and their relative commonness and variety of ecological preferences. They are easy to collect, taxonomically relatively easy, and they are not used by humans (Tuomisto & Ruokolainen 1998).

It has been shown that fern and lycophyte species distributions in the Neotropics reflect the environmental heterogeneity, especially the soil heterogeneity related to exchangeable base cation concentration and soil texture (e.g. Tuomisto & Poulsen 1996, Vormisto et al. 2000, Smith et al. 2001, Tuomisto et al. 2003a,

Duque et al. 2005, Jones et al. 2006, 2013, 2016, Cárdenas et al. 2007, Ruokolainen et al. 2007). It was also shown that fern and lycophyte species richness in Central Amazonia increase with cation content (Costa 2006). Studies carried out in poor and rich soil formations have shown that ferns and lycophytes species composition differs between them (Higgins et al. 2011, Suominen et al. 2015, Tuomisto et al. 2016). Moreover, it was also shown that species composition can be used to produce forest classification maps showing different floristic patterns in inundated forest, terrace forest (of relatively poor soils), intermediate tierra firme forest (of intermediate soil richness) and forest on Pebas formation (of rich soils) (Salovaara et al. 2004). Because the fern and lycophyte flora reflects local scale soil heterogeneity, it can successfully be used to map habitat variability, which can then be used, for example, for wildlife management and conservation planning (Sirén et al. 2013).

Now, it is also evident that the phylogenetic structure of fern communities vary along the edaphic gradients, even within tierra firme forests, suggesting that soil heterogeneity plays an important evolutionary role in the Neotropical rainforests (Lehtonen et al. 2015).

In a similar way that edaphic factors influence fern and lycophyte distribution and floristic composition, the distribution, density, diversity and abundance of some animal populations in the Neotropics have been reported to be affected by soil properties. For instance, Álvarez et al. (2013) found the distribution of some bird populations to be restricted to white-sand forests in western Amazonia, and Pomara et al. (2012, 2014) showed that bird species composition in forests growing on poor soils is different to the species composition of birds living in rich soil forests. Sääksjärvi et al. (2006) mentioned that the species richness and composition of parasitoid wasps was also affected by soil richness. In the case of mammals, Emmons (1984) found their density to be higher on white-water and mixed-water alluvial soils and in volcanic soils than in sandy soils in the Guiana-Shield. Peres (1997) mentioned that the density of folivorous monkeys in western Brazil can be predicted by characteristics of the habitat, such as soil fertility, since folivorous density and biomass were found to be lower in poor than in rich soils. The home range size of the red howler monkey (*Alouatta seniculus* L.) and red-rumped agoutis (*Dasyprocta leporina* L.) have been observed to be influenced by soil fertility (Palacios & Rodriguez 2001) and availability of food (Jorge & Peres 2005), respectively.

At Estación Biológica Quebrada Blanco (EBQB), in northeast Peru (Fig. 1), the behaviour of monkeys, especially tamarins of the genera *Saguinus* Hoffmannsegg and *Leontocebus* Wagner (Fig. 2A), have been extensively studied since the 1980s. However, the forest, inhabited by the monkeys, has not been properly characterised, even though it may have some implications in monkey behaviour. In this thesis, I aimed to investigate the soil conditions at EBQB, and to place them in a broader Amazonian context, based on fern and lycophyte species occurences. Moreover, since edaphic factors seems to affect fern and lycophyte, as well as monkey distribution, I used ferns and lycophytes to assess the relationship between the monkeys' home range size and soil fertility at EBQB.

The basic expectation is that monkeys inhabiting poor soils will display a larger home range than monkeys inhabiting richer soils (Chapter I).

1.3 Fern and lycophyte taxonomy and species delimitation

Over the almost three decades of fern and lycophyte inventories in the Neotropics, the number of apparently undescribed species in the UTU-ART collection has grown almost with every inventory. This is not surprising given that many of the inventoried areas had not been floristically studied before. But as in the case of ferns and lycophytes, there are several other groups that have also unexplored diversity. For instance, Giam et al. (2002), in a study done in tropical areas, calculated that there are over 1700 amphibian and around 80 mammal species still undescribed in the Neotropics. This highlights the importance of taxonomical studies considering that the understanding of biodiversity highly depends on the correct identification of specimens (McNeely 2002, Funk 2006). Regarding the fact that ferns and lycophytes is a group that UTU-ART uses in its inventories for ecological studies, it is important that the taxa in the group have proper names in order to facilitate their use as indicator species.

The delineation of species boundaries is important not only for pure research purposes, but also for social benefits such as conservation planning and management programmes, and of course for facilitating the communication regarding biodiversity between different disciplines and stakeholders (Stuessy 2009). The application of traditional taxonomy – the morphological identification, classification and nomenclature of taxa (Winston 1999) – has had and still has an unquestionable role in delimiting taxon boundaries and proposing classifications. However, the inclusion of molecular, biogeographical and ecological data has been increasingly useful to refine taxon boundaries and classifications that have remained dubious using traditional taxonomy (e.g. Smith et al. 2001, Marcussen 2003, Lehtonen & Tuomisto 2007). In recent years, species molecular delimitation methods, such as Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012) and Bayesian implementation of the Poisson Tree Processes (bPTP) (Zhang et al. 2013), have been developed to validate species delimitation. These two methods have initially been developed to delineate species boundaries in animals, and have increasingly been used, for example, in molluscs (Puillandre et al. 2012), moths (Kekkonen et al. 2015), bees, birds and fish (Ratnasingham & Hebert 2013), butterflies (Toussaint et al. 2016), arthropoda, annelida, chordata, equinodermata, platyhelminthes and cnidaria (Kapli et al. 2017). In this thesis, I applied them in the fern genus *Salpichlaena* to assess their effectiveness in this plant genus.

In this thesis, using integrative taxonomy, I aimed to reveal the hidden taxa within the Neotropical genera *Metaxya* C. Presl and *Salpichlaena* J.Sm. (Fig. 1, Figs. 2B and 2C, respectively, and Fig. 4), thus facilitating their future identification in the field and herbaria. The clarification of taxon limits in these genera will also contribute to their better use as indicator species in the field (Chapters II and III).

1.4 Fern phylogenetics

The development of molecular phylogenetics has revolutionised the understanding of fern relationships (Smith et al. 2006a, Christenhusz & Chase 2014). The first molecular studies on fern phylogeny were based only on one or a few genes from the plastid genome (Hasebe et al. 1994, Manhart 1995, Pryer et al. 1995, Kranz & Huss 1996), yet, these and the subsequent studies have contributed enormously to our understanding of fern relationships. They already proposed, for instance, that lycophytes are a sister group to a clade composed of ferns and seed plants, and that horsetails and whisk ferns belong to ferns (Pryer et al. 2001). Further studies have exposed and resolved the phylogenetic relationships within numerous specific fern groups (see references in PPG I 2016), while others have included a significant number of species in order to provide a general framework of fern phylogeny (e.g. Schuettpelz & Pryer 2007, Lehtonen 2011) and to provide a time frame for fern radiation and diversification (Schneider et al. 2004, Testo & Sundue 2016, Lehtonen et al. 2017). Several studies have discovered new species (e.g. Smith et al. 2001, Hirai et al. 2011), while others have re-circumscribed or segregated new genera (e.g. Smith et al. 2006b, Lehtonen et al. 2010, Hirai et al. 2011, Li et al. 2012). Yet, other studies greatly facilitated fern systematics by providing new primers for sequencing specific genes (e.g. Wolf et al. 1999, Shaw et al. 2005, Small et al. 2005, Korall et al. 2006). All these studies have contributed to elaborate a mostly solid hypothesis of the relationships within the main fern lineages, and to produce more accurate fern classifications, like the most recent classifications proposed by Christenhusz & Chase (2014) and by PPG I (2016).

In the last decade, UTU-ART researchers have also begun to use molecular systematic analyses to shed more light on fern phylogenetics and on the delimitation of new taxa. In their first study, by combining morphological systematics with molecular and ecological data, a new species was described, *Lindsaea digitata* Lehtonen & Tuomisto (Lehtonen & Tuomisto 2007). A year later, the first fern phylogeny of the team was produced when Christenhusz et al. (2008) studied the evolutionary relationships in the neotropical genus *Danaea* (Marattiaceae). Two years later, Lehtonen et al. (2010) produced a global phylogeny for the entire fern family Lindsaeaceae. Since then, the research team has been increasingly involved in studies of fern phylogenetics (Lehtonen 2011, Lehtonen et al. 2012, Christenhusz et al. 2013, Lehtonen 2013, Lehtonen et al. 2013, Weigelt et al. 2015, Lehtonen et al. 2017). These studies, however, were based on only a few molecular markers obtained by Sanger techniques.

Phylogenetic studies based on complete fern plastome genomes began in 2003 with the publication of the first complete fern plastome (*Adiantum capillus-veneris* L.), which was generated using traditional Sanger Sequencing (Wolf et al. 2003), called also 'first-generation sequencing' (Song et al. 2016). Thanks to the development of 'second generation sequencing' or Next Generation Sequencing (NGS) techniques (Egan et al. 2012, Buermas & den Dunnen 2014), the generation of complete fern plastomes has rapidly increased (e.g. Gao et al. 2013, Grewe et al. 2013, Raman et al. 2016, Labiak & Karol 2017, Wei et al. 2017). NGS is becoming more popular because, unlike Sanger sequencing, NGS techniques

produce a massive amount of sequences (high-throughput), and it has a lower cost (Song et al. 2016). Yet, the number of fern plastome produced to date, 130 (http://www.paulwolflab.com/data-protocols/fern_plastome_list), is still smaller in comparison with more than 6000 angiosperm plastomes (Leitch & Leitch 2013). This difference responds to the complexity and to the large size of the fern genome (mean size 10616 Mb) in comparison to the angiosperm genome (mean size 6383 Mb) (Nakazato et al. 2008). The increasing availability of fern plastome data has not only improved our understanding on fern relationships, but also revealed how the fern plastome structure has changed over the evolutionary history, and exposed structural differences in plastid genome among the fern clades and between the ferns and other plant groups.

In this study, I applied NGS techniques to generate eight new complete fern plastomes of the genera: *Davallia* Sm. (Fig. 2D), *Lindsaea* Dryand. ex Sm., *Lomariopsis* Fée, *Nephrolepis* Schott, *Oleandra* Cav., *Pecluma* M.G.Price, *Saccoloma* Kaulf., and *Tectaria* Cav. (Fig. 4). These genera were chosen because they belong to clades with unresolved relationships in the most recent fern classification (PPG I 2016). Using the newly generated plastomes, together with a set of previously published plastomes, I aimed to resolve the Polypodiineae relationship as well as the relationships of Saccolomatineae and Lindsaeineae. Moreover, I aimed to investigated the plastome structural evolution and presence of Mobile Open Reading Frames in Fern Organelles (MORFFO) throughout the fern phylogeny (Chapter IV).

2 MATERIALS AND METHODS

2.1 Data sources

For chapter I, ferns and lycophytes were collected by myself at Estación Biológica Quebrada Blanco (EBQB), in Peru (Fig. 1), in 2010. The data was collected following the sampling method described in Tuomisto & Ruokolainen (1994) and Tuomisto et al. (2003b). Fern and lycophyte species presence and abundances were registered along 11 transects of 5 m width and 500-1400 m length. All the fern and lycophyte individuals larger than 10 cm, and growing at a height less than 2 m above the ground, were registered. Samples of all the species found were collected and deposited in the herbarium of the Universidad Nacional de la Amazonía Peruana (AMAZ, herbarium acronyms according to Thiers continuosly updated), and duplicates were sent to the herbarium of the University of Turku (TUR). Identification of the specimens were done during the collecting process and verified later at TUR. In the same transects, 20 soil samples (1-3 per transect) were gathered from the top 5 cm of the mineral soil (after removing the layer of organic material). Each sample had approximately 200 g of soil and was composed of 5 subsamples taken within a circular area of 5 m in diameter. Samples were analysed for the concentration of exchangeable bases (Ca, Mg, K and Na) and Al. In addition, values of fern and lycophyte soil preferences were obtained from the western Amazonia UTU-ART dataset, which contains data collected in more than 200 sampling site in the Neotropics.

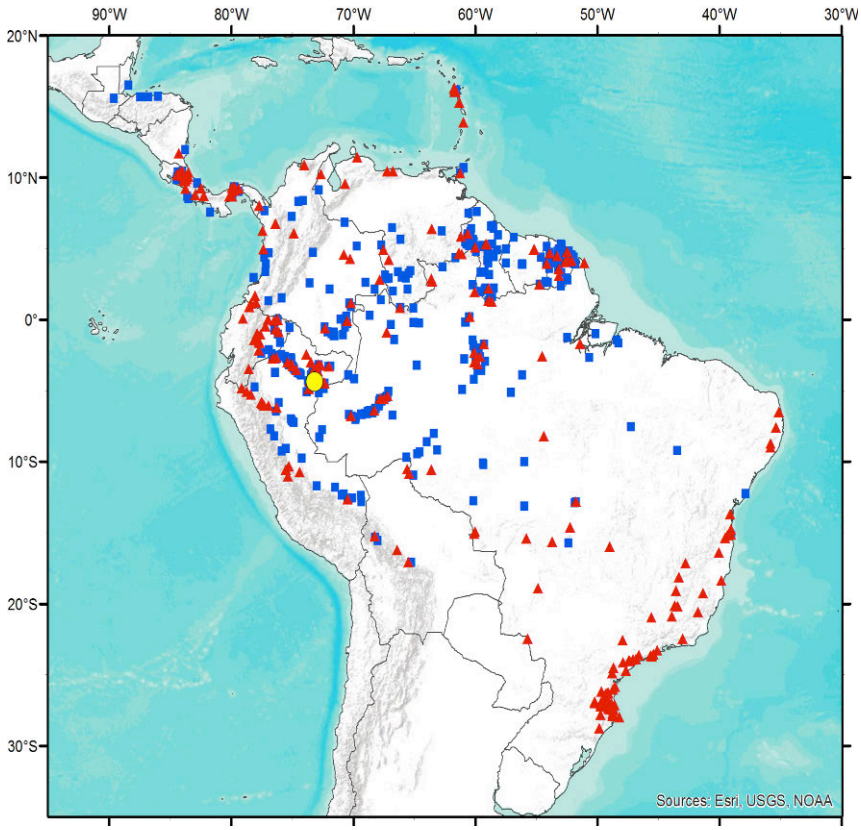

Figure 1. Map showing the origin of the data. Estación Biológica Quebrada Blanco refered to in chapter I (yellow circle), *Metaxya* specimens studied in chapter II (blue squares) and *Salpichlaena* specimens studied in chapter III (red triangles)

The monkey data used in chapter I was collected by researchers from German Primate Center and University of Göttingen at EBQB, between 2004 and 2011. The movements of three groups of tamarins, *Leontocebus nigrifrons* I. Geoffroy Saint-Hilaire (Callitrichidae, Primates) (Fig. 2A), were georeferenced with a GPS devices (Culot et al. 2011, Lledo-Ferrer et al. 2011) from leaving their sleeping site in the early morning (around 6:00 h) until returning to a sleeping site in the afternoon (around 16:00 h) (Smith et al. 2007).

For chapter II and III, I registered the morphological characters of *Metaxya* and *Salpichlaena* specimens mainly at the TUR herbarium, where a complete set of the UTU-ART collections is kept. Additionally, I collected an important part of the data during my visits to AAU, K, P, US and USM herbaria. Quantitative data (e.g. length of fronds, length and width of pinnae and pinnules, number of lateral pinnae and pinnules, number of sori per vein) and qualitative data (e.g. lamina texture, shape of apex, disposition of sori, shape of scales) were gathered from 67 and 283 herbarium specimens for chapters II and III, respectively. Comparative morphological studies of specimens (Fig. 3) was done mainly at the TUR hebarium, but also during visits to AAU, INPA, K, P, US, USM and Z.

Additionally, around 1674 digital images from AAU, ALCB, AMAZ, B, BHCB, BM, CAY, COL, CR, CVRD/VALE, F, FLOR, FURB, GH, HUEFS, INPA, K, LE, MO, NY, P, RON, SP, SPF, UC, UEC, US, USM and Z herbaria were studied.

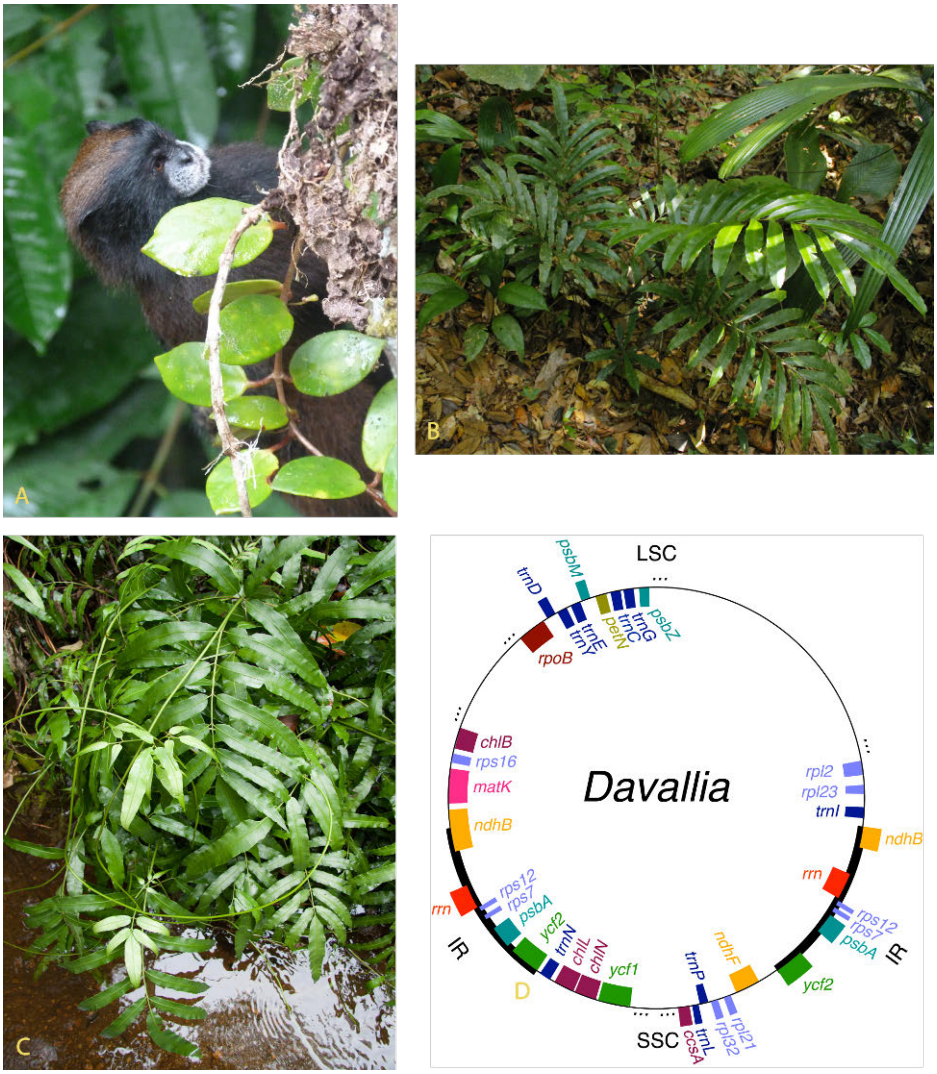

Figure 2. Elements representing each chapter of the thesis. A. *Leontocebus nigrifrons*, B. *Metaxya scalaris*, C. *Salpichlaena hookeriana* and D. *Davallia* plastome. Photos: A: Lucia H. Bartecki, B: Samuli Lehtonen, C: Hanna Tuomisto.

DNA material used in the molecular phylogenetic analyses in chapters II and III were mostly obtained from the UTU-ART collection. Some DNA samples were taken from herbarium material during my visit to AAU, a few came from the Helsinki Botanical Garden and the Royal Botanic Gardens, Kew, and a few were provided by colleagues. From the living plants, a leaf fragment of about three square centimeters was removed from a voucher intended to become a herbarium specimen and dried in a small plastic bag containing silicagel. From

the herbarium samples, a small piece of about one square centimeter was detached for DNA extraction, taking care that informative characters of the specimen were not destroyed.

Figure 3. Morphological study of *Salpichlaena* specimens. Photo: Hanna Tuomisto

Information on geographical distribution of *Metaxya* and *Salpichlaena* taxa (chapters II and III, respectively) was extracted both from the western Amazonia UTU-ART dataset and from the labels of the studied herbarium specimens. In chapter II, data on the distribution of the genus *Metaxya* along edaphic gradients was obtained from previous studies on fern ecology in Amazonia (Tuomisto et al. 2003a, 2003b, 2014 & Higgins et al. 2011).

For chapter IV, DNA samples for the generation of the new plastomes were obtained mainly from the western Amazonia UTU-ART collection, and a few samples were obtained from the Helsinki Botanical Garden and the Royal Botanic Gardens, Kew. For the molecular and phylogenetic analysis, the produced plastome sequences were used together with an extensive amount of open data downloaded from GenBank.

2.2 Laboratory procedures, data processing and data analysis

2.2.1 Characterisation of saddle-back tamarin's (*Leontocebus nigrifrons*) home ranges

The home range size and position of three groups of saddle-back tamarins (*Leontocebus nigrifrons*) were calculated in chapter I based on georeferenced

data from the years 2004, 2007, 2008, 2009 and 2011. The calculations were made individually for every year and for every monkey group in order to investigate how variation in their home range changes over time. Two different methods were used: the 95% kernel utilisation distribution and the simpler 95% minimum convex polygon (MCP) as implemented in R package (R Development Core Team 2013). These two methods were used because they are widely used in studies of mammals home range estimation (Laver & Kelly 2008, Boyle et al. 2009, Gregory 2017). However, for the estimation of the combined home range size of three monkey groups that have data for the same three years (2004, 2007 and 2008), solely 95% kernel was used. The 95% kernel was used instead of 95% MCP, because the 95% kernel was reported to present home ranges better than MCP in previous studies (Worton 1987). Furthermore, it was also reported that MCP is subject to unpredicted and considerable bias (Börger et al. 2006) and Nilsen et al. (2008) cautioned its use for interspecific comparison with little variation in home range size. The three polygons, produced by 95% kernel method (one for each monkey group), were used for the analysis on fern and lycophyte species richness and soil concentration calculations.

Fern and lycophyte species richness was calculated for each home range as the average number of species encountered in the transect subunits (5 m x 25 m) within each home range polygon. Soil conditions were calculated for each home range and for all the subunits inventoried based on the presence of fern and lycophyte species. First, the weight averaging approach (ter Braak & van Dam 1989) was used to calculate the cation optima of the fern and lycophyte species at EBQB, based on the western Amazonian reference UTU-ART dataset of soils and fern and lycophyte species. Then, based on the predicted cation optima, soil cation concentration was calculated for each subunit at EBQB. Finally, the predicted and measured soil cation concentration values were correlated to evaluate the quality of the predictions.

2.2.2 Laboratory procedures and sequences generation

Total genomic DNA was extracted for chapters II, III and IV. DNA was obtained from silicagel dried material, and in chapter II also from herbarium specimens. For chapter II, *matK*, *rbcL*, and *rps4* genes and *trnG-trnR* intergenic spacer, all from the plastid genome, were amplified and sequenced; and for chapter III, *rbcL*, *rpoC1* and *rps4* genes and *rps4-trnS*, *trnH-psbA* and *trnG-trnR* intergenic spacers from the plastid genome, and *pgiC* gene from the nuclear genome, were amplified and sequenced. The chosen markers represent slow and fast evolving regions of the genome and were used in combination in order to improve the phylogenetic resolution of the studied taxa. All the used markers have been already used in fern systematics, although *rbcL*, *rps4*, *rps4-trnS*, *matK* and *trnG-trnR* more extensively (Small et al. 2005, Nagalingum et al. 2007, Kuo et al. 2011, Chao et al. 2014, Bauret et al. 2017) than *rpoC1* or *pgiC* (Dong et al. 2016, Wang et al. 2016, Jorgensen & Barrington 2017). Most of the used plastid markers are located in the Large Single Copy (LSC) of the plastome, with the exception of *trnH-psbA*, which is located in the Inverted Repeat (IR) in the studied taxa. The

specific PCR conditions and primers used can be found in the respective chapters.

For chapter IV, DNA was fragmented into an average size of 250-350 bp and sequenced with paired-end 101 bp reads using Illumina HiSeq 2500 platform (for more details see Data production in chapter IV). The assembly of the trimmed reads into contigs was done by using reference genomes and by *DeNovo* methods. The reference genomes used (*Lepisorus clathratus* Ching, KY419704 and *Pseudophegopteris aurita* (Hook.) Ching, NC_035861) were chosen because they may have a similar genome structure as my target species, since they all belong to the suborder Polypodiineae, thus the mapping would result in better matches. Obtained contigs were assembled into longer contigs and then into complete genomes in Geneious 11.0.5, using the De Novo Assembly tool and manually edited when necessary. In the produced genomes, large single copy (LSC), small single copy (SSC) and inverted repeats (IR) were identified by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and DOGMA (Wyman et al. 2004) and later annotated with DOGMA (for more details see Data production in chapter IV).

2.2.3 Phylogenetic analyses

The Sanger sequences produced in chapters II and III were examined, edited and compiled in PhyDE version 1.0 (Müller et al. 2010). The sequences were aligned with default parameters using MAFFT version 7.149 (Katoh & Standley 2013), after which individual markers were concatenated for the analyses using SequenceMatrix (Meier et al. 2006). In chapter IV, using Geneious, 84 protein coding sequences were extracted from the eight newly produced plastome sequences and an additional 41 fern, three seed plant and one lycophyte plastomes downloaded from GenBank. The sequences were aligned as above and poorly aligned positions were removed with Gblocks v0.91b.

Phylogenetic trees were inferred using maximum likelihood (ML), Bayesian inference and parsimony methods in chapter II and IV, and using solely Bayesian analysis in chapter III. Bayesian and ML are model-based methods that provide a statistical framework for phylogenetic reconstruction. ML looks for the optimal tree which is the one that has the highest probability of producing the observed data under the given evolutionary model (Felsenstein 1973). Instead, Markov Chain Monte Carlo methods in Bayesian inference draw a sample from the posterior distribution of the tree hypothesis, thus allowing estimation of the probability of the trees themselves (Yang & Rannala 1997). The posterior probability of trees is computed based on prior probabilities, such as prior distribution of possible trees, using a model of evolution. Unlike Bayesian inference and ML, parsimony is not a model-based method. It looks for the most parsimonious tree which is the one that requires the fewest character state changes (Barton et al. 2007).

In chapter II, the ML analysis was done using the parallel Pthreads-version of RAxML 7.0.3 (Stamatakis 2006, Ott et al. 2007), and Bayesian analysis was

performed using MrBayes version 3.2.3 (Ronquist et al. 2012). Sensitivity of the resulting tree was also assessed under the parsimony optimality criterion by performing a sensitivity analysis (Wheeler 1995) using direct optimisation (Wheeler 1996) as implemented in the program POY 5.1.1 (Wheeler et al. 2015). POY is a flexible platform which is able to reconstruct a phylogenetic hypothesis from unaligned sequences (Varón et al. 2013). During the tree search, the sequence homologies (ie. the alignment) are dynamically optimised under the selected transformation costs. Six different transformation-cost combinations were applied in this study: indel opening as set 0 or 3, transversions cost to 1 or 2, transitions cost to 1 or 2 and indel extension cost to 1. Results from these analyses were pooled together as sensitivity plots with the program Cladescan (Sanders 2010).

In chapter III, the phylogenetic information present in sequence length variation was included in the analysis by applying simple gap coding (Simmons & Ochoterena 2000). Gap coding data were included, because insertions and deletions resulting in sequence length variation are generally considered to be phylogenetically informative (Giribet & Wheeler 1999, Simmons et al. 2001). The best-fitting evolutionary model for each marker was selected under Bayesian Information Criterion (BIC) using jModelTest-2.1.10 (Darriba et al. 2012). Phylogenetic trees were inferred first independently for each marker with MrBayes version 3.2 (Ronquist et al. 2012) and then combined for final analysis, with the exception of nuclear data, which were analysed separately because of the conflict with the plastid data.

In chapter IV, ML and Bayesian analyses were performed for partitioned and unpartitioned data matrices. Data was partitioned either by sites or by genes. For the partitioning by sites, a new method developed by Rota et al. (2018) was applied. The method uses Tree-Independent Generation of Evolutionary Rates (TIGER) (Cummins & McInerney 2011) to estimate the rate of evolution of each site, and then a python script RatePartitions (Rota et al. 2018) to group all the sites of the dataset into partitions according to the evolutionary rates of the sites. This method intends to create a more realistic way of partitioning the data than an earlier method based on evolutionary rates using *k*-means clustering (Frandsen et al. 2015), which had the problem of placing all the invariable sites into one partition (Baca et al. 2017). RatePartition adds slowly evolving sites into the partition of "invariable sites" using a simple formula and a used defined division factor *d* (Rota et al. 2018). Rota et al. (2018) highlight the importance of the addition of slow evolving sites with the premise that although there are "invariable sites" in the analysed dataset, those "invariable sites" can vary in the time. Partitionfinder v1.1.0 (Lanfear et al. 2012) was used to calculate BIC-scores for the partitioned data schemes. For the unpartitioned data BIC-score was calculated with jModelTest 2.1.10 (Guindon & Gascuel 2003, Darriba et al. 2012). The best partitioning strategy was selected based on the BIC-scores. The most parsimonious tree was inferred using TNT v1.5 (Goloboff & Catalano 2016).

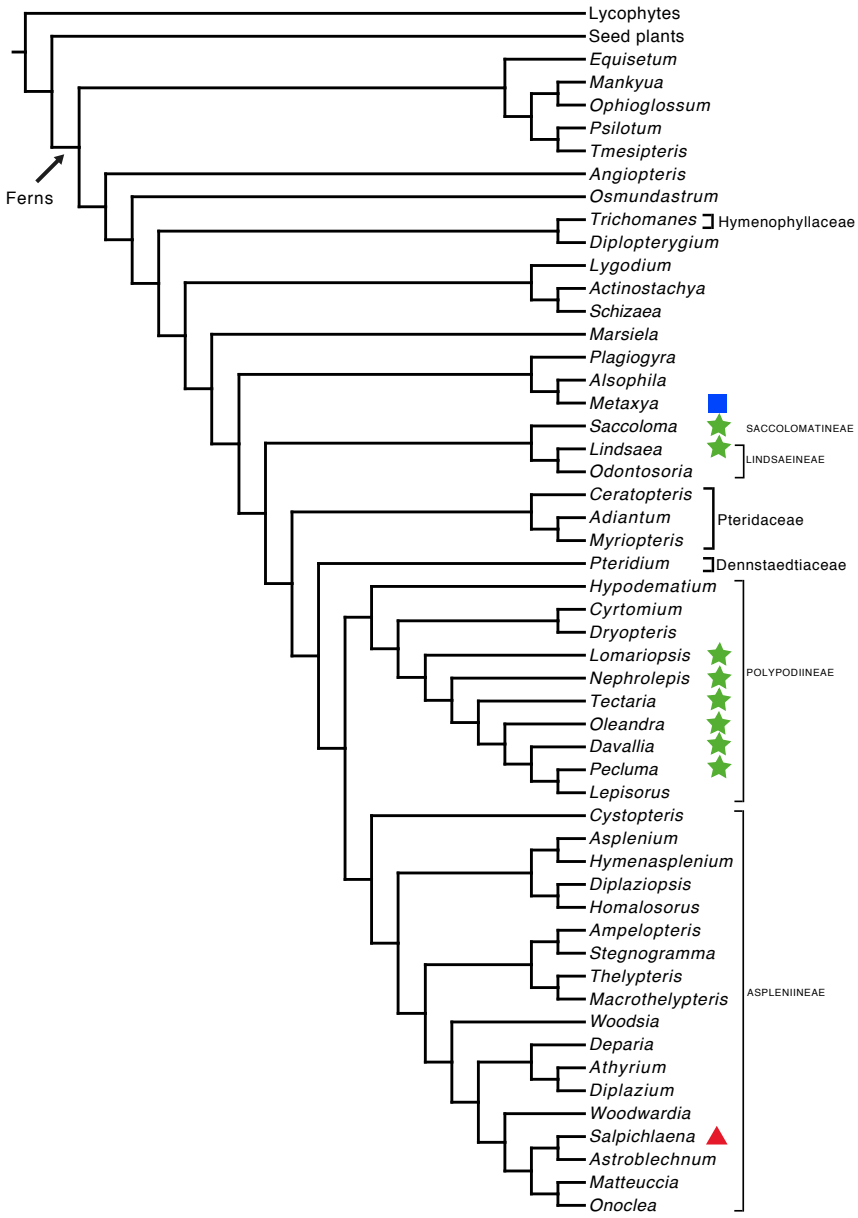

Figure 4. Cladrogram of the studied taxa. Genus *Metaxya* studied in chapter II (blue square), genus *Salpichlaena* studied in chapter III (red triangle), genera from wich new plastomes were generated (green stars) and all the remaining fern taxa were studied in chapter IV. The cladogram was built based on the plastome phylogeny generated in chapter IV. Location of *Metaxya* and *Salpichlaena* in the cladogram is based on PPG I (2016)

2.2.4 Molecular species delimitation

In chapter III, in addition to morphological and phylogenetic methods, two molecular species delimitation methods were applied to the plastid sequences in order to investigate the taxon boundaries in the fern genus *Salpichlaena*. The Automatic Barcode Gap Discovery (ABGD) method assumes that the degree of sequence divergence is larger between than within species, so that interspecific and intraspecific variations do not overlap (Puillandre et al. 2012). Hence, the species boundary can be observed as a barcode gap in pairwise differences between sequences that belong to different species (Puillandre et al. 2012). Since the evolutionary rate varies between plastid regions, the method was applied independently to each molecular marker. The second molecular species delimitation method was the Bayesian implementation of the Poisson Tree Processes model (bPTP) (Zhang et al. 2013). This method assumes that the number of substitutions is significantly higher between species than within species and infers the species boundaries from the phylogenetic input tree and the estimated number of substitutions on its branches.

3 RESULTS AND DISCUSSION

3.1 Habitats, soils and tamarin home ranges at Estación Biológica Quebrada Blanco

This study shows that soils at Estación Biológica Quebrada Blanco (EBQB) are rather homogenenus, and in an Amazonian context, they have intermediate to poor fertility (chapter 1). This is evidenced by the fern and lycophyte species composition found at EBQB, which corresponded to species with a preference for intermediate to relatively poor soils, e.g. *Asplenium halli* Hook., *Adiantum tomentosum* Klotzsch, *Lindsaea falcata* Dryand., *Trichomanes bicorne* Hook. and *Trichomanes elegans* Rich. (Tuomisto & Poulsen 1996, Ruokolainen & Tuomisto 1998). However, some species with an affinity for richer soils were found in the south-western part, e.g. *Mickelia nicotianifolia* (Sw.) R.C. Moran, Labiak & Sundue and *Mickelia guianensis* (Aubl.) R.C. Moran, Sundue & Labiak (Tuomisto & Poulsen 1996, Ruokolainen & Tuomisto 1998). This area is periodically inundated by the creek Quebrada Blanco, which deposits richer sediments on the area, thus explaining the occurrence of rich-soil indicator species. This area was also found to have the highest fern and lycophyte species richness.

Results of this study provided only partial support to the initial hypothesis that tamarins inhabiting forests on low fertility soils would occupy a larger home range than tamarins inhabiting forests on higher fertility soils. The group of tamarins inhabiting the poorest area had indeed the largest home range, but the group of tamarins inhabiting the richest area (in the south-western part), did not always have the smallest home range. This could be explained by the fact that the group of tamarins that inhabit the north-western area sometimes crosses the

creek Choroy, located at the most northern part, but their movements beyond the creek have not been registered (Heymann 2014 pers. comm.).

3.2 Fern species delimitation and phylogenetics

Until the studies in chapter II and III were carried out, there was uncertainty about the number of species within the genera *Metaxya* and *Salpichlaena*. The variation of their morphological characters and the incompleteness of hebarium specimens have made the differentiation of the species within these genera difficult. The species delineation done in this study and the description of five new species, three in *Metaxya* and two in *Salpichlaena*, and two new subspecies, in *Salpichlaena*, show that the number of species within these genera was underestimated and at the same time they clarify the number of species within these genera that has been uncertain as a matter of taxonomical discrepancy (Hooker 1846, Hooker & Baker 1874, Tryon & Tryon 1982, Murillo 2001, Giudice 2008). Additionally, this study also reveals the key morphological characters useful for *Metaxya* and *Salpichlaena* taxa identification.

Both the delineation of taxon boundaries and the discovery of new species withing *Metaxya* and *Salpichlaena* were largely facilitated by the use of Integrative taxonomy. Integrative taxonomy, which "aims to delimit the units of life's diversity from multiple and complementary perspectives" (Dayrat 2005), has been successfully used to delimit species boundaries when a single delimitation criterion has failed (Roe & Sperling 2007, Schlick-Steiner et al. 2010). In this context, the combination of morphological, molecular, phylogenetic, biogeographical and ecological data in chapters II and III was found useful in delimiting taxon boundaries in *Metaxya* and *Salpichlaena*.

In chapter II, the genus *Metaxya* was found to contain six distinct entities. Three of them could be associated with the previously described *M. lanosa*, *M. parkeri* and *M. rostrata*, but three had never been recognised before. The molecular phylogenetic tree grouped the specimens into five clades. Four of the clades matched morphologically distinct entities and thus, received both morphological and molecular support as distinct taxa: *M. contamanensis*, *M. elongata*, *M lanosa* and *M. parkeri*.

The fifth clade included both *M. rostrata*, with irregularly to coarsely dentate to almost entire pinna apices with a few teeth, and the presence of 3-4 sori per vein scattered over most of the lamina as diagnostic characters (Figs. 1 and 2 in chapter II), and an undescribed morphotype having dentate pinna apices with step-like rounded teeth to coarse dentation-serration, and the presence of one dense line of sori aside of the midvein (Figs. 1 and 2 in chapter II). However, since these two *Metaxya* morphotypes are morphologically very distinct, and the phylogeny is solely based on plastid data that may be misleading due to possible hybridisation and chloroplast capture (see below the example in *Salpichlaena*), these two morphotypes were accepted as separate species, and the undescribed morphotype was named *M. scalaris*. It should also be noted that despite the small molecular difference (one bp) there is no indication of crossbreeding between

them, even in the area where both co-occur (Map 1 and Fig. 4 in chapter II), supporting their status as distinct species.

Metaxya contamanensis, *M. elongata*, and *M. scalaris* do not overlap with each other in their geographical distribution, since *M. contamanensis* occurs only on the eastern foothills of the Andes, *M. elongata* in Central America and western side of the Andes, and *M. scalaris* in the Guiana Shield. The other three *Metaxya* species overlap somewhat in their geographical distribution and *Metaxya parkeri* and *M. rostrata* may even be found growing in the same places, but specimens with intermediate morphological characters have not been seen. In turn, *M. lanosa* has overlapping geographical distribution with *M. rostrata* and *M. parkeri*, but as a specialist of nutrient-poor sandy soils, it is found in different habitats.

In chapter III, based on integrative taxonomy, the genus *Salpichlaena* was found to contain seven different taxa. Three taxa were clearly different between each other and easy to identify based on one or two key characters: *Salpichlaena hookeriana*, with foliar buds and clear foliar dimorphism; *S. papyrus*, with broad and shapeless costal scales; and *S. volubilis*, with entire to only slightly serrate pinna apices. These three taxa were resolved in three well supported clades in the plastid phylogeny and, despite their partially overlapping geographical distribution, they maintain their morphological and genetic integrity. This validates their recognition as true species. The *Salpichlaena volubilis* clade was formed by four well-supported subclades, recognised in this study as subspecies: subsp. *amazonica*, subsp. *crenata*, subsp. *thalassica* and subsp. *volubilis*. For the morphological identification of these four taxa, the combination of two or three characters are usually needed. However, in some specimens the characters are not so obvious or are missing, such as in the case of incomplete herbarium material represented only by fertile pinnae, making the identification difficult or even impossible.

The *Salpichlaena* phylogenetic tree, based on nuclear data, was poorly resolved but revealing. In this tree, some specimens that appeared in the *S. hookeriana* clade in the plastid tree were resolved into a highly supported clade within *S. volubilis* subsp. *amazonica* clade. We consider that these specimens represent hybrids between *S. hookeriana* (the maternal parent) and *S. volubilis* subsp. *amazonica* (the paternal parent), and that they form a self-sustained population. Additionally, specimens in this group display intermediate morphological characteristics of the putative parents, which provided support to recognise this taxon as a new species, *S. hybrida*.

Aligned sequences of *Metaxya* and *Salpichlaena* showed *matK*, *rbcL*, *rpoC1* and *psbA-trnH* to have lower substitution rates than *trnG-trnR* and *rps4+rps4-trnS* (Table 1). Similar results with ferns have been obtained by Wang et al. (2016), who showed that *rbcL* was less variable than *trnH-psbA*, and the latter less variable than *rps4-trnS*. This result also agrees with Sang et al. (1997), who found that *matK* had a lower substitution rate than *psbA-trnH*, but disagrees with Olmstead & Palmer (1994), who found *matK* to have a higher substitution rate than *psbA*, *rbcL*, and *rpoC1* in angiosperms.

Table 1. Sequence length, number of changes and percentage of markers site variation of the eight produced plastomes

marker	genus	sequence length	number of changes	percentage
rbcL	*Metaxya*	1125	16	1.4
matK	*Metaxya*	772	11	1.4
trnG-trnR	*Metaxya*	1005	26	2.6
rps4+rps4-trnS	*Metaxya*	1030	34	3.3
rpoC1	*Salpichlaena*	404	9	2.2
rbcL	*Salpichlaena*	1134	26	2.3
trnH-psbA	*Salpichlaena*	487	16	3.3
pgiC	*Salpichlaena*	396	19	4.8
rps4+rps4-trnS	*Salpichlaena*	962	69	7.2
trnG-trnR	*Salpichlaena*	873	102	11.7

The molecular delimitation analyses used in chapter III fully agreed with the morphological and phylogenetic analysis only in the recognition of *Salpichlaena hookeriana*. Otherwise, the molecular species delimitation analyses supported different numbers of species (2-8) depending on the used molecular marker (Fig. 1b in chapter III) and prior maximum intraspecific divergence value (P) (Fig. 1c in chapter III). In ABGD, *rps4* + *rps4-trnS* recognised *S. hookeriana* as a species and lumped all the other taxa into a second species. In contrast, *rpoC1* recognised *S. hookeriana*, *S. volubilis* subsp. *thalassica* and *S. volubilis* subsp. *crenata* as species but divided *S. papyrus* into three species and put part of *S. volubilis* subsp. *amazonica* together with *S. volubilis* subsp. *volubilis*. The lack of consensus in the way different markers split *Salpichlaena* taxa may be explained by the presence of higher variation within the sequences of some markers (e.g. *rpoC1*) in comparison with others (e.g. *rps4* + *rps4-trnS*), which in consequence produced higher or lower numbers of taxa, respectively. In bPTP, seven species were recognised within *Salpichlaena*, but only two of them (*S. volubilis* subsp. *amazonica* and *subsp*. *volubilis*) received a Bayesian posterior value ≥95%. bPTP mostly agreed with the delimitation made based on morphological and phylogenetic methods, with the difference that it recognised two species within *S. paryrus* (Fig. 1c chapter III). The variation within *S. papyrus* was also evidenced in the delimitation done by *rpoC1* in ABGD analysis and can be observed in the phylogeny.

3.3 *Metaxya* and *Salpichlaena* biogeography and diversity

The biogeographical patterns observed in *Metaxya* and *Salpichlaena* – with some narrowly distributed species and others with widespread distribution (Map 1 in chapter II and III, respectively) – could be related to biogeographical and environmental history of the Neotropics. The range of *Metaxya elongata* and *Salpichlaena volubilis* subsp. *thalassica*, limited to the western side of the Andes, suggests that the Andes mountain chain is acting as a geomorphological barrier

for those *Metaxya* and *Salpichlaena* taxa. This pattern has been previously observed by Moran (1995) in around 190 fern and lycopytes species. However, the Andes mountain chain does not seem to restrict *Salpichlaena papyrus*, since it can be found from Central America to Central Amazonia, not only in the lowlands but also in mid elevations. The restricted distribution of *Metaxya contamanensis* to the eastern foothills of the Andes may suggest the preference of this species for relatively high elevations and, according to results of soil affinity (Fig. 4 in chapter II), preference to poor soils. In contrast, *Metaxya rostrata* and *S. volubilis* subsp. *amazonica* are distributed mostly in western Amazonia, consistent with their inferred preference for lower elevation and wider soil fertility tolerance (Fig. 4 in chapter II). *Metaxya lanosa*, although widely distributed, is strongly constrained by its preference to poor white-sand soils. The distribution of *Metaxya scalaris* and *Salpichlaena hybrida* is limited to the Guiana shield, together with *S. volubilis* subsp. *crenata*, which also reaches the Caribbean Islands. *Salpichlaena volubilis* subsp. *volubilis*, restricted to the Atlantic forest, seems to be constrained by the drier "cerrado" forest. Among the most widespread *Metaxya* and *Salpichlaena* taxa are *Salpichlaena hookeriana*, occuring throughout Amazonia, *S. paryrus* occurring in Central America and in the borders of Amazonia, and *M. parkeri* occurring throughout Amazonia, the Caribbean Islands and the Atlantic forest.

Both *Metaxya* and *Salpichlaena* are most diverse in north-western Amazonia. Overall, this region has high habitat heterogeneity due to its geomorphological history, which promotes high species diversity (Sombroek 2000, Higgins et al. 2011, Tuomisto et al. 2016). The presence of the Andean mountains increases heterogeneity of habitats along the elevational gradient with different slope exposures, soils and microclimates (Moran 1995). The habitat heterogeneity of this region also responds to the lateral migration of the Amazonian rivers (Salo & Räsänen 1989), which promotes the exposure of different types of sediments of different ages creating a habitat mosaic.

3.4 Fern plastome generation and evolution

The eight newly generated complete fern plastomes shared almost the same gene content and order with only a few differences (see information of New plastomes in Results in chapter IV). Based on Bayesian Information Criterion (BIC), TIGER+RatePartition with d=3.5 was the best data partitioning strategy, and therefore, it was used in the ML and Bayesian analyses. These results are in agreement with Rota et al. (2018), who also found partitioning by sites to perform better than, for instance, partitioning by genes. Thereby, it underlines the importance of taking into account the heterogeneity of the data in the partition, since characters do not evolve uniformly, not even within genes (Brandley et al. 2005, Rota et al. 2018). On the other hand, unpartitioning gave the worst BIC score. Studies on the effect of data partitioning have found that unpartitioned strategies induce some error in the phylogenetic inference, even more than overpartitioning (Brown & Lemmon 2007, Kainer & Lanfear 2015).

However, despite the differences in BIC scores, the different partitioning strategies produced highly similar trees. Moreover, trees produced by Bayesian inference and ML using partitioned data were identical (referred to as "partitioned" in the following paragraph), as were the trees produced using unpartitioned data (referred to as "unpartitioned" in the following paragraph).

All the trees agree on the position of *Saccoloma* (Saccolomatineae) as sister of Lindsaeaceae, thus, this position can be considered resolved. Disagreement between the partitioned and unpartitioned trees was observed only in the position of *Trichomanes* and the order in which Pteridaceae (*Ceratopteris* and *Adiantum*) and Dennstaedtiaceae (*Pteridum*) diverged (Fig. 3A in chapter IV). Results of the parsimony analysis agreed with the unpartitioned tree in the position of *Trichomanes*, but with the partitioned tree in the divergence order of Pteridaceae and Dennstaedtiaceae. Previous studies have not reached a consensus regarding the phylogenetic position of Pteridaceae and Dennstaedtiaceae (Hasebe et al. 1994, Schuettpelz & Pryer 2007, Wolf et al. 2015, Lehtonen 2018), and in this study, their relative positions still varied depending on the analyses.

Equisetum (Equisetaceae) was resolved as sister to all the other ferns by parsimony analysis in contrast to model-based analyses, in which it was resolved as sister to a clade formed by Ophioglossaceae and Psilotaceae. The phylogenetic position of Equisetaceae has been repeatedly investigated, and although its sister relationship to all the other ferns has received support from nuclear (Rothfels et al. 2015, Qi et al. 2018, Shen et al. 2018), combined mitochondrial and plastid (Knie et al. 2015) and plastome (Kim et al. 2014, Labiak & Karol 2017) data analysis, a considerable number of plastome studies (Karol et al. 2010, Grewe et al. 2013, Kim et al. 2014, Ruhfel et al. 2014, Zhong et al. 2014, Lu et al. 2015, Gitzendanner et al. 2018, Kuo et al. 2018, Lehtonen 2018) support its position as sister to Ophioglossaceae and Psilotaceae.

Another discrepancy among parsimony and model-based trees was observed within Polypodiineae. Parsimony resolved Lomariopsidaceae and Nephrolepidaceae as a clade in contrast to model-based analyses, which resolved them as successive lineages leading to Tectariaceae, Oleandraceae, Davalliaceae and Polypodiaceae (Fig. 3A in chapter IV). This relation has not reached a consensus in previous studies based on plastid and nuclear data (Tsutsumi & Kato 2006, Schuettpelz & Pryer 2007, Li et al. 2009, Hennequin et al. 2010, Christenhusz et al. 2013, Liu et al. 2013, Qi et al. 2018). Further investigation needs to be done to resolve this position.

The different plastome analyses on my study could not resolve the problematic nodes previously observed in the fern phylogeny, except for the position of *Saccoloma* (Fig. 3A in chapter IV). The conflicting relationships obtained by using few genes still remained unresolved with the use of complete genomes (Kuo et al. 2018, Lehtonen 2018). It, therefore, seems that the studies for fern phylogeny reconstruction, using complete plastomes, face similar challenges as the studies based on the traditional approach using a few genes. This supports the statement that the addition of more genes (characters) does not improve the accuracy of

phylogenetic reconstruction as much as the addition of more taxa (Graybeal 1998, Hillis et al. 2003). The results suggest that it may be better to update the following fern phylogenies based on a set of informative markers obtained from nuclear, plastid and mitochondrial DNA, rather than based on pure complete plastome data.

On the other hand, the newly generated plastomes were useful for revealing structural rearrangements in the fern plastome. Although previously thought to be evolutionary conservative (e.g. Palmer & Stein 1986, Wolf & Roper 2008), studies on fern plastome structure have revealed it to be dynamic. The insertions, deletions and translocation observed in the fern plastome structure in specific groups have been proposed to have evolutionary implications (e.g. Gao et al. 2009, Wolf et al. 2011, Raman et al. 2016, Logacheva et al. 2017). Robison et al. (2018) reported the presence of Mobile Open Reading Frames in Fern Organelles (MORFFO), which seem to be a dynamic component of fern plastome. MORFFO are often found related to dynamic regions on plastome structure and Robison et al. (2018) mentioned that MORFFOs may appear in regions that are likely to experience inversion, or that maybe the inversions are controlled by MORFFOs. Similarly, in this study, MORFFO elements were found in structurally dynamic areas of the plastome close to sites of inversions or IR borders. A novel finding was the presence of MORFFO elements within the *rps4-psal* region of the LSC. The much higher occurrence of MORFFO elements in Polypodiineae plastomes in contrast to its sister clade Aspleniineae is a highly interesting pattern that although needs further investigation, it could be a distinctive evolutionary signal among these two clades.

4 CONCLUSIONS

In this thesis, I have studied ferns and lycophytes from different perspectives. I have used fern and lycophyte species as indicators of environmental conditions, specifically soil fertility in chapter I. In chapters II and III, I have studied the systematics and phylogeny of two fern genera, and in chapter IV, I have explored the fern plastid genome structure.

Assesing the species distribution of ferns and lycophytes at Estación Biológica Quebrada Blanco (EBQB), it was possible to infer that soils at the EBQB area are mostly uniform and have intermediate to poor fertility (chapter I). However, despite the little soil variation (and therefore productivity variation) found at EBQB, it was possible to observe differences in home range size of saddle-back tamarin, which although not always conclusive, they can be related to the variation of soil fertility in the area. These findings can be useful in future studies on the understanding of tamarin behaviour, not only at EBQB but also in other areas with similar forest conditions.

The three new species found in *Metaxya*, and the newly described two species and four subspecies in *Salpichlaena* (chapters II and III), give more evidence of the unknown biodiversity in the Neotropics, and that the estimated number of around 5000 species of ferns and lycophytes in the Neotropics (Moran 2008) is constantly growing.

In my study, the use of Integrative taxonomy was crucial for the delineation of taxon boundaries. Not all the species boundaries were clear if relying on only one source of evidence, but the combination of them (morphological, phylogenetic, ecological, biogeographic and molecular delimination) facilitated the taxonomic decision making. An example is the case where the phylogenetic approach helped to reveal the hybrid origin species in *Salpichlaena*, which was difficult to distinguish initially based only on morphological approach. Although all the approaches used in my study contributed in some degree to draw the limits between taxa, morphological and phylogenetic analyses were clearly the most conclusive. Still, molecular species delimitation methods also confered certain support to the taxa, and I would recommend their use as a starting hypothesis in studies of species delimitation.

The UTU-ART collections, not to mention other important natural history collections, still have great potential for uncovering new species by applying the same methodology used in this thesis. Families such as Lindsaeaceae, Marattiaceae, Pteridaceae, Tectariaceae and Thelypteridaceae are among the most promising families to find new taxa. Moreover, the potential of the UTU-ART data goes beyond discovering species. The fact that taxonomic species data can be linked to ecological soil data contributes to providing a better understanding of patterns of species distribution in the Neotropics, not only of plants but may be also of other organisims, and to explaining the evolutionary processes that have led to the current species diversity and distribution in the Neotropics.

The generation of complete genomes in the last few decades has greatly improved our understanding of fern plastome structural evolution. My study, in addition to contributing new fern plastome data for eight species, representing six families newly sampled for complete plastomes, has resolved the position of Saccolomatineae in the fern phylogeny (chapter IV). It also brought into light previously unreported rearrangements in fern plastome evolution. The high predominance of MORFFO elements found in the *rps4-psal* region in the Polypodiineae may suggest a distinct and more dynamic plastome evolution in this clade compared to other clades, especially to the sister clade Aspleniineae.

Taxonomical research in the Neotropics, and more generally in most of the biodiversity-rich tropical areas, faces several obstacles, such as difficulties in accessing the areas of interest because of geographical or political issues, and the lack of representative samples from areas of interest and their incompleteness in the natural history collections. Molecular phylogenetic analysis in turn are bounded by the unavailability of samples for DNA extraction and the failure in obtaining good-quality DNA from the available specimens. Some of these difficulties can no longer be overcome, like the incompleteness of existing herbarium samples, or the difficulty for recovering DNA from herbarium samples that are too old or that have been stored in alcohol, damaging their DNA, at least not with the available techniques.

Fortunately, the quality of future herbarium collections can be improved by paying attention to the key characters needed for taxon identification, which are being revealed by new taxonomic studies. In the same way, the accessibility to DNA material is becoming easier thanks to new technologies. Now it is possible to extract DNA from herbarium samples, if the samples still retain intact DNA. Additionally, more researchers are interested in molecular analysis and are collecting DNA samples and making the sequences available in open source repositories. The future access to genetic resources can also be improved by consolidating relationships with the national institutions of provider countries.

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