University of Gothenburg
Faculty of Science
2008

SPATIOTEMPORAL EVOLUTION OF NEOTROPICAL ORGANISMS

NEW INSIGHTS INTO AN OLD RIDDLE

Alexandre Antonelli

Doctoral Thesis

UNIVERSITY OF GOTHENBURG

This thesis will be defended in public at 10.00 A.M. on November 28th, 2008 in the Lecture Hall, Department of Plant and Environmental Sciences, Carl Skottsbergs Gata 22B, Göteborg, Sweden.

Faculty opponent: Prof. Mari Källersjö
Examiner: Prof. Nils Hallenberg

© Alexandre Antonelli, 2008.
All rights reserved. No part of this publication may be reproduced or transmitted, in any form or by any means, without written permission.

Antonelli, A. 2008. Spatiotemporal Evolution of Neotropical Organisms: New Insights into an Old Riddle. Doctoral Thesis. Department of Plant and Environmental Sciences, University of Gothenburg, Göteborg, Sweden.

Cover image: “Romantic view over Nebo”, by Karl Axel Pehrson (1921–2005), Swedish artist and creator of the statuette for the Swedish Film Award Guldbaggen, *The Golden Beetle*. As in all his paintings, the landscape and organisms portrayed are purely imaginary. Courtesy: County Museum of Örebro, Sweden.

ISBN: 978-91-85529-21-6

http://hdl.handle.net/2077/17695

Printed by Geson Hylte Tryck.

To Anna

Contents

Abstract

Nowhere else on Earth are there so many species of plants and animals as in the Neotropical region. Yet, many questions remain concerning the causes underlying such outstanding diversification.

In this thesis, I use a combination of molecular-based methods (phylogenetic inference, molecular dating, biogeographic reconstruction, analyses of diversification and extinction) together with geological, palaeontological, hydrological and climatological evidence to reconstruct the evolution of some Neotropical organisms in space and time. Diversification patterns obtained from case studies in the plant families Rubiaceae, Chloranthaceae and Campanulaceae are compared to published studies of other plants and animals, especially tetrapods (birds, non-avian reptiles, amphibians and mammals).

The uplift of the Northern Andes in the Neogene (~23 Ma to today) is concluded to have played a major role in promoting Neotropical diversification, by fostering allopatric speciation of lowland organisms and inducing adaptive radiations in newly formed montane habitats. In addition, its formation caused the end of a lowland corridor episodically invaded by marine incursions that separated the Northern and Central Andes, enabling the southward dispersal of boreotropical groups already present in northwestern South America.

The fact that most Neotropical plant groups are either Andean-centred or Amazonian-centred is explained by the long-lasting effect of the Palaeo-Orinoco and Lake Pebas as dispersal barriers between these two diversity centres. Finally, a new diversification model is proposed to explain the origin and evolution of organisms in two areas characterized today by unusually high levels of species richness and endemism: the Huancabamba region and western Amazonia. Under this model, speciation is proposed to have occurred over several million years in connection with the recolonization of previously submerged areas, by means of adaptive radiation of founder colonies and secondary contact of previously isolated populations.

KEYWORDS: Neotropics; Biodiversity patterns, Speciation models, Andean uplift, K/T Event; Biogeography, Phylogenetics, Molecular dating; Rubiaceae, Chloranthaceae, Campanulaceae, Tetrapods.

List of original papers

This thesis is based on the following papers. They will be referred to in the text by their Roman numerals.

I. Andersson, L., **Antonelli, A**. (2005) Phylogeny of the tribe Cinchoneae (Rubiaceae), its position in Cinchonoideae, and description of a new genus, *Ciliosemina. Taxon* 54 (1):17–28.

II. **Antonelli, A.** (2008) Higher level phylogeny and evolutionary trends in Campanulaceae subfam. Lobelioideae: Molecular signal overshadows morphology. *Molecular Phylogenetics and Evolution* 46 (1):1–18.

III. **Antonelli, A.,** Quijada-Masareñas, A., Crawford, A.J., Bates, J.M., Velazco, P.M., Wüster, W. (accepted) Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. In: Hoorn, C., Vonhof, H., Wesselingh, F.: *Amazonia, Landscape and Species Evolution: a Look into the Past.* Blackwell publishing.

IV. **Antonelli, A.**, Nylander, J.A.A., Persson, C., Sanmartín, I. (submitted) Tracing the impact of the Andean uplift on Neotropical plant evolution: evidence from the coffee family.

V. **Antonelli, A.**, Sanmartín, I. (submitted) Reconstructing the spatio-temporal evolution of the ancient angiosperm genus *Hedyosmum* (Chloranthaceae) using empirical and simulated approaches.

VI. **Antonelli, A.** (submitted) Convergence not always the case.

Papers I and **II** reprinted with permission.

Introduction

"In England any person fond of natural history enjoys in his walks a great advantage, by always having something to attract his attention; but in these fertile lands teeming with life, the attractions are so numerous, that he is scarcely able to walk at all."

Charles R. Darwin, 19th April 1939, during his stay in Brazil.

The Neotropical region

The word *Neotropic* (from the Greek neos = "new") refers to the tropical region of the American continent, or "New World" – a term coined by Peter Martyr d'Anghiera in 1493 shortly after Christopher Columbus' first voyage to the Americas (O'Gorman 1972). As currently defined (Schultz 2005), the Neotropic ecozone extends from central Mexico in the north to southern Brazil in the south, i.e. including Central America, the Caribbean islands and most of South America.

Geologically, the Neotropics are distributed across three tectonic plates: the North American, the Caribbean and the South American (Fig. 1), each with a very different geological history. The South American and African plates remained fused in the giant palaeocontinent of Gondwana for hundreds of millions of years until its final breakup about 100 million years ago (Ma; Scotese 2001). The fact that most tropical soils are extremely scarce of nutrients reveals long-lasting weathering conditions.

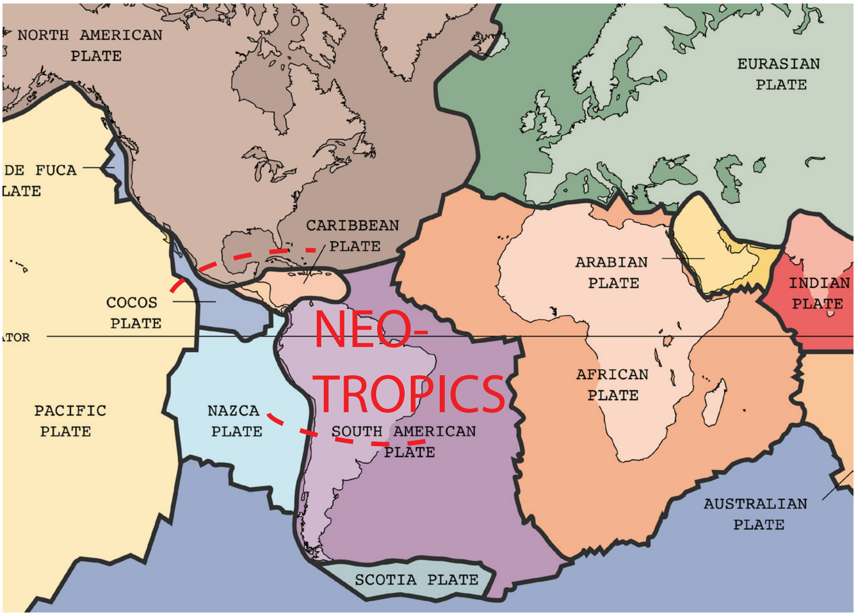


Figure 1. The Neotropical region extends from central Mexico in the north to southern Brazil in the south, thus occupying the North American, the Caribbean and the South American tectonic plates. Many hypotheses of diversification discussed in this thesis derive ultimately from the geological dynamics of these plates: the separation of Africa and South America, the rise of the Andes and the reconnection of South America with a land mass after 90 million years of isolation. (Source: Wikimedia Commons).

Precipitation and annual mean temperatures are generally high, but there is great regional variation. Whereas some places in South America have amongst the highest precipitation rates in the world (such as Quibdo in western Colombia, with almost 9000 mm annually), other regions are extremely dry. Perhaps surprisingly, it is in South America that the driest place on Earth is to be found: Calama, in the subtropical Atacama Desert of Chile, where no rain has yet been recorded (Kricher 1997). In the Amazon Basin, precipitation ranges between 1500 and 3000 mm annually, averaging around 2000 mm in central Amazonia (Salati and Vose 1984).

Before human devastation, the Amazonian rain forest accounted for about one third of the entire South American continent. Aside from Amazonia, there are other terrestrial biomes in the Neotropics that are noteworthy for their size and ecological importance:

- the seasonally dry tropical forests (SDTF) covering most of the Brazilian highlands and scattered areas in Central and South America (Lavin et al. 2006);
- the Atlantic rain forest of eastern Brazil;
- the Chocó region of northwestern South America;
- the Llanos floodplain adjacent to the Orinoco river in northern South America;
- the semi-arid Caatinga of northeastern Brazil.

Neotropical biodiversity

Species numbers in the Neotropics are astounding. Comprising around 90 – 110 000 species of seed plants, the Neotropical region alone is home to about 37% of the world's total number of species. In fact, this is probably more than tropical Africa (30 000 – 35 000 spp.) and tropical Asia and Oceania combined (40 000 – 82 000 spp.; Thomas 1999; Govaerts 2001).

Global patterns of animal diversity seem to be similar to the one shown by plants. Species richness is strongly correlated among amphibians, birds and mammals, even after correcting for differences in area (Grenyer et al. 2006; Lamoreux et al. 2006; Fig. 2). Reptile diversity is also significantly correlated to other vertebrates, although this correlation is more moderate (Lamoreux et al. 2006). For reptiles and amphibians, it has been statistically demonstrated that the Neotropics are significantly richer in species than expected by chance, as compared to other tropical regions (Wiens 2007; Hong Qian 2008).

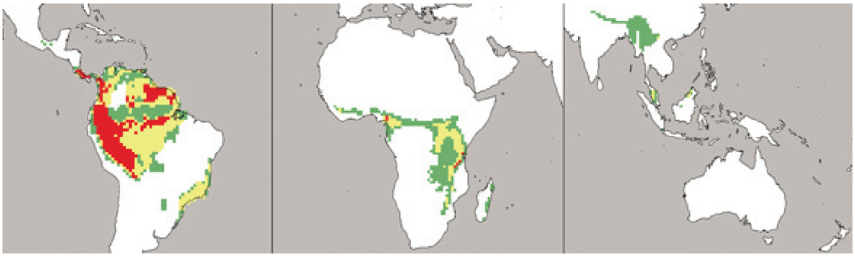


Figure 2. Tropical hotspots of species richness for mammals, birds and amphibians. Red shading shows cells that are hotspots for all three groups, yellow for two groups and green for one group. Hotspots are the richest 5% non-zero cells. Adapted from Grenyer et al. (2006). Reproduced with permission.

Indeed, the Neotropics comprise more than half of all amphibian species in the world (2916 spp.), followed by tropical Australasia (1378 spp.) and tropical Africa (958 spp.; http://www.globalamphibians.org). For birds, the most updated and reliable species count available today (Larsson et al. 2008) clearly shows that tropical Africa contains less species (2048 spp.) and a lower proportion of endemic species (20%) than tropical Asia (2324 spp., 23% endemic). And once again, Neotropical diversity stands out with 3653 bird species documented so far, of which 35% occur nowhere else (Fig. 3). Despite a major wave of extinction among South American mammals some 3.5 Ma (see **Paper III**), recent studies (Grenyer et al. 2006; Schipper et al. 2008) show that the Neotropics still possess more species (1189 spp.) of terrestrial mammals than tropical Africa (1037 spp.). The number of mammal species in tropical Australasia has not been made available.

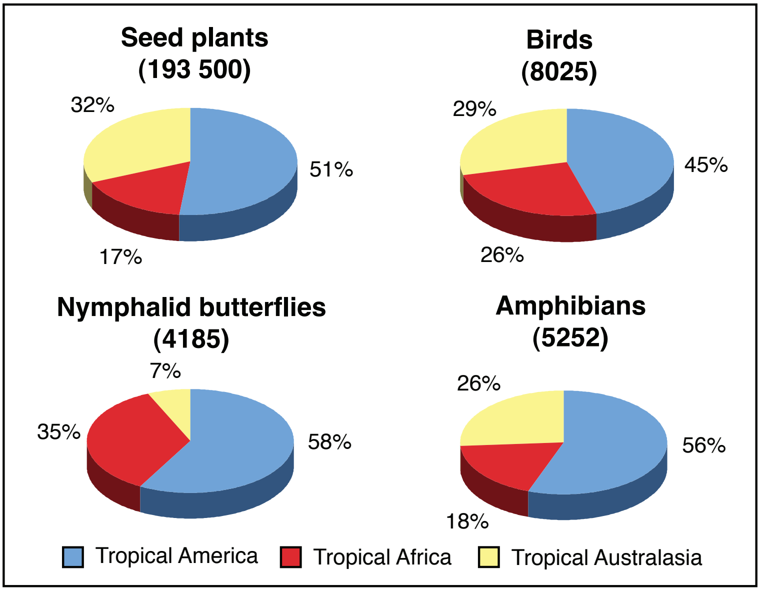


Figure 3. Comparison among the number of tropical species in four organism groups. The estimated total number of tropical species is given within parentheses (see text for data sources).

Among nymphalid butterflies, the Neotropics (with 2433 spp.) are also much more species-rich than the other tropical regions, but in this group tropical Asia (279 spp.) is home to considerably less species than Africa (1473 spp.; Heppner 1991; http://nymphalidae.utu.fi). Other estimations of invertebrate diversity are much more uncertain. Recent studies have argued that the diversity of herbivorous insects (e.g., butterflies, leaf beetles, flies) is a direct function of plant diversity (Novotny et al. 2006; Dyer et al. 2007). A molecular phylogenetic study recently demonstrated that a single plant species may support up to 13 species of flies (Condon et al. 2008). Extrapolations from detailed inventories of insect diversity suggest that there may be somewhere between 3 and 30 million species of herbivorous insects (e.g., Fig. 4) in the Neotropics (May 1990).

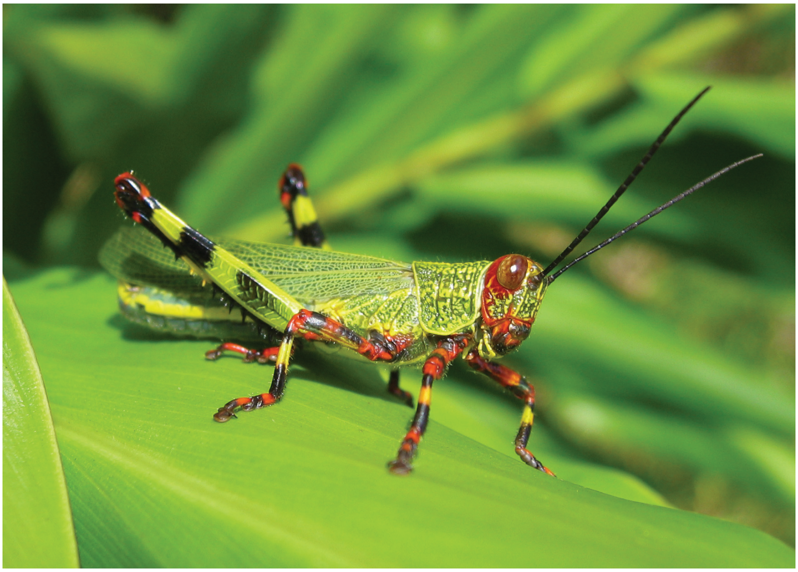


Figure 4. How many species? Many groups of Neotropical insects are still poorly studied, and estimates of species numbers vary greatly – as among herbivorous insects, such as the Brazilian grasshopper portrayed here. ©A.Antonelli.

This ten-fold difference in invertebrate estimations reflects our meagre knowledge of Neotropical insects, but also our insufficient capacity of dealing with genetic and morphological variation in nature. This variation is often continuous between individuals, which contrasts with the categorical grouping of individuals into fixed (discontinuous) taxonomic entities, such as species and genera. It is thus not surprising that what appears to be a single widespread species of butterfly may turn out to be at least 10 groups (should we call them species?) that are genetically distinctive (Hebert et al. 2004).

Ever since the early voyages of renowned explorers, such as Humboldt (1820), Darwin (1845) and Wallace (1852, 1853), the outstanding species richness found today in the Neotropical region has been a major riddle in our understanding of the evolution of life on Earth.

Distributional patterns

Within the Neotropics, species are not distributed randomly. In a seminal paper, Gentry (1982) demonstrated that there are two main patterns of plant distribution, which he termed "Amazonian-centred" and "Andean-centred". Typically, groups that are rich in species in one of these centres are relatively species-poor in the other. This will be termed here *"the Gentry pattern"*.

Andean-centred groups are characterised by having their centres of diversity in northwestern South America and adjacent Central America. Gentry exemplified this pattern with Maas' (1977) diversity map for the genus *Renealmia* (Zingiberaceae; Fig. 5). According to Gentry's (1982) extensive survey, as many as 38% of all Neotropical plant species may belong to Andean-centred groups.

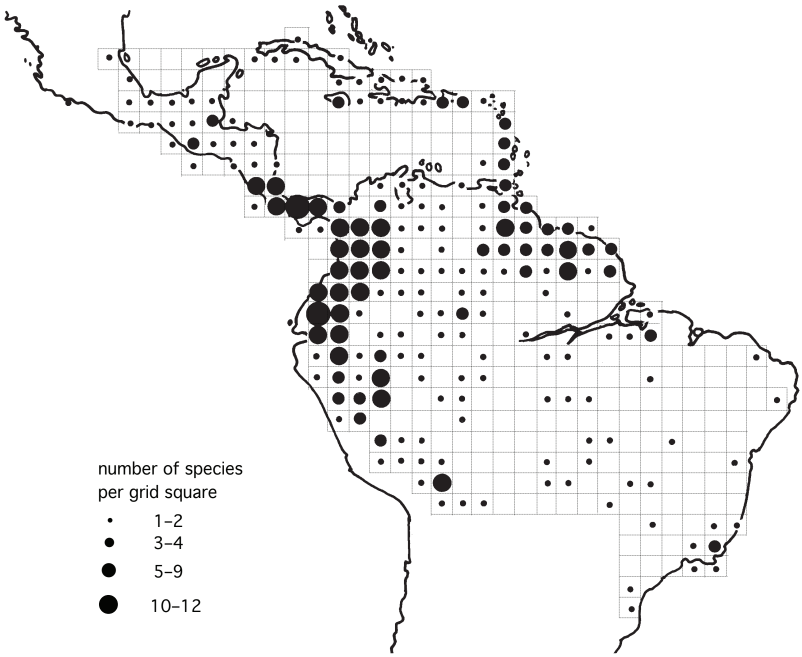


Figure 5. Species diversity of a typical Andean-centred group: the genus *Renealmia* (Zingiberaceae). Redrawn from Maas 1977; the Trinidad grid square, mistakenly left blank by Maas was corrected in accordance with data in the text. Map by L. Andersson (unpublished).

Amazonian-centred groups, on the other hand, are characterised by having a high number of species in the Amazon Basin. Gentry exemplified this pattern using Berg's (1972) diversity map for the tribe Olmedieae (Moraceae; Fig. 6). Amazonian-centred groups, according to Gentry, may comprise some 33% of all Neotropical plant species.

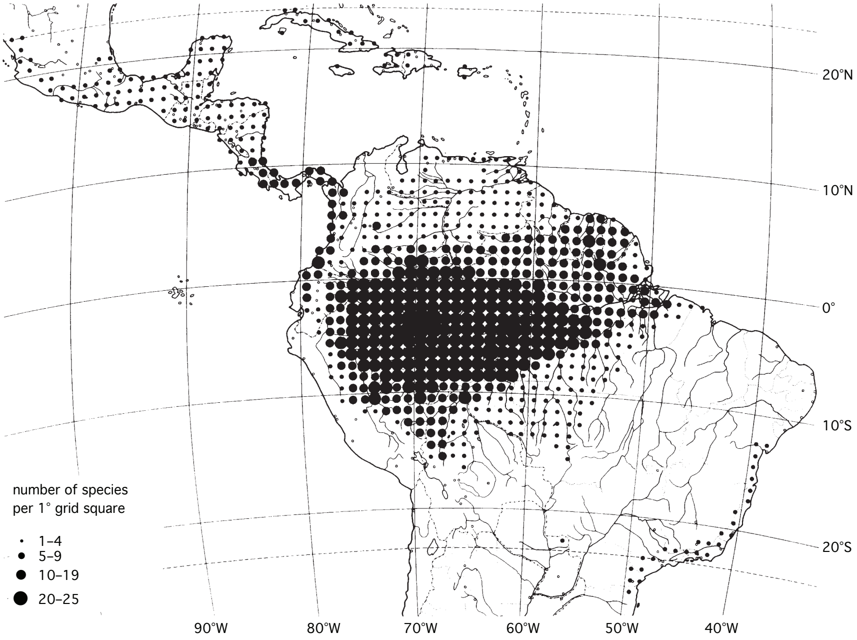


Figure 6. Species diversity of a typical Amazonian-centred group: tribe Olmedieae (Moraceae). Compiled from maps of individual species distributions in Berg (1972) and localities listed by Berg (1998). Map by L. Andersson (unpublished).

Because the great majority of Neotropical plant species (71% in Gentry's survey) belong to either one of these groups, Gentry concluded that *"any explanation of the patterns of evolutionary diversification in these taxa will largely explain the richness of the Neotropical flora"* (Gentry 1982).

Models of diversification

Global patterns of species richness have traditionally been explained in terms of environmental factors (Kreft and Jetz 2007), but lately more integrative explanations have emphasized the role of historical and evolutionary factors in producing diversity (Pennington and Dick 2004; Donoghue 2008; Linder 2008).

Several diversification models have been proposed for the Neotropical region. Here follow some of them and their phylogenetic and temporal predictions. It is important to note that these hypotheses often have similar predictions and it is quite possible that more than one could affect any given lineage (Moritz et al. 2000).

Riverine barriers. From the time of the earliest European biogeographical explorers, it has been apparent that the Amazon River and some of its tributaries separate the ranges of many forest species. Wallace (1853) postulated what we today call the riverine barrier hypothesis. Under this model, widespread Amazonian animals are thought to have split into isolated populations due to the formation of the Amazon River network. Riverine barrier effects have been invoked to explain distribution limits in a number of vertebrate species in the Amazon Basin, including birds (e.g., Bates et al. 2004) and primates (Ayres and Clutton-Brock 1992). Other studies (e.g., Gascon et al. 2000, Aleixo 2004) have shown that at least some major rivers in Amazonia do not appear to have promoted diversification. Bates et al. (2004) proposed that meandering rivers might offer more opportunities for gene flow whereas faster flowing rivers may be stronger barriers.

Pleistocene refugia. During the past 2 million years, the Earth underwent at least twenty major glacial periods when mean global temperatures were at least 4ºC lower than today's (Gates 1993). Based on the observation that the main centres of avian endemism in northern South America are situated in zones that receive today the highest levels of precipitation, Haffer (1969, 1997) suggested that the rainforest cover in Amazonia changed repeatedly in response to global climatic oscillations. According to his hypothesis, lowland forest broke up into isolated refugia during cooler (drier) periods and expanded again during warmer (wetter) interglacials. As a result, allopatric speciation in forest refugia was promoted. This Pleistocene refuge theory gained early support by the demonstration of similar distribution patterns in many taxa. These included reptiles and amphibians (Vanzolini and Willians 1970; Dixon

1979; Lynch 1979), butterflies (Brown 1982) and woody plants (Vuillemier 1970; Prance 1973, 1978, 1982; Andersson 1979). Although there is geological evidence that some parts of Amazonia did become arid during glacial periods (e.g., Ab'Saber 1982), as predicted by the refuge theory, in other areas it has been shown that cooler periods only brought about taxonomic shifts in the forest rather than caused it to fragment (e.g., Bush 2004).

Disturbance-vicariance hypothesis. According to this hypothesis, the major factor triggering diversification in the Neotropics was the temperature fluctuations themselves, rather than aridification and physical fragmentation of lowland forests as proposed under the refuge theory (Colinvaux 1993; Bush 1994). The late Neogene climatic oscillations would have caused recurrent displacement of taxa towards lower or higher altitudes (during cool and warm periods, respectively). The effects of such displacements would have been most notable in the peripheral parts of Amazonia, which would have served as crossroads for the invasions and counter-invasions of montane and lowland species. Fierce competition between these species could result in directional selection and eventually lead to speciation. The hypothesis thus predicts a higher level of endemism in the peripheries of Amazonia as compared to its core, implying a diversity gradient.

Marine incursions. In the Miocene, sea rises of about 100 m above the present level have been suggested to cause large parts of lowland Amazonia to become submerged (Hoorn 1993, 1994; Hoorn et al. 1996; Hoorn and Vonhof 2006), a hypothesis supported by patterns of fish biogeography and phylogeny (Lovejoy et al. 2006). By using topographic maps to identify areas above that altitudinal limit, Nores (1999) argued that during periods of marine incursions, two large islands in northeastern South America existed around present day Guianas. Additionally, a large number of smaller islands and archipelagos would have been formed along the coastal lowlands of northeastern South America and the southern periphery of the Amazon Basin. Because the regions where these major islands would have been formed today contain a high level of endemism among birds, Nores postulated that recurrent marine incursions in Amazonia may have increased the opportunities for allopatric speciation and thus could represent a major force driving diversification in the Neotropics. However, recent evidence (Miller et al. 2005; Müller et al. 2008) strongly indicate that sea level fluctuations have been of considerably lower amplitudes than those assumed for the elaboration of this diversification model (Haq et al. 1987; see Fig. 13 in Results and Discussion).

Andean uplift. Extending over 7000 km along the western coast of South America, the Andean Cordillera constitutes the largest mountain chain in direct connection with a tropical rain forest. Its uplift can be traced back to the Cretaceous (~100 Ma; Milnes 1987), and is thought to have proceeded from south to north and from west to east (Taylor 1991). In the central and northern Andes, most of the uplift took place in the last 25 Ma, with some segments of the Eastern Cordillera in the northern Andes having risen as recently as 5 – 2 Ma (Gregory-Wodzicki 2000; Garzione et al. 2008).

The Andean uplift may have promoted speciation in several ways: ***i)*** by creating previously non-existent Neotropical montane and premontane habitats, favouring morphological and physiological adaptation of lowland taxa; ***ii)*** by producing geographic vicariance, and consequently genetic isolation, between populations on both sides of the emerging mountains; ***iii)*** by favouring allopatric speciation among montane taxa, separated by deep valleys and impassable ridges and peaks. Newly formed lineages along the eastern Andes could then have moved into Amazonia and contributed to lowland diversity. Past exchanges between the Amazonian lowlands and the eastern slopes of the Andes have included range extension of plants presently confined to moderate altitudes into the lowlands during Pleistocene cold phases (Colinvaux et al. 1996).

Objectives

Since the publication of Gentry's (1982) comprehensive account on Neotropical plant diversity and its possible causes, the advent of molecular phylogenetics in the past two decades has given us new tools for addressing questions on the origin and evolution of organisms across space and time.

Advances in the fields of phylogenetic inference, molecular dating, historical biogeography and statistical modelling can now be combined to provide clues on ancestral areas and divergence times (Sanmartín et al. 2001; Sanderson 2002; Donoghue and Smith 2004; Ree et al. 2005; Drummond et al. 2006), the tempo and mode of lineage diversification, i.e., the interplay between extinction and speciation, adaptive radiation and widespread extinction (McKenna and Farrell 2006; Weir 2006; Rabosky and Lovette 2008), and the putative correlation between historical patterns of diversification and morphological and range evolution (Moore and Donoghue 2007).

This project takes advantage of these recent developments in order to reconstruct the spatiotemporal evolution of some Neotropical lineages. Departing from detailed studies on the plant families Rubiaceae, Campanulaceae and Chloranthaceae, the biogeographic patterns found in these groups are then contrasted with what is currently known about diversification of Amazonian tetrapods and other plant and animal groups.

This thesis aims at advancing our understanding on how and when the Gentry pattern was formed, and how the different speciation models proposed for the Neotropical region have contributed to the present-day levels of species richness.

Material and methods

Study groups

Given the huge size of Neotropical biodiversity, any attempt at generalization has to be based on extrapolations of the results found for a few groups of organisms. This practical limitation has important implications for the choice of taxonomic group to be studied. The following considerations have been taken into account for choosing groups in this thesis:

– *Geographic distribution:* only if a taxon occurs in the region of interest will it be possible to draw conclusions regarding the origin and evolution of the region's biota. Groups containing species distributed over several geographic areas and in several habitats have therefore been chosen, rather than narrowly distributed taxa;

– *Access to suitable material for sequencing:* some taxa may be taxonomically and biogeographically interesting, but if they are too poorly represented in modern collections it may be impossible to obtain DNA of good quality for molecular analyses;

– *Taxonomic knowledge:* it can be very time-consuming (although in several ways rewarding) to work with groups that have never been revised taxonomically. Moreover, revisions, checklists and local floras are indispensable for compiling distributional data for biogeographic analyses;

– *Suitable calibration points*: good fossils are exceedingly rare and limited to a few plant and animal groups. However, whenever available, they constitute an invaluable source of information for performing molecular dating analyses.

For animals (**Paper III**), this project has only aimed at summarizing the results from previously published studies, whereas plants were studied much more thoroughly (but fewer groups considered). Based on the considerations above, the following plant families were selected for this project:

Rubiaceae (Papers I and IV)

The Rubiaceae (coffee or madder family) is the fourth largest family of flowering plants, with some 13 100 species in more than 600 genera (Govaerts et al. 2007). The family has a cosmopolitan distribution, but its highest diversity is confined to the tropics.

Three subfamilies are commonly recognized: Rubioideae, Ixoroideae and Cinchonoideae (Bremer et al. 1999). Based on supertree analyses, Ixoroideae and Cinchonoideae have been proposed to constitute a monophyletic clade (Robbrecht and Manen 2006), but this conclusion is disputed (C. Persson pers. comm.). Rubioideae is pantropical, but probably originated in the Old World (Manen et al. 2006). The distribution of the subfamily Ixoroideae is concentrated to the Palaeotropics (Andreasen and Bremer 2000), whereas Cinchonoideae, with the exception of tribe Naucleeae, is predominantly Neotropical.

In the Neotropics, Cinchonoideae is represented by the tribes Cinchoneae and Isertieae. These tribes comprise some 130 species of small trees and shrubs divided into eleven genera and occur in a wide range of habitats. Some species of *Cinchona* are economically important as a source of quinine, which is industrially used to flavour tonic water and as an effective medicine against malaria.

The distribution of Isertieae is concentrated to the lowlands of the Amazon basin and eastern Guianas, whereas Cinchoneae species are mainly confined to the highland and montane habitats of the Northern and Central Andes, reaching up to 3300 m. Widespread species in these two tribes occur also in the West Indies, Central America, the Guiana Shield and southeastern South America. Their wide geographic distributions thus offer good prospects for investigating the evolutionary history of species in nearly all Neotropical ecosystems.

Although the fossil record of Rubiaceae is scarce, there is at least one genus in Cinchonoideae (*Cephalanthus*) with a well-documented fossil record from the Late Eocene (~35 Ma) and onwards in Denmark, Germany and western Siberia (see **Paper IV** for references).

Chloranthaceae (Paper V)

The Chloranthaceae is a small family of flowering plants, comprising some 65–70 species in four genera (Heywood et al. 2007). They are disjunctly distributed in the Old and New World: *Chloranthus*, *Sarcandra* and *Ascarina* are confined to the Palaeotropics, including east Asia (*Chloranthus* and *Sarcandra*) and Australasia (*Ascarina*), while the vast majority of *Hedyosmum* species occurs in montane habitats of Central and South America and the West Indies, but with a single species in southeastern Asia (*H. orientale*). Among the four genera, *Hedyosmum* is by far the most species rich (~45–50 spp.; Todzia 1988, 1993).

Because of its phylogenetic position near the root of the angiosperms, and its extensive and old fossil record extending back to the Early Cretaceous (~110 Ma; Friis et al. 1997; Eklund et al. 2004), the Chloranthaceae has figured prominently in studies on the origin and early diversification of flowering plants.

Relatively recent taxonomic revisions of *Hedyosmum* (Todzia 1988, 1993), which include detailed accounts on geographic distributions, together with the possibility of calibrating the phylogeny of Chloranthaceae using multiple fossil constraints, have made *Hedyosmum* an adequate model group for studying plant evolution in the montane areas of the Neotropics.

Campanulaceae (Papers II and VI)

The Campanulaceae (the bluebell or *Lobelia* family) comprises some 84 genera and nearly 2400 species (Lammers 2007). The group has a cosmopolitan distribution and is present in a wide array of habitats, from tropical rain forest to tundra. The variety of life forms ranges from dwarf herbs shorter than 2 cm to trees up to 15 m tall.

As currently circumscribed (Lammers 1998a), the family is divided into five subfamilies: Campanuloideae, Lobelioideae, Nemacladoideae, Cyphioideae and Cyphocarpoideae. Of these, Lobelioideae is the largest subfamily, comprising 28 genera in about 1200 species, half of which are native to South America. It is noteworthy that the six largest genera contain almost 80% of the species, and that eight genera are monotypic.

In the Neotropics, the Lobelioideae are mainly represented by the genera *Burmeistera, Centropogon, Lobelia, Lysipomia* and *Siphocampylus*, together accounting for some 600 species (Lammers 2007). Most of them occur in montane and pre-montane habitats, with a centre of diversity in the Northern Andes, where they are ecologically important as a nectar source for birds and bats (Muchhala 2003, 2006a, 2006b; Lammers 2007).

Positive aspects for studying Neotropical lobelioids derive mainly from the great number of Neotropical species in the subfamily, which offers the potential of discovering new species, proposing taxonomic rearrangements and investigating plant evolution in montane habitats. Nevertheless, a disadvantage lies in the fact that the fossil record of the Campanulaceae is very scarce (Lammers 2007): only three fossils have been documented so far, all assigned to subfamily Campanuloideae. Molecular dating of the family should therefore be complemented by the use of indirect calibrations, such as geological events and age estimations obtained from large-scale studies.

Sequence regions

It was desirable to obtain phylogenetic resolution both at the family level as well as among species and genera. Relatively conservative and relatively fast-evolving sequence regions have therefore been used in different combinations (Table 1).

Table 1. Sequence regions used for the plant families investigated.

Family	Nuclear DNA	Chloroplast (plastid) DNA				
	ITS*	*rbc*L*	*rps*16	*trn*L–F*	*mat*K*	*ndh*F
Campanulaceae	X	X		X		X
Chloranthaceae	X	X	X			
Rubiaceae	X	X	X	X	X	X

(*) For these regions, new primers have been developed for DNA amplification and/or sequencing (see respective papers for details).

The *rbc*L gene has proved in numerous studies to offer sufficient information for well-supported resolution at higher taxonomic levels (e.g., Chase et al. 1993; Savolainen et al. 2000). It has also the advantage that a large number of useful sequences are available from GenBank.

The *mat*K gene is known from some families to offer more phylogenetic information than the *rbc*L (e.g., Gentianaceae; Thiv et al. 1999).

The *ndh*F gene is 1.5 times longer than *rbc*L and may contain three times more phylogenetic information (Kim and Jansen 1995). It has been used at different taxonomic levels, ranging from the intrageneric level (Källersjö and Ståhl 2003) to studies of major lineages of asterids (Albach et al. 2001; Olmstead et al. 2000).

The *rps*16 intron (e.g., Andersson and Rova 1999; Andersson 2002) and the *trn*L–F intergenic spacer (e.g., Persson 2000; Rova et al. 2002) have been shown in earlier studies to provide resolution within tribes of Rubiaceae.

Among the sequence regions used in this thesis, ITS is by far the most employed one. A quick search in GenBank revealed that at present (August 2008) more than 230 000 ITS sequences have been deposited in that database, which can be compared with the ~10 000 *rps*16 sequences (Fig. 7).

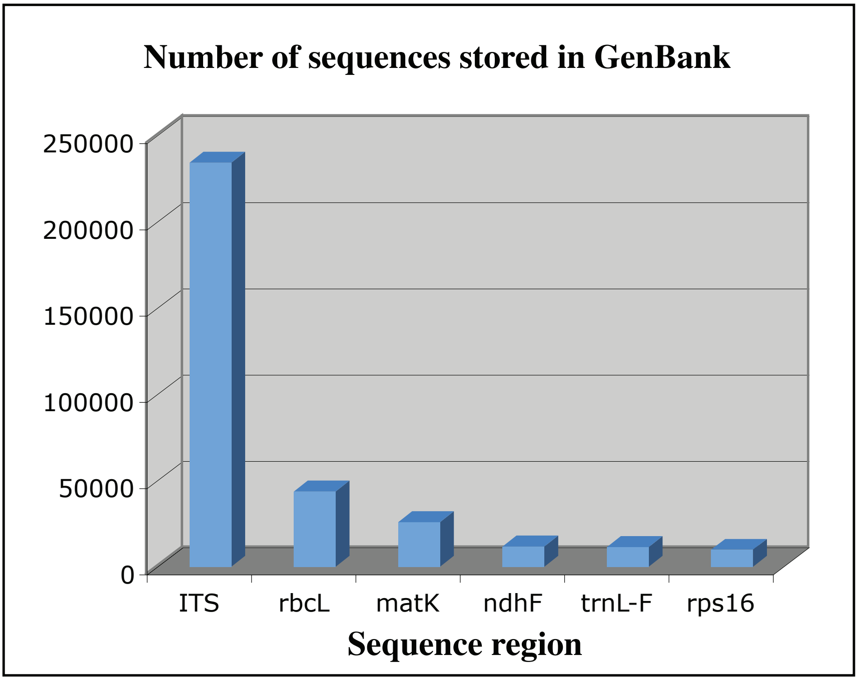


Figure 7. Number of sequences stored in GenBank for the sequence regions used in this project.

The popularity of the ITS is doubtlessly due to that fact that it is one of the most easily amplified and fastest evolving sequence regions available, often providing phylogenetic information for resolving relationships within genera (Shaw et al. 2005, 2007; Hughes et al. 2006). Its use also experienced an upturn when it was proposed as the universal DNA barcoding region for plants and fungi (Kress et al. 2005; Kress and Erickson 2008; Lahaye et al. 2008). Moreover, it has been useful for species-level identification in a wide range of situations, such as the identification of biodiversity hotspots (Kress et al. 2005; Kress and Erickson 2008; Lahaye et al. 2008). ITS is also widely used in mycological studies for species identification of ectomycorrhizae and as support when describing new species (e.g., Kõljalg 2005, Larsson and Örstadius 2008).

Nevertheless, it has been shown that the use of ITS is not entirely unproblematic: tandem repeats, harbouring of pseudogenes in various states of decay and incomplete homogenization are all phenomena which may influence phylogenetic and divergence time estimations (Álvarez and Wendel 2003). When using ITS for these purposes, it is therefore important to compare the phylogenetic results obtained with ITS against those obtained using plastid regions. Strongly supported incongruences and/or multiple PCR bands may be indicative of incomplete lineage sorting and/or hybridization.

Phylogenetic inference

Phylogenetic trees were reconstructed using both maximum parsimony as implemented in TNT (Goloboff et al. 2000) and PAUP* (Swofford 2002) as well as Bayesian inference of phylogeny as implemented in MrBayes (Huelsenbeck and Ronquist 2001).

When MrBayes was used, MrModelTest 2.2 (Nylander 2004) was employed to choose the best evolutionary model for each sequence region. Following the recommendation some works (e.g., Posada and Buckley 2004), the evolutionary models chosen by the Akaike Information Criterion implemented in MrModelTest were incorporated in the input file to MrBayes. The software Tracer (Drummond et al. 2006) was used to determine when the tree parameters stabilized. Jackknife support values (Farris et al. 1996) were estimated in PAUP* under the maximum parsimony criterion.

Incongruence among gene partitions was assessed by means of the Incongruence Length Difference (ILD) test (Farris et al. 1994) using PAUP* and by comparing the topologies obtained using each sequence region separately.

Age estimations

Ever since Zuckerkandl and Pauling (1965) suggested a correlation between the number of genetic mutations in DNA molecules and elapsed time, there has been a large interest in using molecular phylogenies to date the origin and diversification of organisms.

In recent years, molecular dating methods have advanced from a strict molecular clock to a more “relaxed” clock approach that allows mutation rates to vary within a phylogeny, thus providing more realistic estimates of divergence times (Sanderson 2002; Thorne and Kishino 2002; Drummond et al. 2006).

Several works have reviewed these methods and compared their performance (e.g., Linder et al. 2005; Renner 2005; Ricklefs 2007). In data simulations, different algorithms have been shown to perform differently well in response to problems such as incomplete taxon sampling and distance of node to calibration point, producing largely discrepant age estimates (Linder et al. 2005).

In this thesis, divergence times were estimated using Penalized Likelihood (Sanderson 2002) in **Papers IV, V** and **VI**, as well as Bayesian dating (Drummond et al. 2006) in **Paper VI.** In both cases, the effects of phylogenetic uncertainty were taken into account by independently dating a large sample of trees with varying topology. Both methods have the advantage of enabling direct calibration on one or more nodes of a phylogeny. However, a major difference is that Penalized Likelihood assumes that rates are auto-correlated (inheritable), whereas in Bayesian dating each branch is allowed to evolve at its own rate.

It may be virtually impossible to empirically identify which dating method is “best”, as the fossil record will never be sufficiently complete to allow a direct verification of the results obtained. Indeed, *absence of evidence is not evidence of absence* (G. Nelson pers. comm.), and the results from molecular dating analyses should therefore always be seen as hypothetical. In the same way as phylogenetic estimation, they represent hypotheses to be further tested and refined with the inclusion of more taxa, more calibration points and improved dating algorithms. Despite these caveats, molecular dating analyses offer unique opportunities for assessing the biogeographic history of organisms.

A commonly used exploratory tool for examining the temporal evolution of clades is the lineages through time (LTT) plot, first proposed by Nee et al. (1992). Its principle is simple: the number of lineages in a molecular chronogram (or its logarithm) is plotted as a function of time. Although this graphical method has limited statistical value in testing hypotheses (Nee et al. 1992; Paradis et al. 2004), it has been widely used in the literature for visualizing otherwise complex chronograms (see Results and Discussion for examples of LTT plots and an alternative way of constructing similar diagrams).

Analyses of extinction

One way of using LTT plots in a statistical framework is by comparing the fit of an empirical LTT curve with curves generated by a stochastic model (Rabosky 2006). Under the pure birth (Yule) model, the number of lineages increases exponentially through time with a constant speciation rate (Yule 1924). Under a general birth-death model, there is a speciation rate (b) and an extinction rate (d) parameter, and the net diversification rate ($b - d$) is constant through time (Yule 1924; Nee 2001, 2004).

To further test the effect of extinction in shaping the temporal diversification of a clade, arbitrarily defined extinction events can be modelled and incorporated in data simulations. The simulated chronograms can then be compared with chronograms generated from empirical data. A detailed discussion on the use of these methods is presented in **Paper V**. In that study, it is tested whether any of three extinction scenarios may have shaped the empirical chronogram of the Chloranthaceae: a mass extinction at the end of the Cretaceous (65.5 Ma; the K/T event), a mass extinction 35 Ma (the Late Eocene cooling event), or sporadic and less destructive extinction events taking place randomly in time.

Biogeographic reconstruction

Optimization algorithms

Biogeographic methods based on parsimony can be divided into tree-fitting methods that search for the best area cladogram for a set of trees (Brooks 1985; Page 1994; Sanmartín and Ronquist 2004) and character-optimization methods that optimize ancestral areas onto the nodes of a phylogeny (Ronquist 1997; Maddison and Maddison 1992). The latter are more appropriate for regions such as the Neotropics, with a reticulate history of connecting and disappearing barriers that would be difficult to represent in a hierarchical, branching area cladogram (Ronquist

1997; Sanmartín 2007; Wesselingh et al. in press). The two most popular character-optimization methods are Fitch Parsimony Optimization, implemented in the software Mesquite v. 2.0.1 (Maddison and Maddison 2007) and Dispersal-Vicariance analysis (Ronquist 1997), implemented in the software DIVA (Ronquist 2001).

These two methods are based on different models of character evolution. Fitch Optimization constrains ancestors to be restricted to single areas and models range evolution from ancestor to descendant as a change in character state, equivalent to dispersal between single areas. It thus favours a dispersalist explanation. In contrast, DIVA allows widespread distributions at ancestral nodes. Although the maximum number of areas can be constrained in DIVA, single-area ancestors are not allowed and widespread distributions are always divided at speciation events by vicariance. Moreover, in Fitch optimization, biogeographic changes (i.e., dispersal events from one single area to another) are optimized onto the branches subtending from speciation events as in character evolutionary models, whereas in DIVA dispersal events are optimized onto the branches leading to the vicariance speciation event, i.e., dispersal leads to vicariance but it is not directly associated with cladogenesis (Sanmartín 2007).

A problem with these two methods, and in general with most biogeographic methods is that they do not automatically incorporate phylogenetic uncertainty into biogeographic reconstructions. Ancestral areas and biogeographic events are optimized onto a single, fully bifurcated tree, when in reality phylogenetic trees are seldom fully resolved. This phylogenetic uncertainty may bias the results much more than the optimization criterion itself, as biogeographic inferences done using parsimony and maximum likelihood on the same tree usually yield similar results (Ree et al. 2005; Inda et al. 2008; but see Xiang and Thomas 2008 for a different view). One way to incorporate phylogenetic uncertainty into parsimony-based biogeographic inference is to reconstruct ancestral area distributions over a sample of trees with varying topology (Huelsenbeck and Imennov 2002; Ronquist 2003; Nylander et al. 2008).

Here, both these methods were used to infer ancestral distributions and biogeographic events: DIVA was used in **Papers IV** and **V** and Fitch parsimony in **Papers V** and **VI**. In all cases, phylogenetic uncertainty was taken into account.

Operational areas

Delimitation of operational areas in biogeographic studies usually aims at representing natural areas of endemism, but there is no general consensus on how this should be objectively done (Linder 2001; Lomolino 2005). In this project, the areas for the biogeographic analyses were defined by considering ***i)*** areas of endemism, and ***ii)*** geological history and features that may have acted as general barriers to dispersal (Hausdorf and Hennig 2003; Sanmartín 2003). In order to facilitate comparisons and create a useful framework for a wide range of future biogeograhic analyses, it was also attempted to maximize congruence with other biogeographic studies in the Neotropics (e.g., Cracraft 1988; Morrone 1994; Posadas et al. 1997; Katinas and Morrone 1999).

Based on these criteria, eight operational areas were recognized within the Neotropics (Fig. 8):

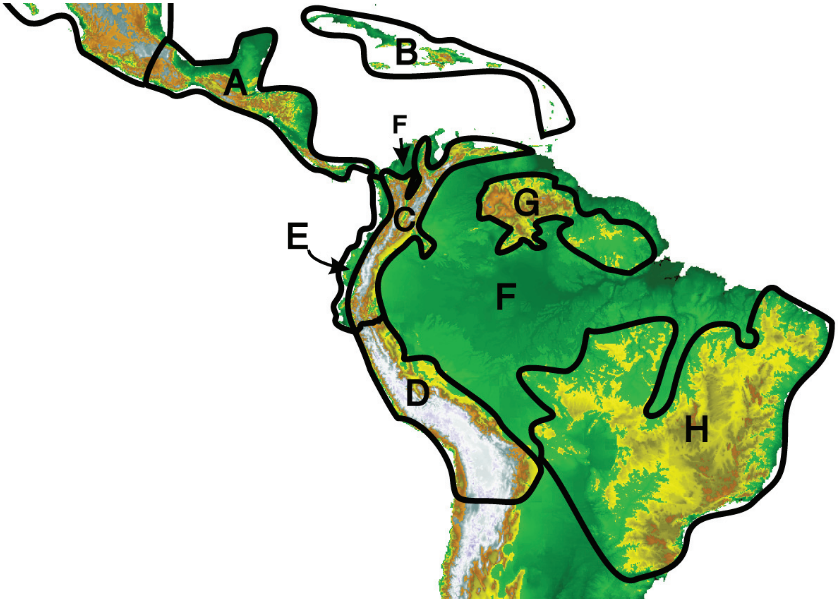


Figure 8. Operational areas used in the biogeographic analyses. A: Central America, B: West Indies, C: Northern Andes, D: Central Andes, E: Chocó, F: Amazonia, G: The Guiana Shield, H: Southeastern South America. Map from the National Geophysical Data Center (http://www.ngdc.noaa.gov).

A – Central America**,** ranging from southern Mexico (Veracruz, Oaxaca, Tabasco, Campeche, Yucatán and Quintana Roo) south to Panama. Although this region has a complex geological history and its land and island connections to South America are still prone to discussion, Central America was long isolated from South America until the uplift of the Panama Isthmus 3.5 Ma (Taylor 1991; Briggs 1994).

B – West Indies, excluding Trinidad and Tobago, which are geologically and biologically more related to South America than to the other Caribbean islands.

C – Northern Andes (10° N – 5° S), ranging from Venezuela and Colombia to northernmost Peru (Piura, Cajamarca and Amazonas), from elevations higher than 500 m. This area is roughly the same as the Páramo recognized in other biogeographic studies (e.g., Morrone 1994; Posadas et al. 1997; Katinas and Morrone 1999), except that occurrences in this area have been arbitrarily coded beginning at altitudes lower than the ones generally adopted.

D – Central Andes (5° S – 18° S), ranging from Peru (San Martín and La Libertad) southwards to the Tropic of Capricorn, from elevations higher than 500 m. This area corresponds roughly to the commonly recognized Puna or Altiplano (e.g., Morrone 1994; Posadas et al. 1997; Katinas and Morrone 1999).

E – The Chocó area, comprising areas west of the Andes and below 500 m in Colombia (Chocó, El Valle, Cauca and Nariño), Ecuador and Peru (Tumbes, Piura). This area is usually recognized by bird biogeographers as a centre of endemism (e.g., Cracraft 1988; Brumfield and Capparella 1996).

F – Amazonia, comprising lowland areas (< 500 m) in Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela, Guyana, Suriname and French Guiana, and including the islands immediately off the South American coast.

G – The Guiana Shield, including the elevated (> 500 m) areas in northeastern South America, parts of Venezuela, Guyana, Suriname, French Guiana and Brazil. It corresponds to the Guianan Bedrock region (Donato 2006).

H – Southeastern South America, mostly comprising the Brazilian Shield, but also including the lowlands in eastern Brazil and the Rio Paraná drainage. This area corresponds to the pre-Cambrian Brazilian Bedrock formation (Donato 2006).

Results and Discussion

Phylogenetic and taxonomic implications

The inferred relationships among the plant species studied in this thesis demonstrate the need of amendments to the classification of all groups. Moreover, several previously unknown or poorly supported relationships receive phylogenetic support in the current analyses.

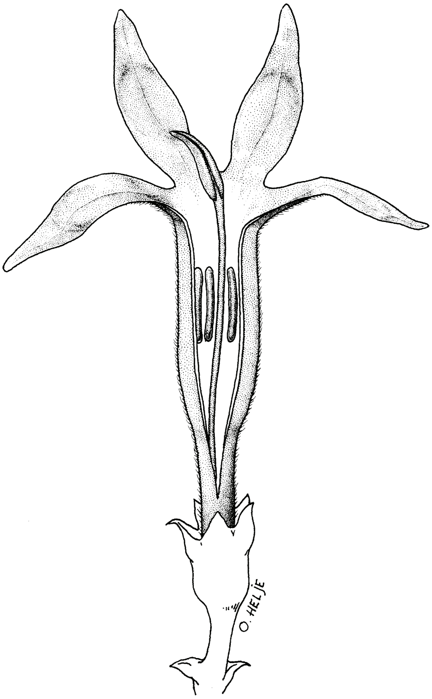


Figure 9. Ciliosemina A. Antonelli, dissected flower. Artwork: O. Helje.

Rubiaceae. Papers I and **IV** show that tribe Naucleeae is probably the sister group of a clade comprising all other taxa in subfamily Cinchonoideae. Within this subfamily, the Neotropical tribes Cinchoneae and Isertieae are monophyletic and strongly supported as sister groups.

In **Paper I**, it is argued that the genus *Remijia* is not monophyletic with the inclusion of *R. pedunculata.* Constraining *Remijia* to be monophyletic results in trees that are 5 steps longer than the most parsimonious trees obtained. The non-monophyly of *Remijia* as traditionally circumscribed is further supported by the Bayesian analysis conducted in **Paper IV**, where *R. pedunculata* appears as sister to genus *Ladenbergia* with 0.71 Bayesian posterior probability. Although none of these analyses provide strong support for the phylogenetic placement of *Remijia pedunculata* within tribe Cinchoneae, they both indicate that this species is best treated in a separate genus.

These molecular-based results are in good agreement with the observation that *Remijia pedunculata,* and the morphologically very similar *R. purdieana,* are conspicuously distinct from all other *Remijia* species. Given these considerations, the new genus *Ciliosemina* A. Antonelli (Fig. 9) is proposed in **Paper I** to accommodate these two species.

Campanulaceae. In **Paper II**, the phylogenetic analyses of subfamily Lobelioideae show the cosmopolitan genus *Lobelia*, traditionally circumscribed to include over 400 species, to be clearly polyphyletic. The single morphological character used to distinguish *Lobelia* from all other lobelioid genera is a corolla tube cleft dorsally to the base. However, an optimization of this character state on ancestral nodes of the Lobelioideae phylogeny indicates that this represents a plesiomorphic condition in the subfamily, thus not reflecting evolutionary relationships within the group.

It is shown for the first time that the Neotropical shrubs *Centropogon, Burmeistera* and *Siphocampylus* are sister to the tiny Andean endemic *Lysipomia*. Each of the genera *Centropogon, Siphocampylus* and the Hawaiian endemic *Cyanea* appear as non-monophyletic in the parsimony and Bayesian analyses. The non-monophyly of *Centropogon* and *Siphocampylus* is further supported by a morphological optimization of fruit types on ancestral nodes, revealing that the character used for discerning between these genera (capsules vs. berries) is highly homoplasious in the subfamily. Nevertheless, a SH test (Shimodaira and Hasegawa 1999) indicates that constraining these genera to form monophyletic clades prior to running a Bayesian analysis produces trees that are not significantly less likely than the unconstrained Bayesian tree. The monophyly of these genera should therefore be further tested by the addition of more taxa and faster evolving sequence regions.

A very unexpected result from **Paper II** is that the giant lobelioids in genera *Lobelia, Trematolobelia, Brighamia, Delissea, Cyanea* and *Clermontia* form a clade, strongly supported in all phylogenetic analyses. The evolutionary implications of this relationship are discussed in detail in **Paper VI**. It is shown that the giant habit in these genera, long thought to be the result of convergent evolution, has in fact only evolved once in a shared African ancestor. The current worldwide distribution of the clade (which includes the Hawaiian islands, southeastern Brazil, tropical Africa and Sri Lanka) is inferred to have been attained through transoceanic dispersals in the last 30 Ma.

These results demonstrate that the Lobelioideae are in strong need of a taxonomic revision. Unfortunately, the low number of species analyzed (69 out of 1200+), combined with the extreme morphological variation shown by these species (see **Papers II** and **VI** for discussion and illustrations), testifies to the huge molecular and morphological work required to achieve a complete and stable classification. Awaiting such efforts from the scientific community, no taxonomic rearrangements are proposed here.

Chloranthaceae. The phylogenetic analyses performed in **Paper V** show the genus *Hedyosmum* to be monophyletic with strong support, but the two sections in the genus containing more than one species (sections Microcarpa and Macrocarpa) need to be recircumscribed in order to become monophyletic. The same applies to the subgenus *Hedyosmum*, which is paraphyletic as currently circumscribed. Section Artocarpoides (containing a single species, *H. mexicanum*) is strongly nested within a clade representing section Macrocarpa. Since Macrocarpa was proposed earlier than Artocarpoides (Todzia 1988), this means that *H. mexicanum* should be transferred to Macrocarpa and the name Artocarpoides abandoned.

Before a new classification of the genus is proposed, it would be desirable to sequence a few additional species in order to ascertain their phylogenetic position (see **Paper V** for an expanded discussion). This work is currently in progress (H. Kong et al. in prep.).

Climatic fluctuations, biotic interchanges, or rise of the Andes?

As outlined earlier, several models of speciation have been proposed for the Neotropics, and one way of testing them is by estimating the absolute time of diversification in various groups of organisms.

Figure 10 shows lineages through time (LTT) plots for a relatively large (N = 55) sample of molecular chronograms (Antonelli et al. in prep.).

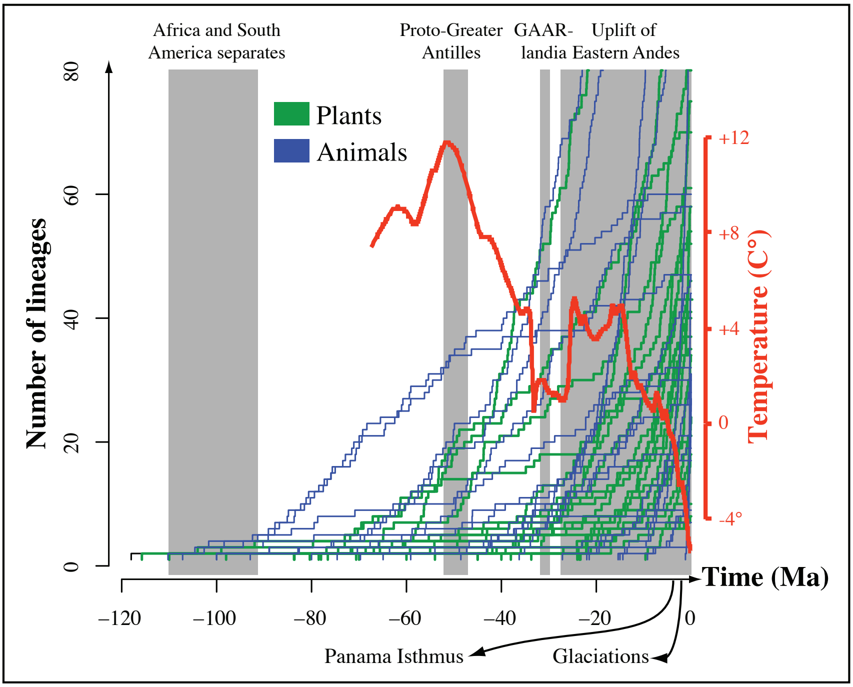


Figure 10. Temporal evolution of plant and animal groups containing Neotropical lineages. The graph shows lineages through time (LTT) plots for 22 plant and 23 animal groups, gathered from this and other studies (Antonelli et al. in prep.). The shaded boxes represent the approximate duration of main geological and geographic events suggested to have fostered the dispersal and radiation of Neotropical organisms. GAARlandia: Greater Antilles and Aves Ridge. Global temperature curve from Zachos et al. (2001).

As with any LTT plot, the curves shown in Fig. 10 invariably increase with elapsed time, despite the varying number of extant species and crown ages (see below for an alternative idea on how to construct similar graphs). However, each curve behaves differently in terms of when and how fast lineage splits took place, as revealed by their shape (steep slopes, flattened curves, etc).

This graph alone goes a long way in addressing Gentry's famous question on whether present-day Neotropical floristic diversity is a result of floristic "inheritance" from Gondwana, phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or a product of the Andean orogeny (Gentry 1982).

Indeed, a visual inspection of Fig. 10 makes evident that at least two of these hypotheses could be readily discarded: ***i)*** prior to the separation of Africa and South America, very few lineages occurring today in the Neotropics existed. This suggests that the biotic inheritance from Gondwanaland contributed minimally to attain the present levels of Neotropical diversity; and ***ii)*** the onset of climatic changes which led to the establishment of glaciations in the Late Pliocene and Pleistocene (~2 Ma; Gates 1993) occurred when most lineages splits had already taken place.

As concluded by Rull (2008), the long-held idea that Pleistocene climate changes accounted for most of present-day species richness in the Neotropics no longer holds. This applies both to the refuge theory (Haffer 1969) and the disturbance-vicariance hypothesis (Colinvaux 1993; Bush 1994).

Biotic interchanges

Most biotic migration between North and South America probably took place after the formation of the Panama Isthmus ~3.5 Ma (Briggs 1994; Taylor 1991; Stehli and Webb 1985). However, limited biotic interchange between these landmasses may have been facilitated earlier by the proto-Greater Antilles in the Early Eocene (~50 Ma) and by the Greater Antilles and the Avies Ridge (GAARlandia) around the Eocene/Oligocene boundary (33–35 Ma; Iturralde-Vinent and MacPhee 1999). Plants and animals adapted to short-distance dispersal could have used those islands as "stepping stones". Indeed, this pathway has been suggested for several plant families, such as Malpighiaceae (Davis et al. 2002), Annonaceae (Pirie et al. 2006; Richardson et al. 2004), Valerianaceae (Bell and Donoghue 2005), Adoxaceae (Winkworth and Donoghue 2005), and is suggested here for Rubiaceae (**Paper IV**). It has also been suggested for a wide variety of tetrapod lineages (see **Paper III** and references therein). Groups that inhabited the tropical vegetation present in southern Laurasia during the warm periods of the

Eocene are commonly called *boreotropical* elements (Lavin and Luckow 1993).

These studies provide substantial evidence that plants and animals migrated into South America from the north, but this does not necessarily mean that the overall species numbers in South America increased. The migration of North American species into South America may have led to the extinction of certain South American species, thus reducing the net increase of species in South America. This has been widely documented by the fossil record of mammals, which shows that the invasion of North American mammals led to the extinction of many South American mammal species (Stehli and Webb 1985). Although the reasons for this extinction are still a matter of debate, some of the hypotheses proposed (competition, lower fitness, or pathogens; Ferigolo 1999; Stehli and Webb 1985) could apply to other animal and perhaps even plant taxa.

Indeed, for most LTT curves in Fig. 10 there is no clear pattern of accelerated diversification following the three biotic corridors proposed between North and South America (the Proto-Greater Antilles, GAARlandia and Panama Isthmus). However, it is possible that the few chronograms that do seem to show a sudden increase in lineage accumulation in connection with the establishment of these corridors did so as a consequence of habitat expansion (e.g., lowland taxa adapting to montane and pre-montane Andean areas; Hughes and Eastwood 2007) or other ecological shifts (e.g., the occupation of unexplored niches; Verola et al. 2007). So even if biotic interchange between North and South America did not directly foster diversification, it certainly contributed to Neotropical biodiversity by enabling the establishment of lineages that later radiated in South America.

Climate vs. geology

Looking again at Fig. 10, it is easy to understand why most molecular dating studies of animals and plants have evoked the Andean uplift as a possible candidate for triggering Neotropical diversification (e.g., von Hagen and Kadereit 2003; Burns and Naoki 2004; Wiens 2004; Bell and Donoghue 2005; Hughes and Eastwood 2006; Moore et al. 2006; Pirie et al. 2006; Roberts et al. 2006; Weir 2006; Brumfield et al. 2007; Brumfield and Edwards 2007; Gamble et al. 2008; Patterson and Velazco 2008). However, many such suggested correlations may have been hastened, as it is difficult to confidently discern between the influence of the Andean uplift and other synchronous palaeogeological and palaeoclimatic events (e.g., climatic fluctuations; see temperature curve in Fig. 10). Therefore a formal and combined inference of the spatial *and* temporal evolution of each clade of interest may be essential for disentangling the role of each of these potential factors underlying diversification.

Another practical issue while seeking for correlates of diversification derives from the fact that many groups have wide distributions, occurring outside the actual area of interest. In a cosmopolitan plant family such as the Rubiaceae, plotting LTT curves for the whole molecular chronogram (i.e. also palaeotropical clades) will inevitably obscure a pattern caused by an event taking place only in South America, such as the Andean orogeny. Following this reasoning, even if diversification in Rubiaceae were to be directly linked to global climatic changes, the only way of discerning between geological and climatologic correlates of diversification in South America may be to restrict the LTT curves to lineage splits occurring in that area.

To further illustrate this problem, a narrower LTT analysis was conducted in the following way. *First*, the main Neotropical clades in families Rubiaceae, Chloranthaceae and Campanulaceae whose most recent common ancestors (MRCAs) have been inferred to occur in South America were identified, and their estimated crown ages and ancestral areas compiled from **Papers I** – **VI** (Table 2). In the case of the Campanulaceae, the MRCA of the clade comprising *Lysipomia, Siphocampylus, Burmeistera* and *Centropogon* (the "LSBC" clade) was reconstructed only as the "Neotropics", as the lack of internal resolution in the group precluded the use of smaller operational areas in the biogeographic analysis. However, the great majority of species in those genera occur in the Andes (Lammers 1998b, 2007), suggesting that most diversification took place there. *Second*, all species not belonging to those major clades were manually pruned from the original molecular chronograms, while keeping the remaining branch lengths unaltered. *Finally*, LTT curves were generated for these clades and plotted against the two synchronous events that were most likely to promote diversification in South America: temperature fluctuations and the Andean uplift (Fig. 11). Unfortunately, there is no temperature curve for South America readily available, so the curve depicted in Fig. 11 represents global mean values. As global climatic changes have largely influenced tropical vegetation at a regional level (Morley 2000), there should be a tight correlation between global and regional temperature variations.

Table 2. Estimated time and geographic origin of major Neotropical clades examined in this project. Clades marked with (*) are plotted in Fig. 11.

Clade	**Species sampled**	**Estimated total**	**Range of MRCA**	**Crown group age [95% CI]**
RUBIACEAE: Subfamily Cinchonoideae (**Papers I** and **IV**)				
Tribe Cinchoneae	22	118	Northern Andes	28.6 [22.9–35.1]
Tribe Isertieae	9	15	Lowland Amazonia	25.0 [19.9–31.4]
*Tribes Cinchoneae + Isertieae	31	133	Northern Andes + Lowland Amazonia	45.2 [12.0–18.9]
CHLORANTHACEAE: *Hedyosmum* (**Paper V**)				
*Subgen. *Tafalla* (-*H. arborescens*)	18	37	Northern Andes[1]	15.2 [9.99–26.6]
CAMPANULACEAE: Subfamily Lobelioideae (**Papers II** and **VI**)				
**Lysipomia, Siphocampylus, Burmeistera, Centropogon*	16	580	Neotropics	37.7 [30.7–44.1
Brazilian giant *Lobelia*	2	10	Neotropics	7.89 [4.91–11.5]

[1] The Northern Andes were inferred to have been the only ancestral area of this clade in the Fitch optimizations, whereas the DIVA-Bayes results indicate that this ancestor also reached the Central Andes and Central America.

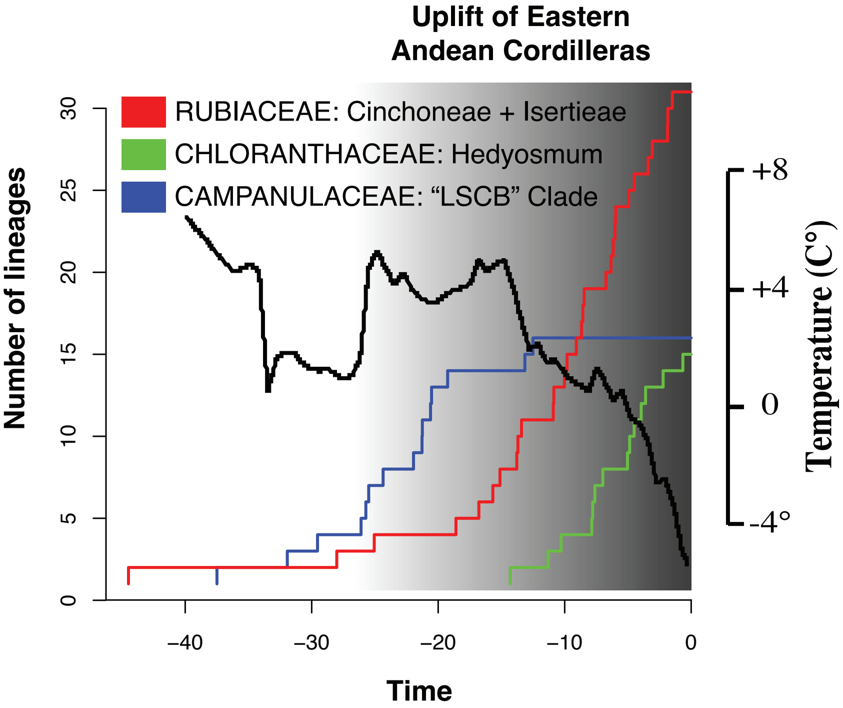


Figure 11. Temporal evolution (LTT plots) of the main clades of South American species studied in this project, in relation to global temperature fluctuations (black curve) and the Andean uplift. Mean ages used; all dating estimations performed using Penalized Likelihood.

No methodology has yet been developed to statistically test the two competing hypotheses of diversification outlined in Fig. 11 (climate changes vs. Andean uplift). Yet, it seems clear for the clades depicted here that ***i)*** diversification does not seem to consistently follow the temperature curve. For instance, no effects on the LTT curves of Rubiaceae and Campanulaceae can be seen in connection with the sudden temperature decrease some 33 Ma; ***ii)*** most diversification occurred during the uplift of the Eastern Andean Cordilleras.

Besides this temporal evaluation of competing hypotheses of diversification, it is important to consider *where* most divergence in these groups took place, as inferred under biogeographic analyses. As outlined in Table 2, at least in the case of tribe Cinchoneae (Rubiaceae) and *Hedyosmum* subgenus *Tafalla* (Chloranthaceae), and presumably even in the case of the “LSBC” clade (see discussion above), nearly all diversification events were inferred to have taken place in the Northern Andes.

Combined, these spatial and temporal results suggest a causal connection between the uplift of the Northern Andes and diversification in the groups studied. The role of global temperature fluctuations in fostering diversification in these groups appears, therefore, to have been minimal.

A highway to the south

From the Eocene to the Middle Miocene (~40 – 12 Ma), episodic marine incursions from the Pacific have been suggested to have penetrated a lowland corridor separating the Northern from the Central Andes at the latitude of southern Ecuador/northern Peru (~3–5° S), sometimes referred to as the "Western Andean Portal" (Fig. 12–I; Hoorn 1993; Hoorn et al. 1995; Steinmann et al. 1999; Hungerbühler et al. 2002; Santos et al. 2008). Many other terms appear in the literature, which all seem to refer to the same area: Huancabamba Depression, Huancabamba Deflexion, Marañón Portal, Guayaquil Gap, Northern Peruvian Low and Pirua Divide (Weigend 2004). The marine incursions are suggested to have ended in connection with the uplift of the Eastern Cordilleras of the Central and Northern Andes from the Middle Miocene onwards (13–11 Ma; Taylor 1991; Hoorn et al. 1995; Hungerbühler et al. 2002), allowing the Northern and Central Andes to be permanently connected by highlands (Hoorn 1993). This presumably created a new dispersal route between the Northern and Central Andes for a variety of organisms adapted to montane conditions.

The biogeographic reconstruction of the Rubiaceae (**Paper IV**) corroborates the existence of a dispersal barrier between the Northern and the Central Andes coincident with the Western Andean Portal ("WAP"), and indicates the persistence of this barrier until the Middle Miocene. The end of the WAP is then observed as at least five independent migrations from the Northern to the Central Andes within the genera *Cinchona* and *Ladenbergia* (Fig. 1 in **Paper IV**). All four events are dated as occurring around the Middle/Late Miocene (12-10 Ma), almost simultaneously with the suggested uplift of the WAP based on geological data (Taylor 1991; Hoorn 1993; Hoorn et al. 1995; Hungerbühler et al. 2002).

Similarly, the biogeographic reconstruction of the Chloranthaceae (**Paper V**) indicates that there may have been up to nine colonization events from the Northern to the Central Andes within *Hedyosmum* (Fig. 3A in **Paper V**). However, these dispersals are inferred to have started more recently than in the Rubiaceae. The mean age of the first diversification leading to a dispersal across the WAP (the split between *H. angustifolium* and *H. scabrum*) is estimated to 5.05 Ma, with 95% confidence intervals ranging between 2.16 Ma and 11.5 Ma (**Paper V**). When compared to the Rubiaceae, this temporal difference (most notable for mean age estimates, but minimal when considering confidence intervals) could have a biological explanation, such as the time it took until suitable habitats were formed in the uplifted WAP for the colonization of *Hedyosmum* ancestors. On the other hand, it could also be the result of an underestimation of the lineage splits in the Chloranthaceae (e.g., due to the use of too young fossils for calibrating the phylogeny).

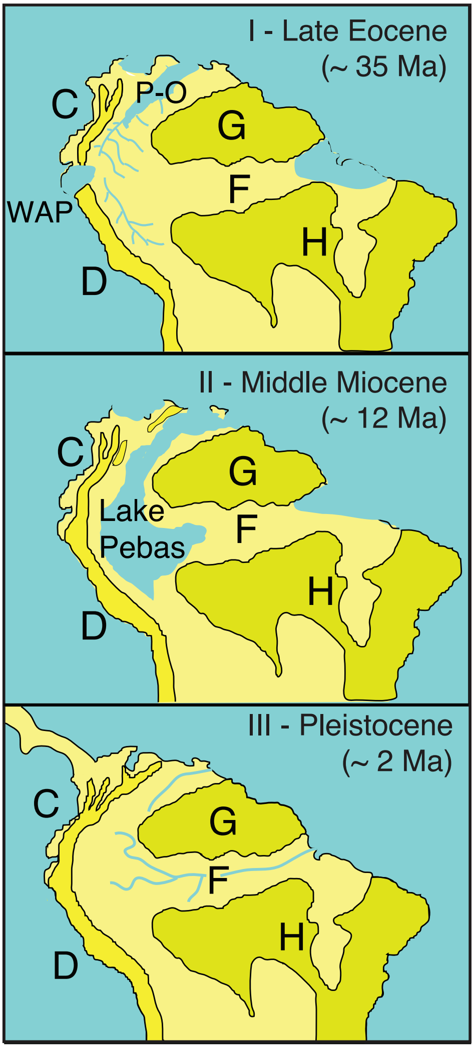


Figure 12. Simplified geophysical and hydrological history of South America in the last 35 Ma (adapted from **Paper IV**). **I**: A northwards flowing fluvial system (the Subandean River System or palaeo-Orinoco, "P-O") dominates the drainage of western Amazonia and the foreland Andean basins. A lowland corridor invaded by marine settings (the Western Andean Portal, WAP) blocks most biotic dispersal between the Northern and Central Andes. **II:** Lake Pebas covers large parts of western Amazonia. The WAP is uplifted, and a "highway to the south" established for boreotropical elements. **III:** Lake Pebas is completely drained, and the dispersal of terrestrial organisms can take place across most of South America. Area codings as in Fig. 8. Light yellow, lowlands; dark yellow, highlands; blue, aquatic settings.

A third group that appears to have been affected by the WAP is the genus *Gunnera* (Gunneraceae). The genus is mainly distributed in the southern Hemisphere, with about 44 species occurring in Central and South America (Mora-Osejo et al. in prep.). A biogeographic analysis of the family (Antonelli et al. in prep.) clearly corroborates the role of the WAP as a dispersal barrier. But contrary to the Rubiaceae and the Chloranthaceae, *Gunnera* ancestors are inferred to have used the uplifted WAP for dispersing northwards, from the Central to the Northern Andes.

These results demonstrate for the first time how and when the WAP influenced the distribution of three modern groups of plants. In addition, if the patterns shown by the Rubiaceae and the Chloranthaceae are proven to be general, this would suggest that many boreotropical elements could not disperse further southwards immediately after their arrival in northwestern South America. These taxa may have been confined to the Northern Andes for several million years, until a "highway to the south" was established by the uplift of the WAP. This scenario could be further tested by reconstructing the spatiotemporal evolution of groups that today exhibit a disjunction between the Northern and Central Andes (see below for examples).

Despite the evident role of the WAP in shaping present-day Andean distributions, several questions remain unanswered concerning its nature, geographic extent and duration (Lundberg et al. 1998; Wesselingh et al. in press; see supplementary information in **Paper IV** for a thorough discussion on the various ideas proposed).

Formation of the Gentry pattern

To date, no studies have attempted to explain the formation of the striking distributional pattern between Amazonian-centred and Andean-centred groups, shown by some 70% of all Neotropical plant species (Gentry 1982; Figs. 5 and 6) – called here the Gentry pattern.

Gentry's own idea was that this pattern arose by differentiation from an originally more uniform pattern, and that the uniform pattern was a remnant of a more or less continuous West Gondwanan rain forest flora. Based on these premises, species richness of Andean-centred groups in northwestern South America was explained as a consequence of evolutionary radiation in certain lineages in response to environmental changes caused by the Andean orogeny. Gentry did not discuss at all how or when a diversity centre would have been formed in the central Amazon basin.

Distributional disjunctions of organisms are commonly formed by the influence of a geographic barrier, but no such barrier exists today between the Andes and the Amazon Basin. It would therefore be natural to assume that ecophysiological constraints, such as those necessary for surviving the harsh montane climates, acted instead as barriers for lowland organisms, at the same time as taxa already adapted to montane habitats were unable to compete with lowland-adapted taxa at lower altitudes. However, as emphasized by Gentry (1982), while the representation of Andean-centred taxa is highest in the Andes, most species occur along the base and slopes of mountains, rather than at high altitudes. It seems therefore plausible to assume that, if no geographic barrier existed between the Andes and Amazonia, biotic interchange between these two areas would have taken place continuously, which should in turn have promoted speciation through gradual adaptation to slightly different altitudes (Smith et al. 1997). Several examples of this scenario have been documented in the Neotropics, for plants and animals (e.g., Eriksson 1993; Mallet 1993; Colinvaux et al. 1996). However, if this was the case for most taxa, no clear geographic pattern should be observed today separating Andean-centred and Amazonian-centred groups.

The solution to this problem may need to be sought in the past. From the time South America began separating from Africa in the Cretaceous until the end of the Oligocene (c. 112 – 24 Ma), a fluvial system referred to as the palaeo-Orinoco dominated the drainage of northwestern Amazonia and the foreland Andean basins towards Lake Maracaibo, on the Caribbean coast (Wesselingh and Hoorn in press; Fig 12–I). Then, in the Early Miocene (c. 23 Ma), geotectonic changes in the Amazon Basin associated with the ongoing uplift of the Eastern Cordillera in the Central Andes caused western Amazonia to gradually become submerged, from south to north and from west to east (Wesselingh and Hoorn in press). This process created a huge (> 1 million km^2) system of long-lived lakes and wetlands between at least 17 to 11 Ma, known as "Lake Pebas" or the "Pebas Sea" (Lundberg et al. 1998; Wesselingh et al. 2002; Wesselingh 2006; Wesselingh and Salo 2006; Wesselingh et al. in press), connected to Caribbean marine realm in the north (Fig. 12–II). From the Late Miocene onwards (11–7 Ma), there was a new period of rapid mountain uplift, affecting mainly the Eastern Andean Cordilleras (Irving 1975; Taylor 1991). This presumably caused the areas west of the Guiana Shield to emerge, which closed the Caribbean connection of the palaeo-Orinoco, shifted the drainage of the Amazon Basin eastwards and lead to the demise of Lake Pebas (Wesselingh and Salo 2006;

Wesselingh and Hoorn in press; Wesselingh et al. in press) (Fig. 12–III). However, aquatic conditions seem to have persisted in western-central Amazonia until at least 7 Ma, when the modern Amazon system came into place (Wesselingh et al. in press).

Remarkably, there is an almost perfect match between the hydrological scenario outlined here (Fig. 12) and the distributional patterns depicted in Figs. 5 and 6. It seems clear that the "missing" barrier needed for creating the split between Andean-centred and Amazonian-centred groups could have been constituted by Lake Pebas and (although less effectively) its predecessor, the palaeo-Orinoco. The inference (Figs. 10 and 11) that most Neotropical diversification has taken place concomitantly with the existence of these aquatic settings in western Amazonia further corroborates this idea, as well as the biogeographic reconstruction presented for the Rubiaceae (**Paper IV**). In that analysis, the reestablishment of land connections between the Andes and Amazonia is evidenced by at least seven independent colonization events in Cinchoneae and Isertieae from the Late Miocene onwards.

According to the biogeographic reconstructions of the Rubiaceae and the Chloranthaceae (**Papers IV** and **V**), regions outside the 'core' areas of the Gentry pattern (i.e., the Andes and Amazonia) have been colonized several times and from different regions. Most notably, these inferences indicate that many widespread distributions of extant species have been attained recently, especially during the Pliocene and Pleistocene (~5 Ma until today). Dispersals in this period were presumably facilitated by the prevailing terrestrial settings, since Lake Pebas had already drained and the Amazon river system had established its present course (Wesselingh and Hoorn in press). Moreover, it could be hypothesized that if the forest cover in Amazonia successively contracted and expanded during the Pleistocene as suggested by Haffer (1969), this may have led to an increase in the dispersal ability of many species by means of natural selection (Darwin 1859). For instance, species that randomly developed lighter or winged seeds would have enhanced chances of escaping aridification, becoming at the same time more prone to attain wide distributions.

Amazonia. The palaeogeographic scenario described here concerning the development of wetlands in the Amazon Basin implies that all plant species adapted to dry land conditions (*terra firme*) in present-day western Amazonia have gained their current distribution after the draining of Lake Pebas (i.e., in the last ~11 Ma; Wesselingh and Hoorn in press). Similarly, *in situ* speciation in those terrestrial groups could not have taken place prior to that event.

These results may seem controversial given the fact that western Amazonia is characterized today by an outstanding number of endemic plant species, of which many belong to long-lived woody families not expected to speciate fast. In a recent study using large molecular chronograms from five angiosperm families, Smith and Donoghue (2008) showed that there is a strong correlation between growth mode and speciation rates: herbs generally diversify much more rapidly than shrubs and trees. However, the few molecular dating studies that have dealt with a representative number of Amazonian trees have presented very contrasting results. Two of the largest genera of Neotropical trees have been inferred to have diversified recently: *Inga* (Fabaceae, 300 spp), which is estimated to have started its diversification either 9.8 Ma (based on cpDNA) or 1.6 Ma (based on nrDNA; Richardson et al. 2001); and *Guatteria* (Annonaceae, 265 spp), where the crown age for all Neotropical taxa (including two large Amazonian subclades) is estimated at 7.4 ± 1.4 Ma (Erkens et al. 2007). These results fit well into the palaeogeographic scenario for Amazonia described here, and suggest that Amazonian trees may have evolved at an unusual rate as compared to other angiosperms (Smith and Donoghue 2008) and many other Neotropical plants and animals (Figs. 10 and 11).

Amazonia has been traditionally seen as a homogeneous and stable environment since the separation of South America from Africa, where species could diversify "undisturbed" (e.g., Raven and Axelrod 1974). The results presented here, however, highlight the importance of discerning between the geological, hydrological and biotic meanings of the word "stability". Although areas of lowland tropical forest have existed continuously in northern South America ever since the Palaeocene or Early Eocene (~56 Ma; Burnham and Johnson 2004), the Amazonian region itself has had a highly dynamic history of wetland systems and mountain uplift in the areas presently occupied by the Andes.

WAP/Huancabamba. Many studies have recognized the Western Andean Portal or Huancabamba Depression as an important biogeographic barrier separating the Northern and Central Andes. In plants, this barrier has been demonstrated to separate species groups in families such as Campanulaceae (Ayers 1999), Calceolariaceae (Molau 1988), Tropaeolaceae (Sparre and Andersson 1991), Loasaceae, Passifloraceae, Grossulariaceae (Weigend 2002, 2004), Clusiaceae (M.H.Gustafsson, pers. comm.) and Alstroemeriaceae (Hofreiter and Rodríguez 2006). In animals, Vuilleumier (1968) showed such a disjunction for populations of the bird superspecies *Asthenes flammulata* (Furnariidae), Nores (2004) defined the region north of the WAP (the northwestern Andes) as a general area of bird endemism, and Cortés-Ortiz (2003) defined the WAP as the southern limit of the monkey *Alouatta palliata*. Moreover, this region (the Río Marañon in Peru) has long been recognized as the turnover point between the Northern and Central Andean regions of bird endemism (Stotz et al. 1996).

However, other studies have called into question the role of the region as a barrier, arguing instead that the Huancabamba–Amotape zone (between 3°S and 7°S; Weigend 2004) should be primarily regarded as a region of high endemicity. For plants, a high endemism level in the area has been demonstrated for families Onagraceae, Solanaceae, Asteraceae, Loasaceae and Alstroemeriaceae (Berry 1982; Young and Reynel 1997; Hensold 1999; Weigend 2002, 2004). For the *Passiflora lobbii* complex, Grossulariaceae and Loasaceae, it has been shown that levels of diversity are six to eight times higher there than in the Central and Northern Andes (Weigend 2004). Several animal groups exhibit the same pattern, such as birds, lizards and frogs (see Weigend et al. 2005 and references therein).

The solution to this controversy may be sought in the history of the area. While the WAP was a lowland corridor episodically invaded by sea (Fig. 12–I), it acted as a dispersal barrier for montane taxa, which lead to the formation of endemism centres both north and south of the barrier. When the WAP uplifted and the lowland corridor disappeared (~13–11 Ma; Taylor 1991; Hoorn et al. 1995; Hungerbühler et al. 2002), the zone became available for biotic colonization and speciation (although environmental constraints, such as varying precipitation levels in different regions of the valley, may have inhibited the dispersal and colonization of certain organisms for longer periods).

A unified model. There are several parallels between western Amazonia and the Huancabamba region. For example, both regions are today characterized by remarkably high numbers of species, many of which are endemic to each region; both were episodically covered by water during millions of years, and eventually drained at about the same time; both are located at about the same latitude. Together, these two regions may account for a significant percentage of the total number of Neotropical plant species. It seems therefore plausible to assume that the drainage of these areas was directly linked to subsequent bursts of speciation.

The observation by Weigend (2004) that the characteristic species and genera from the Huancabamba zone *"form close ties both along the Andes (north – south) and across the Andes (east – west)"* supports the idea that immigrant taxa arrived from various sides. If so, speciation could have taken place in two ways: ***i)*** by peripatric speciation of small "founder populations" – new colonies being established by the dispersal of one to a few individuals of a species into a new area (Templeton 1980). By chance, the new population may be genetically and morphologically distinct from the parental population, evolving into a different species. Eventually, the great heterogeneity of soils in western Amazonia (Salo et al. 1986) and the great variety of habitats in the Huancabamba region (Weigend 2002) induced some of these founder populations to undergo *adaptive radiation* in connection with the uptake of new niches (e.g., Seehausen 2004); ***ii)*** by sympatric speciation via hybridization and polyploidization. This would have been possible if populations of a common ancestral species had been long separated by water, during which time they differentiated but did not develop barriers to interbreeding. When these populations came in contact after the draining, hybrid species were formed. Numerous studies have demonstrated the role of this "secondary contact" model (Stebbins 1984, 1985) in promoting speciation in the Northern Hemisphere of both plants (e.g., Comes and Kadereit 1998; Abbot and Brochmann 2003) and animals (e.g., Avise and Walker 1988; Hansson et al. 2008), but so far they have only focused on Pleistocene glaciation dynamics.

Two additional considerations may be evoked to explain the exceedingly high number of species in Western Amazonia and the Huancabamba region, via the speciation mechanisms described above:

First, the relatively rapid draining of these areas could be likened to the rising of oceanic islands such as those being constantly formed in the Hawaiian archipelago (Clague 1996). The theory of island biogeography (MacArthur and Wilson 1963) postulates that species numbers on islands vary as a function of island area, immigration rates (directly associated with distance from the nearest land mass) and extinction rates. Following this analogy, an unusually high number of species is just what should be expected for these regions: both constitute very large "islands"; immigration rates were probably very high, considering the direct connection with the surrounding species-rich biotas; and extinction rates for many groups were presumably lower there than it would have been in already well-developed ecosystems, where fierce competition minimizes new colonisations for most taxa (Salo et al. 1986).

Second, it seems reasonable to assume that the draining of these areas did not occur only once, but repeatedly over a long period of time. In both regions, the water level must have followed global sea level fluctuations, which have varied greatly both in the long and short terms (Fig. 13; Miller et al. 2005; Müller et al. 2008). If species formed during each draining period survived subsequent phases of water refilling, species numbers in these two regions should steadily increase. In western Amazonia, ice-melting in the Andes probably caused seasonal floods as well, which must have increased the disturbance of the area (e.g., following years of unusually high snow accumulation) but were of insufficient duration for promoting speciation.

The two speciation mechanisms suggested here, founder populations/adaptive radiation and secondary contact, predict that species numbers and ploidy levels should increase near a historical water line and decrease at higher and lower altitudes. For western Amazonia, this prediction finds some support in the observation from several studies that more elevated areas in the lowland forest (usually termed uplands) are considerably more rich in species than areas located at lower elevations (see Dumont et al. 1990 and references therein). The most species-rich sites investigated so far in western Amazonia, containing up to 300 tree species per hectar (Gentry 1988; Valencia et al. 1994), were situated above 140 m.

So far, diversification models dealing with forest cover and river dynamics in the Neotropics have focused on the role of allopatric speciation taking place during periods when populations were isolated by water or dry environments during the Pleistocene (~2 – 0 Ma; Haffer 1969; Räsänen et al. 1987), sometimes much more recently (~0.1 Ma; Salo et al. 1986). In the few cases where evidence for so recent lineage splits has been presented, morphological differentiation has often been insufficient for the recognition of taxonomic species (see **Paper III** for references). Conversely, under the model proposed here, diversification is suggested to have occurred mainly during the *recolonization* of previously flooded areas (Fig. 14). In addition, since this process was presumably more linked to sea level fluctuations than to temperature oscillations, it may have taken place over a considerably longer period of time, several million years. Thus, this model could be expected to have played a more important role in fostering the diversification of morphologically recognizable species than Pleistocene models.

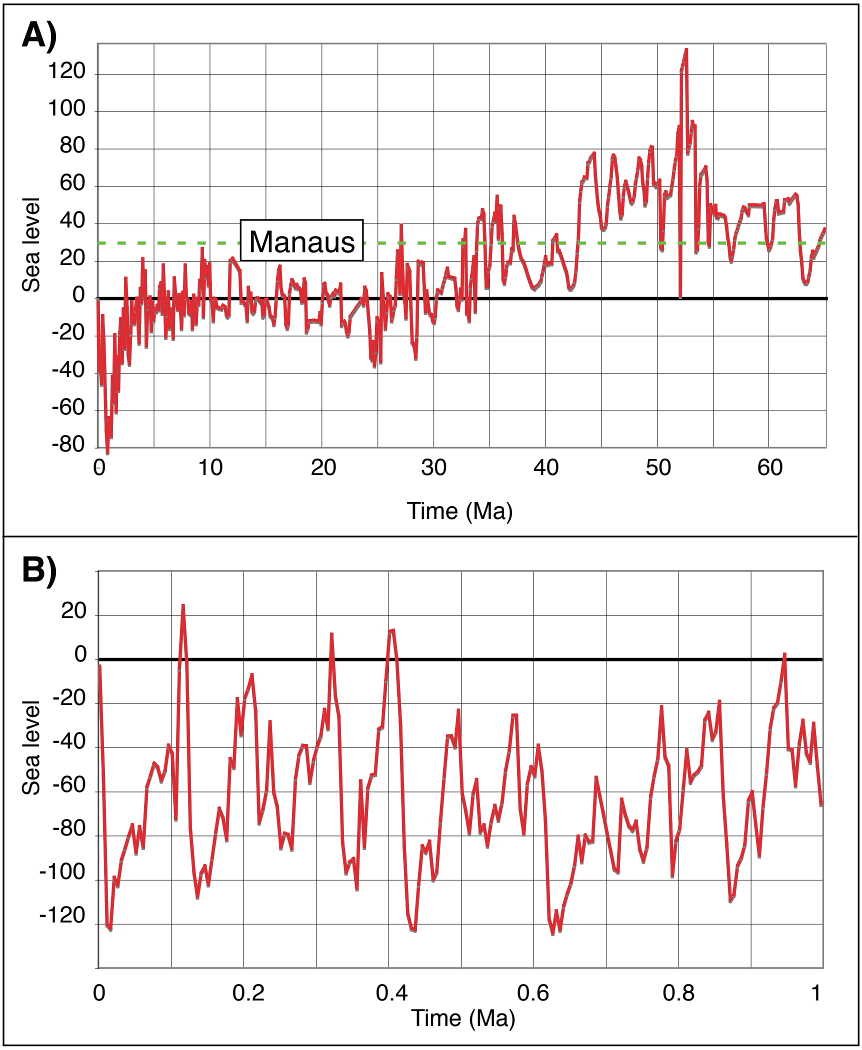


Figure 13. Global sea-level fluctuations in the Cenozoic (A) and in the last million years (B), compared to today's level (0 m). The altitude of Manaus (located approx. 1200 km from the Atlantic sea) is given as reference. Curves compiled from the supplementary information of Miller et al. (2005).

It is noteworthy that 25 out of 35 hotspots of biodiversity in the world include regions situated just a few meters above the present-day sea level (Mittermeier et al. 2004), such as the Chocó in northwestern South America and the Atlantic rain forest of Brazil. Could sea level fluctuations have contributed to species diversification in these areas, by sporadically causing the flooding and draining of large areas, followed by biotic recolonization and radiation?

Figure 14. Decreasing water levels exposes land areas, as here in Amazonia 2005. Events such as these may have been important for triggering diversification in Western Amazonia, the Huancabamba region and other lowland areas, such as the Chocó. (© Greenpeace/Ana Claudia Jatahy. Reproduced with permission).

The role of extinction

It has been suggested that woody magnoliids, to which Chloranthaceae are closely related, were significantly affected by the mass extinction event at the Cretaceous/Palaeogene boundary (65.5 Ma), when the Earth was hit by an asteroid just north of the Yucatán coast in Mexico (Alvarez et al. 1980; Upchurch Jr 1989). This is commonly known as the "K/T event". As many as one third of all angiosperm taxa represented by fossil pollen failed to survive the K/T event in North America (Nichols 2007; Nichols and Johnson 2008). Later, the Terminal Eocene cooling event (35 Ma; e.g., Zachos et al. 2001) extirpated many evergreen plant lineages that once formed part of the boreotropical flora, replacing them by hardwood deciduous groups in Europe and North America (the "mixed-mesophytic forest"; Tiffney 1985).

Surprisingly, no significant influence from either of these two events could be detected in the temporal analyses of the Chloranthaceae (**Paper V**). In fact, the K/T event, widely assumed to have been one of the most destructive mass extinctions of all times (Raup and Sepkoski 1982), was the one that worst described the empirical chronogram of the family (see Fig. 6A in **Paper V**). Instead, the data simulations performed suggest that the Chloranthaceae have been subject to a high and constant extinction rate.

Assuming these results are not methodologically biased, one plausible way of explaining why the Chloranthaceae did not become severely affected by the K/T event is that their ancestors survived in biological refugia far from the site of impact. Indeed, McLoughlin et al. (2008) recently demonstrated that in higher latitudes in the Southern and Northern Hemispheres, the influence of the impact was minor compared to regions nearer it. This possibility is supported by the biogeographic inference of the Chloranthaceae under the Fitch optimization, which indicates that the most recent common ancestor of genus *Hedyosmum* may have been restricted to Australasia (Fig. 3A in **Paper V**). However, it should be noted that this result is only poorly represented in the tree sample analyzed, and only obtained in the Fitch optimization.

Methodological considerations

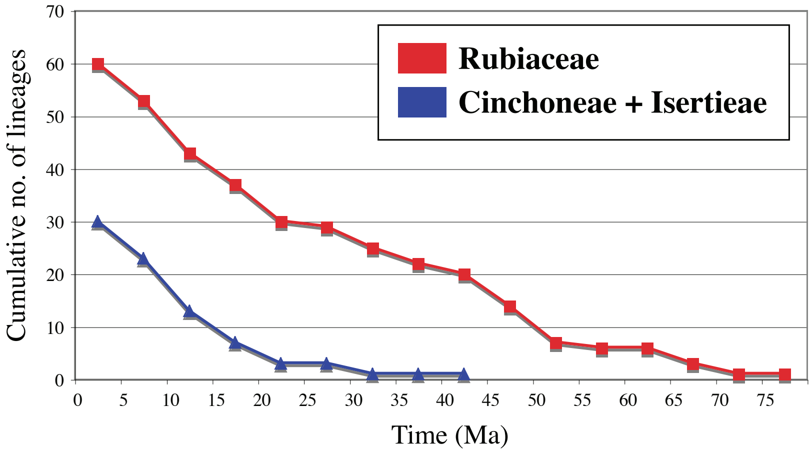
An alternative to LTT plots

One well-known and often discussed aspect of lineages through time (LTT) plots is the tendency of plotted curves to steadily increase in relation to time, which complicates the visual interpretation of the tempo of diversification of the chronogram studied (Emerson et al. 2001). This contrasts with the original aim of these graphs, which was to investigate macro-evolutionary processes such as sudden bursts of radiation or periods of no diversification (be they due to extinction or evolutionary stasis; see **Paper V**).

An alternative to LTT graphs would be to plot non-cumulative curves of lineage splits through time, conveniently termed "LSTT" plots. Discrete periods of time may be defined a priori, and the number of lineage splits within each time period summed across the entire molecular chronogram. An example of how this could be done is illustrated in Fig. 15. LSTT plots emphasize the changes (increase and decrease) in cladogenesis, which are often obscured in conventional LTT plots.

Instead of using fixed periods of time, the intervals in the X-axis could alternatively be chosen to represent geological and climatic events, but in that case the number of lineage splits for each period must be statistically corrected in relation to the absolute duration of the period. A third alternative would be to not use discrete time intervals, but rather plot a trace (from 0 to 1) at the exact time of a lineage split. Such a plot should normally look like a comb that has lost many of its teeth; phases with accentuated diversification would be seen as a concentration of traces ("teeth") along the time axis. Ideally, LSTT plots could be implemented in the APE package (Nee et al. 1992; Paradis et al. 2004), and should allow the simultaneous plotting of chronograms for different taxa as well as alternative chronograms for the same phylogeny.

A) Lineages through time (LTT) plot



B) Lineage splits through time (LSTT) plot

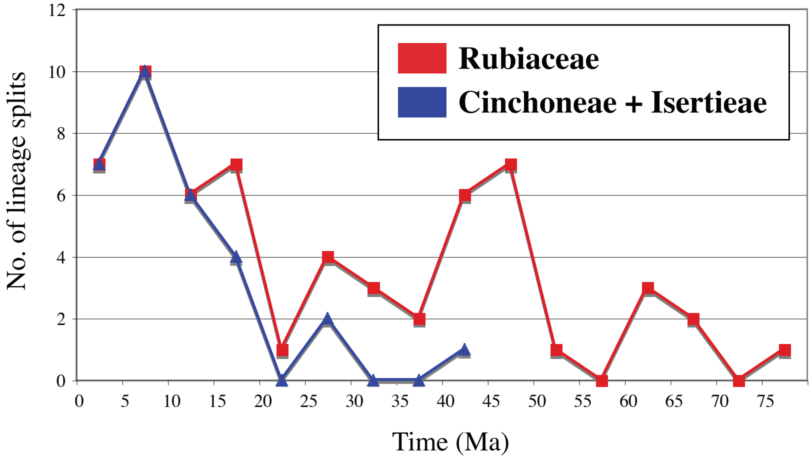


Figure 15. An empirical comparison between **A)** traditional LTT plots and **B)** the alternative proposed here, termed LSTT. Both graphs aim at providing a visual way of exploring the tempo of diversification of clades, but LSTT plots facilitate the identification of periods of sudden increases and decreases in diversification. Discrete time intervals of 5 Ma each were used for computing both graphs. Chronograms from **Paper IV**.

Conclusions

The key findings of this thesis can be summarized as follows:

Plylogeny and taxonomy

- Family Rubiaceae: tribes Cinchoneae and Isertieae (subfam. Cinchonoideae) are monophyletic and sister to each other. A new genus (*Ciliosemina*, comprising two species) is proposed to make *Remijia* monophyletic.

- Family Campanulaceae: *Lobelia* is highly polyphyletic. *Lysipomia* is sister to a clade comprising *Centropogon, Siphocampylus* and *Burmeistera.* The giant lobelioids from the Hawaiian Islands, Brazil, Africa and Sri Lanka constitute a clade.

- Family Chloranthaceae: within *Hedyosmum*, subgenus *Hedyosmum*, sections Microcarpa and Macrocarpa are all non-monophyletic.

Diversification of Neotropical organisms

- Very few groups are old enough to have existed in Gondwana by the time South America separated from Africa. Most tropical lineages with an palaeotropical origin, such as Rubiaceae, have therefore reached the Neotropics by transoceanic dispersal or via the boreotropical route around the Eocene climatic optimum (~50 Ma).

- Most diversification events leading to the present-day levels of species richness in the Neotropics occurred during the Cenozoic (65–0 Ma), with a marked increase in the Neogene (23–0 Ma).

- The inferrence of biogeographic events in families Rubiaceae, Chloranthaceae and Campanulaceae, in combination with a thorough review of molecular studies of Amazonian tetrapods indicate that the uplift of the Eastern Andean Cordilleras in the last ~23 Ma:

  - fostered allopatric speciation of lowland organisms, by acting as a dispersal barrier;
  - induced the adaptation to various montane environments by lowland and pre-montane ancestors, resulting in large radiations;
  - caused the end of a long-lasting dispersal barrier between the Northern and Central Andes, creating a "highway to the south" for boreotropical organisms that had already colonized northwestern South America;
  - was associated with the formation (~23–17 Ma) and dismissal (~11–7 Ma) of a huge wetland system in western Amazonia (Lake Pebas), which largely shaped the present distribution of lowland taxa.

- The distributional pattern shown by Neotropical plants into Andean-centred and Amazonian-centred taxa (here called "the Gentry pattern") was probably formed by the long-lasting effect of Lake Pebas and its predecessor, the Palaeo-Orinoco river, as a dispersal barrier for terrestrial taxa.

- In order to explain the high levels of endemism and species richness in the Huancabamba region and western Amazonia, two regions that were long submerged, a new diversification model is proposed. Under this model (termed here "draining land"), speciation has occurred in connection with the recolonization of newly drained land areas, by means of adaptive radiation of founder populations and secondary contact of previously separated populations.

- The K/T event did not cause noticeable extinction in Chloranthaceae.

Abbreviations and time scale

DIVA – Dispersal-vicariance analysis (a method of biogeographic inference).

K/T – Cretaceous/Paleogene boundary. This is a common denomination deriving from the German *Kreidezeit* and the traditionally recognized Tertiary period, which corresponds today to the Paleogene and Neogene periods (see Fig. 16 below).

LTT – Lineages through time (a diagram for visualizing chronograms).

Ma – Million years. Ma derives from Mega-annum and means a point in time; the adverb "ago" is usually omitted.

MRCA – Most recent common ancestor.

WAP – Western Andean Portal.

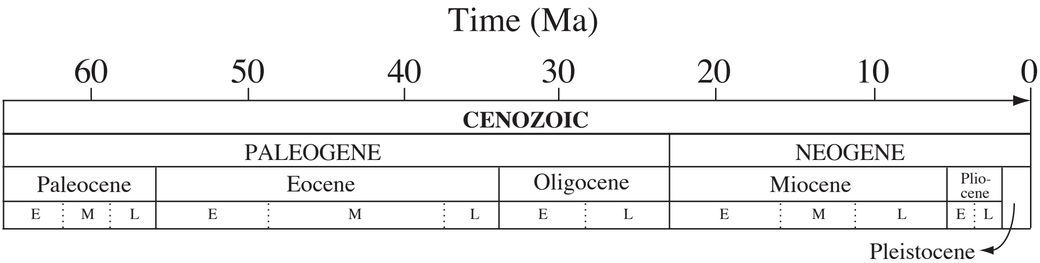


Figure 16. A geologic time scale for the Cenozoic (65.5 – 0 Ma), showing Periods (Paleogene and Neogene) and Epochs (Paleocene, Eocene, Oligocene, Miocene, Pliocene and Pleistocene). E: Early, M: Middle, L: Late. Adapted from Gradstein et al. 2004.

References

Abbott, R. J., and C. Brochmann. 2003. History and evolution of the arctic flora: In the footsteps of Eric Hultén. Molecular Ecology 12:299-313.

Ab'Saber, A. N. 1982. The palaeoclimate and paleoecology of Brazilian Amazonia. Biological Diversification in the Tropics: 41-59.

Albach, D. C., P. S. Soltis, D. E. Soltis, and R. G. Olmstead 2001. Phylogenetic analysis of asterids based on sequences of four genes. Annals of the Missouri Botanical Garden 88:163-212.

Aleixo, A. 2004. Historical diversification of a “terra-firme” forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. Evolution 58: 1303-1317.

Álvarez, I., and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Molecular Phylogenetics and Evolution 29:417-434.

Alvarez, L. W., W. Alvarez, F. Asaro, and H. V. Michel. 1980. Extraterrestrial cause for the Cretaceous-Tertiary extinction. Science 208:1095-1108.

Andersson, L. 1979. Multi-layered distribution patterns and the hypothesis of rain forest refugia. Botaniska Notiser 132(2): 185-190.

Andersson, L. 2002. Relationships and generic circumscriptions in the Psychotria complex (Rubiaceae, Psychotrieae). Systematics and Geography of Plants 72: 167-202.

Andersson, L., and J. H. E. Rova. 1999. The rps16 intron and the phylogeny of the Rubioideae (Rubiaceae). Plant Systematics and Evolution. 214: 161-186.

Andreasen, K., and B. Bremer. 2000. Combined phylogenetic analysis in the Rubiaceae-Ixoroideae: Morphology, nuclear and chloroplast DNA data. American Journal of Botany 87:1731-1748.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society B: Biological Sciences 265:457-463.

Ayers, T. 1999. Biogeography of Lysipomia (Campanulaceae), a high elevation endemic: An illustration of species richness at the Huancabamba Depression, Peru. Arnaldoa 6:13-28.

Ayres, J. M., and T. H. Clutton-Brock. 1992. River boundaries and species range size in Amazonian primates. American Naturalist 140: 531-537.

Bates, J. M., J. Haffer, and E. Grismer. 2004. Avian Mitochondrial DNA sequence divergence across as headwater stream of the Rio Tapajos, a major Amazonian river. Journal of Ornithology 145:199-205.

Bell, C. -D., and M. J. Donoghue. 2005. Phylogeny and biogeography of Valerianaceae (Dipsacales) with special reference to the South American valerians. Organisms Diversity and Evolution 5:147-159.

Berg, C. C. 1972. Moraceae - Part 1. Olmedieae and Brosimeae. Flora Neotropica Monographies 7: 1-228.

Berg, C. C. (1998) Moraceae (excl. *Ficus*). In: G. Harling & L. Andersson (eds.), Flora of Ecuador 60: 1-126.

Berry, P. E. 1982. The systematics and evolution of Fuchsia sect. Fuchsia (Onagraceae). Annals of the Missouri Botanical Garden 69:1-198.

Bremer, B., R. K. Jansen, B. Oxelman, M. Backlund, H. Lantz, and K. J. Kim. 1999. More characters or more taxa for a robust phylogeny - Case study from the coffee family (Rubiaceae). Systematic Biology 48:413-435.

Briggs, J. C. 1994. The genesis of Central America: biology versus geophysics. Global Ecology and Biogeography 4:169-172.

Brooks, D. R. 1985. Historical ecology: a new approach to studying the evolution of ecological associations. Annals of the Missouri Botanical Garden 72:660-680.

Brown Jr, K. S. 1982. Historical and ecological factors in the biogeography of aposematic neotropical butterflies. American Zoologist 22(2): 453-471.

Brumfield, R. T., and A. P. Capparella. 1996. Historical diversification of birds in Northwestern South America: A molecular perspective on the role of vicariant events. Evolution 50:1607-1624.

Brumfield, R. T. and S. V. Edwards 2007. Evolution into and out of the Andes: A Bayesian analysis of historical diversification in Thamnophilus antshrikes. Evolution 61: 346-367.

Brumfield, R. T., J. G. Tello, Z. A. Cheviron, M. D. Carling, N. Crochet, and K. V. Rosenberg. 2007. Phylogenetic conservatism and antiquity of a tropical specialization: Army-ant-following in the typical antbirds (Thamnophilidae). Molecular Phylogenetics and Evolution 45: 1-13.

Burnham, R. J., and K. R. Johnson. 2004. South American palaeobotany and the origins of neotropical rainforests. Philosophical Transactions of the Royal Society B: Biological Sciences 359: 1595-1610.

Burns, K. J., and K. Naoki. 2004. Molecular phylogenetics and biogeography of Neotropical tanagers in the genus Tangara. Molecular Phylogenetics and Evolution 32: 838-854.

Bush, M. B. 1994. Amazonian speciation: A necessarily complex model. Journal of Biogeography 21: 5-17.

Bush, M. B., M. R. Silman, and D. H. Urrego. 2004. 48,000 Years of Climate and Forest Change in a Biodiversity Hot Spot. Science 303:827-829.

Chase, M. W., D. E. Soltis, R. G. Olmstead, D. Morgan, D. H. Les, B. D. Mishler, M. R. Duvall, R. A. Price, H. G. Hills, and Y. L. Qiu. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 80:528-580.

Clague, D. A. 1996. The growth and subsidence of the Hawaiian-Emperor volcanic chain. The origin and evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: pattern and processes.

Colinvaux, P. A. 1993. Pleistocene biogeography and diversity in tropical forests of South America. In: GoldBlatt P (ed) Biological relationships between Africa and South America. Yale University Press, New Haven, pp 473-499.

Colinvaux, P. A., P. E. De Oliveira, J. E. Moreno, M. C. Miller, and M. B. Bush. 1996. A long pollen record from lowland Amazonia: forest and cooling in glacial times. Science 274: 85-88.

Comes, H. P., and J. W. Kadereit. 1998. The effect of quaternary climatic changes on plant distribution and evolution. Trends in Plant Science 3:432-438.

Condon, M. A., S. J. Scheffer, M. L. Lewis, and S. M. Swensen. 2008. Hidden neotropical diversity: Greater than the sum of its parts. Science 320:928-931.

Cortés-Ortiz, L., E. Bermingham, C. Rico, E. Rodriguez-Luna, I. Sampaio, and M. Ruiz-Garcia. Molecular systematics and biogeography of the Neotropical monkey genus, Alouatta. Molecular Phylogenetics and Evolution 26: 64-81.

Cracraft, J. 1988. Deep-History Biogeography: Retrieving the Historical Pattern of Evolving Continental Biotas. Systematic Zoology 37:221-236.

Darwin, C. R. 1845. Journal of researches into the natural history and geology of the countries visited during the voyage of H.M.S. Beagle round the world, under the Command of Capt. Fitz Roy, R.N. 2d edition. London: John Murray.

Darwin, C. R. 1859. On the Origin of Species by Means of Natural Selection.

Davis, C. C., C. D. Bell, S. Mathews, and M. J. Donoghue. 2002. Laurasian migration explains Gondwanan disjunctions: Evidence from Malpighiaceae. Proceedings of the National Academy of Sciences of the United States of America 99:6833-6837.

Dixon, J. R. 1979. Origin and distribution of reptiles in lowland tropical rainforests of South America. Pp 217-240 In: Duellman, W. W. (ed) The South American Herpetofauna: its origin, evolution, and dispersal. Museum of Natural History, University of Kansas, Lawrence, Kansas.

Donato, M. 2006. Historical biogeography of the family Tristiridae (Orthoptera, Acridomorpha) applying dispersal-vicariance analysis. Journal of Arid Environments 66:421-434.

Donoghue, M. J. 2008. A phylogenetic perspective on the distribution of plant diversity. Proceedings of the National Academy of Sciences 105:11549-11555.

Donoghue, M. J., and S.-A. Smith. 2004. Patterns in the assembly of temperate forests around the Northern Hemisphere. Philosophical Transactions of the Royal Society of London B Biological Sciences 359:1633-1644.

Drummond, A. -J., S.-Y. W. Ho, M.-J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology 4:699-710.

Dumont, J. F., S. Lamotte, and F. Kahn. 1990. Wetland and upland forest ecosystems in Peruvian Amazonia: Plant species diversity in the light of some geological and botanical evidence. Forest Ecology and Management 33-34:125-139.

Dyer, L. A., M. S. Singer, J. T. Lill, J. O. Stireman, G. L. Gentry, R. J. Marquis, R. E. Ricklefs, H. F. Greeney, D. L. Wagner, H. C. Morais, I. R. Diniz, T. A. Kursar, and P. D. Coley. 2007. Host specificity of Lepidoptera in tropical and temperate forests. Nature 448:696-699.

Eklund, H., J.-A. Doyle, and P.-S. Herendeen. 2004. Morphological phylogenetic analysis of living and fossil Chloranthaceae. International Journal of Plant Sciences 165:107-151.

Emerson, B. C., E. Paradis, and C. Thébaud. 2001. Revealing the demographic histories of species using DNA sequences. Trends in Ecology and Evolution 16:707-716.

Eriksson, R. 1993. Systematics of the Cyclanthaceae, especially *Sphaeradenia* and *Chorigyne*. Ph.D. thesis. Department of Systematic Botany, University of Göteborg, 16 pp.

Erkens, R. H. J., L. W. Chatrou, J. W. Maas, T. van der Niet, and V. Savolainen. 2007. A rapid diversification of rainforest trees (*Guatteria*; Annonaceae) following dispersal from Central into South America. Molecular Phylogenetics and Evolution 44:399-411.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315-319.

Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12:99-124.

Ferigolo, J. 1999. Late Pleistocene South-American land-mammal extinctions: The infection hypothesis. Quaternary of South America and Antarctic Peninsula 12, 279-310.

Friis, E. M., P. R. Crane, and K. R. Pedersen. 1997. Fossil history of magnoliid angiosperms. Evolution and diversification of land plants:121-156.

Gamble, T., A. M. Simons, G. R. Colli, and L. J. Vitt. 2008. Tertiary climate change and the diversification of the Amazonian gecko genus Gonatodes (Sphaerodactylidae, Squamata). Molecular Phylogenetics and Evolution 46: 269-277.

Garzione, C. N., G. D. Hoke, J. C. Libarkin, S. Withers, B. MacFadden, J. Eiler, P. Ghosh, and A. Mulch. 2008. Rise of the Andes. Science 320:1304-1307.

Gascon, C., J. R. Malcolm, J. L. Patton, M. N. F. Da Silva, J. P. Bogart, S. C. Lougheed, C. A. Peres, S. Neckel, and P. T. Boag. 2000. Riverine barriers and the geographic distribution of Amazonian species. Proceedings of the National Academy of Sciences of the United States of America 97: 13672-13677.

Gates, D. M. 1993. Climate change and its Biological Consequences, Sunderland, MA (United States); Sinauer Associates. 280 pp.

Gentry, A. H. 1982. Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? Annals of the Missouri Botanical Garden 69:557-593.

Gentry, A. H. 1988. Tree Species Richness of Upper Amazonian Forests. Proceedings of the National Academy of Sciences 85:156-159.

Goloboff, P., S. Farris, and K. Nixon. 2000. TNT (Tree analysis using New Technology) ver. 1.0. Published by the authors. Tucumán, Argentina.

Govaerts, R. 2001. How many species of seed plants are there? Taxon 50:1085-1090.

Govaerts, R., D. G. Frodin, M. Ruhsam, D. M. Bridson, and A. P. Davis. 2007. World Checklist and Bibliography of Rubiaceae. Royal Botanic Gardens, Kew.

Gradstein, F. M., Ogg, J. G., Smith, A. G., et al. 2004. A Geologic Time Scale 2004. Cambridge University Press, Cambridge.

Gregory-Wodzicki, K. M. 2000. Uplift history of the Central and Northern Andes: A review. Bulletin of the Geological Society of America 112:1091-1105.

Grenyer, R., C. D. L. Orme, S. F. Jackson, G. H. Thomas, R. G. Davies, T. J. Davies, K. E. Jones, V. A. Olson, R. S. Ridgely, P. C. Rasmussen, T.-S. Ding, P. M. Bennett, T. M. Blackburn, K. J. Gaston, J. L. Gittleman, and I. P. F. Owens. 2006. Global distribution and conservation of rare and threatened vertebrates. Nature 444:93-96.

Haffer, J. 1969. Speciation in Amazonian forest birds. Science 165: 131-137.

Haffer, J. 1997. Alternative models of vertebrate speciation in Amazonia: An overview. Biodiversity and Conservation 6: 451-476.

Hansson, B., D. Hasselquist, M. Tarka, P. Zehtindjiev, and S. Bensch. 2008. Postglacial Colonisation Patterns and the Role of Isolation and Expansion in Driving Diversification in a Passerine Bird. PLoS ONE 3.

Haq, B. U., J. Hardenbol, and P. R. Vail. 1987. Chronology of fluctuating sea levels since the Triassic. Science 235:1156-1167.

Hausdorf, B., and C. Hennig. 2003. Biotic Element Analysis in Biogeography. Systematic Biology 52:717-723.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America 101:14812-14817.

Hensold, N. 1999. Las angiospermas del departamento de cajamarca, Perú. Arnaldoa 6:141-184.

Heppner, J. B. 1991. Faunal regions and diversity of Lepidoptera. Tropical Lepidoptera 2 (Suppl. 1):1-85.

Heywood, V. H., R. K. Brummitt, A. Culham, and O. Seberg. 2007. Flowering plant families of the world. Royal Botanic Gardens, Kew, Richmond.

Hofreiter, A., and E. Rodríguez. 2006. The Alstroemeriaceae in Peru and neighbouring areas. Revista Peruviana de Biología 13:5-69.

Hong Qian, R. E. R. 2008. Global concordance in diversity patterns of vascular plants and terrestrial vertebrates. Ecology Letters 11:547-553.

Hoorn, C. 1993. Marine incursions and the influence of andean tectonics on the miocene depositional history of northwestern Amazonia: Results of a palynostratigraphic study. Palaeogeography Palaeoclimatology Palaeoecology 105:267-309.

Hoorn, C. 1994. Miocene Palynostratigraphy and Paleoenvironments of Northwestern Amazonia: Evidence for Marine Incursions and the Influence of Andean Tectonics:156.

Hoorn, C. and H. Vonhof 2006. Neogene Amazonia: introduction to the special issue. Journal of South American Earth Sciences 21: 1-4.

Hoorn, C., C. G. M. Paxton, W. G. R. Crampton, P. Burgess, L. G. Marshall, J. G. Lundberg, M. E. Rasanen, and A. M. Linna. 1996. Miocene deposits in the Amazonian foreland basin. Science 273:122-125.

Hoorn, C., J. Guerrero, G. A. Sarmiento, and M. A. Lorente. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23:237-240.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Huelsenbeck, J. P., and N. S. Imennov. 2002. Geographic Origin of Human Mitochondrial DNA: Accommodating Phylogenetic Uncertainty and Model Comparison. Systematic Biology 51:155-165.

Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences of the United States of America 103:10334-10339.

Hughes, C. E., R. J. Eastwood, and C. D. Bailey. 2006. From famine to feast? Selecting nuclear DNA sequence loci for plant species-level phylogeny reconstruction. Philosophical Transactions of the Royal Society B: Biological Sciences 361:211-225.

Humboldt, A. 1820. Voyage aux regions equinoxiales du Nouveau Continent. Ed. N. Mazé, Paris 318.

Hungerbühler, D., M. Steinmann, W. Winkler, D. Seward, A. Egüez, D. E. Peterson, U. Helg, and C. Hammer. 2002. Neogene stratigraphy and Andean geodynamics of southern Ecuador. Earth-Science Reviews 57: 75-124.

Inda, L. A., J. G. Segarra-Moragues, J. Müller, P. M. Peterson, and P. Catalán. 2008. Dated historical biogeography of the temperate Loliinae (Poaceae, Pooideae) grasses in the northern and southern hemispheres. Molecular Phylogenetics and Evolution 46:932-957.

Irving, E. M. 1975. Structural evolution for the northernmost Andes, Colombia. United States Geological Survey 846:1-47.

Iturralde-Vinent, M. A., and R. D. E. MacPhee. 1999. Paleogeography of the Caribbean region: implications for Cenozoic biogeography. Bulleting of the American Museum of Natural History 238:1-95.

Katinas, L., and J.-J. Morrone. 1999. Track analysis reveals the composite nature of the Andean Biota. Australian Journal of Botany 47:111-130.

Kim, K-J., and Jansen, R.K. 1995. ndhF sequence evolution and the major clades in the sunflower family. Evolution 92, 10379-10383.

Kreft, H., and W. Jetz. 2007. Global patterns and determinants of vascular plant diversity. Proceedings of the National Academy of Sciences of the United States of America 104:5925-5930.

Kress, W. J., and D. L. Erickson. 2008. DNA barcodes: Genes, genomics, and bioinformatics. Proceedings of the National Academy of Sciences of the United States of America 105:2761-2762.

Kress, W. J., K. J. Wurdack, E. A. Zimmer, L. A. Weigt, and D. H. Janzen. 2005. Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America 102:8369-8374.

Kricher, J. C. 1997. A Neotropical Companion: An Introduction to the Animals, Plants, and Ecosystems of the New World Tropics. Princeton Univ Press.

Källersjö, M., and B. Ståhl. 2003. Phylogeny of the Theophrastaceae (Ericales s.l.). International Journal of Plant Sciences 164, 579-591.

Kõljalg, U., K-H. Larsson, K. Abarenkov, R. H. Nilsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Höiland, R. Kjöller, E. Larsson, T. Pennanen, R. Sen, A. F. S. Taylor, U. Tedorsoo, T. Vrålstad, and B. M. Ursing. 2005 UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166:1063-1068

Lahaye, R., M. Van Der Bank, D. Bogarin, J. Warner, F. Pupulin, G. Gigot, O. Maurin, S. Duthoit, T. G. Barraclough, and V. Savolainen. 2008. DNA barcoding the floras of biodiversity hotspots. Proceedings of the National Academy of Sciences of the United States of America 105:2923-2928.

Lammers, T.G., 1998a. Nemacladoideae, a new subfamily of Campanulaceae. Novon 8: 36-37.

Lammers, T. G. 1998b. Review of the Neotropical endemics Burmeistera, Centropogon, and Siphocampylus (Campanulaceae: Lobelioideae), with description of 18 new species and a new section. Brittonia 50, 233-262.

Lammers, T. G. 2007. World checklist and bibliography of Campanulaceae. Kew, UK: Royal Botanical Gardens.

Lamoreux, J. F., J. C. Morrison, T. H. Ricketts, D. M. Olson, E. Dinerstein, M. W. McKnight, and H. H. Shugart. 2006. Global tests of biodiversity concordance and the importance of endemism. Nature 440:212-214.

Larsson, E., L. Örstadius. 2008. Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycological Research 112:1165-185.

Larsson, L., G. Ekström, E. Larsson, and M. Gandemo. 2008. Birds of the World.

Lavin, M., and M. Luckow. 1993. Origins and relationships of tropical North America in the context of the boreotropics hypothesis. American Journal of Botany 80: 1-14.

Lavin, M., R. T. Pennington, G. P. Lewis, and J. A. Ratter. 2006. Neotropical Savannas and Seasonally Dry Forests: Plant Diversity, Biogeography and Conservation.

Linder, H. P. 2001. On areas of endemism, with an example from the African restionaceae. Systematic Biology 50:892-912.

Linder, H. P. 2008. Plant species radiations: Where, when, why? Philosophical Transactions of the Royal Society B: Biological Sciences 363:3097-3105.

Linder, H. P., C. R. Hardy, and F. Rutschmann. 2005. Taxon sampling effects in molecular clock dating: An example from the African Restionaceae. Molecular Phylogenetics and Evolution 35:569-582.

Lomolino, M. V. 2005. Biogeography (3rd edn). Sinauer Associates.

Lovejoy, N. R., J. S. Albert, and W. G. R. Crampton. 2006. Miocene marine incursions and marine/freshwater transitions: evidence from Neotropical fishes. Journal of South American Earth Sciences, 21: 5-13.

Lundberg, J. G., L. G. Marshall, J. Guerrero, B. Horton, M. Malabarba, F. Wesselingh, L. R. Malabarba, R. E. Reis, R. P. Vari, and Z. M. S. Lucena. 1998. Phylogeny and classification of Neotropical fishes, eds Malabarba, LR, Reis RE, Vari RP, Lucena ZM, Lucena CAS (Edipucrs, PortoAlegre) pp 13-48.

Lynch, J. D. 1979. The amphibians of the lowland tropical forests. The South American Herpetofauna: Its Origin, Evolution, and Dispersal. University of Kansas Museum of Natural History Monography 7: 189-215.

Maas, P. J. M. 1977. Renealmia (Zingiberaceae-Zingiberoideae). Flora Neotropica Monographies 18: 1-218.

MacArthur, R. H., and E. O. Wilson. 1963. An equilibrium theory of insular zoogeography. Evolution 17:373-387.

McLoughlin, S., R. J. Carpenter, G. J. Jordan, and R. S. Hill. 2008. Seed ferns survived the end-Cretaceous mass extinction in Tasmania. American Journal of Botany 95:465-471.

Maddison, W. P., and D. R. Maddison. 1992. MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer, Sunderland, MA.

Maddison, W. P., and D. R. Maddison. 2007. Mesquite: a modular system for evolutionary analysis. Version 2.01. Available at: mesquiteproject. org.

Mallet, J. 1993. Speciation, raciation, and color pattern evolution in *Heliconius* butterflies: Evidence from hybrid zones. Hybrid Zones and the Evolutionary Process. Pp. 226-260. Oxford University Press.

Manen, J. F., S. Dessein, P. De Block, and E. Robbretch. 2006. Towards an evolutionary scenario for the coffee family (Rubiaceae, angiosperms): further insights from a supertree. Third International Rubiaceae Conference, published abstract. Scripta Botanica Belgica 40:47.

May, R. M. 1990. How many species? Philosophical Transactions - Royal Society of London, B 330:293-304.

McKenna, D. D., and B. D. Farrell. 2006. Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. Proceedings of the National Academy of Sciences of the USA 103:10947.

Miller, K. G., M. A. Kominz, J. V. Browning, J. D. Wright, G. S. Mountain, M. E. Katz, P. J. Sugarman, B. S. Cramer, N. Christie-Blick, and S. F. Pekar. 2005. The phanerozoic record of global sea-level change. Science 310:1293-1298.

Milnes, A. G. 1987. Tectonic evolution of the southern Andes, Tierra del Fuego: A summary. in: J.-P. Schaer and J. Rodgers (eds.). The Anatomy of Mountain Ranges. Princeton University Press, Princeton. Pp. 173-177.

Mittermeier, R. A., P. R. Gil, M. Hoffman, J. Pilgrim, T. Brooks, C. G. Mittermeier, J. Lamoreux, and G. A. B. da Fonseca. 2004. Hotspots Revisited: Earth's Biologically Richest and Most Endangered Terrestrial Ecoregions. CEMEX.

Molau, U. 1988. Scrophulariaceae. Part I. Calceolarieae. Flora Neotropica Monographies 47:1-326.

Moore, B. R., and M. J. Donoghue. 2007. Correlates of diversification in the plant clade dipsacales: Geographic movement and evolutionary innovations. American Naturalist 170:S28-S55.

Moore, W. S., A. C. Weibel, and A. Agius. 2006. Mitochondrial DNA phylogeny of the woodpecker genus Veniliornis (Picidae, Picinae) and related genera implies convergent evolution of plumage patterns. Biological Journal of the Linnean Society 87: 611-624.

Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: An integrated molecular approach. Annual Review of Ecology and Systematics 31: 533-563.

Morley, R. J. 2000. Origin and Evolution of Tropical Rain Forests. John Wiley & Sons, Chichester.

Morrone, J. J. 1994. On the Identification of Areas of Endemism. Systematic Biology 43:438-441.

Muchhala, N. 2003. Exploring the boundary between pollination syndromes: Bats and hummingbirds as pollinators of Burmeistera cyclostigmata and B. tenuiflora (Campanulaceae). Oecologia 134:373-380.

Muchhala, N. 2006a. Nectar bat stows huge tongue in its rib cage. Nature 444:701-702.

Muchhala, N. 2006b. The pollination biology of Burmeistera (Campanulaceae): Specialization and syndromes. American Journal of Botany 93:1081-1089.

Müller, R. D., M. Sdrolias, C. Gaina, B. Steinberger, and C. Heine. 2008. Long-term sea-level fluctuations driven by ocean basin dynamics. Science 319:1357-1362.

Nee, S. 2001. Inferring speciation rates from phylogenies. Evolution 55:661-668.

Nee, S. 2004. Extinct meets extant: simple models in paleontology and molecular phylogenetics. Paleobiology 30:172-178.

Nee, S., A. O. Mooers, and P. H. Harvey. 1992. Tempo and mode of evolution revealed from molecular phylogenies. Proceedings of the National Academy of Sciences of the United States of America 89:8322-8326.

Nichols, D. J. 2007. Selected plant microfossil records of the terminal Cretaceous event in terrestrial rocks, western North America. Palaeogeography, Palaeoclimatology, Palaeoecology 255:22-34.

Nichols, D. J., and K. R. Johnson. 2008. Plants and the K-T boundary. Cambridge University Press.

Nores, M. 1999. An alternative hypothesis for the origin of Amazonian bird diversity. Journal of Biogeography 26(3): 475-485.

Nores, M. 2004. The implications of Tertiary and Quaternary sea level rise events for avian distribution patterns in the lowlands of northern South America. Global Ecology and Biogeography 13:149-161.

Novotny, V., P. Drozd, S. E. Miller, M. Kulfan, M. Janda, Y. Basset, and G. D. Weiblen. 2006. Why Are There So Many Species of Herbivorous Insects in Tropical Rainforests? Pp. 1115-1118. Science.

Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Nylander, J. A. A., U. Olsson, P. Alström, and I. Sanmartín. 2008. Accounting for Phylogenetic Uncertainty in Biogeography: A Bayesian Approach to Dispersal-Vicariance Analysis of the Thrushes (Aves: Turdus). Systematic Biology 57:257-268.

O'Gorman, E. 1972. The invention of America: an inquiry into the historical nature of the New World and the meaning of its history. Westport, Conn., Greenwood Press.

Olmstead, R. G., K. Kim, R. K. Jansen, and S. J. Wagstaff. 2000. The Phylogeny of the Asteridae sensu lato Based on Chloroplast *ndh*F Gene Sequences. Molecular Phylogenetics and Evolution 16 (1): 96-112.

Olmstead, R. G., H. J. Michaels, K. M. Scott, and J. D. Palmer. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of rbcL. Annals of the Missouri Botanical Garden 79: 249-265.

Page, R. D. M. 1994. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10:155-173.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20:289-290.

Patterson B. D., and P. M. Velazco. 2008. Phylogeny of the rodent genus Isothrix (Hystricognathi, Echimyidae) and its diversification in Amazonia and the eastern Andes. Journal of Mammalian Evolution 15: 181-201.

Pennington, R.T., and C. W. Dick. 2004. The role of immigrants in the assembly of the South American rainforest tree flora. Philosophical Transactions of the Royal Society of London Series B Biological Sciences 359:1611-1622.

Persson, C. 2000. Phylogeny of Gardenieae (Rubiaceae) based on chloroplast DNA sequences from the rps16 intron and trnL(UAA)-F(GAA) intergenic spacer. Nordic Journal of Botany 20: 257-269.

Pirie, M. D., L. W. Chatrou, J. B. Mols, R. H. J. Erkens, and J. Oosterhof. 2006. 'Andean-centred' genera in the short-branch clade of Annonaceae: Testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. Journal of Biogeography 33:31-46.

Posada, D., and T. R. Buckley. 2004. Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches Over Likelihood Ratio Tests. Systematic Biology 53:793-808.

Posadas, P., J. M. Estévez, and J. J. Morrone. 1997. Distributional patterns and endemism areas of vascular plants in the Andean subregion. Fontqueria 48:1-10.

Prance, G. T. 1973. Phytogeographic support for the theory of Pleistocene forest refuges in the Amazon basin, based on evidence from distribution patterns in Caryocaraceae, Chrysobalanaceae, Dichapetalaceae and Lecythidaceae. Acta Amazonica 3(3): 5-28.

Prance, G. T. 1978. The origin and evolution of the Amazon flora. Interciencia 3(4): 207-222.

Prance, G. T. 1982. A review of the phytogeographic evidences for Pleistocene climate changes in the neotropics. Annals of the Missouri Botanical Garden 69(3): 594-624.

Rabosky, D. L. 2006. Likelihood methods for detecting temporal shifts in diversification rates. Evolution 60:1152-1164.

Rabosky, D. L., and I. J. Lovette. 2008. Explosive evolutionary radiations: decreasing speciation or increasing extinction through time? Evolution 62(8): 1866-1875.

Raup, D., and J. Sepkoski. 1982. Mass extinctions in the marine fossil record. Science 215: 1501-1503.

Raven, P. H., and D. I. Axelrod. 1974. Angiosperm biogeography and past continental movements. Annals of the Missouri Botanical Garden 61:539-673.

Ree, R.-H., B.-R. Moore, C.-O. Webb, and M.-J. Donoghue. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59:2299-2311.

Renner, S. S. 2005. Relaxed molecular clocks for dating historical plant dispersal events. Trends in Plant Science 10:550-558.

Richardson, J. E., L. W. Chatrou, J. B. Mols, R. H. J. Erkens, and M. D. Pirie. 2004. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. Philosophical Transactions of the Royal Society of London Series B Biological Sciences 359:1495-1508.

Richardson, J. E., R. T. Pennington, T. D. Pennington, and P. M. Hollingsworth. 2001. Rapid diversification of a species-rich genus of Neotropical rain forest trees. Science 293: 2242-2245.

Ricklefs, R. E. 2007. Estimating diversification rates from phylogenetic information. Trends in Ecology and Evolution 22:601-610.

Robbrecht, E., and J. F. Manen. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of Coptosapelta and Luculia, and supertree construction based on rbcL, rps16, trnL-trnF and atpB-rbcL data. a new classification in two subfamilies, Cinchonoideae and Rubioideae. Systematics and Geography of Plants 76:85-146.

Roberts, J. L., J. L. Brown, R. v. May, W. Arizabal, R. Schulte, and K. Summers. 2006. Genetic divergence and speciation in lowland and montane peruvian poison frogs. Molecular Phylogenetics and Evolution 41: 149-164.

Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Systematic Biology 46:195-203.

Ronquist, F. 2001. DIVA 1.2. Computer programme distributed by the author. Available from http://www. ebc. uu. se/systzoo/research/diva/diva. html.

Ronquist, F. 2003. Parsimony analysis of coevolving associations. Tangled trees. Phylogeny, cospeciation, and coevolution. University of Chicago Press, Chicago, IL:22-64.

Rova, J. H. E., P. G. Delprete, L. Andersson, and V. A. Albert. 2002. A trnL-F cpDNA sequence study of the Condamineeae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae. Amererican Journal of Botany 89: 145-159.

Rull, V. 2008. Speciation timing and neotropical biodiversity: The Tertiary-Quaternary debate in the light of molecular phylogenetic evidence. Molecular Ecology 17:2722-2729.

Räsänen, M. E., J. S. Salo, and R. J. Kalliola. 1987. Fluvial perturbance in the western Amazon basin: Regulation by long-term sub-Andean tectonics. Science 238:1398-1401.

Salati, E., and P. B. Vose. 1984. Amazon Basin: A System in Equilibrium. Science 225:129-138.

Salo, J., R. Kalliola, I. Häkkinen, Y. Mäkinen, P. Niemela, M. Puhakka, and P.D. Coley. 1986. River dynamics and the diversity of Amazon lowland forest. Nature 322: 254-258.

Sanderson, M. J. 2002. Estimating Absolute Rates of Molecular Evolution and Divergence Times: A Penalized Likelihood Approach. Molecular Biology and Evolution 19:101-109.

Sanmartín, I. 2003. Dispersal vs. vicariance in the Mediterranean: Historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). Journal of Biogeography 30:1883-1897.

Sanmartín, I. 2007. Event-based biogeography: Integrating patterns, processes, and time. Pp. 135-159 in M. C. Ebach, and R. Tangney, eds. Biogeography in a Changing World. Crc Press-Taylor and Francis Group, Boca Raton.

Sanmartín, I., and F. Ronquist. 2004. Southern Hemisphere biogeography inferred by event-based models: Plant versus animal patterns. Systematic Biology 53:216-243.

Sanmartín, I., H. Enghoff, and F. Ronquist. 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. Biological Journal of the Linnean Society 73:345-390.

Santos, C., C. Jaramillo, G. Bayona, M. Rueda, and V. Torres. 2008. Late Eocene marine incursion in northwestern South America. Palaeogeography, Palaeoclimatology, Palaeoecology 264:140-146.

Savolainen, V., M. F. Fay, D. C. Albach, A. Backlund, M. van der Bank, K. M. Cameron, S. A. Johnson, M. D. Lledo, J. C. Pintaud, and M. Powell. 2000. Phylogeny of the eudicots: a nearly complete familial analysis based on rbcL gene sequences. Kew Bulletin 55:257-309.

Schipper, J., J. S. Chanson, F. Chiozza, et al. 2008. The Status of the World's Land and Marine Mammals: Diversity, Threat, and Knowledge. Science 322:225-230.

Schultz, J. 2005. The Ecozones Of The World: The Ecological Divisions Of The Geosphere. Springer.

Scotese, C. R. 2001. Atlas of Earth History. Paleomap Project, Arlington, Texas. Volume 1, Paleogeography.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution 19:198-207.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, and R. L. Small. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92:142-166.

Shaw, J., E. B. Lickey, E. E. Schilling, and R. L. Small. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94:275.

Shimodaira, H. and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution 16: 1114-1116.

Smith, S. A., and M. J. Donoghue. 2008. Rates of Molecular Evolution Are Linked to Life History in Flowering Plants. Science 322:86-89.

Smith, T. B., R. K. Wayne, D. J. Girman, and M. W. Bruford. 1997. A role for ecotones in generating rainforest biodiversity. Science 276: 1855-1857.

Sparre, B., and L. Andersson. 1991. A taxonomic revision of the Tropaeolaceae. Opera Botanica 108.

Stebbins, G. L. 1984. Polyploidy and the distribution of the arctic-alpine flora: new evidence and a new approach. Botanica Helvetica 94:1-13.

Stebbins, G. L. 1985. Polyploidy, hybridization, and the invasion of new habitats. Annals of the Missouri Botanical Garden 72: 824-832.

Stehli, F. G. and S. D. Webb. 1985. The Great American Biotic Interchange. Plenum Press, London.

Steinmann, M., D. Hungerbühler, D. Seward, and W. Winkler. 1999. Neogene tectonic evolution and exhumation of the southern Ecuadorian Andes; a combined stratigraphy and fission-track approach. Tectonophysics 307: 255-276.

Stotz, D. F., J. W. Fitzpatrick, T. A. Parker, and D. K. Moskovits. 1996. Neotropical Birds: Ecology and Conservation. University of Chicago Press.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version.

Taylor, D. W. 1991. Paleobiogeographic relationships of Andean angiosperms of Cretaceous to Pliocene age. Palaeogeography, Palaeoclimatology, Palaeoecology 88: 69-84.

Templeton, A. R. 1980. The theory of speciation via the founder principle. Genetics 94 (4): 1011-38.

Thiv, M., L. Struwe, and J. W. Kadereit. 1999. The phylogenetic relationships and evolution of the Canaria laurel forest endemic Ixanthus viscosus (Aiton) Griseb. (Gentianaceae): evidence from matK and ITS sequences, and floral morphology and anatomy. Plant Systematics and Evolution 218: 299-317.

Thomas, W. W. 1999. Conservation and monographic research on the flora of Tropical America. Biodiversity and Conservation 8:1007-1015.

Thorne, J. L., and H. Kishino. 2002. Divergence times and evolutionary rate estimation with multilocus data. Systematic Biology 51:689-702.

Tiffney, B. H. 1985. Perspectives on the origin of the floristic similarity between eastern Asia and eastern North America. Journal of the Arnold Arboretum 66:73-94.

Todzia, C. A. 1988. Chloranthaceae: Hedyosmum. Flora Neotropica Monographies 48:1-139.

Todzia, C. A. 1993. New Species of Hedyosmum (Chloranthaceae) from Northern South America. Novon 3:81-85.

Upchurch Jr, G. R. 1989. Terrestrial environmental changes and extinction patterns at the Cretaceous-Tertiary boundary, North America. Mass Extinctions; processes and evidence. Columbia University Press. New York, NY, United States:195-216.

Valencia, R., H. Balslev, and G. Paz Y Miño C. 1994. High tree alpha-diversity in Amazonian Ecuador. Biodiversity and Conservation 3:21-28.

Vanzolini, P. E. and E. E. Williams. 1970. South American anoles: The geographic differentiation and evolution of the Anolis chrysolepis species group (Sauria, Iguanidae). Arquivos de Zoologia 19(1-2): 1-298.

Verola, C. F., J. Semir, A. Antonelli, and I. Koch. 2007. Biosystematic studies in the Brazilian endemic genus *Hoffmannseggella* H.G. Jones (Orchidaceae: Laeliinae): a multiple approach applied to conservation. Lankesteriana 7 (1-2): 419-422.

von Hagen, K. B., and J. W. Kadereit. 2003. The diversification of Halenia (Gentianaceae): ecological opportunity versus key innovation. Evolution 57:2507-2518.

Vuilleumier, F. 1968. Population Structure of the Asthenes Flammulata, Superspecies (Aves: Furnariidae). Museum of Comparative Zoology.

Vuilleumier, F. 1970. Insular Biogeography in Continental Regions. I. The Northern Andes of South America. The American Naturalist 104(938): 373-388.

Wallace, A. R. 1852. On the monkeys of the Amazon. Proceedings of the Zoological Society of London 20:107-110.

Wallace, A. R. 1853. A Narrative of Travels on the Amazon and Rio Negro, with an account of the native tribes, and observations on the climate, geology, and natural history of the Amazon Valley. London: Reeve.

Weigend, M. 2002. Observations on the biogeography of the Amotape-Huancabamba Zone in northern Peru. Botanical Review 68:38-54.

Weigend, M. 2004. Additional observations on the biogeography of the Amotape-Huancabamba Zone in Northern Peru - Defining the south-eastern limits. Revista Peruviana de Biología 11:127-134.

Weigend, M., E.F. Rodríguez, and C. Arana. 2005. Bosques relictos del NO de Perú y SO de Ecuador. Revista Peruviana de Biología 12(2): 185 - 194.

Weir, J. T. 2006. Divergent timing and patterns of species accumulation in lowland and highland Neotropical birds. Evolution 60:842-855.

Wesselingh, F.P., M. E. Räsänen, G. Irion, H. B. Vonhof, R. Kaandorp, W. Renema, L. Romero Pittman, and M. Gingras. 2002. Lake Pebas: a palaeoecological reconstruction of a Miocene, long-lived lake complex in western Amazonia. Cainozoic Research 1:35-81.

Wesselingh, F.P. 2006. Miocene long-lived lake Pebas as a stage of mollusc radiations, with implications for landscape evolution in western Amazonia. Scripta Geologica 133:1-17.

Wesselingh, F.P., and J. A. Salo 2006. A Miocene perspective on the evolution of the Amazonian biota. Scripta Geologica 133:439-458.

Wesselingh, F.P., and M. C. Hoorn. In press. A geological perspective on the development of Amazonian fish faunas. In: Albert, J. & Reis, R. (eds.). Historical Biogeography of Neotropical Freshwater Fishes.

Wesselingh, F. P., M. C. Hoorn, S. B. Kroonenberg, J. Lundberg, H. H. Vonhof and H. Hooghiemstra. In press. On the origin of Amazonian landscapes and biodiversity: a synthesys. *In:* Hoorn., C. et al. (eds.). Amazonia, landscape and species evolution.

Wiens, J. J. 2004. Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. Evolution 58: 193-197.

Wiens, J. J. 2007. Global patterns of diversification and species richness in amphibians. American Naturalist 170.

Winkworth, R.-C., and M.-J. Donoghue. 2005. Viburnum phylogeny based on combined molecular data: Implications for taxonomy and biogeography. American Journal of Botany 92:653-666.

Xiang, Q. Y. J., and D. T. Thomas. 2008. Tracking character evolution and biogeographic history through time in Cornaceae-Does choice of methods matter? Journal of Systematics and Evolution 46:349-374.

Young, K., and C. Reynel. 1997. Huancabamba region, Peru and Ecuador. Centres of Plant Diversity: A Guide and Strategy for Their Conservation 3:465-469.

Yule, G. U. 1924. A mathematical theory of evolution, based on the conclusions of Dr. JC Willis. Philosophical Transactions of the Royal Society B: Biological Sciences 213:21-87.

Zachos, J., H. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292: 686-693.

Zuckerkandl, E., and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. Evolving Genes and Proteins: 97-166.

Svensk populärvetenskaplig sammanfattning

I mitt avhandlingsprojekt har jag använt mig av DNA-sekvenser för att spåra *när* och *var* några växtgrupper bildades. Jag har jämfört mina resultat med andra studier av växter och djur och försökt identifiera gemensamma orsaker till varför det har utvecklats så många arter i just tropiska Amerika. Mina resultat tyder på att Anderna fungerat som en ”artpump” varifrån nya arter kunde sprida sig till resten av kontinenten. Dessutom har Andernas bildning orsakat stora förändringar i Amazonas och därigenom påverkat artbildningen även där.

Nedan ger jag en kort introduktion till ämnet, berättar hur jag gått tillväga i min undersökning och presenterar de viktigaste resultaten.

Bakgrund

Ingen annanstans på jorden finns det så många arter av växter och djur som i tropiska Amerika (Neotropis, eller den Neotropiska regionen som det också kallas). Här finns lika många växtarter som i hela tropiska Afrika och Sydostasien tillsammans: runt 100 000 arter. Mönstret upprepar sig vad gäller antalet arter av fåglar, grod- och kräldjur, däggdjur, praktfjärilar – i alla dessa grupper, och förmodligen många andra, är antalet arter betydligt högre i Neotropis än i andra tropiska områden.

Flera orsaker har föreslagits för att förklara denna uppseendeväckande artrikedom. Nedan följer de tre viktigaste teorierna, som jag har försökt testa under mitt projekt.

En förklaring skulle kunna vara att många djur och växter “följde med” den sydamerikanska kontinenten när denna bröt sig loss från den afrikanska för 100 miljoner år sedan. Dessa arter skulle sedan ha haft mycket lång tid på sig att ge upphov till nya arter (diversifiera) på denna stora tropiska ö. När Sydamerika till slut kom i kontakt med Nordamerika, i samband med att Panamanäset reste sig ur havet för 3,5 miljoner år sedan, kunde många djur och växter sprida sig söderut, vilket bidrog ytterligare till den sydamerikanska artrikedomen.

En annan viktig händelse i Sydamerikas historia som antas ha lett till att många arter bildades är Andernas utveckling. Den Andinska bergskedjan sträcker sig över 500 mil längs Sydamerikas västkust och är därmed världens enda storskaliga bergsformation i direkt anslutning till en låglandsregnskog, Amazonas. Trots dess imponerande längd och höjd (många bergstoppar når över 6 000 m.ö.h.) är Anderna geologiskt sett unga. Största delen av norra Anderna bildades under de senaste 20 miljoner åren – att jämföra med den skandinaviska fjällkedjan som började bildas för ca 70 miljoner år sedan. Andernas uppkomst skapade nya bergsmiljöer där djur och växter anpassade sig efter nya förhållanden och bildade på så sätt nya arter. När olika populationer av en art blev isolerade från varandra, pga barriärer i form av höga berg eller djupa dalgångar, blev de med tiden alltmer olika varandra, och var till slut inte längre samma art.

En helt annan teori om varför det finns så många arter i just Amazonas går under namnet *refugieteorin.* Den har att göra med de stora klimatförändringarna som drabbat jorden under de senaste 3 årmiljonerna, då 20 istider skapade stora glaciärer på norra halvklotet. Trots att Sydamerika aldrig blev täckt av is, antar man att nederbördsmängden minskade under istiderna. Detta ledde i sin tur till att den sydamerikanska regnskogen krympte till mindre fläckar och savanner bredde ut sig mellan dessa “regnskogsöar” – även kallade *refugier*. När istiden så småningom nådde sitt slut blev jorden åter varmare och nederbörden i tropikerna ökade, regnskogen expanderade åter och täckte stora arealer. Under den tid som varje istid pågick hann en del populationer av vissa arter förändras mycket i sina respektive refugier. När regnskogen senare blev sammanhängande kunde inte dessa populationer längre reproducera sig sinsemellan – nya arter hade bildats. Denna process antas ha upprepats många gånger och hos så många olika växt- och djurgrupper att den länge ansågs vara den mest avgörande orsaken bakom Amazonas artrikedom.

Metoder

DNA-tekniken är idag ett oumbärligt verktyg för att studera evolutionen av världens biologiska mångfald. Genom att mäta hur DNA-sekvenserna från olika arter skiljer sig åt kan man med hjälp av avancerade analysmetoder uppskatta släktskapen mellan dessa arter. Skillnaderna kan även användas för att uppskatta hur långt tillbaka i tiden arterna skiljdes åt från en gemensam förfader. Dessutom kan analyser som utgår ifrån arternas nuvarande geografiska utbredning ge en bra uppskattning om förfaderns utbredning. Det är just sådana analyser som varit kärnan i mitt projekt.

Eftersom det är praktiskt omöjligt att DNA-sekvensera alla arter i Neotropis har jag valt att studera tre växtgrupper i detalj, för att sedan resonera kring hur tillämpliga resultaten från varje grupp kan vara för andra växter och djur. Två av artiklarna i min avhandling handlar därför om familjen Rubiaceae (kaffe- och mårväxter), två om familjen Campanulaceae (klockväxter), och ett om familjen Chloranthaceae (som saknar svenskt namn). Dessutom har jag och några medarbetare i ett bokkapitel sammanställt resultaten från ett stort antal studier om däggdjur, fåglar, grod- och kräldjur i Amazonas.

Viktiga resultat och slutsatser

Teorier som bör förkastas – De flesta växt- och djurgrupper som finns i Neotropis idag tycks vara alldeles för unga för att ha funnits på den tid då Afrika och Sydamerika bildade en gemensam kontinent. Därför var det inte så många afrikanska grupper som kunde “följa med” när Sydamerika bröt sig loss från Afrika, eller så har de flesta som gjorde det dött ut under senare tid. Merparten av de grupper som har ett afrikanskt eller asiatiskt ursprung och som idag finns i Neotropis kan därför antas ha kommit genom s.k. långdistansspridning över Atlanten (t.ex. genom att frön fastnar på fåglars fötter eller att djur följer med stormvirke). Ett annat sätt, som i min avhandling föreslås ha varit fallet för familjen kaffeväxter, var att först sprida sig till Europa, sedan till Nordamerika, och till slut söderut till Sydamerika. Avståndet mellan dessa landytor har inte alltid varit lika stort som idag, och vid flera tillfällen tror man att det funnits tillfälliga öförbindelser som underlättade spridningen över havet (och till slut Panamanäset). Denna spridningsväg styrks av både europeiska växtfossil och den uppskattade tiden för migrationen, vilken sammanföll med en av jordens varmaste perioder (för runt 50 miljoner år sedan då den globala medeltemperaturen var ca 12°C varmare än idag). Då täcktes stora delar av södra Nordamerika och Europa av tropisk skog, vilken utgjorde en lämplig spridningsväg för tropiska växter och djur från Afrika och Asien.

Tidpunkten för de flesta artbildningar visar tydligt att refugieteorin som beskrivs ovan inte kan förklara mer än en bråkdel av den nuvarande biologiska mångfalden i Neotropis. Den största diversifieringen skedde istället under de senaste 30 årmiljonerna, så när istiderna började fanns redan de flesta nu levande arter. Detta bevis borde sätta punkt på en långlivad teori.

Teori som får stöd – Den avgörande orsaken till den stora artrikedomen i Neotropis tycks vara Andernas formation. Tidpunkten för diversifieringarna i de växtgrupper jag har studerat i detalj, och även för många andra växt- och djurgrupper, sammanfaller väl med denna händelse. Dessutom visar mina resultat att förfäderna till vissa artgrupper befann sig just i norra Anderna då nya arter bildades. Tillsammans tyder dessa resultat på att det funnits en stark koppling mellan norra Andernas formation och artbildning. En sådan koppling kan inte hittas mellan temperaturfluktuationer och artbildning. Troligtvis har artbildning i Anderna skett av de orsaker som angetts ovan – isolering av populationer och anpassning till nya miljöer i samband med uppresning av bergskedjan.

Från Anderna spred sig många arter vidare till andra områden i Neotropis, som Amazonas, Karibien, Centralamerika och östra Sydamerika. Mina resultat är de första som tyder på att spridningen söderut längs med Anderna möjliggjordes först när norra och centrala Anderna kom i kontakt med varandra för ca 10 – 12 miljoner år sedan. Dessförinnan hade en lång havsvik fungerat som en effektiv spridningsbarriär mellan dessa områden. Ofta ledde spridningar till att nya arter bildades, i takt med att de fyllde nya ekologiska funktioner. På detta sätt kan man betrakta Anderna som en sorts "artpump" till resten av den Neotropiska regionen. Naturligtvis delar inte alla växt- och djurgrupper samma evolutionära historia, men eftersom uppemot 40 % av alla Neotropiska växtarter tillhör Andinska grupper kan denna modell troligen förklara en stor del av den nuvarande artrikedomen.

En ny teori – Ända sedan Sydamerika skiljde sig från Afrika dränerades västsluttningarna av Anderna och västra Amazonas norrut mot Karibiska havet med hjälp av Palaeo-Orinoco floden. I samband med en ny fas av bergsbildning för ca 20 miljoner år sedan försvårades dock vattendräneringen norrut, vilket sakta ledde till en gigantisk översvämning av hela västra Amazonas och bildningen av *Pebas*-sjön. Man uppskattar att Pebassjön, som existerade mellan 17 och 11 miljoner år sedan, som mest täckte en yta på ca 1 000 000 km^2, dvs mer än Sverige och Norge tillsammans. Så småningom ledde ytterligare geologiska förändringar till att Pebassjön dränerades och den nuvarande Amazonfloden bildades, för ca 7 miljoner år sedan.

Det som är märkligt med västra Amazonas är att det där man idag finner den absolut största artrikedomen i världen (åtminstone vad gäller antalet trädarter), trots att området varit täckt av vatten under så lång tid. I ett område på 100 x 100 m har man hittat så många som 300 olika arter av träd (att jämföra med ca 35 vilda trädarter i Sverige). Hur har så många trädarter kunnat bildas på så kort tid, ca 10 miljoner år? Ur ett mänskligt perspektiv kan detta tyckas vara obegripligt länge, men nya studier har visat att artbildningen här har gått mycket snabbare än på de flesta andra ställen.

Flera andra artrika ställen i Neotropis (t.ex. Chocó och Huancabamba) och i resten av världen förmodas ha varit tidvis täckta av havsvatten i samband med globala fluktuationer i havsnivån (som mest uppemot ca 120 m över och under den nuvarande nivån).

Jag föreslår att en rimlig förklaring till den stora artrikedomen på dessa ställen skulle vara att artbildning har skett i samband med *återkoloniseringen* av dessa områden omedelbart efter att de blivit dränerade. En stor yta som frigörs i Amazonas blir snabbt koloniserad av växter och djur från den närliggande skogen. Av slumpmässiga skäl kommer en del av dessa små populationer att vara avvikande från de flesta individer i den stora skogspopulationen. Konkurrensen på denna nya yta är i regel svagare är mitt i den täta skogen, och här finns många outnyttjade miljöer. Denna situation gynnar nya anpassningar och därmed artbildning.

Dessutom kommer populationer som länge varit isolerade pga vattnet att mötas när vattnet försvinner. Har de under tiden hunnit bli så avvikande från varandra att de inte längre kan korsa sig betyder detta att artbildning har skett. Kan de däremot fortfarande reproducera sig trots skillnaderna kan korsningarna vara så olika föräldrapopulationerna att de bör betraktas som nya arter (s.k. hybrider).

Dessa två processer – artbildning genom avknoppning från en moderpopulation och anpassning till den nya miljön, samt artbildning genom isolering och korsning – skulle kunna ha bidragit till uppkomsten av en väsentlig del av arterna i dessa låglandsområden. Dessutom har denna process troligen upprepats ett stort antal gånger, i samband med att vattennivån höjdes och sänktes.

Denna teori kombinerar allmänt accepterade ekologiska modeller, men skiljer sig från andra teorier (t.ex. refugieteorin) genom att lägga tonvikt på återkolonisering snarare än skogsfragmentering. Dessutom sträcker den sig mycket längre tillbaka i tiden (tiotals årmiljoner) än refugieteorin. Det bör poängteras att jag inte har några starka bevis på att artbildning verkligen gått till på det sätt som jag föreslår; framtida studier kommer förhoppningsvis att klargöra hur viktig denna process har varit i verkligheten. Teorin är inte heller avsedd att ”ersätta” andra teorier (t.ex. Andernas roll som ”artpump”), utan snarare beskriva artbildningen i områden som legat under vatten och som idag är synnerligen artrika.

Meteoritnedslag och utdöende – Ett av de största massutdöendena i jordens historia skedde för 65,5 miljoner år sedan då en meteorit slog ned i Mexikanska golfen. Flodvågor mellan 50 och 100 m höga svepte över södra Nordamerika, tryckvågor utplånade allt liv på långt håll, miljontals ton stoft kastades ut i atmosfären och jorden kom in i en lång natt. Troligen ledde mörkret till att mycket begränsad fotosyntes kunnat ske under flera år. Resultatet var förödande: ca 75 % av alla arter antas ha dött ut, däribland alla dinosaurier förutom fåglarna.

Eftersom en av växtgrupperna som jag har studerat (familjen Chloranthaceae) är en av de äldsta kända blomväxterna, med 110 miljoner år gamla fossiler, har jag och en medarbetare försökt uppskatta till vilken grad den drabbades av nedslaget. Genom datasimuleringar kunde vi visa att nedslaget inte haft någon som helst märkbar effekt på dessa växter. Detta var ett förvånansvärt resultat, vilket dock har en del stöd från tidigare studier. Mina resultat tyder på att vid tidpunkten för nedslaget befann sig dessa växter i Asien, där nedslaget troligen inte haft lika omedelbar påverkan som i Amerika. Denna hypotes har tidigare lagts fram för trädormbunkar från Tasmanien, och här finner den stöd från blomväxter i Asien.

Resumen en Español

En ninguna otra región de la Tierra existen tantas especies de plantas y animales como en la Región Neotropical. Sin embargo, a pesar de esta riqueza, no se conocen todavía cuáles son los mecanismos ecológicos y/o evolutivos responsables de tan extraordinaria diversidad.

La región tropical de Sudamérica es la región más rica del planeta en número de especies de plantas: un tercio de todas las plantas con flores se encuentran en el Neotrópico. Desde antiguo los científicos han intentado explicar el origen de esta riqueza en términos de factores ecológicos como una mayor tasa de luminosidad, temperatura y humedad que habrían favorecido la diversificación biológica. Recientemente, se han avanzado explicaciones más integradoras, de carácter histórico, según las cuales la extraordinaria biodiversidad de los Neotrópicos sólo puede explicarse en términos de la historia evolutiva de cada uno de los organismos que la forman. Por ejemplo, la hipótesis del "conservatismo neotropical" sugiere que la región neotropical es más rica en especies simplemente porque más linajes se originaron y diversificaron allí debido a su estabilidad climática (se trata de una región cercana al Ecuador) y a la tendencia de las especies a conservar su "nicho ecológico" a lo largo del tiempo evolutivo (el conjunto de condiciones en las que la especie puede mantener sus poblaciones).

Una explicación alternativa es la "hipótesis boreotropical" según la cual muchos linajes de plantas neotropicales que ahora presentan su centro de diversidad en los trópicos, como la familia de las Anonáceas o el orden Malpighiales, se habrían originado en la antigua Laurasia (Europa y Asia) como parte de la "flora boreotropical"– una combinación de bosques deciduos con elementos tropicales de hoja perenne que no se parece a ningún otro bosque actual y que existió a comienzos del Terciario (65–50 millones de años), y desde allí migrado a Norte América a través de la conexión Atlántica ("Thulean Land Bridge") o el Estrecho de Bering. La migración a Sudamérica probablemente se produjo a través de la conexión caribeña (proto-Antillas).

Un punto en común de todas estas hipótesis es el papel clave atribuido al levantamiento de los Andes desde finales del Terciario (20 millones de años, Mioceno) en la rápida diversificación de la flora neotropical. Según estas hipótesis, los Andes Neotropicales habrían actuado tanto como ruta de dispersión hacia el sur para linajes “boreotropicales” (procedentes de Norte América), como un agente de especiación en linajes de altitud, por ejemplo favoreciendo la creación de nuevos nichos ecológicos en las cimas y laderas de las montañas o mediante especiación alopátrica (especiación por aislamiento geográfico) al aislar geográficamente a las poblaciones en los valles. Aproximadamente, 22500 especies de plantas (7.5% del total) son endémicas de la región tropical de los Andes.

En esta investigación doctoral, se utilizan datos moleculares (secuencias de ADN) en combinación con métodos de inferencia filogenética, datación molecular, reconstrucción biogeográfica, y modelos macroevolutivos de extinción y diversificación, así como evidencia geológica, paleontológica, climatológica, e hidrológica, para reconstruir la evolución espacio-temporal de algunos grupos de organismos Neotropicales que destacan por su diversidad, historia evolutiva, o adaptaciones morfológicas.

Este estudio confirma que el levantamiento tectónico de la Cordillera Andina Neotropical durante ejerció un papel crucial en la generación de la extraordinaria diversidad biológica en los Neotrópicos. Por un lado, se favoreció la especiación alopátrica en organismos de llanuras y tierras bajas, y por otro lado se promovieron radiaciones adaptativas en los nuevos hábitats formados en las cimas y laderas de las montañas. Asimismo, el levantamiento de la cordillera tropical Andina contribuyó al cierre del llamado “Portal Occidental de los Andes” en el Mioceno Superior (Orogenia Quechua I, 13 millones de años), una región de tierras bajas periódicamente invadida por incursiones marinas desde el Pacifico a la altura del sur de Ecuador, que habría actuado como barrera geográfica impidiendo la migración entre los Andes del Norte y los Andes Centrales durante parte del Paleógeno y Neógeno (50–13 millones de años). El levantamiento de esta región permitió la dispersión hacia el sur de linajes boreotropicales. El cierre del “Portal Occidental” por el levantamiento andino también favoreció la formación de un sistema lacustre, el Lago Pebas, que ocupaba la Cuenca amazónica occidental, y que reforzó el aislamiento entre los Andes al oeste y las llanuras amazónicas al este.

El hecho de que la mayor parte de las plantas neotropicales presenten su centro de diversidad en la región andina (“Andean-centreed”) o en la llanura amazónica (“Amazonian- centreed”) se explica por la existencia de dos barreras geográficas, el sistema fluvial del Paleo-Orinoco y el Lago Pebas, que habrían actuado como barreras dispersivas e impedido la migración biótica y flujo genético entre estos dos centros de biodiversidad hasta finales del Neogeno (10–8 millones de años).

Finalmente, se propone un nuevo modelo de diversificación para explicar el origen y evolución de las especies en dos áreas caracterizadas por extraordinarios niveles de riqueza de especies y endemismo en la Región Neotropical: la Región de Huancabamba y la Amazonia occidental. Según este modelo, su extraordinaria biodiversidad se explicaría por un incremento en la tasa de especiación relacionado con la recolonización de áreas previamente sumergidas, en Huancabama por incursiones marinas desde el Pacífico y en Amazonia occidental por el Lago Pebas. La disponibilidad de nuevos nichos ecológicos y la falta de competición habría favorecido procesos de radiación adaptativa en las especies colonizadoras, seguido de contacto secundario de las poblaciones previamente aisladas.

En conclusión, el escenario biogeográfico aquí postulado muestra un extraordinario paralelismo entre la historia paleogeográfica de la Región Neotropical y los principales eventos de cladogénesis en linajes neotropicales. El levantamiento de los Andes fue el evento mas importante en la historia biogeográfica de Sudamérica. No sólo favoreció la diversificación en linajes de altitud al crear nuevos nichos ecológicos en las laderas y cimas de las montañas, sino que también afectó a los linajes amazónicos al modificar el sistema de drenaje de la Cuenca Amazónica dando lugar a la formación de nuevos lagos y cambiando el curso de los ríos.

Acknowledgements

This PhD in figures:

Seconds elapsed since the project began : 170 899 200
Meters cycled back and forth to work : 6 600 000
DNA nucleotides analysed : 786 247
Electronic mails written : 4317
Specimens collected : 1805
Children produced : 3
Moon trips : 0

Doing a PhD is great fun, but also a lot of hard work. Fortunately, I was always surrounded by fantastic people at the institute, during my travels and at home.

Thanks to… (not in a particular order)

- my great supervisors **Claes Persson**, for all the time you spent on me and our mad trip to Central America – it's actually amazing we survived; **Roger Eriksson**, for your meticulous comments on my manuscripts and enthusiastic culinary discussions – I hope we'll have a *cochinillo asado* together someday; **Isabel Sanmartín**, for the tremendous assistance you've given me the last years, for writing the Spanish summary of this thesis, and for introducing me to the *Mazapán de Toledo*; **Bengt Oxelman**, for your encouragement and advice, and for resurrecting systematic biology in Göteborg; **Lennart Andersson**, not longer among us, for being the first one talking about "biogeography" to me, for our trips to South America, and for teaching me to take a break while Adobe Illustrator initializes.

- my great colleagues and friends at the department: **Mats Töpel**, for every day giving me a great start at work and for our conspiracy plans; **Vivian Aldén**, for the tremendous help at the lab during all these years,

and for teaching me Skånska; **Gunilla Bok**, for all the years we shared the office and your endless knowledge on how to solve all sorts of problems, from choosing cement quality to childhood stomachaches; **Martin Ryberg,** for always having some piece of good advice to share, and showing me that vegan food can actually look quite appealing; **Henrik Nilsson**, for solving all sorts of computer headaches, our joint projects and your weird presents after you'd been abroad; **Elisabet Sjökvist**, for critically reading this thesis, and changing curtains in Martin's office; **Bente Eriksen,** for our nice discussions, your endless patience when compiling the teaching schemes, and your delicious and healthy Danish cakes; **Nils Hallenberg**, for our biogeographic discussions and joint projects, and for been the first one who got me interested in systematics; **Thomas Appelqvist,** for pouring over your field expertise, for our joint orchid project, and for never lacking a smile and an infamous joke during your daily walks in the corridor; **Sven Toresson** and **David Allbrand,** for tirelessly providing all kinds of practical assistance, for your engagement with environmental issues, and for always buying cinnamon rolls to the breaks; **Anne-Cathrine Scheen**, for carefully reading and commenting on this thesis, and for your friendship ever since our stressful trip to Crete; **Uno Eliasson**, for your encouragement and various support during this project, and for regularly sharing your astronomic knowledge; **Martin Eriksson**, for inviting me to participate in your exciting research; **Anna Petri,** for commenting on the Swedish summary of this thesis, and for bringing music to our office; **Jenny Klingberg**, for making lazy people cycle more, and always being so friendly; **Inga Groth**, **Kristina Andersson,** and **Ingela Lyck** for a lot of administrative support; **Ellen** and **Karl-Henrik Larsson,** for your engagement in systematics; **Christina Wising, Ingrid Skoglund,** and **Saramma Varkey** at the library, for getting hold of the world's most obscure journals in a matter of hours; **Karin Lindblad**, for giving me my first employment in a research project, and for taking me to Latjnajaure when I should instead be going on my honeymoon; **Ulf Molau,** for our discussions on Huancabamba, and for giving a live demonstration on how lemmings commit mass suicide; **Gunnar Harling**, for telling me old stories about Indians and jaguars in Ecuador; **Sven Fransén**, for always showing up during school holidays and tipping on expansive books; **Arne Strid,** for taking us to Greece twice and teaching us the important difference between ouzo and raki; **Magnus Neuendorf,** for tirelessly showing me around in the greenhouse and pouring fantastic stories about tropical plants; **Claes Gustafsson**, **Grazyna Bochenek**, **Henrik Aronsson, Robert Björk, Robert Daun, Åslög Dahl, Biljana**

Jokic, Thorsten Elfström, Ewa Batorowicz, Erik Ljungstrand, Sara Brosché, Maria Ernfors, Björn Nordén, Marianne Hall, Dellilah Lithner, Stephan Andersson, Cecilia Petternsson, Per Weslien, Heidi Paltto, Håkan Plejel, Hans and **Margareta Ryberg, Göran Wallin, Anna Wik, Annika Jägerbrand,** and all other brilliant and friendly people in the building, for making the work at the department highly enjoyable.

- **Lovisa Gustafsson, Kaisa Carlgren, Marion Jannes, Lena Dahlström** and **Johan Blomby**, for your enthusiasm in your Master's projects, and for making me realize how fun it is to be a supervisor.

- **Thomas Lammers**, for all the invaluable help you've given me with the Campanulaceae over the years, and for always replying to my messages within 30 minutes (at most). I'm looking forward to meeting you in person someday! **Johan Nylander**, for your broad skills and capacity of overcoming all statistical and computational problems, and for been such a nice guy to talk to at various times; **James Doyle**, for great advice concerning the fossil history and evolution of Chloranthaceae, and for introducing me to the amazing world of palaeobotany (and not least palaeobotanists). **Livia Wanntorp**, for sharing a great deal of personal advice, getting me involved with the amazing Gunneraceae, and commenting on this thesis. **Frank Wesselingh** and **Carina Hoorn**, for providing me with invaluable updates on the geological and palaeontological history of South America, commenting on my manuscripts, and editing the Amazonian book. **Toby Pennington,** for excellent discussions on Neotropical diversification, commenting on the convergence manuscript, and various other help.

- **Luiza Kinoshita**, for your invaluable support and encouragement, and for always making me feel at home at Unicamp. Also **Christiano Verola, Lidyanne Aona, Leonardo Meireles, Maria do Carmo Amaral**, **Volker Bittrich, Washington Marcondes, Kikyo Yamamoto, Fernando** and **Eliana Martins, Flávio Santos, George Shepherd, João Semir** and **Jorge Tamashiro**, some of the people who have made Unicamp to one of Brazil's best university.

- **Adrián Quijada-Mascareñas, Alejandra Jaramillo, Alexandre Muellner, Ana Odete, André Simões, Andrea Weeks, Andreas Tribsch, Andrew Crawford, Andrew Rambaut, Anna Oldermark, Anthony Friscia, Argelia Cuenca, Arnaud Mouly, Bertil Ståhl,**

Birgitta and **Kåre Bremer, Bozo Frajman, Burtom Lim, Cajsa Andersson, Camila Ribas, Carlos Jaramillo, Catarina Ekenäs, Charlotte Taylor, Chris Yesson, Christian Puff, Christian Uhink, Christopher Witt, Cristina Miyaki, David Orme, Elisabeth Eriksson, Elmar Robbrecht, Else Marie Friis, Eric Knox, Frederic Delsuc, Fredrick Barker, Gareth Nelson, Germinal Rouhan, Gerald Carr, Guilhem Mansion, Heidi Meudt, Henry Hooghiemstra, Hervé Sauquet, Hongzhi Kong, Itayguara Costa, James Patton, Jan Ohlson, Jan Schipper, Jelle van Zweden, Jens Klackenberg, Joakim Kadereit, Johan Rova, John Bates, John Hafner, John Lundberg, John Wiens, Jonna Bjuhr, Julia Borg, Jürg Schönenberger, Kalle Ruokolainen, Karin Tremetsberger, Karol Marhold, Kathleen Kay, Katya Romoleroux, Kenitiro Suguio, Kenneth Campbell, Kevin Edwards, Kit Tan, Kjell Bolmgren, Klaus-Peter Koepfli, Kristina Mellström, Lars Chatrou, Lars Larsson, Lena Struwe, Magnus Popp, Malin Laggren, Maria Backlund, Marie-Stéphanie Samain, Mats Gustafsson, Matthew Lavin, Maximillian Weigend, Michael Andersson, Michael Braby, Michael Hardman, Michael Knapp, Michael Pirie, Michael Sanderson, Milton Groppo, Mitsuru Arai, Nathan Muchhala, Nico Cellinese, Niklas Wahlberg, Niklas Wikström, Nina Rønsted, Ole Seberg, Oliver Pybus, Paúl Velazco, Per Erixon, Peter Bengtson, Peter Linder, Peter Uetz, Philipp Trenel, Piero Delprete, Rich Warshauer, Richard Abbott, Richard Ree, Robert Brumfield, Ruy Bemerguy, Santiago Madriñán, Scott Mori, Scott Steppan, Sergio Pereira, Silane Silva, Silvana Combita, Silvana Godoy, Steve McLoughlin, Steve Wagstaff, Susanne Renner, Sylvain Razafimandimbison, Taylor Feild, Thiago Conforti, Thierry Sempere, Thomas Denk, Tiina Särkinen, Tina Ayers, Tod Stuessy, Torsten Eriksson, Ulf Swenson, Vanessa Plana, Victor Rodrizuez, Wolfgang Wüster, Zuzana Musilova,** and **you-whom-I-forgot,** for scientific discussions and/or sharing invaluable research material. Thanks for making life as a scientist exciting even though so much time is spent in front of a computer screen!

- **Daniel Santamaría, Álvaro Herera, Nelson Zamora, Daniel Solano,** for great assistance while in Costa Rica, **Carmen Galdames** and **Mireya Correa** while in Panama, and **Otar Abdaladze** and **George Nakhutsrishvili** while in Georgia.

- The staff at Göteborg's Natural History Museum (**Torkel Hagström, Göran Nilsson, Göran Andersson, Ted von Proschwitz, Tor-**

sten Nordander), Västsvenska Entomologklubben (**Johnny Lindquist, Lars Gambréus, Bengt Carlsson, Bertil Andrén**), Universeum (**Ramiro Fuentes, Mikael Olsson**), the Zoological Department (**Urban Olsson, Per Sundberg, Jenny Eklöf, Lisa Matamoros, Christer Erséus, Emma Vodoti, Susanne Viker, Malin Strand, Helena Wiklund, Anna Ansebo, Pierre De Wit,** and previously **Johan Eklöf, Jens Rydell**) for a great deal of zoological and conservational inspiration.

- The herbarium curators and staff at **UEC** (Brazil); **SCZ** (Panama); **JBSD** (Dominican Republic); **InBio** (Costa Rica); **S** (Sweden); **P** (France); **K, RBGE** (United Kingdom); **MA** (Spain); **TBI** (Georgia); **Z** (Switzerland), for letting me access your plant collections.

- The **Swedish Research Council**, the **Royal Swedish Academy of Sciences**, **Royal Society of Arts and Sciences in Göteborg**, **Kungliga och Hvitfeldtska Stiftelsen, Adlerbertska Stipendiestiftelsen, Stiftelsen Paul och Marie Berghaus donationsfond, Helge Ax:son Johnsons Stiftelse, University of Gothenburg** and the **Systematics Association**, for generous grants.

- **Simon Häggbom, Gustavo Meyer, Olof Helje, Rossano Ramos, Humberto Ito, Johan Dahlberg, Stefan Pettersson, Nadia Karasawa, Peter Wirdenäs, Eleonor Eliasson,** for your friendship.

- **Jan, Martin, Erik, Linnea, Oscar, Johan, Jacob, David, Fredrik, Alma, Moa, Linus, Helene, Linda**, for making me a member of the Sveide family, and especially **Mona** and **Rolf** for everything you have helped us with during these years, and for caring so much for our children.

- **Nadirá, Cláudia, Marcelo, Fernando, Therezinha, Alberto, Jair, Beth, Elissa, Tarcísio, Steve, Eduardo, Fabiana, Lia,** for being my family, with everything that implies.

- **Maria, Clara** and **Gabriel**, for being the most wonderful children a father could ever wish for.

- **Anna,** for everything you have put up with, for giving birth to the most wonderful children a father could ever wish for, and for loving me.