

Neotropical diversification: historical overview and conceptual insights

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Abstract

Studying the causes of biological diversification and the main environmental drivers involved is useful not only for the progress of fundamental science but also to inform conservation practices. Unraveling the origin and maintenance of the comparatively high Neotropical biodiversity is important to understand the global latitudinal biodiversity gradients (LBGs), which is one of the more general and conspicuous biogeographical patterns on Earth. This chapter reviews the historical development of the study of Neotropical diversification, in order to highlight the influence of methodological progress and to identify the conceptual developments that have appeared through history. Four main steps are recognized and analyzed, namely the discovery of the LBGs by pioneer naturalists, the first biogeographic studies, the inception of paleoecology and the recent revolution of molecular phylogeography. This historical account ends with an update of the current state of the study of Neotropical diversification and the main conceptual handicaps that are believed to slow progress towards a general theory on this topic. Among these constraints, emphasis is placed on (i) the shifting from one paradigm to another, (ii) the extrapolation from particular case studies to the whole Neotropics, (iii) the selection of biased evidence to support either one or another hypothesis, (iv) the assumption that Pleistocene diversification equals to refuge diversification, and (v) the straightforward inference of diversification drivers from diversification timing. The main corollary is that the attainment of a general theory on Neotropical diversification is being delayed by conceptual, rather than methodological causes. Some solutions are proposed based on the Chamberlin's multiple-working-hypotheses scheme and a conceptual research framework to address the problem from this perspective is suggested.

1. Introduction

This paper reviews the historical development of research on the origin of Neotropical biodiversity under a conceptual perspective, in an attempt to identify the main factors that have hindered the attainment of a holistic theory on this subject. The Neotropics, or the American tropics, are among the most biodiverse regions in the world. A synthetic perspective on the origin and maintenance of such diversity would be relevant to understand the global Latitudinal Biodiversity Gradients (LBGs), one of the most pervasive global macroecological patterns on Earth (Lomolino et al 2010; Cox et al 2016). In addition, understanding how extant biodiversity and its major geographical patterns have been generated and sustained is needed for better informed conservation and restoration practices (Winter et al 2013; Dietl et al 2015).

The review emphasizes the environmental drivers and the evolutionary processes that have contributed to the origination of Neotropical species, ecological mechanisms favoring species' coexistence and biodiversity maintenance will be addressed when appropriate but are treated in more depth elsewhere (Wright 2002; Mittelbach et al 2007; Cannon and Lerdau 2015; Usinowicz et al 2017). Following the definitions of Pielou (1975), the measure of diversity employed in this paper is species diversity, that is, the number of species in a given geographical context. Other measures such as ecological diversity, which also considers the relative abundance of each species, are not analyzed. Species richness is here considered at local (α -diversity) and regional (γ -diversity) levels; diversity trends along environmental gradients or across regions or ecosystems/biomes (β -diversity) and other spatial patterns (Anderson et al 2011; Wiegand et al 2017) are not under scrutiny.

Technical or specialized jargon and concepts have been avoided or appropriately explained, due to the transdisciplinary character of the topic, the variety of research fields and methodological approaches involved and the general interest that the topic may have for a wide scientific audience, including advanced students. However, basic knowledge on general disciplines (as for example geology, evolution, ecology, biogeography or genetics, among others) is required. It should also be stressed that, given the great amount of literature available on Neotropical diversification and the continued publication of numerous papers on this subject, the literature provided here is by no means exhaustive but a selection aimed to be, at least, representative. The use of papers on diversification studies of particular taxonomic groups and/or geographical regions has been kept to a minimum; emphasis is placed on general studies, revisions, meta-analyses and other synthetic views that address the problem of Neotropical diversity from more general perspectives. It should also be stressed that evidence-based studies and models are preferred. Top-down modelling approaches using flawed biological assumptions, as for example species' equivalence –i.e., the purported irrelevance of specific biological traits and particular niche features- to favor previously established stochastic processes (e.g., Colwell and Hurt 1994, Hubbell 2001, Scheffer et al 2018), are not analyzed. This paper is mainly a personal approach to the problem and uses data, concepts and arguments already published by the author elsewhere, in which case the corresponding references are provided in order to avoid self-plagiarism and eventual double-publication claims (Gutiérrez and Block 2013; Rosenzweig and Schnitzer 2013). This is the only reason why own author's citations are overrepresented with respect to others.

The paper has been subdivided into four sections for more clarity. In order to provide a worldwide perspective, the first part briefly summarizes the issue of the LBGs and their potential causes. The second part emphasizes the comparatively higher Neotropical biodiversity with respect to other tropical areas and how this has been framed in global conservation strategies. The third section provides a historical account on the development of hypotheses about Neotropical diversification and the nature of the evidence used to propose and to test them. The

fourth section emphasizes several conceptual and methodological constraints that have slowed the progress towards a synthesis on the origin and maintenance of Neotropical diversity and proposes some potential solutions. Finally, some hints are provided on the possible future research trends on Neotropical diversification, with emphasis on the mentioned conceptual constraints.

2. The latitudinal biodiversity gradients

The occurrence of Earth's biodiversity gradients from the species-rich tropics to the almost barren poles is a classical biogeographical topic since the late 18th century, when the first biogeographical explorations took place (Lomolino et al 2010). Johann Forster, who travelled around the world with the famous Captain James Cook, was the first to note the latitudinal trends in plant diversity attributing this fact to the increased intensity of heat towards the tropics (Forster 1778). Since then, the LBGs have been recognized in many terrestrial and marine organisms, as well as in total biodiversity patterns, and is now considered a first-order biogeographical pattern (Rosenzweig 1995; Willig et al 2003; Hildebrand 2004; Krug et al 2009; Yasuhara et al 2012). The LBGs do not seem to be only a present-day-only feature; paleontological studies have shown that similar patterns were already present during several Paleozoic and Cenozoic phases, especially those characterized by global cooling or 'icehouse' phases. Contrarily, the Mesozoic 'greenhouse' phase seems to have been characterized by reverse patterns characterized by depleted tropical biodiversity and extra-tropical richness peaks (Mannion et al 2014). Understanding the environmental drivers and the ecological and evolutionary processes and mechanisms involved in the generation and maintenance of the LBGs is still challenging (Mittelbach et al 2007; Lomolino et al 2010; Brown 2014).

In general terms, the LBGs are considered to be generated and maintained by coupled ecological and evolutionary processes. Geographical differences in origination (speciation), extinction and dispersal (*s. l.*) over evolutionary timescales seem to have been essential (Krug et al 2009; Mannion et al 2014). Ecological mechanisms related to predation, competition, colonization ability, mating behavior, germination, differential growth or habitat heterogeneity, among others, would have been responsible for minimizing extinction and favoring species coexistence thus contributing to the maintenance of a high biodiversity (Wright 2002; Mittelbach et al 2007; Stein et al 2014; Cannon and Lerda 2015; Usinowicz et al 2017). Stebbins (1974) asked whether the tropics are more diverse due to higher speciation rates (cradle hypothesis) or lower extinction rates (museum hypothesis). Others consider that net diversification –the balance between speciation and extinction- is higher in the tropics (Mittelbach et al 2007). In addition to speciation and extinction, dispersal has also been considered crucial to explain the LBG (Jablonski et al 2006). Whatever the case, the environmental drivers and the ecological and evolutionary processes involved in speciation, extinction and dispersal trends in both time and space are the object of intense debate.

Some hypotheses favor the dominant action of single factors of worldwide extent, as for example the energy-gradient hypothesis, according to which, the increasing solar energy from the poles to the equator would be responsible for increased tropical productivity thus promoting higher diversity (Connel and Orias 1964; MacArthur 1965). Others emphasize the combined action of environmental and ecological factors by assuming that higher tropical diversity is due to more stable climates, which favor ecological interaction and niche diversification, whereas lower extra-tropical diversity is due to the depleting action of harsh climates, notably the Pleistocene glaciations (Fischer 1960; Pianka 1966; Slobodkin and Sanders 1969). The time-area hypothesis proposes that the tropics have accumulated more species than the temperate zones because they

are bigger and have remained in its present geographical location for more time (Farrel et al 1992; Ricklefs and Schluter 1993). The niche-conservatism hypothesis considers that tropics are both a cradle and a museum and, as a consequence, their older species largely retain their ancestral traits and niches (Wiens and Donoghue 2004). The out-of-the-tropics hypothesis contends that the tropics are more diverse because most taxa originated in the tropics and migrated toward higher latitudes but still remaining in the tropics (Jablonski et al 2006; Jansson et al 2013). At local and regional scales, migration –the dispersal across hospitable terrains (Pielou 1979)- can be important, either as species' input (immigration) or output (emigration) (Gaston and Spicer 2005).

Until recently, these and other hypotheses about the origin and maintenance of the LBGs were tested using mainly present-day biogeographical evidence (sometimes coupled with paleontological and paleoecological data) and theoretical modelling. Yet, the recent development of molecular phylogenetic tools has revolutionized the field. Empirical testing using these new methods has supported a number of the existing hypotheses or combination of them (Brown 2014; Kerhoff et al 2014; Antonelli et al 2015; Fine 2015; Schluter 2016) but the debate continues. According to Hurlbert and Stegen (2014) and Jablonski et al (2017), the tendency to focus on single causal factors and processes is blocking progress towards a general LBG theory, which needs a more synthetic and integrative approach. Such synthesis should consider habitat and niche features, ecological interactions, evolutionary trends and biogeographical shifts, as well as their variability along environmental gradients and across temporal scales. Whittaker et al (2001) and Willis and Whittaker (2002) question the possibility of attributing global biodiversity patterns to a single causal explanation and propose a hierarchical framework that considers the more influential processes and their corresponding spatio-temporal scales (Table 1).

3. The Neotropics

In the American continents, the LBG is particularly apparent (Fig. 1). This paper is concerned with the tropical part of these continents, the ecozone known as the Neotropics (Schultz 2005), lying between the tropics of Cancer and Capricorn (Fig. 2). Therefore, the term 'Neotropical' used here refers to the Neotropics and should not be confused with the Neotropical biogeographic realm, encompassing not only the tropical Americas but also the whole South America (Lomolino et al 2010). Defined in this way, the Neotropics range from central Mexico and the Greater Antilles, to the north, to the southern end of Bolivia, to the south. The Neotropics exhibits a complex topography, ranging from the sea level to above 5000 elevation (Fig. 2) and encompasses a varied range of biomes, from the driest deserts to the more humid forests on earth (Fig. 3).

The amazing biodiversity of the Neotropics, as compared to the rest of the planet, is well illustrated by higher plants, which have been considered as surrogates of general biological diversity. For example, it has been estimated that Amazonian forests contain more tree species diversity in a square kilometre than do all the temperate forests of Europe, North America, and Asia combined (Usinowicz et al 2017). The Neotropics has also been considered the more species-rich tropical region, with ca. 100,000 species of seed plants, almost 40% of the world's total (Antonelli and Sanmartín 2011). These patterns are similar for many animal species including amphibians, mammals, birds, butterflies and reptiles, among others (Antonelli et al 2015). Therefore, the Neotropics could be considered the most biodiverse region of the world and, therefore, a well suited place to study the drivers, processes and mechanisms involved in the generation of the LBGs.

The Neotropics is also a keystone region for biodiversity conservation as manifested, for example, in the occurrence of six of the 25 world's biodiversity hotspots considered as conservation priorities, namely the Caribbean, the Mesoamerican, the Atlantic forest, the Cerrado,

the Tropical Andes and the Chocó/Darién/Western Ecuador areas (Fig. 4). A hotspot has been defined as a region holding exceptional biodiversity levels, mainly of vascular plants and vertebrates, and suffering exceptional rates of habitat loss (Myers et al 2000). The Amazon rainforests are not included in this classification because, despite their amazing biodiversity, they have not been considered to be under exceptional risk of habitat loss. However, given the current rates of rainforest destruction in Amazonia, this region might eventually be included in the list. If so, the whole Neotropics would be considered a mega-biodiversity hotspot. In addition, the Neotropics encompasses six of the 17 world's countries defined as megadiverse, namely Brazil, Colombia, Ecuador, Mexico, Peru and Venezuela (Fig. 5). As a whole, these 17 countries account for ~70% of the total world's biodiversity and bear at least 5000 endemic species, thus deserving special conservation priorities (Mittermeier et al 1997).

Therefore, although the purely academic study of the ecological and evolutionary causes of the high Neotropical biodiversity makes sense by itself, it also has fundamental practical significance in the face of direct (e.g., deforestation and other forms of habitat destruction) and indirect (notably anthropogenic global warming) threats resulting from human activities. This makes of Neotropical biodiversity research a central activity, from basic and applied perspectives, towards a more sustainable use of natural resources.

4. Historical outline

For more clarity, the historical account on the study of the origin of Neotropical biodiversity has been subdivided into four main periods: 1) the discovery of the American LBG by pioneer naturalists, 2) the first attempts to explain this LBG using present-day biogeographical patterns, 3) the incoming of paleoecological evidence to the investigations and 4) the appearance and the rapid development of molecular phylogenetic and phylogeographic methods. This does not mean that these approaches have replaced one another through history, as a number of them have eventually been combined to obtain a broader picture. This has been especially true in the last years, when some synthetic approaches have been attempted, which is briefly discussed at the end of this section.

4.1. The beginnings

Few decades after the discovery of the general LBGs by Johann R Forster (1778), between 1799 and 1804, Alexander von Humboldt did similar observations in the New World. He noted an increase not only in the number of plant species but also in “structure, grace of form, and mixture of colors, as also in perpetual youth and vigor of organic life” (von Humboldt 1850) towards the tropics, and he attributed this fact to climatic constraints (Hawkins 2001). Humboldt also realized that the latitudinal floristic gradients could be observed at more local scales in the form of elevational gradients across mountains (Lomolino et al 2010). Charles Darwin (1839) and Alfred R Wallace (1853) also highlighted the luxuriance of plant and animal life in the Neotropics, especially in Amazonia. Wallace (1878) pointed out that these pioneer observations not only revealed a major biogeographical pattern, but also implicitly hypothesized about its potential causes, with emphasis on the increasing climatic severity from the tropics to the poles and the resulting intensification of the struggle for the existence (Lomolino et al 2010).

Until the 1970s, the Neotropics –and the tropics, in general- were considered climatically stable areas that have been free from the influence of Pleistocene glaciations. In this context, diversification theories considering only rather static latitudinal energy gradients like the present ones dominated the scene. This view changed when widespread evidence of Pleistocene climatic

shifts in tropical areas started to accumulate (Damuth and Fairbridge 1970; Emiliani 1971; Ab'Saber 1982). In the Neotropics, these environmental changes were considered to have fostered biotic diversification, mainly through sea-level changes, temperature-driven elevational migrations of montane ecological belts, and intense moisture changes in the lowlands (Simpson 1971). The first general theories about Neotropical diversification considering environmental instability appeared in the third quarter of the 20th century and emphasized the role of speciation, extinction and dispersal under the influence of climate changes and topographic and paleogeographic shifts derived from continental drift.

4.2. Biogeographical inferences

The first hypotheses consisted of evolutionary inferences from extant biogeographical patterns. Based on the present-day distribution of lowland Amazon rainforests' avifauna and the assumption of severe climatic aridity during the Plesitocene glaciations, Haffer (1969) proposed that these forests would have been fragmented into isolated patches, or refugia, in a sea of non-forested vegetation, which would have favored speciation by vicariance. More humid interglacial phases would have caused forest re-expansion and coalescence, thus promoting sympatric speciation and hybridization along secondary contact zones. The same model was proposed shortly after by Vanzolini and Williams (1970), while working on lizards. These trends would have repeated several times during the Pleistocene and, although eventual extinctions were not dismissed, this recurrence would have led to forest faunas significantly more diverse than the pre-Pleistocene ones. Based on the diversity and endemism patterns of Amazonian birds and the modern distribution of precipitation, Haffer (1969) identified nine areas as potential candidates for Pleistocene glacial refugia. According to this author, the refuge hypothesis would be applicable to other animal groups such as insects, amphibians, reptiles and mammals. Further biogeographical analyses on these and other groups of organisms (e.g. butterflies and some plant families) were considered to be consistent with the refuge hypothesis predictions and this diversification model became paradigmatic (Prance 1982; Whitmore and Prance 1987). The combination of paleoclimatic, pedological, geomorphological and biogeographical evidence resulted in the mapping of the potential distribution of the presumed refugia for the lowland Neotropics (Fig. 6). A similar diversification scenario was proposed for the avifauna of the Andes, where interglacial upward migration of mountain belts would have led to the isolation of high-mountain biomes thus favoring vicariance, whereas glacial downward migration would have fostered coalescence and gene flow (Simpson 1971). Again, the main supporting evidence were present-day biogeographical patterns.

According to Raven and Axelrod (1974), Pleistocene climate changes would have been important in the shaping of present-day Neotropical biodiversity, but pre-Pleistocene paleogeographic changes linked to continental drift were considered to be essential for the formation of modern biotas. These authors discussed the topic using the worldwide distribution of angiosperms and vertebrates, and their migrational possibilities during the Cenozoic, in the context of a changing physical context characterized by (i) dramatic changes in land-sea geographical patterns, (ii) the uplift of new cordilleras and (iii) other consequences of plate tectonics potentially responsible for the waxing and waning of migration pathways and barriers. The debate between the relevance of either Pleistocene climate changes or pre-Pleistocene (mostly Cenozoic) tectonic events on the shaping of Neotropical biodiversity started with Gentry (1982), who explicitly raised the dilemma in the title of its influential paper. Using the biogeographical patterns of the extant angiosperm flora, mainly at genera and family levels, this author concluded that the Pliocene closure of the Panama Isthmus and the Mio-Pliocene Andean

uplift were the most significant events for the origin of the high Neotropical diversity (Fig. 7). The Panama Isthmus (Fig. 2) would have provided the pathway for northern temperate taxa to colonize South America in the so called Great American Biotic Interchange (GABI) (Marshall et al 1982). The Andean orogeny would have created new montane habitats that allowed northern biotas to persist and migrate southward. According to Gentry (1982), “The evolutionary phenomena associated with the Andean uplift account for almost half of the total Neotropical flora and are thus largely responsible for the excess floristic richness of the Neotropics” (p. 589). Pleistocene climate changes, although discussed, were not considered to have the same importance as the mentioned tectonically-driven processes (Fig. 7). This author considered that co-evolutionary relationships with pollinators played a prominent role in Neotropical plant evolution.

4.3. The inception of paleoecology

In the late 1980s, Quaternary paleoecological evidence was added to the debate. After an extensive literature review, Colinvaux (1987) did not find enough evidence to support glacial aridity during the Last Glacial Maximum (LGM), occurred ca. 21,000 years ago. In addition, further palynological analyses documented the continuity of lowland Amazon forests during the LGM, which contradicted the predictions of the refuge hypothesis concerning forest fragmentation (Colinvaux et al 1996). Instead, these forests would have changed their composition due to downward migration of montane species caused by cooling, moderate precipitation reduction and atmospheric CO₂ depletion (Bush 1994). Acting together, these phenomena could have provided the physical basis to explain the observed biogeographical patterns without the need for the refuge hypothesis (Colinvaux et al 2000). This was called the disturbance-vicariance hypothesis. However, this view was not shared by all paleoecologists working on the Neotropics and the initial Haffer’s idea of potential glacial forest refugia on current Amazon areas of high precipitation remained (van der Hammen and Hooghiemstra 2000). A debate settled that still persists nowadays between the defenders and the detractors of the refuge hypothesis.

The proponents of the disturbance-vicariance hypothesis vividly combated the refuge hypothesis and emphasized the importance of the Andean uplift and the stability of the Amazon forests through the Cenozoic (Colinvaux and De Oliveira 2001). These authors noted that the modern Amazon basin was formed during the Miocene, when the Andean uplift caused a major shift in the drainage patterns of northern South America. Indeed, until the early Miocene, most of the drainage of the present western Amazon region was directed to the north but in the late Miocene, the uplift that shaped the modern Andes drastically changed this pattern and formed the huge Amazon and Orinoco river systems draining to the Atlantic Ocean (Hoorn et al 1995). In addition, some preliminary palynological data suggested that many taxa of the modern Amazon forests were already present in the middle Miocene and climates were warm tropical (Hoorn 1994). This, combined with their own evidence of forest continuity during the last glaciation, led Colinvaux and De Oliveira (2001) to propose that present-like Amazon rainforests had been present and stable since the middle Miocene, in agreement with the museum hypothesis of the high Neotropical biodiversity, as a consequence of reduced extinction due to high environmental stability. The refuge hypothesis was considered useful to explain modern biogeographical patterns in temperate areas but unlikely for the Neotropics (Willis and Whittaker 2000; Bush and De Oliveira 2006). The Neogene origin of modern Amazon rainforests was considered to be supported by palynological and paleobotanical data (Jaramillo et al 2010).

Besides the refuge hypothesis, the disturbance-vicariance hypothesis and the hypotheses emphasizing tectonically-driven processes such as the Andean uplift or the closure of the Panama

Isthmus, other existing proposals for Neotropical diversification involving Pleistocene and Neogene drivers and processes were summarized by Haffer (1997). According to the river hypothesis, the ancestral widespread and uniform populations of Amazonian animals were fragmented and isolated by the progressive development of the Amazon fluvial network during the Neogene and the early Quaternary, thus favoring allopatric speciation. A variant is the river-refuge hypothesis, according to which forest refugia would have been separated by non-forested vegetation and also by rivers, or by both, depending on the case. The gradient hypothesis predicted parapatric speciation across step environmental gradients and was assumed to be active in marginal populations even in the absence of physical disconnection. Nores (1999) observed a consistent pattern of high-diversity spots in areas above 100 m elevation in the Amazon lowlands and hypothesized that sea-level increases of this magnitude would have isolated these areas thus favoring vicariance. A variant of the refuge hypothesis is the vanishing refuge model, according to which, refugial areas should not necessarily be constant through time and may experience habitat changes thus providing new adaptive opportunities (Damasceno et al 2014). Another possibility suggested by Rull et al (1989) contends that Neotropical biota would have survived the cold and possibly arid glacial climates in small but diverse and widespread clusters, or microrefugia, under special local microclimatic conditions (see also Rull 2009, 2010).

4.4. The molecular revolution

In the last decades, the methodological refinement of molecular DNA phylogenetic methods has revolutionized the study of biological diversification by providing phylogenetic trees for extant species that can be calibrated in time units and mapped, thus providing spatiotemporal evidence on speciation events. In the Neotropics, the first studies of this type found a contrasting pattern between the Andes and the Amazon lowlands. In the Andes, many endemic bird species were of recent origin, probably due to the habitat complexity created by the relatively recent uplift. In the Amazon lowlands, on the contrary, many endemisms were Neogene phyletic relicts, which was considered to support the hypothesis of the long-standing Neotropical stability (Fjelds  1994). Similar patterns found in other animal taxa (amphibia, reptiles and small mammals) were used to question the refuge hypothesis for the lowland Amazon rainforest faunas (Moritz et al 2000). In other biomes, however, elements of both Neogene and Pleistocene diversification were recognized (Pennington et al 2004; Carnaval and Bates 2007). During the 1990s and the early 2000s, molecular phylogenetic and phylogeographic studies on particular groups of Neotropical organisms proliferated and a variety of diversification drivers –i.e., glacial aridity, temperature and CO₂ depletion, Andean uplift or the Panama closure- were proposed according to the case and the geographical area (Rull 2008 and references therein). In spite of this dramatic increase in the amount of evidence to unravel the origin of Neotropical diversity, the polarization between the Pleistocene refuge hypothesis and the Neogene tectonic hypotheses continued. A synthetic effort seemed thus pertinent to organize and understand this body of knowledge in the search for potential regularities.

A first meta-analysis was performed on >1400 species (105 genera and other supra-specific lineages) with dated molecular phylogenies encompassing the whole Neotropics –a unusual feature in previous literature, mainly concerned with the Amazon basin- covering a wide range of terrestrial and marine organism groups (amphibians, arachnids, birds, corals, echinoderms, fishes, insects, mammals, mollusks, reptiles and plants) (Rull 2008). These species originated in a continual manner since the Eocene-Oligocene until the Pleistocene and no diversification bursts were observed at any particular time (Fig. 8). Nearly the half of these species emerged during the Neogene, while the other half were of Pleistocene origin (Fig. 9). This suggested that extant

Neotropical biodiversity originated through a diversity of drivers and evolutionary mechanisms. Therefore, it was proposed that the dual simplistic Neogene-Pleistocene controversy did not make sense as the current Neotropical biodiversity was likely the result of a complex interplay of ecological and evolutionary processes across spatial and temporal scales, initiated by Neogene tectonic events and continued under the action of Pleistocene climatic changes (Rull 2011a).

A further meta-analysis on almost 190 Amazon genera of plants and animals (insects, fishes, amphibians and birds) with dated phylogenies found that most of these genera emerged during the Neogene (Fig. 10) and concluded that current Neotropical biodiversity patterns originated before the Pleistocene and that the Mio-Pliocene Andean uplift was a paramount diversification driver (Hoorn et al 2010). This study used the dating of the initial diversification event of each genus represented by the crown node or the node of the crown group, which is the monophyletic clade that contains all extant members of the genus (Quental and Marshall 2010). Several types of geological, paleontological, paleoecological and paleoclimatic evidence was provided in support of this Neogene hypothesis (Hoorn and Wesselingh 2010). According to this view, the Andean uplift not only changed drastically the drainage and climatic patterns of northern South America, but also created new montane environments and a complex topography that provided new opportunities for *in situ* speciation and a dispersal barrier promoting vicariance at both sides of the cordillera (Antonelli et al 2010; Hoorn et al 2013). Pleistocene diversification was explicitly dismissed (Antonelli and Sanmartín 2011). The same team of authors argued that Miocene diversification was fundamental for the origin of extant Neotropical biodiversity and contended that “the Quaternary was a time of distribution shifts, but can no longer be considered a time of diversification in Amazonia”, rather, “the Quaternary should possibly be considered as a period of net loss of biodiversity” (Wesselingh et al 2010, p 421). This return to the Neogene paradigm was further debated on the basis of conceptual and methodological features (Hoorn et al 2011, Rull 2011b), which will be discussed later on. Rull (2014), using recent molecular phylogeographic evidence of intense Pleistocene diversification amongst many Andean lineages, insisted on the combined action of mountain building and climatic change in the shaping of current Neotropical biodiversity. The potential effect of Pleistocene climate changes were illustrated for two extra-Andean mountain complexes such as the Atlantic Forest, in the southwestern Brazilian coasts (Fig. 3), and the Guayana Highlands (Fig. 2), between the Orinoco and the Amazon basins (Rull 2004, 2005; Carnaval and Bates 2007; Carnaval and Moritz 2008).

Another meta-analysis (Turchetto-Zolet et al 2013) of molecular phylogeographies of ca. 480 South American species of algae, plants, invertebrates, fishes, amphibians, reptiles, birds and mammals including many tropical lineages, reported again similar percentages of Pleistocene and pre-Pleistocene diversification thus reinforcing the idea that the current diversity patterns of the Neotropics were shaped by the combination of Pleistocene climatic oscillations and Mio-Pliocene tectonic events (Fig. 11). Moreover, the idea of the Pleistocene as a time of extinction was not supported by empirical evidence. Estimating extinction using molecular phylogenetic data is problematic as it deals with the genome of living organisms (e.g., Quental and Marshall 2010; Pyron and Burbrink 2013). Extinction trends through time are better approached using the paleoecological record. Using this type of evidence, Willis and Bhagwat (2009) highlighted that, in plants, there is a single extinction documented for the entire Pleistocene, whereas in animals, the more significant extinctions recorded are the disappearance of large mammals during the Late Pleistocene-Early Holocene, which have been related to climate changes and human activities. Palaeoecological evidence has shown that, rather than extinction, Pleistocene glacial-interglacial changes have promoted large scale migrations and range shifts, which have been decisive for the shaping of present-day biogeographical patterns and the assembly of extant communities (Willis & Bhagwat, 2009). This, combined with the mounting molecular phylogenetic evidence of

Pleistocene speciation, suggested that the Pleistocene was a time of net diversification in the Neotropics thus supporting the view of a continuous diversification trend since the Neogene to the Pleistocene (Rull 2012a).

Molecular phylogenetic results have also been used to suggest inter-continental long-distance dispersal, a process not contemplated in the classical studies previously mentioned about the role of migration in the shaping of Neotropical diversity (Raven and Axelrod 1974; Gentry 1982). For example, Cody et al (2010) found evidence that plants would have been able to cross the Isthmus of Panama region well before its closure, which suggested that the GABI started earlier for plants likely due to the possibility of long-distance dispersal across marine barriers (but see also Carrillo et al 2015). A fascinating idea is the possibility of trans-oceanic dispersal between Africa and South America, which is also supported by a number of molecular plant phylogenies and would have been important for the assembly of present-day Amazonian rainforest flora (Pennington and Dick 2004; Weeks et al 2014). Antonelli et al (2015) contended that the Neotropics has been an engine for global plant diversity, as species' emigration from the American tropics has largely exceeded immigration during the Cenozoic. This would support, in part, the out-of-the-tropics hypothesis for the occurrence of LBGs. Dispersal ability has been proposed as an important diversification mechanism even within the Neotropics. For example, Smith et al (2014) argued that landscape change is not needed to explain the current biogeographical patterns of Neotropical birds at species level, which is more consistent with a model that combines persistence and dispersal across the landscape matrix followed by allopatric speciation. Similar results were obtained by Dexter et al (2017) for several genera of Amazonian trees and Prates et al (2016a, 2017) for lizards, although in this last case, dispersal seem to have been triggered by Pleistocene climate changes.

The amount of molecular phylogenetic studies on Neotropical taxa has greatly increased since the beginning of this century (Fig. 12) thus providing new opportunities for synthetic approaches. Some attempts in this direction have been made in the last years. For example, Hughes et al (2013) recognized the complexity of Neotropical diversification but insisted on the Neogene paradigm by arguing that a cluster of geological and climatic events occurred during this period –i.e., the Late Miocene global cooling, the latest Andean uplift occurred 9-10 Ma and the formation of the Panama landbridge ca. 3.5 Ma (Fig. 7)- coincided with the preponderance of Mid to Late Miocene crown ages of species-rich Neotropical clades thus suggesting that the Miocene was “a pivotal time for the establishment and diversification of the modern Neotropical flora and that a very large fraction of extant Neotropical plant species diversity has arisen within the last 10 million years” (p. 13). These authors suggested that niche conservatism and high speciation rates, rather than low extinction rates, would have been fundamental for Neotropical diversification (museum hypothesis). Pleistocene diversification was not addressed.

A cursory examination of the great amount of studies on Neotropical diversification suggests that different taxonomic groups would have been submitted to different drivers, processes and mechanisms across time and space, which might lead some to believe that there is no a general explanation for the high Neotropical biodiversity. The identification of potential general patterns would require a comprehensive Neotropical database of molecular phylogenetic studies at species level and a reanalysis of older phylogenetic trees using the latest-generation dating methods (Rull 2011a). An attempt in this direction is a recent synthesis based on a very large worldwide plant database (ca. 22,600 species) and updated methods of chronological calibration found that the comparatively higher Neotropical diversity was associated with higher speciation and extinction rates than in tropical Asia and Africa (Antonelli et al 2015). This was interpreted in terms of a higher species turnover and shorter average species longevity, which was preliminary attributed to the considerable landscape dynamics that has affected the Neotropics

since the Miocene, as well as to differences in biome size, niche space and climatic history, with respect to other tropical areas. Using a similar approach, the same research team performed a meta-analysis on 4450 species of varied taxonomic categories and biomes from the whole Neotropics and concluded that Amazonia was the main source of Neotropical biodiversity, as >2800 lineages originated in this region and dispersed to other Neotropical regions, especially to Mesoamerica (Antonelli et al 2018). Again, biotic turnover among regions was considered the main diversification factor. According to these authors, such biotic interchange would have been active during the last >60 million years with a general increase towards the present.

Together with these recent synthetic attempts, more and more case studies on a wide range of Neotropical organisms subjected to a variety of diversification drivers and ecological constraints appear almost every week in specialized journals and edited books. A number of these studies insist on the Neogene-Pleistocene controversy and try to make generalizations while others do not but this debate is still alive. A general impression is that the Neogene paradigm has experienced a revitalization whereas the Pleistocene paradigm has been comparatively underrated, some even consider that “the effects of Quaternary climatic changes on Neotropical rain forest vegetation remain something as a black hole in Neotropical biogeography” (Cavers and Dick 2013, p 616). This could be due to the difficulty of demonstrating a causal relationship between past fragmentation and extant diversity patterns and also to the unfeasibility of attributing speciation events to particular Pleistocene shifts using the current tools for dating phylogenetic trees (Pennington and Dick 2010). A further effort is needed for data storing, processing and integration of the great amount of information on Neotropical diversification that is produced every year. But merely the sum of all the available information will not suffice to attain a holistic perspective and a change of strategy seems necessary. Hughes et al (2013), suggested to study diversification patterns separately in different biomes to include not only geological and climatic drivers but also the ecological constraints involved in the assembly of present-day biotas. Examples of such procedure on the assembly of a wide range of Neotropical forested and non-forested biomes are Simon et al (2009), Pennington et al (2010), Särkinen et al (2012), Erickson et al (2014), Willis et al (2014) and Honorio Coronado et al (2015), among others.

5. Conceptual and methodological insights

The history of research on Neotropical diversification has been more than a succession of hypotheses and theories trying to explain new evidence provided by novel methodological tools and the improvement of existing ones. There is a conceptual background behind all of the debates and discussions mentioned above that is worth to be highlighted. Such theoretical developments have not necessarily proceeded parallel to the methodological improvements; some concepts and views have maintained through time, others have been replaced and others have been revitalized with the advent of new evidence that was previously unavailable. This section briefly discusses the more relevant conceptual developments, with emphasis on those that have hindered the advancement towards a synthetic theory of Neotropical diversification. Such account might seem irrelevant as many of the topics discussed would be considered by some as obvious, even trivial issues. However, a number of anachronistic views and fruitless debates still persist in the study of Neotropical diversification that should be revised if a truly holistic diversification framework is to be achieved. The main flawed approaches discussed here are: 1) the shifting from one paradigm to another, 2) the extrapolation from particular case studies to the whole Neotropics, 3) the selection of different lines of evidence –notably different taxonomic categories- to support either one or another hypothesis, 4) the implicit assumption that Pleistocene diversification equals to refuge diversification and 5) the straightforward inference of diversification drivers from diversification

timing (Rull 2012a, 2013, 2015). These drawbacks are briefly discussed and several potential solutions are suggested.

5.1. Paradigm shifting

As discussed in the historical section, the biogeographically-based Pleistocene refuge hypothesis was paradigmatic until the late 1980s (Whitmore and Prance 1987), when paleoecological data started to be used to reject such explanation in favor of the hypothesis of an assumed stability and continuity of the Amazon rainforests since the middle Miocene until the present (Colinvaux and De Oliveira 2000). Further molecular phylogenetic studies were used to dismiss Pleistocene diversification, supporting the Neogene hypothesis, and advocated for tectonically-driven events such as the Andean orogeny or the closure of the Panama Isthmus as the main diversification drivers (Hoorn et al 2010). This shift from the refuge hypothesis paradigm to the Neogene paradigm was radical and many scholars considered that these hypotheses were not only opposed but also excluding. It not unusual that the defenders of either one or another of these hypotheses highlight the evidence needed to support their preferred explanation ignoring contradictory evidence. In the late 20th century, the refuge hypothesis was the favorite explanation but in the last decades, the Neogene hypothesis seems to be preferred. Shifting from one paradigm to another is unlikely to result in progress towards a better understanding of Neotropical diversification (Rull 2013). In light of the available evidence, the continued debate between the Pleistocene and the Neogene paradigms as excluding explanations has been considered rather simplistic, outdated and sterile (Rull 2008).

5.2. Unwarranted generalizations

Another flawed approach is to use case studies of particular geographical regions and/or groups of organisms to try to develop general theories on Neotropical diversification. There are some taxonomic groups whose diversification patterns are consistent with a particular type of diversification driver, as for example the refuge hypothesis (Haffer 1969), the microrefuge hypothesis (Rull and Montoya 2014), the vanishing refuge hypothesis (Damasceno et al 2014), the disturbance-vicariance hypothesis (Noonan and Gaucher 2005), the river hypothesis (Ribas et al 2011), the Andean uplift (Hughes and Eastwood 2006) or the closure of the Panama isthmus (Cody et al 2010), among others. But this does not mean that each of these drivers can be extrapolated to the whole Neotropics. The high physiographic, environmental and biotic heterogeneity of the Neotropics, together with the long history of paleogeographic reorganizations and paleoclimatic changes (Hoorn and Wesselingh 2010), precludes any generalization from selected case studies. In this context, it is noteworthy that the Amazon lowlands, especially the rainforests they support, have been the object of the majority of Neotropical diversification studies and there is the danger of considering these environments as representatives of the whole Neotropics. For example, multidisciplinary studies including GIS modelling, paleoecology and molecular phylogenetics (Carnaval and Moritz 2008; Carnaval et al 2009, 2014; Porto et al 2013) seem to be consistent with the existence of a complex patterns of Pleistocene forest refugia in the so called Atlantic forest, situated at the south-eastern Brazilian coasts (Fig. 3). Therefore, any extrapolation from the Amazon or the Atlantic forest models to the whole Neotropics would be unwarranted. The tendency to infer general trends from particular examples was more frequent in earlier times and has notably decreased with time and the flourishing of diversification studies but, unfortunately, it has not been yet completely eradicated. It should also be highlighted that most studies on Neotropical diversification have been carried out

on terrestrial organisms and the validity of the conclusions obtained for marine environments is not granted.

5.3. Taxonomic resolution

Choosing the appropriate taxonomic category is essential in diversification studies. Moritz (1994) defined the Evolutionary Significant Unit (ESU) as the lower taxonomic unit needed to represent the actual genetic variability associated with a distinct evolutionary potential and considered the ESU as the preferred category for conservation purposes (Moritz 2002). Usually, ESUs coincide with species or sub-specific categories, which are the commonly used to define current biodiversity patterns. More recent approaches based on Bayesian phylogenetics and phylogeography (BPP) methods have been used for species delimitation as an alternative to ESUs (Fujita and Leaché 2010). Both ESUs and BPP methods emphasize the suitability of specific and sub-specific categories for biodiversity assessment. Supra-specific categories, as for example genera, are unreliable biodiversity descriptors as they may contain a wide range of internal diversity, from one to thousand or more species (Rull 2012a). Therefore, unraveling the origin and maintenance of current Neotropical biodiversity should be preferably addressed using species and sub-specific units, that is, ESUs. Paleontological and paleoecological studies have important limitations in this sense, as identification at species level is rare. However, molecular phylogenetic studies are able to attain specific and sub-specific resolution thus rightly reproducing true genetic variability relevant for evolutionary potential. In spite of this, a number of phylogenetic and phylogeographic studies on the origin of Neotropical diversity are performed using genera and other supra-specific categories (e.g., Hoorn et al 2010). Using species or genera makes a great difference in the final results obtained, as the second underestimate recent diversification and, therefore, the evolutionary origin of extant ESUs. A good example of using either one or another taxonomic category can be found in the Gimnosperm order of the Cycadales, commonly called cycads.

The cycads have been traditionally considered as 'living fossils', as their extinct representatives with almost the same morphological characteristics as the living ones are present in 280 million years old Permian rocks. However, a recent study has revealed that most of the ca. 300 extant cycad species originated during the last 12 million years (Nagalingum et al 2011). The more diverse Neotropical genus of cycads is *Zamia*, with almost 80 species. Nagalingum et al (2011) studied 35 of these species and found that this genus separated from *Microzamia* during the Oligocene and started to diversify in the Late Miocene (Fig. 13). This would suggest that some event occurred during the Late Miocene would have been the responsible for the diversification of this genus. But a closer examination reveals that only one species, the oldest one, is of Miocene origin, whereas 10 species originated during Pliocene and the remaining 24 emerged in the Pleistocene (Rull 2012b). Therefore, further Pliocene and Pleistocene events would have been involved for the origin of most studied species. The lesson is that using crown dating alone, the Neogene paradigm would be favored (e.g., Hoorn et al 2010, Hughes et al 2013), whereas using species dating, the idea of a complex diversification process involving Miocene, Pliocene and Pleistocene drivers and processes seems more realistic (Rull 2011a, 2012a). In summary, crown dating is useful to identify the initial diversification event and its possible causes, whereas species dating is needed to understand the origin of the full range of extant diversity, at the ESU level, and infer the potential drivers involved (Rull 2013).

Taxonomic resolution is also important in paleoecology. For example, the palynological evidence used to support to the Neogene origin of modern Amazon rainforests (Jaramillo et al 2010) is weak as is not based on actual species but on fossil species with assumed taxonomic

affinities with extant families and genera. Besides the reliability of such affinities, the fragmentary nature and the low taxonomic resolution of the fossil record is not enough to firmly assess that modern Amazon forests originated in the Neogene and remained constant through time (see also Prates et al 2015).

5.4. Pleistocene diversification and refugia

There is also a tendency, among a number of researchers, to assume, explicitly or not, that Pleistocene diversification should have followed the model of the refuge hypothesis. Indeed, many defenders of this hypothesis implicitly accept that demonstrating Pleistocene diversification supports refuge diversification (e.g., Garzón-Orduña et al 2014), whereas many critics believe that dismissing Pleistocene diversification automatically rejects diversification on glacial refugia (e.g., Hoorn et al. 2010). However, as has been explained in the historical section, there are other diversification models for the Pleistocene as for example the disturbance-vicariance hypothesis, the river hypothesis and its variants, the gradient hypothesis, the vanishing refuge hypothesis or the microrefugia hypothesis, among others (Haffer 1997, Nores 1999, Rull 2009, Antonelli and Sanmartín 2011, Antonelli et al 2010; Damasceno et al, 2014; Rull and Montoya 2014). Therefore, when Pleistocene diversification is supported by empirical data, a careful examination of the potential mechanisms involved should be addressed, rather than relying only in the refuge model.

5.5. Diversification timing and drivers

It is also common to deduce eventual diversification drivers and mechanisms simply from the chronological coincidence between the age of diversification of a given lineage and its species and the occurrence of some specific or singular geological or environmental events. This is the case, for example, of the studies that observe a diversification event at the time of the Andean uplift or the occurrence of Pleistocene glaciations and automatically link the biodiversity increases with these phenomena. Examples are numerous as this is a usual procedure. In essence, this is not a flawed procedure but it should only be considered the first step of a more in-depth research needed to identify the ecological and evolutionary processes and mechanisms unequivocally relating the geological/environmental shift with the diversification trends observed. For example, the Pliocene was a time of intense environmental shifts and paleogeographic reorganizations including the latest Andean uplift, the closure of the Panama Isthmus and the onset of the global cooling that ended in Pleistocene glaciations. These events occurred in a narrow temporal window between ca. 4 and ca. 3 million years before present (Coates et al 1992, Mora et al 2010, McKay et al 2013), a time interval that could be accommodated within the statistical error of phylogenetic tree dating. Therefore, the increasing speciation trend occurred during the Pliocene (Figs. 8, 10 and 11) could be attributed to any of these events or a combination of them but the exact drivers, processes and mechanisms involved cannot be evaluated using solely this chronological coincidence.

5.6. Potential solutions

An obvious solution is to avoid the above shortcomings but this seems to be more difficult than expected given their persistence in time. Some conceptual tools addressed to provide new comprehensive theoretical frameworks may be helpful. A first step could be the adoption of an open-minded attitude able to address the full spatio-temporal complexity of the problem. One possibility is the use of the multiple working hypotheses framework proposed more than a century ago by Thomas Chamberlin (1890). According to this author, we humans are prone to attribute a

phenomenon to a single cause and tend to erect premature or precipitate explanations turning into ruling theories that direct research. Indeed, when a ruling theory is established, most efforts are oriented to finding evidence to either support or reject it, depending on individual interests. This is what happened with the refuge theory for Neotropical diversification. Alternatively, a potential explanation for empirical observations can be considered as a working hypothesis to be tested, rather than a ruling theory. According to Chamberlin (1890), however, the risk of a working hypothesis becoming a controlling idea remains high, as it is occurring at present with the Neogene paradigm for Neotropical diversification. The multiple working hypotheses framework seeks to explore any explanation possible for the observed phenomena and to develop every testable hypothesis in relation to the possible causes. This approach promotes thoroughness, suggests lines of inquiry that might otherwise be overlooked and develops the habit of parallel and complex thought (Chamberlin 1890). This approach would seem trivial but it is surprisingly unusual in the study of Neotropical diversification (Rull 2013), where the Pleistocene and the Neogene ruling theories are still alive and remain as excluding options for many, especially those who ignore or explicitly dismiss Pleistocene diversification (e.g., Antonelli and Sanmartín 2011, Wesselingh et al 2010; Hughes et al 2013).

Using the multiple hypotheses approach requires at least six conceptual steps to relate the extant biodiversity patterns with their corresponding speciation drivers, processes and mechanisms (Rull 2015) (Fig. 14). First, the research question should be clear, in order to focus the type of study to be developed and the more suitable empirical evidence to be seek. The second step is to use empirical data to estimate the speciation timing and establish the more likely chronological correlations with the candidate environmental drivers. In order to avoid circularity, it is essential that the corresponding phylogenies have been dated with events different from these drivers (Baker et al 2014). For example, we cannot calibrate a phylogenetic tree with the age of the closure of the Isthmus of Panama and then try to test the influence of this event on speciation. The next step is critical and is the analysis of the potential speciation patterns and processes, that is, the more likely speciation modes and their geographical expression. Using the same example, the closure of the Panama Isthmus reversed the evolutionary trends in terrestrial and marine organisms leading to allopatric speciation in the sea and gene flow in the land. Finally, speciation is not instantaneous but proceeds through a variety of microevolutionary mechanisms involving genotypic changes at the population level occurring within the community that, in turn, is changing via ecological succession. For example, in the case of speciation across and along the Panama Isthmus, ecological processes and genetic mechanisms associated with reproductive isolation and gene flow should have been more relevant than, for instance, genetic drift. Addressing all these points with empirical data in each single study would be difficult but any suggestion about potential relationships between speciation timing and causes should be evaluated in light of the possible speciation modes and their geographical expression, as well as the ecological processes and genetic mechanisms possibly involved. Some models trying to integrate all these elements to explain Neotropical diversity are in progress (Graham et al 2014; Collevatti et al 2015, Beheregaray et al 2015) but studies across different spatial, temporal and biological scales are still poorly addressed.

Any conceptual change must accommodate the continued methodological improvements in the disciplines that provide empirical evidence for data bases and meta-analyses, notably paleoecology and molecular phylogenetics. One of the most decisive paleoecological drawbacks is taxonomic resolution as expressed in the difficulty of attaining the species level in fossil identification (Rull 2014). Some advances in this venture include the introduction of biomarker analysis, as for example DNA analysis of well-preserved fossils (i.e., ancient DNA) and also the study of free sedimentary DNA and other molecules that allow identifying the presence of non-

fossilized organisms (Brown and Barnes 2015, Key et al 2017, Marx 2017). Another recent innovation is the use of Fourier transform infrared spectroscopy (FTIR), a promising technique to increase taxonomic resolution, especially in palynology (Jardine et al 2017). Regarding molecular phylogenetics, constant progresses are made in dating phylogenetic trees (Laurin 2012, Pyron and Burbrink 2013, Hipsley and Müller 2014, van Tuinen and Torres 2015), which makes necessary the reconsideration of earlier data using old dating methods -as for example those assuming constant mutation rates and extrapolating them among different taxonomic groups- in order to make them comparable with latest studies using updated dating methods. Ideally, data bases should contain the raw phylogenetic information needed for dating, rather than solely the ages obtained when this information was produced. In this way, the ages of species and the clades they form could be constantly updated and homogenized. It would be especially useful to increase dating resolution and minimize statistical errors, in order to attain a higher precision in the dating of diversification events. This would contribute to enhance the reliability of chronological coincidences between speciation and geological/environmental events thus improving the formulation and testing of causal hypotheses, especially in more recent times. The possibility of reliably estimating extinction using molecular phylogenetics would be important to obtain a more complete picture of this parameter, which estimation currently relies on the fragmentary and taxonomically imprecise fossil record. New approaches using Bayesian approaches combined with biogeographic and paleontological models are under way (Sanmartín and Meseguer 2016). Finally, it should be stressed that increasing the number, quality and interdisciplinarity of raw data to be stored in databases is not enough for fruitful collaboration among the different disciplines that participate in the study of Neotropical diversification.

6. Future directions

Usually, a section with this title is attempted to discuss potential methodological developments useful for raising new hypotheses and/or improving and enhancing the empirical basis for their testing. In the case of Neotropical diversification, methodological research is undoubtedly very important and some hints have already been provided in the former section on potential solutions. However, as it has been seen in the historical section, methodological progress alone has not been enough thus far to develop a general theory on the origin and maintenance of Neotropical biodiversity and this is not expected to occur in the near future without a change in the current conceptual framework of most studies. In other words, progress towards a general theory of Neotropical biodiversity would be hampered by conceptual, rather than methodological constraints. In this sense, two aspects seem to be of paramount importance, namely the need for truly multidisciplinary approaches and the abandonment of premeditated ruling theories. Rather than pursuing evidence for either supporting or rejecting biased and incomplete hypotheses, an open-minded multiple working hypotheses framework including evolutionary, ecological, biogeographical and geological considerations, among others, is urgently needed. Recent synthetic attempts using phylogenetic and phylogeographical evidence are strongly biased towards the Neogene paradigm and ignore or explicitly dismiss the abundant body of evidence on Pleistocene diversification. This seems to be a reaction to the past attachment of many studies to the refuge hypothesis, which has been erroneously considered by many as a synonym of Pleistocene speciation.

The multidisciplinary nature of the team works addressing these aspects of the problem should not be restricted to the combination of different research fields but also of different points of view on the problem. Research teams should be open to approaches different from the paradigms they defend; otherwise, the origin and maintenance of Neotropical diversity will remain

enigmatic, no matter the money invested, the amount of people involved and the variety of research disciplines participating in its study. The Neotropical mystery will hardly be solved by big projects with the participation of large and multidisciplinary teams, whose researchers are engaged in a single paradigm or ruling theory. Figure 14 is an attempt to provide a basis for a conceptual framework based on the multiple working hypotheses idea. It is hoped that this could be improved, perhaps even deeply modified, in the future in order to include all the available possibilities in the progress towards a holistic theory of Neotropical diversification.

7. Final remarks

The idea of a general theory of Neotropical diversification, as well as for the origin of the LBGs, is underlying most studies on this topic. However, it could be asked whether such general theory really exists or it is just a human expectancy. Biodiversity gradients are complex features and the fact that we describe this pattern with a single term does not mean that it is due to a single factor. As mentioned above, different diversification drivers and mechanisms have produced different diversity patterns across regions and taxonomic groups. Does this mean that there is no a general explanation for the high Neotropical biodiversity and we should go case by case, biome by biome or region by region? (Prates et al 2016b). It is hard to escape the idea that a general pattern should have a general explanation. The question is whether it is possible to accommodate all spatial heterogeneity, ecological disparity and temporal variability into a single conceptual framework. One of the more general approaches is the Stebbins' (1974) cradle vs. museum model and its variants, based on the occurrence of latitudinal gradients in speciation and extinction rates. However, such a general approach is primarily descriptive and does not provide causal explanations. The same is true for other general approaches as for example the energy-gradient hypothesis or the climate-stability hypothesis (McGlone 1996). On the contrary, specific case studies on particular taxonomic groups and/or geographical regions suggest fairly detailed, although very heterogeneous, causal diversification relationships. Thus, there seems to be an inverse relationship between generality and explanatory power that would prevent to attain a satisfactory explanation of general validity.

But progresses towards a unifying theory with sufficient explanatory power are worth to be done. Among the options available to date, a combination of the hierarchical framework of Willis and Whittaker (2002) (Table 1) and the multiple hypothesis framework of Chamberlin (1890), as expressed in Fig. 14, might be useful to address the issue of Neotropical diversification. Under such approach, the clear definition of the research question and its taxonomic, spatial and temporal scope appears as a fundamental premise to progress in the right direction. Alternative ways of thinking could also help. For example, the usual question: why the Neotropics is so diverse?, which has directed the research to date, implicitly assumes that lower extra-tropical diversity is the norm and the comparatively higher tropical diversity requires an explanation. Such fallacy is a cultural bias resulting from the extrapolation of ecological and evolutionary principles obtained in temperate regions to the tropics, whose study started later and was initially conducted by scholars from temperate countries (Raby 2017). In the reverse situation, lower extra-tropical diversity would have been the unusual feature to be explained.

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Table 1. A hierarchical framework for processes influencing biodiversity (simplified from Willis and Whittaker 2002).

Spatial scale	Species richness	Environmental variables	Temporal scale
Local	Within communities and habitat patches	Fine-scale biotic and abiotic interactions	1-100 years
Landscape	Between communities	Soils, elevation, peninsula effect	100-1000 years
Regional	Within continents	Radiation budget, water availability, area, latitude	Last 10,000 years
Continental	Across continents	Pleistocene glacial/interglacial cycles, mountain-building events, aridification	Last 1-10 million years
Global	Between continents and geographical realms	Continental drift, sea-level changes	Last 10-100 million years

References

Ab'Saber AN (1982) The paleoclimate and paleoecology of Brazilian Amazonia. In: Prance G (ed) Biological diversification in the tropics. Columbia Univ Press, New York, p 41-59

Anderson MJ, Crist TO, Chase JM et al (2011) Navigating the multiple meanings of α diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14:19-28

Antonelli A, Sanmartín I (2011) Why are there so many plants species in the Neotropics? *Taxon* 60:403-414

Antonelli A, Quijada-Mascareñas A, Crawford AJ et al (2010) Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. In: Hoorn C, Wesselingh F (eds) Amazonia: landscape and species evolution. A look into the past. Wiley-Blackwell, Chichester, p 386-404

Antonelli A, Zizka A, Silvestro D et al (2015) An engine for global plant diversity: highest evolutionary turnover and emigration in the American tropics. *Front Genet* 6:130, doi 10.3389/fgene.2015.00130

Antonelli A, Zizka A, Antunes F et al (2018) Amazonia is the primary source of Neotropical biodiversity. *Proc Natl Acad Sci USA* 115:6034-6039.

Baker PA, Fritz SC, Dick CW et al (2014) The emerging field of geogenomics: constraining geological problems with genetic data. *Earth-Sci Rev* 135:38-47

Beheregaray LB, Cook GM, Chao NL, Landguth EL (2015) Ecological speciation in the tropics: insights from comparative genetic studies in Amazonia. *Front Genet* 5:477, doi 10.3389/fgene.2014.00477

Brown JH (2014) Why are there so many species in the tropics? *J Biogeogr* 41:8-22

Brown TA and Barnes IM (2015) The current and future applications of ancient DNA in Quaternary science. *J Quat Sci* 30:144-153

Bush MB (1994) Amazonian speciation: a necessarily complex model. *J Biogeogr* 21:5-17

Bush MB, De Oliverira PE (2006) The rise and fall of the Refugial Hypothesis of Amazon speciation: a paleoecological perspective. *Biota Neotrop* 6:1, doi 10.1590/S1676-06032006000100002

Cannon CH, Lerda (2015) variable mating behaviors and the maintenance of tropical biodiversity. *Front Genet* 6:183, doi 10.3389/fgene.2015.00183

Carnaval AC, Bates JM (2007) Amphibian DNA shows marked genetic structure and tracks Pleistocene climate change in northeastern Brazil. *Evolution* 61:2942-2957

Carnaval AC, Moritz C (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *J Biogeogr* 35:1187-1201

Carnaval AC, Hickerson MJ, Haddad CFB et al (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science* 323:785-789

Carnaval AC, Waltari E, Rodrigues MT et al (2014) Prediction of phylogeographic endemism in an environmental complex biome. *Proc R Soc B* 281:20141461

Carrillo JD, Forasiepi A, Jaramillo C, Sánchez-Villagra MR (2015) Neotropical mammal diversity and the Great American Biotic Interchange: spatial and temporal variation in South America's fossil record. *Front Genet* 6:451, doi 10.3389/fgene.2014.00451

Cavers S, Dick CW (2013) Phylogeography of neotropical trees. *J Biogeogr* 40:615-617

Chamberlin TC (1890) The method of multiple working hypotheses. *Science (old series)* 15:92-96 (reprinted 1965, 148:754-759)

Coates AG (1992) Closure of the isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. *Geol Soc Am Bull* 104:814-828

Cody S, Richardson JE, Rull V, Ellis C, Pennington RT (2010) The Great American Biotic Interchange revisited. *Ecography* 33:326-332

Cohen KM, Finney SC, Gibbard PL, Fan J-X (2013) The ICS International Stratigraphic Chart. *Episodes* 36:199-204

Colinvaux PA (1987) Amazon diversity in light of the paleoecological record. *Quat Sci Rev* 6:93-114

Colinvaux PA, De Oliveira PE (2001) Amazon plant diversity and climate through the Cenozoic. *Palaeogeogr Palaeoclimatol Palaeoecol* 166:51-63

Colinvaux PA, De Oliveira PE, Bush MB (2000) Amazonian and neotropical plant communities on glacial time-scales: the failure of the aridity and refuge hypothesis. *Quat Sci Rev* 19:141-169

Colinvaux PA, De Oliveira PE, Moreno JE, Miller MC, Bush MB (1996) A long pollen record from lowland Amazonia: forest and cooling in glacial times. *Science* 274:85-88

Collevatti RG, Terribile LC, Diniz-Filho JAF, Lima-Ribeiro MS (2015) Multimodel inference in comparative phylogeography: an integrative approach based on multiple lines of evidence. *Front Genet* 6:31, doi 10.3389/fgene.2015.00031

Colwell RK, Hurt GC (1994) Non-biological gradients in species richness and a spurious Rapoport effect. *Am Nat* 144:57-595

Connell JH, Orias E (1964) The ecological regulation of species diversity. *Am Nat* 98:399-414

Cox CB, Moore PD, Ladle R (2016) *Biogeography: an ecological and evolutionary approach*. Wiley-Blackwell, Chichester

- Damasceno R, Strangas ML, Carnaval AC et al (2014) Revisiting the vanishing refuge model of diversification. *Front Genet* 5:353, doi 10.3389/fgene.2014.00353
- Damuth JE, Fairbridge, RW (1970) Equatorial Atlantic deep-sea arkosic sands and ice-age aridity in tropical South America. *Bull Geol Soc Am* 81:189-206
- Darwin C (1839) *Journal of the researches into the geology and natural history of various countries visited by HMS Beagle from 1832 to 1836*. H Colburn, London
- Dexter KG, Lavin M, Torke BM et al (2017) Dispersal assembly of rain forest tree communities across the Amazon basin. *Proc Natl Acad Sci USA* 114:2645-2650
- Dietl GP, Kidwell SM, Brenner M et al (2015) Conservation paleobiology: leveraging knowledge of the past to inform conservation and restoration. *Annu Rev Earth Planet Sci* 43:79-103
- Emiliani C (1971) The amplitude of Pleistocene climatic cycles at low latitudes and the isotopic composition of glacial ice. In: Turekian KK (ed) *The Late Cenozoic glacial ages*. Yale Univ Press, New Haven, p 183-197
- Erickson DL, Jones FA, Swenson NG et al (2014) Comparative evolutionary diversity and phylogenetic structure across multiple forest dynamics plots: a mega-phylogeny approach. *Front Genet* 5:358, doi 10.3389/fgene.2014.00358
- Farrell BD, Mitter C, Futuyma DJ (1992) Diversification at the insect-plant interface. *BioScience* 42:34-42
- Fine PVA (2015) Ecological and evolutionary drivers of geographic variation in species diversity. *Annu Rev Ecol Evol Syst* 46:369-392
- Fischer AG (1960) Latitudinal variation in organic diversity. *Evolution* 14:64-81
- Fjelds  J (1994) Geographical patterns of relict and young species of birds in Africa and South America and implications for conservation priorities. *Biodivers Conserv* 3:207-226
- Forster JR (1778) *Observations made during a voyage made round the world, on physical geography, natural history and ethic philosophy*. G Robinson, London
- Fujita MK, Leach  AD (2010) A coalescent perspective on delimiting and naming species: a reply to Bauer et al. *Proc R Soc B*, doi 10.1098/rspb.2010.1864
- Garz n-Ordu a JJ, Benetti-Longhini JE, Brower AVZ (2014) Timing the diversification of the Amazonian biota: butterfly divergences are consistent with Pleistocene refugia. *J Biogeogr* 41:1631-1638
- Gentry AH (1982) Neotropical floristic diversity: phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Ann Missouri Bot Gard* 69:557-593

- Gaston KJ, Spicer JI (2005) *Biodiversity, an introduction*. Blackwell, Oxford
- Graham CH, Carnaval AC, Cadena CD et al (2014) The origin and maintenance of montane diversity: integrating evolutionary and ecological processes. *Ecography* 37:1-9
- Gutiérrez RJ, Block B (2013) Self-plagiarism in publishing. *J Wildlife Manag* 77:1487-1488
- Haffer J (1969) Speciation in Amazonian forest birds. *Science* 165:131-137
- Haffer J (1997) Alternative models of vertebrate speciation in Amazonia: an overview. *Biodiv Cons* 6:451-476
- Hawkins BA (2001) Ecology's oldest pattern? *Trends Ecol Evol* 16:470
- Hildebrand H (2004) On the generality of the latitudinal diversity gradient. *Am Nat* 163:192-211
- Hipsley CA, Müller J (2014) Beyond fossil calibrations: realities of molecular clock practices in evolutionary biology. *Front Genet* 5:138, doi 10.3389/fgene.2014.00138
- Honorio Coronado EN, Dexter KG, Pennington RT et al (2015) Phylogenetic diversity of Amazonian tree communities. *Divers Distrib* 21:1295-1307
- Hoorn C (1994) An environmental reconstruction of the paleo-Amazon river system (Middle-Late Miocene, NW Amazonia). *Palaeogeogr Palaeoclimatol Palaeoecol* 112:187-238
- Hoorn C, Wesselingh F (2010) *Amazonia: landscape and species evolution. A look into the past*. Wiley-Blackwell, Chichester
- Hoorn C, Guerrero J, Sarmiento GA, Lorente MA (1995) Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* 23:237-241
- Hoorn C, Mosbrugger V, Mulch A, Antonelli A (2013) Biodiversity from mountain building. *Nat Geosci* 6:154
- Hoorn C, Wesselingh FP, ter Steege H et al (2010) Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330:927-931
- Hoorn C, Wesselingh FP, ter Steege H et al (2011) Origins of biodiversity-response. *Science* 331:399-400
- Hubbell SP (2001) *The unified neutral theory of biodiversity and biogeography*. Princeton Univ Press, Princeton
- Hughes C, Eastwood R (2006) Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. *Proc Natl Acad Sci USA* 103:10334-10339
- Hughes CE, Pennington RT, Antonelli A (2013) Neotropical plant evolution: assembling the big picture. *Bot J Linn Soc* 171:1-18

Hurlbert AH, Stegen JC (2014) On the processes generating latitudinal richness gradients: identifying diagnostic patterns and predictions. *Front Genet* 5:420, doi 10.3389/fgene.2014.00420

Jablonski D, Roy K, Valentine JW (2006) Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. *Science* 314:102-106

Jablonski D, Huang S, Roy K, Valentine JW (2017) Shaping the latitudinal diversity gradient: new perspectives from a synthesis of paleobiology and biogeography. *Am Nat* 189:1-12

Jansson R, Rodríguez-Castañeda G, Harding LE (2013) What can multiple phylogenies say about the latitudinal diversity gradient? A new look at the tropical conservatism, out of the tropics, and diversification rate hypotheses. *Evolution* 67:1741-1755

Jaramillo C, Hoorn C, Silva SAF et al (2010) The origin of the modern Amazon rainforest: implications of the palynological and paleobotanical record. In: Hoorn C, Wesselingh F (eds) *Amazonia: landscape and species evolution. A look into the past*. Wiley-Blackwell, Chichester, p 317-334

Jardine PE, Abernethy FAJ, Lomax BH et al (2017) Shedding light on sporopollenin chemistry, with reference to UV reconstructions. *Rev Palaeobot Palynol* 238:1-6

Kerhoff AJ, Moriarty PE, Weiser MD (2014) The latitudinal richness gradient in New World woody angiosperms is consistent with the tropical conservatism hypothesis. *Proc. Natl Acad Sci USA* 111:8125-8130

Key FM, Posth C, Krause J et al (2017) Mining metagenomic data sets for ancient DNA: recommended protocols for authentication. *Trends Genet* 33:508-520

Krug AZ, Jablonski D, Valentine JW, Roy K (2009) Generation of earth's first-order biodiversity pattern. *Astrobiology* 9:113-124

Laurin M (2012) Recent progresses in paleontological methods for dating the tree of life. *Front Genet* 3:130, doi 10.3389/fgene.2012.00130

Lomolino MV, Riddle BR, Brown JH, Whittaker RJ (2010) *Biogeography*. Sinauer, Sunderland

MacArthur RH (1965) Patterns of species diversity. *Biol Rev* 40:510-533

Mannion PD, Upchurch P, Benson RBJ, Goswami A (2014) The latitudinal diversity gradient through deep time. *Trends Ecol Evol* 29:42-50

Marshall LG, Webb SD, Sepkoski JJ, Raup DM (1982) Mammalian evolution and the great American biotic interchange. *Science* 215:1351-1357

Marx V (2017) Genetics: new tales from ancient DNA. *Nature Methods* 14:771-774

- McGlone MS (1996) When history matters: scale, time, climate and tree diversity. *Glob Ecol Biogeogr Lett* 5:309-314
- McKay R, Naish T, Carter L et al (2012) Antarctic and Southern Ocean influences on Late Pleistocene global cooling. *Proc natl Sci USA* 109:6423-6428
- Mittelbach GG, Schemske D, Cornell HV et al (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol Lett* 10:315-331
- Mittermeier RA, Mittermeier CG, Robles P (1997) Megadiversity: earth's biologically wealthiest nations. CEMEX, Mexico
- Mora A, Baby P, Roddaz M et al (2010) tectonic history of the Andes and sub-Andean zones: implications for the development of the Amazon drainage basin. In: Hoorn C, Wesselingh F (eds) Amazonia: landscape and species evolution. A look into the past. Wiley-Blackwell, Chichester, P 39-60
- Moritz C (1994) Defining 'evolutionarily significant units' for conservation purposes. *Trends Ecol Evol* 9:373-375
- Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Syst Biol* 51:238-254
- Moritz C, Patton JL, Schneider CJ, Smith TB (2000) Diversification of rainforest faunas: an integrated molecular approach. *Annu Rev Ecol Syst* 31:533-563
- Mutke J, Bathlott W (2005) Patterns of vascular plant diversity at continental to global scales. *Biol Skr* 55:521-531
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853-858
- Nagalingum NS, Marshall CR, Quental TB et al (2011) Recent synchronous radiation of a living fossil. *Science* 334:796-799
- Noonan BP, Gaucher P (2005) Phylogeography and demography of Guyanan harlequin toads (*Atelopus*): diversification within a refuge. *Mol Ecol* 14:3017-3031
- Nores M (1999) An alternative hypothesis for the origin of Amazonian bird diversity. *J Biogeogr* 26:475-485
- Olson DM, Dinerstein E, Wikramanayake ED et al (2001) Terrestrial ecoregions of the world: a new map of life on Earth. *BioScience* 51:933-938
- Pennington RT, Dick CW (2004) The role immigrants in the assembly of South American rainforest flora. *Phil Trans R Soc London* 359:1611-1622

- Pennington RT, Dick CW (2010) Diversification of the Amazon flora and its relation to key geological and environmental events: a molecular perspective. In: Hoorn C, Wesselingh F (eds) Amazonia: landscape and species evolution. A look into the past. Wiley-Blackwell, Chichester, p 373-385
- Pennington RT, Prado LM, Pendry CA, Pell SK, Butterworth CA (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. *Phil Trans R Soc London* 359:515-537
- Pennington RT, Lavin M, Särkinen T et al (2010) Contrasting plant diversification histories within the Andean biodiversity hotspot. *Proc Natl Acad Sci USA* 107:13783-13787
- Pianka ER (1966) Latitudinal gradients in species diversity: a review of concepts. *Am Nat* 100:33-46
- Pielou EC (1975) *Ecological diversity*. John Wiley & Sons, New York
- Pielou EC (1979) *Biogeography*. Wiley, New York
- Porto TJ, Carnaval AC, da Rocha PLB (2013) Evaluating forest refugia models using species distribution models, model filling and inclusion: a case study with 14 Brazilian species. *J Biogeogr* 19:330-340
- Prance GT (1982) *Biological diversification in the tropics*. Columbia Univ Press, New York
- Prates I, Rodrigues MT, Melo-Sampaio PR, Carnaval AC (2015) Phylogenetic relationships of Amazonian anole lizards (*Dactyloa*): taxonomic implications, new insights about phenotypic evolution and the timing of diversification. *Mol Phylogenet Evol* 82:258-268
- Prates I, Rivera D, Rodrigues MT, Carnaval AC (2016a) A mid-Pleistocene rainforest corridor enabled synchronous invasions of the Atlantic Forest by Amazonian anole lizards. *Mol Ecol* 25:5174-5186
- Prates I, Xue AT, Brown JL, Alvarado-Serrano DF et al (2016b) Inferring responses to climate dynamics from historical demography in neotropical forest lizards. *Proc Natl Acad Sci USA* 113:7978-1985
- Prates I, Melo-Sampaio PR, Drummond LO et al (2017) Biogeographic links between southern Atlantic Forest and western South America: rediscovery, re-description, and phylogenetic relationships of two rare montane anole lizards from Brasil. *Mol Phylogenet Evol* 113:49-58
- Pyron RA, Burbrink FT (2013) Phylogenetic estimates of speciation and extinction rates for testing ecological and evolutionary hypotheses. *Trends Ecol Evol* 28:729-736
- Quental TB, Marshall CR (2010) Diversity dynamics, molecular phylogenies need the fossil record. *Trends Ecol Evol* 25:434-441
- Raby M (2017) The colonial origins of tropical field stations. *Am Sci* 105:216-223

Raven PH, Axelrod DI (1974) Angiosperm biogeography and past continental movements. *Ann Missouri Bot Gard* 61:539-673

Ribas CC, Aleixo A, Nogueira ACR et al (2011) A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proc R Soc B* 279:681-689

Ricklefs RE, Schluter D (1993) Species diversity: regional and historical influences. In: Ricklefs RE, Schluter D (eds) *Ecological communities: historical and geographical perspectives*. Univ Chicago Press, Chicago, p 350-363

Rozenzweig ML (1995) *Species diversity in space and time*. Cambridge Univ Press, Cambridge

Rozensweig M, Schnitzer AE (2013) Self-plagiarism: perspectives for librarians. *Coll Res Libr* 74:492-494

Rull V (2004) Biogeography of the 'Lost World': a palaeoecological perspective. *Earth-Sci Rev* 67:125-137

Rull V (2005) Biotic diversification in the Guayana Highlands. *J Biogeogr* 32:921-927

Rull V (2008) Speciation timing and neotropical biodiversity: the Tertiary-Quaternary debate in the light of molecular phylogenetic evidence. *Mol Ecol* 17:2722-2729

Rull V (2009) Microrefugia. *J Biogeogr* 36:481-484

Rull V (2010) On microrefugia and cryptic refugia. *J Biogeogr* 37:1623-1627

Rull V (2011a) Neotropical biodiversity: timing and potential drivers. *Trends Ecol Evol* 26:508-513

Rull V (2011b) Origins of biodiversity. *Science* 331:398-399

Rull V (2012a) Palaeobiodiversity and taxonomic resolution: linking past trends with present patterns. *J Biogeogr* 39:1005-1006

Rull V (2012b) Cycad diversification and tropical biodiversity. *Coll Bot* 31:103-106

Rull V (2013) Some problems in the study of the origin of neotropical biodiversity using palaeoecological and molecular phylogenetic evidence. *Syst Biodiv* 11:415-423

Rull V (2014) Biodiversity, mountains and climate change. *Coll Bot* 33:75-79

Rull V (2015) Pleistocene speciation is not refuge speciation. *J Biogeogr* 42:602-609

Rull V, Montoya E (2014) *Mauritia flexuosa* palm swamp communities: natural or human-made? A palynological study of the Gran Sabana region (northern South America) within a neotropical context. *Quat Sci Rev* 99:17-33

- Rull V, Schubert C, Aravena R (1989) Palynological studies in the Venezuelan Guayana Shield: preliminary results. *Curr Res Pleist* 5:54-56
- Sanmartín I, Meseguer AS (2016) Extinction in phylogenetics and biogeography: from timetrees to patterns of biotic assemblage. *Front Genet* 7:35, doi 10.3389/fgene.2016.00035
- Särkinen T, Pennington RT, Lavin M et al (2012) Evolutionary islands in the Andes: persistence and isolation explain high endemism in Andean dry tropical forests. *J Biogeogr* 39:884-900
- Scheffer M, van Nes EH, Vergnon R (2018) Toward a unifying theory of biodiversity. *Proc Natl Acad Sci USA* 115:639-641
- Schluter D (2016) Speciation, ecological opportunity, and latitude. *Am Nat* 187:1-18
- Schultz J (2005) *The ecozones of the world: the ecological divisions of the biosphere*. Springer, Berlin
- Simon MF, Grether R, de Queiroz LP et al (2009) Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution and adaptations to fire. *Proc Natl Acad Sci USA* 106:20359-20364
- Simpson B (1971) Pleistocene changes in the flora and fauna of South America. *Science* 173:771-780
- Slobodkin LB, Sanders HL (1969) On the contribution of environmental predictability to species diversity. In: Woodwell GM, Smith HH (eds) *Ecological systems*. Brookhaven Symp Biol 22:82-93
- Smith BT, McCormack JE, Cuervo AM et al (2014) The drivers of tropical speciation. *Nature* 515:406-409
- Stein A, Gerstner K, Kreft H (2014) Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. *Ecol Lett* 17:866-880
- Stebbins GL (1974) *Flowering plants: evolution above the species level*. Belknap Press, Cambridge
- Turchetto-Zolet AC, Pinheiro F, Salgueiro F, Palma-Silva C (2012) Phylogeographical patterns shed light on evolutionary process in South America. *Mol Ecol* 22:1193-1213
- Usinowicz J, Chang-Yang CH, Chen YY et al (2017) Temporal coexistence mechanisms contribute to the latitudinal gradient in forest diversity. *Nature* doi 10.1038/nature24038
- van der Hammen T, Hooghiemstra H (2000) Neogene and Quaternary history of vegetation, climate, and plant diversity in Amazonia. *Quat Sci Rev* 19:725-742
- van Tuinen M, Torres CR (2015) Potential for bias and low precision in molecular divergence time estimation of the Canopy of Life: an example from aquatic bird families. *Front Genet* 6:203, doi 10.3389/fgene.2015.00203

Vanzolini PE, Williams EE (1970) South American anoles: the geographical differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arq Zool São Paulo* 19:1-298

von Humboldt A (1850) Views of nature: or contemplation on the sublime phenomena of creation. Harrison & Sons, London

Wallace AR (1853) A narrative of travels on the Amazon and Rio Negro. Dover, New York

Wallace AR (1878) Tropical nature and other essays. Macmillan, New York

Weeks A, Zapata F, Pell SK et al (2014) To move or to evolve: contrasting patterns of intercontinental connectivity and climatic niche evolution in "Therebintaceae" (Anacardiaceae and Burseraceae). *Front Genet* 5:409, doi 10.3389/fgene.2004.00409

Wesselingh F, Hoorn C, Kroonenberg SB et al (2010) On the origin of Amazonian landscapes and biodiversity: a synthesis. In: Hoorn C, Wesselingh F (eds) Amazonia: landscape and species evolution. A look into the past. Wiley-Blackwell, Chichester, p 421-431

Whitelock LM (2002) The cycads. Timber Press, Portland

Whitmore TC, Prance GT (1987) Biogeography and Quaternary history in tropical Latin America. Oxford Univ Press, New York

Whittaker RJ, Willis KJ, Field R (2001) Scale and species richness: towards a general, hierarchical theory of species diversity. *J Biogeogr* 28:453-470

Wiegand T, Uriarte M, Kraft NJB et al (2017) Spatially explicit metrics of species diversity, functional diversity, and phylogenetic diversity: insights into plant community assembly processes. *Annu Rev Ecol Evol Syst* 48:329-351

Wiens JJ, Donoghue MJ (2004) Historical biogeography, ecology and species richness. *Trends Ecol Evol* 19:639-644

Willig MR, Kaufman DM, Stevens RD (2003) Latitudinal gradients of biodiversity: Pattern, process, scales and synthesis. *Annu Rev Ecol Evol Syst* 34:273-309

Willis KJ, Whittaker RJ (2000) The refugial debate. *Science* 287:1406-1407

Willis KJ, Whittaker RJ (2002) Species diversity-scale matters. *Science* 295:1245-1247

Willis KJ, Bhagwat, SL (2009) Biodiversity and climate change. *Science* 326:806-807

Willis CG, Franzone BF, Xi Z, Davis CG (2014) The establishment of central American migratory corridors and the biogeographic origins of seasonally dry tropical forests in Mexico. *Front Genet* 5:433, doi 10.3389/fgene.2014.00433

Winter M, Devictor V, Schweiger O (2013) Phylogenetic diversity and nature conservation: where are we? *Trends Ecol Evol* 28:199-204

Wright JS (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130:1–14

Yasurara M, Hunt G, Dowsett HJ et al (2012) Latitudinal species diversity gradient of marine zooplankton for the last three million years. *Ecol Lett* 15:1174-1179

Figure captions

1. Latitudinal distribution of species richness among vascular plants (left) and vertebrates (right) across the American continents. Vascular plant biodiversity is expressed in species number per unit area (adapted from Mutke and Bathlott, 2005) and vertebrates as the total number of species, which is maximum at the brown side of the color spectrum and minimum at the blue side of the color spectrum (adapted from Mannion et al 2014).
2. Topographic/bathymetric map of the Neotropics with the main physiographical regions. AL – Amazon Lowlands, BE – Brazil East Coast Range, BH – Brazilian Highlands, CA – Central Andes, Cam – Central America, CS – Caribbean Sea, GA – Greater Antilles, GH – Guiana/Guayana Highlands, GM – Gulf of Mexico, LA – Lesser Antilles, MP – Mexican Plateau, NA – Northern Andes, OB – Orinoco Lowlands, PI – Panamá Isthmus, PL – Pantanal, YP – Yucatán Peninsula. Base map: NOAA National center for Environmental Information (<https://ngdc.noaa.gov/mgg/topo/globega2.html>).
3. Map of the Neotropics and its major biomes. Redrawn from Olson et al (2001).
4. Biodiversity hotspots (green areas) of the Neotropics, according to Myers et al (2000). The Amazon rainforests are also indicated although they have not yet been considered a biodiversity hotspot (see text). Modified from an original map of Wikimedia Commons (https://en.wikipedia.org/wiki/Biodiversity_hotspot), licensed by Creative Commons 3.0.
5. Megadiverse countries (green areas, yellow text) of the Neotropics according to Mittermeier et al (1997). Sovereign states from the Lesser Antilles: Antigua & Barbuda (AB), Barbados (B), Dominica (D), Grenada (G), Saint Kitts and Nevis (SK), Santa Lucia (SL), Saint Vincent and the Grenadines (SV), Trinidad and Tobago (TT).
6. Overlapping patterns of assumed glacial forest refugia areas, as deduced from paleoclimatic, pedological and geomorphological evidence, and present-day endemism centers of plants, butterflies and birds. Redrawn from Whitmore and Prance (1987).
7. Geochronological units of the International Stratigraphic Chart (Cohen et al 2013, updated 2015), and the main tectonic and climatic events discussed in the text, according to Hoorn et al 2010). The first peaks of north-central Andean building occurred at the Oligocene/Miocene transition (ca. 23 Ma) but the most intense pulses took place in the Middle Miocene (ca. 12 Ma) and the Early Pliocene (ca. 4.5 Ma). The closure of the Panama Isthmus occurred during the Pliocene (ca. 3.5 Ma).
8. Continuous Neotropical diversification trends since the Miocene until the Pleistocene, as shown by a meta-analysis of >1400 species belonging to 105 groups (genera and other supra-specific lineages). The brown solid line represents the age of the initial speciation event for each group sorted chronologically. The column plot displays the amount of these groups that initiated their speciation in each epoch, in both absolute numbers (orange bars, left scale) and rates, expressed as groups per million years (green bars, right scale). Mio – Miocene, Plio – Pliocene, Ple – Pleistocene). Redrawn from Rull (2013).
9. Percentages of species originated either during Pleistocene (blue bars) or pre-Pleistocene (orange bars) times using the same data set as in Fig. 8. Redrawn from Rull (2008).
10. Histogram and normalized density function of the ages of initial diversification events (crown nodes) of 189 Amazon living genera including plants, insects, fishes, amphibians and birds. Redrawn from Rull (2011a) after raw data from Hoorn et al (2010).
11. Timing of lineage divergence of South American taxa based on phylogeographic studies published until 2011. Note that diversification amounts (expressed in percentages) are similar for Pleistocene and pre-Pleistocene times. Redrawn from Turchetto-Zolet et al (2013).

12. Number of phylogeographical studies published between 1994 and 2010 on South American organisms, most of them from tropical areas. Redrawn from Turchetto-Zolet (2013).
13. Time-constrained phylogenetic tree for 35 species of the cycad genus *Zamia*, which diverged from *Microcycas* in the Oligocene. CN – Crown node, LM – Late Miocene, Plio – Pliocene, Ple – Pleistocene. Redrawn from Nagalingum et al (2011). Geographical distribution according to Whitelock (2002).
14. Schematic view of the conceptual steps discussed in the text for making inferences about the origin of biodiversity using paleoecological and phylogenetic evidence. Redrawn from Rull (2015).

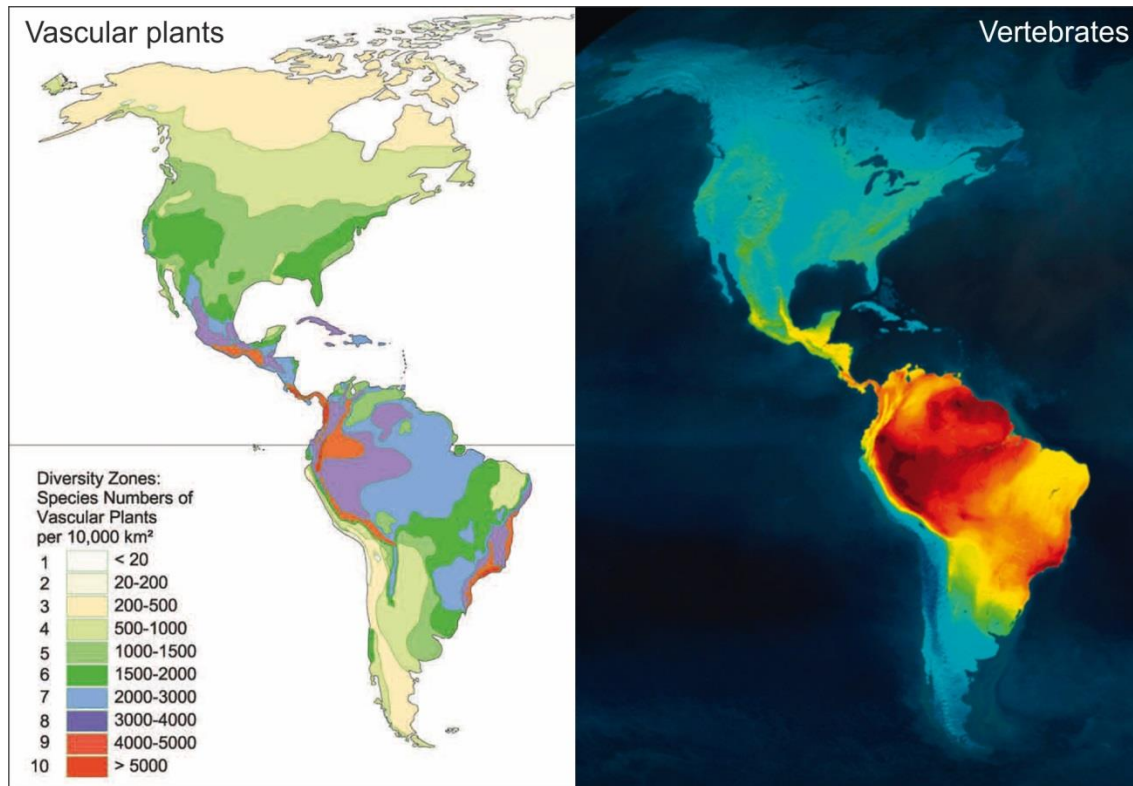


Figure 1

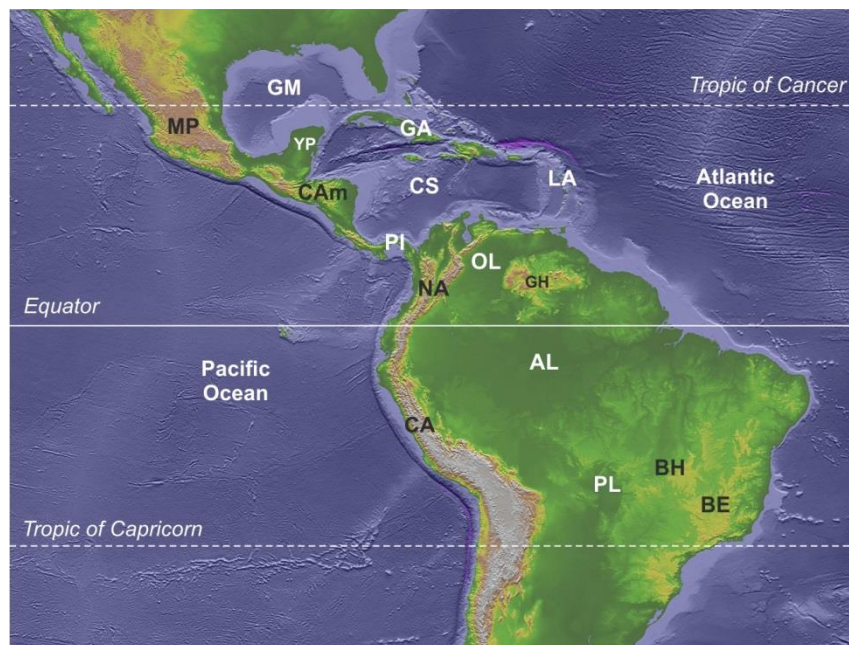


Figure 2

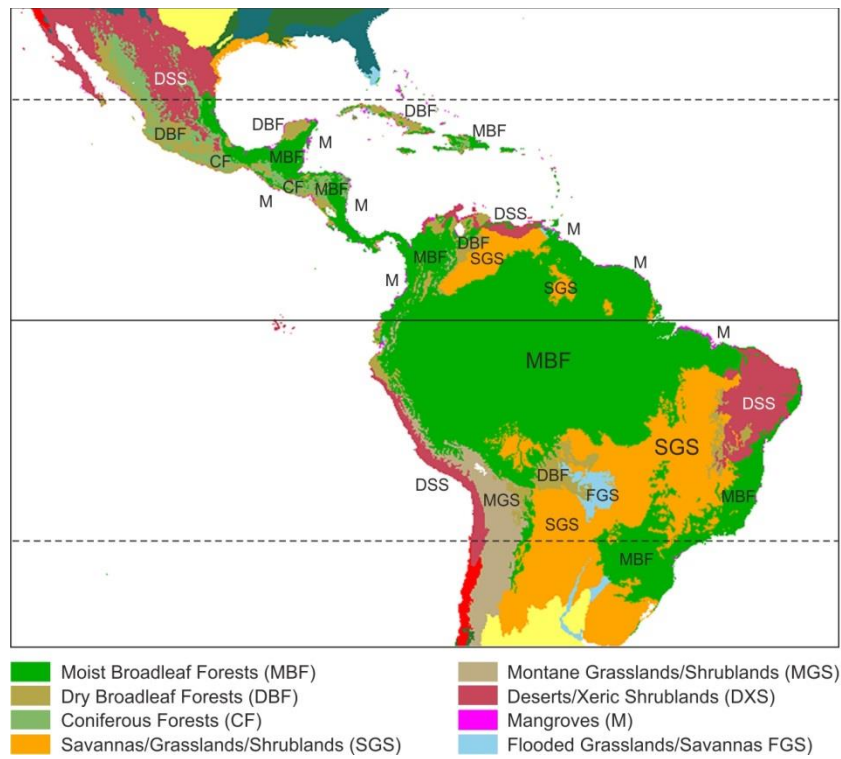


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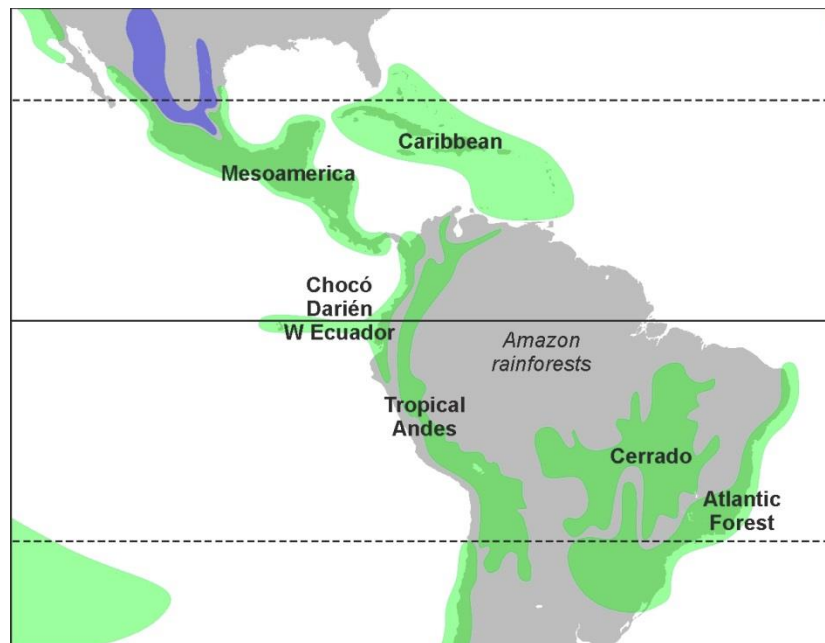


Figure 4



Figure 5

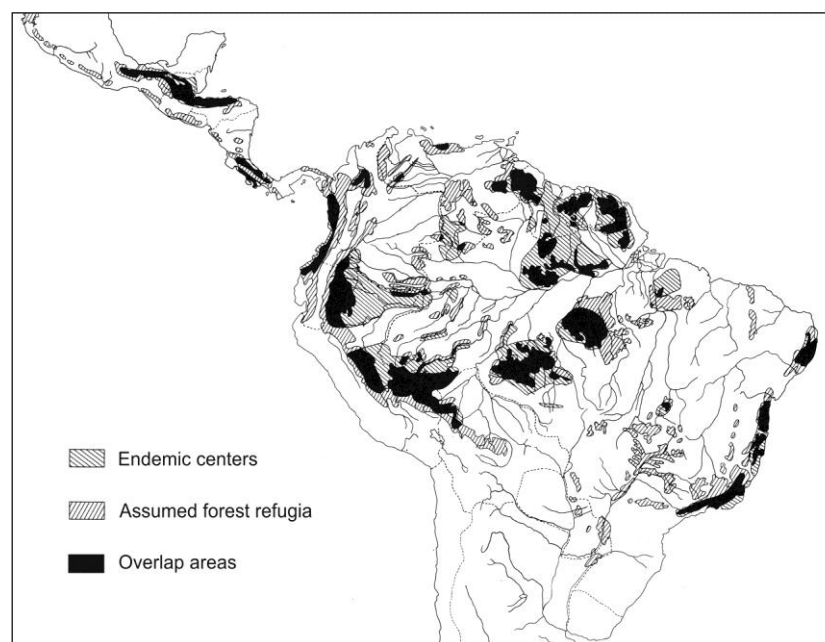


Figure 6

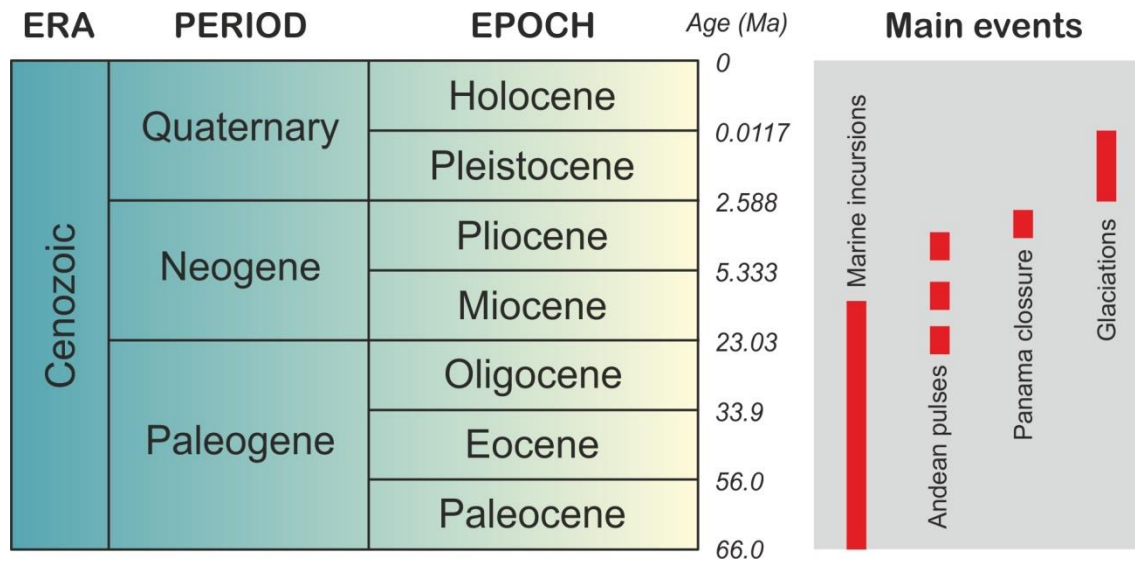


Figure 7

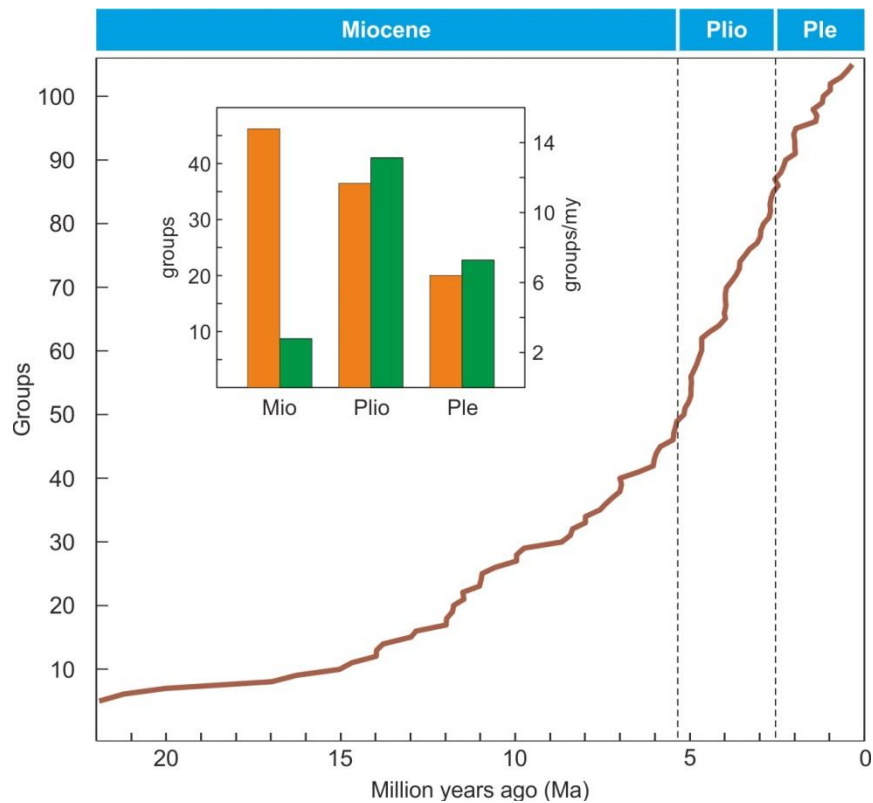


Figure 8

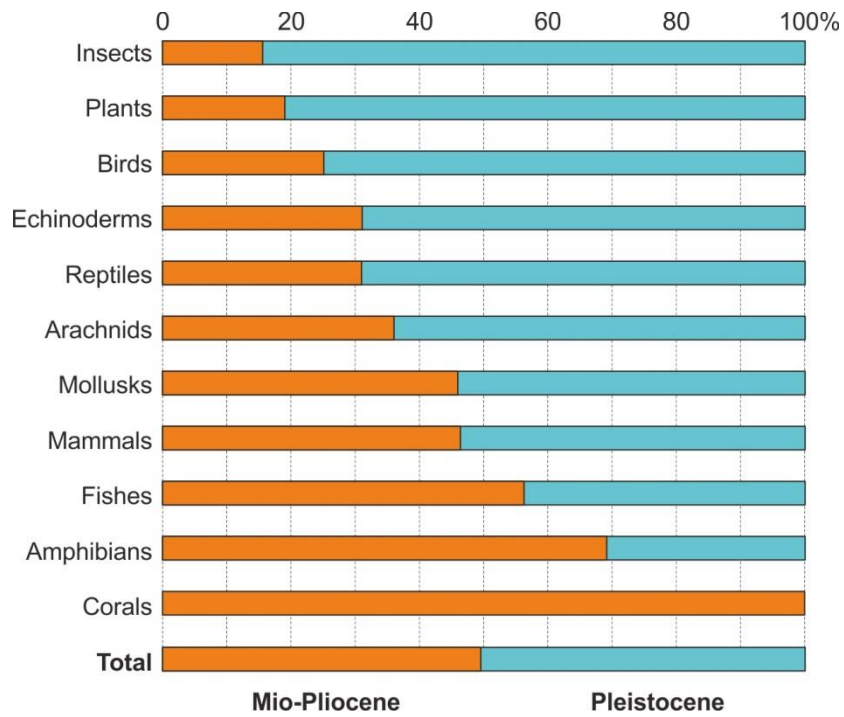


Figure 9

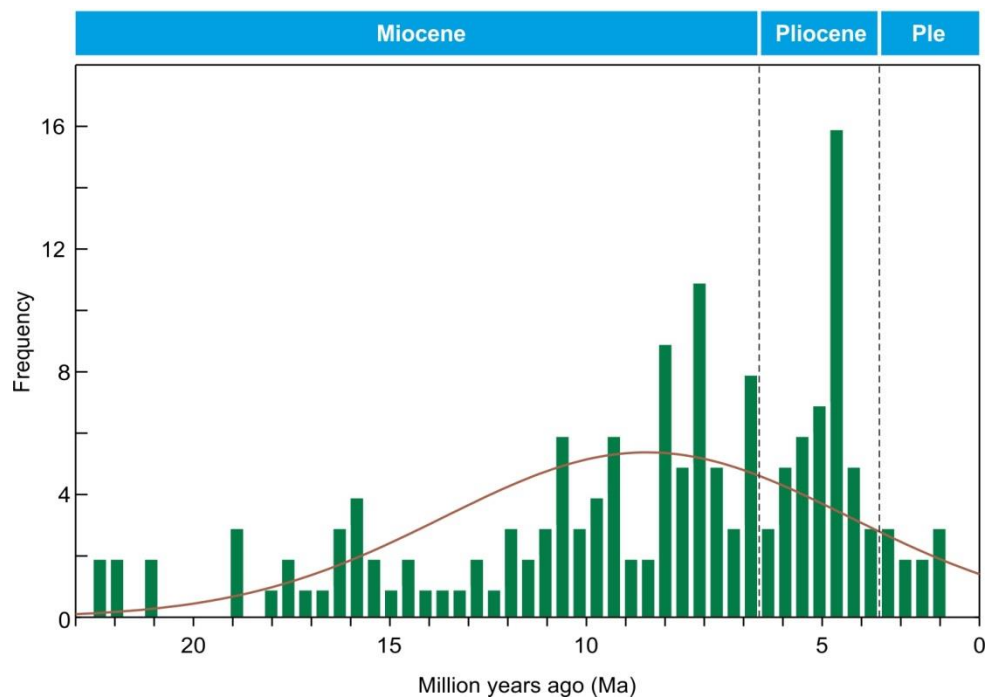


Figure 10

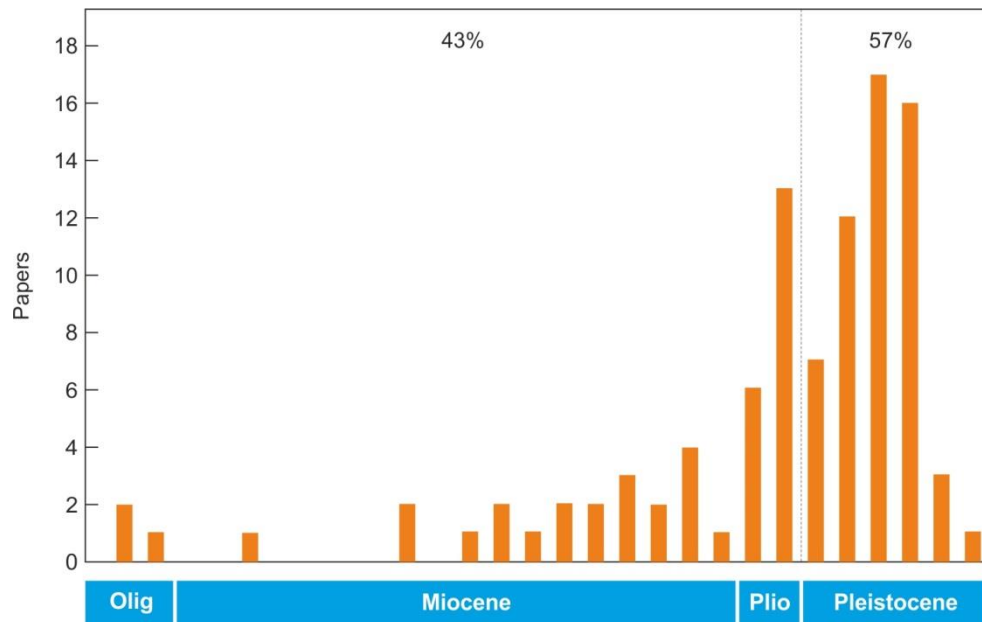


Figure 11

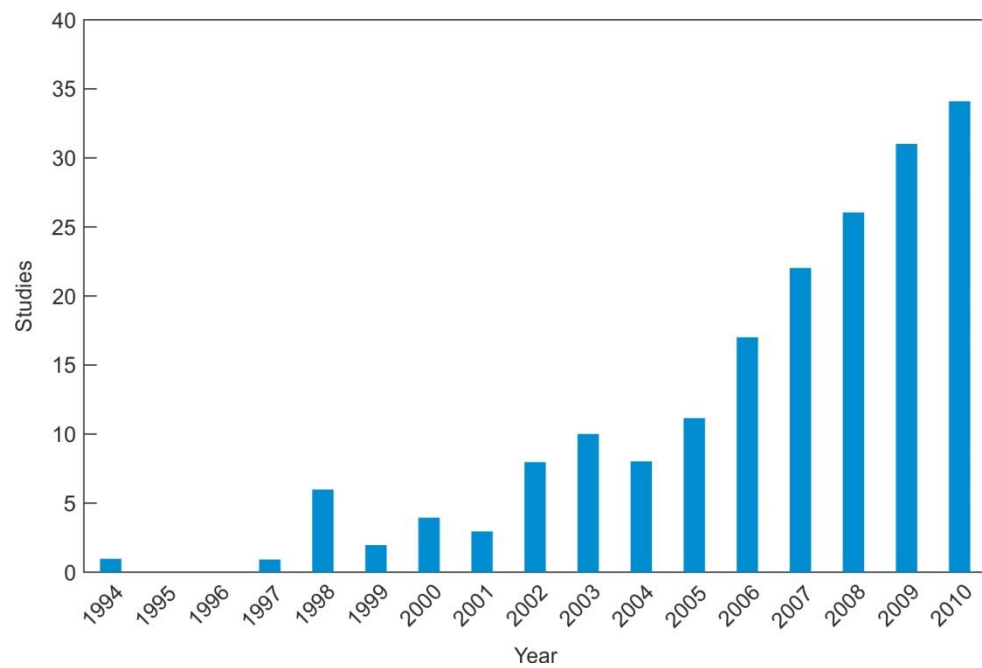


Figure 12

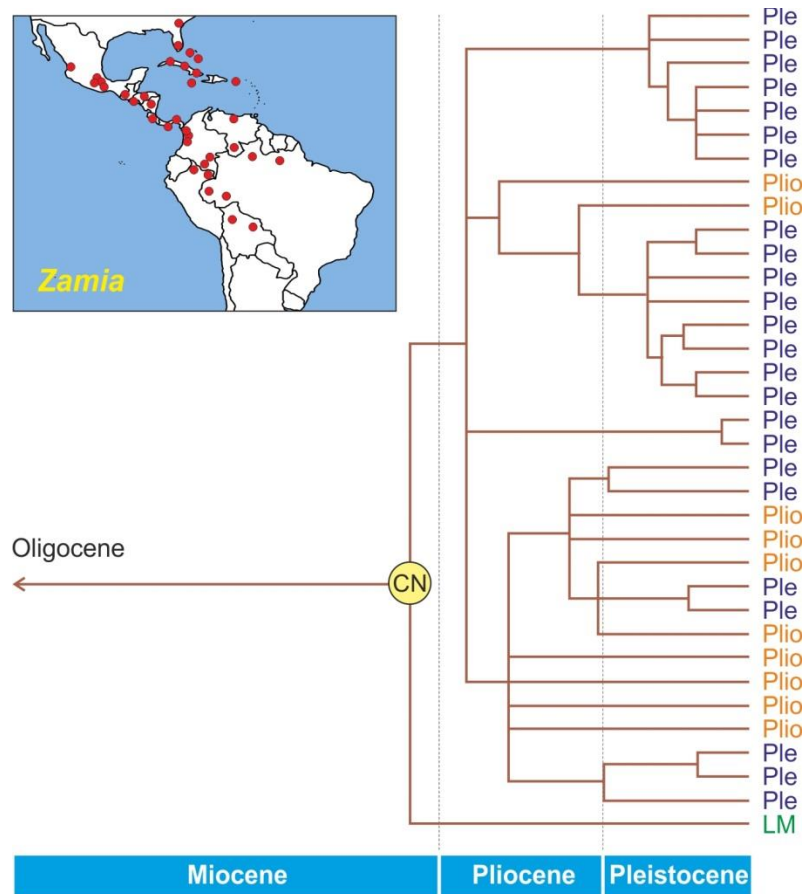


Figure 13

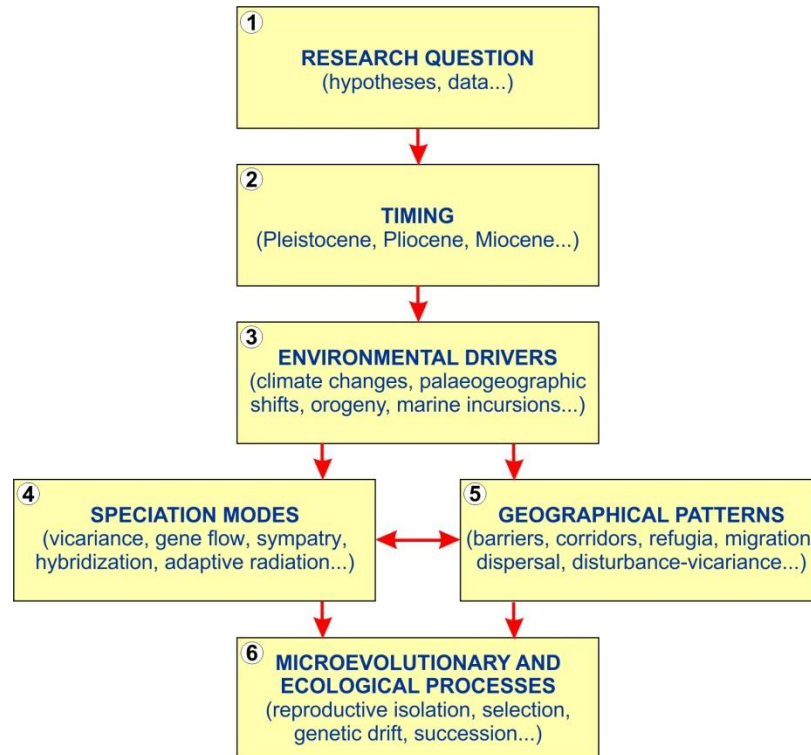


Figure 14