Investigating patterns and rates of species evolution in tropical rainforests using the pantropical family of the Annonaceae (Magnoliales)

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MASTER'S DISSERTATION

INVESTIGATING PATTERNS AND RATES OF SPECIES EVOLUTION IN TROPICAL RAINFORESTS USING THE PANTROPICAL FAMILY OF THE ANNONACEAE (MAGNOLIALES)

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ENGLISH SUMMARY

Tropical rainforests are known for their outstanding floral and faunal diversity across both local and global scales. These forests contain an estimated 50% of the global terrestrial biodiversity, while covering c. 7% of the Earth's surface encompassing equatorial areas in the Neotropics, Africa and the Indo-Pacific, making it the most biodiverse terrestrial biome on Earth. However, the patterns and drivers behind the tropical diversity remain ambiguous and hotly debated. Understanding this is fundamental for understanding and predicting climate change induced diversity loss, which is critical for conservation efforts regarding the current declining tropical diversity. The pantropical plant family of the Annonaceae consists of flowering trees, shrubs and lianas and is mostly found in tropical and subtropical lowland forests. Analysing the family of the Annonaceae is important to test hypotheses explaining the origin and maintenance of the elevated diversity in the tropics. This can be done by unravelling the evolutionary history of the family and taking it as a proxy for the evolution of the whole biome. Annonaceae are quite suitable for this due to its early divergence time, biodiversity and its pantropical distribution. The aim of this study is to analyse the evolutionary history of Annonaceae by assessing diversification rates and patterns through time and subsequently link the identified patterns to an evolutionary scenario or a combination of scenario's proposed by Meseguer et al. (2020). This was carried out by using BAMM together with a time-calibrated phylogenetic tree, obtained by using RaxML in combination with BEAST, based on 819 species and five different genome regions (*rbcL*, *matK*, *ndhF*, *trnL-F* and *psbA-trnH).* Due to the convergence problems obtained in the BEAST and BAMM analysis, conclusions cannot be drawn with certainty in this research. However, results in this research hint at the diversification rate pattern through time within Annonaceae, as well as clades with elevated diversification rates. Nonetheless, congruence with previous diversification rate analyses regarding Annonaceae, support our results. Heterogeneity in regard to diversification rate patterns through time were found between the subfamilies. Our results indicate that diversification rates within the family were slowly increasing, almost constant until 40 million years ago when major rate increases occurred. These were subsequently followed by diversification rate decelerations up to the present, but this phenomenon is most probably due to biases in diversification rate analyses as the "push of the past". Our results show that the increase in diversification rates is most likely the result of elevated speciation rates rather than reduced extinction rates or a combination of both. Six diversification rate shifts were identified: at the node comprising the whole family, in *Guatteria, Duguetia, Goniothalamus,* the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* and in the clade comprising the tribes Malmeeae, Miliuseae, Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae. Therefore, the evolutionary history of the Annonaceae coincides with a combination of two scenario's proposed by Meseguer et al. (2021). A mixed model of a gradual increase scenario (Sc. 1), more specifically a 'Cradle & Museum' subscenario. Followed by an exponential increase scenario (Sc. 2), more specifically a 'Recent Cradle' subscenario from the Late Eocene onwards. In this mixed model environmentally stable conditions in tropical environments, and thus low constant extinction rates, preserve ancient lineages and accumulate them through time as well as lineages resulting from relatively recent radiations linked to elevated speciation rates. The diversification rate pattern through time as well as the diversification rate shifts found within the Annonaceae could potentially be linked to several biogeographical events such as: Indian rafting, the Paleogene cooling and subsequent boreotropical disruption, several Miocene Paleotropical vicariance events, the flooding of Lake Pebas, the Andean uplift and the closure of the Panama isthmus. Likewise several morphological innovations such as shifts regarding the pollination system and seed dispersal unit as well as shifts towards a lianescent habit, circadian pollinator trapping and androdioecy could potentially have played an important role regarding the diversification of Annonaceae.

NEDERLANDSE SAMENVATTING

Tropische regenwouden staan bekend om hun enorme diversiteit. Deze wouden bevatten naar schatting 50% van de biodiversiteit op aarde, terwijl ze ongeveer 7% van het aardoppervlak innemen. Ze omvatten equatoriale gebieden in de Neotropen en de Paleotropen, waardoor ze het meest biodiverse terrestrische bioom zijn op aarde. De patronen en drijvende krachten achter de tropische diversiteit blijven echter onduidelijk en zijn het onderwerp van verhitte discussies. Inzicht hierin is van fundamenteel belang om het door klimaatverandering veroorzaakte verlies aan diversiteit te kunnen begrijpen en voorspellen, wat van cruciaal belang is om de huidige afnemende tropische diversiteit in stand te houden. De pantropische plantenfamilie van de Annonaceae bestaat uit bloeiende bomen, struiken en lianen en wordt vooral aangetroffen in tropische en subtropische laaglandbossen. Onderzoek binnen de familie van de Annonaceae is belangrijk om hypothesen te toetsen die de oorsprong en het behoud van de hogere diversiteit in de tropen verklaren. Dit kan men doen door de evolutionaire geschiedenis van de familie te ontrafelen en haar als proxy te nemen voor de evolutie van het gehele bioom. Annonaceae zijn hier heel geschikt voor vanwege de vroege divergentieperiode, de biodiversiteit en de pantropische verspreiding. Het doel van deze studie is om de evolutionaire geschiedenis van de Annonaceae te analyseren door de diversificatiesnelheden en -patronen door de tijd heen te achterhalen en vervolgens de geïdentificeerde patronen te koppelen aan een evolutionair scenario of een combinatie van scenario's voorgesteld door Meseguer et al. (2020). Dit werd uitgevoerd met behulp van BAMM samen met een gedateerde fylogenetische boom, verkregen via RaxML in combinatie met BEAST, op basis van 819 soorten en vijf verschillende genoomregio's (*rbcL, matK, ndhF, trnL-F* en *psbA-trnH*). Als gevolg van de convergentieproblemen bij de BEAST- en BAMM-analyse kunnen in dit onderzoek geen conclusies met zekerheid worden getrokken. De resultaten van dit onderzoek wijzen echter op het patroon van diversificatie doorheen de tijd binnen Annonaceae, alsook op clades met een verhoogde diversificatiegraad. Desalniettemin ondersteunt de congruentie met eerdere diversificatieanalyses, met betrekking tot Annonaceae, onze resultaten. Heterogeniteit met betrekking tot diversificatiepatronen door heen de tijd heen werd aangetroffen tussen de subfamilies. Onze resultaten geven aan dat de diversificatiesnelheden binnen de familie langzaam toenamen, bijna constant tot 40 miljoen jaar geleden, toen grote snelheidsverhogingen optraden. Deze werden dan gevolgd door vertragingen in diversificatie, maar dit verschijnsel is waarschijnlijk te wijten aan vertekeningen in de analyses zoals bijvoorbeeld de "push of the past". Onze resultaten tonen aan dat de toename in diversificatie het meest waarschijnlijk het resultaat is van verhoogde speciatiesnelheden in plaats van verminderde extinctiesnelheden of een combinatie van beide. Er werden zes verschuivingen in de diversificatiesnelheid vastgesteld: in de node die de hele familie omvat, in *Guatteria*, *Duguetia*, *Goniothalamus*, de clade die *Desmos*, *Dasymaschalon*, *Friesodielsia* en *Monanthotaxis* omvat, alsook in de clade die de stammen Malmeeae, Miliuseae, Maasieae, Fenerivieae, Dendrokingstonieae en Monocarpieae omvat. Daarom valt de evolutionaire geschiedenis van de Annonaceae samen met een combinatie van twee scenario's voorgesteld door Meseguer et al. (2021). Een gemengd model van een scenario van geleidelijke toename (Sc. 1), meer bepaald een 'Cradle & Museum' subscenario. Gevolgd door een exponentieel toenamescenario (Sc. 2), meer specifiek een 'Recent Cradle' subscenario vanaf het Late Eoceen. In dit gemengd model, zorgen stabiele omgevingsomstandigheden, en dus lage/constante extinctiesnelheden, dat soorten behouden blijven en accumuleren ze door de tijd heen, evenals soorten die het resultaat zijn van relatief recente radiaties gekoppeld aan verhoogde speciatiesnelheden. Het diversificatiepatroon doorheen de tijd en de diversificatieverschuivingen die binnen de Annonaceae zijn gevonden, kunnen mogelijk in verband worden gebracht met verschillende biogeografische gebeurtenissen, zoals: Indische rafting, de Paleogene afkoeling en de daaropvolgende Boreotropische verstoring, verschillende Paleotropische vicariantiegebeurtenissen in het Mioceen, de overstroming van het Pebas meer, het vormen van het Andesgebergte en de afsluiting van de landengte van Panama. Ook verschillende morfologische vernieuwingen zoals verschuivingen in het bestuivingssysteem en de zaadverspreidingseenheid, alsook verschuivingen naar een liaan-groeiwijze, circadiane bestuivingsvallen en androdioecy zouden een belangrijke rol kunnen hebben gespeeld bij de diversificatie van de Annonaceae.

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ABSTRACT

Understanding the main drivers behind the elevated tropical diversity in regard to extratropical regions is fundamental for understanding and predicting the current declining diversity within the tropics. The Annonaceae is a pantropical plant family consisting of flowering trees, shrubs and lianas. Unravelling the evolutionary history of Annonaceae is important for exploring future hypotheses regarding the drivers and patterns of diversification of tropical angiosperms and taxa linked to them. In this study the evolutionary history of Annonaceae is analysed by assessing diversification rates and patterns through time using a time-calibrated phylogenetic tree based on 819 species and five different genome regions (*rbcL*, *matK*, *ndhF*, *trnL-F* and *psbA-trnH).* Our results regarding the evolutionary history of the Annonaceae coincides with a combination of two scenario's proposed by Meseguer et al. (2021). A mixed model of a gradual increase scenario (Sc. 1), more specifically a 'Cradle & Museum' subscenario. Followed by an exponential increase scenario (Sc. 2), more specifically a 'Recent Cradle' subscenario from the Late Eocene onwards. Six diversification rate shifts were identified: at the node comprising the whole family, in *Guatteria, Duguetia, Goniothalamus,* the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* and in the clade comprising the tribes Malmeeae, Miliuseae, Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae. Indian rafting, the Paleogene cooling and subsequent boreotropical disruption, several Miocene Paleotropical vicariance events, the flooding of Lake Pebas, the Andean uplift and the closure of the Panama isthmus as well as shifts regarding the pollination system and seed dispersal unit and shifts towards a lianescent habit, circadian pollinator trapping and androdioecy could potentially have played an important role regarding the diversification of Annonaceae.

Guatteria crassipes. An endemic plant recollected in the Colombia Bio Programme, joint Expedition to Boyacá
© RBG Kew, Mauricio Diazgranados (POWO, 2019)

INTRODUCTION

Tropical rainforests are known for their outstanding floral and faunal diversity across both local and global scales. These forests contain an estimated 50% of the global terrestrial biodiversity, while covering c. 7% of the Earth's surface encompassing equatorial areas in the Neotropics, Africa and the Indo-Pacific, making it the most biodiverse terrestrial biome on Earth (Hill & Hill, 2001; de la Estrella et al., 2017; Eiserhardt et al., 2017). While there is some dispute regarding the biomes defining criteria, it is mostly identified by warm-humid climates with high mean monthly temperatures, 2000 mm of annual precipitation and the absence of a pronounced dry season (Eiserhardt et al., 2017). Tropical rain forests are biotically typified by an angiosperm-dominated, closed, multi-layered canopy consisting of an abundance of trees, vines, lianas and epiphytes (Hill & Hill, 2001; Eiserhardt et al., 2017).

The gap in biological diversity between the tropics and other terrestrial biomes has been known for a long time (Wallace, 1878; Fischer, 1960; Pianka, 1966; Janzen, 1967; Moreau & Bell, 2013). The Latitudinal Diversity Gradient (LDG), or the increase in biological diversity from polar to equatorial latitudes, is one of the oldest recognized biogeographical patterns. It is observed across most of the Earth's diversity with some exceptions, for example temperate peaks in the salamander and frog diversity (Willig et al., 2003; Mittelbach et al., 2007; Mannion et al., 2014). Despite the long-time recognition of this biogeographical diversity trend and the ecological, biogeographical and phylogenetic research regarding tropical clades, the patterns and drivers behind the tropical diversity remain ambiguous and hotly debated (Brown, 2014; de la Estrella et al., 2017). Understanding this is fundamental for e.g. understanding and predicting climate change induced diversity loss, which is critical for conservation efforts regarding the current declining tropical diversity (Mannion et al., 2014).

While trying to unravel the preliminary history behind the tropical diversity observed today, a debate has risen regarding the main drivers behind the elevated tropical diversity in regard to extratropical regions that cause the generation and maintenance of the diversity in these tropical regions. Proposed explanations range from hypotheses concerning spatial and temporal scales, environmental change, niche and habitat diversification, species interactions, genetic differentiations, productivity, niche portioning/conservatism, energy balance to temporal variation in speciation and extinction rates (Hill & Hill, 2001; Willig et al., 2003; Currie et al., 2004; Allen et al., 2006; Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008)

All of the proposed hypotheses regarding this topic can be roughly subdivided into three classes, which are accompanied by a different general background mechanism explaining the elevated tropical diversity (Mittelbach et al., 2007). The first class, containing historical hypotheses, state that the elevated tropical diversity is the result of greater time for diversification and accumulation within the tropics (Hill & Hill, 2001; Stephens & Wiens, 2003; Mittelbach et al., 2007; Brown, 2014; Eiserhardt et al., 2017; Vasconcelos et al., 2021) (see Box 1).The second class comprises the ecological hypotheses that state that the tropics have a higher carrying capacity or maximum amount of species in comparison with extratropical regions (Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008; Eiserhardt et al., 2017) (see Box 2). The last mechanism explaining the diversity-gap states that the tropics have an elevated diversification rate in regard to non-tropical areas, these are appointed as evolutionary hypotheses (Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008; Eiserhardt et al., 2017; Vasconcelos et al., 2021) (see Box 3).

Most of the many proposed hypotheses are not mutually exclusive, however, but a generally accepted combination of hypotheses integrated into one explanation still remains ambiguous to this day (see Box 4). Many of the proposed hypotheses have little empirical support, are speculative, circular, too specific, complex or impossible to test or have been proven to be inadequate to explain the ubiquity and magnitude of the patterns. Some authors however have tried to narrow down and audit the proposed explanations, and some have even tried to synthesise them into one coherent explanation (Willig et al., 2003; Brown, 2014; Eiserhardt et al., 2017).

BOX 1: HISTORICAL HYPOTHESES

Variation in time, greater time for diversification and accumulation of tropical species

The general idea of the class of 'historical hypotheses' dates back to Wallace (1878). These point out that the cause of the elevated tropical diversity in regard to extratropical regions are differences in time rather than differences in diversification rates or ecological limitations on the overall species richness (Hill & Hill, 2001; Stephens & Wiens, 2003; Mittelbach et al., 2007; Brown, 2014; Eiserhardt et al., 2017; Vasconcelos et al., 2021). These state that tropical environments had a relatively longer undisturbed history for diversification and hence accumulation of species in comparison with extratropical ones that have undergone more pronounced climatic changes during their history (e.g. Pleistocene glaciations) (Hawkins, 2003; Arita & Vazquez-Dominguez, 2008). The tropics had more time for speciation, immigration and co-evolution as a result of the uninterrupted warm, wet and aseasonal climate regimes throughout most of history with far less frequent climatic perturbations due to extreme conditions such as periods of continental glaciation. Therefore, the elevated tropical diversity is simply the result of differences in the past geological, climatic and evolutionary events between temperate and tropical regions, and thus the legacy of both short and long term tropical climatic stability that allowed for more effective time to accumulate more lineages (time-for-speciation effect; Stephens & Wiens, 2003) and on average more species per lineage (Hill & Hill, 2001; Mittelbach et al., 2007; Brown, 2014, Eiserhardt et al., 2017). Additional support for the idea that tropical environments had more diversification time results from phylogenetic research showing that many temperate taxa are nested within tropical clades and thus once originated within tropical environments (Ricklefs, 2005; Jablonski et al., 2006; Mittelbach et al., 2007). As for example trees within major orders of the Fagales and Rosales (Ricklefs, 2005).

Proposed hypotheses that can be placed within or linked with this class are the stability-time hypothesis (Sanders, 1968; Hill & Hill, 2001) and the phylogenetic niche conservatism hypothesis (Wiens & Donoghue, 2004; Brown, 2014).

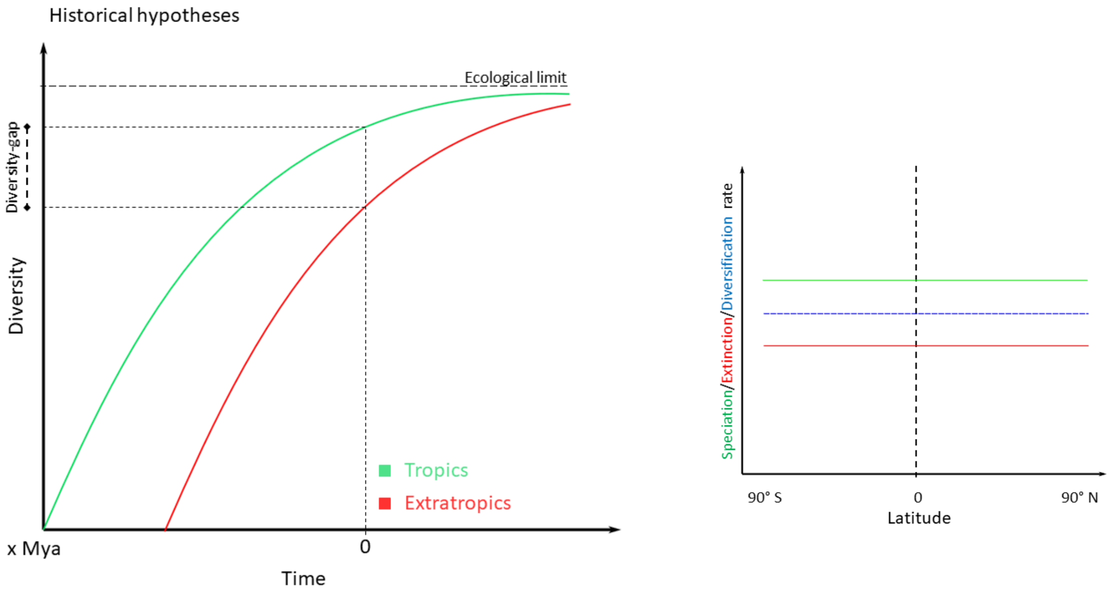

Figure 1. Hypothetical graphs demonstrating the general idea behind historical hypotheses. Own visualization of graphs within Mittelbach et al. (2007) and Mannion et al. (2014).

BOX 2: ECOLOGICAL HYPOTHESES

Variation in carrying capacity, greater diversity carrying capacity within the tropics

Within ‘ecological hypotheses’ the proposed mechanisms all fall back on the idea of ecological carrying capacity limitations. These hypotheses state that the gap in diversity between tropical and temperate regions is caused by the fact that the maximum of species that can coexist within a region are placed upon different levels (Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008; Eiserhardt et al., 2017). They state that the gap is not a consequence of differences in diversification rate or time of origination between tropical and extratropical regions, but that ecological and abiotic characteristics in these regions determine the upper limits of species coexistence. This implies that the levels of speciation and immigration decrease and/or the levels of extinction increase with an increasing species richness, due to the fact that species richness impacts ecological opportunities, levels of competition, population sizes etc. (Rabosky, 2009; Eiserhardt et al., 2017). This was appointed as negative diversity dependence by Rabosky & Hurlbert (2015) or diversity-dependent cladogenesis. An example of such diversity-dependent diversification rate slowdowns is found in the Protieae of the Burseraceae (Fine et al., 2014). As the upper limit is reached, speciation and extinction rates have a more or less equal magnitude with species originating and disappearing at an even pace (Eiserhardt et al., 2017).

Phylogenetic data on Annonaceae have been tested in regard to diversity-dependent diversification models, these showed unrestrained diversification in certain clades indicating very high or even absent ecological limits in at least some clades within tropical regions (Couvreur et al., 2011; Eiserhardt et al., 2017).

Many propositions and empirical tests have been put forward regarding environmental variables, correlated with these ecological limitations. With the emphasis on the environmental differences between tropical and temperate regions. Examples are net primary production, temperature, area, spatial heterogeneity, predation, competition etc. (Willig et al., 2003; Wiens et al., 2006). In some cases correlations have been found (e.g. net primary production (Brown, 2014)). These hypotheses are limited however, because of the fact that they do not explain the difference in processes that account for the change in species richness between tropical and extratropical regions such as speciation, extinction and dispersal (Wiens & Donoghue, 2004; Wiens et al., 2006).

One of the proposed drivers that limit species richness is productivity. This is based on the net primary production concept or the amount of energy captured, transformed and transitioned into living matter through an ecosystem per unit of area and time. The higher this amount, the more resources available in an area, the more species that can capture a sufficient amount to maintain viable healthy genetic populations, which reduces extinction (Hill & Hill, 2001; Willig et al., 2003; Brown, 2014). In other words, it determines the maximum capacity of life a region can support, and sets constraints on the maximum abundance and biomass of all species within that region. The net primary production in a terrestrial region is mainly determined by the annual solar radiation and is limited by water availability (Willig et al., 2003; Brown, 2014). Tropical rain forests, as mentioned above, have high average annual temperatures, which is mainly the result of the more oblique angle of solar radiation in tropics, leading to an increased receival of solar energy. This is paired with heavy rainfall and short or absent dry seasons (Eiserhardt et al., 2017). These climatic conditions lead to an overall higher net primary production in comparison with temperate regions increasing the potential maximum of species richness (Hill & Hill, 2001; Brown, 2014). These lead to a higher overall species richness in tropical regions over time as you can see in figure 2. Hypotheses that can be placed in this category are the species energy theory (Wright, 1983) and its extensions and the productivity hypothesis (Hutchinson, 1959).

Other proposed drivers regarding ecological limitations not explained here are kinetics (Willig et al., 2003; Brown, 2014), niche relationships (Hill & Hill, 2001; Brown, 2014), spatial relationships (Willig et al., 2003; Willig & Bloch, 2006; Brown, 2014), disturbance (Hill & Hill, 2001) etc. However, a sole driver behind the carrying capacity of a region is not realistic. They are all integrated in a complex interplay of factors instead of being alternative, mutually exclusive drivers behind ecological limitations for certain regions (Hill & Hill, 2001; Willig et al., 2003; Brown, 2014).

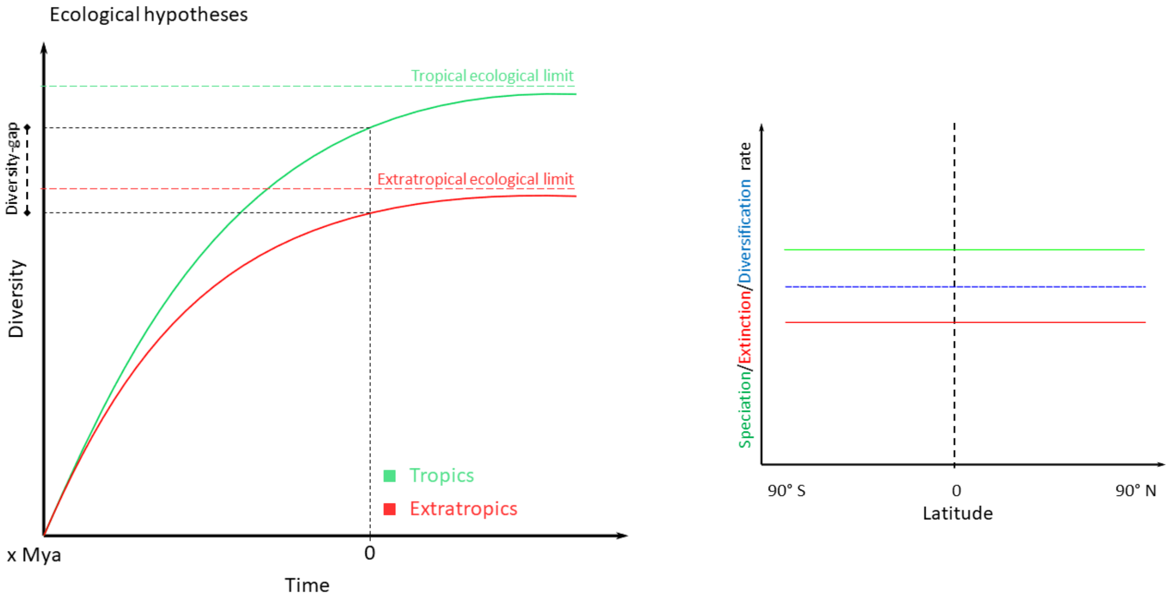

Figure 2. Hypothetical graphs demonstrating the general idea behind ecological hypotheses. Own visualization of graphs within Mittelbach et al. (2007) and Mannion et al. (2014).

BOX 3: EVOLUTIONARY HYPOTHESES

Variation in diversification rate, greater diversification rate within the tropics

Another class of hypotheses regarding the elevated tropical diversity emerged only later when it was fuelled by the increased availability of molecular, palaeontological and biogeographical data and the computational power for its analyses. Furthermore, the historical and ecological hypotheses proved inadequate to explain the ubiquity and magnitude of the observed diversity patterns, and the evolutionary dynamics which underpin them (Jablonski et al, 2006; Mittelbach et al., 2007).

Evolutionary hypotheses contend that the tropics have an elevated diversity because of a higher net diversification rate, which is determined by extracting the net extinction rate from the net speciation rate or the balance of speciation and extinction over time as stated by Pontarp & Wiens (2017). This idea is based on the understanding that, if taxa are biome-restricted (Crisp et al., 2009), the species diversity within a biome should be fundamentally dependent on the biome-specific speciation and extinction rates.

And subsequently if these biome-specific speciation and/or extinction rates are very different, which is likely due to the rate dependency on biome-diverging abiotic and biotic factors, biomes should accumulate different numbers of species given an equal timeframe (Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008; Eiserhardt et al., 2017; Vasconcelos et al., 2021).

These evolutionary hypotheses state that tropical regions have a higher overall diversification rate and therefore accumulate species faster through time. This elevated diversification rate can present a couple of things due to its cumulative nature. It can be achieved through an elevated speciation rate, through a depressed extinction rate or through a combination of both (Donoghue & Sanderson, 2015). These different ways in achieving elevated diversification rates is a main point of discussion within this class of hypotheses and was appointed as the ‘Cradle vs. Museum’ debate. ‘Cradles’ refer to the elevated speciation-side of the debate and consequently ‘Museums’ to the side believing that a depressed extinction rate causes the difference in diversification rate. In both models the net species diversification rate in the tropics is higher than in other biomes, explaining the elevated species-richness in tropical rain forests (Mittelbach et al., 2007; Arita & Vazquez-Dominguez, 2008; Mannion et al, 2014; Eiserhardt et al., 2017; Meseguer et al., 2021; Vasconcelos et al., 2021).

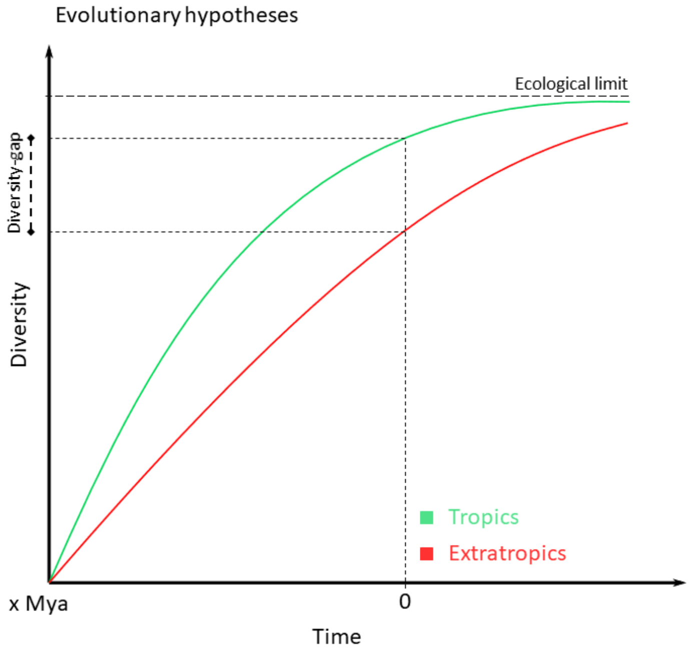

Figure 3. Hypothetical graph demonstrating the general idea behind evolutionary hypotheses. Own visualization of graph within Mittelbach et al. (2007).

‘Cradles’ vs ‘Museums’

‘Cradles’ are systems with sets of characteristics maximizing the potential for diversification. ‘Cradle’-like systems are environmentally unstable in time which increases the chance of fragmentation and reproductive isolation, booming the potential for speciation. Another criterion linked to these systems is that they are heterogeneous across space, meaning that they are diverse in environmental conditions on a small scale, increasing the chance of geographic isolation and the overall extent and variation of external selective pressures regarding the whole area, which will both boom the potential for speciation as well. Areas that score well on both of these criteria are for example areas with extensive climatic or/and orogenic cycles, areas with an ecotonal/transitional nature, areas with occasional fires or droughts … As mentioned before, these areas have an elevated potential for speciation and consequently have an elevated diversification rate even though species in these environmental unstable areas will experience an elevated extinction rate as well (Stebbins, 1974; Vasconcelos et al., 2021).

Recent interpretations derogate from Stebbins (1974), as described above. They leave out the also increasing extinction rate and state that 'Cradle'-like systems have an elevated speciation rate, while the extinction rate does not vary latitudinally (Mannion et al, 2014; Eiserhardt et al., 2017; Vasconcelos et al., 2021).

Support for the tropics being evolutionary 'Cradles' comes from species radiations in response to recent climatic, tectonic or biotic events (e.g. Pleistocene glaciation, Pliocene Panama isthmus bridging) observed in geographical patterns of species distribution, richness and endemism (McKenna & Farrel, 2006).

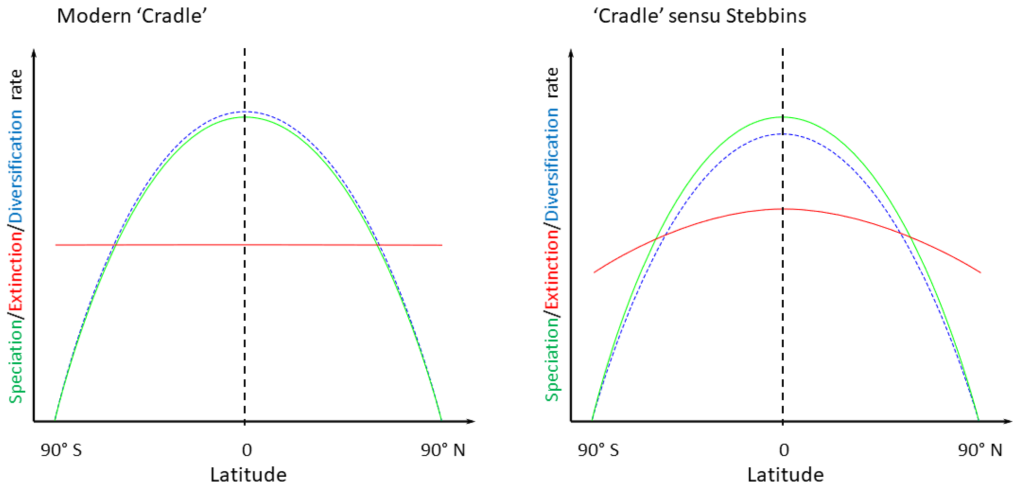

Figure 4. Hypothetical graphs demonstrating the general idea behind 'Cradles'. Own visualization of graphs within Mannion et al. (2014).

'Museums' however were characterized by Stebbins with the direct opposite criteria of his 'Cradles'. 'Museums' are systems where lineages are preserved through time. As time accumulates in these areas, species richness will as well. 'Museum'-like systems are environmentally stable through time, reducing the chance of fragmentation and reproductive isolation of populations within these systems, diminishing the potential of extinction. These systems are less heterogeneous across space, decreasing the chance of geographic isolation as well as degrading the amount and variation of external selective pressure, which also diminishes the potential of extinction in these areas. These criteria however diminish the rise and accumulation of adaptations and thus decrease the overall speciation rate within these systems. Consequently, lineages can persist for longer periods of time and species accumulate as time proceeds in these regions due to the lowered extinction rate, explaining the overall elevated diversification rate (Stebbins, 1974; Vasconcelos et al., 2021). The interpretations of 'Museums' have been altered over time. In recent literature, they are mainly considered systems with decreased extinction rates while the speciation rates are constant in relation to latitude (Mannion et al, 2014; Eiserhardt et al., 2017; Vasconcelos et al., 2021).

Support for a 'Museum'-like evolutionary history comes from paleontological evidence suggesting that the evolutionary radiations leading to the current tropical crown diversity are a result of global warming events, Cenozoic maximum global temperatures and latitudinal expansions during the late Paleocene and/or early to middle Eocene (McKenna & Farrel, 2006).

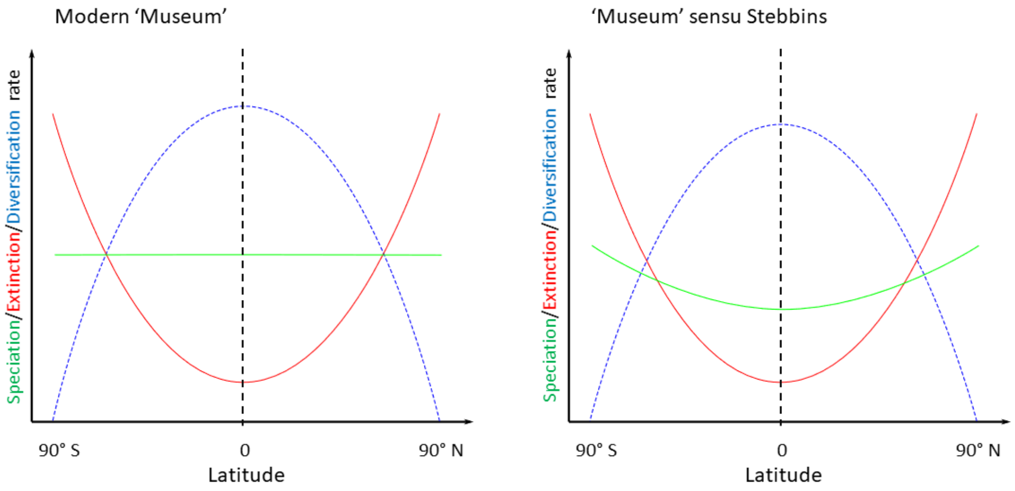

Figure 5. Hypothetical graphs demonstrating the general idea behind 'Museums'. Own visualization of graphs within Mannion et al. (2014).

'Cradles' and 'Museums', both fluctuating over time and taxa

Many authors state that 'Cradles' and 'Museums' probably are not mutually exclusive and that the true mechanism behind the elevated tropical diversity is a combination of both (McKenna & Farrel, 2006; Arita & Vazquez-Dominguez, 2008; Koenen et al., 2015; Eiserhardt et al., 2017; Meseguer et al., 2021; Vasconcelos et al., 2021).

This reconciliation of both can be the result of two things. The first one is based upon the fact that environmental characteristics within regions fluctuate over time and space, for example the drastic environment changing climatic cycles in the Pleistocene also known as the Milankovitch cycles. Consequently, selective pressures within a region will fluctuate over time and space as well. Therefore, as these environmental characteristics shift, the ability of specific traits to boost speciation or to reduce extinction will shift over time and space as well. With this in mind, regions that for example first function in a 'Cradle'-like manner for a certain species can over time shift into a 'Museum'-like functioning region for that species and so on. Phylogenetic studies analysing habitat transitions have found frequent transitions from regions functioning as 'Museums' to regions functioning in a 'Cradle'-like manner (Vasconcelos et al., 2021) as for example in Donoghue & Edwards (2014) where they conclude that biome shifts are common in plant evolution.

Secondly, due to the differential distribution of traits among lineages within a region, speciation and extinction rates may vary within and among lineages that experience similar environmental characteristics. As such, certain environmental characteristics within a region can function as a 'Cradle' for certain taxa while functioning as a 'Museum' for other taxa at the same time. In addition, as mentioned in the first point, the combination of 'Cradle'-like and 'Museum'-like lineages may shift in time and space with the fluctuating environmental characteristics as well.

Evidence for combinations of 'Cradles' and 'Museums' within tropical lineages is found in for example tropical *Cephaloleia*-beetles, where both 'Cradle' and 'Museum'-like timing and rate characteristics were found when analysing their diversification history (McKenna & Farrel, 2006). Other examples of combinations are found in Meliaceae (Koenen et al., 2015).

Other evolutionary hypotheses

'Out of the tropics'-model (OTT)

Jablonski et al. (2006) proposed another evolutionary hypothesis regarding the elevated tropical diversity called the 'Out of the tropics'-model (OTT). They expanded 'Cradles' and 'Museums' with large-scale changes in the spatial distributions of taxa. These temporal spatial changes include climatic change induced geographic range shifts of species and geographical species distributions encompassing both tropical and extratropical areas.

Following the OTT-model, tropics are both a 'Cradle' and a 'Museum' with most taxa first originating in the tropics and secondly migrating towards higher latitudes without losing their original tropical distributions. The OTT-model states that the tropics have higher speciation rates, lower/equal extinction rates and lower immigration rates in regard to extratropical regions. As such the OTT-model suggests that the tropical elevated diversity results from mechanisms augmenting tropical diversification rates and mechanisms determining species specific geographical range limits (Jablonski et al., 2006; Arita & Vazquez-Dominguez, 2008; Mannion et al., 2014).

'Geographic area hypothesis'

Another hypothesis regarding the latitudinal difference in species richness that can be classified within the evolutionary hypotheses is the geographic area hypothesis proposed by Terborgh (1973) and Rosenzweig (1995). This hypothesis is based upon the same findings regarding geographic area as one of the ecological limits mentioned above within the class of the ecological hypotheses. The geographic area hypothesis states that due to the vast amount of geographic area in tropical regions, as a result of the increasing latitudinal circumferences and fewer disjunct zones in comparison with extratropical zones, speciation rates are elevated and extinction rates diminished in regard to these of extratropical regions. This is based upon the thought that taxa that occupy large geographic ranges are more likely to experience geographic isolation and thus allopatric speciation, due to a higher likelihood of barrier formation, as well as higher likelihoods to experience niche refugia and larger population sizes. These factors elevate the tropical speciation and lower the extinction rate, who elevate the tropical net diversification rate explaining the latitudinal increase of species richness towards tropical regions (Willig & Bloch, 2006).

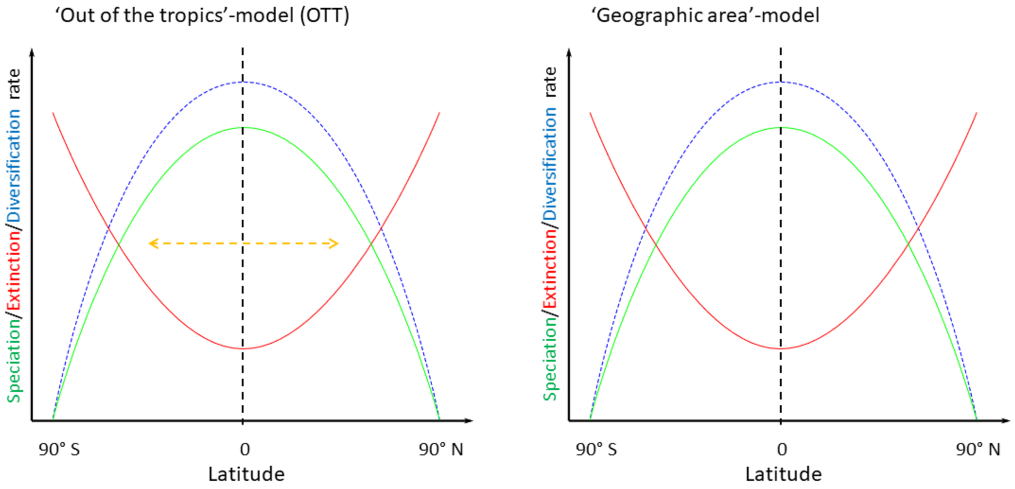

Figure 6. Hypothetical graphs demonstrating the general idea behind the 'Out of the tropics'-model and the 'Geographic area' model. Own visualization of graphs within Mannion et al. (2014). Yellow arrow indicates geographic range shifts.

BOX 4: INTEGRATED HYPOTHESES

The general idea behind the three classes of hypotheses mentioned above are different but do often overlap regarding the underlying empirical evidence and arguments. The most realistic scenario leading to an elevated tropical diversity present throughout the history of the earth is determined by differences in time, ecological diversity limiting factors and diversification rates between tropical and extratropical regions. Without integration it is difficult to fully explain comprehensive biogeographical patterns as the latitudinal biodiversity gradient due to the complex interplay of factors. Integrated approaches determining how historical and ecological variables influence evolutionary processes such as speciation, extinction as well as dispersal and vice versa are needed to fully explain the ubiquity and magnitude of the latitudinal species richness gradient and its encompassing elevated tropical diversity (Hill & Hill, 2001; Willig et al., 2003; Wiens et al., 2006; Wiens & Donoghue, 2004; Eiserhardt et al., 2017; Pontarp & Wiens, 2017).

Some authors have tried to put forward integrated models regarding the latitudinal diversity gradient encompassing the general ideas behind historical, ecological and evolutionary hypotheses. Examples of such approaches are the 'Tropical conservatism hypothesis' (Wiens & Donoghue, 2004) or the model proposed by Pontarp & Wiens (2017).

'Tropical conservatism hypothesis'

Wiens & Donoghue (2004) summarized and elaborated earlier proposed hypotheses into one model labeled the 'tropical conservatism hypothesis'. The hypothesis is based on three fundamental concepts, the first one is the idea that most clades, especially the ones with a high current tropical diversity, originated in tropical regions and have spread towards higher latitudes either recently or not. In this manner, tropical taxa had more time for speciation and thus accumulation of taxa, explaining an elevated tropical diversity. This concept coincides with the idea behind historical hypotheses. The second concept states that tropical regions were more extensive until 30-40 million years ago, explaining the elevated tropical origination rate of species in comparison with temperate latitudes which coincides with the idea behind the geographic area hypothesis, where diversity is positively linked to geographic extent. The third and last concept is based upon the idea that many taxa are adapted to tropical climates and that adaptations regarding temperate climates (e.g. winter temperatures below 0°C) are only possible to appear in a small number of these taxa, which limits their dispersal and persistence towards and within temperate regions. This concept coincides with the phylogenetic niche conservatism hypothesis (Wiens & Donoghue, 2004; Brown, 2014).

Pontarp & Wiens (2017)

Pontarp & Wiens simulated evolutionary radiations while tracking species richness and ecological conditions over time, to find out the influence of diversification rate components, time and ecological carrying capacities on diversity patterns and each other. The study concludes that the three classes of hypotheses aren't mutually exclusive with carrying capacities rather being ecological factors of influence regarding diversification rates and time for accumulation rather than being a sole explanation for the seen diversity pattern. Limited carrying capacities reduce the overall speciation rate, elevate the overall extinction rate and thus reduce the diversification rate. They also increase the time needed for the colonization of the corresponding regions and thus reduce the time to accumulate species there. The study also states that diversification rate explanations and time-for-speciation explanations aren't competing paradigms to explain diversity patterns when considering the compete time-scale. They are both true but work upon different levels of the timescale. They suggest that the time-for-speciation effect has the most influence on shorter time-scales, for example the time-difference in colonizing certain regions with earlier colonized regions comprising more species. But when all regions are eventually colonized, regions with higher diversification rates will eventually overtake the time-for-speciation induced diversity differences, suggesting that diversification rates are most influential on larger temporal scales. These ideas explain the contrasting views and the corresponding empirical evidence regarding the origin of the latitudinal diversity gradient in literature and is an example of an integrated view regarding the latitudinal gradient (Pontarp & Wiens, 2017).

APPROACH

Old-fashioned subdivision

The three-way subdivision in hypotheses regarding the origin and maintenance and overall evolutionary history of the elevated tropical diversity is deprecated in comparison with the current integrative literature on this topic. This is shown in the fourth class of the integrated hypotheses, these explanations combining mechanisms of historical, ecological and evolutionary hypotheses started to pop-up more and more in the near past to now (e.g Wiens & Donoghue, 2004; Pontwarp & Wiens, 2017). This need for more integration in hypotheses applies to the division of the three classes as well as to the subdivision within each class such as the 'Cradle' vs 'Museum' dichotomy. These current subdivisions are limited in explaining the complex combination of variables underlying the origin and maintenance of the temperature to tropical gradient in diversity. As pointed out by Meseguer et al. (2021) they are inferior in explaining the occurrence of ancient and species-poor lineages as well as the decline in diversity of certain clades. Paraphrasing Vasconcelos et al. (2021), metaphors linked to simple binary dichotomies have probably outlived their usefulness, focusing on the processes rather than the metaphor will advance research on this topic.

Approach

Before we can make conclusions about the differences regarding the prior evolutionary history causing the increasing diversity gradient towards the tropics, we first need to determine in what manner/trend (gradual, exponential, saturating,..) tropical rainforests accumulated species over time. When the major species accumulating trend(s) can be identified, as well as those of extratropical regions, it will be easier and more substantiated to pinpoint factors causing an elevated tropical diversity in comparison with extratropical ones. This can be done by unravelling the evolutionary history, thus assessing diversification (speciation & extinction) rates through time out of molecular dated phylogenetic trees (Erkens et al., 2012; Xue et al., 2020) for large tropical-confined clades, such as the Annonaceae and take them as a proxy for the evolutionary history of the tropical biome. Identified clades with diversification rate shifts can afterwards be linked with various abiotic and biotic explanations. However, due to the many biases that creep into these analyses it is important to compare results between various clades achieved in a similar way (see biases below).

In response to the need of a more integrated, comprehensive picture Meseguer et al. (2021) proposed four alternative evolutionary scenarios regarding the diversification history of the Neotropics, gradual increase, exponential increase, saturated increase and waxing and waning. In this thesis I want to tackle the same approach as Meseguer et al., but elaborate the scenarios over the whole tropical biome. Thus regions in the Neotropics and Paleotropics (Africa as well as Asian-Pacifical regions). When regularly adopted in diversification analyses, these scenarios will facilitate comparisons regarding the evolutionary history of different tropical and extratropical clades. Below an overview is given of the proposed scenarios.

Scenario 1: Gradual increase (Sc. 1)

The first scenario assumes gradual increase in diversity or species gradually accumulating through time or a constant diversification rate over time. This constant/gradual trend of species accumulation can arise out of two different combinations of speciation and extinction rates over time. The first one being constant speciation and extinction rates over time, appointed as the 'Cradle and Museum'-model. The second way in achieving this gradual trend, appointed as the 'Recent Cradle & Ancient Museum'- model, is a parallel increase in speciation and extinction rates over time, therefore a constant diversification rate. In lineages appointed with this diversification scenario, a positive relationship should be found between clade age and size due to the fact that older clades will have more species if they diversify at a constant rate, this has been found in some tropical lineages. (Derryberry et al., 2011). This scenario is associated with the long-term environmental stability aspect of previous hypotheses regarding the tropical diversity (Meseguer et al., 2021).

Scenario 2: Exponential increase (Sc. 2)

The second scenario assumes an exponential increase in diversity, or species accumulating in pulses through time or a non-constant diversification rate over time. This exponential trend of species accumulation can also arise out of two combinations of speciation and extinction rates over time. The first one is a combination of constant extinction rates with increasing speciation rates over time, appointed as the 'Recent Cradle'-model. The second combination is one of constant speciation rates with decreasing extinction rates over time, which is appointed as the 'Recent Museum'-model. Pulses of species accumulation have been identified, for example the elevation of the Andes in the Neotropics (Esquerre et al., 2019; Meseguer et al., 2021).

Scenario 3: Saturated increase (Sc. 3)

The third scenario assumes a saturated increase in diversity, or species accumulating slower towards the present than in the past, ultimately reaching a diversity plateau or a non-constant diversification rate over time. This saturating trend of species accumulation can arise again from two combinations of speciation and extinction rates. The first one is the combination of constant extinction rates with decreasing speciation rates towards the present, appointed as the 'Ancient Cradle' model. The second option, is the combination of constant speciation rates with increasing extinction rates towards the present, appointed as the 'Ancient Museum'-model. Species accumulate slower towards the present, hence diversification rates decline towards the present ultimately reaching a diversity plateau where speciation and extinction rates are equal. This trend is associated with the ecological limit-aspect of previous hypotheses. A trend of an early burst in diversification followed by decelerations of diversification has been identified in some Neotropical lineages (Meseguer et al., 2021).

Scenario 4: Waxing and Waning (Sc. 4)

The last scenario assumes declines in diversity after periods of expansion or a non-constant diversification rate over time. As in the third scenario of a saturated increase in diversity, diversification rates decrease towards the present, however this scenario differs from the latter due to the fact that in this scenario the extinction rates exceed the speciation rates and thus diversity is lost towards the present, which is not the case in the third scenario. Again this scenario can be due to two combinations of speciation and extinction rates. The first one is the combination of constant extinction rates with decreasing speciation rates through time, appointed as the 'Ancient Cradle & Recent Decline'-model. The second option is the combination of constant speciation rates with increasing extinction rates towards the present, which was appointed as the 'Ancient Museum & Recent Decline'-model. This fourth scenario of declinations in diversity after expansions, is called 'Waxing & Waning' (Meseguer et al., 2021).

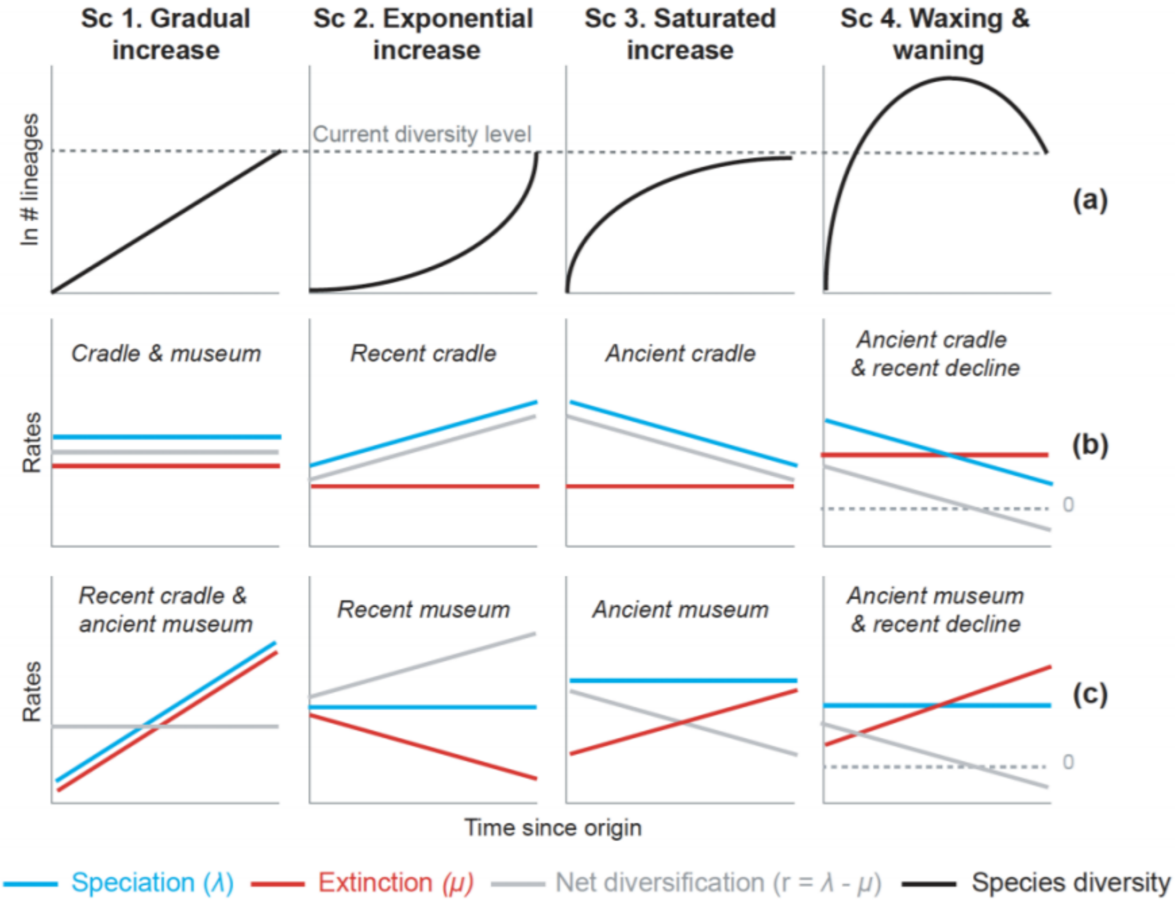

Figure 7. Graphs obtained from Meseguer et al. (2020) demonstrating the general idea behind the different scenarios and subscenarios. Species richness through time (a), alternative evolutionary processes that could generate the corresponding patterns (b, c).

MODEL GROUP - ANNONACEAE

The Annonaceae is a pantropical plant family consisting of flowering trees, shrubs and lianas mostly found in tropical and subtropical lowland forests (Couvreur, 2009; Xue et al., 2020). The family is part of the Magnoliids, containing around 2440 species in 109 genera (Chatrou et al., 2012; Erkens et al., 2012; Rainer & Chatrou, 2021), making it the most biodiverse family of the early-divergent Magnoliales. The Annonaceae comprise four subfamilies with more than 95% of the species placed in the two largest subfamilies, namely the Annonoideae and Malmeoideae which are sister clades. Informally these subfamilies have been named the Long Branch Clade (LBC) and the Short Branch Clade (SBC) respectively due to the different rates of molecular evolution, resulting in branch length differences. The remaining species are placed in the Anaxagoreoideae and the Ambavioideae which are the two most basal clades of the family (Chatrou et al., 2012; Richardson et al., 2014; Hoekstra et al., 2017; Xue et al., 2020)

Analysing the family of the Annonaceae is important to test hypotheses explaining the origin and maintenance of the elevated diversity in the tropics by unravelling the evolutionary history of the family and taking it as a proxy for the evolution of the whole biome as mentioned above. Annonaceae are quite suitable for this due to its early divergence time, biodiversity and its pantropical distribution, making them an excellent model group for unravelling diversification patterns within the tropical biome (Couvreur et al, 2011; Punyasena et al., 2008; Xue et al., 2020).

Many phylogenetic studies, molecular dating analyses, studies regarding molecular character evolution and biogeography focussing on the Annonaceae led to a comprehensive knowledge of the evolution of the family. This provides a good foundation for further research on the limited known diversification patterns and their plausible drivers (Doyle et al., 2004; Scharaschkin & Doyle, 2005; Erkens et al., 2007; Takahasi et al., 2008; Zhou et al., 2009; Couvreur et al., 2011; Pirie & Doyle, 2012; Chatrou et al., 2012; Guo et al., 2017, Xue et al., 2020).

Regarding diversification patterns within Annonaceae, three papers have been published (Couvreur et al., 2011; Erkens et al., 2012; Xue et al., 2020). Couvreur et al., (2011) were the first to study diversification rate patterns through time in Annonaceae using a maximum likelihood approach. They tested if the family diversified constant through time or if Annonaceae had undergone variable diversification rate changes throughout their history. Results showed a slow, nearly constant diversification through time with low extinction rates.

Erkens et al. (2012) tackled this using a different approach by identifying clades with elevated diversification rates. In contrast with Couvreur et al. (2011) some clades were identified with such rates after a period of slow, nearly constant diversification. These two studies however were limited in taxon sampling which could be a source of noise in diversification rate analyses (Sauquet & Maggalon, 2018). Xue et al. (2020) performed diversification analyses on a more comprehensive dataset, trying to reduce noise obtained from this limited taxon sampling. Their results were congruent with Erkens et al. (2012), obtaining slow, nearly constant diversification rates through time followed by elevated diversification rates from the Miocene (c. 25 Ma) onwards.

Understanding diversification of Annonaceae is important for exploring future hypotheses regarding the drivers and patterns of diversification of tropical angiosperms as well as for understanding diversification patterns through time in taxa dependent on this Annonaceae such as various beetle families (Massoni et al., 2015).

AIM

The aim of this study is to analyse the evolutionary history of Annonaceae by assessing diversification rates and patterns through time using a phylogenetic approach and subsequently link the identified patterns to an evolutionary scenario or a combination of scenarios proposed by Meseguer et al. (2020) as described above. Additionally following questions will be addressed in this thesis: (1) Do diversification rate patterns through time differ between the four subfamilies within the Annonaceae? (2) Are diversification rate shifts present within Annonaceae? (3) Which clades are identified with diversification rate shifts? (4) What is the timing and mode of the identified diversification rate shifts? (5) Which plausible abiotic and biotic mechanisms proposed in literature could explain the observed diversification rate shifts?

MATERIALS AND METHODS

Taxon/DNA region sampling and Alignment

We selected five different genome regions commonly used in phylogenetic research regarding Annonaceae; *rbcL*, *matK*, *ndhF*, *trnL-F* and *psbA-trnH*. Adopting a super-matrix approach, the assemblage of this dataset builds further on a representative family-wide molecular dataset regarding Annonaceae, provided by Lars Chatrou. The pre-aligned markers were critically checked, reviewed and selected regarding missing data, gaps and overall useability for this study using Geneious Prime v.2021.1.1 (http://www.geneious.com; Kearse et al., 2012.). Afterwards each marker was supplemented with additional sequences from more recent, genus-specific published datasets such as Tang et al. (2015) (*Goniothalamus*, 67 sequences), Thomas et al. (2015) (*Artabotrys*, 18 sequences), Couvreur (2009) (*Monodora*, 14 sequences; *Isolona*, 16 sequences), Ghogue et al. (2017)

(*Annickia*, 3 sequences; *Sirdavidia solanonna*, *Polyceratocarpus parviflorus*, *Piptostigma,* 5 sequences), Saunders et al. (2011) (*Fenerivia*, 8 sequences; *Fitzalania heteropetala*, *Polyalthia*, 13 sequences), Gosline et al. (2019) (*Mischogyne*, 6 sequences) and 84 sequences of *Monanthotaxis* provided by Lars Chatrou. The sequences were downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank) and combined with the corresponding markers. Per region, the added sequences were automatically aligned using the MAFFT v.7.450 plugin (Katoh, 2002; Katoh & Standley, 2013) in Geneious Prime. Each aligned marker was manually edited in Geneious Prime and Aliview v. 1.x if necessary. Subsequently, the markers were concatenated in Geneious Prime. Sequences were reviewed regarding missing data, duplicate data and were excluded or combined if needed. The taxonomy of the included species was reviewed and if needed corrected following KewScience (http://www.plantsoftheworldonline.org) and Annonbase (Rainer & Chatrou, 2006). Five species regarding four other families within the Magnoliales namely *Degeneria vitiensis* (Degeneriaceae), *Eupomatia bennettii* (Eupomatiaceae), *Galbulimima belgraveana* (Himantandraceae), *Liriodendron chinense* and *Magnolia Kobus* (Magnoliaceae) and one species out of the Laurales namely *Persea americana* (Lauraceae) were used as outgroups. The final concatenated dataset consisted of 6280 positions comprised in five markers; *rbcL, matK, ndhF, trnL-F* and *psbA-trnH*, with 16%, 26%, 50%, 10%, 29% missing data respectively. Including 813 ingroup sequences, sampling ten species of Ambavioideae (1.2%), five species of Anaxagoreoideae (0.6%), 530 species of Annonoideae (65,2%), and 268 species of Malmeoideae (32.9%).

Phylogenetic analyses

Maximum likelihood analyses using RAxML v.7.0.4 (Stamatakis, 2014) were run on the VSC-HPC node skitty server provided by Ghent University. A Bayesian approach for the phylogenetic analysis would be more elegant in combination with the subsequent divergence time estimation using Beast which is also based on Bayesian statistics. However due to time constraints as a result of convergence problems, a Maximum likelihood approach was obtained. This was also done by previous diversification rate analyses on Annonaceae (Xue et al., 2020).

PartitionFinder2 (Lanfear et al., 2017) was used to assign the best evolutionary model to our markers in the dataset. For all markers a general time-reversible nucleotide substitution model with gamma distributed rates and a proportion of invariant sites was obtained (GTR+G+I).

Divergence time estimation

The ultrametric tree was obtained using BEAST v1.10.4 (Suchard et al., 2018) provided by the CIPRES Science Gateway (Miller et al., 2010). The analysed dataset was partitioned as described above and analysed using a GTR+G model for each partition as determined by Partitionfinder2 (Lanfear et al., 2017). Analyses were run using a lognormally distributed uncorrelated relaxed molecular clock and a speciation yule process for the tree prior (Gernhard, 2008). The ML tree out of the previously mentioned RaxML analyses was used as input starting tree and was used to fix the tree topology as well. Each of the two independent MCMC chains was run for the 168 hours which comprised c. 56 million generations per independent run. Convergence and burn-in were afterwards checked and determined using Tracer v.1.7.1 (Rambaut et al., 2018).

Uniform priors were used to constrain the Annonaceae crown and root node of the phylogenetic tree. The root node was constrained between 156 Mya, which is the overlapping estimated age of the Mesangiospermae (Smith et al., 2010; Bell et al., 2010; Zeng et al., 2014), and 112 Mya, the estimated age of *Endressinina brasiliana* (Early Cretaceous; Mohr & Bernardes-de-Oliveira, 2004), a fossilized flowering shoot, which was proposed as a reliable Magnoliales fossil for calibration by Pirie and Doyle (2012). The crown node was constrained between 112 Mya, as explained above, and 89 Mya, the estimated age of *Futabanthus asamigawaensis* (Late Cretaceous; Takahashi et al., 2008) a fossilized flower which shows features consistent with the Annonaceae crown group (Pirie and Doyle, 2012). The usage of *Endressinina* and *Futabanthus* in uniform priors regarding the crown and root nodes follows calibration scheme CS1 sensu Thomas et al. (2015).

Due to the number of tips in the phylogenetic tree and intensive sampling, the output files were computationally too big to use regular BEAST applications as LogCombiner and TreeAnnotator. Files were therefore analysed in R v3.6.1 in RStudio v1.2.5001 (R Core Team, 2020; RStudio Team, 2019) using the package coda (Plummer et al., 2006) for fragmenting and editing the posterior files using a 'divide and conquer' strategy. Results were summarized using the median age values of each node, as median values are more robust against outliers in regard to mean values. Variation was incorporated by including highest density credible intervals (HDI) of the posterior values on each node. HDI were chosen over other options such as the equal-tailed intervals (ETI), because it penalizes the outliers more. The maximum clade credibility tree was searched using the R package phangorn (Schliep, 2011). 20% was discarded as burn-in.

Diversification rate analysis

Diversification rate analyses were performed using the Bayesian Analysis of Macroevolutionary Mixtures software (BAMM) v. 2.5.0 (Rabosky et al., 2014). Priors for the analyses were obtained using the BAMMtools v. 2.1.7 (Rabosky et al., 2014) 'setBAMMpriors' function. The analysis ran for 40 million generations with a sampling frequency of every 100,000 generations. 10% of the output was discarded as burn-in. Post burn-in files were analysed using the R package BAMMtools ver. 2.1.7 (Rabosky et al., 2014).

The credible set of shift configurations was searched and a phylorate plot was generated using 'Plot.bammdata'. Diversification rate shifts were added using the 'Addbammshifts' function. Rate through time plots regarding speciation, extinction and diversification rate using the 'PlotRateThroughTime' function in BAMMtools were generated for the Annonaceae, specific subfamilies and for clades identified with the occurrence of a diversification rate shift by BAMM.

Additionally, a lineage through time (LTT) plot was generated using the R package phytools (Revell, 2012).

RESULTS

Phylogenetic analyses and divergence time estimation

Crown nodes of the subfamilies and genera are well supported in the obtained RaxML-phylogenetic tree (fig. 8).

The divergence time estimation using BEAST had trouble converging. Some ESS-values were in the final analysis below 200. In general, these have to be at least 200, though they were above 100 which is not ideal but acceptable for large datasets as this one. However, the ESS-value for the likelihood, one of the more important values, was in the combined analysis 2869. Also the MCMC-analyses of both runs reached a plateau. These indicate convergence of the analysis.

The origination node of the Annonaceae was inferred at c. 98 Mya. The crown nodes of the Annonoideae and the Malmeoideae were inferred at c. 82 Mya and c. 51 Mya respectively. The crown nodes of the smaller subfamilies as the Ambavioideae and the Anaxagoreoideae were inferred at c. 81 Mya and c. 36 Mya respectively. As explained above, variation was incorporated by including a highest density credible interval (HDI) of the posterior values on each node (see appendix).

Diversification rate analysis

The ESS-values of the log likelihoods and the number of rate shifts were 215.1749 and 175.6896 respectively. In general, these have to be at least 200, where 200 is already on the low side but reasonable for large datasets as this one. Hence, the ESS-values of the log likelihoods are acceptable. The ESS-values for the number of rate shifts are however below 200, but these might be reasonable as well due to the converging difficulties obtained in the divergence time estimation and the number of tips present in our dataset. Priors determined by the 'setBAMMpriors' function can be found in the appendix.

Phylorate plot

BAMM detected a diverse amount of diversification rate shifts across the Annonaceae ranging from 2 to 11 shifts. 6 diversification rate shifts were detected most frequently with a posterior distribution of 0.33 and 5 diversification rate shifts second mostly with a posterior distribution of 0.32. Other amounts of detected diversification rate shifts had posterior distributions of 0.15 and below. 809 distinct shift configurations were found. The best distinct shift configuration, thus the configuration with the highest posterior probability or the one that was sampled most often, had a posterior probability of only 2%. The credible set of shift configurations is given.

BAMM identified 6 clades with accelerated diversification rate shifts in the credible set of shift configurations (see further). An accelerated diversification rate shift occurred at the node of the clade comprising the whole family of the Annonaceae. Within the Annonoideae, 4 clades with accelerated diversification rates were detected: *Guatteria, Duguetia, Goniothalamus* and the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* Within the Malmeoideae 1 diversification rate acceleration was detected at the start of the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae. All of the detected diversification rate accelerations within the family were followed by subsequent rate slowdowns.

Rate-through-time plots

Family wide net diversification rates show almost constant, very slowly increasing diversification rates (up to c. 0.06) from the start up to around 40 Mya when they start to accelerate significantly (up to c. 0.09). Around 15 Mya the diversification rates decelerate again up to the present. When analysing the rate-through-time-plots of the four subfamilies different diversification rate patterns are seen. The Annonoideae start with a very slow increase, until around 35 Mya when the rates start to accelerate.

Only until around 25 Mya the diversification rate acceleration is significantly larger (up to c. 0.08). Around 15 Mya the diversification rates decelerate again up to the present as seen in the whole family. In the Malmeoideae a different diversification rate pattern is observed. Around 40 Mya, only c. 10 Mya after their divergence, the diversification rates accelerate significantly up to c. 0.14. Around 30 Mya the diversification rates decelerate until the present. In the smaller subfamilies of the Anaxagoreoideae and the Ambavioideae no major diversification rate accelerations or decreases are observed. The Ambavioideae has a very slow diversification rate increase (up to c. 0.05) from their origination to around 50 Mya when they stay constant until the present. The Anaxagoreoideae have low constant diversification rates from start to present. When looking at the speciation and extinction rate patterns of the whole family and the subfamilies, we see that the speciation rates follow the same pattern as the diversification rates whereas the extinction rates start close to 0 and stay more or less constant over time in all groups. Mean speciation and extinction rates for the Annonaceae were estimated respectively with 90% highest posterior density (HPD) between 0.081 - 0.093 and 0.003 - 0.017. Mean speciation and extinction rates for the subfamilies were estimated with 90%-HPD between 0.089 - 0.113 and 0.001 - 0.027 respectively for the Malmeoideae, 0.076 - 0.090 and 0.003 - 0.018 respectively for the Annonoideae, 0.054 - 0.072 and 0.0 - 0.016 respectively for the Ambavioideae and between 0.028 - 0.071 and 0.0 - 0.018 respectively for the Anaxagoreae.

When plotting rate through time curves for clades identified with the occurrence of a diversification rate shift different patterns were identified. *Guatteria* and *Duguetia* showed similar diversification rate curves through time, in both rates accelerated rapidly around 15 Mya (up to c. 0.17 and c. 0.16 respectively) and were quickly followed by a diversification rate slowdown up to the present. *Goniothalamus* shows in comparison with other diversification patterns of identified clades, no rapid rate accelerations. Instead, diversification rates remain almost constant around c. 0.05 from the crown node at c. 26 Mya up to c. 10 Mya, when diversification rates slowdown slowly. The fact that *Goniothalamus* still is identified as a diversification rate shift within the Annonaceae, is probably due to the long time between the stem and crown node of the genus. In the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* diversification rates accelerated rapidly around 26 Mya (up to 0.15). Afterwards the rates experience several small rate accelerations and slowdowns until around 15 Mya when diversification rates slow down up to the present. Diversification rates rapidly accelerated around 40 Mya in the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae (up to c. 0.14). The acceleration is followed around 30 Mya by a slow diversification rate decrease up to the present. Again when looking at the speciation and extinction rate patterns in these clades, it is seen that the diversification rates are mainly dependent on the speciation rates instead of the extinction rates.

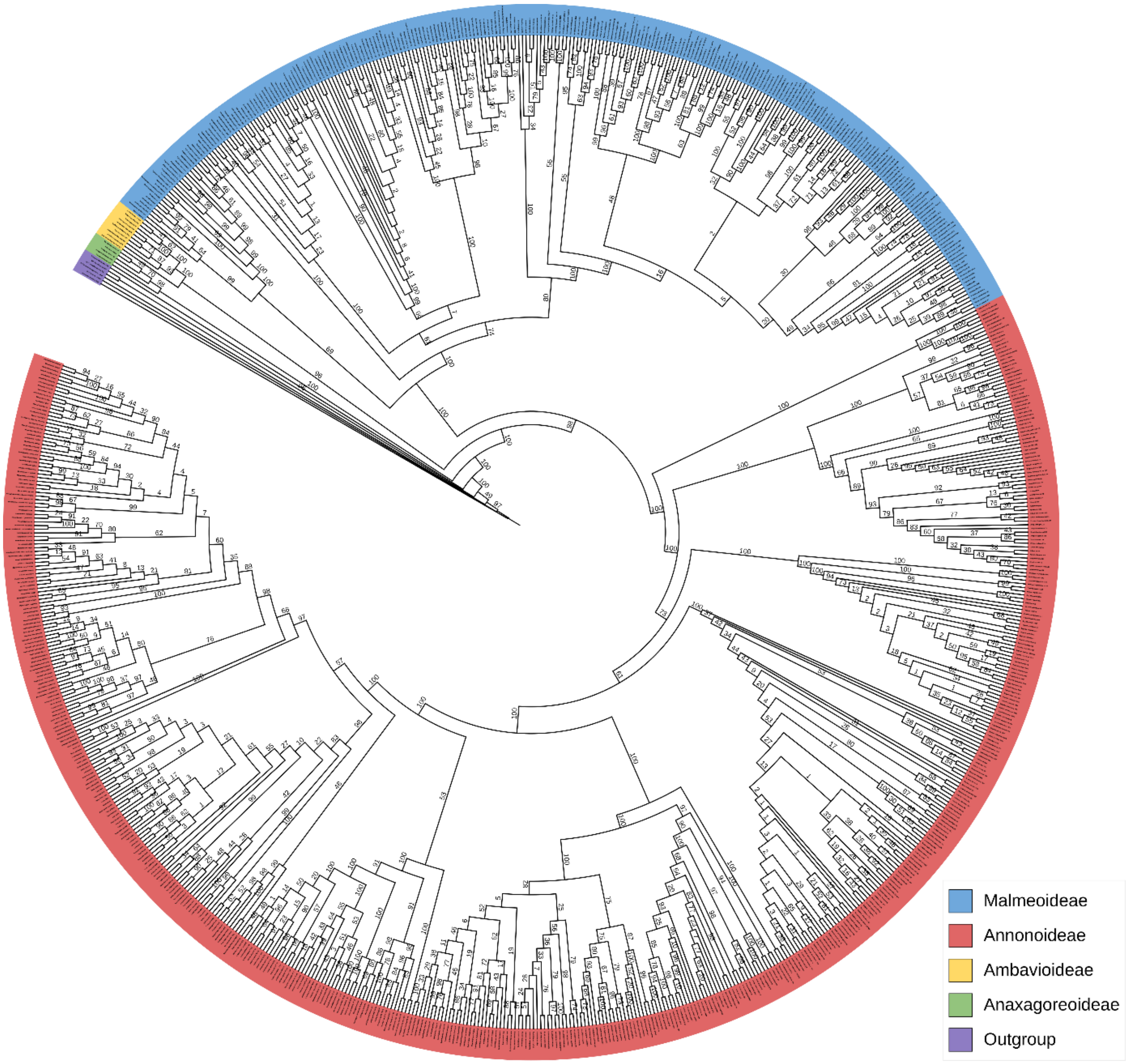

Figure 8. Maximum likelihood tree of the Annonaceae obtained through RaxML. Numbers indicate bootstrap values. Colors indicate the four subfamilies and the outgroup, as specified in the legend.

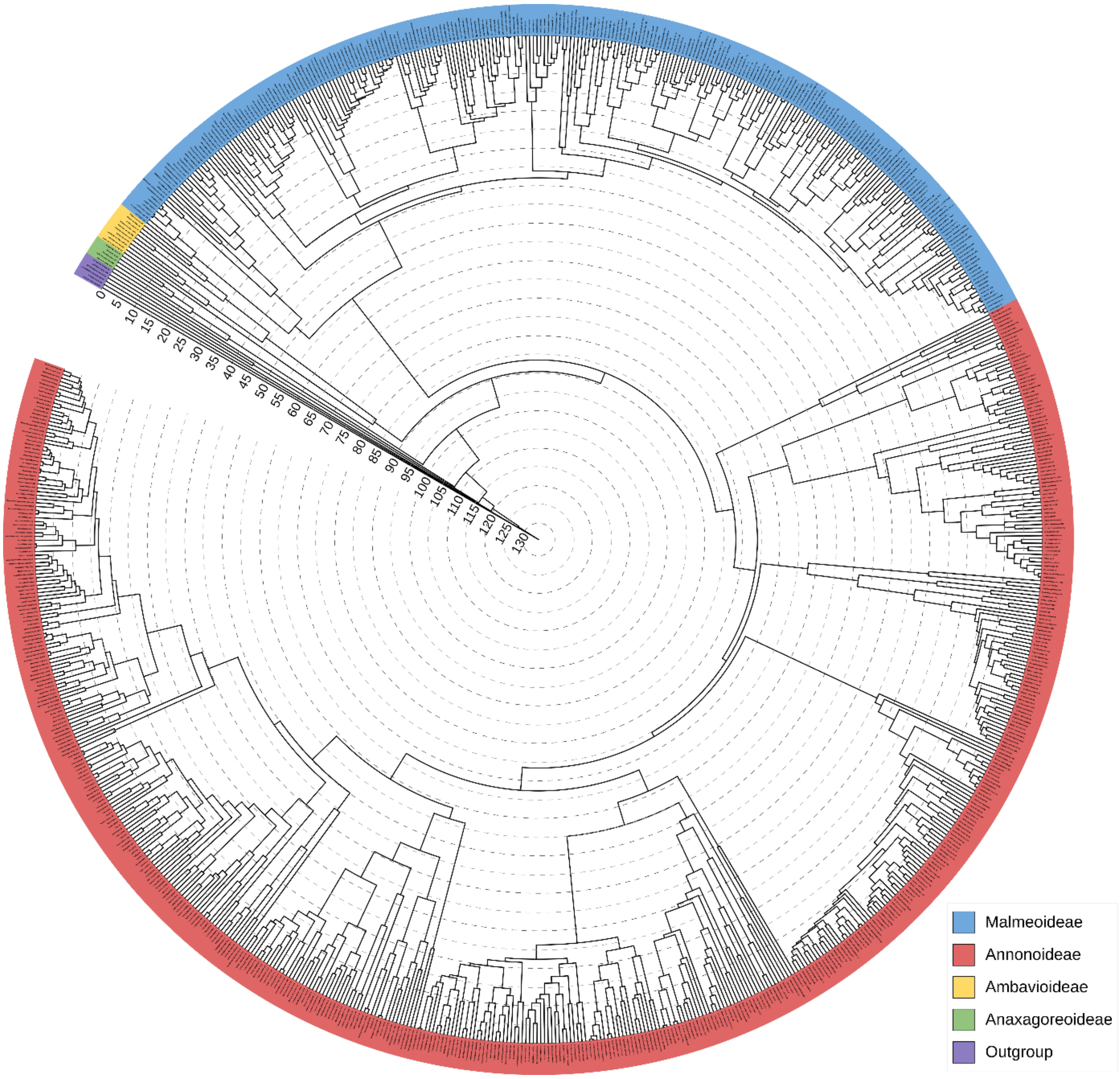

Figure 9. Maximum clade credibility chronogram of the Annonaceae obtained through BEAST. Numbers indicate time in million years. Colors indicate the four subfamilies and the outgroup, as specified in the legend.

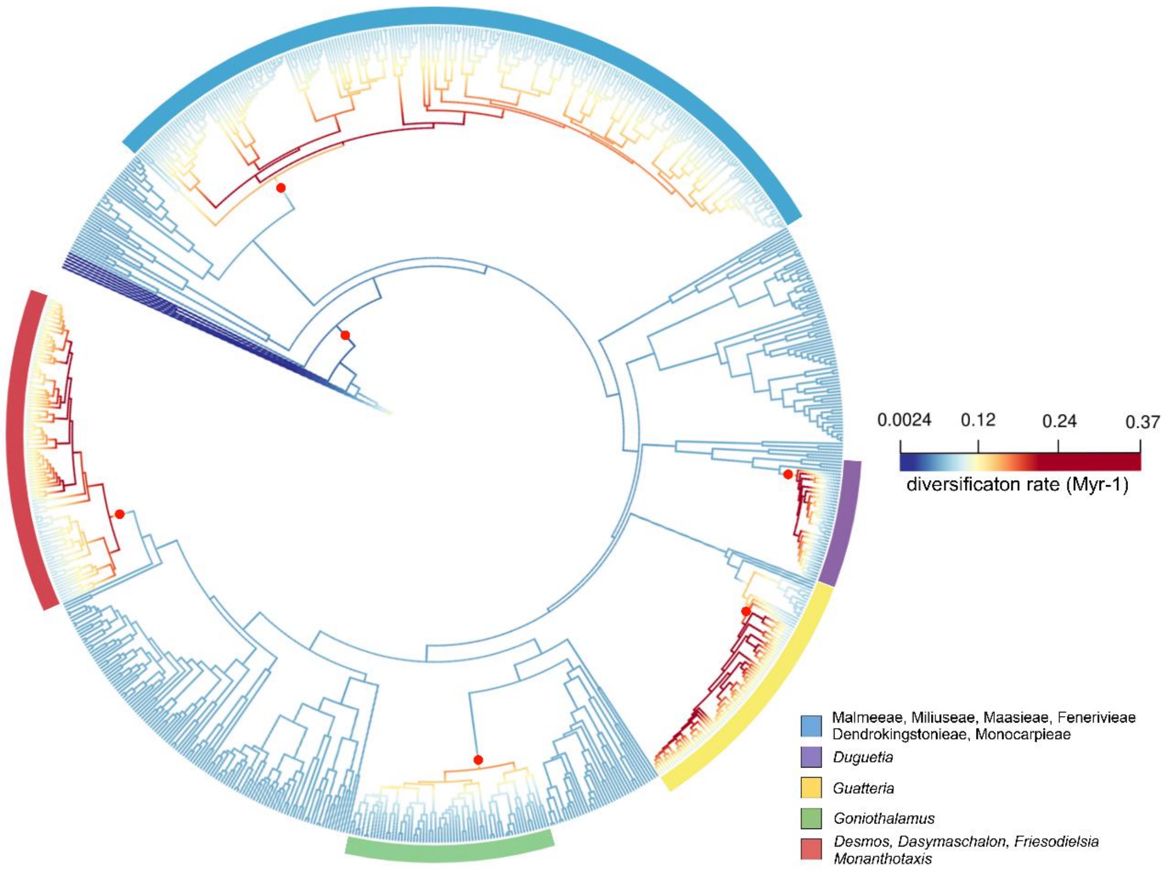

Figure 10. Credible set of shift configurations/Phylorate plot of the Annonaceae obtained through BAMM. Branches are colored according to the diversification rate (Myr^{-1}). Red dots indicate diversification rate shifts. Colors indicate clades identified with a diversification rate shift as specified in the legend.

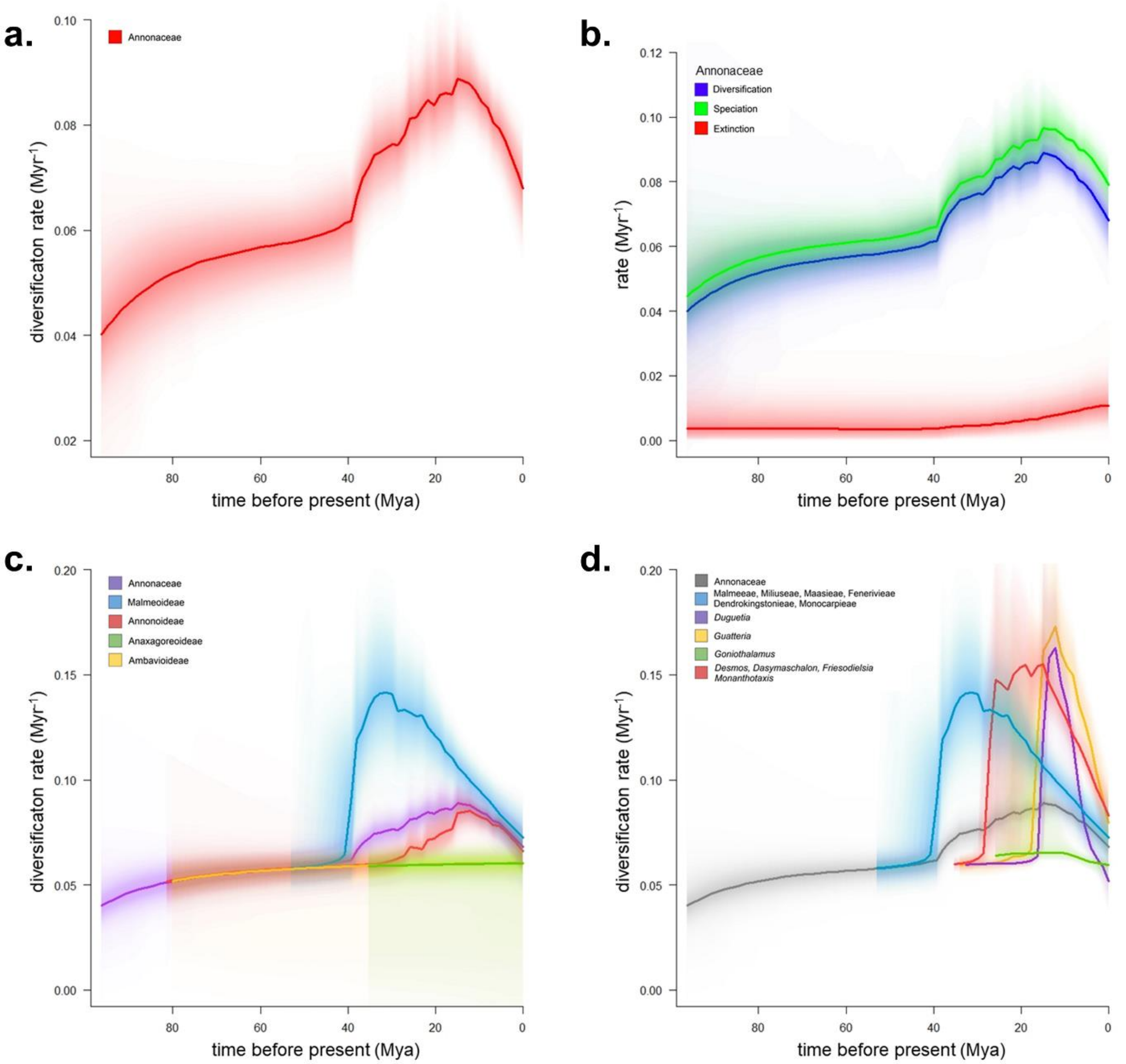

Figure 11. Net diversification rate through time in Annonaceae obtained through BAMM (a), net diversification (blue), speciation (green) and extinction (red) rate through time in Annonaceae obtained through BAMM (b), net diversification rate through time in the Annonaceae (purple), Malmeoideae (blue), Annonoideae (red), Anaxagoreoideae (green) and Ambavioideae (yellow) obtained through BAMM (c). net diversification rate through time of the clades identified with diversification rate shifts. Annonaceae (grey), Malmeeae, Miliuseae, Maasieae, Fenereriveae, Dendrokingstonieae and Monocarpieae (blue), *Duguetia* (purple), *Guatteria* (yellow), *Goniothalamus* (green) and *Desmos*, *Dasymaschalon*, *Friesodielsia* and *Monanthotaxis* (red).

Accumulation of lineages through time

The log. lineage through time (LTT) plots based on the dated phylogenetic tree of the Annonaceae obtained in the divergence time estimation shows a relatively linear overall trend of lineage accumulation until c. 40 Mya, when lineages started to accumulate exponentially up to the present.

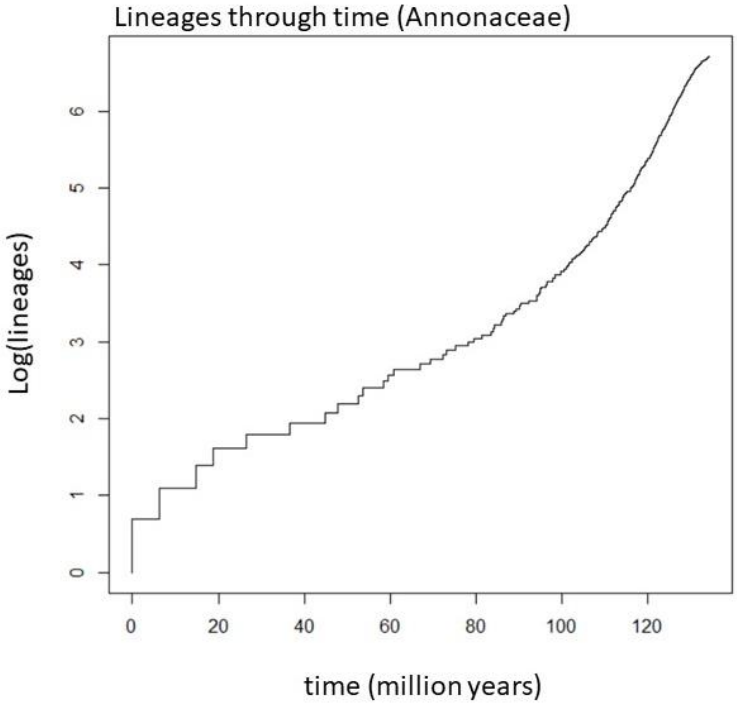

Figure 12. Log. lineage through time plot (LTT) of the Annonaceae obtained through the R package phytools.

DISCUSSION

Phylogenetic analyses & divergence time estimation

The resulting topology of the RaxML-phylogenetic tree of 819 taxa is largely congruent with previous family-wide phylogenetic studies regarding Annonaceae (Chatrou et al., 2012; Guo et al., 2018).

Xue et al. estimated the crown node of the Annonaceae at c. 94 Mya, Annonoideae at c. 83 Mya, Malmeoideae at c. 42 Mya, Ambavioideae at c. 78 Mya and Anaxagoreoideae at c. 28 Mya. Thus our results are largely congruent with the divergence time estimation obtained by Xue et al. (2020), except maybe for the crown node of the Malmeoideae, which in this study was estimated c. 9 million years older. Our results were also congruent with other studies in which divergence time estimations were performed on Annonaceae, as for example Thomas et al. (2017).

The divergence time estimation had, however, trouble converging indicated by low ESS-values. This can be linked with several factors such as missing data, number of generations the analysis ran and the prior model used for the divergence estimation.

The last factor is linked with a general problem found in literature regarding dating in Annonaceae (Pirie & Doyle, 2012). Most of the species within the family can be found in the two major subfamilies namely the Annonoideae and Malmeoideae or respectively referred to in literature as the long branch clade (LBC) and the short branch clade (SBC), due to the difference in branch lengths between the two subfamilies. This difference linked to differences in substitution rates, which is higher in the Annonoideae (Hoekstra et al., 2017), causes molecular dating in Annonaceae to be a challenge. As only one molecular clock can be used for the whole divergence time estimation in BEAST, the analysis adopts the substitution rate of the most sampled subfamily. In many studies as in this one, the Annonoideae contain the most species and thus substitution rates of the Annonoideae are adopted for the molecular clock used in the dating of the whole phylogenetic tree. This bias results in a younger age estimation of the Malmeoideae crown node in comparison with models were the Malmeoideae substitution rate is adopted. If the Malmeoideae are dominant regarding species-sampling, the Annonoideae crown node gets pushed to older age estimates (Pirie & Doyle, 2011). Clade-specific heterogeneity in molecular substitution rates have shown to challenge molecular dating in other clades within angiosperms (Beaulieu et al., 2015). Thus, models allowing clade-specific heterogeneity in molecular substitution rates should be a solution to the molecular dating problems occurring in Annonaceae (Beaulieu et al., 2015) as also found in our results. Furthermore, more data and more generations within each run of the analysis can help.

Diversification rate analysis

Diversification through time in Annonaceae

The diversification rate pattern observed through time of the Annonaceae coincides with a combination of the patterns seen in the two major subfamilies of the family. The early rate acceleration seen in the Late Eocene within the Annonaceae is driven by the rapid diversification rate acceleration at that time in the Malmeoideae. Later when the rates within the Malmeoideae starts to slow down around the Late Eocene – Late Oligocene, diversification rates in the Annonoideae start to accelerate driving the further acceleration of the whole family up to the Middle Miocene where diversification rates slow down up to the present. Previous papers regarding the Annonaceae diversification rate through time found constant or slowly increasing rates across the family until the Middle Oligocene - Early Miocene, 25 Mya (Couvreur et al., 2011; Xue et al., 2020). Afterwards, Xue et al. (2020) found significant diversification rate increases, which coincide with the major subfamilies Annonoideae and the Malmeoideae that start to diversify rapidly around that time. Following Xue et al., the Ambavioideae diversified more recently within the Miocene due to radiations within the *Cyathocalyx, Drepananthus* clade in South East Asia. Also Erkens et al. (2012) found major diversification rate accelerations after a long period of constant diversification in Annonaceae. Couvreur et al. (2011) found in contrast slowly increasing, almost constant diversification rates through time across lineages in the family, with a few rate increases after 25 Mya but no major diversification rate shifts. However, this can be assigned due to several factors in their methodology as incomplete species sampling and arbitrary thresholds, as stated by Xue et al. (2020). However, some differences are seen while comparing our results. For example the timing of the shifts observed in the Malmeoideae. In Xue et al. rates within the Malmeoideae started to accelerate around 25 Mya as in the Annonoideae, due to the fact that in Xue et al. (2020) rates only really start to accelerate in the Miliuseae-tribe. In comparison with our results, when rates start to accelerate at an earlier node within the Malmeoideae. Because of the fact that the accelerations in these major subfamilies drive the shape of the curve seen in the Annonaceae, rates in the Annonaceae only start to accelerate in the Late Oligocene in regard to the Late Eocene as found in our results. Also significant diversification rate shifts observed in the Ambavioideae by Xue et al., were not seen in our results. Differences in results can be explained by differences in taxon sampling, genome-region sampling and prior settings, which are factors known to influence diversification rate analyses as well as the linked divergence time estimations (Rabosky et al., 2014; Sauquet et al., 2017; Sauquet & Magallón, 2018; Meyer & Wiens, 2018).

Another difference is the observed diversification rate slows down up to the present. These were not detected by Xue et al., where rates keep accelerating up to the present. However, the observed pattern of high rates of early diversification within clades (referring to the clades identified with diversification rate shifts influencing the overall diversification patterns seen in the family and subfamilies) and subsequent slowdowns in diversification rates to the long-term average as the present is approached is congruent with patterns resulting from biases associated with diversification rate analyses explained by Budd & Mann (2018), appointed as the “push of the past”. With this bias in mind associated with the results of Xue et al. (2020) and with the shape of our lineage through time plot, we can assume that the found slowdowns are a bias of our analysis and will not be seen as the real evolutionary scenario within Annonaceae.

Our log. lineage through time plot shows a linear trend of lineage accumulation through time indicating gradual accumulation of lineages associated with constant diversification rates through time (Couvreur et al., 2011), until c. 40 Mya when lineages started to accumulate exponentially indicating elevated diversification rates. Thus the log. lineage through time plot indicates a gradual increase scenario followed by an exponential increase scenario from the Late Eocene onwards, as already proposed by Meseguer et al. (2021).

In general, our results, being a combination of slowly increasing — almost constant — diversification rates with major rate increases between the Late Eocene-Middle Miocene, coincide with results obtained in Erkens et al. (2012) and Xue et al. (2020) regarding Annonaceae. This non-homogeneous diversification mode indicated by the presence diversification rate shifts across lineages was also found by Massoni et al. (2015) while analysing diversification rate shifts within the whole Magnoliidae. They found general increases of diversification rates through time within Magnoliales, the order comprising the Annonaceae. Other examples of major tropical plant clades indicated with non-constant diversification rates through time due to relatively recent rate accelerations are the Cactaceae (Arakari et al., 2011), Fabaceae (Koenen et al., 2013), Malpighiales (Xi et al., 2012), Meliaceae (Koenen et al., 2015) and Calophyllaceae (Cabral et al., 2021). This was also found in some tropical animal clades, e.g. in the tropical leaf beetle genus *Cephaloleia* (McKenna & Farrel, 2006) and in the Neotropical golden orbweavers, genus *Nephila* (Turk et al., 2021).

Thus the evolutionary history of Annonaceae through time as shown by our results and as found in previous studies, is a gradual accumulation scenario, indicated by linear lineage accumulation through time associated with almost constant diversification rates (see also Couvreur et al., 2011). This was followed by a rapid increase in lineages between the Late Eocene-Middle Miocene, 40 to 15 million years ago, due to increased diversification rates across lineages in the family. This increase in diversification rates is most likely the result of increased speciation rates rather than reduced extinction rates or a combination of both. Extinction rates stayed constant throughout the history of the Annonaceae whereas speciation rates stayed constant as well until the Late Eocene–Middle Miocene when accelerations start to occur as with the diversification rates, this was also observed in the results obtained by Xue et al. (2020). However, the trouble converging of the analysis indicated by the low ESS-values, must be kept in mind.

Therefore, the evolutionary history of the family of the Annonaceae coincides with a combination of two scenarios proposed by Meseguer et al. (2021). A mixed model of a gradual increase scenario (Sc. 1), more specifically a 'Cradle & Museum' subscenario due to constant speciation and extinction rates through time. Followed by an exponential increase scenario (Sc. 2), more specifically a 'Recent Cradle' subscenario from the Late Eocene onwards, due to elevated speciation rates instead of lowered extinction rates. Thus, timing and rate patterns within the evolutionary history of the Annonaceae hint at a combination of 'Cradle' and 'Museum' models simultaneously through time. In this mixed model, environmentally stable conditions in tropical environments, and thus low constant extinction rates, preserve ancient lineages and accumulate them through time as well as lineages resulting from relatively recent radiations linked to increased speciation rates.

Identified diversification rate shifts

As mentioned, BAMM identified 809 distinct shift configurations, with only a posterior probability of 2% for the best distinct shift configuration. This combined with the low ESS-values obtained indicate convergence problems in the analysis. This can be explained by the large number of tips used in the analysis, as the total number of distinct shift configurations is convergent with the number of branches in a phylogenetic tree. Because it is not possible to show all shift configurations found, a credible set of shift configurations is given. This is the set of shifts that accounts for 95% probability of the data. It is searched by identifying all distinct shift configurations, here 809, and rank them by posterior probability. Starting with the most probable configuration, these are summed until a configuration of shifts is obtained that accounts for 95% of the data. Thus, it must be kept in mind that the shift configuration shown is a cumulative set of configurations instead of the configuration that is most sampled (Rabosky et al., 2014).

Five diversification rate shifts were detected within the two major subfamilies of the Annonaceae. One at the node comprising the whole family. Four within the Annonoideae and one within the Malmeloideae. In the Annonoideae, the shifts were found in the genera *Guatteria, Duguetia* and *Goniothalamus* as well as at the node of the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* Whereas in the Malmeoideae, the diversification rate shift was identified in a major clade comprising the tribes Malmeeae, Miliuseae and four smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae.

Erkens et al. (2012) found significant diversification rate accelerations in Annonaceae at the base of the major clades Annoneae-Monodorae-Uvariea and Miliuseae-Monocarpieae-Malmeeae, but also near the smaller tribe Miliuseae and genera such as *Goniothalamus*, *Isolona*, *Monodora* and *Stenanona* using a topological and temporal method. Xue et al. (2020) found in comparison to this study, twelve diversification rate accelerations in the Annonaceae using BAMM and seven using turboMEDUSA. However the results of the two methods were generally consistent with each other. Xue et al. found shifts using BAMM in the genera *Annona, Artabotrys, Asimina, Drepananthus, Duguetia, Goniothalamus, Guatteria, Uvaria,* Xylopia, the tribes Miliuseae and Malmeeae and the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthothaxis.* Thus, except for shifts found in smaller clades comprising genera as *Annona, Artabotrys, Asmina, Uvaria* and *Xylopia*, the diversification rate shifts found in our study are in general concordant with the findings of Xue et al., *Drepananthus* was not included in our dataset. Our results regarding the clades with diversification rate shifts were not concordant with results obtained by Erkens et al. (2012). This could however be assigned to lower species sampling as our species sampling is more congruent to the sampling in Xue et al. (2020) as stated before.

The found diversification rate shifts in the Annonoideae and Malmeoideae differ in timing but not in overall magnitude. In Malmeoideae, the identified clade accelerates rapidly in the Late Eocene (c. 40 Mya) from c. 0.05 to c. 0.14, whereas the clades identified in the Annonoideae all peak in the Late Oligocene, Early to Middle Miocene (c. 25 – 15 Mya) from c. 0.05 to c. 0.15-0.17. Congruent with our results, rapid diversification in the Miocene of the genus *Guatteria* (Annonoideae) was identified by Erkens et al. (2007). Potentially rapid recent diversification has been identified in *Goniothalamus* (Erkens et al., 2012), but this was partly rejected by Thomas et al. (2017) who found older crown group divergence in the genus with lower diversification rates in comparison with other radiations within Annonaceae, which is congruent with our results.

As already mentioned, the diversification rates within the Malmeoideae and Annonoideae largely drive the diversification rate accelerations of the whole family. In addition to that, when comparing the diversification rate patterns through time between the major subfamilies and the patterns of the seen diversification rate shifts within them, they largely coincide with each other. Therefore, the diversification rate shift seen in the Late Eocene of the Malmeoideae is driven by the rate acceleration at that time observed in the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae. The diversification rate acceleration seen in the Annonoideae are at first driven by the rate accelerations within *Goniothalamus* and the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* In the Miocene, when these rates started to slow down until the present, rates in *Guatteria* and *Duguetia* started to accelerate driving the further diversification rate acceleration in the Annonoideae. Until the Middle Miocene, when diversification rates peaked and subsequently slowed down until the present. These correlations are handy for analysing the plausible drivers behind the diversification rate increases of the family or more specific subfamilies at certain times in their evolutionary history, as we know the morphology and biogeographical distribution of these clades (see further).

Plausible causal mechanism of the seen diversification rate accelerations

Establishing causal mechanisms behind identified diversification rate shifts is difficult due to the large amount of factors proven to have an impact on speciation and extinction rates simultaneously in both the opposite or the same direction (Donoghue & Sanderson, 2015; Massoni et al., 2015). Also many of these factors are most probably correlated with each other. The effect of a trait on diversification of a lineage is dependent on other taxa, other traits within the organism and the environmental characteristics present (Donoghue & Sanderson, 2015; Massoni et al., 2015). For example Donoghue & Sanderson (2015) state that a certain trait primarily decreasing the extinction rate in a lineage can become a trait that primarily increases the speciation rate along the branch due to a certain event changing the environmental characteristics present. Thus this context dependency implies that evolutionary changes aren't fundamentally needed for shifts in diversification rate. These can be caused by changing environmental characteristics locally or by the migration of lineages in other environments (Donoghue & Sanderson, 2015).

Another bias in regard to assigning causes to diversification rate shifts is the plausibility that certain shifts only occur when a combination of several traits or environmental characteristics is present, due to the additive downstream effects of sequential changes along a branch in the phylogenetic tree. This last factor and the context dependency of mechanisms lead to the possibility that the seen diversification shift happens several nodes after the timing of where an evolutionary change happened, which is called evolutionary lag or delayed shifts (Donoghue & Sanderson, 2015; Massoni et al., 2015). These explained biases complexes the assignment of biotic and abiotic causal mechanisms to seen diversification rate shifts, and show that one must always act very cautious while searching for these correlations. A solution for this is to take as many as possible potential factors into account while doing this. Donoghue & Sanderson (2015) proposed in reaction to this new terminology such as 'key synnovation', which refers to the combination of two traits promoting diversification rates. And 'key confluence' referring to the combination of 'innovations' and 'synnovations' with environmental changes/geographic movements promoting diversification rates.

Massoni et al. (2015) stated that speciation and extinction rates in Magnoliidae are potentially influenced by climatic shifts, variation in the available area, variation in the geographic distribution and relationships regarding pollinators. Many plausible biotic and abiotic drivers of diversification were proposed for the Annonaceae (e.g. Erkens et al., 2012; Saunders, 2012; Richardson et al., 2004; Thomas et al., 2015; Thomas et al., 2017; Xue et al., 2020). However, Erkens et al. (2012) stated that clades identified with elevated diversification rates in Annonaceae are difficult to link to proposed mechanisms indicating that hypotheses regarding random or nearly random cladogenesis must be kept in mind. Below some of these plausible biogeographical and morphological drivers congruent with the timing and clades of the seen diversification rate accelerations are given. But with in mind that one must always act very cautious while assigning mechanisms to seen diversification rate shifts, as explained above due to biases as context dependency and additive downstream effects of traits, etc.

Plausible underlying biogeographical drivers

The sudden increase in diversification rates, and thus as stated in speciation rates, around 40 to 25 million years ago could be assigned to major events in the biogeographical history of Annonaceae. Below a summary is given of plausible vicariance events that could be at the basis of the seen diversification rate increases in the family and the tropical biome as a whole.

'Indian raft' hypothesis

India separated from Madagascar c. 90-85 Mya in the Late Cretaceous and drifted North-Eastwards across the Indian ocean with a subsequent collision with continental South-East Asia. The timing of this event is however disputed, proposed timings range from 50 to 35 Mya based on geodynamical and palynological data (Thomas et al., 2014 and references therein). This collision may have facilitated biotic dispersal and later establishment from the African to the Asian continent by lineages rafting on the drifting Indian plate, which could have spiked diversification rates after the collision (Couvreur et al., 2011; Thomas et al., 2014). Fossilized seeds and wood from Annonaceae are proven to be present on the Indian Plate prior to the eventual collision (Couvreur et al., 2011; Thomas et al., 2014 and references therein), indicating that this mechanism is a plausible driver of diversification in Annonaceae.

Only one diversification rate shift found in our results is consistent with the timing regarding this event namely the diversification rate acceleration, and thus as mentioned speciation rate acceleration, found in the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae of the Malmeoideae. Hence, the Indian rafting could be a plausible causal mechanism behind the observed diversification rate shift. Thomas et al. (2014) supports this hypothesis by stating that rafting of Malmeoideae ancestors on the Indian plate seems plausible. In contrast, Couvreur et al. (2011) supported boreotropical dispersal, see below, over Indian rafting as the driver of distribution patterns seen in Malmeoideae using ancestral area reconstructions.

Paleogene cooling and the Boreotropical disruption

Due to a warming trend (Early Eocene Climatic Optimum, EECO) in the late Paleocene-Early Eocene (Richardson et al., 2004) climatic conditions supported tropical vegetation at higher latitudes and Annonaceae lineages and other tropical taxa were widely distributed around the northern mid-latitudes in a biome called the Boreotropical forests during the late Paleocene-Eocene. These widespread northern Boreotropical forests acted as a frost-free and humid climate and vegetation corridor for the exchange of lineages between tropical forests in the Americas, Africa and South-East Asia in the Paleocene and Eocene (Couvreur et al., 2011; Thomas et al., 2015). In the late Eocene global temperatures dropped (Morley, 2003; Richardson et al., 2004; Couvreur et al., 2011) which peaked drastically around the Eocene-Oligocene boundary (ca. 34 Mya; Thomas et al., 2015). Due to this cooling trend, frost-sensitive Boreotropical vegetation were replaced with frost-tolerant temperate taxa (Morley, 2003), and tropical taxa went extinct or receded to equatorial regions. Due to this Boreotropical disruption, these Eurasian latitudes stopped functioning as a corridor for intercontinental biotic exchange (Thomas et al., 2015) and could have spiked diversification rates through vicariance, the geographic isolation of populations, in the Oligocene. Afterwards biotic exchange between continents has been restricted to rare long-distance dispersal events. This in combination with the fact that the primary dispersal agents of Annonaceae are mammals explains the generic endemism pattern seen in Annonaceae, with most genera either being Neotropical or Paleotropical (comprising African and Asian-Pacific regions) (Richardson et al., 2004). Lineages splitting up at a time consistent with the boreotropical disruption has been found in several tropical plant families as mentioned in Couvreur et al. (2011) such as the Malpighiaceae (Davis et al., 2002), Meliaceae (Muellner et al., 2006), Rubiaceae (Antonellli et al., 2009) and Melastomataceae (Renner et al., 2001).

In our results only 1 diversification rate shift found is consistent with the timing regarding the Boreotropical disruption namely the diversification rate acceleration found in the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae of the Malmeoideae. Thus the Paleogene cooling and consequent boreotropical disruption could be a plausible driver behind the seen diversification and underlying speciation rate acceleration. The mentioned clade comprises genera of the Neotropics and Paleotropics, which could support the boreotropical disruption explanation, being a major global biogeographical event. This is supported by previous research suggesting that distribution patterns within Malmeoideae (Richardson et al., 2004; Couvreur et al., 2011) and within Andean-centred genera of the Malmeoideae (Pirie et al., 2006) are the result of geodispersal across the Boreotropics. However, this was also stated for clades within the Annonoideae (Erkens et al., 2007; Erkens et al., 2009; Couvreur et al., 2011; Thomas et al., 2017).

Post-boreotropical biogeographical events

Paleotropics

During the early to middle Miocene (23-12 Mya) the Afro-Arabian plate collided with the Iranian and Anatolian plates, this in combination with the Middle Miocene Climatic Optimum (17-15 Mya) possibly formed suitable climate and vegetation corridors between Africa and Southwest Asia where biotic exchange and range expansion via overland dispersal was feasible for tropical taxa. From the late middle Miocene onwards the climate became cooler, drier and more seasonal again inducing the expansion of drought-adapted vegetation in these areas. This combination of overland dispersal and subsequent geographic isolation of Paleotropical taxa due to habitat fragmentation could have induced vicariance events and spiked diversification rates within the Miocene (Thomas et al., 2015).

Many dispersal-vicariance patterns are occurrent in tropical Asia, but they remain poorly understood. Examples are: Miocene dispersal events across water bodies in Malesia due to plate convergence and subsequent land emergence; Long distance dispersal of taxa via stepping stones between the Philippines and Taiwan; Dispersal of angiosperms across the deep-water channel of the Makassar Straits between Borneo and Sulawesi from the early Miocene onwards (Thomas et al., 2017).

Of the clades identified with a diversification rate shift within the Annonoideae, two are Paleotropical (POWO, 2019) and congruent with the timing of this biogeographical event namely the clade leading to *Goniothalamus* and the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* Therefore, this biogeographical event could be a plausible driver for the diversification and underlying speciation rate acceleration observed in the results. Supporting this, Thomas et al. (2015) identified the majority of the inferred Paleotropical vicariance events in his analysis are clustered in the Miocene as for example splits in the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* Thomas et al. (2017) suggested that the distribution of *Goniothalamus* is linked with dispersal events across waterbodies in tropical Asia during a Miocene timeframe as explained above.

Long-distance transoceanic dispersal could have also played a significant role in establishing the current diversity within the Paleotropics. However these are hard to link with specific diversification events because these could have occurred at any point in time (Thomas et al., 2015).

Neotropics

Around the middle Miocene (ca. 17-11 Mya) the western Amazon was flooded by Lake Pebas, this flooding may have formed a Northwest to West dispersal barrier for tropical biota. The vicariance event due to the flooding but also the occurrence of new forested habitat due to the drainage of the Lake in the late Miocene may have spiked diversification rates in tropical lineages (Pirie et al., 2018).

The Andean uplift started from the early Miocene onwards, but with most of the orogeny occurring in the late Miocene and Pliocene through intense bursts. The current elevation of the Andes was reached 6 million years ago (Meseguer et al., 2021). Through the Andean orogeny an east-west dispersal barrier for neotropical taxa was established between the sides of the elevational range geographically isolating populations and thus may have spiked vicariance events in the Neogene (Pirie et al., 2018). Also the initial and later uplift could have established ecological opportunities in new tropical habitats/climate regimes at different elevational degrees which may have boosted diversification rates as well as the vicariance events as described above. The Andean uplift is considered in literature as one of the main drivers in the elevated diversification of Neotropical plant lineages (Meseguer et al., 2021).

Due to the westward dispersal of the North and South American plates and the eastward dispersal of the Caribbean plate combined with subsequent uplift events, the Panama isthmus closed in the late Miocene-Pliocene allowing biotic exchange along a land bridge between North, Central and South America (Morley, 2003; Pirie et al., 2018). The formed continuous land bridge between North and South-America during the Pliocene, enabled easy migration for taxa into new environments which could have spiked diversification rates. However, taxa could have migrated and diversified rapidly before the complete closure of the Isthmus across the submerged land bridge via stepping stones (Erkens et al., 2011).

Results show that diversification rates spiked in *Duguetia* and *Guatteria* around 15 million years ago. *Duguetia* and *Guatteria* are neotropical genera, except for 4 species of *Duguetia* which are distributed in Western-Africa (POWO, 2019). Therefore, the Neotropical biogeographical events as described above could be a plausible driver of the seen diversification and underlying speciation rate acceleration observed in these clades. Erkens et al. (2011) stated that migration of *Guatteria* from Central

America into South America pre-dates the time the Andes worked as an effective dispersal barrier, by concluding that the genus dispersed into the region sometime during the Miocene via the explained stepping-stones. Transoceanic dispersal of *Guatteria* into South America is thought to be a main driver behind the Miocene diversification rate accelerations found in *Guatteria* (Erkens et al., 2007; Erkens et al., 2009).

Plausible underlying morphology drivers

As an extension of diversification analyses some papers also identify traits strongly correlated with elevated diversification rates found in certain clades (Erkens et al., 2012; Xue et al., 2020). These results give an idea about plausible causal mechanisms regarding morphology for the observed diversification rate accelerations. Morphological key innovations may elevate diversification rates through processes as the invasion of new zones free from competitors and/or natural enemies, gaining an increased fitness and consequently outcompeting other clades locally or through the enhancement of conditions in regard to reproductive/ecological specializations (Heard & Hauser, 1995; Gianoli, 2004). Erkens et al. (2007) state that only for a few clades within the Annonaceae, morphological innovations might be linked to elevated diversification rates.

Shifts in pollination systems

Pollination by small beetles belonging to the families Curculionidae (weevils), Nitidulida (sap beetles), Staphylinidae (rove beetles) and Chrysomelidae (leaf beetles) is most common within the family as it is observed in all main clades. In addition to that the plesiomorphic state regarding pollination within Annonaceae is most probably pollination by these small beetles (Saunders, 2012). Only in the two major subfamilies of Annonaceae, the Malmeoideae and Annonoideae, independent shifts to different pollination groups as large beetles, thrips, flies, cockroaches and bees are present. Shifts in pollinator groups usually come together with morphological adaptations in regard to the olfactory and visual cues, presence and/or enclosure and/or size of the pollination chamber and pollen grain size. These specializations, thus only observed in the two major subfamilies, may be a plausible explanation behind the identified diversification rate accelerations only present in the Malmeoideae and Annonoideae in comparison with the other smaller subfamilies where no diversification shifts were identified as well as the overall higher diversity in these major subfamilies (Saunders, 2012).

However, when looking more specific at the pollination systems of our clades identified with diversification rate accelerations, only in *Duguetia* and in the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae of the Malmeoideae other pollinator groups as small beetles are found (shifts to thrips, large scarab beetles in *Duguetia* and to large beetles, thrips, flies and bees in the Malmeoideae). Although, pollination by small beetles still remains the dominant pollination system in these clades. *Goniothalamus*, *Guatteria,* and the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* are all pollinated by small beetles (Saunders, 2012 and references therein).

Lianescent habit

Xue et al. showed that the lianescent habit is associated with elevated diversification rates. This can be the result of the given that lianescent plants can allocate more resources to reproduction, canopy development and stem and root elongation due to the fact that less resources are needed for structural support as xylem. Or the result of a better resistance against water stress due to their enhanced vascular efficiency, giving them an evolutionary advantage in regard to non-lianescent plants in dryer regions (Xue et al., 2020). In our results, only one clade that was identified with a diversification rate acceleration has the occurrence of lianescent species, namely the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* (see Supplementary figure S4A in Xue et al., 2020). This association was also found in Xue et al. (2020) next to the clade comprising *Artabotrys* and the clade comprising *Uvaria*. Thus the occurrence of the lianescent habit could be a plausible morphological driver of the rate accelerations observed in this clade due to the selective advantages as explained above. This hypothesis is supported by Gianoli (2004), who indicated a positive effect of a climbing habit on clade diversification across several plant families. Gianoli hypothesized that the effect could be a result of the favoured resource allocation to leaves and transport tissues at the expense of supportive tissue.

Circadian pollinator trapping

Pollinator trapping or the occurrence of tightly enclosed floral chambers that trap pollinators for certain periods of time which is aligned with their circadian activity rhythms has shown to provide selective advantages (Lau et al., 2017). Selective advantages provided with this mechanism are a broader range of potential pollinators, improved pollination by promoting pollen deposition on the pollinator and a more effective inter-floral pollinator movement (Lau et al., 2017; Xue et al., 2020). Two clades identified with accelerated diversification rates in our results species occurred performing pollinator trapping, namely the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis* and the clade comprising the genus *Gontiothalamus* (see Supplementary figure S4B in Xue et al., 2020). These correlations were also found by Xue et al. (2020) next to the clade comprising *Atrabotrys*. Thus pollinator trapping could be a plausible morphological mechanism behind the seen diversification rate accelerations in these clades. Supporting this, Lau et al. (2017) stated that pollinator trapping is likely to be a plausible key evolutionary innovation for *Goniothalamus* due to the selective advantages mentioned above.

Androdioecy

The occurrence of male flowers and hemaphrodites, or Androdioecy is relatively common in Annonaceae in regard to other families within the Angiosperms (Pang & Saunders, 2014; Xue et al., 2020). This type of reproductive system could provide potential selective advantages by outcrossing species (Xue et al., 2020). Androdioecy is most common within the tribes Miluseae and Malmeeae of the Malmeoidea (see Supplementary figure S4C in Xue et al., 2020) and could provide an explanation of the diversification rate acceleration indicated at the base of the clade comprising the tribes Malmeeae, Miliuseae and 4 smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae in our results. This is supported by Xue et al. (2020), where the highest diversification rates found in their analyses were associated with the occurrence of androdioecy. Thus the occurrence of androdioecy could be a plausible morphological mechanism driving diversification rate accelerations found in the Malmeoideae.

Seed dispersal unit

Different seed dispersal mechanisms occur within the family of the Annonaceae, ranging from single seeded segments that are dispersed sequentially to the dispersal of entire fruits. Xue et al. (2020) associated dispersal units as single-seeded monocarp fragments with the highest diversification rates found, individual monocarp dispersal with the second highest rates and entire fruit dispersal with the third highest diversification rates found in their analyses. In the clades indicated by diversification rate accelerations in our results, single-seeded fragments as dispersal unit are found in species within the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* Entire fruit dispersal is found in the clade comprising *Duguetia* and the clade comprising *Goniothalamus*. Individual monocarp dispersal is a plesiomorphic feature for the entire family (see Supplementary figure S4F in Xue et al., 2020). Thus, seed dispersal mechanisms as single-seeded fragments and entire fruits could be plausible morphological drivers behind diversification rate accelerations seen in the mentioned clades.

Erkens et al. (2012) stated that speciation and thus diversification rate within *Goniothalamus* could be driven by divergent selection on fruit and seed dispersal mechanisms due to the extremely diverse fruit and seed structures found within the genus.

BIASES REGARDING DIVERSIFICATION RATE ANALYSES

BAMM

Recent critical papers questioned the accuracy of BAMM regarding the estimation of speciation, extinction and diversification rates (Moore et al., 2016; Meyer & Wiens, 2018). A solution for these doubts is to compare results obtained by BAMM with results obtained by other methods regarding diversification rate analyses such as turboMEDUSA and Methods-of-moments estimators. Xue et al. (2020) found similar results via the three methods, supporting the working of BAMM. These comparisons have not been performed in this thesis due to time constraints. Additionally, many of the statements in Moore et al. (2016) were refuted in a paper by Rabosky et al. (2017).

Diversification rate analyses

When reconstructing the diversification history of tropical rainforests, with the emphasis on whether the origin of the elevated tropical diversity is due to 'Museum' or 'Cradle'-like circumstances or a combination of both, many studies point out that linages with 'Cradle'-type characteristics are generally younger than the 'Museum'-type (e.g. species with large leaves and entire margins) counterparts. Concluding that the tropical diversity must have originated in a 'Museum'-like environment, while some lineages, or the habitat they thrive in, transitioned into 'Cradle'-like environments. This points out the bias due to the fossil record, interpretations and migration rates causing ambiguous and even wrong conclusions out of diversification history analyses. Little studies mention these biases, but it is very well explained in a paper by Vasconcelos et al. (2021).

The bias is caused by the same mechanisms that separate 'Cradle' and 'Museum'-type systems, namely differences in speciation and extinction rates and their causes. The unstable environment characterizing a 'Cradle'-like system causing its elevated speciation rates also elevates its extinction rates through time as mentioned above. In addition to that, the unstable environmental characteristics also diminish fossil preservation rates in comparison with the stable environmental characteristics of 'Museums'. These two factors lead to the fact that the phylogenetic signal of lineages originating in 'Cradles' erodes much faster through time in comparison with lineages originating in 'Museum'-like environments, leading to a 'Museum'-biased tropical fossil record and current crown lineages regarding their characteristics. Which provokes the wrong idea that 'Cradle'-type originating lineages are generally younger and as a consequence provokes incorrect reconstructions regarding the number and direction of tropical diversification rate shifts and their accompanying habitat transitions. Not only the fossil record biases the reconstructions but also a 'survivorship bias' results in wrong interpretations. This refers to the constant migration of species who diversified in unstable, 'Cradle'-like environments to stable, 'Museum'-like environments with lower extinction rates. And as it were, these species survive/skip the lowered extinction rates in their source environment (Vasconcelos et al., 2021).

Thus, when reconstructing and interpreting the diversification history, one must be cautious with drawing conclusions and must always take into account these biases in their interpretations. Something that is often not the case.

CONCLUSION

Due to the convergence problems obtained in the BEAST and BAMM analysis, conclusions cannot be drawn with certainty in this research. However, results in this thesis hint at the diversification rate pattern through time within Annonaceae, as well as clades with elevated diversification rates. Nonetheless, congruence with previous diversification rate analyses regarding Annonaceae, support our results. Heterogeneity in regard to diversification rate patterns through time were found between the subfamilies. Our results indicate that diversification rates within the family were slowly increasing, almost constant until 40 million years ago when major rate increases occurred. These were subsequently followed by diversification rate decelerations up to the present, but this phenomenon is most probably due to biases in diversification rate analyses as the "push of the past". Thus the evolutionary history of Annonaceae through time is a gradual accumulation scenario followed by a rapid increase in lineages from the Late Eocene onwards. Our results show that the increase in diversification rates is most likely the result of elevated speciation rates rather than reduced extinction rates or a combination of both. Therefore, the evolutionary history of the Annonaceae coincides with a combination of two scenarios proposed by Meseguer et al. (2021). A mixed model of a gradual increase scenario (Sc. 1), more specifically a 'Cradle & Museum' subscenario. Followed by an exponential increase scenario (Sc. 2), more specifically a 'Recent Cradle' subscenario from the Late Eocene onwards. Thus, our results hint at a combination of 'Cradle' and 'Museum' models simultaneously through time. Where environmentally stable conditions in tropical environments, and thus low constant extinction rates, preserve ancient lineages and accumulate them through time as well as lineages resulting from relatively recent radiations linked to increased speciation rates.

Six diversification rate shifts were identified. One at the node comprising the whole family. Four within the Annonoideae: *Guatteria, Duguetia* and *Goniothalamus* and at the clade comprising *Desmos, Dasymaschalon, Friesodielsia* and *Monanthotaxis.* A last diversification rate shift was identified at the node of the major clade comprising the tribes Malmeeae, Miliuseae and four smaller tribes namely Maasieae, Fenerivieae, Dendrokingstonieae and Monocarpieae within the Malmeloideae.

The diversification rate pattern through time as well as the diversification rate shifts found within the Annonaceae could potentially be linked to several biogeographical events such as: Indian rafting, the Paleogene cooling and subsequent boreotropical disruption, several Miocene Paleotropical vicariance events, the flooding of Lake Pebas, the Andean uplift and the closure of the Panama isthmus. Likewise several morphological innovations such as shifts regarding the pollination system and seed dispersal unit as well as shifts towards a lianescent habit, circadian pollinator trapping and androdioecy could potentially have played an important role regarding the diversification of Annonaceae. All with the biases regarding assigning potential drivers to observed diversification rate accelerations kept in mind.

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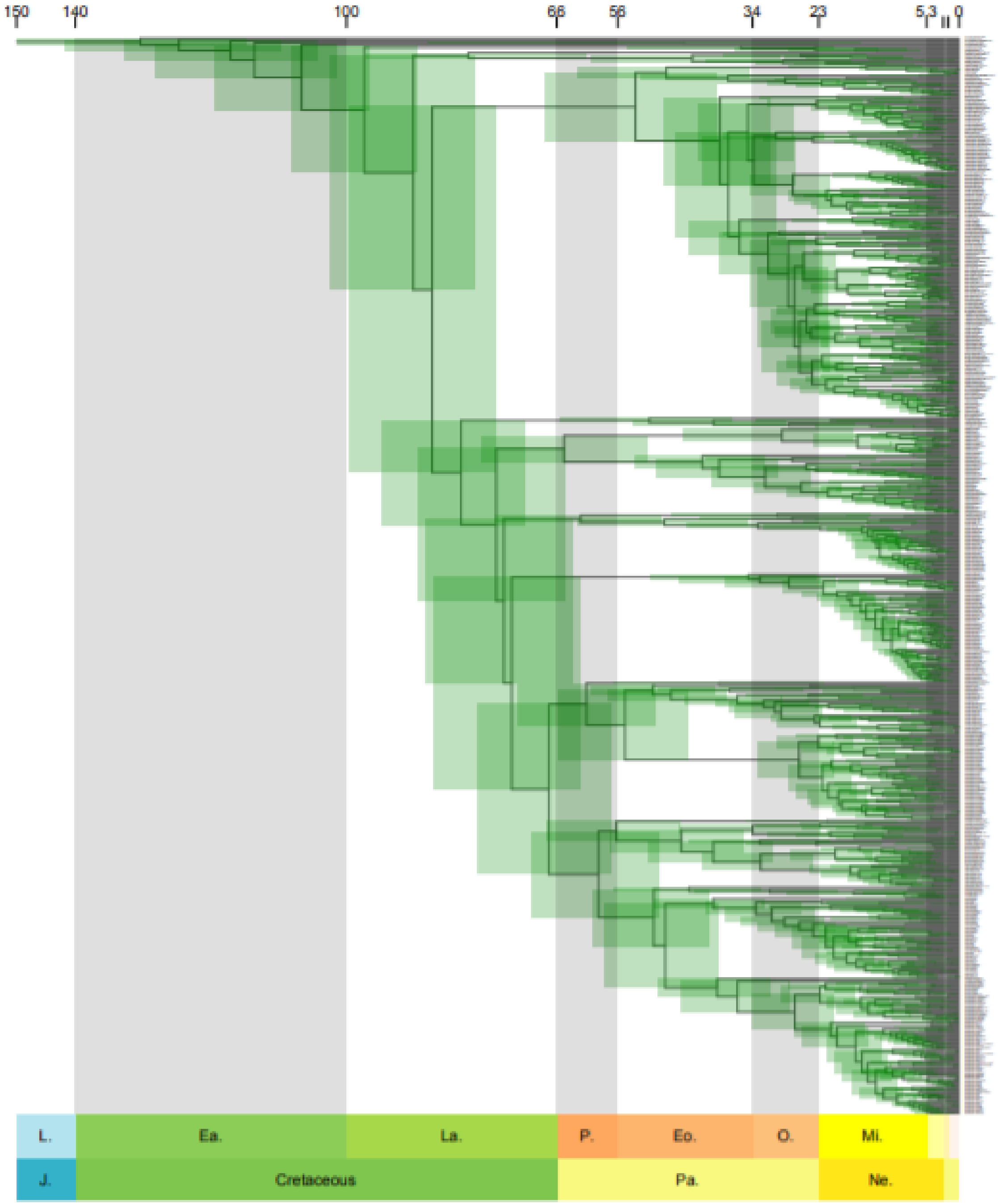
APPENDIX

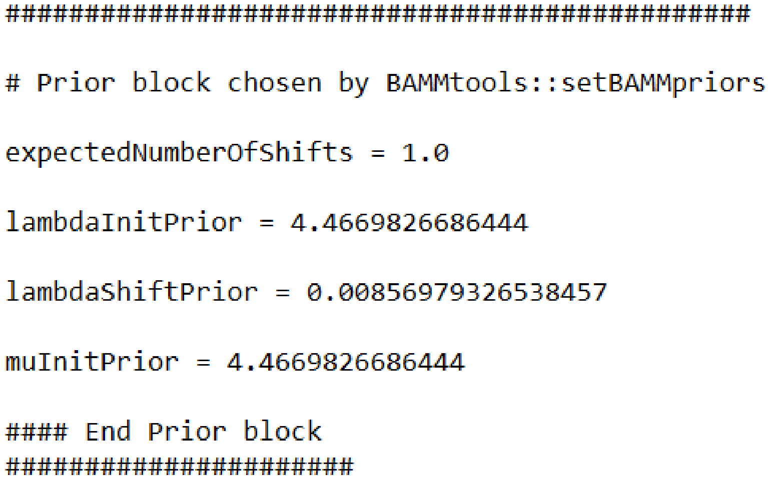
Supplementary Appendix 1.

Tree with nodes ages as median values of the posterior distribution. For each node the age interval (HDI) is plotted.



Supplementary Appendix 2.

Prior block for BAMM analyses determined by BAMMtools, setBAMMpriors



```
###############################################

# Prior block chosen by BAMMtools::setBAMMpriors

expectedNumberOfShifts = 1.0

lambdaInitPrior = 4.4669826686444

lambdaShiftPrior = 0.00856979326538457

muInitPrior = 4.4669826686444

#### End Prior block
######################
```