A peer-reviewed version of this preprint was published in PeerJ on 24 March 2016.

View the peer-reviewed version (peerj.com/articles/1744), which is the preferred citable publication unless you specifically need to cite this preprint.

https://doi.org/10.7717/peerj.1744
Evolution of *Philodendron* (Araceae) species along Neotropical biomes

Leticia Loss-Oliveira, Cassia CMS Sakuragui, Maria de Lourdes Soares, Carlos G Schrago

*Philodendron* is the second most diverse genus of the Araceae, a tropical monocot family with significant morphological diversity along its wide geographic distribution in the Neotropics. Although evolutionary studies of *Philodendron* were conducted in recent years, the phylogenetic relationship among its species remains unclear. Additionally, analyses conducted to date suggested the inclusion of all American representatives of a closely related genus, *Homalomena*, within the *Philodendron* clade. A thorough evaluation of the phylogeny and timescale of these lineages is thus necessary to elucidate the tempo and mode of evolution of this large Neotropical genus and to unveil the biogeographic history of *Philodendron* evolution along the Amazonian and Atlantic Rain Forests, as well as open dry forests of South America. To this end, we have estimated the molecular phylogeny for 68 *Philodendron* species, which consists of the largest sampling assembled to date aiming the study of the evolutionary affinities. We have also performed ancestral reconstruction of species distribution along biomes. Finally, we contrasted these results with the inferred timescale of *Philodendron* and *Homalomena* lineage diversification. Our estimates indicate that American *Homalomena* is the sister clade to *Philodendron*. The early diversification of *Philodendron* took place in the Amazon Forest from Early to Middle Miocene, followed by colonization of the Atlantic Forest and the savanna-like landscapes, respectively. Based on the age of the last common ancestor of *Philodendron*, the species of this genus diversified by rapid radiations, leading to its wide extant distribution in the Neotropical region.
Evolution of *Philodendron* (Araceae) species along Neotropical biomes

Leticia Loss-Oliveira\(^1\), Cassia M. Sakuragui\(^2\), Maria L. Soares\(^3\) and Carlos G. Schrago\(^1\)*

\(^1\)Department of Genetics and \(^2\)Department of Botany, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
\(^3\)Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil

*Address for correspondence:*
Carlos G. Schrago
Universidade Federal do Rio de Janeiro
Instituto de Biologia, Departamento de Genética, CCS, A2-092
Rua Prof. Rodolfo Paulo Rocco, SN
Cidade Universitária
Rio de Janeiro, RJ
CEP: 21941-617
BRAZIL
Phone: +55 21 2562-6397
Phone: +55 21 4063-8278
Email: carlos.schrago@gmail.com

**Running title:** Diversification of *Philodendron*

**Keywords:** biogeography; South America; Amazon; Andes; dispersal; supertree
Abstract

Philodendron is the second most diverse genus of the Araceae, a tropical monocot family with significant morphological diversity along its wide geographic distribution in the Neotropics. Although evolutionary studies of Philodendron were conducted in recent years, the phylogenetic relationship among its species remains unclear. Additionally, analyses conducted to date suggested the inclusion of all American representatives of a closely related genus, Homalomena, within the Philodendron clade. A thorough evaluation of the phylogeny and timescale of these lineages is thus necessary to elucidate the tempo and mode of evolution of this large Neotropical genus and to unveil the biogeographic history of Philodendron evolution along the Amazonian and Atlantic Rain Forests, as well as open dry forests of South America. To this end, we have estimated the molecular phylogeny for 68 Philodendron species, which consists of the largest sampling assembled to date aiming the study of the evolutionary affinities. We have also performed ancestral reconstruction of species distribution along biomes. Finally, we contrasted these results with the inferred timescale of Philodendron and Homalomena lineage diversification. Our estimates indicate that American Homalomena is the sister clade to Philodendron. The early diversification of Philodendron took place in the Amazon Forest from Early to Middle Miocene, followed by colonization of the Atlantic Forest and the savanna-like landscapes, respectively. Based on the age of the last common ancestor of Philodendron, the species of this genus diversified by rapid radiations, leading to its wide extant distribution in the Neotropical region.
Introduction

*Philodendron* is an exclusively Neotropical genus, comprising 482 formally recognized species (Boyce & Croat, 2013). Their geographic distribution range from Northern Mexico to Southern Uruguay (Mayo et al., 1997), consisting mainly of the biomes of the Amazonian and Atlantic Rain Forests and also the open dry forests of South America. According to Olson et al.’s (2001) classification of terrestrial biomes, South American open dry forests are composed of the Cerrado (savanna-like landscapes) and Caatinga biomes (Croat, 1997, Mayo, 1988, Mayo, 1989, Sakuragui et al., 2012a) (Figure 1). *Philodendron* species richness is especially significant in Brazil, where 168 species were described thus far (Sakuragui et al., 2012b).

Although *Philodendron* presents a significant morphological plasticity, wide leaf variation and several types of habits (Sakuragui et al., 2012b, Coelho, 2000), the inflorescence morphology of its representatives is largely conserved. The unisexual flowers in the spadix are clustered in male, female and sterile zones; located at the basal, median and superior portions, respectively (Figure 1b). The spadix, in nearly all of its extension, is surrounded by the spate (Sakuragui, 2001).


Because of the wide geographic range, patterns of distribution along niches, as well as the characteristic morphology, interest in investigating *Philodendron* systematics and evolution has
increased in the last decades (Sakuragui et al., 2005, Mayo, 1986, Grayum, 1996, Croat, 1997).

Morphological and anatomical characters of flowers has been of special interest for phylogenetic analysis due to their high level of variability (Sakuragui, 1998). However, the plasticity and convergence of these characters in *Philodendron* may increase the probability of homoplasies (Mayo, 1986, Mayo, 1989).

Recently, Gauthier et al. (2008) investigated the phylogenetic relationships of *Philodendron* species based on three molecular markers, sampling a total of 49 species. This work comprised the largest taxon sampling of the genus to date. In accordance to previous analysis (Barabé et al., 2002, Mayo et al., 1997), authors questioned the monophyly of *Philodendron*, suggesting the inclusion of all American species of the morphologically similar genus, *Homalomena* Schott, within the Philodendron clade. *Homalomena* species occur in America and Asia and its geographic distribution partly overlaps with *Philodendron* in the Neotropics. The inference of the evolutionary relationships between *Philodendron* and *Homalomena* has a significant biogeographic appeal. If American *Homalomena* species are indeed more closely related to *Philodendron* than to Asian *Homalomena*, a single colonization event should be considered. Unveiling the evolutionary relationships between those lineages is thus necessary to elucidate their origin and subsequent diversification.

Besides phylogeny, several issues regarding *Philodendron* evolution remain unclear. For example, the historical events that led to the wide geographic occurrence along biomes need a thorough analysis. In this sense, investigating the evolutionary affinities of a large sample of *Philodendron* species will shed light on how this lineage diversified along the Amazonian and Atlantic Rain Forests, as well as South American open dry forests biomes, namely, the Cerrado and Caatinga. To this end, we have performed an ancestral area reconstruction of *Philodendron*
and *Homalomena* species and estimated the divergence times from a phylogeny inferred from the largest *Philodendron* dataset composed to date. We were able to address the timing and pattern of *Philodendron* diversification in selected Neotropical biomes with a focus on the evolutionary relationships between the three *Philodendron* subgenera.
Materials and Methods

Taxon and gene sampling

We have sequenced new data for 110 extant species of Philodendron and 16 species of Homalomena of the following molecular markers: the nuclear 18S and external transcribed spacer (ETS); and the chloroplast trnL intron, trnL-trnF intergenic spacer, the trnK intron and maturase K (matK) genes. Additionally, 13 outgroup species were analyzed, comprising the genera Cercestis, Culcasia, Colocasia, Dieffenbachia, Heteropsis, Montrichardia, Nephthytis, Furtadoa and Uropspatha. Outgroup choice was based on the close evolutionary affinity of these genera to Philodendron, as suggested by previous studies. The complete list of species included in this study, the voucher and GenBank accession numbers were listed in Tables 1 and 2 of the Supplementary Material.

Ancestral biome reconstruction is dependent on the estimated phylogeny and the current geographic distribution of sampled species terminals. Thus, taxon sampling may impact the inference of ancestral species distribution along biomes. As indicated in Table 1 (Supplementary Material), we have sampled all P. subg. Meconostigma species; 82 P. subg. Philodendron species and 7 P. subg. Pteromischum species. Our sampling strategy is representative of the current Philodendron diversity. Although ~75% of the sampled species are P. subg. Philodendron in our analysis, ~82% of Philodendron species consist of P. subg. Philodendron (Boyce & Croat, 2013, Sakuragui et al., 2012a).

DNA isolation, amplification and sequencing

Genomic DNA was isolated with QIAGEN DNeasy Blood & Tissue kit from silica-dried or fresh leaves. Primers used for amplification and sequencing were listed in Table 3 of
Supplementary Material. Sequencing reactions were performed in the Applied Biosystems 3730xl automatic sequencer and edited with the Geneious 5.5.3 software.

Alignment and phylogenetic analysis

Molecular markers were individually aligned in MAFFT 7 (Katoh & Standley, 2013) and then manually adjusted in SeaView 4 (Gouy et al., 2010). We estimated individual gene trees (Fig. 1, SM) for each molecular marker in MrBayes 3.2.2 (Huelsenbeck & Ronquist, 2001, Ronquist & Huelsenbeck, 2003) using the GTR + G substitution model. The Markov chain Monte Carlo (MCMC) algorithm was ran twice for 10,000,000 generations, using four chains. Chains were sampled every 100th cycle and a burn-in of 20% was applied. A supertree was estimated from the tree topologies of each molecular marker using the PhySIC_IST algorithm, available at the ATGC-Montpellier online server (http://www.atgc-montpellier.fr/physic_ist/). Only clades with posterior probability ≥ 85% were considered to estimate the supertree. We have used this approach to avoid the impact of missing data in phylogeny estimation (Scornavacca et al., 2008). As PhySIC_IST calculates non-plenary supertrees, it removes taxa with significant topological conflict and/or with small taxon sampling (Scornavacca et al., 2008). The final supertree was thus composed of 89 terminals, as 50 terminals were discarded due to conflicting resolutions.

In order to assess the stability of the (Philodendron + American Homalomena) clade, we have calculated the log-likelihoods of alternative topological arrangements in PhyML 3.0 (Guindon et al., 2009) using the species sampling of the supertree. We have tested the following topologies: (I) (American Homalomena (P. subg. Philodendron + P. subg. Meconostigma); (II) (P. subg. Meconostigma (P. subg. Philodendron + American Homalomena) and (III) (P. subg. Philodendron (P. subg. Meconostigma + American Homalomena). The significance of the
difference in log-likelihoods between topologies was tested with the approximately unbiased (AU) and the Shimodaira-Hasegawa (SH) tests implemented in CONSEL 1.2.0 (Shimodaira & Hasegawa, 2001).

Divergence time inference

Dating _Philodendron_ evolutionary history is difficult mainly because of the scarcity of the fossil record (Loss-Oliveira et al., 2014). For instance, Dilcher and Daghlian (1977), based on fossilized leaves, described a putative _P._ subg. _Meconostigma_ fossil from the Eocene of Tennessee (56.0 – 33.9 Ma). However, Mayo (1991) identified the referred fossil as a _Peltranda_. Thus, we have decided not to use this fossil as calibration information. Alternatively, in order to estimate divergence times, we have assigned a prior on the rate of nucleotide substitution. We were then prompted to infer the evolutionary rates of plastid coding regions of monocots using a large sample of publicly available chloroplast genomes. Nuclear genes were excluded from dating analysis because of the absence of prior information on evolutionary rates.

To estimate monocots substitution rate, we used chloroplast genomes from 154 Liliopsida species retrieved from the GenBank (Table 4). All orthologous coding regions were concatenated into a single supermatrix. Maximum likelihood phylogenetic reconstruction was implemented in RaxML 7.0.3 (Stamatakis, 2006) under GTR model. Molecular dating of monocots (Liliopsida) was conducted under a Bayesian framework, using fossil information obtained from Iles et al. (Iles et al., 2015) (Table 5). Because the number of terminals used was large, rate estimation was conducted with the MCMCTree program of PAML 4.8 package (Yang, 2007) using the approximate likelihood calculation (dos Reis & Yang, 2011) and the uncorrelated model of evolution of rates. In MCMCTree, posterior distributions were obtained via MCMC; chains were sampled every 500th cycle until 50,000 trees were collected. We performed two independent
replicates to check for convergence of the estimates. Calibration information for Liliopsida was entered as minimum and maximum bounds of uniform priors. The estimated mean substitution rate was inferred at 3.26 x 10^{-9} substitutions/site/year (s/s/y). This value is significantly higher than the previous estimate of Palmer (1991), which reported an average substitution rate of 0.7 x 10^{-9} s/s/y for angiosperm platids. As the credibility interval of our estimate was large, we adopted a Gaussian prior for evolutionary rates with a 95% highest probability density (HPD) interval that included maximum and minimum values of our estimate and that of Palmer’s.

Dating analysis of *Philodendron* and *Homalomena* species was performed in BEAST using a relaxed molecular clock with evolutionary rates modeled by an uncorrelated lognormal distribution; the GTR + G model of sequence was applied. MCMC algorithm was ran for 50,000,000 generations and sampled every 1,000th cycle, with a burn-in of 20%.

**Biome shifts**

To unveil how *Philodendron* species colonized the Amazon forest, Atlantic Forest, Cerrado and Caatinga, we conducted a Bayesian Binary MCMC (BBM) (Yu et al., 2012, Ronquist & Huelsenbeck, 2003) implemented in Reconstruct Ancestral State in Phylogenies 2.1b (RASP) software (Yu et al., 2012). The input tree topology was the supertree estimated in PhySIC_IST. BBM chains were ran for 10,000,000 generations and were sampled every 1000th cycle. State frequencies were estimated under the F81 model with gamma rate variation.

Information on the occurrence of each *Philodendron* species along Neotropical biomes was obtained from Sakuragui et al. (2012b) and from the (Team) CATE Araceae (http://araceae.e-monicot.org).
Results

The Homalomena genus was not recovered as monophyletic; the Asian Homalomena clustered within a single group and the American representatives clustered independently, as sister to Philodendron species (Figure 2). Although our analysis failed to support the monophyly of Philodendron with significant statistical support, the topological arrangement in which Philodendron is a monophyletic genus was significantly supported by the AU and SH tests ($p < 0.05$, Figure 3, Table 6SM). Within Philodendron, subg. Meconostigma was recovered as monophyletic (Fig. 2, node D), whereas subg. Philodendron was recovered as polyphyletic (Fig. 2, node E). Finally, the monophyly of P. subg. Pteromischum was not inferred, because Pteromischum species clustered with P. subg. Philodendron species.

We estimated that the last common ancestor (LCA) of Philodendron diversified in the Amazon Forest (Fig. 4, node B) at ca. 8.6 Ma (6.8 – 12.1 Ma) 95% HPD. Thus, we inferred that the LCA of Philodendron diversified from Middle to Late Miocene. This also suggests that the divergence between Philodendron and the American Homalomena occurred in a short period of time after this American lineage diversified from the Asian Homalomena (Figure 4, nodes B and A, respectively).

The earliest events of Philodendron diversification occurred exclusively in the Amazon Forest (e.g., Fig. 4, nodes C, D, E, F). The ancestors of Atlantic Forest lineages were inferred to have been distributed in the Amazon (Fig. 4, nodes I, J and nodes G, H). This pattern of Amazonian ancestry of Atlantic Forest lineages was also observed in some terminal branches. For instance, from node K to P. loefgrenii and from node L to P. imbe.

On the other hand, the majority of Cerrado species evolved from Atlantic Forest ancestors (Fig. 4, nodes J and M; node N to P. rhizomatosum and P. pachyphyllum). In subgenus
Meconostigma, the age of early species diversification into Atlantic Forest was dated at 3.7 Ma (5.6 – 2.7 Ma) (Fig. 4, node J), whereas in the P. subg. Philodendron early lineage diversification occurred at 4.1 Ma (5.5 – 3.0 Ma) (Fig. 4, node J). Therefore, during a period of 5.0 – 6.0 Ma, Philodendron species occupied exclusively the Amazon Forest. The diversification into Cerrado biome occurred later, at approximately 1.7 Ma (3.3 – 1.1 Ma) (Fig. 4, node M).
Discussion

Phylogenetic relationship between Philodendron and Homalomena

In this study, Asian Homalomena was recovered as sister to the (Philodendron + American Homalomena) clade, and Furtadoa mixta clustered with the Asian Homalomena clade. The evolutionary affinities of American Homalomena, P. subg. Meconostigma and P. subg. Philodendron were not strongly supported. However, the topological arrangement in which Philodendron is a monophyletic genus was statistically significant by the AU and SH tests, suggesting the monophyly of Philodendron.

Previous studies have reported conflicting results concerning the monophyly of Philodendron and the phylogenetic status of American Homalomena (Figure 5). For instance, Barabé et al. (2002), based on the trnL intron and the trnL-trnF intergenic spacer, proposed P. subg. Philodendron as a paraphyletic group and was unable to solve the (P. subg. Meconostigma + Asian + American Homalomena) polytomy (Figure 5A). Gauthier et al. (2008) recovered the American Homalomena as sister to Philodendron and the Asian Homalomena as sister to the (American Homalomena + Philodendron) clade, although their Bayesian analysis inferred a paraphyletic Philodendron, with P. subg. Pteromischum sister to the American Homalomena (Figure 5B and 5C, respectively). Alternatively, Cusimano et al. (2011) recovered a monophyletic Philodendron, with Homalomena as sister lineage of Furtadoa (Figure 5D).

Recently, Yeng et al. (2013) estimated the Homalomena phylogeny based on the nuclear ITS marker and also sampled Philodendron species. In the ML and Bayesian trees reported in their study, P. subg. Pteromischum was closely related to the American Homalomena, whereas P. subg. Meconostigma and P. subg. Philodendron were recovered as sister taxa (Figure 5E).
Discrepancies between previous works and our analysis may be due to different choice of phylogenetic methods, markers and taxon sampling. Gauthier et al. (2008) was the only study intended to investigate specifically the systematics of *Philodendron* genus. When compared to their analysis, our study included a larger sampling of taxa and molecular markers with the aim of estimating the phylogeny of *Philodendron* and *Homalomena* species; it is also the first analysis that used a supertree approach to this end.

Our phylogeny characteristically presents short branch lengths within the *Philodendron* clade. The high frequency of polytomies indicates the genetic similarity among terminals, which is further corroborated by the ease in obtaining artificial hybrids between different species. This corroborates a scenario of low genetic differentiation and low reproductive isolation (Carlsen, 2011).

*Philodendron* diversification may also consist of several recent rapid radiation events. Phylogenetic reconstruction under this scenario is challenging, because of a significant amount of substitutions is needed to accumulate within short periods of time (Maddison and Knowles, 2006). However, morphological variation of *Philodendron* is remarkable, which seems contradictory considering the previously discussed features. However, it has been extensively discussed that morphological variation is not a suitable proxy for genetic variation (e.g., Prud’Homme et al., 2011; Houle et al., 2010). Many environmental and epigenetic factors may can increase phenotypic variation even in the absence of DNA sequence variation (Prud’Homme et al., 2011). Evidently, we cannot rule out the possibility that DNA regions that present significant genetic differences between species were not sampled in this work.

*Diversification of* *Philodendron* and *Homalomena*
Although the chronology of *Philodendron* divergence was not extensively focused by previous studies, Nauheimer et al. (2012) analyzed the global history of the entire Araceae family based on a supermatrix composed of 5 chloroplast markers and several well-established calibration points. Their analysis included a single *Philodendron* species and estimated age of the *Philodendron*/Asian *Homalomena* divergence at approximately 20.0 Ma (ranging from 31.0 – 9.0 Ma). This study, however, also included a single species of Asian *Homalomena*.

The wide range of the posterior distribution credibility intervals of Nauheimer et al. (2012) hampers the proposition of putative biogeographic scenarios for the evolution of *Philodendron*, American and Asian *Homalomena*. Differences between their timescale and the divergence times proposed in this study might therefore be due to methodological differences caused by their reduced taxonomic sampling. Nevertheless, both our estimate of the age of the *Philodendron* divergence from Asian *Homalomena* and that of Nauheimer et al. (2012) suggests that this event took place when South America was essentially an isolated continent.

The isolation of the South American continent persisted from approximately 130.0 Ma (Smith & Klicka, 2010) to 3.5 Ma (Vilela et al., 2014), with the rise of the Panamanian land bridge. Therefore, from the Early to Middle Miocene there was no land connection with North America, Asia or Africa (Oliveira et al., 2010). If dispersal, rather than vicariance, is the most plausible hypothesis to explain *Philodendron* and American *Homalomena* colonization of the Neotropics, hypotheses on the possible routes of colonization should be investigated. Based on the continental arrangement during the Miocene, we propose that the dispersal of *Philodendron* and American *Homalomena* ancestor could have followed four possible routes (Figure 6): (1) from Asia to North America through the Bering Strait; (2) from Africa to the Neotropics by
crossing the Atlantic ocean; (3) from Asia to Neotropics by crossing Pacific ocean; and (4) from
Asia to Neotropics, also by crossing the Atlantic ocean.

The Araceae fossil record is currently assigned to Florida, Russia, Germany, United
Kingdom, Venezuela, Yemen, Colombia and Canada (Shufeldt, 1917, Berry, 1936, Bogner et al.,
2005, Wing et al., 2009, Stockey et al., 2007). However, as none of the fossil specimens was
described as closely related to Philodendron or Homalomena, the Araceae fossil record fails to
corroborate any dispersal hypothesis in particular.

Considering route 1, although the Bering Strait have connected Asia to the North
America during most of the Cenozoic period (Butzin et al., 2011), there is no evidence of extant
Philodendron and Homalomena in North America or North Asia. Route 2 involves long distance
oceanic dispersal through ca. 2,000 km – the minimum distance between Africa and the
Neotropics (Oliveira et al., 2010) – through Atlantic paleocurrents, which were probably stronger
than Pacific currents. This hypothesis is congruent with the clustering of Philodendron and
American Homalomena into a single clade, assuming Africa as the center of diversification of
Asian and American Homalomena, as well as Philodendron. However, we should consider that
the last recent common ancestor of Philodendron and Homalomena was distributed in Africa. On
the other hand, this hypothesis is corroborated by the distribution of the extant Philodendron and
Homalomena species. Givnish and colleagues (2004) also suggested two long distance dispersal
events through the Atlantic, but in the opposite direction. Their analysis indicated that
Bromeliaceae and Rapateaceae arose in the Guayana Shield of northern South America and reached
tropical west Africa via long-distance dispersal at ca. 6–8 Ma.

When considering long distance dispersal events, it is crucial to evaluate their viability as
related with the plant’s ability to produce dispersal structures that would tolerate aquatic and
saline conditions for long periods of time (Lo et al., 2014). Although such features have not been
evaluated for Philodendron and Homalomena, some Homalomena species inhabits swamp
forests and open swamps. Thus, features that would favor their survival in waterlogged
environments could also influence their maintenance in seawater.

Although route 3 is geographically unlikely due to the 8,000 km distance between Asia
and the Neotropics through the Pacific Ocean (Oliveira et al., 2010), it cannot be completely
discarded, because it is corroborated by the extant distribution of Homalomena and
Philodendron. Finally, route 4 suggests the dispersal through the Atlantic ocean from Asia to the
Neotropics. This is also an improbable hypothesis, because the African continent would act as a
barrier between Asia and the Neotropics, requiring the dispersal through both the Indian and the
Atlantic oceans.

The extant distribution of Philodendron and Homalomena species and the scarcity of
fossil information challenge the proposition of a scenario for the origin of Philodendron and
American Homalomena in the Neotropics. However, the biological and geographical information
provided to date indicates a long distance oceanic dispersal through the Atlantic, as suggested by
route 2, as the most plausible hypothesis to explain Philodendron and American Homalomena
colonization of the Neotropics.

Early diversification of Philodendron species

According to our analysis, the last common ancestor of Philodendron and the American
Homalomena was distributed in the Amazon Forest about 8.6 Ma (11.1 – 6.8 Ma) during the
Middle/Late Miocene. Interestingly, this time estimate is very close to the age of the divergence
between the (Philodendron/American Homalomena) clade from the Asian Homalomena (Fig. 4,
node A). The Middle and Late Miocene were characterized by wetland expansion into western
Central Amazonia, which fragmented the rainforest and formed extensive wetlands (Jaramillo et al., 2010). According to our analysis, *Philodendron* earliest divergence events took place in this scenario. The Amazon forest, from the Late Miocene to the beginning of Pliocene, was composed of a diverse and well-structured forest. The Amazon river landscape was well established, this probably allowed the extensive development of the Amazonian *terra firme* forest (Jaramillo et al., 2010). This scenario is compatible with the biology of extant species of *Philodendron*, because a well-structured forest would allow the development of epiphyte and hemiepiphyte species, such as *Philodendron*.

**Philodendron diversification along Neotropical biomes**

Our results suggest that *Philodendron* species occurred exclusively at the Amazon forest for ca. 5.0 – 6.0 Ma. During the Pliocene, as result of the glacial cycles, climate cooling and drying permitted the expansion of the open savanna areas, mostly represented by the ‘dry diagonal’, which is constituted by the Caatinga, Cerrado and Chaco biomes. This consisted of a crucial event, because it resulted in the isolation of the Atlantic forest in the east coast of South America (DaSilva & Pinto-da-Rocha, 2013), which is synchronous to the inferred age of the early diversification of *Philodendron* in this biome. This also corroborates the hypothesis that the Atlantic forest taxa present a closer biogeographic relationship with the Amazon forest, as proposed by Amorim and Pires (1996) and Eberhard and Bermingham (2005). After the separation between Atlantic and Amazon Forests during the Pliocene, species dispersal might have been common through the forest patches (DaSilva and Pinto-da-Rocha, 2013).

Roig-Juñent and Coscarón (2001) and Porzecanski and Cracraft (2005) suggested that the Atlantic rainforest also presents similarities in organismal composition with the Cerrado biome. This association would have been a result of dispersal events through gallery forests. The history
of the formation of Cerrado biome is still uncertain (Zanella, 2013, Werneck, 2011), but our
analysis indicated that the ancestors of Philodendron clades from the Cerrado were distributed in
the Atlantic forest. Therefore, we also corroborate the hypothesis of lineage dispersal from the
Atlantic Forest to the Cerrado biome. These events would have occurred after the colonization
the Atlantic Forest by Philodendron species.

Final considerations on Philodendron evolution

Given the significant morphological diversity of Philodendron, its widespread
distribution in the Neotropics and the age of the Araceae family (~140.0 Ma, Nauheimer et al.,
2012), it would be expected that the origin of this genus was older. In sharp contrast, we have
estimated phylogenies with very short branch lengths and very recent divergence times. A
similar scenario was reported by Carlsen and Croat (2013) for Anthurium, which is the most
diverse Araceae genus, and also by Nagalingum and colleagues (2011) for cycads. Therefore, the
inferred tempo and mode of evolution of Philodendron species were reported in several plant
families.

Conclusion

The present work was the first attempt to establish a chronological background for the
diversification of this highly diverse genus and to suggest possible routes of colonization of the
ancestors of Neotropical Philodendron and Homalomena. Philodendron was statistically
supported as a monophyletic genus, sister to American Homalomena by AU and SH tests. The
last common ancestor of Philodendron diversified from the Middle to the Late Miocene in the
Amazon Forest, where the earliest events of Philodendron diversification occurred. Amazon was
also the exclusive biome occupied by *Philodendron* species during a 5.0 – 6.0 million years period. Atlantic Forest lineages of *P.* subg. *Meconostigma* and *P.* subg. *Philodendron* diverged from Amazonian ancestors. On the other hand, the majority of Cerrado species evolved from Atlantic Forest ancestors, from the Late Miocene to the Pliocene.
Acknowledgements

We thank Petrobrás and INPA for allowing field expeditions in their biological reserves. We also
thank Alexandre Antonelli for valuable contributions in the manuscript text.

References

biodiversity estimation. (Bicudo, C. E. M. & Menezes, N. A., eds.). pp. 183-219. CNPq,
Campos do Jordão.

As-Saruri, M. L., Whybrow, P. J. & Collinson, M. E. 1999. Geology, fruits, seeds, and
vertebrates (Sirenia) from the Kaninah Formation (Middle Eocene), Republic of Yemen.
Fossil Vertebrates of Arabia: 443-453

Barabé, D., Bruneau, a., Forest, F. & Lacroix, C. 2002. The correlation between development of
atypical bisexual flowers and phylogeny in the Aroideae (Araceae). Plant Systematics

Museum 83: 335-360.

Albertarum pueri geno. nov. et sp. nov., of Late Cretaceous (late Campanian) age from
the Horseshoe Canyon Formation of southern Alberta, Canada. Canadian Journal of
Earth Sciences 85: 591-598.

number of species in aroid genera. pp. 7-9.

carbon cycle modeling combined with benthic isotope records. Paleoceanography 26.

Carlsen, M. (2011) Understanding the origin and rapid diversification of the genus
Anthurium Schott (Araceae), integrating molecular phylogenetics, morphology and

Genus <I>Anthurium</I> (Araceae) based on Combined Chloroplast and Nuclear DNA.
Systematic Botany 38: 576-588.

survey of findings in the light of recent botanical observations, Vol. 4. pp. British
Museum (Natural History), London.

Reserva Ecológica de Macaé de Cima - Nova Friburgo, Rio de Janeiro, Brasil.
Rodriguesia 51: 21-68.

Chronostratigraphic Chart. Episodes 36 (3).

Croat, T. B. 1997. A revision of Philodendron subgenus Philodendron (Araceae) for Mexico and


Figure legends

**Figure 1A.** Geographic distribution of *Philodendron* species along the Neotropical biomes of Amazon, Atlantic Forest, Cerrado and Caatinga. **B.** *Philodendron* inflorescence and the flower zones.

**Figure 2.** Supertree of *Philodendron* and *Homalomena* species.

**Figure 3.** Phylogeny of *Philodendron* and *Homalomena* corroborated by the approximately unbiased (AU) test.

**Figure 4.** Ancestral biome reconstructions and divergence time estimates of *Philodendron* and *Homalomena* lineages. The epoch intervals followed the international chronostatigraphic chart (Cohen et al., 2015) and are indicated by dashed lines.

**Figure 5.** Phylogenetic relationships between *Philodendron* and *Homalomena* recovered by previous studies. A. Barabé et al. (2002); B. Gauthier et al. (2008) using the maximum parsimony method; C. Gauthier et al. (2008) using Bayesian analysis; D. Cusimano et al. (2011); E. Yeng et al. (2013).

**Figure 6.** Putative dispersal routes of the ancestor of *Philodendron* and American *Homalomena* to the Neotropical region during the Miocene.
Figure 1 (on next page)

Figure 1

A. Geographic distribution of *Philodendron* species along the Neotropical biomes of Amazon, Atlantic Forest, Cerrado and Caatinga. B. *Philodendron* inflorescence and the flower zones.
Figure 2 (on next page)

Supertree of *Philodendron* and *Homalomena* species.
Figure 3

Phylogeny of *Philodendron* and *Homalomena* corroborated by the approximately unbiased (AU) test.
P. subg. Meconostigma

P. subg. Philodendron + Pteromischum

American Homalomena

Asian Homalomena
Figure 4 (on next page)

Figure 4

Ancestral biome reconstructions and divergence time estimates of *Philodendron* and *Homalomena* lineages. The epoch intervals followed the international chronostatigraphic chart (Cohen et al., 2015) and are indicated by dashed lines.
Figure 5 (on next page)

Figure 5

Phylogenetic relationships between *Philodendron* and *Homalomena* recovered by previous studies. A. Barabé et al. (2002); B. Gauthier et al. (2008) using the maximum parsimony method; C. Gauthier et al. (2008) using Bayesian analysis; D. Cusimano et al. (2011); E. Yeng et al. (2013).
Putative dispersal routes of the ancestor of *Philodendron* and American *Homalomena* to the Neotropical region during the Miocene.