A comprehensively sampled reassessment of the molecular phylogeny of the genistoid legumes questions the traditional placement of *Haplormosia*, an African monotypic genus traditionally classified within tribe Sophoreae close to the Asian-American geographically disjunct genus *Ormosia*. Plastid *matK* sequences placed *Haplormosia* as sister to the American-Australian tribe Brongniartieae. Despite a superficial resemblance between *Haplormosia* and *Ormosia*, a re-examination of the morphology of *Haplormosia* corroborates the new phylogenetic result. The reciprocally monophyletic deep divergence of the *Haplormosia* stem lineage from the remaining Brongniartieae is dated to ca. 52 Mya, thus supporting a signature of an old single long-distance dispersal during the early Eocene. Conversely, we estimated a relatively recent long-distance dispersal rooted in the Early Miocene for the Australian Brongniartieae clade emerging from within a grade of American Brongniartieae. The Bayesian ancestral area reconstruction revealed the coming and going of neotropical ancestors during the diversification history of the Brongniartieae legumes in Africa and all over the Americas and Australia.

© 2016 Elsevier Inc. All rights reserved.
et al., 2005; Oliveira-Filho et al., 2013; Pennington and Lavin, 2016).

The most recent review of legume systematics (LPWG, 2013a) highlighted some 51 genera of Papilionoideae that are unsampled in molecular phylogenetic analyses due to the lack of molecular data. By resolving the relationships of these 51 genera, we believe it will also advance the ongoing phylogeny-based classification of the legume family (LPWG, 2013b). Despite our recent efforts in filling in such gaps in the Papilionoideae phylogeny (Cardoso et al., 2012a, 2015; Queiroz et al., 2015; Ramos et al., 2016), the placement and monophyly of several enigmatic genera still remained unanswered as was the case of the African genus Haplormosia Harms. The genus comprises solely Haplormosia monophylla Harms (Fig. 1), which is an evergreen tree or shrub usually growing 6–20 (–35) m tall. The species has a long but fairly narrow distribution along the coast of West and western Central Africa, from Sierra Leone to south Gabon, with an apparent distributional gap from Ghana to Benin. It occurs in tropical lowland evergreen forests, usually along rivers, lagoons, mangroves, or in swamps. In some areas, notably in Sierra Leone and Liberia, it occurs inland along rivers to 100 m elevation. However, in central Africa the distribution is only close to the coast, thus the species exemplifies the “coastal legume species” distribution pattern described by de la Estrella et al. (2012). Haplormosia has papilionate flowers, pinkish to purple petals, essentially free stamens, and leathery becoming woody, single-seeded pods. These features have been cited in traditional classifications that place Haplormosia in tribe Sophoreae, near the species-rich Asian-Neotropical disjunct genus Ormosia (Polhill, 1981, 1994; Pennington et al., 2005). However, Haplormosia lacks the brightly colored seed coat that characterizes most species of Ormosia and has strictly unifoliolate leaves, a feature occurring rarely in Ormosia, hence the generic name of the former.

Molecular phylogenetic analyses have shown that tribe Sophoraeae as traditionally circumscribed is polyphyletic (Pennington
et al., 2001; Wojciechowski et al., 2004; Cardoso et al., 2012a, 2013a). The recent analyses place *Ormosia* in an expanded Genistoid clade, a diverse assemblage of ~95 genera and >2400 species (Cardoso et al., 2012a, 2013a), characterized by the accumulation of quinolizidine alkaloids, a potential synapomorphy for the group (Pennington et al., 2001; Kite and Pennington, 2003; Van Wyk, 2003; Kite et al., 2013; LPWG, 2013a; Wink, 2013). Quinolizidine alkaloids have also been reported in *Haplormosia* (Kinghorn et al., 1988), suggesting that its purported close relationship with *Ormosia* may be real. If so, explanations might be sought for the highly disparate diversifications of the two genera, the ca. 150 species of *Ormosia* more or less evenly split between the American and Asian tropics (one species in tropical Australia) and standing in sharp contrast to the single species of *Haplormosia*. Thus, a time-calibrated phylogeny might resolve the long-standing debate on *Haplormosia* systematics and also reveal if long-distance oceanic dispersal can account for the biogeographic history of the genus, an explanation shown in a myriad of trans-Atlantic clades of plants (Givnish et al., 2004; Renner, 2004; Schrire et al., 2005; Mueller et al., 2006; Särkinen et al., 2007; Zhang et al., 2007; Duchen and Renner, 2010; Michalak et al., 2010; Carvalho and Renner, 2012; Fritsch and Cruz, 2012; Richardson et al., 2014; Thornhill et al., 2015; Bardon et al., 2016; Ruhfel et al., 2016) and animals (de Queiroz, 2005; Antoine et al., 2011; Mitchell et al., 2014; Eilertsen and Malaquias, 2015; Longrich et al., 2015; Rota et al., 2016).

Here we evaluate the phylogenetic position of *Haplormosia* for the first time in a comprehensively-sampled molecular phylogenetic analysis of plastid *matK* sequences from within the main lineages of Papilionoideae. New morphological evidence for the genus is assessed in light of the phylogeny. We also used the molecular dataset to estimate divergence times and ancestral ranges to infer the biogeographic history of this enigmatic genus.

2. Materials and methods

2.1. Taxon sampling and molecular data

Most old collections of *Haplormosia* were not really suited for DNA extraction, but some recent collections from Gabon that were dried immediately or collected into silica gel have changed this situation (Sosef et al., 2006; Harris et al., 2012; van der Maesen and Wieringa, 2013). These new collections provided us with the opportunity to assess the enigmatic placement of *Haplormosia* in a molecular phylogenetic context of the Papilionoideae for the first time. Two sampling strategies were employed. The first dataset combined new *matK* protein-coding gene sequences of *Haplormosia* and several other taxa with 911 previously published *matK* sequences (Cardoso et al., 2015) comprising a broad and dense sample of legume lineages. The *matK* gene has been widely used in legumes to resolve relationships at different taxonomic levels (Hu et al., 2000; Lavin et al., 2001; McMahon and Hufford, 2004; Wojciechowski et al., 2004; Bruneau et al., 2008; Delgado-Salinas et al., 2011; Sirichamorn et al., 2012; Wojciechowski, 2013; Swanepoel et al., 2015).

Since the analyses of *matK* supported the placement of *Haplormosia* within the Genistoid clade, a second sampling strategy with reduced taxon coverage focusing on that clade was employed for the analyses of ITS/5.8S and plastid trnL intron sequences. The ITS dataset included 151 accessions of 146 species and 79 genera, whereas the trnL intron dataset comprised 99 accessions of 88 species and 46 genera. These datasets included taxa representing the broad morphological range of most genera currently recognized in the main lineages of the Genistoid clade (Cardoso et al., 2013a). A smaller *matK* dataset comprising 58 accessions from 51 species and 19 genera, with focus on the *Haplormosia*'s most closely related genistoid lineages, was used to estimate molecular divergence times and to infer biogeographic history.

2.2. Molecular laboratory analyses

The Qiagen Kits (i.e., Qiagen, Santa Clarita, California, USA) were used to isolate genomic DNA from silica-gel dried leaf material or from herbarium specimens. The following set of primers were used to PCR-amplify and sequence the *matK* gene: trnK85F (5′-GTA TCG CA C ATAT GTA TTA TTT GA-3′) and matK4R (5′-CAT TTT CCA AGT ATC GAA G-3′) and matK4La (5′-CTT TCG ATA CTG GGT GAA AGA T-3′) and matK1932R (5′-CAC GCC GGC TTA CTA ATG GG-3′); matK1100L (5′-TTC AGT GGT ACC GAG TCA AAT G-3′) and trnK2R (5′-CCC GGA ACT AGC GAA G-3′) (Hu et al., 2000; Wojciechowski et al., 2004). PCR conditions for *matK* were described in detail by Wojciechowski et al. (2004). The universal forward primer C (5′-CAA ATG CGG TAG ACC CGA CGA G-3′) was used with the reverse primer D (5′-GGG CAT AGA GGC ACT TGA AC-3′) to amplify the trnL intron (Taberlet et al., 1991). PCR conditions for the trnL intron included a 3-min denaturing step at 94 °C, followed by 40 cycles of 1 min at 94 °C (denaturation), 30 s at 50 °C (annealing), 1 min at 72 °C (extension), and further extension for 10 min at 72 °C. The forward primer 17SE (5′-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3′) was used with the reverse primer 26SE (5′-TAG AAT TCC CCG GTT CGC TCG CCT GGA C-3′) to amplify the ITS region (Sun et al., 1994). PCR involved a 5 min denaturing step at 94 °C, followed by 28–30 cycles of 1 min at 94 °C (denaturation); 1 min at 50–52 °C (annealing); 3 min at 72 °C (extension) and further extension for 7 min at 72 °C. Amplified PCR products were purified using 20% solution of polyethylene glycol (PEG) 6000 macrogol. The same set of primers used for the PCR were also used for sequencing, except for the ITS region that was sequenced with the primers 92 (5′-AAG GTT TCC GTA TTA GGA C-3′) (Desfeux and Lejeune, 1996) and ITS4 (5′-TCC GCT TAT TGA TAT GC-3′) (White et al., 1990). Sequencing reactions in both directions were performed using BigDye Terminator kit v.3.1; Applied Biosystems/Life Technologies Corporation, Carlsbad, California, USA. The products of sequencing were analyzed on ABI3730XL sequencers (Applied Biosystems) following the manufacturer's protocol at Universidade Estadual de Feira de Santana or FIOCRUZ, Bahia, Brazil. Table 1 provides GenBank accession numbers and voucher details for the 41 sequences newly generated for this study. All sequences obtained from other studies through GenBank are reported with their associated accession numbers immediately below the taxon names in the original alignments or in the phylogenetic trees that are available as online supplementary data.

2.3. Alignment and phylogenetic reconstructions

Forward and reverse chromatogram reads were assembled with the Staden Package (Staden et al., 1998). To avoid inconsistencies
Haplormosia DNA sequences newly generated for this study, with focus set on the phylogenetically enigmatic African monotypic legume genus *Haplormosia* (in bold). Voucher specimen information, including collection locality, voucher collector and number, and herbarium acronym are provided.

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher details, herbarium</th>
<th>Country, locality</th>
<th>GenBank</th>
<th>GenBank</th>
<th>GenBank</th>
<th>GenBank</th>
<th>GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphidod eiffusi</em> Huber</td>
<td>L.P. de Queiroz 13051 (HUEFS)</td>
<td>Brazil, Pará, Moju</td>
<td>KX584391</td>
<td>KX584391</td>
<td>KX584391</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clathrotropis nitida</em> Harms</td>
<td>H.C. de Lima et al. 2361 (BR)</td>
<td>Brazil, Amazonas, São Gabriel da Cachoeira</td>
<td>KX584394</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clathrotropis macrocarpum</em> D. Cardoso et al. 3348 (HUEFS)</td>
<td>Brazil, Amazonas, São Gabriel da Cachoeira</td>
<td>KX584410</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cyclolobium brasiliense</em> Benth.</td>
<td>B.A.S. Pereira 3701B (HUEFS)</td>
<td>Brazil, Goiás, Niquelândia</td>
<td>KX584390</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Harpylace brasiliana</em> Benth.</td>
<td>D. Cardoso et al. 2510 (HUEFS)</td>
<td>Brazil, Goiás, Planaltina</td>
<td>KX584388</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Harpylace hilariana</em> Benth.</td>
<td>R.M. Harley 28589 (HUEFS)</td>
<td>Brazil, Bahia, Correntina</td>
<td>KX584414</td>
<td>KX584436</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Harpylace lanata</em> L.P. Queiroz</td>
<td>D. Cardoso et al. 1393 (HUEFS)</td>
<td>Brazil, Bahia, Mucugê</td>
<td>KX584415</td>
<td>KX584387</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Harpylace parvifolia</em> H.S. Irwin &amp; Arroyo</td>
<td>L.P. de Queiroz 7530 (HUEFS)</td>
<td>Brazil, Minas Gerais, Grão Mogol</td>
<td>KX584416</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haplormosia monophylla</em> Harms</td>
<td>M.A. van Bergen 167 (WAG)</td>
<td>Gabon, Ogouë-Maritime</td>
<td>KX584395</td>
<td>KX584404</td>
<td>KX584376</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haplormosia monophylla</em> Harms</td>
<td>D.J. Harris et al. 8280 (E)</td>
<td>Gabon, Ogouë-Maritime</td>
<td>KX584396</td>
<td>KX584405</td>
<td>KX584377</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Haplormosia monophylla</em> Harms</td>
<td>D.J. Harris et al. 8717 (E)</td>
<td>Gabon, Ogouë-Maritime</td>
<td>KX584397</td>
<td>KX584406</td>
<td>KX584378</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ormosia bahiensis</em> Monach.</td>
<td>D. Cardoso et al. 2984 (HUEFS)</td>
<td>Brazil, Bahia, Umburanas</td>
<td>KX584399</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ormosia timboensis</em> D.B.O.S. Cardoso, Meireles &amp; H.C. Lima</td>
<td>D. Cardoso et al. 1649 (HUEFS)</td>
<td>Brazil, Bahia, Amargosa</td>
<td>KX584400</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pericopsis angolensis</em> (Baker) Meeuwen</td>
<td>C.H. Bosch 210 (WAG)</td>
<td>Tanzania, Kagera</td>
<td>KX584401</td>
<td>KX584411</td>
<td>KX584383</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pericopsis angolensis</em> (Baker) Meeuwen</td>
<td>R. Dechamps 1560 (WAG)</td>
<td>Angola, Cuanza Norte</td>
<td>KX584402</td>
<td>KX584412</td>
<td>KX584384</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pericopsis laxiflora</em> (Benth. ex Baker) Meeuwen</td>
<td>P. Houngnon 7753 (WAG)</td>
<td>Benin, Atakora, Natitingou</td>
<td>KX584403</td>
<td>KX584413</td>
<td>KX584385</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poecilanthe grandiflora</em> Benth.</td>
<td>J.R. Lemos 131 (HUEFS)</td>
<td>Brazil, Ceará, Aruaba</td>
<td>KX584409</td>
<td>KX584381</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poecilanthe itapuana</em> G.P. Lewis</td>
<td>E.P. Queiroz 63 (HUEFS)</td>
<td>Brazil, Bahia, Esplanada</td>
<td>KX584382</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poecilanthe pfloribena</em> Benth.</td>
<td>O. Barbosa 1 (HUEFS)</td>
<td>Brazil, São Paulo, Bauru</td>
<td>KX584392</td>
<td>KX584392</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poecilanthe subcordata</em> Benth.</td>
<td>L.P. de Queiroz 14493 (HUEFS)</td>
<td>Brazil, Bahia, Formosa do Rio Preto</td>
<td>KX584393</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Poecilanthe ulei</em> (Harms) Arroyo &amp; Rudd</td>
<td>D. Cardoso 891 (HUEFS)</td>
<td>Brazil, Bahia, Tucano</td>
<td>KX584389</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Templetonia retusa</em> (Vent.) R.Br.</td>
<td>T.J. Alford 203 (PERTH)</td>
<td>Australia, Western Australia, Jerdacutup Road along side Jerdacutup River</td>
<td>KX584380</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thinacula incana</em> (J.H. Ross) J.H. Ross</td>
<td>H.I. Aston 2844 (K)</td>
<td>Australia, Western Australia, Great Sandy Desert</td>
<td>KX584408</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Derived from automated multiple alignments, sequences were aligned manually in AliView version 1.17.1 (Larsson, 2014), following the sequence homology criteria of Kelchner (2000) and Simmons (2004). Because the matK matrix contains a high number of indels, we employed the strategy of Wojciechowski et al. (2004) in which homologies are inferred from amino acid translated sequences.

Phylogenetic reconstructions were performed with model-based Bayesian and maximum likelihood methods. The Bayesian analyses were run in MrBayes version 3.2.1 (Ronquist et al., 2012) with GTR+I+G as the best-fit nucleotide substitution model selected for each dataset via the Akaike Information Criterion (AIC), as implemented in MrModelTest version 2.2 (Nylander, 2004). In two independent runs of a Metropolis-coupled Markov Chain Monte Carlo (MCMC) permutation of parameters, eight simultaneous chains were initiated with a random tree and run for 5,000,000 (ITS and trnL intron) or 10,000,000 (matK) generations, with one tree sampled every 5000 or 10,000 generations, respectively. After a burn-in of 25%, non-autocorrelated sampled trees were summarized in a 50% majority-rule Bayesian consensus tree. The program AWTY (http://king2.scs.fsu.edu/CEBProjects/awty/awty_start.php; Nylander et al., 2008) was used to assess topological convergence by visualizing the cumulative split frequencies in the set of posterior trees. The clade frequencies or posterior probabilities (PP) were used as support measures (Huelsnbeek et al., 2002). Maximum likelihood trees were inferred in RAxML v7.2.8 (Stamatakis, 2006), using the evolutionary model GTR+CAT. Branch supports were estimated through 1000 bootstrap (BS) replications (Stamatakis et al., 2008). Visualization and editing of phylogenetic trees was done in FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). All phylogenetic analyses were run via the CIPRES Science Gateway v. 3.3 on-line portal (www.phylo.org) (Miller et al., 2010).

2.4. Divergence time estimation

Molecular divergence times were estimated from the smaller matK dataset with a Bayesian uncorrelated lognormal relaxed clock model (Drummond et al., 2006) implemented in BEAST package version 1.8.2 (A.J. Drummond et al., 2012) via the CIPRES Science Gateway. The BEAST analysis incorporated the same substitution model used in the phylogenetic reconstruction, a random starting
tree, and a Yule speciation process. To obtain absolute ages, lognormal prior age distributions were used on two fossil-calibrated nodes (see Ho, 2007). The root was calibrated at 60 Mya (offset = 60.0, mean = 0.0 and stdev = 1.0) based on fossil leaves and fruits possibly representing Ormosia that are found in Colombia with an estimated age between 60 and 55 Mya (Lavin et al., 2005). This age is indeed close to the crown node of the Genistoids estimated by Lavin et al. (2005) at ca. 56.4 Mya. Fossil leaves and pods from the Middle Eocene of the southeastern USA, with apomorphic traits (e.g. membranous pod valves, numerous seeds that are transversally oriented, a narrow wing on the placental suture, and leaflets variably alternate to opposite) suggesting affinity to Bowdichia Kunth and Diplotropis Benth. (Herendeen and Dilcher, 1990; Lavin et al., 2005), were used to set a calibration of 45 Mya (offset = 45.0, mean = 0.0 and stdev = 1.0) for the crown node of the Leptolobieae clade (sensu Cardoso et al., 2013a or the Bowdichia clade sensu Cardoso et al., 2012a). Here we have biased the calibration towards older ages by fossil-constraining more derived nodes rather than previous dating analyses that have constrained calibration towards older ages by fossil-constraining more derived clade sensu Cardoso et al., 2012a). Here we have biased the calibration towards older ages by fossil-constraining more derived nodes rather than previous dating analyses that have constrained calibration towards older ages by fossil-constraining more derived clades.

The BEAST file was generated in BEAUti v.1.8.2 (A.J. Drummond et al., 2012), with the main lineages (Brongniartieae and Leptolobieae) preset based on the results of Cardoso et al. (2012a, 2012c), but without forcing them to be monophyletic. Two independent runs of MCMC for 100 million generations were implemented, sampling parameters every 5000 generations after a 10% burn-in period. Convergence and stationarity were checked with Tracer v1.6 (Rambaut and Drummond, 2013), and all parameter estimates had ESS (effective sample size) values >200. Independent runs were combined in LogCombiner, and the maximum clade credibility (MCC) tree was generated using the TreeAnnotator. The MCC tree was annotated as a chronogram with median ages and 95% highest posterior density (HPD) intervals of node ages, and visualized with FigTree version 1.4.2.

2.5. Historical biogeographic reconstruction

The package BioGeoBEARS (BioGeography with Bayesian Evolutionary Analysis in R Scripts; Matzke, 2014) as implemented in R 3.2.2 (R Core Team, 2015) was used to reconstruct ancestral areas on internal nodes of Haplormosia and closely related lineages. The analysis was run using the six models available in BioGeoBEARS: DEC, DIVALIKE and BAYAREALIKE; and each of them with a parameter j that corresponds to founder event speciation and allows for long-distance dispersal events. Four geographical areas were defined in the analysis: South America, Mesoamerica, Africa, and Australia. Species distributions were coded as presence and absence in each of the four areas based primarily on literature and herbarium records. We set the maximum number of areas to four, and used only one individual per species. Analyses were implemented without constraints using the BEAST output tree. Likelihood values of each of the six different BioGeoBEARS models were compared using AIC and deltaAIC.

3. Results

All Bayesian and maximum likelihood analyses, regardless of the marker used, supported the placement of Haplormosia with the Genistoid clade (Fig. 2; Appendices S1, S2). However, a close relationship between Haplormosia and Ormosia was not supported. Rather, Haplormosia was supported in analyses of matK as the sister lineage to the American-Australian disjunctly-distributed Brongniartieae legumes (Fig. 2). Its placement with respect to the Brongniartieae was unresolved in the analyses of ITS and the trnl intron (Appendices S2, S3). Additional support was found for a sister relationship between the Leptolobieae clade and the clade comprising Brongniartieae plus Haplormosia.

The Bayesian divergence time estimates (Fig. 3) indicate that Haplormosia started to diverge from the Brongniartieae stem group by the Early Eocene, ca. 52 Mya (HPD 95%: 59.8–42.6 Mya). Within the crown-group Brongniartieae, Poeclinanth Benth. was the first genus to diverge in the Late Eocene, ca. 40.8 Mya (HPD 95%: 48.9–32 Mya).

Our estimation of ancestral areas indicated models DEC+j and DIVALIKE+j as most probable, as evidenced by AIC and deltaAIC (Table 2). According to Burnham and Anderson (2002), when differences are within 2 AIC units competing models are equally probable. However, whilst model DEC+j (Appendix S4) exhibits the highest likelihood (Table 2), it recovered South America plus Africa area in the deepest node, which is not possible as the split of this node occurred at 65–51 Mya, a period when these continents were already separated. Thus, we opted to follow results from the DIVALIKE+j model that inferred South America as ancestral area for the ingroup.

Under DIVALIKE+j model (Fig. 3) our results suggest that the geographical isolation of Haplormosia in Africa came about via long-distance dispersal from a South American ancestor and that its sister group, the neotropical Brongniartieae, originated in South America and spread subsequently into Central America and Australia, with two independent colorizations of Central America. The divergence of a Central American clade of Brongniartia from the Australian lineages was dated to ca. 20 Mya. A more recent independent dispersal event from South America to Central America and the Caribbean in Harpalyce was dated to ca. 15 Mya.

4. Discussion

4.1. Placing Haplormosia in the Papilionoidae phylogeny

The description of the African genus Haplormosia by Harms (1917) to accommodate a single tree species, Haplormosia monophylla, highlighted its morphological resemblance to the species-rich genus Ormosia that is widely disjunct in the Neotropics and tropical Asia, with a single species in tropical Australia. Later morphologically-based classifications placed both genera together in the informal Ormosia group of the morphologically diverse tribe Sophoreae (Polhill, 1981, 1994). The two genera share woody pods and bilaterally symmetrical papilionate flowers with free stamens and pinkish to purple petals completely differentiated into standard, keel, and wings. Additionally, the single-seeded pods very similar to Haplormosia are found in the bulk of Ormosia species. This morphological similarity explains why Polhill’s (1981) taxonomic position of Haplormosia was also maintained in the current classification of all legumes. The recent phylogenetic review of the early-branching papilionoid genera (Cardoso et al., 2013a) likewise considered the putative relationship of Haplormosia with Ormosia even in the absence of molecular data. However, here we have revealed that Haplormosia is part of the early-branching Genistoid clade. Unexpectedly, Haplormosia is not even close to the Genistoid genus Ormosia, rather it appears as sister to the American-Australian Brongniartieae clade (Fig. 2). An ongoing multi locus phylogenetic analysis that has densely sampled the species of Ormosia taxonomically and geographically also did not show any close affinity of that genus with Haplormosia (Tork et al., unpubl. data).
The Genistoids form one of the main lineages within the 50-kb inversion clade of Papilionoideae and contain several genera once classified in the largely polyphyletic tribe Sophoreae. The placement of *Haplormosia* in the Genistoid clade is further corroborated by the accumulation of quinolizidine alkaloids in the seeds (Kinghorn et al., 1988). Quinolizidine alkaloids have proven to be a powerful chemotaxonomic character for the Genistoid legumes. For example, the presence of quinolizidine alkaloids in the leaves and seeds of *Poeclianthe* was the first indication of its affinities with other Brongniartieae genera (Greenwald et al., 1995; a result later supported by molecular data: Crisp et al., 2000; Hu et al., 2000, 2002), rather than with the Dalbergieae (Lavin, 1987), where it...
had been traditionally placed. The co-occurrence of the quinolizidine alkaloids epilupinine and cytisine has been detected in several Brongniartieae genera (e.g. Greinwald et al., 1995, 1996). Whether such types of alkaloids are chemical synapomorphies for an expanded Brongniartieae tribe including the early-diverging sister branch *Haplormosia* will be an interesting topic to investigate further in a phylogenetic context, for the Genistoids, as we have seen increasingly well supported relationships and unexpectedly new generic realignments in the clade (Cardoso et al., 2012a, 2012b, 2015).

Although unrecognized in traditional classifications (Polhill, 1981, 1994; Lewis et al., 2005), there are several morphological features that favor a close relationship between *Haplormosia* and the Brongniartieae genera over a relationship to *Ormosia*, as suggested by the analysis of *matK* sequences (Fig. 2). The unifoliolate leaves, axillary contracted inflorescences, the two upper lobes of the calyx united for almost their entire length, and single-seeded, tardily dehiscent fruits with thick, leathery becoming woody valves that characterize *Haplormosia* are also found variously in Brongniartieae (e.g. in the South American genera *Cyclolobium*, *Limadendron*, and *Tabaroa*; Schrire, 2005; Queiroz et al., 2010). However, such morphological features very likely evolved independently in other Genistoid clades (Crisp et al., 2000). Strikingly, the stamens with distinctly dimorphic anthers (alternately basifix and dorsifix) that are found in most Brongniartieae genera (Thompson, 2010a, 2010b, 2010c; Queiroz et al., 2010; Meireles et al., 2014) are also a notable floral trait of *Haplormosia*, yet went unnoticed or was disregarded in previous taxonomic classifications of the genus.

**Tucker** (1987, 1996) notes that stamen heteromorphism is common in papilionoids. But information on the occurrence of this character across the subfamily is missing and an investigation addressing this is currently under way (Paulino et al., 2016). Paulino et al. (2016) recently presented a comprehensive analysis

![Fig. 3. BEAST-derived chronogram of *Haplormosia* and related Genistoid clades based on *matK* sequences, with ancestral distribution under DIVALIKE+j model as inferred through BioGeoBEARS. Light gray bars on the nodes represent 95% of high posterior density of divergence times. Black diamonds at nodes indicate calibration points. Pie charts at nodes represent relative probabilities of putative ancestral areas, with respective colors according to general distribution of extant species in South America, Mesoamerica, Africa, and Australia. The gray color in pie charts represents the area that includes South America plus Africa. Time scale is in millions of years.]

<table>
<thead>
<tr>
<th>Model</th>
<th>LnL</th>
<th>Numparams</th>
<th>d</th>
<th>e</th>
<th>j</th>
<th>AIC</th>
<th>deltaAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEC</td>
<td>-23.6119</td>
<td>2</td>
<td>0.001154</td>
<td>1.00E-12</td>
<td>0</td>
<td>51.223819</td>
<td>7.216456</td>
</tr>
<tr>
<td>DEC+j</td>
<td>-19.0037</td>
<td>3</td>
<td>1.00E-12</td>
<td>0.012397</td>
<td>0</td>
<td>44.007364</td>
<td>0</td>
</tr>
<tr>
<td>DIVALIKE</td>
<td>-22.5986</td>
<td>2</td>
<td>0.001809</td>
<td>1.00E-12</td>
<td>0</td>
<td>49.197176</td>
<td>5.189812</td>
</tr>
<tr>
<td>DIVALIKE+j</td>
<td>-19.8539</td>
<td>3</td>
<td>1.00E-12</td>
<td>0.014975</td>
<td>0</td>
<td>45.707826</td>
<td>1.700462</td>
</tr>
<tr>
<td>BAYAREALIKE</td>
<td>-37.7094</td>
<td>2</td>
<td>0.001836</td>
<td>0.007252</td>
<td>0</td>
<td>79.418885</td>
<td>3.54E+01</td>
</tr>
<tr>
<td>BAYAREALIKE+j</td>
<td>-20.2689</td>
<td>3</td>
<td>1.00E-07</td>
<td>0.014834</td>
<td>0</td>
<td>46.537719</td>
<td>2.530356</td>
</tr>
</tbody>
</table>
of stamen and anther heteromorphism in two papilionoid legumes \((\text{Cytisus} \text{ and } \text{Lupinus})\). The authors showed that in \text{Lupinus} anther dimorphism is correlated with the mode of pollen presentation \((\text{pump mechanism})\) and that the smaller anthers produce pollen that is sterile and in much lower quantity than in the larger anthers. In \text{Cytisus} division of labor was shown for the first time with some stamens producing pollen for food and others for pollination. Besides the occurrence of stamen heteromorphism among Papilionoideae, detailed investigation of functional aspects, not only of the stamens and anthers, but also of the pollen grains, appears necessary to increase our understanding of evolution of these characters.

Overall, the androecium provides a wide range of systematic characters useful for legume classification on various taxonomic levels \((\text{Tucker}, 1987, 1996; \text{Prener}, 2004, 2013)\). Preliminary studies indicate that the androecium in \text{Harpalyce brasiliensis} at least in some samples \((\text{Poecilanthe itapuana})\) is asymmetric \((\text{G.P.}, \text{pers. obs.})\) with the adaxial stamen of the inner whorl formed either to the left or right of the median plane \((\text{cf. Prener}, 2004)\). It remains to be studied whether \text{Haplormosia} shows the same pattern of stamen initiation and how this fits into the larger picture of androecial symmetry among Brongniartieae and early-branching papilionoid legumes \((\text{Prener}, 2004; \text{Prener} \text{ and Cardoso}, 2016)\).

### 4.2. Biogeographic history of Haplormosia and the closely related Brongniartieae legumes

Vicariance processes involving continental plate tectonics have been put forward to explain the origins of Gondwanan-like intercontinental disjunctions, as seen, for example, in legumes \((\text{Raven} \text{ and Polhill}, 1981)\) and in the giant flightless ratite birds \((\text{Mitchell et al.}, 2014)\). With respect to plants, the Boreotropical hypothesis \((\text{Lavin} \text{ and Luckow}, 1993)\) provided an alternative biogeographical explanation for tropical disjunctions. It predicted that such lineages were once widespread in the Eocene-Early Oligocene tropical forests of the northern hemisphere and that present-day distributions in the tropics resulted from dispersal from the Northern Hemisphere into equatorial zones, with subsequent extinction in the former area due to cooling global climate.

Ensuing advances in time-calibrated phylogenies have greatly impacted our understanding of the temporal context for intercontinental plant disjunctions. In most cases, the finding of relatively recent divergences in these studies favors a much greater role for trans-oceanic dispersal and establishment over vicariance in the biodiversity assemblage of global biomes \((\text{Lavin et al.}, 2004; \text{Pennington} \text{ and Dick}, 2004; \text{Schrire et al.}, 2005; \text{Hughes et al.}, 2013)\). Our divergence estimates also reject diversification by continental vicariance as an explanation for the intercontinental disjunctions in the \text{Haplormosia} plus Brongniartieae clade, as \text{Haplormosia} apparently split off from the remaining Brongniartieae well after the break up of the Gondwana. In fact, numerous dated phylogenies have repeatedly revealed the recency of divergence in transcontinental taxa, regardless of the biological group \((\text{plants or animals in many different clades})\) \((\text{Renner}, 2004; \text{Knapp et al.}, 2005; \text{Schrire et al.}, 2005; \text{Mueliener et al.}, 2006; \text{Särkinen et al.}, 2007; \text{Zhang et al.}, 2007; \text{Duchen} \text{ and Renner}, 2010; \text{Michalak et al.}, 2010; \text{Antoine et al.}, 2011; \text{Thiv et al.}, 2011; \text{Carvalho} \text{ and Renner}, 2012; \text{Fritsch} \text{ and Cruz}, 2012; \text{Mitchell et al.}, 2014; \text{Richardson et al.}, 2014; \text{Sriuchamorn et al.}, 2014; \text{Eiertsen} \text{ and Malaquias}, 2015; \text{Longrich et al.}, 2015; \text{Thornhill et al.}, 2015; \text{Bardon et al.}, 2016; \text{Rota et al.}, 2016; \text{Ruhfeld et al.}, 2016)\), suggesting the apparent ease with which plants have been able to disperse across oceans. Our inferred time frame and ancestral area reconstruction \((\text{Fig. 3})\), where the MRCAs of the Leptolobiaeae-Brongniartieae and \text{Haplormosia}-Brongniartieae clades were inferred with South American distribution and the Mesoamerican and Australian clades are recent radiations from a South American ancestor, reject any assertion that relates the diversification and resulting distribution patterns in the \text{Haplormosia}-Brongniartieae to the break up of the Gondwana \((\text{Arroyo}, 1981; \text{Thompson et al.}, 2001)\). During Early Eocene South America and Africa exhibited a relatively shorter distance between each other \((\text{Appendix S4})\), which might have facilitated overseas dispersal of South American lineages. The ecological predilection of \text{Haplormosia monophylla} for river- and lagoon banks \((\text{D.H. and J.W.}, \text{pers. obs.})\) suggests adaptation for seed dispersal by water, moreover, \text{Haplormosia} pods have been found on Gabonese beaches as drift seeds. Perhaps dispersal by water is an ancestrally inherited trait that could have favored the transoceanic dispersal of its South American ancestor. On the other hand, our competing, yet equally probable hypothesis of ancestral area reconstruction, that posits Africa plus South America as ancestral area \((\text{Appendix S3})\), coupled with the date for the split between \text{Haplormosia} and the Brongniartieae do not necessarily reject the Boreotropical hypothesis or dispersal through the northern boreotropical forests \((\text{Lavin} \text{ and Luckow}, 1993)\). The ancestral area inferred by DEC+I, that is represented by Africa plus South America on extant taxa, can be attributed to ancestral populations of Brongniartieae lineages which might have lived in boreotropical forests during the Eocene and underwent extinction leaving remaining relatives in southern continents.

Phylogenetic patterns of reciprocal monophyly like the amphi-Atlantic \text{Haplormosia}-Brongniartieae clade have been associated mostly with vicariant breaks \((\text{e.g. Cunningham} \text{ and Collins}, 1998; \text{Lavin et al.}, 2000)\). Nonetheless, they can also result from old long-distance dispersal events followed by population coalescence processes acting at the clade level \((\text{Barraclough}, 2010)\). The relatively long stem branches of the reciprocally monophyletic \text{Haplormosia}-Brongniartieae clade indicate that the American-Australian Brongniartieae lineage might have experienced much species turnover without subsequent long-distance dispersal to Africa \((\text{Barraclough}, 2010)\). Indeed, the phylogenetic signature of reciprocal monophyly after long-distance dispersal is commonplace in amphi-Atlantic legume clades, such as the caesalpinoid \text{Hymenaea-Gaibourtia} clade, within the genus \text{Mimoso}, the \text{Dichrostachys-Calliandra} and \text{Afrocalliandra-Calliandra} clades in the mimosoids, and the \text{Amburana-Cordyla-Mildbraediodendron} and \text{Chapmannia-Diphysa-Ormocarpum} clades in the papilionoids \((\text{Lavin et al.}, 2004; \text{Schrire et al.}, 2005; \text{Simon et al.}, 2011; \text{Souza et al.}, 2013; \text{Cardoso et al.}, 2015)\), all of which post-date the split of west Gondwana.

Challenging new geological, fossil, and molecular evidences have pushed the date for the emergence and closure of the Isthmus of Panama back to around 20 Mya, earlier than previous estimates \((\text{Carvalho} \text{ and Renner}, 2012; \text{Bacon et al.}, 2015; \text{Hoorn} \text{ and Flintua}, 2015; \text{Montes et al.}, 2015)\). That extensive terrestrial landscapes with the rise of the Isthmus were already linking North and Central America with South America during the Middle Miocene, however, has recently been critically challenged by an exhaustive review and reanalysis of geological records and paleontological and molecular data of marine organisms \((\text{O’Dea et al.}, 2016)\). Given such more consistent evidence supporting the widely recognized age of approximately 3 Mya for a fully-closed Isthmus, we believe that dispersal of South American ancestors most likely explains the origin of the Mesoamerican Brongniartieae lineages around 20–15 Mya \((\text{Fig. 3})\). Larger oceanic barriers did not prevent dispersal of an \text{Haplormosia} ancestor across the Atlantic because of eastward-flowing sea currents \((\text{Renner}, 2004)\), likewise relatively small oceanic barriers might not have constrained dispersal of Brongniartieae taxa within the Americas.

Why the American-Australian Brongniartieae clade is more diverse than its sister \text{African Haplormosia} lineage, despite both having very old stem ages, is still an open question. Contrasting
diversification patterns involving closely related species-rich neotropical and species-poor African clades has also been shown in many other clades, such as Mimosa (Simon et al., 2011), Afrocallyandra-Calliantra (Souza et al., 2013), and the Swartziioids (Cardoso et al., 2013a). Outside of legumes, flagship examples include the closely-related monocot families Bromeliaceae and Rapateaceae (Givnish et al., 2004). An in-depth look at the distribution of species diversity within the Brongniartieae also reveals an interesting, yet unexplored pattern. Most South American genera are species poor (1–2 spp.) or have at most 10 spp. Conversely, the independent colonization of the Brongniartieae into new continental expanses coincides with high speciation. For example, the genus Brongniartia Kunth underwent crown radiation during the Middle Miocene that resulted in ca. 65 species with exclusive Mesoamerican (southern USA and mostly in Mexico) distribution (Fig. 3). Likewise, the independent dispersal and Miocene diversification of the Brongniartieae in Australia resulted in the second largest genus of the clade, Hovea R.Br. ex W.T.Aiton, which comprises at least 37 species. The Mesoamerican and Australian Brongniartieae are in line with radiations in many instances in different plant families (Bacon et al., 2013; Givnish et al., 2014; Richardson et al., 2014; Schwartz et al., 2015), including the “super radiation” of the papilionoid legume Lupinus in the Andes (C.S. Drummond et al., 2012), in which biogeographic movements or newly colonized territories underlie the acceleration of diversification. A more complete species-level phylogeny of the Brongniartieae is needed to test for possible shifts in diversification rates and to assess whether not just such extrinsic biogeographic factors, but a “key confluence” also involving morphological “synnovation” (sensu Donoghue and Sanderson, 2015) worked in combination to elevate species richness in the Mesoamerican and Australian lineages.

The geographical distribution of the neotropical Brongniartieae and related genera within the Leptolobieae clade in different biomes creates an excellent opportunity to understand the role of phylogenetic niche conservatism (Couvreur et al., 2011; Pennington and Lavin, 2016) or niche evolution (Simon et al., 2009). The distal phylogenetic placements and relatively recent age estimates of the fire-prone, savanna-inhabiting species studied here (Bowdichia virgilioides, Leptolobium elegans, L. dasycarpum, L. brachystachyum, and Staminodianthus racemosus from Leptolobieae; and Harpalyce brasiliiana, H. hilariana, H. lanata, H. parvifolia from Brongniartieae; all of which have stem ages dated less than 10 Mya; Fig. 3) are compatible with the hypothesis that most Cerrado lineages are recent and were derived by niche evolution of rainforest ancestors (Simon et al., 2009).

5. Conclusions and future prospects

Here we have gathered new information for the previously unplaced African monotypic genus Haplormosia in order to fill in the gaps of the early evolution and biogeography of the spectacularly species-rich Papilionoideae radiation of legumes. Divergence by continental vicariance does not account for the sister relationship of Haplormosia and the American-Australian Brongniartieae clade. The time-calibrated phylogeny and ancestral area inference indicated that the Haplormosia monophylla stem-clade originated at ca. 52 Mya in the Early Eocene by long-distance overseas dispersal from a neotropical ancestor into Africa while its neotropical sister subsequently diversified all over the main biomes of the Americas and Australia.

An all-encompassing biogeographic reconstruction of the Brongniartieae clade is hampered by the generally poor resolution at the early-branching nodes of the Australian and Mesoamerican lineages. A comprehensive study of the biogeography and evolution of the Brongniartieae clade is planned in a forthcoming densely-sampled multilocus study that will include multiple accessions of species in order to assess the phylogenetic signature of evolutionary persistence of lineages with different ecologies (Pennington and Lavin, 2016) across the tropical seasonality gradient (Oliveira-Filho et al., 2013).

Even failing short of fully resolving the sister-relationship of Haplormosia upon plastid trnL intron and nuclear ITS sequences, we have provided solid evidence from morphology, phytochemistry, and, most notably, the phylogenetic analysis of the protein-coding matK gene. Haplormosia is not phylogenetically close to the genus Ornmosia of tribe Sophoreae, which seems at odds with the traditional taxonomic view. Rather, it is yet another surprising example of generic realignments that made up the historically complex circumscription of the tribe Brongniartieae (Crisp et al., 2000; Hu et al., 2000, 2002; Queiroz et al., 2010). With the addition of Haplormosia in an expanded concept of tribe Brongniartieae, the group contains 15 genera (Ross and Crisp, 2005; Queiroz et al., 2010; Cardoso et al., 2013a; Meireles and Tozzi, 2014; Meireles et al., 2014; Queiroz et al., unpubl. data). Another recent addition to the Brongniartieae was the Cuba-endemic monotypic genus Behaimia Griseb. (Queiroz et al., submitted for publication). Oddly, the genera of the Brongniartieae clade were formerly placed in at least four separate tribes by Polhill and Raven (1981), namely the Brongniartieae, Bossieae, Sophoreae, and Millettiae. This patchy treatment is not only due to the taxonomic history of Brongniartieae clearly demonstrates the morphological heterogeneity of the tribe. Now that we have increasingly moved towards a better picture of the Brongniartieae phylogeny and systematics, we could explore in the future some emerging diversification-related patterns. For example, except for the more diverse American Brongniartia (ca. 65 species) and Harpalyce (ca. 25), and the Australian Hovea (ca. 37), most Brongniartieae genera are represented by just one or two species, despite being relatively old (most of them diverged well before the Late Miocene, Fig. 3; see the divergence at ca. 19 Mya for the sister monotypic genera Amphiodon Huber from the Amazonian rainforest and Tabaroa L.P. Queiroz, G.P. Lewis & M.F. Wojc. from the Brazilian Caatinga dry woodland). Why the Brongniartieae remained poorly diversified at genus-level and geographically in Africa and South America seems to be a stimulating question to be addressed in the light of a more complete time-calibrated phylogeny densely sampled at the species level.

Acknowledgments

This work received financial support from Sistema Nacional de Pesquisa em Biodiversidade-SISBIOTA (processes CNPq 563084/2010-3 and FAPESB PES0053/2011), Prêmio CAPES de Teses (process 23038.009148/2013-19), and FAPESB (processes PET0039/2012 and APP0037/2016). DC and LPQ acknowledge the Research Productivity Fellowships (processes 306736/2015-2 and 300811/2010-1, respectively) from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the postdoctoral fellowship from CAPES (process BEX 2812/13-4) through the Reflora programme which made possible the study of important collections of Haplormosia and other basal papilionoids at the Royal Botanic Gardens, Kew and Royal Botanic Garden Edinburgh. We very much appreciate the comments and suggestions of two anonymous reviewers. DC also thanks the Rede de Plataformas Tecnológicas for use of its sequencing facility in FILOCRAZ-Bahia. JJW and DJH are grateful for the collaboration and assistance received from the National Herbarium of Gabon (part of IPHAMETRA-CENAREST) during several collecting missions to Gabon.


