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A dated phylogeny of Lardizabalaceae reveals an unusual long-distance dispersal across the Pacific Ocean and the rapid rise of East Asian subtropical evergreen broadleaved forests in the late Miocene

Wei Wang^{a,b}, Xiao-Guo Xiang^c, Kun-Li Xiang^{a,b}, Rosa del C. Ortiz^d, Florian Jabbour^e,
Zhi-Duan Chen^a

^aState Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; ^bUniversity of Chinese Academy of Sciences, Beijing 100049, China; ^cJiangxi Province Key Laboratory of Watershed Ecosystem Change and Biodiversity, Institute of Life Science and School of Life Sciences, Nanchang University, Nanchang 330031, China; ^dMissouri Botanical Garden, 4344 Shaw Blvd, St. Louis, Missouri 63166-0299, USA; ^eInstitut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, Université des Antilles, EPHE, 57 rue Cuvier, CP39, Paris 75005, France

Running title: Spatio-temporal evolution of Lardizabalaceae

*Corresponding author. *E-mail address*: wangwei1127@ibcas.ac.cn

Abstract

Temperate South American-Asian disjunct distribution is the most unusual in organisms, and challenging to explain. Here, we address the origin of this unusual disjunction in Lardizabalaceae using explicit models and molecular data. The family (approximately 40 species distributed in ten genera) also provides an opportunity to explore the historical assembly of East Asian subtropical evergreen broadleaved forests, a typical and luxuriant vegetation in East Asia. DNA sequences of five plastid loci of 42 accessions representing 23 species of Lardizabalaceae (approximately 57.5% of estimated species diversity), and 19 species from all other six families of Ranunculales, were used to perform phylogenetic analyses. By dating the branching events and reconstructing ancestral ranges, we infer that extant Lardizabalaceae dated to the late Cretaceous of East Asia, and that the temperate South American lineage might have split from its East Asian sister-group at *c.* 24.4 Ma. A trans-Pacific dispersal possibly by birds from East Asia to South America is plausible to explain the establishment of the temperate South American-East Asian disjunction in Lardizabalaceae. Diversification rate analyses indicate that net diversification rates of Lardizabalaceae experienced a significant increase around *c.* 7.5 Ma. Our findings suggest that the rapid rise of East Asian subtropical evergreen broadleaved forests occurred in the late Miocene, associated with the uplift of the Tibetan Plateau and the intensified East Asian monsoon, as well as the higher winter temperature and atmospheric CO₂ levels.

Introduction

Intercontinental disjunctions of organisms have fascinated biologists for over a century (Raven and Axelrod, 1974), and understanding how these disjunctions were shaped is a central theme in biogeography (Lomolino et al., 2010). Trans-equatorial Andean-Asian disjunction in which closely related terrestrial organisms are separated by the entire width of the Pacific Ocean is relatively rare. Maekawa (1965) listed 16 angiosperm groups. Molecular phylogenetic analyses added three, i.e. *Gleditsia* (Fabaceae; Schnabel and Wendel, 1998), *Chrysosplenium* (Saxifragaceae; Soltis et al., 2001), and *Gaultheria* (Ericaceae; Lu et al., 2019). The extreme trans-equatorial Andean-Asian distribution is presented by the organisms occurring in temperate South America and Asia. Thorne (1972) considered that this disjunct type was the most unusual and difficult to explain among his sixteen categories of disjunct ranges of intercontinental magnitude.

Lardizabalaceae presents a striking case of temperate South American-Asian disjunct distribution (Fig. 1; Thorne, 1972). The family encompasses ten genera and approximately 40 species (Qin, 1997; Wang et al., 2009). Two monospecific genera, *Lardizabala* and *Boquila*, are endemic to southwestern South America, while the remaining genera and species are restricted to eastern Asia from the Himalayas to Korea and Japan (Fig. 1; Qin, 1997). Several competing hypotheses have been proposed to explain the current distribution pattern of Lardizabalaceae (Fig. 1). (1) “Vicariance” hypothesis, in which the disjunction was caused by the separation of Laurasia and Gondwanaland (Maekawa, 1965; Qin, 1997). This hypothesis implies

that Lardizabalaceae originated and was broadly distributed across the supercontinent before it broke up completely (*c.* 150 Ma; Mao et al., 2012), and the African lineage(s) underwent extinction (Raven and Axelrod, 1974). (2) “Migration” hypothesis, according to which the family was originally Laurasian and then extended to South America through North America and Central America (Raven and Axelrod, 1974; Schuster, 1976; Tiffney, 1993), or through the East Indies, Australia and Antarctica (Tiffney, 1993). This implies that fossils or other evidence for occurrences of this family could be found in geographically intermediate areas. The fossils of *Sargentodoxa* found in the early Miocene and middle Eocene of North America (Tiffney, 1994; Manchester, 1999) seem to support the former migration route. However, this will depend on how *Sargentodoxa* is related to other Lardizabalaceae, especially to the South American genera *Lardizabala* and *Boquila* (Tiffney, 1993). (3) “Rafting” hypothesis, based on which the family originated in Gondwanaland and dispersed to Laurasia through the Indian plate as a vector (Schuster, 1976). This means that the South American taxa would be early divergent in the family. (4) “Long-distance dispersal (LDD)” hypothesis, according to which the current distribution of the family in southern South America is a result of LDD in more recent times (Christenhusz, 2012). However, Thorne (1972) suggested that direct LDD between Chile and eastern Asia was rather unlikely given the vast land discontinuity. These competing hypotheses remain to be tested in a phylogenetic context using explicit models and the absolute age of the temperate South American-Asian disjunction estimated by molecular data.

The overwhelming majority of Lardizabalaceae species are restricted to the East Asian subtropical evergreen broadleaved forests (EBLFs), which mainly occur in $c.$ 23 to 39° N and 97 to 141° E (Song and Da, 2016), extending westward to 95° E within Tibet (e.g., Chayu and Motuo counties in the Yaluzangbujiang region; Tang, 2015). This range is highly congruent with the distribution area of East Asian Lardizabalaceae (Fig. 1). With the exception of the monospecific *Decaisnea*, which is an erect shrub, the remaining Lardizabalaceae plants are lianas (woody climbers) (Qin, 1997). Lardizabalaceae is one of the three most dominant liana families in the East Asian subtropical EBLFs (Cai and Song, 2000), which contribute substantially to the local abundance of woody plants (Cai and Song, 2000; Tang, 2015). The South American *Lardizabala* and *Boquila* also occur in EBLFs (Qin, 1997). Lianas are an important physiognomic and structural component of tropical and subtropical forests (Gentry, 1991; Song and Da, 2016) and can thereby be regarded as an indicator of both biomes (Gentry, 1991; Wang et al., 2012). Lardizabalaceae thus offers a good opportunity to investigate the historical assembly of East Asian subtropical EBLFs.

East Asian subtropical EBLF is recognized as a zonal vegetation type or a climatic climax of the central part of the subtropical zone, which developed under the monsoon climate conditions of East Asia (Wu, 1980; Song and Da, 2016). This vegetation covers large areas of East Asia and harbors high biodiversity and endemism compared to the same latitudinal zones elsewhere on the planet, which are mostly arid and semiarid lands (Tang, 2015). East Asian subtropical EBLFs comprise 2600 genera and approximately 14600 species of seed plants, of which more than

50% are endemic (Wu, 1980). Owing to human activities, primary subtropical EBLFs have been largely destroyed in East Asia, particularly in eastern China (Tang, 2015). Paleovegetational reconstructions and molecular phylogenetics have contributed to our understanding of the origin of East Asian subtropical EBLFs. Some paleovegetations of Yunnan in southwestern China assigned to the Miocene were suggested as reminiscence of subtropical EBLFs (Jacques et al., 2011). Nevertheless, recent U-Pb zircon dates show that these vegetations in southwestern China might have been established by the early Oligocene, *c.* 33 Ma (Linnemann et al., 2017). Dated phylogeny of *Dendrobium* (Orchidaceae), an epiphytic element of Asian EBLFs, has also supported the view that the EBLFs have existed in mainland Asia at least since the beginning of the Oligocene (Xiang et al., 2016). Using Theaceae as a proxy, Yu et al. (2017) suggested that the progressive development of the subtropical EBLFs in East Asia occurred around the Oligocene–Miocene boundary and was driven by the intensification of East Asian monsoon (EAM). Yu et al. (2017) also detected an increase of diversification rate in the Theaceae that occurred in the latest Miocene (5.4–5.7 Ma; crown of *Schima*).

During the Miocene, the climate and vegetation of East Asia, as well as other regions on the planet, were undoubtedly altered dramatically, in association with the Indo-Eurasian collision-induced tectonic activities from the Neogene onward (Zachos et al., 2001; Guo et al., 2002; Liu et al., 2015). In particular, the significant enhancements of the EAM occurred in the Miocene (Guo et al., 2002). However, the historical assembly of East Asian subtropical EBLFs in the Miocene remains unclear.

Here, we reconstruct the most comprehensive phylogenetic tree for Lardizabalaceae to date based on five plastid DNA regions. By integrating phylogenetic, molecular dating and biogeographic methods, we then test the aforementioned four hypotheses explaining of the temperate South American-East Asian disjunct distribution. Finally, we use Lardizabalaceae as a proxy to investigate the assembly history of East Asian subtropical EBLFs, namely we attempt to evaluate whether East Asian subtropical EBLFs might have experienced a rapid rise during the Miocene, associated with the intensified EAM.

Materials and methods

Taxon sampling and DNA sequencing

We sampled 23 species from 40 of Lardizabalaceae (Ranunculales), representing all ten genera recognized in the family (Qin, 1997; Wang et al., 2009). Outgroups included nineteen species covering all other six families of Ranunculales (Table S1). The samples were collected from the field or from herbarium specimens. Based on previous studies (Wang et al., 2009; Sun et al., 2017), the tree was rooted with *Euptelea polyandra* (Eupteleaceae), the earliest-diverging lineage in the order. Five plastid loci were used: *rbcL*, *matK*, and *ndhF* genes, *trnL-F* regions (*trnL* intron, and *trnL* [UAA] 3' exon-*trnF* [GAA] intergenic spacer), and *rpS16* intron. Each species was sequenced for the five DNA loci except for *Kingdonia uniflora*, whose *ndhF* gene is absent (Sun et al., 2017). Voucher information and GenBank accession numbers are listed in Table S1.

Total genomic DNA was extracted from silica-gel-dried leaves or herbarium material using the NuClean PlantGen DNA Kit (CWBIO, Beijing, China). The primers used in this study are listed in Table S2. Sequencing reactions were conducted using the ABI Prism Bigdye Terminator Cycle Sequencing Kit (Applied Biosystems, ABI) and analyzed using ABI 3730xl automated DNA sequencers.

Phylogenetic analyses

Sequences were aligned using CLUSTAL X (Thompson et al., 1997), and further adjusted manually in BioEdit v5.0.9 (Hall, 1999). Five difficult-to-align regions in *trnL-F* (289 sites) and two difficult-to-align regions in *rpS16* (36 sites) were removed from the analyses. The aligned lengths of the *rbcl*, *matK*, *ndhF*, *trnL-F*, and *rpS16* sequences were 1395, 1263, 2136, 1232, and 1236 bp, respectively, resulting in a concatenated 42-taxon alignment of 7262 bp. We first conducted maximum likelihood (ML) searches using RAxML v8.0 (Stamatakis, 2014) for each of the five loci. As there were no significantly conflicting nodes (> 85% bootstrap support), all subsequent analyses were carried out for the concatenated dataset only. Phylogenetic analyses for the concatenated dataset were performed using ML, Bayesian inference (BI), and maximum parsimony (MP) methodologies in RAxML v8.0 (Stamatakis, 2014), MrBayes v3.2.5 (Ronquist et al., 2012), and PAUP* v4.0b10 (Swofford, 2003), respectively. For ML analysis, the GTR + Γ substitution model was assigned for each region. For BI analysis, each region was assigned its own model of nucleotide substitution, as determined by the Akaike Information Criterion (AIC) in jModeltest

v2.1.4 (Posada, 2008). The detailed descriptions of these three analytical methods can be found in Wang et al. (2017).

Divergence time estimation

Divergence times were estimated using BEAST v2.4.0 (Bouckaert et al., 2014). To convert stratigraphic ages into absolute ages, we used the geological time scale of Cohen et al. (2013, updated). Four fossils confidently placed in our tree were selected as minimum-age constraints (Fig. 2). (1) *Decaisnea bornensis*, seeds from the late Oligocene of Germany, corresponds to the extant *Decaisnea* in morphology, including placement and size of the hilum, raphe and micropylar point and in anatomy of the seed coat (Mai and Walther, 1991). The stem group age of *Decaisnea* was given a lower bound of 23.03 Ma (C₁). (2) *Sargentodoxa globosa* from the middle Eocene of Oregon belongs to the extant *Sargentodoxa* based on its ovoid seeds with an oblique hilar facet and a thick seed coat and the placement of the raphe, microphyle, and chalaza (Manchester, 1999). The stem group node of *Sargentodoxa* was given a lower bound of 41.2 Ma (C₂). (3) A fossil flower, *Kajanthus lusitanicus*, was reported from the Early Cretaceous (late Aptian–early Albian, 110 Ma) in the Chicalhão site, western Portugal (Mendes et al., 2014). Phylogenetic analysis suggested that *Kajanthus* was close to *Sinofranchetia*, but given that the fossil could be connected as well to any deeper branch of Lardizabalaceae without increasing the number of reconstructed character changes (Mendes et al., 2014), we used this fossil to constrain the stem group age of Lardizabalaceae (C₃). (4) *Leefructus mirus* described from the Early

Cretaceous Yixian Formation in China (122.6 Ma; Sun et al., 2011). Based on a phylogenetic analysis, *Leeфраctus* was placed as sister to the Ranunculaceae (Wang et al., 2016) and was used to constrain the stem group age of the family (C_4). Given phylogenetic uncertainty of fossil calibrations, we conservatively enforced all four fossil calibrations with a uniform prior distribution (Mao et al., 2012). To avoid overestimation of root age, we set a 131.7 Ma (95% highest posterior density (HPD): 129.7–133.4 Ma) maximum age for the root (C_5), which is the estimated crown group age of eudicots (Magallón et al., 2015), with a normal distribution (Fig. 2: analysis 1). For comparison, we also constrained the root age to be 153 Ma (138–172 Ma) (Fig. 2: analysis 2) based on Smith et al. (2010). This age is highly congruent with those estimated by Harris and Davies (2016) and Salomo et al. (2017) without the oldest tricolpate pollen calibration. To evaluate the impact of the Early Cretaceous *Kajanthus* on divergence times of Lardizabalaceae, dating analyses were also run with the exclusion of this calibration (Fig. 2: analyses 3–4). To minimize rate heterogeneity for the dating analysis, we removed two taxa with long branches (Fig. S1), *Circaeaster agrestis* and *Ranunculus macranthus*, and then conducted the same four dating analyses using the 40-taxon data matrix (Fig. 2: analyses 5–8). We also estimated divergence times by excluding the Early Cretaceous *Leeфраctus* (analyses 9–12).

All dating analyses were performed under an uncorrelated lognormal clock model, a birth-death prior process for incomplete sampling, and the GTR + I + Γ model for each region separately. Two distinct runs were conducted with 50 million generations and trees sampled every 5,000th generations. Convergence and ESS > 200

were assessed using Tracer v1.6 (Rambaut et al., 2014). The maximum clade credibility (MCC) tree, median ages and their 95% HPD were generated using TreeAnnotator v1.8.2 (part of the BEAST package). Among these twelve dating analyses, divergence times for Lardizabalaceae are highly congruent (Figs. 2 and S4; see below Results).

Biogeographic analyses

Lardizabalaceae once occurred in regions outside its present-day distribution range (Fig. 1; Table S3). For example, *Sargentodoxa* was found in North America and Europe (Tiffney, 1993; Wilde and Frankenhauser, 1998; Manchester, 1999; Mai, 2001) and *Decaisnea* was discovered in Europe (Mai, 1980). *Akebia parvisemina* was reported by Mai (2001) from the late Miocene of Germany, but it lacks anatomical details and diagnostic features for extant *Akebia* seeds, therefore its identity is uncertain (Manchester et al., 2009). Four geographical areas were coded to cover distributions of all extant and fossil taxa of Lardizabalaceae (Fig. 1): (A) East Asia, (B) South America, (C) North America, and (D) Europe. Due to the challenges in scoring other under-sampled families for geographic areas, we restricted biogeographic analysis to Lardizabalaceae and its sister Circaeasteraceae (this study; Wang et al., 2009; Sun et al., 2017).

To infer the location of the disjunction between eastern Asian and South American lineages, ancestral areas were reconstructed using the R package BioGeoBEARS v0.2.1 (Matzke, 2013). We tested the three models (DEC,

DIVALIKE, and BAYAREALIKE) including or not a “jump dispersal” parameter J that permits founder-event speciation in the species’s history. Founder-event speciation is the result of a LDD event of individual lineages founding a population outside the ancestral range (Matzke, 2014). The fit for the different models was evaluated using the AIC values. The seeds of Lardizabalaceae are embedded in a sweet, gelatinous, white pulp and can be dispersed by birds and other animals (Qin, 1997), so we did not constrain the availability of connections between areas.

Diversification rate analyses

We used two R packages with the BEAST MCC tree from which all outgroups were pruned to investigate the temporal dynamics of diversification rates of Lardizabalaceae. First, we employed Bayesian analysis of macro-evolutionary mixture (BAMM) implemented in BAMM v2.5 (Rabosky, 2014) to infer speciation and extinction rates, as well as the possible shifts of speciation across the phylogeny. Missing taxa were taken into account by using a fraction of total species in a clade (see details in Fig. S1). We performed two independent runs on the MCC tree with a reversible jump MCMC run of 10 million generations, sampling parameters every 1000th generations. Following the suggestion of Rabosky et al. (2014) for small trees (< 500 tips), we used a Poisson prior value of 1.0 for the ‘ExpectedNumberofShifts’ that implies a null hypothesis of zero rate shifts across the phylogeny. ESS values were calculated in the R package CODA (> 200). The BAMM output files were analyzed by discarding 15% burn-in samples using the R package BAMMtools v2.0.2

(Rabosky et al., 2014). The posterior distribution of the BAMM analysis was used to estimate the distinct rate shift configurations within the 95% credible set. We also used the `plotRateThroughTime` function in BAMMtools to plot speciation, extinction and net diversification rates.

Second, we used MEDUSA (Alfaro et al., 2009) implemented in Geiger v1.3-1 (Harmon et al., 2008) to infer rate shifts. As in the BAMM analysis, the missing taxa were assigned to monophyletic terminal clades (Fig. S1). Because the set of a cut-off value for a significant change in ΔAIC can influence the inferred number of shifts (Alfaro et al., 2009), we selected the model with the lowest AIC score.

Results

Phylogenetic analyses

Three phylogenetic analyses (ML, BI and MP) recovered nearly identical topologies with overall strong node support in particular at the backbone (Fig. S1). Circaeasteraceae containing *Circaeaster* and *Kingdonia* is strongly supported as sister to Lardizabalaceae (MP-BS = 96%, ML-BS = 99%, PP = 1.0). The monophyletic Lardizabalaceae is strongly supported (MP-BS = 100%, ML-BS = 100%, PP = 1.0). Within Lardizabalaceae, *Sargentodoxa* is sister to the remaining taxa of the family, followed by *Decaisnea*, and then *Sinofranchetia*. Each of Lardizabalaceae and Akebieae is supported as monophyletic. *Akebia* is monophyletic with strong support (MP-BS = 92%, ML-BS = 97%, PP = 1.0), whereas *Holboellia*, *Parvatia* and *Stauntonia* are not monophyletic.

Divergence time estimates and biogeographic analyses

Divergence time estimates based on the different ages of the root are highly consistent (analyses 1 vs. 2, 3 vs. 4, 5 vs. 6, and 7 vs. 8). The results with and without the Early Cretaceous *Kajanthus* as a calibration point are congruent (analyses 1 vs. 3, 2 vs. 4, 5 vs. 7, and 6 vs. 8). The inclusion of two long-branch taxa did not alter the results significantly (analyses 1 vs. 5, 2 vs. 6, 3 vs. 7, and 4 vs. 8). A comparison of the time estimates for selected nodes using the eight different analyses (1-8) is shown in Fig. 2. The exclusion of the Early Cretaceous *Leefructus* did not alter the results significantly (analyses 1 vs. 9, 2 vs. 10, 5 vs. 11, and 6 vs. 12). Detailed comparisons among divergence times estimated in analysis 2 and analyses 9–12 are shown in Table S4.

Results from the BEAST analysis 2 with the root set as 153 Ma, three calibrating fossils, and 42 taxa are here reported and were used for biogeographical and diversification rate analyses. The chronogram resulting from the analysis 2 is shown in Fig. 2. Ancestral area reconstructions using the DEC and DIVALIKE models including or not a parameter *J* generated congruent results, although DIVALIKE was identified as the best-fitting model for our data (Table S5). Results from the ancestral area reconstructions conducted for Lardizabalaceae under the DIVALIKE model are indicated in Fig. 3a. The crown age of Lardizabalaceae was estimated at 76.9 Ma (95% HPD: 45.98–109.3). The most recent common ancestor of the family was most likely distributed in East Asia. A dispersal event was inferred from East Asia to South

America (nodes 1/2). The age of node 1 was estimated at 40.61 Ma (95% HPD: 21.62–63.98). The age for the split of the South American Lardizabaleae containing *Boquila* and *Lardizabala* and its East Asian sister group (Akebieae) was estimated at 24.4 Ma (95% HPD: 12.23–41.31; node 2). The crown age of Akebieae was estimated at 7.49 Ma (95% HPD: 3.76–13.77; node 3).

Diversification rate analyses

The phylorate plot of the speciation rate obtained from BAMM analysis indicated diversification heterogeneity within Lardizabalaceae (Fig. 3b). One acceleration of diversification rates was detected at the crown of Akebieae (*c.* 7.5 Ma) by sampling the maximum *a posteriori* configuration with the highest frequency ($f = 0.97$; Fig. 3b), and its net diversification rate is 0.24 species per million years (myr), whereas the background net rate is 0.0446. The rate-through-time plots suggested that the global speciation and net diversification rates of Lardizabalaceae significantly accelerated at the same time (Fig. 4). Comparing pure-birth versus birth-death models, MEDUSA favored the pure-birth model (AICc = 78.66). Under this model, MEDUSA detected one shift in net diversification rates (Fig. 3a) relative to background level in Lardizabalaceae ($r = 0.0179$), i.e. a shift at the crown of Akebieae ($r = 0.3607$).

Discussion

Phylogeny and divergence times of Lardizabalaceae

Our analyses supported Circaeasteraceae as the sister group of Lardizabalaceae, in

agreement with previous phylogenetic studies (e.g., Wang et al., 2009; Sun et al., 2017). Our results suggested that *Sargentodoxa* was sister to all the other Lardizabalaceae, followed by *Decaisnea*, and the South American Lardizabaleae is sister to the East Asian Akebieae. These relationships are in agreement with previous results (Hoot et al., 1995; Wang et al., 2002; Wang et al., 2009), but are usually resolved here with greater support.

Our twelve dating analyses generated highly congruent time estimates for Lardizabalaceae (Figs. 2 and S4). Analysis 2 suggests a crown group age of 76.9 Ma (95% HPD: 45.98–109.3; node 1) for the family, which is older than the estimate of Wefferling and Hoot (2018; 44.88 Ma), but our credibility interval overlapped with the assumptions of Anderson et al. (2005), 66 Ma and 95 Ma inferred by penalized likelihood and nonparametric rate smoothing methods, respectively. Wefferling and Hoot (2018) estimated the split time between Lardizabaleae and Akebieae and the crown age of Akebieae at 18.63 Ma and 7.86 Ma, respectively, which are in agreement with our results: 24.4 Ma (95% HPD: 12.23–41.31; node 2) and 7.49 Ma (95% HPD: 3.76–13.77; node 3). There is no a temporal conflict between the South American lineage and the Tertiary wood record from Patagonia (Schönfeld, 1954), although the exact age of the fossil remains unclear.

A plausible explanation of the temperate South American-East Asian disjunction in Lardizabalaceae

Our study suggests a late Oligocene origin of the temperate South American-East

Asian disjunct distribution in Lardizabalaceae, and eastern Asia as the source for colonization of temperate South America. Several proposed hypotheses that might explain the cause of the extreme trans-pacific disjunction in the family are discussed below:

1. One possibility is that Lardizabalaceae was distributed across the supercontinent before the complete separation of Laurasia and Gondwanaland (Maekawa, 1965; Qin, 1997). Our estimated crown group age for the family (*c.* 76.9 Ma) is obviously younger than the timing of their separation (*c.* 150 Ma; Mao et al., 2012). We find no data in the published fossil records indicating that Lardizabalaceae ever occurred in Africa, as would be expected if its current distribution was to be attributed to vicariance event between Laurasia and Gondwanaland (Raven and Axelrod, 1974). Thus, the vicariance hypothesis between Laurasia and Gondwanaland could be rejected.

2. The present-day distribution of Lardizabalaceae can come into existence if the family originated in Gondwanaland and then spread to Laurasia through the Indian plate (Schuster, 1976). However, our biogeographical analyses show that Lardizabalaceae originated in eastern Asia (Fig. 3a), in agreement with most authors' views (e.g., Raven and Axelrod, 1974; Tiffney, 1993; Wu et al., 2003). Moreover, our phylogenetic analyses indicate that the South American Lardizabaleae is nested within the eastern Asian taxa (Fig. S1). Additionally, the estimated age of 24.4 Ma for the divergence of Lardizabaleae from the East Asian ancestor also makes it impossible to invoke the Indian plate as a vector for this group, which would have required an

estimated date of divergence at least as early as 65 Ma (McLoughlin, 2001).

3. Another potential explanation for the present-day distribution of Lardizabalaceae has to do with two likely migration routes. One is the migration through the boreotropical route to North America and then southward to South America (Raven and Axelrod, 1974; Schuster, 1976; Tiffney, 1993). Our dating analysis indicates that the split between the South American lineage and its eastern Asian group occurred at 24.4 Ma, which is younger than the timing of disruption of the boreotropical flora, *c.* 34 Ma (Collinson, 1992). Moreover, no fossils or other evidence for occurrences of Lardizabalaceae were found in Central America and northern South America. Thus, this northern migration route seems just unlikely.

An alternative migration route was through the East Indies, Australia, and Antarctica to South America. The split time between the South American and East Asian groups was estimated at 24.4 Ma, which is slightly younger than the opening of the Drake Passage between South America and Antarctica (*ca.* 30–35 Ma; McLoughlin, 2001). Since the late Eocene, global climate was cooling (Zachos et al., 2001), thermophilic taxa could therefore not migrate through Antarctica (Lian et al., 2019). Furthermore, no fossil representative of this family was found in the geographically intermediate areas. This route also appears unlikely.

4. The direct trans-pacific LDD from East Asia to temperate South America (Fig. 3a) seems to be more parsimonious and plausible than the other competing hypotheses. Fruits of Lardizabalaceae are berries or fleshy follicles filled with whitish and sweet gelatinous pulp (Fig. 1), which are highly favored by birds and other animals (Qin,

1997). The edible fruits of *Stauntonia*, *Akebia* and *Lardizabala* are consumed by humans and even sold on local markets (Christenhusz, 2012). In the family, seeds with hard seed coat are usually small (< 1 cm; Qin, 1997), and can therefore be carried for long distances by animals. Recently, a mid-Miocene LDD event from East Asia to temperate South America was found in *Gaultheria* (13.57–15.11 Ma; Ericaceae; Lu et al., 2019), whose fruits are also favored by birds. By using miniature archival geolocating tags, Shaffer et al. (2006) found that a small seabird, the sooty shearwater (*Ardenna grisea*, Procellariidae), can fly across the entire Pacific Ocean. Bird-mediated LDD has been shown to be the best explanation for the establishment of the bipolar disjunction, another huge geographical discontinuity (e.g., Popp et al., 2011; Lewis et al., 2014).

Diversification of East Asian subtropical EBLFs in the late Miocene

Our analyses show that extant Lardizabalaceae date to the Late Cretaceous of East Asia (Fig. 2), but a significant increase of net diversification rates of the family occurred relatively recently, beginning in the late Miocene of East Asia, *c.* 7.5 Ma (95% HPD: 3.76–13.77; Fig. 3). The timing of the rapid diversification within Lardizabalaceae is similar to those of other representative plant groups in East Asian subtropical EBLFs, such as woody bamboos (*c.* 7.2 Ma; Arundinarieae, Poaceae; Zhang et al., 2016) and *Quercus* sect. *Cyclobalanopsis* (*c.* 8.3 Ma; Fagaceae; Deng et al., 2017). Additionally, several elements of East Asian subtropical EBLFs, such as *Camellia* (*c.* 6.8 Ma; Theaceae; Yu et al., 2017); *Coptis* (*c.* 6.5 Ma; Ranunculaceae;

Xiang et al., 2018), and *Sabia* (c. 7.9 Ma; Sabiaceae; Yang et al., 2018), also appear to have diverged during the same period. We therefore suggest that the late Miocene was a relevant period for the assembly and evolution of modern East Asian subtropical EBLFs.

Several potential factors might have been responsible for the diversification burst of East Asian subtropical EBLFs during the late Miocene (Fig. 4). The rise of East Asian subtropical EBLFs has been attributed to intensified EAM (Yu et al., 2017), which was in turn related to the uplift of the Tibetan Plateau (Liu et al., 2015). The accelerated uplift of the Plateau, especially its northeastward expansion could have enhanced the EAM, as well as the aridification of inland Asia (An et al., 2006). Thermochronometry and magnetostratigraphy studies revealed that the northeastern Tibetan Plateau experienced apparent tectonic activities in 8–7 Ma (Shen et al., 2017; Fang et al., 2019), in agreement with the intensification of the EAM inferred from dust accumulation rate in northern China (Guo et al., 2002), that can bring high precipitation in summer. The χ_{fd} /HIRM (frequency-dependent magnetic susceptibility/hard isothermal remanent magnetization) record also suggests intensified East Asian summer monsoon precipitation during c. 8.5 to 7 Ma (Nie et al., 2017). The relationship between rainfall and diversification rates of plant groups in East Asian subtropical EBLFs remains unknown, but based on the distribution maps of 11,405 woody species in China, Wang et al. (2011) indicated that annual rainfall is the best single water-related predictor for the species richness of woody plants in China.

The major limiting factor for the distribution of East Asian subtropical EBLFs is low temperature in the winter (Song and Da, 2016). Although cooling has been a general trend from the mid-Miocene Climatic Optimum onwards (Zachos et al., 2001), palynological data in Southwest China indicate that the temperature, especially the mean temperature of the coldest month, has gradually increased since the Middle Miocene (Zhang et al., 2012). Similar to the temperature, atmospheric CO₂ levels have an effect on biodiversity in humid, warm forests (Royer and Chernoff, 2013; Lian et al., 2019). High atmospheric CO₂ levels occurred in the late Miocene (Tripathi et al., 2009), which might also have driven the rapid rise of East Asian subtropical EBLFs during that period.

Conclusions

Molecular dating, together with phylogenetic and biogeographical analyses, indicates a late Oligocene origin of the temperate South American-East Asian disjunct distribution in Lardizabalaceae. Our findings suggest that a bird-mediated LDD event across the Pacific is the most plausible explanation for the present-day disjunct distribution of Lardizabalaceae between temperate South America and East Asia, and possibly also in other plant groups exhibiting a temperate South American-East Asian disjunction. In addition, our data suggest that a burst of diversification of East Asian subtropical EBLFs occurred in the late Miocene, promoted by the intensified EAM and other environmental factors. This study may provide new insights into the historical assembly of East Asian subtropical EBLFs.

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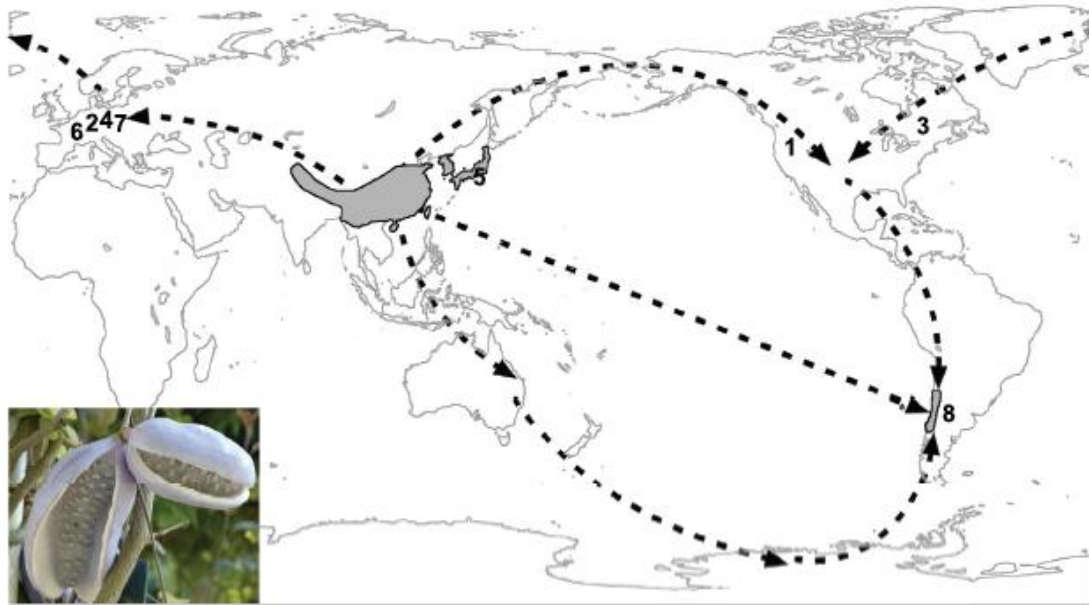


Fig. 1. Modern (grey areas) and fossil (numbers) distribution of Lardizabalaceae. 1-6 represent fossils of *Sargentodoxa*: 1-2, middle Eocene; 3, early Miocene; 4, middle Miocene; 5, early late Miocene; 6, late Miocene–Pliocene. 7 represents fossil of *Decaisnea*, late Oligocene. 8 represents fossil of *Lardizabaloxylon lardizabaloides*, Tertiary. The numbers correspond to those in Table S3. The dashed lines indicate the main hypothesized colonization routes towards South America (see details in Text). Image of berries full of whitish and sweet gelatinous pulp of *Akebia quinata* is presented in the low left, photograph by W Wang.

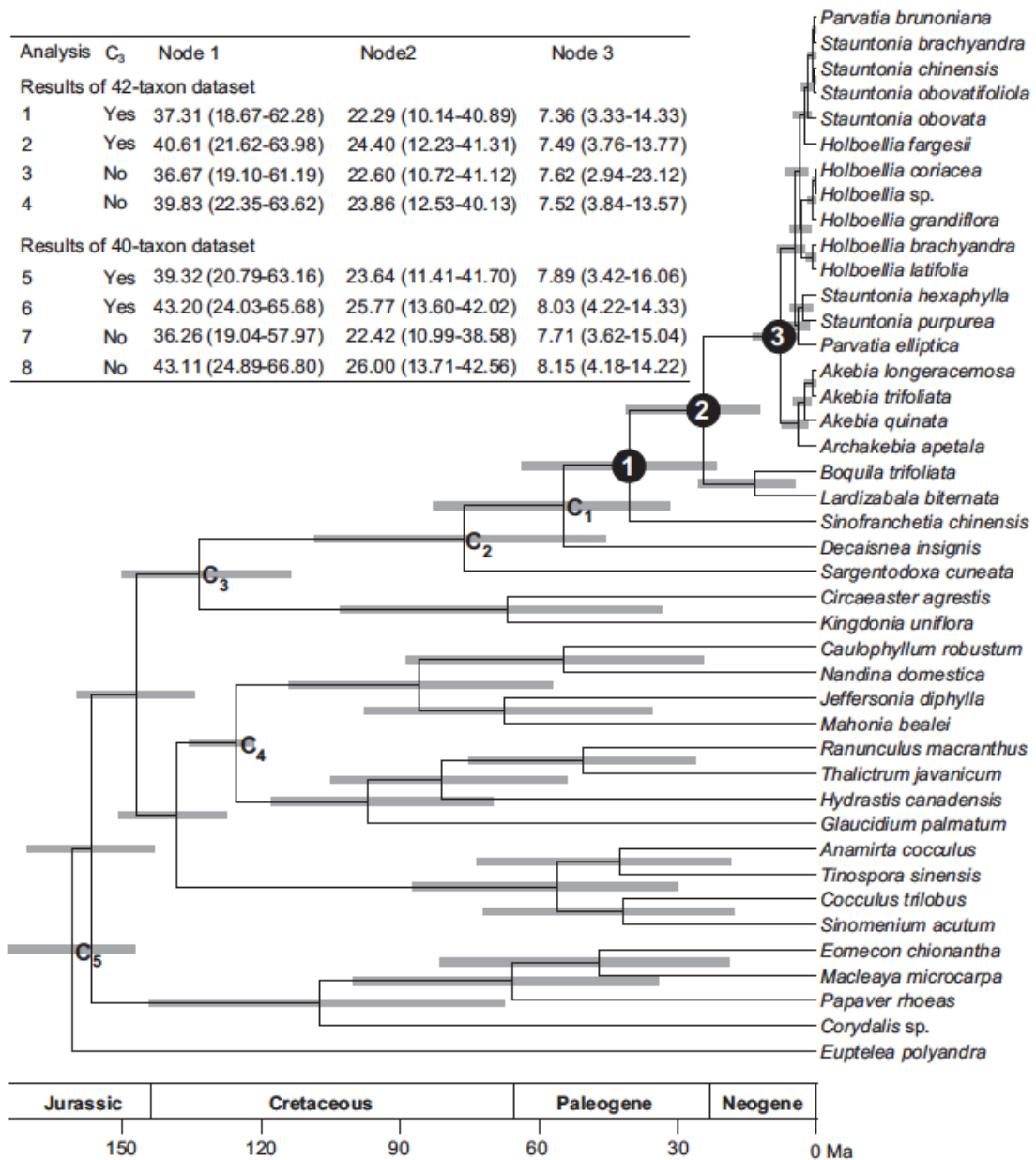


Fig. 2. Time-calibrated phylogeny of Lardizabalaceae using all four fossil calibration points, 42 taxa, and root constrained to 153 Ma (analysis 2). Gray bars indicate 95% highest posterior density intervals. The C₁-C₅ indicate the locations of calibration points. Nodes of interests were marked as 1–3 in circles. A comparison of the time estimates for selected nodes using the eight different analyses (1-8) is presented in the upper left. Yes, calibration included; No, calibration not included.

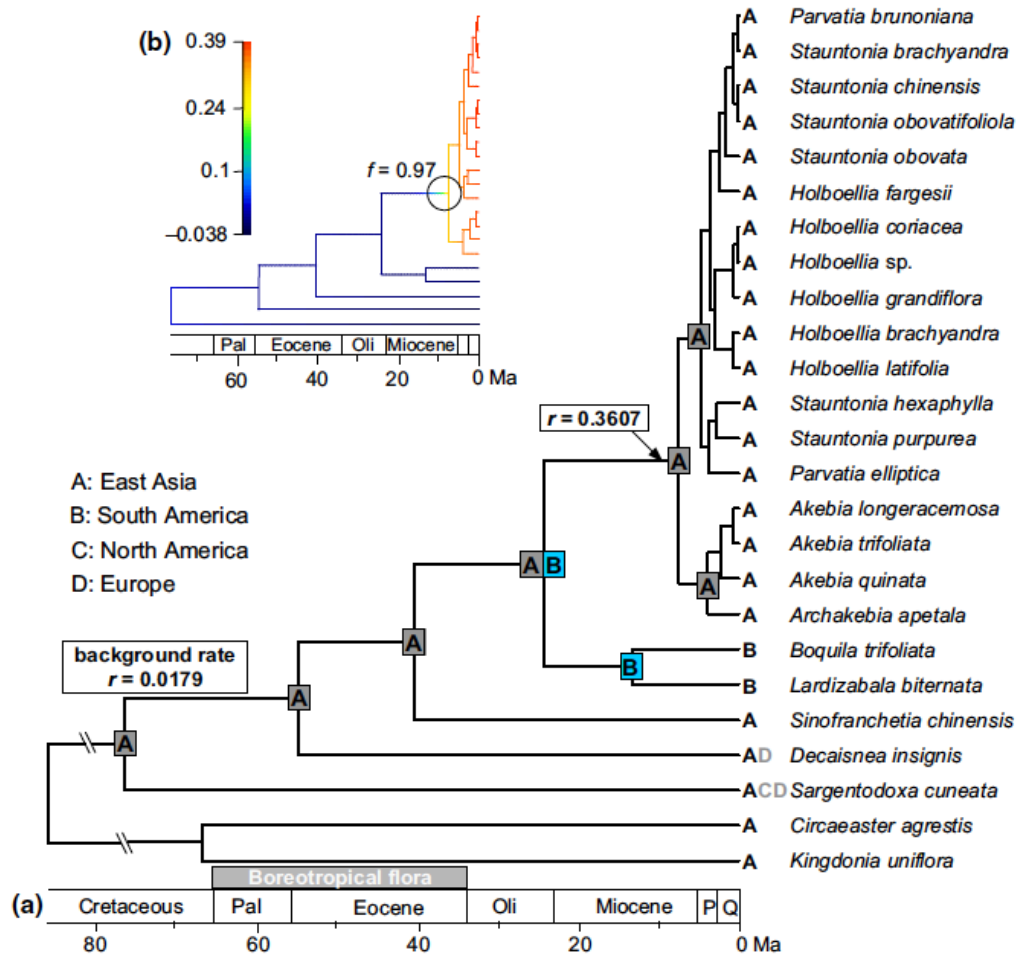


Fig. 3. Combined biogeographic and diversification rate analyses of Lardizabalaceae.

(a) Ancestral area reconstructions for Lardizabalaceae with BioGeoBEARS under the DIVA-like model. Areas of tip species are shown left of taxa names, where fossil areas are in grey. (b) Phylorate inferred from BAMM analysis under the prior of ‘ExpectedNumberofShifts’ as 1. Branches are color-coded according to speciation rates (lineages/myr). The black circle indicates the position of the unique shift in the maximum sampled *posterior* configuration. Changes in diversification rates (r) estimated under the MEDUSA birth–death likelihood model with relative extinction set to zero are indicated at the appropriate node. Dated phylogeny of Lardizabalaceae is derived from the BEAST analysis 2.

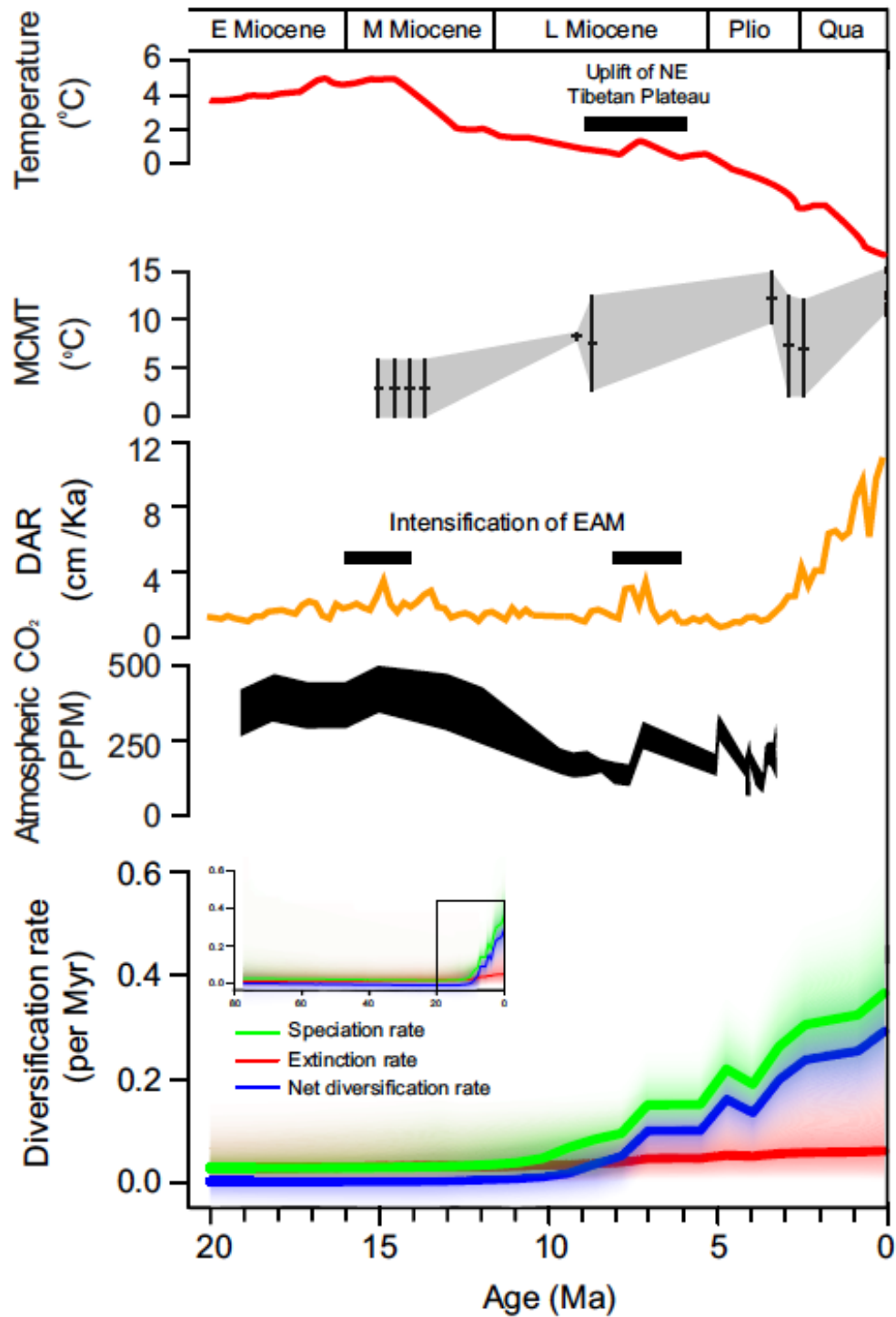


Fig. 4. Potential factors responsible for the diversification burst of East Asian subtropical evergreen broadleaved forests during the late Miocene. Uplift of northeastern Tibetan Plateau demonstrated by thermochronometry studies (reviewed by Shen et al., 2017), global temperature inferred from $\delta^{18}\text{O}$ levels in benthic

foraminifera (Zachos et al., 2001), the mean temperature of the coldest month (MCMT) reconstructed from palynological data in Yunnan, southwest China (Zhang et al., 2012), intensification of the East Asian monsoon (EAM) inferred from dust accumulation rate (DAR) in northern China (Guo et al., 2002), and atmospheric CO₂ measured from boron/calcium ratios of surface-dwelling foraminifera compared with climate records (Tripathi et al., 2009). For Lardizabalaceae, speciation, extinction and net diversification rates through time were obtained from the BAMM analysis carried out under the 'ExpectedNumberofShifts' as 1 based on the maximum clade credibility (MCC) tree of scenario 2 of BEAST analyses. Color density shading denotes confidence in rate reconstructions.