Origin and evolution of the genus *Piper* in Peninsular India

Sandeep Sen[a,b,c,d,*], Selvadurai Dayanandan[c,d], Thomson Davis[e], Rengaian Ganesan[a], M.R. Jagadish[a], P.J. Mathew[e], Gudasalamani Ravikanth[a]

[a] Suri Sehgal Center for Biodiversity and Conservation, Ashoka Trust for Research in Ecology and the Environment (ATREE), Bangalore, India
[b] Manipal Academy of Higher Education, Manipal, Karnataka, India
[c] Department of Biology and Center for Structural and Functional Genomics, Concordia University, Sherbrooke Street West, Montreal, Quebec H4B1R6, Canada
[d] Quebec Center for Biodiversity Science, 1205 Dr. Penfield Avenue, Montreal, Quebec H3A1B1, Canada
[e] Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Trivandrum, India

ARTICLE INFO

ABSTRACT

The evolution of Peninsular Indian biodiversity has been a fascinating topic of research due to historical connections of this region to the ancient Gondwanaland. We investigated the phylogeny and historical biogeography of nearly all extant species of the genus *Piper* reported from the region to assess the biogeographical origins and test mechanisms of lineage diversification (dispersal, vicariance and *in situ* radiation) of this highly diverse genus of angiosperms commonly found in the understory of evergreen forests. The phylogeny of 21 species of *Piper* reported from Peninsular India was reconstructed for the first time, which included three new putative species from the Western Ghats. We used BEAST for the divergence time estimations (using three constraints), and ancestral range estimations were performed with the dated phylogenetic tree using BIOGEOBEARS. Divergence dating analysis revealed that the genus *Piper* originated during lower Cretaceous around 110 Ma [95% highest posterior density (HPD): 116–105 Ma] and colonized Peninsular India five times independently, from Southeast Asia starting from the Oligocene. The two major dispersals into India occurred during the periods of 27.3 Ma (95% HPD: 35.8–19.9.) and 15.5 Ma (95% HPD: 24.9–7.11). This was followed by rapid radiations in some lineages with subsequent back dispersals to Southeast Asia. Our study indicates that dispersals from Southeast Asia led to the arrival of *Piper* to Indian subcontinent following the Indo-Eurasian collision. Members of *Piper* have colonized and diversified within the climatically stable habitats of Peninsular India. Furthermore, the present study provides evidence for the Miocene overland dispersal of *Piper* species to Africa from South Asia.

1. Introduction

Home to four biodiversity hotspots; India is one of the most biologically diverse countries in the world. This rich biodiversity is a result of long-term evolution under a complex geological history and a tropical climate. The Indian subcontinent has experienced a variety of climatic conditions during its geological history as compared to any other geographic region in the world (Mani MS 1974, Samanth and Mohabey 2009, Prasad et al., 2009). Once a part of the supercontinent Gondwana, Peninsular India separated from Africa and started drifting away from Madagascar by late Cretaceous (99-66 Ma) and collided with Eurasia during 55–42 Ma (Briggs, 2003) followed by final suturing between 45 and 35 Ma (Ali and Aitchison, 2008; Metcalfe, 2013). During the drifting, its vegetation underwent colossal changes (Morley, 2000). The Indo-Eurasian collision had a profound impact on biotic evolution through dispersal of biota into and out of India (Mani, 1974; Koehler and Glaubrecht, 2007). Such dispersals also enabled several Gondwanan African elements to reach Eurasia through the Indian plate (known as the 'biotic ferry model') (Dayanandan et al., 1999; Rutschmann et al., 2004; Datta-Roy and Karanth, 2009). Thus, studies of Peninsular Indian biota play a vital role in understanding the biogeographical origins of several palaeotropical floras and in revisiting hypotheses on the origin of high species diversity in the region.

In Peninsular India, biodiversity is concentrated in two main centers, the Western Ghats –Sri Lanka biodiversity hotspot and the Eastern Ghats which support strikingly higher levels of floral and faunal endemism (Mani, 1995). The origin of high endemism and unique distribution patterns intrigued many evolutionary biologists to formulate and test several biogeographical hypotheses (Gower et al., 2016; Agarwal and Karanth, 2015; Agarwal et al., 2014; Datta-Roy et al.,

2012). These studies provide insights into the biogeographical forces, dispersal pathways, and the rate of biotic exchanges that influenced the evolution of extant biodiversity in Peninsular India. For example, a biogeographic analysis of Asian *Eutropics* (Squamata: *Scincidae*) provided support to the "into India" and "out of India" dispersal events after the collision of the Indo-Eurasian plate (Datta-Roy et al., 2012). Gower et al. (2016), suggested that the wet zone contraction within India during Miocene and Pleistocene climatic fluctuations drove the ancient divergence of Caecilian genus *Gegeneophis* in the Western and Eastern Ghats. Another study by Biju and Bossuyt (2003) revealed that the frog genus *Nasikabatrachus* (Sooglossidae), endemic to the Western Ghats, is a Gondwanan relict, thus establishing an older biotic link between the Indian subcontinent with Seychelles to the early Cretaceous. Similarly, a recent study by Joshi and Edgecombe (2019) suggest that the evolutionary history of Peninsular Indian centipede *Ethmostigmus* (Scolopendridae) was shaped by the Gondwanan breakup. Most of the biogeographic studies in the last decade within Peninsular India have focused mainly on faunal components (e.g., Agarwal and Karanth, 2015; Bossyut and Milinkovitch, 2001; Datta-Roy et al., 2012; Bansal and Karanth, 2010) whereas the biogeography of floral elements remains relatively under explored. However, some biogeographical patterns have emerged from few key studies on plant lineages. Studies on Asian Dipterocarps (Dayanandan et al., 1999) and Crypteroniaceae (Conti et al., 2002; Rutschmann et al., 2004) imply that these groups reached the Asian mainland via the rafting Peninsular India from Gondwana, followed by 'out of India' dispersals. A recent biogeographic analysis of *Impatiens* (Surveswaran et al., 2009) further confirms India-Africa connections. In addition, the isolation of the Indian plate after it split from Madagascar and its current isolation from the rest of Eurasia by the Himalayas, led most of the dispersed elements to undergo *in-situ* diversification. This was in response to the availability of diverse niches resulting from events such as volcanic activity and the Himalayan uplift (Karanth, 2015; Agarwal and Karanth, 2015; Joshi and Karanth 2013, Surveswaran et al., 2009; Van Bocxlaer et al., 2009; Koehler and Glaubrecht, 2007).

Although several biogeographical processes such as into and out of India, biotic ferry model, and island model have been widely used to explain the assembly of endemic species within the Indian sub-continent; the examples based on plant lineage analyses represent a mere 6 out of 21 published datasets. Thus, studies encompassing several floral elements are required for a comprehensive understanding of the historical dispersal pathways and rate of exchange of floristic diversity within and across Peninsular India. Here, we chose *Piper* L., a strikingly diverse pan-tropical genus, consisting of shrubs, treelets, climbers and hemi-epiphytes (restricted to the neotropics) (Dyer and Palmer, 2004; Jaramillo et al., 2008; Wanke et al., 2007) to test biogeographical hypotheses pertaining to the evolution of endemic biodiversity within the Peninsular India.

Globally, *Piper* (Piperaceae) contains ca. > 1500 species, with most of its diversity concentrated in the Neotropics (Jaramillo et al., 2008). A recent study by Ulloa et al. (2017), estimated that the New World alone has 1804 species of *Piper,* and the *Piper* in Southeast Asia consists of ~ 600 species (Martínez et al., 2015). The species in this clade include herbs, shrubs and climbers with several economically important species such as *P. nigrum* L. (black pepper), *P. betel* L. and *P. longum* L. Recently, Asmarayani (2018) proposed new infrageneric groupings in *Piper* with a comprehensive sampling from Southeast Asia, thus advancing the taxonomic understanding from this region. Still, little is known about the phylogenetic relationships among the Peninsular Indian *Piper* species. In contrary, the African continent harbors fewer species of *Piper*. *Piper capense* L.f., a member of the South Pacific clade (Jaramillo et al., 2008) is a shrub with bisexual flowers that show floral morphological similarity to Neotropical *Piper*, whereas *P. borbonense* (Miq.) C.DC. and *P. guinense* Shumach. & Thonn. are dioecious vines that resemble members of Southeast Asian clade (Smith et al., 2008). The low number of species in this region is quite unusual as Africa was once dominated by tropical rainforests and higher plant diversity is expected. The aridification during the Neogene may have resulted in extinction of several species (Whitmore, 1998).

The Peninsular Indian component of *Piper* consists of ~ 21 species that are predominantly distributed in the understorey of tropical evergreen forests with disjunct distribution (Sen et al., 2016) in the Northeastern Himalayan region, the Western Ghats and the Eastern Ghats. A total of eighty four species were reported from the entire Indian region till date (Mukherjee, 2018). Earlier phytogeographic studies hypothesized that Peninsular Indian *Piper* originated from Gondwanan ancestral forms that reached the Indian subcontinent during mid-Cretaceous (Ravindran, 2003). For example, the presence of *P. barberi* *Gamble*, an endemic species in the southern Western Ghats have characters unusual to Paleotropical *Piper*. *Piper barberi* is shown to have reticulately veined leaves and long dangling peduncles similar to Neotropical *Piper,* especially the pinnately nerved leaves at the entire length of its blade, is one typical character common in South American *Piper*. Considering these morphological affinities to Neotropical species, *P. barberi* is speculated to have reached India before splitting of the Deccan plate from the Gondwana (Babu et al., 1992; Anand and Rao, 2000). Furthermore, the *Piper* species in Peninsular India are polyploids and diploid forms are reported from South America (Ravindran, 2003). Thus, presence of species with morphological affinities to the taxa in Neotropics, and their disjunct distribution within the Indian plate, make *Piper* an ideal candidate to test the existing hypotheses such as "into" and "out of India" pertaining to the evolution of biodiversity in Peninsular India.

The two alternative hypotheses, whether the Peninsular Indian *Piper* is of Gondwanan or Malayan origin, have not been tested within a dated phylogenetic framework. Thus, we reconstructed the phylogeny of *Piper* species in India and estimated the divergence times and ancestral ranges to (1) infer the biogeographic origins of endemic biodiversity within Peninsular India and (2) test the hypothesis if the extant species of *Piper* in Peninsular India derived from Gondwanan elements.

2. Materials and methods

2.1. Taxon sampling and DNA sequencing

Leaf samples of *Piper* species were collected from the Western Ghats, Eastern Ghats, Northeast India and the Andaman and Nicobar Islands (Fig. 1; Table1). Our collection included 17 out of 18 described species and 3 putative new species from the Western Ghats, 3 species from the Andaman and Nicobar islands, 1 from the Eastern Ghats and 8 from the Himalayas. A total of 49 accessions representing 32 species were used in the present study. Multiple accessions were sampled for the widely distributed species. Collected leaf samples were stored at −20 °C. The total genomic DNA was extracted following a modified CTAB method (Sambrook & Russel, 2001). The nuclear ITS, internal transcribed spacer of the rDNA (ITSA 5′ GGAAGGAGAAGTCGTAACAAGG 3′ ITSB 5′ CTTTTCCTCCGCTTATTGATATG 3′) and the chloroplast intron *psbJ-petA* (*psbJ*-IGSF 5′ATTCCGCATTGGGCTCATC3′ *psbJ*-IGSF 5′GAAACAG TTTGAGAAGGTTCA3′) were PCR amplified and sequenced for the present study. We chose these genomic regions based on earlier studies, which showed suitability of these regions for resolving species level phylogeny of *Piper* (Jaramillo et al., 2008; Martínez et al., 2015). The PCR conditions for amplifying ITS region consisted of initial denaturation at 94 °C for 4:00 min followed by 35 cycles of 94 °C for 1:00 min, 59 °C for 1:00 min, 72 °C for 1:00 min and final extension of 72 °C for 07:00 min. The *psbJ-petA* intron was amplified through initial denaturation at 94 °C for 3:00 min followed by 35 cycles of 95 °C for 1:00 min, 55 °C for 1:00 min, 72 °C for 2:00 min and the final extension of 72 °C for 10:00 min. The amplified PCR products were sequenced at Scigenome, Cochin, India. The sequences were deposited in GenBank (Table 1).
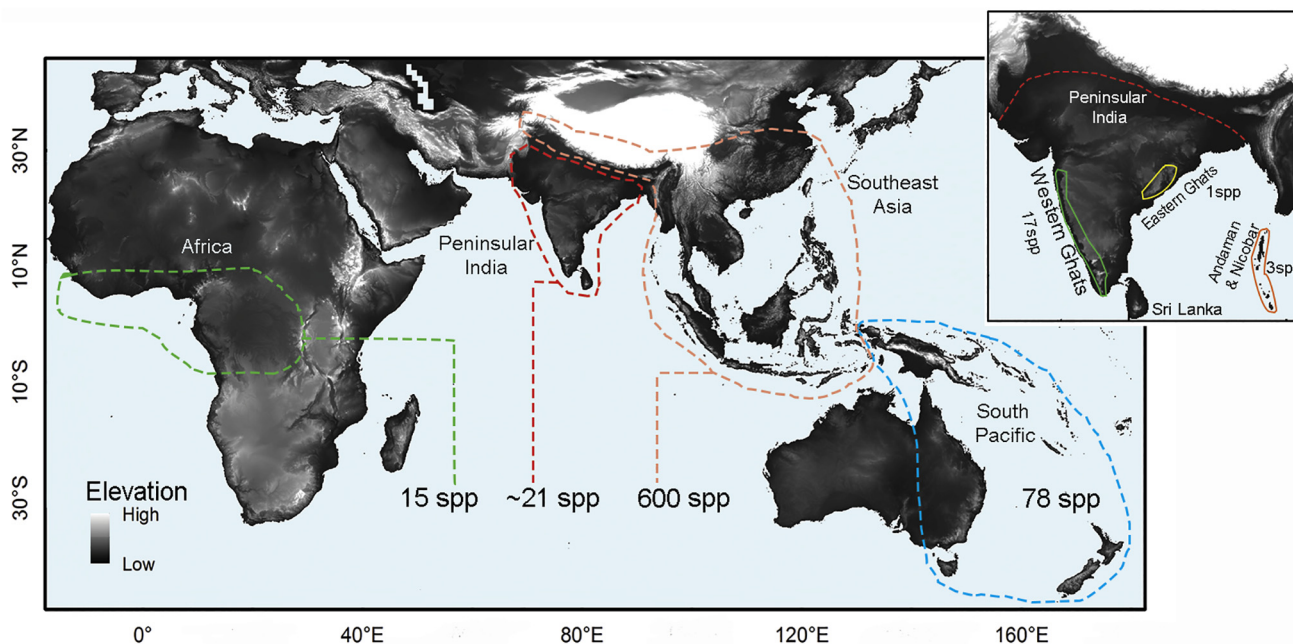
Fig. 1. Map showing geographic locations of biogeographic zones featured in this study. Inset: Peninsular India including Andaman Island and the number of taxa sampled from each region. The Western Ghats, Eastern Ghats and Sri Lanka are part of the Peninsular India.

2.2. Phylogenetic analysis

Both forward and reverse sequences were obtained for multiple accessions per species where possible, while for the species from Himalayas, Andaman Islands and six species from Peninsular India only one accession were sequenced. We retrieved sequences reported by Jaramillo et al. (2008) and ITS sequences from Asmarayani (2008) from the GenBank to increase the taxonomic and geographic coverage for phylogenetic reconstruction, which included representative taxa from Africa (2 species), Neotropics (142 species), Southeast Asia (146 species) and South Pacific (8 species) (Table S1.1). The sequence quality was checked using the program Geneious v 9.1.6 and then aligned using MAFFT v7.213 (Katoh and Standley, 2013) following L-INS-I (accurate) alignment option. The gaps were treated as missing data. From the *psbJ* - *petA* alignment two randomly inverted stretches with identical sequences from positions 453–467 and 853–875 were removed from further analysis following Jaramillo et al., 2008. These regions are reported to have identical sequences between distantly related taxa which can result in spurious tree topologies. The JMODELTEST v2.1.7 software program (Darriba et al., 2012) was used to identify the best fitting nucleotide substitution model for two genes based on the Akaike information criterion (AIC) scores. Maximum likelihood (ML) analysis was performed using the program RAxML (Stamatakis, 2006; Stamatakis et al., 2008) independently on the chloroplast, nuclear and the combined DNA sequence datasets. A rapid bootstrap analysis under ML was carried out using 1000 bootstrap replicates to estimate the node support using RAXML-HPC v.8 implemented on the CIPRES science gateway {http://www.phylo.org}. Analysis based on *psbJ-petA* alone did not provide enough resolution compared to the nuclear phylogeny. This was mainly due to the lack of *psbJ-petA* sequences for the newly sequenced species from Asmarayani (2018) and we treated this as missing data in further analysis. However, *psbJ-petA* region alone resolved the three major clades in *Piper*. Hence, all further phylogenetic analysis was performed on the combined dataset. The software program Mr. Bayes v3.2.6 (Ronquist et al., 2012) on CIPRES (Miller et al., 2010) was used to perform the Bayesian phylogenetic analysis. Two independent runs of Markov Chain Monte Carlo (MCMC) for ten million generations with sampling every 1000 generations were performed on the concatenated data matrix. The chain convergence was assessed based on the average standard deviation of split frequencies and estimated sample size (ESS) values. A majority rule consensus tree was then calculated after discarding the first 20% trees as burn-in. Additionally, a neighbour-net analysis was performed on the plastid data alone after removing the missing data using the program SplitsTree (Huson and Bryant, 2006)

2.3. Divergence time estimation

The divergence time was estimated in BEAST v 1.8.3 (Drummond et al., 2012) implemented in CIPRES Science gateway V.3.3 (Miller et al., 2010) using the combined dataset. We partitioned the datasets by gene and an un-correlated lognormal relaxed clock model was employed after unlinking the substitution models and clock models, and the Yule model was selected as the speciation prior. We used fossil data to calibrate the phylogenetic trees and estimate the divergence times. A maximum diversification age for *Piper* was constrained using a uniform distribution prior between 105 Ma and 117 Ma following the age estimated by Naumann et al. (2013) and recommended by Martínez et al. (2015). We used two fossil calibrations. The first one from the Neogene sediments of fossil seeds from the Neotropics with a minimum age of 6.5 Ma with high similarity to *P. alatabaccum* Trel. & Yunck., which is found in the Amazon and belongs to the Neotropical clade *Ottonia* (Callejas 2002). The calibration point was set within the *Piper* section, *Ottonia* at MRCA of *P. alatabaccum* and *P. bartilingianum* (Miq.) C.DC. by setting a log-normal prior offset of 6.5 Myr and standard deviation equal to 1 (see, Molina-Henao et al., 2016). A second calibration point based on the late Cretaceous fossil records of *P. margaritae* Martínez-A., was set to the stem node of the *Piper* section *Schilleria* clade. A log-normal prior offset of 67 Myr and a standard deviation equal to 1 was set based on Martínez et al., 2015; (see Fig. S2.1). A starting tree with branch lengths satisfying the age priors was developed using penalized likelihood approach Sanderson (2002) implemented in the R package APE' 3.1.4 (Paradis et al., 2004). Analyses were performed twice for 100 million generations in BEAST, sampling every 10, 000 generations to ensure proper mixing of MCMC chains. Independent runs were combined using LOGCOMBINER 1.8.2 (Rambaut and Drummond,

Table 1

The geographic location of *Piper* samples used in the study along with corresponding herbarium and GenBank accession numbers.

Species	Herbarium No	GenBank Accession Numbers		Locality
		ITS	*psbJ-petA*	
P. argyrophyllum Miq.1	TBGT70364	MH128799	MH151875	Periyar, Western Ghats
P. attenuatum Buch.-Ham.ex Miq.1	TBGT71726	MH128789	–	Agasthyamalai, Western Ghats
P. attenuatum Buch.-Ham.ex Miq.2	ATREE5902	MH128790	–	Gundia, Western Ghats
P. attenuatum Buch.-Ham.ex Miq.3	ATREE5903	MH128791	MH151873	BR Hills, Western Ghats
P. attenuatum Buch.-Ham.ex Miq.4	ATREE5904	MH128792	MH151874	Agumbe, Western Ghats
P. barberi Gamble	TBGT70305	MH128798	MH151872	Schenduruni, Western Ghats
P. galeatum (Miq.) C. DC. 1	TBGT71703	MH128774	–	Periyar, Western Ghats
P. galeatum (Miq.) C. DC. 2	TBGT71767	MH128775	MH151876	Agasthyamalai, Western Ghats
P. galeatum (Miq.) C. DC. 3	TBGT71777	MH128776	MH151877	Sholayar, Western Ghats
P. galeatum (Miq.) C. DC. 4	ATREE5089	MH128777	MH151878	Periyar, Western Ghats
P. hookeri Miq.	ATREE5905	MH128793	MH151881	Sirsi, Western Ghats
P. hymenophyllum Miq.1	TBGT71704	MH128784	MH151883	Periyar, Western Ghats
P. hymenophyllum Miq.2	TBGT70335	MH128785	MH151882	Agasthyamalai, Western Ghats
P. hymenophyllum Miq.3	ATREE5098	MH128786	MH151870	BR Hills, Western Ghats
P. hymenophyllum Miq.4	ATREE5099	MH128787	–	Coorg, Western Ghats
P. hymenophyllum Miq.5	ATREE5100	MH128788	–	KMTR, Western Ghats
P. mullesua Buch.-Ham. ex.D. Don 1	ATREE5080	MH109308	MH151885	Nilgiri
P. mullesua Buch.-Ham. ex.D. Don 2	TBGT71722	MH109309	MH151886	Wythiri, Western Ghats
P. mullesua Buch.-Ham. ex.D. Don 3	TBGT71737	MH109310	MH151887	Marayoor, Western Ghats
P. mullesua Buch.-Ham. ex.D. Don 4	ATREE5083	MH109311	MH151884	Brahmagiri, Western Ghats
P. nigrum L. 2	TBGT71704	MH128802	–	Ranni, Western Ghats
P. nigrum L.1	TBGT71747	MH128801	MH151893	Moozihyar, Western Ghats
P. pedicellatum C. DC.	TBGT71798	MH128805	–	Andaman and Nicobar (TBGRI)
P. pseudonigrum Velayudhan & Amalraj	TBGT71742	MH128782	MH151865	Periyar, Western Ghats
P. relictum Lekhak, S. S. Kambale & S. R. Yadav.	ATREE5095	MH128783	MH151859	Anshi, Western Ghats
P. rhytidocarpum Hook.f.	ATREE5915	MH128806	MH151866	Sikkim, Eastern Himalayas
P. ribesioides Wall.	TBGT71796	MH128803	MH151858	Andaman and Nicobar (TBGRI)
P. sarmentosum Roxb.	TBGT71797	MH128804	MH151864	Andaman and Nicobar (TBGRI)
P. schmidtii Hook. f.1	ATREE5062	MH151919	MH151892	Korkunda, Western Ghats
P. schmidtii Hook.f.2	TBGT71759	MH151920	–	Marayoor, Western Ghats
P. spp EG	ATREE5913	MH128794	MH151867	Eastern Ghats
P. spp1 WG	ATREE5054	MH107116	MH151868	Nilgiris, Western Ghats
P. spp2 WG	ATREE5053	MH128795	MH151871	Nilgiris, Western Ghats
P. spp3 WG	ATREE5093	MH128781	MH151869	BR Hills
P. spp4 WG	TBGT71783	MH128800	–	Idukky, Western Ghats
P. spp NE1	ATREE5919	–	MH151861	Arunachal, Eastern Himalayas
P. spp NE11	ATREE5918	MH137039	MH151863	Mizoram, Eastern Himalayas
P. spp NE2	ATREE5917	MH137038	MH151857	Meghalaya, Eastern Himalayas
P. spp NE5	ATREE5920	–	MH151856	Meghalaya, Eastern Himalayas
P. spp NE6	ATREE5921	–	MH151855	Eastern Himalayas
P. spp NE8	ATREE5916	MH137037	MH151862	Sikkim, Eastern Himalayas
P. trichostachyon (Miq.) C.DC. 1	TBGT71780	MH128778	MH151888	Coorg, Western Ghats
P. trichostachyon (Miq.) C.DC. 2	TBGT70342	MH128779	MH151889	Periyar, Western Ghats
P. trichostachyon (Miq.) C.DC. 3	ATREE5092	MH128780	–	Agasthyamalai
P. wightii Miq.1	ATREE5051	MH128796	MH151891	Nilgiri, Western Ghats
P. wightii Miq.2	ATREE5057	MH128797	MH151890	Nilgiri, Western Ghats
P. colubrinum (Miq.) C. DC.	TBGT71799	MH128772	MH151860	TBGRI garden
P. hapnium Buch.-Ham.	ATREE5085	MH128773	MH151879	Palaruvi, Western Ghats
P. hapnium Buch.-Ham.	ATREE5086	MH128773	MH151880	Neryamangalam, Western Ghats
P. umbellatum L.	–	MK478914	MK491945	Sirsi, Western Ghats

2010) after evaluating the ESS values. The program Tracer v1.7 (Rambaut et al., 2018) were used to visualize MCMC chain length convergence and remove the burn in values (25%) to sample the stationary phase of each runs. We used the software TreeStat (part of the BEAST program package) to calculate the distribution of node ages from a set of 1000 posterior trees to check uncertainties in divergence date estimates. Maximum clade credibility (MCC) tree and the 95% confidence intervals of nodes were calculated using the software Tree Annotator V1.8 (part of the BEAST program package). We set the options to mean height and a burn-in period of 25%. The final trees were visualized and edited using the program Fig Tree V1.4.2 (Rambaut, 2016).

2.4. Ancestral area estimations

We designated four broad areas: 1. Peninsular India (I) covering areas south of Vindhya-Satpura ranges and Sri Lanka 2. Southeast Asia

(S) consisting of the Himalayas, China, Malaysia, Indonesia, Philippines along with the Andaman and Nicobar Islands 3. Africa (A) comprising African tropics, Madagascar and the adjacent Reunion Islands and 4. South Pacific (P), which incorporates Pacific Islands and New Zealand (Fig. 1) to estimate the ancestral areas. The ancestral area estimations were performed in R (R Core Team, 2015) package BioGeoBEARS 0.21 (Matzke, 2012, 2013). 'BioGeoBEARS' combines three major analytical frameworks in biogeography, a modified form of S-DIVA (Ronquist, 1997) implemented as DIVALIKE, the BAYAREALIKE model modified from BAYAREA (Landis et al., 2013), and dispersal extinction cladogenesis model (DEC) (Ree and Smith, 2008). Besides, three variants of these models allowing for founder event speciation i.e DEC + J, DIVALIKE + J and BAYAREALIKE + J were analyzed separately. The biogeographic analysis in BioGeoBEARS was performed using the BEAST MCC tree. The species belonging to Neotropical clade and the outgroups were pruned prior to the analysis. We performed ancestral area estimations by defining five time frames in concordance with major plate

tectonic events i.e. (1) 110–55 Ma (splitting of India from rest of Gondwana); (2) 55–30 Ma (collision between India and Eurasia leading to biotic exchanges, Africa remains disconnected with Eurasia); (3) 30–20 Ma (Africa connected to Eurasia due to the closure of Tethys sea) (4) 20–14 Ma (biotic exchange between Africa and Eurasia) (5) 14–0 Ma (opening of red sea disconnecting Africa with rest of Eurasia). The dispersal probabilities were set based on the connectivity and distance of different land masses during each time slices (see Fig S2.2 for the dispersal multiplier matrix for each time slices).

3. Results

3.1. Phylogenetic analysis

The final combined dataset consisted of two genomic regions, the nuclear ITS (800 bp) and the chloroplast intron *psbJ-petA* (807 bp). The models of sequence evolution selected were TVM + I + G for ITS and TVM + G for *psbJ-petA*. The DNA sequences representing 24 species of *Piper*, which include three new putative species from the Western Ghats and eight species from the Northeastern Himalaya were newly added to GenBank. The phylogenetic trees were rooted on several outgroup taxa such as *Manekia naranjoana*, *Manekia sydowii*, *Zippleia begonifolia*, *Hottuniya cordata*, *Saururus chinensis* and seven species of *Peperomia* (Table S1). Both maximum likelihood (ML) and Bayesian inference (BI) resulted in congruent tree topologies with three major clades corresponding to the Neotropical, South Pacific and South Asian regions (pp = 1, BS > 95).

The *Piper* species from Africa and the Peninsular Indian regions were nested within the larger South Asian clade (Fig. 2). Our combined analysis of ITS and *psbJ-petA* and the ITS data set alone (Fig S2.4) are in agreement with the 12 infrageneric groups proposed by Asmarayani (2018). The species of Western Ghats were recovered in *Piper sensu Miquel* (1844) s.str, clade W1 and clade W2 respectively within the larger South Asian clade (Fig. 2). The Peninsular Indian species which are mostly climbers attached to the tree trunks with ivy like roots, fused and globose bracts, and round fruits were included in the clade *Piper sensu Miquel* (1844) s.str (pp = 1 BS = 88). This clade contains *P. nigrum* (black pepper), one of the commercially valuable spice crops, and its putative parents *P. galeatum* (Miq.) C.DC. and *P. trichostachyon* (Miq.) C.DC. *as per* Ravindran 2000. Interestingly, this clade also consists of the morphologically distinct species *P. schmidtii* Hook.f. and *P. barberi* Gamble (pp = 1, BS = 88). The two newly included members in this group viz *P. spp 4* and *P. spp 3* are potential new species which are woody climbers with fused and globose bracts found in the Western Ghats hotspot. The African species *P. borbonense* (Miq.) C.DC and *P. guineense* Schumach. &Thonn. were nested within the W6 clade. Other climbing species of *Piper* in the Sri Lanka, Western and Eastern Ghats with peculiarly longer spikes, free bracts, and oval fruits were nested within the W2 clade (pp = 1, BS = 87). This includes *P. hookeri* Miq., *P. hymenophyllum* Miq., *P. attenuatum* Buch.-ham ex Miq., *P. argyrophyllum* Miq. and two putative new species from the Western Ghats, along with other four species from Northeast India. Within this group, polytomies are found in both tree-building methods, with extremely short internal branch lengths. *Piper wightii* Miq. an endemic from Western Ghats appears closer to other South East Asian species *P. ploysyphoum* C.DC., *P. kadsura* (Choisy), *P. arboricola* C.DC., *P. hancei* Maxim. and *P. chaudocanum* C.DC.. The members of clade W1 (BS = 63) are characterized by their infructescences size which generally vary from 1.5 to 30 cm., and consist of both shrubs and climbing species. *Piper hapnium* Buch.- Ham., endemic to Western Ghats, appear closer to *P. mullesua* in our analysis. In Indian subcontinent, *P. mullesua* Buch.-ham. Ex.D.Don has a wide distribution range from Peninsular India to parts of Southeast Asia, both species are climbers with erect cylindrical and globose spikes respectively.
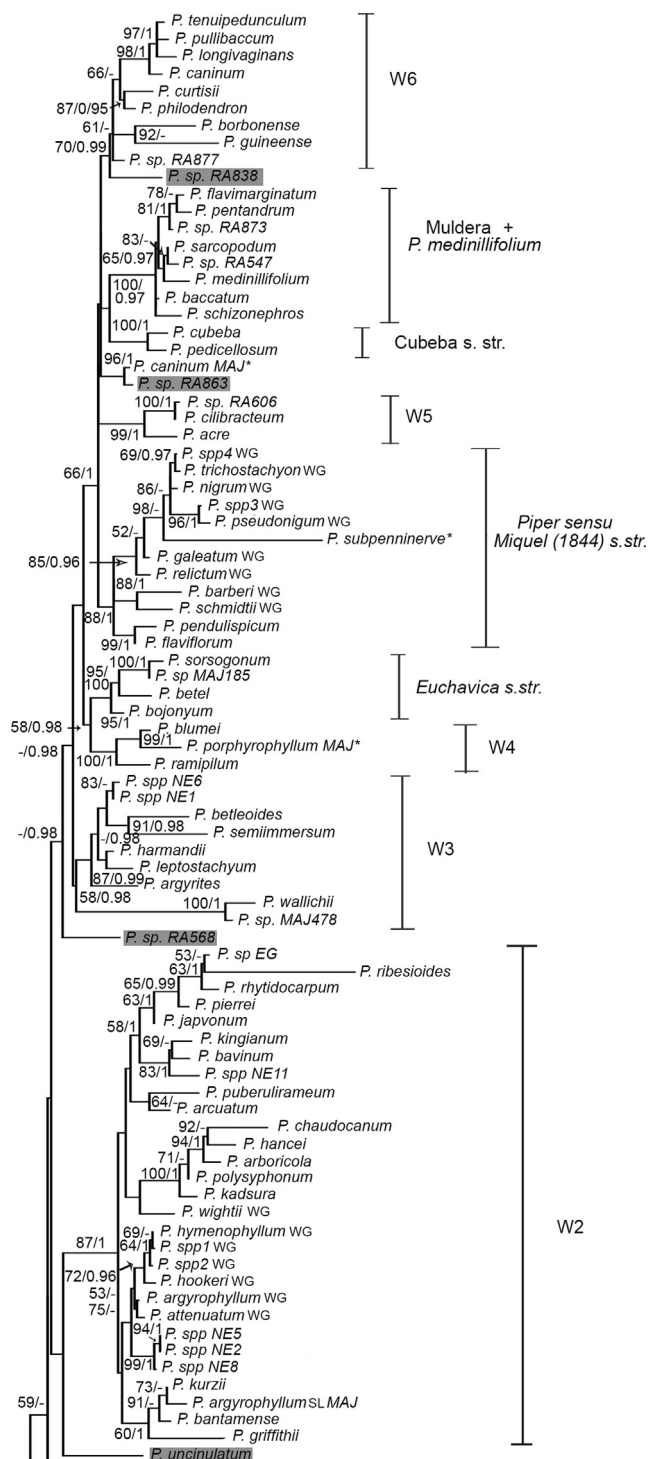

Fig. 2. Phylogenetic position of the Peninsular Indian *Pipers* within a global phylogeny derived from the combined dataset is highlighted in green. Support values for the internal branches represent maximum likelihood bootstrap values (MLBS) and Bayesian posterior probabilities (BPP). Values below MLBS 50 and BPP 0.95 are not shown. NE' indicates species from the Northeast India, EG: Eastern Ghats SL: Sri Lanka and AN: Andaman and Nicobar Islands. * indicates species whose relationships are not well established in this study compared to the earlier phylogenetic hypothesis proposed by Asmarayani 2008. Species without consistent affiliation are highlighted. Clades W1-W6, Cubeba, Muldera, Euchavica and *Piper* sensu represents the species distributed to the West of Wallace line and E1-E5 represents groups distributed to the East of Wallace line.

Fig. 2. (continued)

3.2. Divergence time estimation and biogeographic analysis

Our divergence time tree estimates using BEAST retrieved three major lineages: *Piper s.s.* (Asian tropics), *Macropiper* (South Pacific) and the Neotropical clade. The maximum clade credibility tree estimated using three calibrations suggested *Macropiper* as sister to the Asian clade, which is in agreement with the topology generated by Asmarayani (2018) and Jaramillo et al. (2008). Our divergence analysis showed that *Piper* originated during the lower Cretaceous 110 Ma (HPD: 116–105). Stem age of all three major lineages was dated back to upper Cretaceous, and the stem age of *Piper s.s* and *Macropiper* was estimated as 83.4 Ma (HPD: 97.5–68.8). The age of *Piper s.s* was estimated to be 71.3 Ma (HPD: 83.9–57.3), and the clade *Macropiper* was estimated to have originated around 60.4 Ma (HPD: 83.2–37.6). All major endemic radiations in India occurred during the Oligocene (< 35Mya; Figs. 3 and 4). The tropical African species, *P. guineense* and *P. borbonense,* diverged from their sister taxa in South Asia around 22.3 Ma (HPD: 29.7–14.6) (Figs. 3, S2.1 and Table S1.2). The distribution of node ages calculated from a set of 1000 random trees from the posterior distribution have not shown substantial variation from the node age estimated by the MCC tree. Overall, AIC values suggested DEC + J and DIVALIKE + J model (AIC = −116.99) as the best model (Table 2). Here, we focus our discussions on the results from DEC model since (1) the *j* parameters are used to model jump dispersals in Island systems and (2) few methodological issues associated with the DEC + J model (Ree and Sanmartin, 2018). The ancestral area reconstructions (Fig. 4) indicated several dispersal pathways, a) between India and Southeast Asia and b) between Southeast Asia and Africa (see Figs. S3.1–S3.5 for results from DEC + J and other models). In all models, Southeast Asia was the ancestral area for Peninsular Indian *Piper* and its sister groups. The ancestors of these lineages arrived from Southeast Asia and further diversified within. Our analysis also indicates back dispersals out of Peninsular India into Southeast Asia. The ancestral areas for the African species included in our study were estimated to be from Southeast Asia, with a notable exception of *P. capense* in the Southpacific clade, a shrub with habit and floral characters similar to Neotropical species. A previous study by Smith et al., (2008) suggests a likely introduction of this species to Africa.
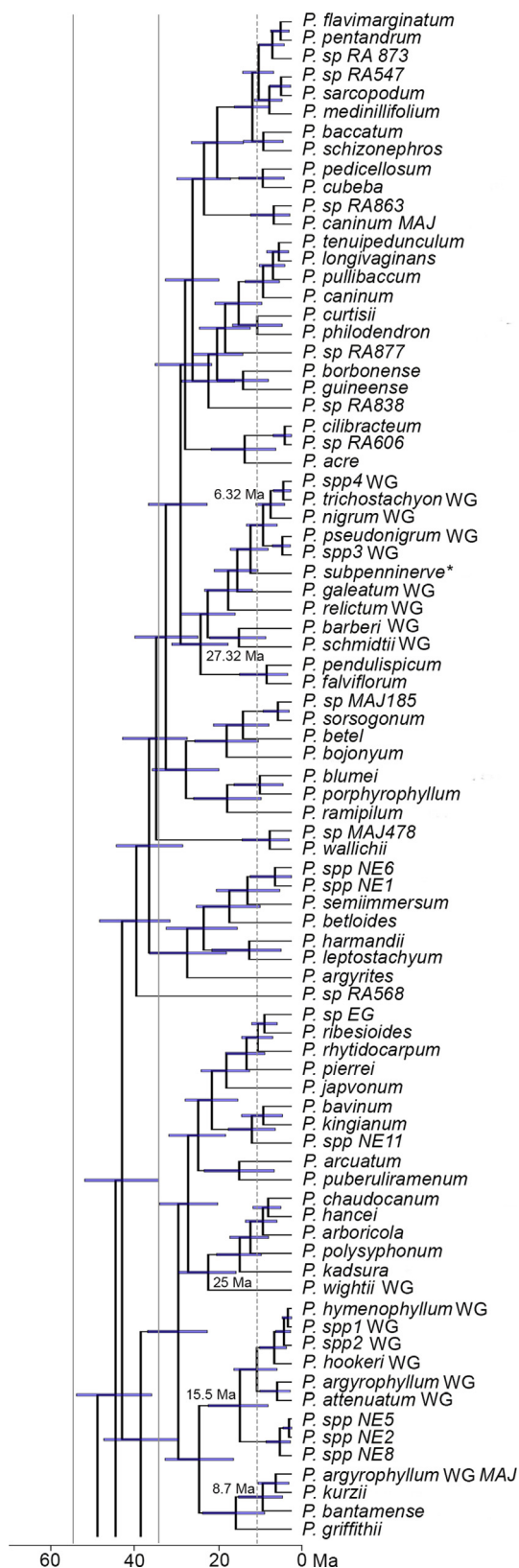
Fig. 3. Chronogram of *Piper* derived from nuclear and chloroplast dataset using BEAST. The horizontal blue lines indicate 95% highest posterior density for node ages. The numbers correspond to important nodes and radiation within Peninsular India. The values represent node ages and 95% HPD of important nodes discussed in this study. The vertical lines indicate the presence of Eocene land bridges and the dotted lines indicates the onset of monsoon in Peninsular India. Inset: *Piper wightii*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. Phylogenetic analysis and divergence dating

The results presented here represent the most comprehensive phylogenetic analysis to date of *Piper* species in South Asia covering over 90% of species in Peninsular India. The previous phylogenetic studies of *Piper* included limited representation from the Indian subcontinent (Jaramillo et al., 2008; Smith et al., 2008; Asmarayani 2018). Our analysis, which includes 18 endemic species from Peninsular India, suggests their assignment within three major clades in the recent *Piper* phylogeny (Asmarayani, 2018). Our phylogeny is in agreement with the infrageneric groups suggested by Asmarayani (2018). But, in our topology, *P. quinqueangualatum* (accession numbers ITS: AF275208, *psbJ-petA*: EU581546) were found nested with the E5 clade and sister to *P. austrocaledonicum*. Similar relationships are supported by the phylogenetic hypothesis suggested by Jaramillo et al. (2008), but these sequences were not represented in the phylogeny of Asmarayani (2018). Moreover, Asmarayani (2018) identified *P. subpenninerve* as *P. nigrum*. However, it is noteworthy to mention that *P. nigrum* is naturally distributed in the wet evergreen forest of Western Ghats hot spot from 8°N to 14°N (Sen et al., 2016) and the sequences used in this study were from the wild *P. nigrum*. In our analysis *P. subpenninerve* is well supported and does not appear sister to *P. nigrum* and hence we retain the name as *P. subpenninerve*. However, we recommend interpreting this relationship with great caution. Because, considering that Asmarayani (2018) examined the voucher specimen and identified this as *P. nigrum*, there is a possibility that this collection represents a cultivar or an accidental sampling of a cultivated individual found in the wild conditions. Furthermore, we notice taxonomic uncertainties within *P. argyrophyllum*, *P. caninum* and *P. abbreviatum* sequences from Jaramillo et al. (2008). We refrain from explaining these uncertainties and also acknowledge that the taxonomic complexity in this genus is much higher and further investigation is required to resolve these relationships. Yet another interesting aspect of our phylogeny is the topological conflicts between the nuclear and plastid phylogenies even after the addition of several new species (see Jaramillo et al., 2008 for previous results). Several studies in angiosperm lineages have established this pattern where a nuclear tree is in agreement with the morphology based grouping where the chloroplast topologies is in accordance with its geographical distribution (Nauheimer et al., 2012; Rieseberg and Soltis 1991). Our analyses also support this view to a great extent; our nuclear phylogeny recovered the morphological and geographical grouping proposed in *Piper*, while the analysis of plastid data alone reflects a geographical structure (Fig. S2.3). This structuring of chloroplast (maternally inherited) DNA based topology could be due to limited genetic exchange via seed dispersal, while the congruence between nuclear phylogeny (biparently inherited) and morphology can be explained by higher level of genetic exchange via pollen transfer (Asmarayani, 2018). Hybridization is yet another factor which could possibly explain these topological conflicts (see Pérez-Escobar et al., 2017 and references therein). However little is known about hybridization in Peninsular Indian *Piper* species.

Our study further provides molecular evidence for the first time that *P. nigrum*, one of the widely used spice plants, originated in the Western Ghats during the Late Miocene 6.3 Ma (HPD: 10.7–2.13). This finding is of particular importance because this species is widely cultivated throughout South Asia and South America, and has had several speculations about its geographical areas of origin (Ravindran, 2003). The recent rapid radiation of *Piper* species in Peninsular India is characterized by limited variability in DNA sequences and similar morphological and ecological characteristics among species. Species such as *P. attenuatum*, *P. hymenophyllum* and *P. argyrophyllum* have strikingly similar morphology and ecological requirements. Our analysis did not provide enough support for delimiting these species (pp < 0.95, BS < 50). This could be due to recent rapid radiations, and our
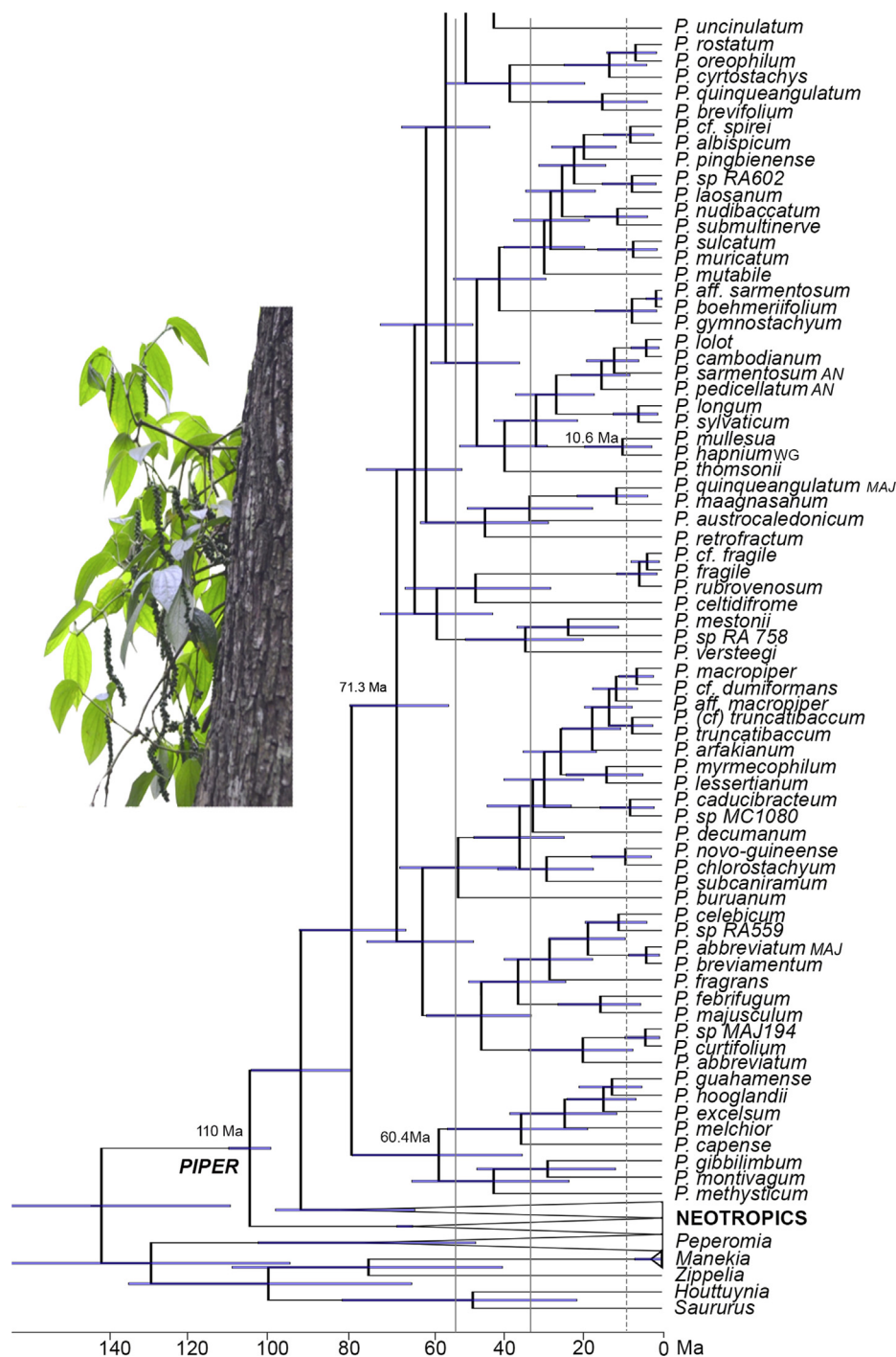
Fig. 3. (*continued*)

molecular divergence dates are in agreement with this. However, these species show some contrasting morphological features which can be used for their identification. For example, *P. hymenophyllum* is pubescent compared to the other two species, and are also distributed in the higher altitudes. Similarly, *P. argyrophyllum* exhibits bullate structure in the leaf compared to *P. attenuatum* and *P. hymenophyllum*. However, more sequence information is required to resolve the relationships within these species complexes. *Piper umbellatum*, one of the commonly found species in secondary habitats of the Western Ghats is known to be biogeographically widespread and occurs in Asia, Pacific Islands, Africa and Neotropics. *Piper umbellatum sequenced* from the Western Ghats clustered with samples from the Neotropical clade (Jaramillo et al., 2008). This species is considered as an accidental introduction to India

(Smith et al., 2008). Moreover, *P. umbellatum* is known to have medicinal properties and could be one possible reason for its introduction to this region (Roersch, 2010). Our molecular dates predict a lower cretaceous origin for *Piper* and in general predict older dates when compared to the molecular ages of the earliest flowering plant lineages using 22 fossils by Salomo et al. (2017). This could be due to use of two fossils within genus *Piper* in our analysis, where their calibration scheme did not use any fossils specific to genus *Piper*.

4.2. Origin of Peninsular Indian Piper: dispersal or vicariance?

The intracontinental crown clade ages of *Piper* ranges from 33 to 15 Ma, pointing to Oligocene - Miocene diversification. The collision
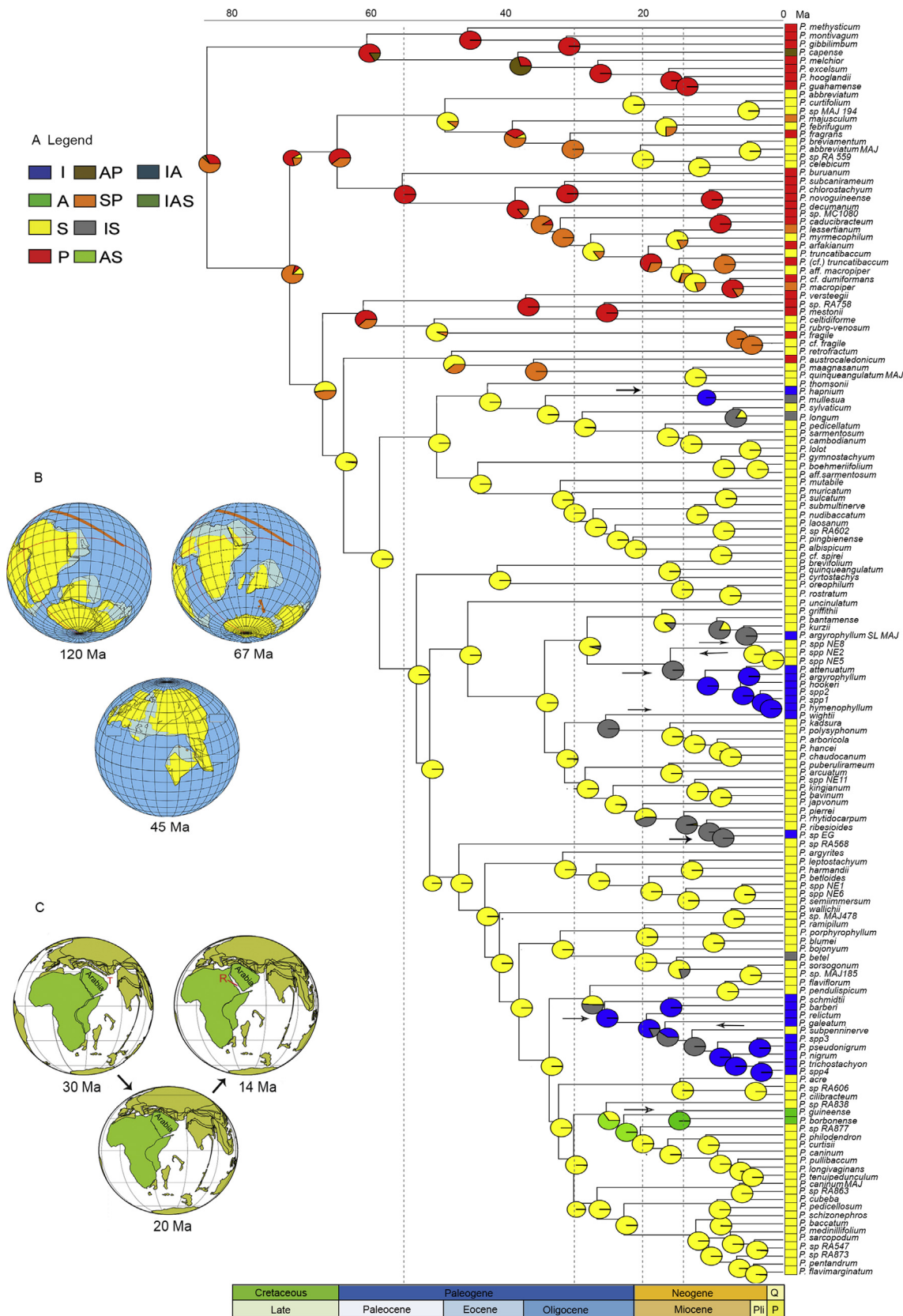
Fig. 4. Ancestral range estimations of *Piper* from BIOGEOBEARS. I = Peninsular India, A = Africa, S = Southeast Asia and P = South Pacific. (a) Colors correspond to the 4 defined geographical regions and other ancestral areas in this study. (b) Indicates the relative position of Peninsular India from late cretaceous to Eocene (c) Indicates closure of Tethys (T) by Arabian Peninsula and the opening of red sea (R) in the middle Miocene. Pie charts indicate the probabilities of different ancestral areas for the respective nodes under the DEC model. The vertical dotted lines indicate five different time slices in the ancestral area estimations. '→' represent major dispersal events discussed in the study. '←' indicates dispersal back to Southeast Asia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Summary table showing results from ancestral range estimations using BIOGEOBEARS with six different models applied to the maximum clade credibility tree of *Piper*. The selected model is highlighted in bold. See main text for the detailed explanation.

Model	−LnL	No. of parameters	d	e	j	AICc	p
DEC	−116.992	2	31×10^{-4}	1×10^{-12}	0	238.063	4.6×10^{-8}
DEC + J	−102.05	3	13×10^{-4}	1×10^{-12}	0.026	210.064	
DIVALIKE	−116.992	2	31×10^{-4}	1×10^{-12}	0	238.063	9×10^{-7}
DIVALIKE + J	−102.053	3	13×10^{-4}	1×10^{-12}	0.026	210.264	
BAYAREALIKE	−157.358	2	28×10^{-4}	82×10^{-4}	0	318.796	5.1×10^{-22}
BAYAREALIKE + J	−110.831	3	11×10^{-4}	1×10^{-7}	0.035	227.822	

d, rate of dispersal; *e*, rate of extinction; *j*, relative probability of founder-event speciation; *p*, indicates significance between each model pairs with and without the *j* parameter

between the Indian plate and the Eurasian plate is known to have facilitated biotic exchange throughout the Eocene- Oligocene period. The warm, per humid conditions, supporting diverse rainforest vegetation were found across the Indian subcontinent through most of the Late Eocene (39–36 Ma) and Oligocene (36–25 Ma) period. Our molecular based divergence date estimates are thus in line with most of the geological studies that date Indian- Eurasian collision at around 50 Ma (Ali and Aitchison, 2008; Najman et al., 2010). Similar habitat conditions at that time period might have facilitated the species to track its niche and colonize Peninsular India during Indo-Eurasian contact.

The initial lineages that gave rise to *Piper* arrived from Southeast Asia during the Oligocene and then diversified *in-situ* in Peninsular India. This *in-situ* diversification occurred primarily in two clades that gave rise to the endemic species in the Western Ghats and further dispersed back from Peninsular India to Southeast Asia. Recently, Klaus et al. (2016) suggested that the number of dispersals between mainland India and Southeast Asia peaked around the mid-Miocene climatic optimum and started decreasing from 14 Mya, and the finding of the present study is in agreement with this view. The change from more seasonal monsoonal climate in the early Miocene to wet climate in the Middle Miocene resulted in the expansion of rainforests in Peninsular Indian region (Morley, 2000). This change could have strongly influenced the speciation in this group during the mid to late Miocene in *Piper* (Fig. 3). We infer several independent back dispersals out of India to Southeast Asia during the Miocene. The increased aridification in mainland India during Miocene may have been a factor that promoted back dispersals of *Piper* species. During this period, warmer climate aided the establishment of widespread evergreen forests across northern India and resulted in the establishment of a rain forest belt along Himalayan foothills in the region of present-day Myanmar (Burma) and Bhutan facilitating the dispersal of its ancestors (Klaus et al., 2016). However, from 14 Ma onwards there is notable decrease in the biotic exchange between India and Southeast Asia, likely due to the establishment of a widespread arid climate. In addition, our ancestral range estimations for, *P. subpenninerve* from Malaysia showed an Indo Southeast Asian origin 12.3 Ma (18.3–7), which appears sister to *Piper* species from Peninsular India in the clade *Piper* sensu Miquel (1844) s.str, indicating a vicariance of its ancestral range during mid Miocene climatic optimum. However, we consider this as a back dispersal since the models other than DEC suggest a likely back dispersal.

The burst of species in the clades *Piper sensu* Miquel (1844) s.str and the clade W2 of *Piper* in Peninsular India draws special attention. We identified species with short internal branch lengths indicative of a recent speciation. Their divergence time (< 10 Ma) coincides with intensification of monsoon climate with seasonal weather conditions in Peninsular India. This monsoon driven climate change might have given enough opportunities for these groups to diversify within the wet evergreen forest of Western Ghats; creating novel habitats and forming insular environments which can promote allopatric speciation by limiting migrations. Similarly, monsoon -driven climate change mediated diversification patterns were also observed in unrelated taxon such as *Cyrtodactylus* (Agarwal and Karanth, 2015) and Sitana (Deepak and Karanth, 2018) in Peninsular India.

The two African species (*P. borbonense* and *P. guineense*) found nested within the Southeast Asian group suggests their dispersal 'into Africa' (Jaramillo et al., 2008; Smith et al., 2008). Our divergence time dating and ancestral area estimations support the overland migration between Eurasia and Africa around 20 Ma, and in agreement with several other studies. For example, Popp et al. (2008) suggest that *Lychnis* (Caryophyllaceae) reached Africa via the Arabian Peninsula, and *Uvaria* (Annonaceae) was found dispersed 'out-of Africa' and further aided in the radiation of Primates in Eurasia during the Miocene (Zhou et al., 2012). Another study by Yu et al. (2014) shows that *Isodon* (Lamiaceae) reached Africa by an overland migration from Asia during the early Miocene, which was disrupted as a result of the opening of the Red Sea during the middle Miocene. Although other competing hypothesis exist (Conti et al., 2002; Yuan et al., 2005, etc.), our analysis and divergence time estimate supports the view of an overland migration of *Piper* 'into Africa' from Asia during early Miocene via the Arabian Peninsula.

The ancestral range estimations further suggest the Southeast Asia as the likely ancestral area to the species distributed towards the east of Wallace line. We observed several instances of *in situ* radiations, back dispersals and range extensions for *Piper* species in this region. For example, *P. austrocaledonicum,* a species found in New Caledonian Islands have showed a Southeast Asian origin in all major biogeographic models. These dates agree with the long-distance dispersal hypothesis proposed by Murienne (2009) explaining the evolution of Caledonian biogeographic patterns. However, much larger taxon sampling from the Pacific and Southeast Asian *Piper* species are required to elucidate the factors behind the origin and evolution of *Piper* in these regions.

5. Conclusion

The results of our biogeographic analysis indicate that *Piper* species arrived to India following Indo-Eurasian collision during the Eocene. These arrivals were followed by *in situ* diversification as well as back dispersals out of India. Our study further indicates *P. nigrum* originated *in situ* within the wet-evergreen forest of Western Ghats during Miocene. Even though these results are robust, we also acknowledge certain caveats in this study. Our phylogenetic trees lacked resolution within the Southeast Asian clade, even after inclusion of several species from Peninsular India. Higher representation of the species from Northeastern India and Southeast Asia along with more sequence data is required for an improved understanding of the phylogeny and the evolutionary process underlying the diversification of this group.

Author contributions

SS and GR conceived the idea. SS, SD and GR designed the experiment. SS, GR, TD, RG, JMR and MPJ did the field-work. SS, JMR and TD compiled the data. RG, TD, SS and MPJ identified the specimens. SD and GR provided the guidance. GR, SD and RG provided the lab

facilities. SS performed the analysis and completed the writing with subsequent contributions from other authors.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2019.05.033.

References

Agarwal, I., Karanth, K.P., 2015. A phylogeny of the only ground-dwelling radiation of *Cyrtodactylus* (Squamata, Gekkonidae): diversification of *Geckoella* across peninsular India and Sri Lanka. Mol. Phylogenet. Evol. 82, 193–199.

Agarwal, I., Bauer, A.M., Jackman, T.R., Karanth, K.P., 2014. Insights into Himalayan biogeography from geckos: a molecular phylogeny of *Cyrtodactylus* (Squamata: Gekkonidae). Mol. Phylogenet. Evol. 80, 145–155.

Ali, J.R., Aitchison, J.C., 2008. Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma). Earth Sci. Rev. 88, 145–166.

Anand, A., Rao, C.S., 2000. A rapid in vitro propagation protocol for Piper barberi Gamble, a critically endangered plant. In Vitro Cell. Dev. Biol.-Plant 36 (1), 61–64. https://doi.org/10.1007/s11627-000-0014-6.

Asmarayani, R., 2018. Phylogenetic relationships in Malesian-Pacific *Piper* (Piperaceae) and their implications for systematics. Taxon 67, 693–724.

Babu, K.N., Nair, R.R., Johnson, G.K., Ravindran, P.N., 1992. *Piper barberi* Gamble- are description of the species with a note on the karyotype. J. Spices Aromatic Crop. 1, 88–93.

Bansal, R., Karanth, K.P., 2010. Molecular phylogeny of *Hemidactylus* geckos (Squamata: Gekkonidae) of the Indian subcontinent reveals a unique Indian radiation and an Indian origin of Asian house geckos. Mol. Phylogenet. Evol. 57, 459–465.

Biju, S.D., Bossuyt, F., 2003. New frog family from India reveals an ancient biogeographical link with the Seychelles. Nature 425, 711–714.

Bossuyt, F., Milinkovitch, M.C., 2001. Amphibians as Indicators of Early Tertiary "Out-of-India" dispersal of vertebrates. Science 292, 93–95.

Briggs, J.C., 2003. The biogeographic and tectonic history of India. J. Biogeogr. 30, 381–388.

Callejas, R., 2002. Micromorfología de las semillas en el género Piper. In: Rangel, J.O., Aguirre, J., Andrade, M.G. (Eds.), Libro de Resúmenes Octavo Congreso latinoamericano y Segundo Colombiano de Botánica. Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Colombia, 435 pp.

Conti, E., Eriksson, T., Schönenberger, J., Sytsma, K.J., Baum, D.A., 2002. Early Tertiary out-of-India dispersal of *Crypteroniaceae*: evidence from phylogeny and molecular dating. Evolution 56, 1931–1942.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772.

Datta-Roy, A., Karanth, K.P., 2009. The Out-of-India hypothesis: what do molecules suggest? J. Biosci. 34, 687–697.

Datta-Roy, A., Singh, M., Srinivasulu, C., Karanth, K.P., 2012. Phylogeny of the Asian *Eutropis* (Squamata: Scincidae) reveals an 'into India' endemic Indian radiation. Mol. Phylogenet. Evol. 63, 817–824.

Dayanandan, S., Ashton, P.S., Williams, S.M., Primack, R.B., 1999. Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast rbcL gene. Am. J. Bot. 86, 1182–1190.

Deepak, V., Karanth, P., 2018. Aridification driven diversification of fan-throated lizards from the Indian subcontinent. Mol. Phylogenet. Evol. 120, 53–62.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Dyer, L.A., Palmer, A.D. (Eds.), 2004. Piper: A Model Genus for Studies of Phytochemistry, Ecology, and Evolution. Kluwer academic/Plenum Publishers, New York, pp. 117–134.

Gower, D.J., Agarwal, I., Karanth, K.P., DattaRoy, A., Giri, V.B., Wilkinson, M., San Mauro, D., 2016. The role of wet zone fragmentation in shaping biodiversity patterns in peninsular India: insights from the caecilian amphibian *Gegeneophis*. J. Biogeogr. 43, 1091–1102.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Jaramillo, M.A., Callejas, R., Davidson, C., Smith, J.F., Stevens, A.C., Tepe, E.J., 2008. A phylogeny of the tropical genus *Piper* using ITS and the chloroplast intron *psbJ–petA*. Syst. Botany 33, 647–660.

Joshi, J., Karanth, P., 2013. Did southern Western Ghats of peninsular India serve as a refugia for its endemic biota during the Cretaceous volcanism? Ecol. Evol. 10, 3275–3282.

Joshi, J., Edgecombe, G.D., 2019. Evolutionary biogeography of the centipede genus Ethmostigmus from Peninsular India: testing an ancient vicariance hypothesis for Old World tropical diversity. BMC Evol. Biol. 19.

Karanth, K.P., 2015. An island called India: phylogenetic patterns across multiple taxonomic groups reveal endemic radiations. Curr. Sci. 108, 1847–1851.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Klaus, S., Morley, R.J., Plath, M., Zhang, Y.P., Li, J.T., 2016. Biotic interchange between the Indian subcontinent and mainland Asia through time. Nat. Commun. 7, 12132.

Koehler, F., Glaubrecht, M., 2007. Out of Asia and into India: on the molecular phylogeny and biogeography of the endemic freshwater gastropod *Paracrostoma* Cossmann, 1900 (Caenogastropoda: Pachychilidae). Biol. J. Linnean Soc. 91, 627–651.

Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. Syst. Biol. 62, 789–804.

Mani, M.S., 1995. Biogeography in India. Surya Publications, Dehra Dun, India.

Mani, M.S., 1974. Biogeography of Peninsula. In: Ecology and biogeography in India. Dr W Junk Publishers, The Hague, pp. 614–646.

Martínez, C., Carvalho, M.R., Madriñán, S., Jaramillo, C.A., 2015. A Late Cretaceous *Piper* (Piperaceae) from Colombia and diversification patterns for the genus. Am. J. Bot. 102, 273–289.

Matzke, N.J., 2012. Founder-event speciation in BioGeoBEARS package dramatically improves likelihoods and alters parameter inference in Dispersal-Extinction-Cladogenesis (DEC) analyses. Front. Biogeogr. 4, 210.

Matzke, N.J., 2013. BioGeoBEARS: Biogeography with Bayesian (and Likelihood) evolutionary analysis in R scripts. Retrieved from http://cran.r-project.or/web/packages/BioGeoBEARS/ (last accessed 20 December 2017).

Metcalfe, I., 2013. Gondwana dispersion and Asian accretion: tectonic and palaeogeographic evolution of eastern Tethys. J. Asian Earth Sci. 66, 1–33.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Institute of Electrical and Electronics Engineers (Ed.), Proceedings of the gateway computing environments workshop (GCE). IEEE Xplore, New Orleans, LA, pp. 1–8.

Molina-Henao, Y.F., Guerrero- Chacòn, A.L., Jaramillo, M.A., 2016. Ecological and geographic dimensions of diversification in *Piper* subgenus Ottonia: a Lineage of Neo tropoical rain forest shrubs. Syst. Botany 41, 253–262.

Morley, R.J., 2000. Origin and Evolution of Tropical rain Forests. John Wiley & Sons.

Mukherjee, P.K., 2018. Nomenclatural notes on Piper Linn. (Piperaceae) from India II. Phytotaxa 338, 17–32.

Murienne, J., 2009. Testing biodiversity hypothesis in New Caledonia using phylogenetics. J. Biogeogr. 36, 1433–1434.

Najman, Y., Appel, E., Boudagher Fadel, M., Bown, P., Carter, A., Garzanti, E., Parrish, R., 2010. Timing of India Asia collision: geological, biostratigraphic, and palaeomagnetic constraints. J. Geophys. Res.: Solid Earth 115 (B12).

Nauheimer, L., Boyce, P.C., Renner, S.S., 2012. Giant taro and its relatives: a phylogeny of the large genus Alocasia (Araceae) sheds light on Miocene floristic exchange in the Malesian region. Mol. Phylogenet. Evol. 63, 43–51.

Naumann, J., Salomo, K., Der, J.P., Wafula, E.K., Bolin, J.F., Maass, E., Wanke, S., 2013. Single-copy nuclear genes place haustorial Hydnoraceae within Piperales and reveal a Cretaceous origin of multiple parasitic angiosperm lineages. PLoS One 8 (11), e79204.

Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.

Pérez-Escobar, O.A., Gottschling, M., Chomicki, G., Condamine, F.L., Klitgård, B.B., Pansarin, E., Gerlach, G., 2017. Andean mountain building did not preclude dispersal of lowland epiphytic orchids in the Neotropics. Sci. Rep. 7, 4919.

Popp, M., Gizaw, A., Nemomissa, S., Suda, J., Brochmann, C., 2008. Colonization and diversification in the African 'sky islands' by Eurasian Lychnis L. (Caryophyllaceae). J. Biogeogr. 35, 1016–1029.

Prasad, V., Farooqui, A., Tripathi, S.K.M., Garg, R., Thakur, B., 2009. Evidence of Late Palaeocene-Early Eocene equatorial rain forest refugia in southern Western Ghats. India. J. Biosci. 34, 777–797.

R Core Team, 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Available at http://www.R-project.org/ (last accessed March 2017).

Rambaut, A., 2016. Figtree, a graphical viewer of phylogenetic trees. Retrieved from http://tree.bio.ed.ac.uk/software/figtree (accessed 20 December 2017).

Rambaut, A., Drummond, A.J., 2010. LogCombiner version 1.6.1 [computer program] http://beast.bio.ed.ac.uk.

Rambaut, A., Drummond A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Tracer v1.7, Available from http://tree.bio.ed.ac.uk/software/trace.

Ravindran, P.N. (Ed.), 2003. Black pepper: Piper nigrum. CRC Press.

Ree, R.H., Sanmartín, I., 2018. Conceptual and statistical problems with the DEC + J model of founder-event speciation and its comparison with DEC via model selection. J. Biogeogr. 45, 741–749.

Ree, R.H., Smith, S.A., 2008. Lagrange: software for likelihood analysis of geographic range evolution. Syst. Biol. 57, 4–14.

Rieseberg, L.H., Soltis, D.E., 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trends Plants 5, 65–84.

Roersch, C.M., 2010. *Piper umbellatum* L.: a comparative cross-cultural analysis of its medicinal uses and an ethnopharmacological evaluation. J. Ethnopharmacol. 131, 522–537.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Rutschmann, F., Eriksson, T., Schönenberger, J., Conti, E., 2004. Did *Crypteroniaceae* really disperse out of India? molecular dating evidence from rbc L, ndh F, and rpl 16 intron sequences. Int. J. Plant Sci. 165, S69–S83.

Salomo, K., Smith, J.F., Feild, T.S., Samain, M.S., Bond, L., Davidson, C., Wanke, S., 2017. The emergence of earliest angiosperms may be earlier than fossil evidence indicates. Syst. Bot. 42, 607–619.

Sambrook, J.R., Russell, D., 2001. Molecular cloning: a laboratory manual. Q. Rev. Biol. 76, 348–349.

Samanth, B., Mohabey, D.M., 2009. Playnoflora from Deccan volcano-sedimentary sequence (Cretaceous-Palaeogene transition) of central India: implications for spatio-temporal correlation. J. Biosci. 34, 811–823.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Sen, S., Gode, A., Ramanujam, S., Ravikanth, G., Aravind, N.A., 2016. Modeling the impact of climate change on wild *Piper nigrum* (Black Pepper) in Western Ghats, India using ecological niche models. J. Plant Res. 129, 1033–1040.

Smith, J.F., Stevens, A.C., Tepe, E.J., Davidson, S., 2008. Placing the origin of two species-rich genera in the late Cretaceous with later species divergence in the tertiary: a phylogenetic, biogeographic and molecular dating analysis of *Piper* and *Peperomia* (*Piperaceae*). Plant Syst. Evol. 275, 9.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 22, 2688–2690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771.

Surveswaran, S., Kamble, M.Y., Yadav, S.R., Sun, M., 2009. Molecular phylogeny of *Ceropegia (Asclepiadoideae, Apocynaceae)* of Indian Western Ghats. Plant Syst. Evol. 281, 51–63.

Ulloa, C.U., Acevedo-Rodríguez, P., Beck, S., Belgrano, M.J., Bernal, R., Berry, P.E., Jørgensen, P.M., 2017. An integrated assessment of the vascular plant species of the Americas. Science 358, 1614–1617.

Van Bocxlaer, I., Biju, S.D., Loader, S.P., Bossuyt, F., 2009. Toad radiation reveals into-India dispersal as a source of endemism in the Western Ghats-Sri Lanka biodiversity hotspot. BMC Evol. Biol. 9, 131.

Wanke, S., Jaramillo, M.A., Borsch, T., Samain, M.S., Quandt, D., Neinhuis, C., 2007. Evolution of *Piperales* -*mat*K gene and *trn*K intron sequence data reveal lineage specific resolution contrast. Mol. Phylogenet. Evol. 42, 477–497.

Whitmore, T.C., 1998. An Introduction to Tropical Rain Forests, second ed. Oxford University Press, Oxford.

Yu, X.Q., Maki, M., Drew, B.T., Paton, A.J., Li, H.W., Zhao, J.L., Li, J., 2014. Phylogeny and historical biogeography of *Isodon* (Lamiaceae): rapid radiation in south-west China and Miocene overland dispersal into Africa. Mol. Phylogenet. Evol. 77, 183–194.

Yuan, Y.M., Wohlhauser, S., Möller, M., Klackenberg, J., Callmander, M.W., Küpfer, P., 2005. Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean Basin resulted from long distance dispersal and extensive radiation. Syst. Biol. 54, 21–34.

Zhou, L., Su, Y.C., Thomas, D.C., Saunders, R.M.K., 2012. 'Out of Africa' dispersal of tropical floras during the Miocene climatic optimum: evidence from *Uvaria* (*Annonaceae*). J. Biogeogr. 39, 322–335.