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A phylogenetic approach toward the understanding of disjunct distributions of plant taxa in Western Ghats and northeastern India

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Abstract

There are many Indian plant taxa with disjunct distributions in northeastern India and Western Ghats, raising several systematic and biogeographic questions. Such questions are best addressed in a phylogenetic context as presented in this review of nine genera (*Arisaema* Mart., *Begonia* L., *Ceropegia* L., *Hoya* R. Br., *Impatiens* L., *Indigofera* L., *Rubus* L., *Strobilanthes* Blume and *Vitis* L.). These genera were chosen because they contain species that are endemic and occur in either northeastern India or Western Ghats, and at least one species is included in global phylogenetic analyses. This review reveals that even with limited sampling within India, the phylogenetic studies are consistent with present understanding of the Indian flora: that it contains Chinese, South East Asian and African influences, and that the collision of India with Asia played a crucial role in the dispersal of some taxa into India. Some of these dispersals occurred soon after the collision (e.g., *Arisaema* at 45 ma), while others were more recent (*Begonia* at 15 ma from SE Asia). Other dispersals occurred between India and Africa (e.g., *Begonia* at 13.75 ma and *Indigofera* 13 ma). We analyzed new sequences of ITS and *atpB-rbcL* regions from nine species of *Impatiens*, combined with existing data. Our analysis of *Impatiens* suggests that there were not only dispersals from China and SE Asia into India, but also dispersals out of India into Africa and back into China and SE Asia. Furthermore, as in the case of *Indigofera*, the Indian and African species diverged at the most 10 million years ago, ruling out an ancient Gondwanan distribution.

Keywords: Biogeography, *Impatiens*, India, Phylogeny

Introduction

The Western Ghats and northeastern India are among the biodiversity hotspots of the world. These two regions are among the wettest regions in India, receiving over 250 cm of rainfall per year, and contain taxa showing disjunct distributions. Disjunctions are distributions in which closely related organisms are geographically widely separated; that is, their distribution is not continuous. Such cases are reported in plants (*Hoya* R. Br.: Mani, 1974; Wanntrop *et al*., 2006), birds (*Hieraaetus kienerii* G. de Sparre: Ali, 1935; Gaston & Zacharias, 1996), reptiles (*Draco* L.: Jayaram, 1974), fishes (*Chela* Hamilton: Jayaram, 1974) and amphibians (*Limnonectes* Fitzinger: Jayaram, 1974), mammals (*Martes gwatkinsi* Horsfield: Kurup, 1974) and insects (*Ceriagrion rubiae* Laidlaw: Joshi & Kunte, 2014). In these disjunct distributions, taxa of the Western Ghats have been suggested to represent Palaeogene and Pleistocene relicts (Mani, 1974; Prasad *et al*., 2009).

This disjunct pattern raises questions regarding the history of these distributions, that is: *What is the shared history of species in the two regions?*; *Which species share common ancestors?*; *Where did the ancestor come from?*; *Did this ancestor split into southwestern and northeastern Indian lineages and, if so, when?*. To fully answer these questions we need to answer questions regarding the history of the two regions, that is: *What is the biogeographical history of the disjunct wet regions?*; *What geological/climate events were responsible for the disjunction?*; *When did the disjunction occur?*; *Do the geological events satisfactorily explain the taxonomic disjunction, that is, do the patterns as well as timing of events coincide?*.

There are two models that have been put forward to explain the discontinuous distribution of taxa in Western Ghats and northeastern India. One model suggests that dispersal via corridors followed by extinction in the corridors have caused disjunctions (Ripley & Beehler, 1990). An early explanation was the Satpura hypothesis, according to which species from Eastern Himalaya colonized peninsular India through the Vindhya-Satpura ranges (Hora, 1949). Other areas that might have acted like corridors are the Brij area, which lies to the north of the Satpura range (Dilger, 1952), and the Aravali range (Mani, 1974). A second model suggests that the current disjunctions reflect relicts of a once continuous distribution. The fossil record suggests that some taxa in northeastern India may have become extinct because of such changes in climate due to the upliftment of Himalayas. For example, a fossil leaf similar to that of *Poeciloneuron indicum* Bedd., an endemic species to the Western Ghats, is found in the Oligocene sediments of Tinsukia, Assam. *Poeciloneuron indicum* is currently not found in northeastern India, and is inferred to have become extinct there (Srivastava & Mehrotra, 2013). It is also possible that species in northeastern India and Western Ghats were introduced independently from different regions; an additional hypothesis that we pose here.

However, it is extremely difficult to distinguish among the above models based just on present day spatial distributions of Indian flora. If we knew the phylogenetic relationships of the disjunct taxa we could test these models effectively. This is because each explanation or model has definite phylogenetic predictions (Donoghue & Moore, 2003; Karanth, 2003).

The geographical historical facts are relatively well-known: India was once covered by continuous tropical humid forests that in recent times have been fragmented into isolated patches due to climatic changes (Mani, 1974). Fossil evidence suggests that from Upper Cretaceous and Late Palaeocene (100–50 ma) onward until recently, India was much wetter than today and covered by humid forests (Meher-Homji, 1977; Chauhan, 2002; Prasad *et al.*, 2006). Three factors played a major role in the onset of drier climate in the peninsular region the northward movement of the Indian plate, the upliftment of the Himalaya and the Western Ghats (Meher-Homji, 1983; Karanth, 2003). The change in tropical flora to savanna grasses during Pliocene (Upper Siwalik, 5.1–1.6 ma) indicates that there was drying due to seasonal onset of dry climate (Patnaik & Prasad, 2016).

Phytogeographic studies reveal affinities of the Indian flora with those of neighbouring regions such as China, SE Asia, and Europe. The present day Indian flora consists of original Gondwanan elements (e.g., *Ochrocarpus longifolius* Benth. & Hook.f. [Calophyllaceae], and *Casuarina* L. [Casurinaceae]) enriched by the dispersal of plant species from neighbouring regions with which India has land connections (Lakhanpal, 1970; Mani, 1974). One of the dominant floristic groups consists of tropical Asiatic elements that have largely Indochinese and Malayan affinities. Tropical Asiatic elements are found on the lower slopes of the Himalaya and also in the southern peninsula. These species range from being continuously distributed to disjunct isolates. For example, *Acacia arabica* (Lam.) Willd. (Fabaceae) is distributed continuously in most parts of India. *Pittosporum glabratum* Lindl. (Pittosporaceae) extends from Hong Kong to Khasi hills in Assam. *Willoughbeia* Roxb. (Apocynaceae) is a primarily Malayan genus that occurs in Assam, Myanmar, and Sri Lanka. Temperate elements derived from Europe and Asia are restricted to the Himalaya, Khasi hills and Anamalai hills (e.g., *Clematis* L. and *Caltha* L. [Ranunculaceae]) occur in the Himalaya and also in Western and northeastern India. Steppes elements of central Asiatic origin (e.g., *Tauscheria* L. [Lasiocampidae] and *Euclidium* L. [Brassicaceae]), are found in the higher ranges of Western Himalaya. Western Himalaya also has some Mediterranean elements, species of southern European and North Africa, which have occasionally extended to the Western Ghats. For example, *Fagonia arabica* L. (Zygophyllaceae) occurs in Sind, Punjab and the Western Ghats. Tropical African and Malagasy elements are found in the peninsular flora, e.g., *Lawsonia inermis* L. (Lythraceae) which occurs in India, Central Asia and Indomalesian regions (Mani, 1974; Negi, 1993; Hajra *et al.*, 1996).

The high diversity of plants in India is a result of its equatorial positioning and, later on, connections with neighbouring regions and especially the collision of India with the Asian plate in Eocene (65–34 ma) (Jaeger *et al.*, 1989; Butler, 1995; Aitchison & Davis, 2004). Palaeontological support for this is found, for example, in the family Melastomataceae, suggested to enter into India from Asia during Miocene (Renner *et al.*, 2001; Dutta-Roy & Karanth, 2009). This is an example where taxa had entered India after India collided with the Asian plate. Conversely, *Sterculia* L. (Malvaceae), which was present in India but absent in Myanmar in the Paleogene, is reported from Myanmar later, in the Neogene (Srivastava & Mehrotra, 2010). This shows that *Sterculia* may have dispersed out of India into South East Asia (SE Asia) in the Neogene, exemplifying "Out of India" movement as (Dutta-Roy & Karanth, 2009). For evaluation of geographic history using modern methods, knowledge of phylogenetic history is essential (Cracraft, 1988; Donoghue & Moore, 2003; Karanth, 2003; Ronquist & Sanmartin, 2011). However, relatively little is understood about the history of Indian taxa in terms of phylogenetic relationships, though much is known about their taxonomic and geographic affinities.

The overall objective of this study is to evaluate existing phylogenetic studies for their ability to address the biogeographic question: What is the history of disjunction of taxa (specifically endemic species of disjunct genera) in the Western Ghats and northeastern Indian regions? The first aim of this study is to investigate this question examining nine genera for which global phylogenetic analyses already exist: *Arisaema* Mart. (Araceae), *Begonia* L. (Begoniaceae), *Ceropegia* L. (Apocynaceae), *Hoya* R. Br. (Apocynaceae), *Impatiens* L. (Balsaminaceae), *Indigofera* L. (Fabaceae), *Rubus* L. (Rosaceae), *Strobilanthes* Blume (Acanthaceae), and *Vitis* L. (Vitaceae); the second aim is to use one of these genera, *Impatiens*, as a case-study for further investigation and to present results of a preliminary phylogenetic analysis using a total of 15 Indian species including 11 new sequences.

Impatiens is a species-rich genus that comprises *c.* 1000 species (Janssens *et al.*, 2009). The family Balsaminaceae is composed of two genera *Impatiens* and the monotypic *Hydrocera* Blume ex Wight & Arn. *Impatiens* has 3 free sepals and 5 petals, of which 4 are fused into 2 lateral pairs, while *Hydrocera* has 5 free sepals 5 free petals. The fruit of *Impatiens* is an explosively dehiscent capsule, while that of *Hydrocera* is a pseudo-berry. *Impatiens* is found along the streams, while *Hydrocera* occurs in semi-aquatic habitats in still waters. *Impatiens* species grow in tropical and subtropical montane forests between 500 and 800 m. The species are typically found at margins of streams and cannot withstand persistent drought and direct sunlight (Fischer, 2004). Recent studies report around 210 species in India, 103 of which are endemic to the Western Ghats (Dessai & Janarthanam, 2011; Bhaskar, 2012). There have been phylogenetic studies of the genus, but include just a few southern Indian species. Those studies suggest that *Impatiens* is a relatively new genus estimated to have originated *c.* 22.5 ma ± 5.6 ma in South China (Bremer *et al.*, 2004; Yuan *et al.*, 2004; Janssens *et al.*, 2006, 2007, 2009). The genus rapidly diversified in SE Asia during the Early Miocene, coinciding with global cooling and subsequent glacial oscillations. Himalayan species may have had their origin in Southwest China during Late Miocene (Janssens *et al.*, 2009).

Materials and Methods

Evaluation of nine disjunct genera

An extensive survey of the literature revealed nine genera with disjunct endemic species in the Western Ghats and northeastern India whose phylogenetic study included at least one Indian species: *Arisaema* (Renner *et al.*, 2004), *Begonia* (Forrest *et al.*, 2005), *Ceropegia* (Bruyns *et al.*, 2015), *Hoya* (Wanntrop *et al.*, 2006), *Impatiens* (Janssens *et al.*, 2009), *Indigofera* (Schrire *et al.*, 2009), *Rubus* (Alice & Campbell, 1999), *Strobilanthes* (Moylan *et al.*, 2004), and *Vitis* (Tröndle *et al.*, 2010). We evaluated the systematic and biogeographic insights obtained in each case.

Phylogenetic study of *Impatiens*

Taxon sampling

We collected nine species of *Impatiens* in Goa, Bangalore and Meghalaya (Voucher specimens at DUH) and new sequences obtained were submitted to NCBI (**Appendix 1**). Sequences of 71 species were taken from Janssens *et al.* (2006). These included eight Indian species and outgroup species, *Hydrocera triflora* (L.) Wight & Arn. (Balsaminaceae), *Norantea guianensis* Aubl., *Souroubea* sp. (Marcgraviaceae) and *Pelliciera rhizoporae* Planch. & Triana (Tetrameristaceae).

DNA extraction and amplification

Total genomic DNA was isolated from fresh and silica dried leaves using a CTAB protocol (Aras *et al.*, 2003) modified by adding 3% PVP-40 to the extraction buffer to bind phenolic compounds. Primers *ITS5* [5′-GGAAGTAGAAGTCGTAACAAGG-3′] and *ITS4* [5′-TCCTCCGCTTATTGATATGC-3′] (White *et al.*, 1990) were used for standard PCR amplification of the nuclear ribosomal internal transcribed spacer (ITS) region in 25 μl volume. The *ITS* reaction mixture contained 2.5 μl 10× PCR buffer, 1.5 μl dNTPs (10 pmol), 1.5 μl $MgCl_2$ (25 mM), 2 μl (10 pmol) each of forward and reverse primers, 0.2 μl *Taq* polymerase (5 units/μl, 1 μl (10–20 ng) genomic DNA and H_2O to make up the total volume to 25 μl. To amplify the *atpB-rbcL* spacer the following primers were designed and used for amplification: *Imp-atpB* [5′AGCCGCAGGACCAGAAGTAG3′] and *Imp-rbcL* [5′TGGTGACATAAATCCCTCCCTA 3′]. The *atpB-rbcL* spacer reaction mixture contained 2.5 μl 10× PCR buffer, 2 μl dNTPs (10 pmol), 1.5 μl–2.5 μl $MgCl_2$ (25 mM), 1 μl (10 pmol) each of forward and reverse primers, 0.2 μl *Taq* polymerase (5 units/

μl), 1 μl (10–20 ng) genomic DNA and H_2O to make total volume 25 μl. The PCR amplifications were performed in a BIORAD thermal cycler programmed for 95°C for initiation, followed by 35 cycles of 45 sec at 94°C, 45 sec at 55°C–57°C for *ITS* and 50°C–52°C for *atpB-rbcL* spacer, 1 min at 72°C, with a final extension period of 7 min at 72°C. The amplified products were run on 1% agarose gels, visualized, excised, purified using Gel PCR DNA extraction Kit (Geneaid) and sequenced at the Central Instrumentation Facility, University of Delhi, South Campus using Applied Biosystems 3730 Genetic Analyzer.

Data Analyses

The newly obtained sequences were combined with sequences obtained from TreeBase, an online database of published phylogenetic datasets and trees (www.treebase.org, study number S1443: Janssens *et al*., 2006). Sequences of the two regions *ITS* and *atpB-rbcL* spacer were aligned, both separately and combined, using the programme Maaft-Q (Katoh & Standley, 2013; www.ebi.ac.uk/Tools/msa/mafft/). The aligned output in fasta form was converted to the appropriate format (either nexus or Phylip) for phylogenetic estimation. The best fit substitution model (GTR) with gamma correction for rate heterogeneity (G) was selected using the likelihood ratio test as implemented in ModelTest 3.06 (Posada & Crandall, 1998). To determine whether the data could be combined, it is necessary to evaluate the heterogeneity of the data, i.e., whether they produce differing and conflicting phylogenetic signals. If significantly different, the data would have to be analysed separately (Huelsenbeck & Bull, 1996; Cunningham, 1997). This test was conducted using the stepping stone method in MrBayes ver. 3.2 (Huelsenbeck & Ronquist, 2001).

Phylogenetic Analysis

Both datasets were analysed separately using ML and Bayesian methods. The ML analyses were done using software RaxML (Stamatakis, 2006; https://www.phylo.org). The analyses were run with the following parameters: GTR+G, 1000 bootstrapping replications. Bayesian phylogenetic analyses were done using MrBayes ver. 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Four chains (one cold, three heated), were initiated using autoclose option and stop value less than 0.01 and 25% burnin. The analyses were run on The CIPRES Science Gateway V. 3.3 (https://www.phylo.org/). The resulting trees were analysed using the programme FigTree (http://tree.bio.ed.ac.uk/software/figtree) (Rambaut, 2009).

Ancestral Area Reconstruction

On the basis of the presence of at least one endemic species the distribution of *Impatiens* was coded as follows: China (A), SE Asia (B), Europe (C), India (D), Africa (E), and America (F). The analyses were done on 1000 trees sampled from the posterior distribution obtained by BEAST analyses. We used parsimony as applied in the programme S-DIVA implemented in RASP (Yu *et al*., 2015). S-DIVA overcomes problems of uncertainties in phylogeny; the frequencies of an ancestral range at a node in most parsimonious reconstructions of ancestral states are averaged over all trees and each possible ancestral range at a node is weighted by the frequency at which the node occurs among all trees. These ancestral ranges at each node were reconstructed for *Impatiens*; preliminary analyses were also done for *Ceropegia, Hoya, Indigofera, Strobilanthes* and *Vitis*. The original *Begonia* study included area reconstructions, which were used in discussion. Analyses were not conducted for other genera because either their phylogenetic trees contained very few Indian species (*Rubus*) or the phylogenetic trees were unresolved and thus could not be used (*Arisaema*).

Estimation of divergence times

Divergence times were estimated using Bayesian methods as applied in BEAST ver. 1.75 (Drummond *et al*., 2012). The desired input file, in XML format, was obtained using the programme BEAUTi (part of the BEAST package) to convert the nexus format aligned file. The programme generates a distribution of phylogenetic trees on which the time estimates are done. The same nucleotide substitution model (GTR+G) and the following settings were applied: Uncorrelated Log-normal clock model (Relaxed Clock, to handle heterogeneity of molecular rates), Yule tree prior (assumes a steady rate of speciation, 1, and no extinction). The MCMC protocol was set up for 8 million generations. To estimate time, calibration is needed; however reliable fossils are not available for Balsaminaceae, therefore we used secondary calibration, i.e., dates from previous, more inclusive studies that included *Impatiens* and *Hydrocera* (Bremer *et al*., 2004; Janssens *et al*., 2009). We defined the two most common recent ancestors (mrca), or nodes, that we wanted to calibrate: one, the mrca of *Impatiens* and *Hydrocera* and two, the mrca of *Impatiens* (the clade consisting of all *Impatiens* species). The dates used were 30.7 ma (SD

= ± 8.6 ma) for the divergence between *Impatiens* and *Hydrocera* and 22.5 ma (SD = ± 5.6 ma) for the crown group of *Impatiens* (Bremer *et al*., 2004; Janssens *et al*., 2009). The output tree file contains trees sampled in BEAST in nexus format. It was analysed in TreeAnnotator (part of the BEAST package) to get the maximum clade credibility tree, which was examined in FigTree to obtain the time estimates. The BEAST log file was analysed in Tracer to estimate the Standard deviation of the node ages. The Highest Probability Density (HPD 95%) was estimated for nodes.

Results

Nine disjunct genera of Western Ghats and northeastern India

In the following section we review phylogenetic studies of nine genera: *Arisaema*, *Begonia*, *Ceropegia, Hoya, Indigofera, Rubus, Strobilanthes*, *Vitis* and *Impatiens* to see what these studies reveal about the biogeography and systematics of these genera.

Arisaema Mart. (Araceae) includes *c*. 170 species all around the world (Mabberley, 2008). Of these, 16 are endemic to India, and of which, 12 have disjunct distributions in the Western Ghats and the northeastern India (Singh *et al.,* 2015). The remaining four species occur only in the Western Himalaya. A global phylogenetic study of this genus included 81 species of the *Arisaema* (Renner *et al*., 2004). The sampling from India and Africa was limited, including just six species from India: *Arisaema album* N.E. Br., *A. tortuosum* (Wall.) Schott (Meghalaya), *A. speciosum* (Wall.) Mart. (Arunachal Pradesh), *A. propinguum* Schott (West Bengal), *A. jaquemontii* Blume (Himachal Pradesh) and *A. murrayi* (J. Graham) Hook. (Maharashtra). The phylogenetic tree has a basal polytomy of many lineages, of which just those relevant to our discussion are shown (**Fig. 1**). As is obvious, this prevents definitive reconstructions of ancestral ranges. Five of the Indian species occur in two different clades: *A. album* and *A. ostiolatum* H. Hara (northeastern India) are closely related to Chinese and SE Asian species, while *A. speciosum* (northeastern India) is most closely related to African and Chinese species, suggesting at least two dispersals. In India, the earliest introduction has been estimated at 45 ma based on an analysis of the family that included just one species of *Arisaema* (Nauheimer *et al.,* 2012). This coincides with the collision of India with Eurasia (65–34 ma). Therefore, the dispersal and divergence of *Arisaema* in India may have taken place after the collision of the subcontinent with Eurasia.

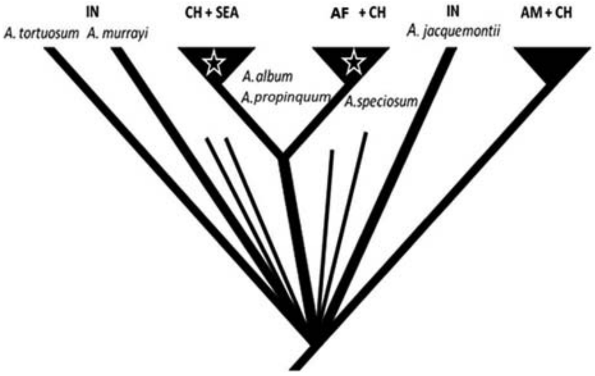

Fig. 1. Summary of phylogenetic patterns of the geographic distribution of species of *Arisaema*, after Renner *et al.* (2004). [IN – India, SEA – South East Asia, AF – Africa, CH – China, AM – America].

Begonia L. (Begoniaceae) is a large genus containing *c*. 1400 species around the world (Forrest *et al*., 2005; Mabberley, 2008). Asian *Begonia* species are divided into 20 sections and contain *c*. 750 species, of which 56 species are in India (Uddin, 2007). Not all these 56 species are ascribed to sections. The global phylogenetic study of Forrest *et al.* (2005) contained 64 species of *Begonia* including 6 Indian species: *B. dipetala* Graham (Tamil Nadu), *B. floccifera* Bedd. (Kerala), *B. malabarica* Lam. (Tamil Nadu), *B. sikkimensis* A. DC. (Arunachal Pradesh), *B. sizemoreae* Kiew (northeastern India) and *B. palmata* D. Don (Arunachal Pradesh). Phylogenetic analyses of plastid and nuclear sequences provide evidence for the non-monophyly of Asian sections (Thomas, 2010).

Indian Begonias are nested in three different positions in a large Asian clade (**Fig. 2**). One species,

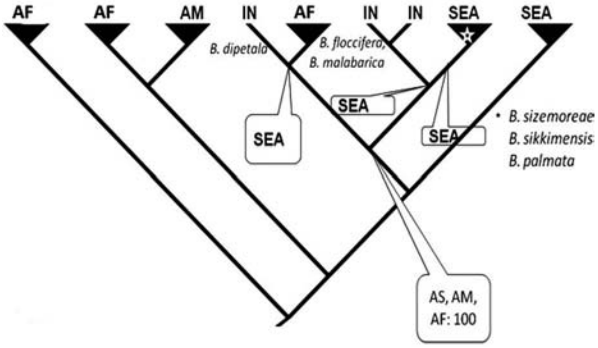

Fig. 2. Summary of phylogenetic patterns of the geographic distribution of species of *Begonia*, after Thomas *et al*. (2012). Nodes discussed in the text are marked with ancestral ranges reconstructed by parsimony (S-DIVA), number denotes marginal probability of the reconstruction. [SEA – South East Asia, AF – Africa, AM – America].

B. dipetala occurs in clade with two African species, *B. floccifera* and *B. malabarica* form a clade that is sister to a clade of SE Asian species. The third Indian clade consists of *B. sikkimensis*, *B. sizemoreae* and *B. palmata*, and is nested within this SE Asian clade. The diverse positions of Indian species imply at least three separate origins of Indian species indicating at least three separate dispersals: one, *B. dipetala; B. floccifera* and *B. malabarica*; two, *B. sikkimensis* and three, *B. palmata* and *B. sizemoreae* (**Fig. 2**). The genus was suggested to have diversified sometime between Late Cretaceous (*c.* 65 ma) and early Neogene (*c.* 15 ma) (Goodall-Copestake *et al.*, 2009), with origin of the Asian clade at *c.* 16 ma (Thomas *et al.*, 2012). The earliest dispersal into India from SE Asia occurred soon after that (*c.* 15 ma) (**Fig. 2**). The relationship of *B. dipetala* with African species *B. socotrana* Hook.f. and *B. samhaensis* M. Hughes & A.G. Mill., shows a connection between India and Africa. However, this phylogenetic relationship is not likely to be the result of Gondwanan affinity because their common ancestor is dated at Miocene (13.75 ma), which is much more recent than the collision of India with Eurasia (65–34 ma). A comprehensive understanding of the diversification of *Begonia* in India requires much more sampling.

Ceropegia L. (Apocynaceae: Ceropegieae) has around 200 species (Meve, 2002). There are *c.* 50 species in India, of which 33 are endemic to the country (Surveswaran *et al.*, 2009). Endress & Bruyns (2000) placed *Ceropegia* and *Brachystelma* R. Br. under the tribe Ceropegieae, but Surveswaran *et al.* (2009) and Bruyns *et al.* (2015) showed that *Brachystelma* is nested within *Ceropegia*. Thus, the tribe Ceropegieae is monophyletic, but the genus *Ceropegia* is paraphyletic.

Here we discuss the geographic distributions of a broadly defined *Ceropegia* complex according to these phylogenetic results (**Fig. 3**). Indian Ceropegias are closely related to SE Asian *Brachystelma* within an Afro-Asian clade. From Africa a lineage may have dispersed into India and SE Asia (as revealed by our S-DIVA analyses, **Fig. 3**). There are no time estimates for *Ceropegia*; the earliest fossil record for Apocynaceae is Early Miocene (*c.* 53 ma) (Magallon & Sanderson, 2001). Based on this calibration the divergence time of the tribe Ceropegieae was estimated to be at around 25 ma and the divergence time of the *C. juncea* (Western Ghats), estimated at around 10 ma (Rapini *et al.*, 2007). Other divergence times estimated included *C. saxatilis* Jum. & H. Perrier and *Stapelia glanduliflora* Masson (nested within *Ceropegiaeae*) at *c.* 5 ma and 1.5 ma, respectively; these calibrations could be used in the future to infer divergence times of other Indian species.

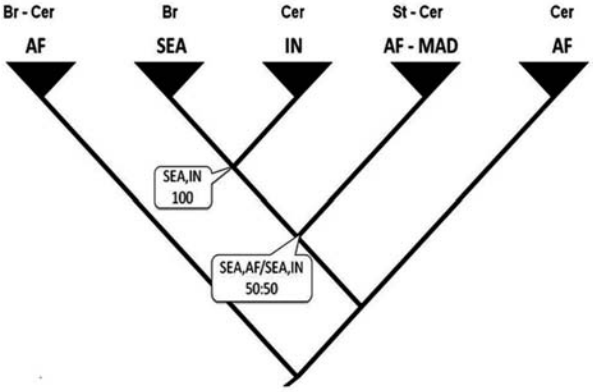

Fig. 3. Summary of phylogenetic patterns of the geographic distribution of species of *Ceropegia (sensu lato)*, after Bryuns *et al.* (2015). Nodes discussed in the text are marked with ancestral ranges reconstructed by parsimony (S-DIVA), numbers denote marginal probabilities of reconstructions. Star represents *Ceropegia.* [IN – India, SEA – South East Asia, AF – Africa, MAD – Madagascar, Br – *Brachystelma*, Cer – *Ceropegia*, St – Stapeliads].

Hoya R. Br. (Apocynaceae: Marsenieae) contains *c.* 200 species around the globe (Mabberley, 2008) and 28 species and 1 variety are in India (Jagtap & Singh, 1999; Karthikeyan *et al.*, 2009). Recently *Hoya* has been subdivided into 14 or 16 sections (Burton, 1985, 1995, 1996; Kloppenburg, 2001a, b). A recent phylogenetic analysis of plastid and nuclear markers in *Hoya* includes 36 species of which four are Indian: *Hoya serpens* Hook.f., *H. edeni* King ex Hook.f., *H. retusa* Dalzell and *H. pauciflora* Wight (Wanntorp *et al.*, 2006). Indian species occur in two separate clades. One clade consists of Indian, Australian and SE Asian species. The other clade consists of Indian and Australian species (**Fig. 4**). The most recent common ancestor of Indian species in each clade was Australian, Indian or SE Asian; ultimately these descended from a SE Asian species. The plastid and nuclear trees are in conflict and, while the authors do not seem to have discussed this point, the topologies suggest hybridization events. Given this reticulate pattern, it is difficult to speculate on dispersal patterns involved.

No molecular dating has been done for *Hoya.* Using the earliest fossil record of family Apocynaceae (Miocene, *c.* 53 ma) as age constraint, the divergence time between *Hoya australis* and close relatives *Dischidia bengalensis* Colebr. and *Micholitzia obcordata* N.E. Br. was estimated to be *c.* 15 ma by Rapini *et al.* (2007). These dates can be used as secondary calibrations to estimate the divergence times of *Hoya* species.

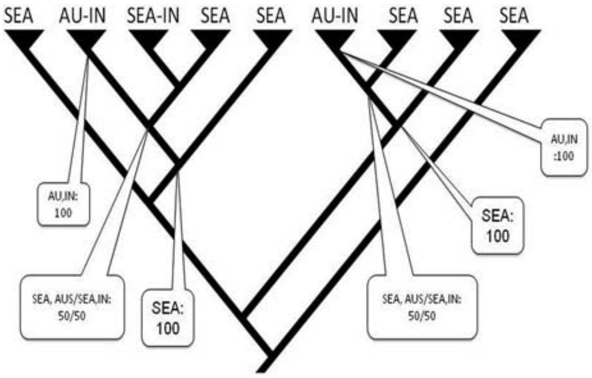

Fig. 4. Summary of phylogenetic patterns of the geographic distribution of species of *Hoya*, after Wanntrop *et al*. (2006). Nodes discussed in the text are marked with ancestral ranges reconstructed by parsimony (S-DIVA), numbers denote marginal probabilities of reconstructions. [IN – India, SEA – South East Asia, AU – Australia].

Indigofera L. (Fabaceae) is a genus of *c.* 730 species having a worldwide distribution (Mabberley, 2008), and there are *c*. 60 species in India (Sanjappa, 1992). Phylogenetic study of 92 *Indigofera* species shows that the genus is monophyletic and consists of four major clades that the authors refer to as palaeotropical, pantropical, Cape and Tethyan (Schrire *et al*., 2009). This study included 12 Indian species: *I. mysorensis* Rottler ex DC., *I. angulosa* Edgew., *I. cassioides* Rottler ex DC., *I. dalzellii* T. Cooke, *I. glandulosa* J.C. Wendl., *I. nigrescens* Kurz ex King & Prain, *I. uniflora* Buch.-Ham. ex Roxb., *I. trita* L.f. (Western Ghats), *I. himalayensis* Ali, *I. heterantha* Wall. ex Brandis, *I. hebepetala* Benth. ex Baker (Himalaya) and *I. atropurpurea* Buch.-Ham. ex Hornem. (northeastern India). *Indigofera mysorensis* and *I. uniflora* are in a clade (IN1) with species from SE Asia, nested in the palaeotropical clade (**Fig. 5**). Within the pantropical clade is nested a clade (IN2) made up of species from Himalaya, Western Ghats and northeastern India -- *I. himalayensis, I. heterantha, I. hebepetala, I. nigrescens, I. cassioides* and *I. atropurpurea*. Nested within this clade are the Nepalese species *I. dosua* Buch.-Ham. ex D. Don and Chinese species *I. amblyantha* Craib. The remaining Indian species fall in the Tethyan clade, which consists of two major clades. *Indigofera glandulosa* falls in one of these clades (IN3) and is closely related to Asian-Australian species, while *I. dalzellii, I. angulosa* and *I. trita* fall in three different places within the other major clade. All the relationships indicate biogeographic connections mainly with African and other Asian species. There were at least three dispersals of *Indigofera* species into India from Africa (including Socotra). The origin of *I. glandulosa* is uncertain. The dates of the paleotropical and Tethyan clades are estimated to *c.* 13 ma, so the dispersals of ancestral IN1 and IN3 must have occurred after that date (Schrire *et al*., 2003; Schrire *et al*., 2009).

Rubus L. (Rosaceae) is found everywhere on the globe except Antarctica and contains *c*. 250 species (Mabberley, 2008), of which *c*. 75 species are estimated to occur in India (Dash *et al*., 2016). The most recent global phylogenetic systematic treatment of *Rubus* included 56 species in 12 subgenera (Alice & Campbell, 1999). In general, the monophyly of subgeneric groups was not supported. The study included only one Indian (Himalayan) species, *Rubus nepalensis*, (Hook.f.) Kuntze, which was found to be sister to the Japanese species, *R. pectinellus* Maxim. This close relationship of the Himalayan and Japanese species might be the result of either long distance dispersal or dispersal through China (followed by extinction in China). It has been assumed that *Rubus* originated in China, because of the high species diversity in that area, but phylogenetic results do not support this hypothesis (Alice & Campbell, 1999). Rosales originated *c*. 76 ma (Wikstrom *et al*., 2001) – or even earlier – 96–85 ma (Wang *et al*., 2009); Rosaceae diverged *c*. 69 ma and Rosoideae *c*. 54 ma (Smedmark *et al*., 2003). These dates can be used to infer divergence time estimates of *Rubus* in India, which would be less than 54 ma (as Rosideae diverged at *c*. 54 ma). At present insufficient sampling of Indian species

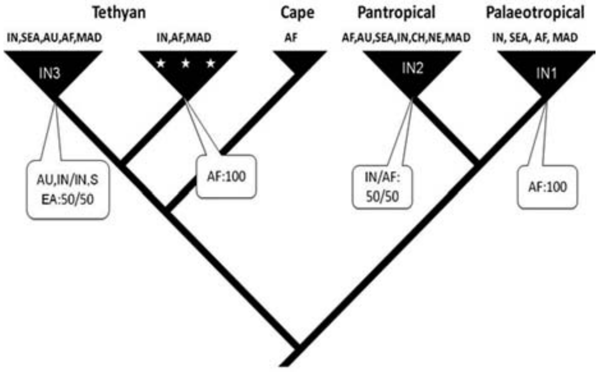

Fig. 5. Summary of phylogenetic patterns of the geographic distribution of species of *Indigofera*, after Schrire *et al*. (2009). Nodes discussed in the text are marked with probabilities of reconstructions. [IN – India, SEA – South East Asia, AF – Africa, MAD – Madagascar, CH – China, NE – Nepal, AU – Australia, IN1 – *I. mysorensis* and *I. uniflora*, IN2 – *I. heterantha, I. hebepetala, I. cassioides, I. atropurpurea,* and *I. nigrescence*, IN3 – *I. glandulosa*, stars represent *I. angulosa, I. trita,* and *I. dalzellii*].

precludes any further comments on the history and biogeography of *Rubus*.

Strobilanthes Blume s.l. (Acanthaceae) is a genus of *c*. 400 species (Mabberley, 2008), of which 149 species occur in India where it has a disjunct distribution (Karthikeyan *et al.*, 2009). A phylogenetic study by Moylan *et al*. (2004) included 30 species from Himalaya and southern India. The genus was found to be non-monophyletic, forming a well-supported clade with five other genera *Hemigraphis* Nees, *Stenosiphonium* Nees, *Aechmanthera* Nees, *Sericocalyx* Bremek. and *Clarkeasia* J.R.I. Wood (Moylan *et al*., 2004). This group of genera has been proposed to comprise a greatly expanded *Strobilanthes* s.l. (Moylan *et al*., 2004). Overall, *Strobilanthes* s.s. is a basally paraphyletic genus within which are nested the other genera mentioned.

Two species of *Hygrophila* R. Br. (Malay Peninsula) are sister to two major clades, of which one clade consists of southern Indian/Sri Lankan species with *Stenosiphonium* and species of *Sericocalyx* nested within. The other clade consists of a basal paraphyletic group of mainly Javanese and Himalayan species, within which is embedded in a clade consisting of *Hemigraphis*, other *Aechmanthera* and *Clarkeasia* (**Fig. 6**). *Strobilanthes* may have originated or, at least, diversified in peninsular India; its sister group (*Hemigraphis* spp.) and one of putative sisters of this larger clade (*Hygrophila*) occur in the Indomalesian region, therefore an origin and later extinction in the SE Asian region cannot be ruled out. This is especially the case because bootstrap support for the major clades is poor. There were dispersals from SE Asia and also back to SE Asia. There are no fossils dates of *Strobilanthes.* However, *Hygrophilla* has been estimated as 20.4 ma old by Tripp & McDade (2014). These estimates could be used as secondary calibrations in future analyses.

Vitis L. (Vitaceae) contains *c*. 65 species in the world (Mabberley, 2008) and 2 species and 1 variety in India (Shetty & Singh, 2000). They are mostly confined to temperate regions and grow as lianas. A recent molecular phylogenetic study of Vitaceae includes 30 species of *Vitis*, of which 3 were from India (*V. flexuosa* Thunb., *V. jaquemontii* R. Parker and *V. quinquangularis* Rehder). The study suggests that the genus *Vitis* is monophyletic and identifies two sister clades corresponding to subgenera *Vitis* L. and *Muscadinia* (Planch.) Small, congruent with the morphological classification (Tröndle *et al*., 2010). Of the Indian species *V. flexuosa*, which occurs in northeastern India, is closely related to *V. cinerea* (Engelm.) Millardet, a North American species, while *V. jaquemontii* (Himachal Pradesh) and *V. quinquangularis* (northeastern India) are closely related to Chinese *Vitis.* The published phylogeny does not include the other Indian species and it is too premature to say anything about the origin of disjunctions in India. Recent study on family Vitaceae has shown that *Vitis* is an old world genus that may have arisen *c*. 37.3 ma (Liu *et al*., 2015).

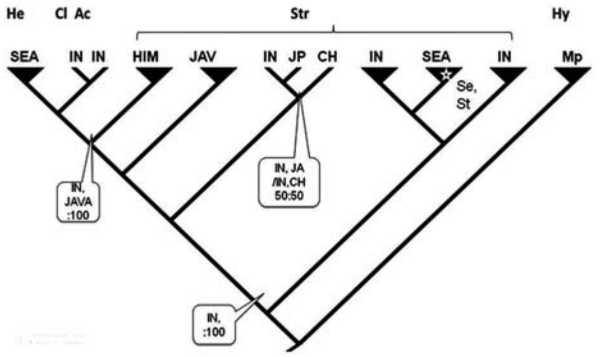

Fig. 6. Summary of phylogenetic patterns of the geographic distribution of species of *Strobilanthes*. Nodes discussed in the text are marked with ancestral ranges reconstructed by parsimony (S-DIVA), with number denoting marginal probabilities of reconstructions. [IN – India, HIM – Himalaya, JAV – Java, CH –China, He – *Hemigraphis*, Cl – *Clarkeasia*, Ac – *Acmenthera*, Str – *Strobilanthes*, Hy – *Hygrophila*].

Impatiens (Balsaminaceae) The present phylogenetic study included 82 taxa (ingroup and outgroup), of which 9 are newly added Indian species (11 accessions) of *Impatiens* (including *I. majumdarii* Ghara & Ghora from the north eastern state, Meghalaya) thus making a total of 16 Indian species. Two datasets were generated, *ITS* and *atpB-rbcL*, by combining new sequences with published sequences. The molecular models GTR+I+G and TVM+G were found to be best fitted for *ITS* and *atpB-rbcL* spacer sequences, respectively. Heterogeneity was insignificant, as inferred from the homogeneity test ($p = 0.01$) and the stepping stone analyses for data partition (marginal likelihood for unpartitioned data, -8653.77, was higher than for the partitioned data, -8657.55), allowing us to analyse the combined data. The combined *ITS* and *atpB-rbcL* multiple sequence alignment consisted of 2131 characters.

Impatiens species in India occur in multiple clades

Both ML and Bayesian analyses of individual datasets resulted in similar topologies and we show just the ML topology here (**Fig. 7**). Clearly,

Fig. 7. Strict consensus bootstrap tree resulting from Maximum Likelihood (ML) analyses of the 82 taxa combined *ITS* and *atpB-rbcL* spacer dataset. Bootstrap values are reported above the branches. Blue branches represent Indian species.

the genus *Impatiens* is monophyletic, but Indian *Impatiens* do not form a clade as also seen in previous studies (Janssens, 2006, 2007, 2009). The 16 species sampled from India occur in four different clades, two more than in previous studies (Janssens *et al.*, 2009; **Fig. 7**). Clade (A), including *I. glandulifera* Royle, a Himalayan species, is separate from the other Indian species and sister to the southern Chinese species, *I. chungtienensis* Y.L. Chen; the European species, *I. parviflora* DC. is also in this well-supported clade. The second clade (B) includes *I. viscida* DC., *I. campanulata* Wight and *I. clavicornu* Turcz. from southern India (BS = 100%). These species are closely related to southern Chinese species, but with low bootstrap support (46%). The third clade (C) includes *I. kleiniiformis*, *I. minor* (DC.) Bennet, *I. gardneriana* Wight ex Hook.f. and *I. majumdarii* from India (BS = 84%). *Impatiens majumdarii*, a northeastern Indian species, is sister to a group of southern Indian species. The species of clade C are most closely related to African species. The fourth clade (D), contains *I. balsamina* L., *I. scabriscula* B. Heyne ex Wall., *I. pendula* B. Heyne ex Wight & Arn., *I. pulcherrima* Dalzell, *I. cuspidata* Wight & Arn., *I. latifolia* L. and *I. leschenaultii* (DC.) Wall., African species and the Chinese species *I. aureliana* Hook.f.

Impatiens viscida and *I. campanulata* (Section Sub-Umbellatae: Bhaskar, 2012) form a clade with *I. clavicornu* (Section Scapigerae: Bhaskar, 2012). *Impatiens chinensis* L. and *I. latifolia* occurred in different clades, but are classified in section Enantiophyllon (Warburg & Reiche, 1895). These results are well-supported by bootstrap values. Thus, these molecular phylogenetic results are not congruent with some aspects of the taxonomy of the genus. However, much more sampling needs to be done before conclusive statements can be made regarding this point.

Multiple dispersals between India and Africa; India and China

The results of analyses using S-Diva suggest 12 dispersal and 11 vicariance events **(Fig. 8)**. The common ancestor of clade A probably was distributed in China, Europe and India (marginal probability 100%); this ancestor itself was derived from a Chinese species (marginal probability 100%). Thus, the Himalayan species *I. glandulifera* may have been derived directly from a Chinese ancestor or indirectly via an European ancestor. Clade B, an entirely Indian clade, was derived from a Chinese-Indian (marginal probability 100%) species that gave rise to this Indian clade and its Chinese sister clade. Clade C contains six Indian species, derived from a common ancestor that was distributed in India (marginal probability 100%), and itself probably descended from an African-Indian species (marginal probability 100%). This Indian clade gave rise to *I. platypetala* Lindl., a SE Asian species. Clade D contains African and Indian species, again derived from an ancestral African-Indian species (marginal probability 91.4%) that, ultimately, had an African ancestor (marginal probability 95%). Clades B, C and D shared a common ancestor that may equally likely have occurred in India-Africa, India-China or India-Africa-China. According to this analysis, the ultimate ancestor of *Impatiens* may have occurred in China (marginal probability 85.5%).

Dispersals into India occurred between 11 and 4.5 ma

The species of *Impatiens* in India occur in four different clades that originated from species widespread either in India-Africa or in India-China or in India-Africa-China (**Fig. 8**). According to divergence time analyses of the present data, the common ancestor of Clade B is estimated to be around 8 ma (95% HPD: 2.96–13.26 ma), and the common ancestor of Clade A is estimated to be around 4.5 ma (Clade A, HPD: 1.41–9.06 ma). This implies that dispersal/s between India and China were at least as old as 8 ma and at least as recent as 4.5 ma. These age estimates are slightly older than that suggested by previous analyses that estimate dispersals into India as recent as 6.5 to 2 ma from China (Janssens *et al.*, 2009). Mid Miocene climatic optima (17–11 Ma) are considered as warm and humid globally (Zachos *et al.*, 2001). Dispersal of biota between China and India through the Himalayan corridor could have taken place during that period. Similarly, in the present study, dispersal between India and Africa is estimated to be at least as old as 10.5 ma (Clade C, HPD: 5.06–15.5 ma) and as recent as 7.6 ma (Clade D, HPD: 4.07–11.38 ma). At *c.* 14 ma India was wetter than at present; climatic fluctuation and drying started at *c.* 5 ma to 1 ma (Patnaik & Prasad, 2016). In particular, the northwestern part of the country and adjoining regions into Africa are supposed to have been humid until *c.* 10 ma (Herrera *et al.*, 2016). The upper limits of the estimates of exchanges between India and Africa, at 15.5 to 11.38 ma (Clades C and D, respectively) support the possibility of dispersal of *Impatiens* between these regions during this wet period. It must be kept in mind that the present estimates are constrained by the fact that they are based on secondary calibrations.

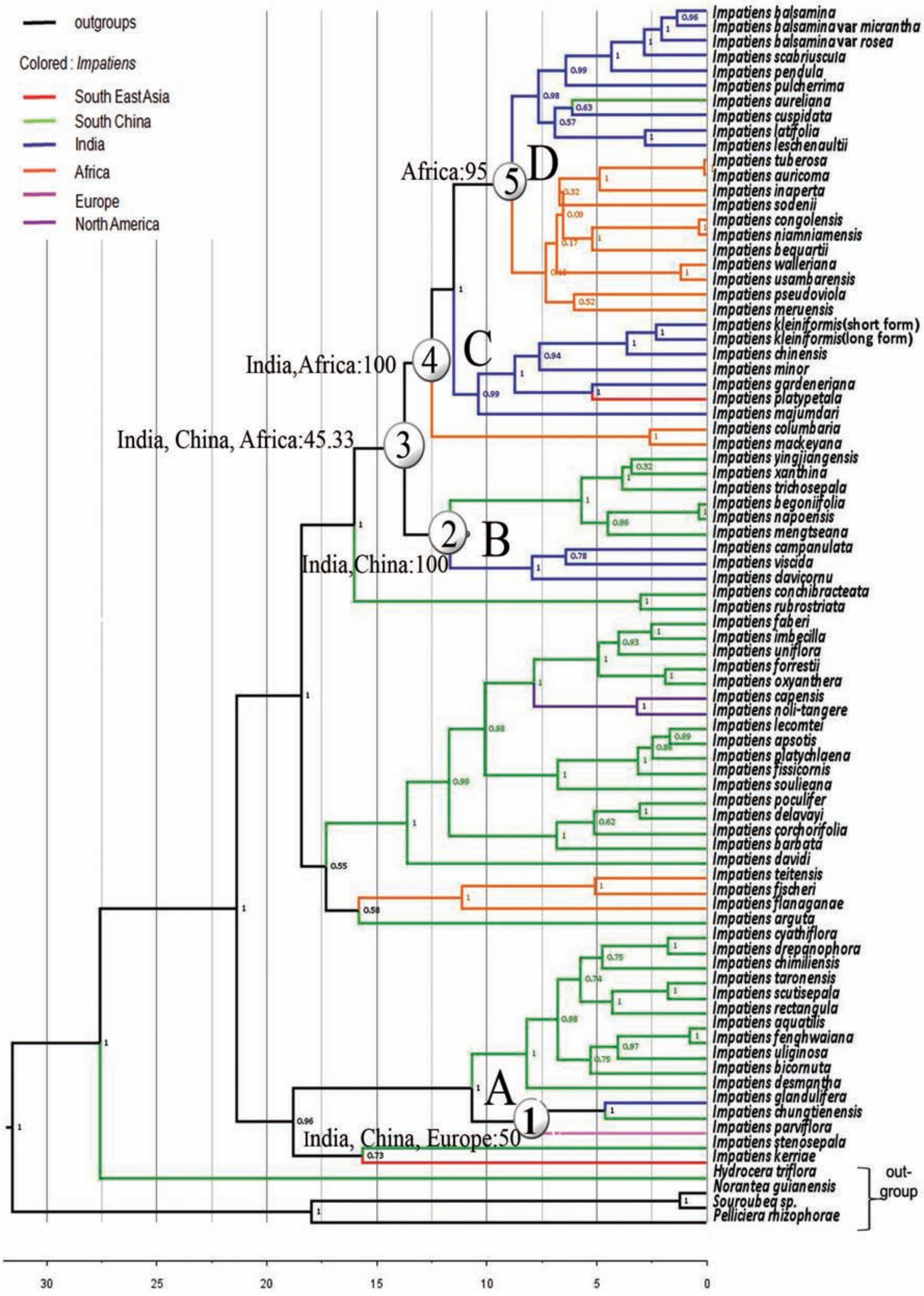

Fig. 8. Bayesian chronogram (generated using software BEAST) based on the combined *ITS* and *atpB-rbcL* dataset. Different colours indicate different areas of distribution as indicated by the colour legend. Nodes discussed in the text are marked with most probably ancestral ranges as reconstructed by maximum parsimony (S-DIVA), with number denoting marginal probabilities of reconstructions.

A tentative history of Impatiens in India

An Indochinese-European ancestor (1) (**Fig. 8**) dispersed into Himalaya at around 4.5 ma during Pliocene (Clade A). This is the earliest introduction into India either directly or via Europe. Indo-Africa-Chinese ancestor (3) diverged to give two descendants (Ancestor 2 and Ancestor 4). Ancestor 2 may have dispersed into India at around 8 ma (Clade B). An Indo-African descendant of Ancestor 4 dispersed into India at *c.* 10.5 ma to give rise to Clade C. The other, African, descendant of 4 dispersed to India at around 7.6 ma to give rise to Clade D.

The last two dispersal events could have taken two possible routes. One by long transoceanic dispersal as seen in the case of Melastomataceae (Renner *et al*., 2001). The second possibility is over land – when the ancestor of Clade C migrated from Africa to India (15.5–5.06 ma), climatic conditions were wet in the Indo-African region (Mid Miocene climatic optima) (Zachos *et al*., 2001). However, the only documented cases of such dispersal are of birds (Boere *et al*., 2006). As these times of divergence are very recent, it is impossible that the African and Indian species share an ancient Gondwanan connection.

Dispersal events were not only restricted into India. Clearly there has been dispersal out of India into China (as in Clade D) and into SE Asia (Clade C). Dispersals have taken place within India as well. Our study includes one species from northeastern India, *I. majumdarii*, which is nested within a group of southern Indian species. This result shows that at least one group of southern Indian *Impatiens* shares common ancestry with northeastern *Impatiens.* So, this suggests that either there has been dispersal from northeastern India to Western Ghats or vice-versa, or that in the past there might be a continuous dispersal route between the two regions for the distribution of *Impatiens*. This could be said clearly only after enough sampling of *Impatiens* is done in future phylogenetic studies.

Conclusions

As seen in the above nine examples, phylogenetic study answers not only systematics questions but also biogeographic ones. Our literature survey (2012–13) on disjunct genera revealed just nine studies that included at least one Indian species. Our analysis of phylogenetic studies of these genera is consistent with the general consensus that the Indian flora contains Chinese, SE Asian and African and European influences. The collision of India with Eurasia (65–34 ma) may have played a crucial role in the dispersal of some taxa into India. Some of these dispersals occurred soon after the collision (e.g., *Arisaema* at 45 ma), while others were more recent (*Begonia* at 15 ma from SE Asia). Dispersals occurred between India and Africa, but do not represent ancient Gondwanan connections (e.g., *Begonia* at 13.75 ma and *Indigofera* 13 ma). In other cases, we cannot assign times, but can say that dispersal occurred from Africa or SE Asia (*Ceropegia*), SE Asia (*Hoya*, *Strobilanthes*) and China (*Vitis*).

Impatiens is one of the largest genera of angiosperms and contains more than 200 species in India; of these only 16 species India are included in the present phylogenetic study. Even this limited sampling has shown that there have been dispersals not only into India but also out of India. The close phylogenetic relationship of some Indian *Impatiens* with African species is a recent connection that might have occurred through of long distance dispersal. A comprehensive history and classification of Indian *Impatiens* will be possible by including more Indian *Impatiens* species in future phylogenetic studies.

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Appendix 1: List of species, with collection and sequence information (NCBI accession numbers for atpB-rbcL and ITS regions)

Impatiens gardeneriana Wight, Bengaluru, India, *Bhaskar* 892, *atpB-rbcL* KX271231, ITS KX261217; *Impatiens pendula* B. Heyne ex Wight & Arn., Bengaluru, India, *Bhaskar* 852(a), *atpB-rbcL* KX271230, ITS KX261216; *Impatiens balsamina* L. var. *rosea* (Lindl.) Hook.f., University of Delhi, India, *Renu* DE006, *atpB-rbcL* KX271229, ITS KX271229; *Impatiens balsamina* L. var. *micrantha* Hook.f., Delhi, India, *Renu* DE007, *atpB-rbcL* KX271228, ITS KX261210; *Impatiens chinensis* L., Kerala, India, *Renu* KE001, *atpB-rbcL* KX271232, ITS KX261218; *Impatiens kleiniformis* Sedgw. (long form), Goa, India, Geeta Go004, *atpB-rbcL* KX271233, ITS KX261219; *Impatiens kleiniformis* Sedgw. (short form), Goa, India, *Geeta* Go008, *atpB-rbcL* KX271238, ITS KX261211; *Impatiens majumdarii* Ghara & Ghora, Shillong, India, *Renu* SH005, *atpB-rbcL* KX271234, ITS KX261215; *Impatiens minor* (DC.) Bennet, Goa, India, *Geeta* Go003, *atpB-rbcL* KX271235, ITS KX261214; *Impatiens pulcherrima* Dalzell, Goa, India, Geeta Go011, *atpB-rbcL* KX271236, ITS KX261213; *Impatiens scabriuscula* (G. Don) B. Heyne ex Wall., Bengaluru, India, *Bhaskar* 850, *atpB-rbcL* KX271237, ITS KX261212

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