



Molecular Phylogeny of Balsams (Genus *Impatiens*) Based on ITS Regions of Nuclear Ribosomal DNA Implies Two Colonization Events in South India

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ABSTRACT

The present investigation is the first attempt of its kind to analyze the phylogenetic association of *Impatiens* species of South India by placing them in the global Internal Transcribed Spacer (ITS) phylogeny of *Impatiens*. Representative samples from all the seven sections of genus *Impatiens* reported from South India were collected. Total genomic DNA was extracted from fresh plant leaf and Polymerase Chain Reaction (PCR) was carried out using ITS specific forward and reverse primers. Forty seven sequences from representatives of seven sections of *Impatiens* species from South India and 45 *Impatiens* sequences from other centers of *Impatiens* diversity were obtained from GenBank and used to reconstruct the evolutionary relationships within the genus *Impatiens*. Maximum Parsimony (MP) analysis was conducted by using PAUP*v.4.0b10. Bayesian Inference (BI) analysis was carried out in MrBayes v.3.2.2. Molecular phylogeny of South Indian *Impatiens* spp. based on ITS sequences showed the same association as that of morphological taxonomy. This study including all representative extant sections of South India, indicates that South India was colonized from Southeast Asia by two independent dispersal events i.e., once by ancestral species of sections *Scapigeriae*, *Epiphyticae*, *Tomentosae*, *Sub-Umbellatae* and *Racemosae* and another time by ancestral species of sections *Microsepalae* and *Annuae*.

1. INTRODUCTION

The family Balsaminaceae comprises only two genera, *Impatiens* and *Hydrocera*. The genus *Impatiens* is a large genus consisting of more than 1000 species and is mainly distributed in mountain areas of old world tropics and subtropics [1]. The genus *Hydrocera* has a single species, *Hydrocera triflora*, a semi-aquatic herb native to the Indo-Malaysian countries [2]. Other generic names, for example, *Petalonema*, *Semeiocardium* and *Impatientella* are confirmed to be synonyms of the genus *Impatiens* [3, 4]. The species of *Impatiens* are significant as ornamentals, medicinals and experimental plant materials in botanical research [5].

Five biodiversity hotspots for *Impatiens* have been identified that is Southeast Asia, Southern India and Sri Lanka, Tropical Africa, Madagascar and the Eastern Himalayas. Several novel species are recognized in these regions every year [6, 7, 8]. A minute number of *Impatiens* species are represented in the temperate areas in Europe, Northern China and North America [1] with no indigenous species in South America and Australia [2]. High proportions of endemism are related with these hotspots, for example, nearly 91% of the Southern Indian species and almost all the native species of Madagascar are endemic [4]. In India, the concentration of *Impatiens* species is amazingly local and occurs in two well-defined regions, viz., the Himalaya in the North and the Western Ghats mountain ranges in the South. The genus *Impatiens* contains more than 210 species in India, over half of which occurs in the Western Ghats and at least 103 species of *Impatiens* are endemic to the Western Ghats [5]. Several hypotheses related to the origin of *Impatiens* spp were proposed based on species diversity, karyological data, morphological similarity etc. [9, 10].

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Bhaskar [11] suggested that South India contains majority of the phylogenetically old species and Western Ghats is the places of origin of *Impatiens* considering the concentration of diploids, primitive radial pollen grains and shrubby habit. Phylogenies of *Impatiens* species based on ITS sequences of Yuan *et al.* [2] revealed that extant *Impatiens* species are of Southeast Asian origin, from where it dispersed to boreal Eurasia and North America, to central Asia and Eastern Europe via the Himalayas, and to India and Africa.

Janssens *et al.* [1], *atpB-rbcL* spacer sequences phylogenetics suggested that *Impatiens* originated in South China from which it colonized the nearby regions and afterwards dispersed to North America, India, Africa, the Southeast Asian peninsula, and the Himalayan area. Based on morphological characters combined with sequence data from three genetic regions, including nuclear ribosomal ITS, plastid *atpB-rbcL* and *trnL-F*, Yu *et al.* [12] presented a new classification of *Impatiens*, in which *Impatiens* is divided into two subgenera, subgenus *Clavicarpa* and subgenus *Impatiens*.

The subgenus *Impatiens* is further subdivided into seven sections. In the published data of phylogeny and biogeography of Balsaminaceae inferred from ITS sequences of Yuan *et al.* [2] and phylogenetics of Balsaminaceae using chloroplast *atpB-rbcL* spacer sequences of Janssens *et al.* [1] contained only a few samples of *Impatiens* species from South India. Hence this can be considered as the first work on the molecular phylogeny of *Impatiens* species of South India including representative species from all seven sections of the genus *Impatiens* from South India.

2. MATERIALS AND METHODS

2.1 Taxon Sampling

Representative samples from the seven sections of *Impatiens* species were collected from different locations of Western Ghats. The plants were identified in the field with help of the taxonomic monograph on *Impatiens* L. (Balsaminaceae) of Western Ghats, South India [5].

The specimens were collected and brought to the lab for further molecular analysis. The plants were authenticated by Dr. Jomy Augustine (Taxonomist, Department of Botany, St. Thomas College, Palai, Kottayam, Kerala, India). Voucher specimens were deposited in the Herbarium of St. Thomas College (Palai, Kerala, India). The details of sample collection are summarized in Table 1.

2.2 Molecular Protocols

Total genomic DNA was extracted using Gen Elute Plant Genomic DNA Miniprep Kit (Sigma Aldrich, St. Louis, USA). For PCR amplification, OrionX h-Taq PCR Smart Mix (Origin, India) was used.

The primers used for the amplification of the ITS gene were ITS 4- 5'-GGAAGTAGAAGTCGTAACAAGG-3' and ITS 5- 5'-TCCTCCGCTTATTGATATGC-3' [13]. The temperature

profile consisted of 2 min initial denaturation at 95° C followed by 35 cycles of 30 s denaturation at 95° C, 30 s primer annealing at 50.9° C, 1 min extension at 72° C and a final extension of 8 min at 72° C.

Amplification reactions were carried out in an Agilent Sure Cycler 8800 (Agilent Technologies, USA). PCR products were sequenced (Scigenome Labs Pvt. Ltd., Cochin, Kerala, India) in ABI Cycle sequencer.

2.3 Data Matrices and alignment

ITS sequences were initially aligned using the CLUSTALW [14] algorithm in BioEdit V. 7.0.5.2 [15] applying the default parameters for gap opening and gap extension. Non- informative or ambiguous gaps were not coded. Only the indel coded data matrices were used for phylogenetic analyses.

2.4 Phylogenetic analysis

All sequences generated in this study were subjected to a search in BLAST (NCBI) against the GenBank nucleotide database and submitted to GenBank (Table 1). *H. triflora* was kept as out group (GenBank Accession No. AY348853). Sequences of *Impatiens* species from five diversity hotspots were collected from GenBank (Table 2). The sequences were multiple aligned using the CLUSTALW [14] program using BioEdit 7.0.5.2 [15].

Maximum parsimony (MP) analyses were conducted using PAUP*v.4.0b10 [16]. Heuristic searches were performed on a gap-coded data set with 10 replicates of the random addition option and one tree held at each step during stepwise addition. Branch-swapping algorithm was tree-bisection-reconnection (TBR) and steepest descent option off. Initial 'MaxTrees' setting was 100 and 'MulTrees' option was in effect. Characters were equally weighted and character states were specified to be unordered. Supports for various nodes were determined through 1000 bootstrap replications.

The Akaike information criterion (AIC) implemented in the program j-modelTest version 2.1.5 [17] was used to choose substitution models that best fit the dataset through hierarchical likelihood ratio tests and to estimate the transition-transversion rate ratio, gamma shape parameters and base frequencies under the best supported model.

Bayesian Inference (BI) analyses were carried out in MrBayes v.3.2.2 [18] in two independent runs, each with one heated chain and one cold chain and each run consisted of Markov Chain Monte Carlo (MCMC) methods to estimate the posterior distribution of model parameters for 1 lakh generations. Convergence occurred when standard deviation (SD) of split frequencies fell below 0.05; the first 25% of MCMC generations were discarded as burn-in. Posterior probability values were used to estimate branch support. Trees were visualized by Fig Tree, Tree Figure drawing tool version 1.4.2 [19].

Table 1: Sampled species used in this study with Location, Voucher No. and GenBank accession No.

SI. No.	Species name with Section	Location*	Voucher No. of sample deposited	GenBank Accession Number
Section: <i>Scapigerae</i>				
1	<i>I. acaulis</i>	Agasthyamala Biosphere Reserve	S.P.P.4862	KR011120
2	<i>I. levingei</i>	Eravikulam National Park	S.P.P.4854	KT225490
3	<i>I. modesta</i>	Eravikulam National Park	S.P.P.4857	KT225493
4	<i>I. pandata</i>	Eravikulam National Park	S.P.P.4856	KT225494
5	<i>I. scapiflora</i>	Vagamon	S.P.P.4502	KJ685214
Section: <i>Epiphyticae</i>				
6	<i>I. jerdoniae</i>	Wayanad	S.P.P.4525	KT225492
7	<i>I. violacea</i>	Wayanad	S.P.P.4858	KT225495
Section: <i>Annuae</i>				
8	<i>I. chinensis</i>	Munnar	S.P.P.4545	KF804101
9	<i>I. dalzellii</i>	Eravikulam National Park	S.P.P.4852	KT225491
10	<i>I. debilis</i>	Wayanad	S.P.P.4544	KF804102
11	<i>I. gardneriana</i>	Wayanad	S.P.P.4520	KF719156
12	<i>I. herbicola</i>	Neryamangalam	S.P.P.4505	KF719158
13	<i>I. ligulata</i>	Wayanad	S.P.P.4530	KF719159
14	<i>I. lucida</i>	Munnar	S.P.P.4519	KF719160
15	<i>I. minor</i>	Neryamangalam	S.P.P.4504	KF719162
16	<i>I. oppositifolia</i>	Eravikulam National Park	S.P.P.4855	KT254232
17	<i>I. raziana</i>	Eravikulam National Park	S.P.P.4851	KT254235
18	<i>I. rufescens</i>	Wayanad	S.P.P.4542	KT254236
19	<i>I. tomentosa</i>	Agasthyamala Biospher Reserve	S.P.P.4861	KT254238
Section: <i>Microcepalae</i>				
20	<i>I. bababudenensis</i>	Anamudi Hills	S.P.P.4548	KT254226
21	<i>I. balsamina</i>	Munnar	S.P.P.4517	KF804100
22	<i>I. cuspidata</i>	Munnar	S.P.P.4509	KF719153
23	<i>I. dasysperma</i>	Neryamangalam	S.P.P.4506	KJ685213
24	<i>I. flaccida</i>	Vagamon	S.P.P.4516	KT254228
25	<i>I. floribunda</i>	Wayanad	S.P.P.4521	KF719155
26	<i>I. latifolia</i>	Eravikulam National Park	S.P.P.4549	KT254231
27	<i>I. mysorensis</i>	Wayanad	S.P.P.4534	KF719164
28	<i>I. pulcherrima</i>	Eravikulam National Park	S.P.P.4853	KT254234
29	<i>I. scabriuscula</i>	Wayanad	S.P.P.4531	KF719168
30	<i>I. walleriana</i>	Munnar	S.P.P.4518	KF804104
Section: <i>Tomentosae</i>				
31	<i>I. henslowiana</i>	Munnar	S.P.P.4508	KT254230
32	<i>I. johnii</i>	Wayanad	S.P.P.4543	KF804103
33	<i>I. neo-munronii</i>	Wayanad	S.P.P.4523	KF719165
Section: <i>Sub-Umbellatae</i>				
34	<i>I. anaimudica</i>	Anamudi Hills	S.P.P.4550	KT254225
35	<i>I. cordata</i>	Munnar	S.P.P.4515	KT254227
36	<i>I. disotis</i>	Wayanad	S.P.P.4528	KF719154
37	<i>I. goughii</i>	Neryamangalam	S.P.P.4510	KF719157
38	<i>I. grandis</i>	Wayanad	S.P.P.4524	KT254229
39	<i>I. travancorica</i>	Eravikulam National Park	S.P.P.4547	KT254239
40	<i>I. umbellata</i>	Agasthyamala Biospher Reserve	S.P.P.4859	KT254240
41	<i>I. uncinata</i>	Wayanad	S.P.P.4529	KT254241
42	<i>I. viscida</i>	Munnar	S.P.P.4533	KF719166
43	<i>I. viscosa</i>	Munnar	S.P.P.4538	KF697232
Section: <i>Racemosae</i>				
44	<i>I. maculata</i>	Devikulam	S.P.P.4507	KF719161
45	<i>I. phoenicea</i>	Wayanad	S.P.P.4527	KT254233
46	<i>I. tangachee</i>	Eravikulam National Park	S.P.P.4860	KT254237
47	<i>I. wightiana</i>	Wayanad	S.P.P.4522	KF719167

*All locations in Kerala, India.

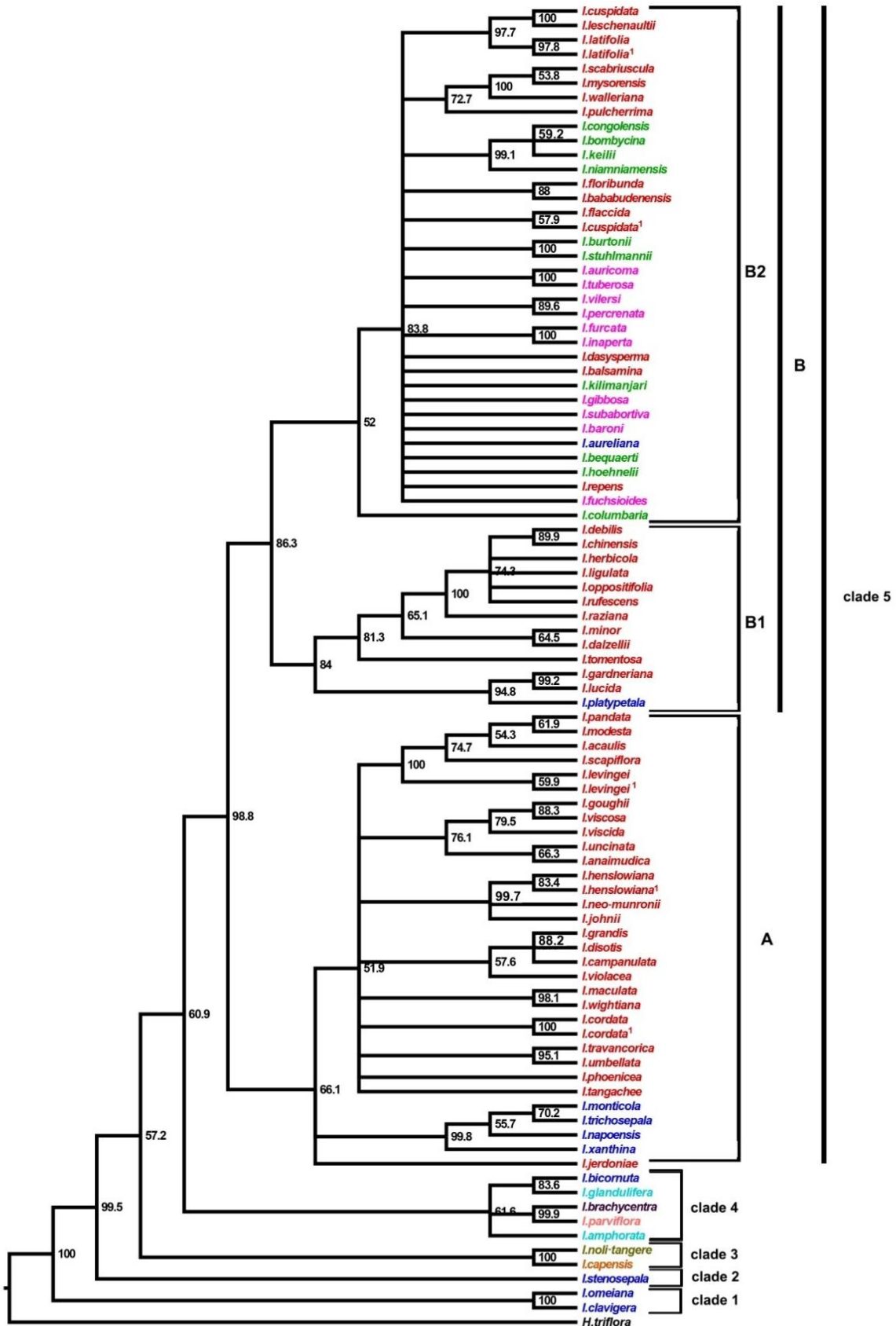
Table 2: Details of sequences obtained from GenBank.

SI. No.	Place of origin and Species name	Genbank Accession Number
	Sri Lanka	
1	<i>H. triflora</i> (out group)	AY348853
	East and Southeast Asia	
2	<i>I. aureliana</i>	AY348747
3	<i>I. bicornuta</i>	AY348754
4	<i>I. clavigera</i>	HQ718766
5	<i>I. monticola</i>	AY348810
6	<i>I. napoensis</i>	AY348811
7	<i>I. omeiana</i>	KC905505
8	<i>I. platypetala</i>	AY348819
9	<i>I. stenosepala</i>	AY348835
10	<i>I. trichosepala</i>	AY348843
11	<i>I. xanthina</i>	AY348850
	Africa	
12	<i>I. bequaerti</i>	AY348753
13	<i>I. bombycina</i>	AY348755
14	<i>I. burtonii</i>	AY348757
15	<i>I. congolensis</i>	AY348766
16	<i>I. columbaria</i>	AY348764
17	<i>I. hoehnelii</i>	AY348792
18	<i>I. keilii</i>	AY348798
19	<i>I. kilimanjari</i>	AY348800
20	<i>I. niarniamensis</i>	AY348812
21	<i>I. stuhlmannii</i>	AY348836
	Madagascar	
22	<i>I. auricomma</i>	AY348748
23	<i>I. baroni</i>	AY348751
24	<i>I. fuchsioides</i>	AY348785
25	<i>I. furcata</i>	AY348786
26	<i>I. gibbosa</i>	AY348787
27	<i>I. inaperta</i>	AY348797
28	<i>I. percrenata</i>	AY348817
29	<i>I. subabortiva</i>	AY348837
30	<i>I. tuberosa</i>	AY348844
31	<i>I. vilersi</i>	AY348848
	South India and Sri Lanka	
32	<i>I. campanulata</i>	AY348758
33	<i>I. cordata</i> ¹	AY348768
34	<i>I. cuspidata</i> ¹	AY348769
35	<i>I. henslowiana</i> ¹	AY348790
36	<i>I. latifolia</i> ¹	AY348801
37	<i>I. leschenaultii</i>	AY348803
38	<i>I. levingei</i> ¹	AY348804
39	<i>I. repens</i>	KC905524
	The Himalayas	
40	<i>I. amphorata</i>	AY348740
41	<i>I. glandulifera</i>	AY348788
	Central Asia and Europe	
42	<i>I. brachycentra</i>	AY348756
43	<i>I. parviflora</i>	AY348816
	Boreal Eurasia and North America	
44	<i>I. nolintangere</i>	AY348813
45	<i>I. capensis</i>	AY348759

3. RESULTS

ITS gene sequences of forty-five *Impatiens* species, including all the five biodiversity hotspots, were obtained from the NCBI database. A total of forty-seven sequences of South Indian *Impatiens* were generated in the present study including all the seven sections of the genus *Impatiens*. *H. triflora* was selected as outgroup in all the analyses. MP analysis of the ITS data matrix included 92 taxa and 503 characters. Out of the 503 characters, 198 were constant and 86 variable characters, which were parsimony-uninformative. The parsimony-informative characters

were 219. The strict consensus of the optimal tree kept by heuristic searches and the bootstrap clade support (when greater than 50%) was showed (Figure 1). The MP strict consensus tree was moderately resolved. The resolved lineages of *Impatiens* were grouped into five clades. Southeast Asian *Impatiens* species *viz.*, *I. clavigera* and *I. omeiana* formed a basal clade 1 with high bootstrap support (BSS) of 100%. *I. stenosepala* (Southeast Asian origin) was resolved as a highly supported clade 2 with strong BSS of 99.5%. The North American species, *I. capensis* showed close affinity with the boreal temperate Eurasian species, *I. noli-tangere* (100%) forming clade 3 with weak BSS of 57.2%.



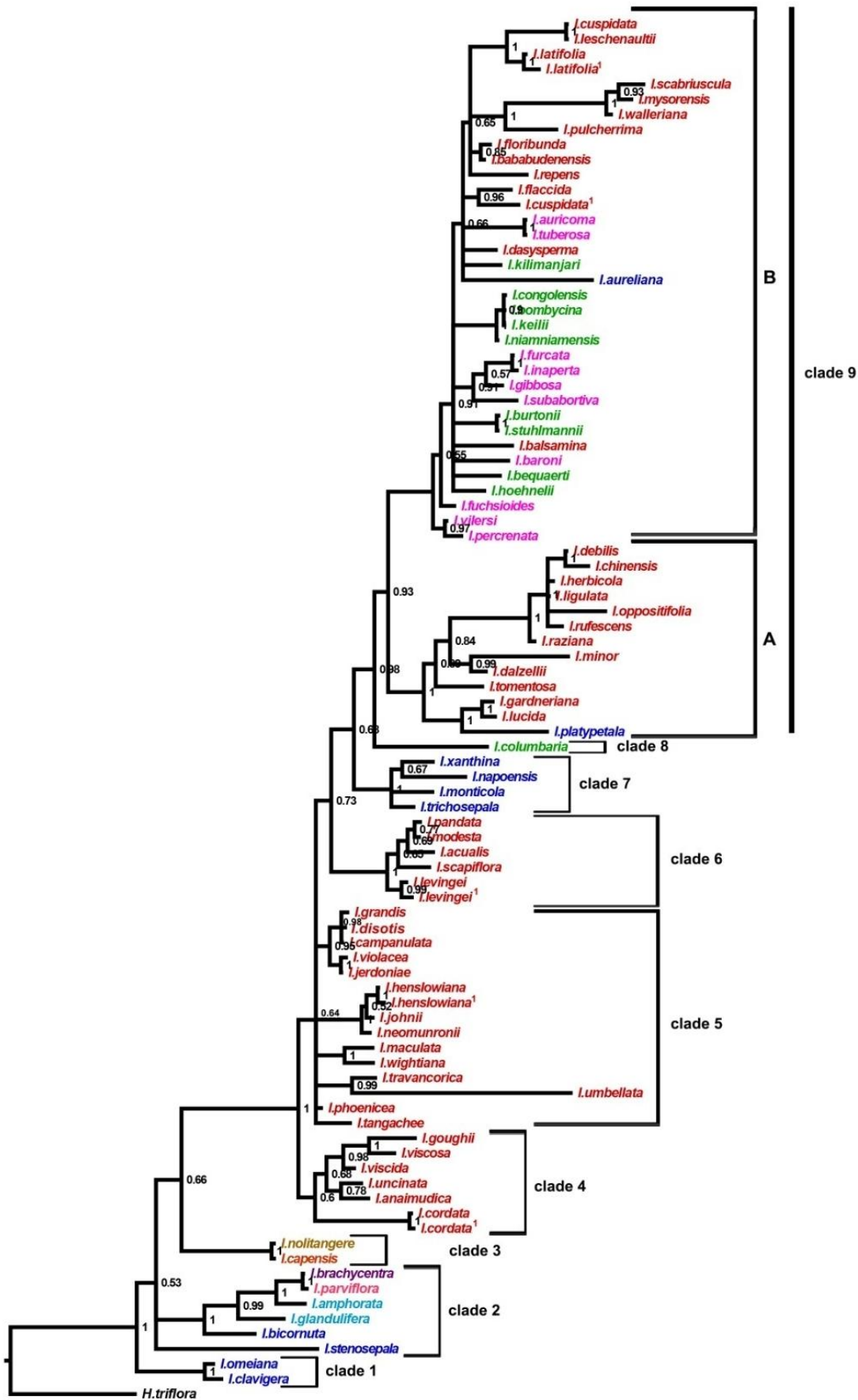


Fig. 2: Bayesian consensus phylogram based on nuclear ITS gene. The numbers by the nodes indicate bayesian posterior probabilities greater than 0.5.

The Eastern European species, *I. parviflora* and the central Asian species, *I. brachycentra* were grouped together. The Southeast Asian species, *I. bicornuta* and the Himalayan species *I. glandulifera* were also grouped together. These two groups showed affinity to the Himalayan species *I. amphorata* and formed clade 4 with moderate BSS of 60.9%. Clade 5 was a large clade with high BSS of 98.8% and was divided into two sub clades i.e., A and B. The sub clade A showed a grouping of Southeast Asian species (*I. xanthina*, *I. napoensis*, *I. trichosepala* and *I. monticola*) and the South Indian species of sections *Epiphyticae* (*I. jerdoniae* and *I. violacea*), *Sub-Umbellatae* (*I. umbellata*, *I. travancorica*, *I. cordata*, *I. disotis*, *I. grandis*, *I. anaimudica*, *I. uncinata*, *I. viscida*, *I. viscosa* and *I. goughii*), *Scapigeriae* (*I. levingei*, *I. scapiflora*, *I. acaulis*, *I. modesta* and *I. pandata*), *Racemosae* (*I. tangachee*, *I. phoenicea*, *I. wightiana* and *I. maculata*) and *Tomentosae* (*I. johnii*, *I. neo-munronii* and *I. henslowiana*) with BSS of 66.1%. This sub clade contained sequences of South Indian species from NCBI, GenBank namely, *I. cordata*¹, *I. campanulata*, *I. henslowiana*¹ and *I. levingei*¹. The sub clade B was divided into sub clade B1 and B2 with BSS of 86.3%. The sub clade B1 (BSS 84%) contained the South Indian species of section *Annuae* (*I. lucida*, *I. gardneriana*, *I. tomentosa*, *I. dalzellii*, *I. minor*, *I. raziana*, *I. rufescens*, *I. oppositifolia*, *I. ligulata*, *I. herbicola*, *I. chinensis* and *I. debilis*) and only one South east Asian species *I. platypetala*. The sub clade B2 comprised of all the selected African, Madagascan species and also South Indian (section *Microsepala*) species with only one Southeast Asian species (*I. aureliana*) with weak BSS of 52%. This sub clade contained African species like *I. columbaria*, *I. hoehnelii*, *I. bequaerti*, *I. kilimanjari*, *I. stuhlmannii*, *I. burtonii*, *I. niarniamensis*, *I. keilii*, *I. bombycina* and *I. congolensis*. The Madagascan species were *I. fuchsoides*, *I. baroni*, *I. subabortiva*, *I. gibbosa*, *I. inaperta*, *I. furcata*, *I. percrenata*, *I. vilersi*, *I. tuberosa* and *I. auricoma*. This clade also included South Indian species from NCBI like *I. repens*, *I. cuspidata*¹, *I. latifolia*¹ and *I. leschenaultii*. The species of section *Microsepala* found in this clade were *I. balsamina*, *I. dasysperma*, *I. flaccida*, *I. bababudenensis*, *I. floribunda*, *I. pulcherrima*, *I. walleriana*, *I. mysorensis*, *I. scabriuscula*, *I. latifolia* and *I. cuspidata*.

A general time reversible model of evolution with invariant sites and a gamma distribution (GTR+I+G) was selected using jModeltest 2.1.5 [17]. This model was used for the Bayesian Inference (BI) analysis. The BI tree had well-resolved topology and the overall relationships are depicted in almost the same way as in the MP analysis (Figure 2). The resolved lineages of *Impatiens* species were grouped into nine clades. Southeast Asian *Impatiens* species (*I. clavigera* and *I. omeiana*) formed clade 1 with strong BPP of 1.00. The clade 2 included the Southeast Asian species (*I. stenosepala* and *I. bicornuta*), the central Asian species (*I. brachycentra*), the Eastern European species (*I. parviflora*), and the Himalayan species (*I. amphorata* and *I. glandulifera*) with weak BPP of 0.53. Clade 3 is grouped with the boreal temperate Eurasian species (*I. noli-tangere*) and the North American species (*I. capensis*) with moderate BPP of 0.66. Species of section *Sub-*

Umbellatae (*I. cordata*, *I. anaimudica*, *I. uncinata*, *I. viscida*, *I. viscosa* and *I. goughii*) formed clade 4 with strong BPP of 1.00. Clade 5 included species of sections *Racemosae* (*I. tangachee*, *I. phoenicea*, *I. wightiana* and *I. maculata*), *Sub-Umbellatae* (*I. umbellata*, *I. travancorica*, *I. disotis* and *I. grandis*), *Tomentosae* (*I. neo-munronii*, *I. johnii* and *I. henslowiana*) and *Epiphyticae* (*I. jerdoniae* and *I. violacea*). In this clade, *I. jerdoniae* and *I. violacea* of section *Epiphyticae* grouped together with high BPP value 1.00. The clade 6 comprised only species of section *Scapigeriae* (*I. levingei*, *I. scapiflora*, *I. acaulis*, *I. modesta* and *I. pandata*) with BPP 0.73. Clade 7 included only the Southeast Asian species (*I. trichosepala*, *I. monticola*, *I. napoensis* and *I. xanthina*) with BPP of 0.68. African species (*I. columbaria*) formed clade 8 with BPP of 0.98. Clade 9 (BPP 0.93) was divided into sub clade A and sub clade B. The sub clade A contained species of section *Annuae* and only one Southeast Asian species (*I. platypetala*) with strong BPP value 1.00 as same as in MP (sub clade B1) analysis. The sub clade B included African, Madagascan and the South Indian species (section *Microsepala*) with strong BPP value 1.00 as same as in MP (sub clade B2) analysis.

4. DISCUSSION

4.1 Biogeographic Implications of the ITS Phylogenies

Balsaminaceae showed an interesting distributional pattern, with five biodiversity hotspots located in Africa, Madagascar, South India and Sri Lanka, the Himalayas and Southeast Asia [2]. Based on species diversity and karyological data, Jones and Smith [9] suggested that *Impatiens* originated in the Himalayan region and dispersed to other areas. Grey-Wilson [10] developed a hypothesis suggesting that Balsaminaceae originated in Western Gondwana and spread to Southeast Asia through Madagascar and India. From Southeast Asia and the adjacent Sino-Himalayan area, *Impatiens* diversified secondarily into two lineages: one lineage radiating to the temperate Eurasian areas and North America, and the other radiating to tropical and subtropical areas of Southeast Asian islands. Grey-Wilson [10] rejected the possibility of an overland migration between Africa and India.

Phylogenies of *Impatiens* species based on ITS sequences of Yuan *et al.* [2] revealed that extant *Impatiens* species are of Southeast Asian origin, from where it dispersed to boreal Eurasia and North America, to central Asia and Eastern Europe, via the Himalayas, and to India and Africa. Janssens *et al.* [2] *atpB-rbcL* spacer sequences based phylogeny suggested that *Impatiens* originated in South China from which it colonized the nearby regions and afterwards dispersed to North America, Africa, India, the Southeast Asian peninsula and the Himalayan area. Maximum-likelihood based ancestral area reconstruction of Janssens *et al.* [20] illustrated that the vast majority of the current diversification of *Impatiens* has originated in Asia. He also suggested that Southwest China was clearly reconstructed as the ancestral area for the earliest-diverging lineages.

Phylogenetic analyses of nuclear ITS data provides well-supported trees that give a good hypothesis of evolutionary

relationships in the genus *Impatiens*. Biogeographic analysis onto the ITS phylogenies revealed that Southeast Asia as the ancestral area of extant *Impatiens* species. All phylogenies clearly illustrate the Southeast Asian origin of the genus *Impatiens*. Yuan *et al.* [2] (based on ITS phylogeny) suggested that North American species *I. capensis* and boreal Eurasian species *I. noli-tangere* dispersed from Southeast Asia. In this study, these two species grouped together with strong bootstrap support (100%). Yuan *et al.* [2] also suggested that the central Asian species *I. brachycentra* and European species *I. parviflora* may have been dispersed from Southeast Asia via the Himalayas. In this study, these species assembled with high support value and showed relationship with Himalayan species. These results are in confirmation with the findings of Yuan *et al.* [2]. Janssens *et al.* [1] suggested that only one speciation event from Southwest China accounts for the diversity of *Impatiens* in North America. ITS phylogeny of Yuan *et al.* [2] also suggested that Africa was colonized at least twice from Southeast Asia and Madagascan *Impatiens* showed clearly an African origin. Janssens *et al.* [1] based on *atpB-rbcL* phylogeny suggested that Madagascan species originated from an African ancestor. Studies of Janssens *et al.* [20] clearly illustrated that African continent was colonized from Southwest China in three independent dispersal events and Madagascan species was derived from a single colonization event. In this study, all the Madagascan and the African species occurred into the same sub clade. Due to insufficient sampling and less resolved clades, it is not possible to conclude the colonization events of African and Madagascan species from this study.

ITS phylogeny of Yuan *et al.* [2] contained only nine species from India and Sri Lanka. Of which some species (*I. campanulata*, *I. hookeriana*, *I. cordata*, *I. henslowiana* and *I. levingei*) showed Southeast Asian connection and others (*I. cuspidata*, *I. parasitica*, *I. latifolia* and *I. leschenaultii*) showed African connections. From this results Yuan *et al.* [2] concluded that South India and Sri Lanka had two origins and not commended the colonization events of this region. But Janssens *et al.* [1] *atpB-rbcL* based phylogeny confirmed that South India was colonized at least twice, once by an East Asian ancestor, another time by an ancestor with African affinities. Biogeographic reconstructions of Janssens *et al.* [20] revealed that *Impatiens* dispersed into South India via two independent colonization events from Southwest China.

In this study, all the analyses show that South Indian species belonged to recent lineages of *Impatiens*. South Indian species contained clade 5 (in MP) was obviously separated into two sub clades A and B. Of the total sampled South Indian species, some species grouped with Southeast Asian species (sub clade A in MP analysis) and some species grouped with African species (sub clade B in MP and BI analyses). The present study contained species, *I. cordata*, *I. henslowiana* and *I. levingei* showing Southeast Asian connection and *I. cuspidata* and *I. latifolia* showing African connection confirming the study of Yuan *et al.* [2]. From these phylogenies it is clearly understand that South India was colonized by two independent dispersal events

i.e., once by Southeast Asian ancestor and another time by an ancestor with African affinities.

4.2 Implications of the ITS phylogenies on infrageneric classification of *Impatiens*

Impatiens is taxonomically considered as one of the most difficult genera of angiosperms, primarily due to the delicate yet hypervariable structure and fragile nature of its flowers. It is extremely difficult to examine dried specimens if prepared conventionally [3, 10]. The first modern general treatment for the genus is the important revision of the African taxa by Grey-Wilson [10], which distinguished six informal infrageneric groups for the African species for practical diagnosis only. Based on morphological and molecular data sets, Yu *et al.* [12] presented a new classification of *Impatiens*. In which, *Impatiens* is divided into two subgenera, subgenus *Clavicarpa* and subgenus *Impatiens*. The subgenus *Impatiens* is further sub divided into seven sections such as *Semeiocardium*, *Tuberosae*, *Racemosae*, *Impatiens*, *Scorpioidae*, *Fasciculatae* and *Uniflorae*.

The taxonomic treatments of South Indian *Impatiens* of Bhaskar [5] classified South Indian species under seven sections i.e., *Scapigeriae*, *Epiphyticae*, *Annuae*, *Microsepalae*, *Tomentosae*, *Sub-Umbellatae* and *Racemosae*. Based on the present molecular phylogenetic study, most of the species of each sections form monophyletic association with strong bootstrap support. This study confirms the morphological classifications of Bhaskar [5].

Grey-Wilson [10] observed several morphological similarities among species endemic to Africa and South India. Grey-Wilson [10] also suggested a close affinity between African and South Indian taxa and even a possible migration route connecting these two areas. Species of section *Microsepalae* (sub clade B1 in MP and sub clade B in BI) was included in the same sub clade of African and Madagascan species. In all the analyses, the species of section *Annuae* formed a separate sub clade (sub clade B2 in MP and sub clade A in BI) with strong support. All phylogenies revealed that these two sections showed sister clade relationships. These results demonstrate that species of section *Microsepalae* and *Annuae* shows African affinities. This proves the Grey-Wilson's [10] observation of morphological similarities among species endemic to Africa and South India. MP analysis (sub clade A) clearly indicates that species of sections *Scapigeriae*, *Epiphyticae*, *Tomentosae*, *Sub-Umbellatae* and *Racemosae* showed Southeast Asian relationship. It is pointed out that these sections dispersed from Southeast Asia. This study including all representative extant sections of South India, indicates that South India was colonized from Southeast Asia by two independent dispersal events i.e., once by ancestral species of sections *Scapigeriae*, *Epiphyticae*, *Tomentosae*, *Sub-Umbellatae* and *Racemosae* and another time by ancestral species of sections *Microsepalae* and *Annuae*, having African affinities.

5. CONCLUSION

The biogeographical elucidation based on this study is mainly in accordance with the conclusion of Yuan *et al.* [2] The

results based on all phylogenetic analyses reveal that mainland Southeast Asia is the original place of extant *Impatiens* species and from where it dispersed to other places like boreal Eurasia and North America, central Asia and Eastern Europe, via the Himalayas, and to Africa and India. But our conclusion is in opposition with Jones and Smith's [9] implication of *Impatiens* originated in the Himalayan region, Grey-Wilson's [10] proposition of dispersal from an assumed West Gondwana origin and spread to Southeast Asia and the adjacent Sino-Himalayan region and Bhaskar's [11] suggestions of the genus *Impatiens* originated from Western Ghats. This study, including all the representative extant sections of South India, indicates that South India was colonized by two independent dispersal events from Southeast Asia i.e., once by ancestral species of sections *Scapigerae*, *Epiphyticae*, *Tomentosae*, *Sub-Umbellatae* and *Racemosae* and another time by ancestral species of sections *Microsepalae* and *Annuae*.

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