Phylogenetic Analyses of Molecular Data and Reconstruction of Morphological Character Evolution in Asian Impatiens Section Semeiocardium (Balsaminaceae)

Authors: Ruchisansakun, Saroj, Niet, Timotheüs van der, Janssens, Steven B., Triboun, Pramote, Techaprasan, Jiranan, et al.

Source: Systematic Botany, 40(4) : 1063-1074

Published By: The American Society of Plant Taxonomists

URL: https://doi.org/10.1600/036364415X690102

Systematic Botany (2015), 40(4): pp. 1063–1074

DOI 10.1600/036364415X690102
Date of publication December 15, 2015

Phylogenetic Analyses of Molecular Data and Reconstruction of Morphological Character Evolution in Asian *Impatiens* section *Semeiocardium* (Balsaminaceae)

Saroj Ruchisansakun,[1,2,8] Timotheüs van der Niet,[1,2] Steven B. Janssens,[3] Pramote Triboun,[4] Jiranan Techaprasan,[5] Thaya Jenjittikul,[6] and Piyakaset Suksathan[7]

[1]Naturalis Biodiversity Center, Darwinweg 4, 2333 CR, Leiden, The Netherlands.
[2]Leiden University, Darwinweg 4, 2333 CR, Leiden, The Netherlands.
[3]Botanic Garden Meise, Nieuwelaan 38, BE-1860, Meise, Belgium.
[4]Thailand Institute of Scientific and Technological Research, 35 Mu 3, Khlong Ha, Khlong Luang, Pathum Thani, 12120, Thailand.
[5]National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Khlong Nueng, Khlong Luang, Pathum Thani, 12120, Thailand.
[6]Department of Plant Science, Faculty of Science, Mahidol University, 272 Rama VI, Ratchathewi, Bangkok, 10400, Thailand.
[7]Garden Department, Queen Sirikit Botanic Garden, P.O. Box 7, Mae Rim, Chiang Mai, 50180, Thailand.
[8]Author for correspondence (saroj.ruchisansakun@naturalis.nl)

Communicating Editor: Sven Buerki

Abstract—Phylogenetic analyses have revealed extensive evolutionary lability of morphological characters in the large genus *Impatiens*. This potentially complicates taxonomic classifications, especially if these are based on analyses which include a limited number of characters and taxa. In this study we focus on the systematics of previously poorly represented *Impatiens* from Southeast Asia, a hotspot of species diversity, using an expanded taxon sampling. Specifically we implement phylogenetic analyses of DNA sequences from the nuclear ITS and plastid atpB-rbcL intergenic spacer and reconstruct the evolution of several morphological characters to study *Semeiocardium*, a taxon which has been recognized as a distinct genus, subgenus, and section in previous taxonomic treatments. Both Bayesian and Parsimony analyses demonstrated that *Impatiens* subg. *Semeiocardium* is not monophyletic. Thirteen representatives are part of a large clade, which also includes many other Southeast Asian taxa. Connate lateral united petals are a synapomorphy of this clade, while a four-locular ovary and connate outer lateral sepals are not. *Impatiens muscicola* and *I. santisukii*, which were previously classified under *I.* subg. *Semeiocardium*, do not belong to the clade with connate lateral united petals or to *I.* sect. *Semeiocardium*. Due to ambiguous combinations of morphological characters we could not determine whether sampled species belonged to *I.* sect. *Semeiocardium*. In addition to the characters that were used to diagnose *I.* sect. *Semeiocardium*, we identify connate lateral united petals as a taxonomically useful character to distinguish an additional monophyletic clade in *I.* sect. *Semeiocardium*. Further research is needed to identify diagnostic characters for the sister clade comprising *I. stenosepala* and its allies.

Keywords—Asia, *atpB-rbcL* intergenic spacer, ITS, morphology, synapomorphy, taxonomy.

Balsaminaceae comprises over 1,000 species, mainly distributed in the Old World tropics and subtropics, with only few species occurring in northern hemisphere temperate regions (Grey-Wilson 1980a; Yuan et al. 2004; Janssens et al. 2009). The family is characterized by spurred zygomorphic flowers with fused stamens surrounding ovary and stigma. Several genera have been described within Balsaminaceae (e.g. Nieuwland and Lunnel 1916; Perrier de la Bâthie 1927; Peter 1928), but currently only *Impatiens* and the monospecific *Hydrocera* are recognized as valid genera (Rao et al. 1986; Grey-Wilson 1989a). *Impatiens* differs from its sister genus in having fused lateral petals and an explosive seed capsule whereas *Hydrocera* is characterized by five free petals and an indehisent pseudoberry. *Impatiens* occurs mainly in montane areas of the tropics and subtropics of the Old World (tropical Africa, Madagascar, South India and Sri Lanka, Sino-Himalaya and Southeast Asia) (Fig. 1). In contrast, *Hydrocera* is native to Indo-Malayan region, where it occurs as a semi-aquatic plant in stagnant water around sea level (Grey-Wilson 1980b; Ramadevi and Narayana 1990).

The morphological classification of Balsaminceae is supported by molecular data, which showed that no combinations of previously recognized genera were mutually monophyletic (Yuan et al. 2004; Janssens et al. 2006; Yu et al. 2015). The same molecular systematic studies also showed that the most comprehensive infrageneric treatment of *Impatiens* from Warburg and Reiche (1895), largely based on phyllotaxy, inflorescence type, and spur length, is artificial (Yuan et al. 2004; Janssens et al. 2006). The discordance between morphological and molecular data has complicated the taxonomic subdivision of the large genus *Impatiens*. However, by combining molecular and morphological data in a phylogenetic analysis implementing a total-evidence approach, a new global infrageneric classification of *Impatiens* was proposed (Yu et al. 2015). According to this new classification *Impatiens* is divided into *I.* subg. *Clavicarpa* S. X. Yu ex S. X. Yu & Wei Wang, characterized by a four-locular ovary and one ovule per locule, and *I.* subg. *Impatiens*, characterized by a five-locular ovary (rarely four) and many ovules per locule. Within *I.* subg. *Impatiens*, seven sections are delimited.

The largest concentration of *Impatiens* species is found in China and Southeast Asia, including Myanmar, Thailand, Laos, Cambodia, Singapore, Malaysia, Indonesia, the Philippines, Timor-Leste, Brunei, and Papua New guinea (Hooker 1874; Shimizu 1970; Grey-Wilson 1989b; Kress et al. 2003; Chen et al. 2008). Taxonomic treatment of species from this region has been problemetic, mainly due to insufficient studies. One of the sections of Yu et al. (2015), *Impatiens* section *Semeiocardium* (Zoll.) S.X. Yu & Wei Wang, has been particularly problematic. The taxonomic status, delimition, and diagnostic characters of *I.* sect. *Semeiocardium* have all frequently changed (Bleeker 1858; Backer 1935; Grey-Wilson 1989a; Utami 2009; Yu et al. 2015). This taxon was originally described by Zollinger as a monotypic genus with *Semeiocardium arriensii* Zoll., from the

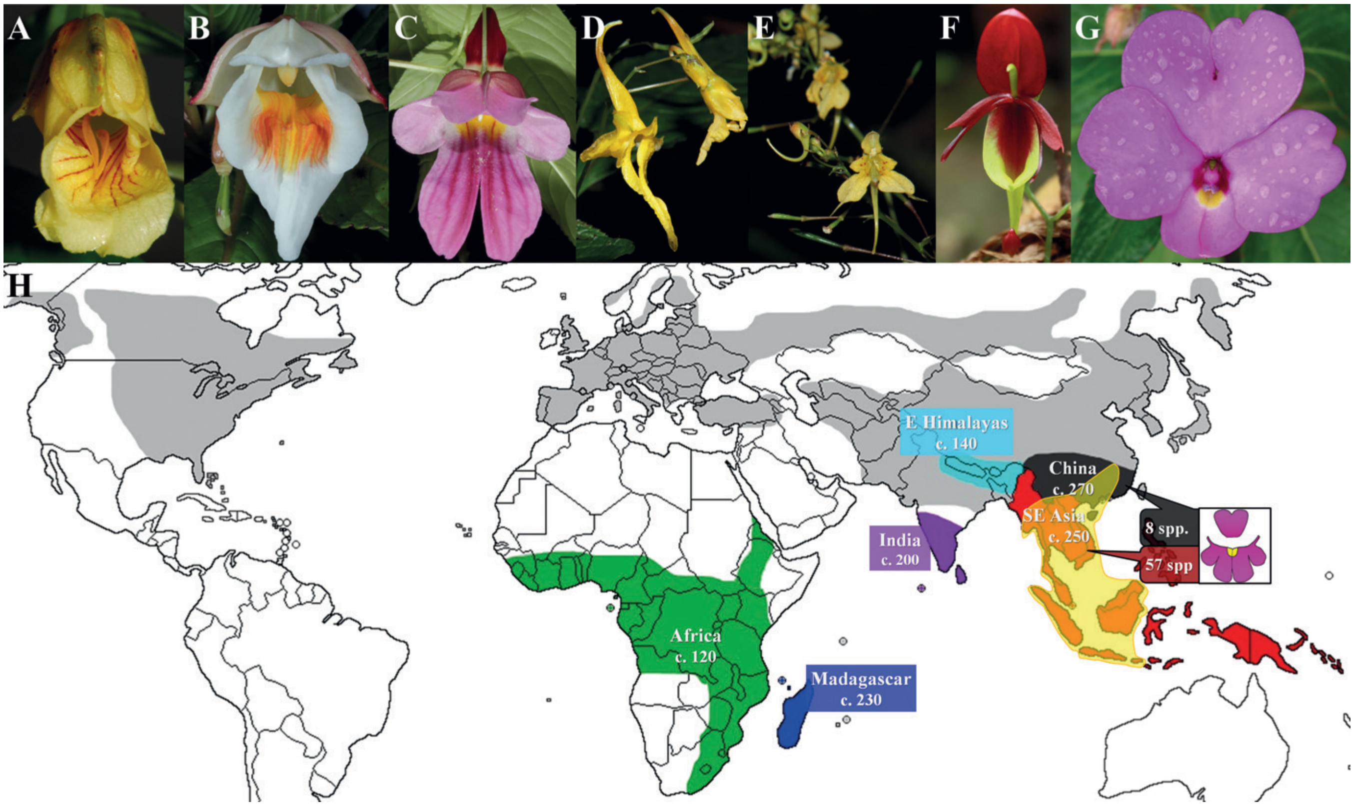

FIG. 1. A–G. Representatives of *Impatiens* from each clade in this study. A. *I. damrongii* (clade 1). B. *I. kerriae* (CLUP clade). C. *I. psittacina* (CLUP clade). D. *I. longiloba* (clade 2). E. *I. racemosa* (clade 2). F. *I. hians* (clade 3). G. *I. phuluangensis* (clade 3). H. The distribution of *Impatiens*: the grey area shows the distribution of a small number of widespread species. The other colors represent one of the five *Impatiens* hotspots. Estimated species number is given. The yellow area represents the distribution of species from the CLUP clade, the callout bullets show the number of species with connate lateral united petals in China and SE Asia respectively.

Indonesian Madura island, as the type species (Bleeker 1858). However, Zollinger produced only a short and ambiguous description with a wrong diagnosis (Bleeker 1858; Backer 1935; Grey-Wilson 1989a), based on an incomplete type specimen. In addition, the type specimen also contained plant parts of a Polygalaceae (Backer 1935). Backer (1935) re-discovered *S. arriensii*, confirmed the generic status of *Semeiocardium*, and included a more detailed description based on living material. Grey-Wilson (1989a) summarized the three characters that had been used by Bleeker (1858) and Backer (1935) to distinguish *Semeiocardium* from *Impatiens*: 1) the outer lateral sepals enveloping the lower sepal and spur are connate as opposed to free, 2) the lateral united petals are connate as opposed to free, and 3) the ovary is four-locular as opposed to five-locular. He concluded that these character states are not unique to *Semeiocardium,* but can also be observed in other species such as *I. harmandii* Hook. f. from Thailand with connate outer lateral sepals, *I. kerriae* Hook. f. from Thailand with connate lateral united petals, and various Southeast Asian *Impatiens* species with four-locular ovaries (Grey-Wilson 1989a). As a result, Grey-Wilson synonymized *Semeiocardium* with *Impatiens* (Grey-Wilson 1989a). Utami (2009) changed the status of *Semeiocardium* to subgeneric level as *Impatiens* subgenus *Semeiocardium* (Zoll.) N. Utami, based on studies of floral morphology and seed coat ornamentation. She included the type species of the genus *Semeiocardium*, *I. arriensii* (Zoll.) T. Shimizu, together with 19 other species distributed from South China to South Indonesia that all possess a four-locular ovary, connate lateral united petals, and a pilose or granulate and finely granular seed coat with long hairs (Utami 2009). In the phylogenetic analysis of Yu et al. (2015) a monophyletic clade with brick-shaped seed as synapomorphies, including eight *Impatiens* species, was re-defined as *I.* sect. *Semeiocardium* within *I.* subg. *Impatiens*. In the diagnostic key of Yu et al. (2015) species are classified as belonging to *I.* sect. *Semeiocardium* if they possess a four-locular ovary, many ovules per carpel, and obconical capsules.

Although the molecular systematic studies in *Impatiens* performed to date have included a broad species sampling, in any study less than 3% of representatives from Southeast Asia were included (Yuan et al. 2004; Janssens et al. 2006, 2007, 2009; Yu et al. 2015). Given the evolutionary lability of morphological characters, robust taxonomic classifications require that a large number of species be included to test whether a particular diagnostic character, or combination of characters, can be used to recognize monophyletic taxa. Based on the lack of SE Asian taxa in molecular systematic studies, more taxa from this area need to be included in a phylogenetic analysis. The aim of this study was to: 1) test the monophyly of *I.* subg. *Semeiocardium* and *I.* sect. *Semeiocardium* using molecular phylogenetics, and 2) reconstruct the evolution of the diagnostic morphological characters, which have been used throughout the taxonomic history of *Semeiocardium*. We expanded the taxon sampling of previous phylogenetic studies in *Impatiens* by adding a large number of Asian taxa, including many representatives of the former *I.* subg. *Semeiocardium*.

Materials and Methods

Taxon Sampling—In total, 164 accessions of Balsaminaceae (one *Hydrocera*, the remainder *Impatiens*) were included in our analysis. We expanded the previous data set of Yuan et al. (2004) and Janssens et al. (2006) with 76 new accessions (Appendix 1). The majority of the new accessions are from Southeast Asia and they include 15 out of 20 recognised species from *I.* subg. *Semeiocardium*. Many newly added taxa await formal description as new species, and are here indicated with informal names between quotation marks. *Marcgravia umbellata, Souroubea* sp., *Norantea guianensis* (Marcgraviaceae), and *Pelliciera rhizophorea* (Tetrameristaceae s. l.), are used as outgroup (Bremer et al. 2002; APG II 2003; APG III 2009). *Pelliciera rhizophorea* was used to root the tree in the phylogenetic analyses.

Molecular Protocols—Genomic DNA was extracted from fresh or silica-dried leaf material and herbarium specimens using a reduced CTAB protocol (Doyle and Doyle 1987). The nuclear ribosomal internal transcribed spacer ("ITS" hereafter) and the plastid *atpB-rbcL* intergenic spacer ("*atpB-rbcL*" hereafter) were amplified following Yuan et al. (2004) and Janssens et al. (2006). The total PCR reaction volume was 50 μl and the reaction mix, modified from Techaprasan et al. (2010), contained 1 × PCR buffer (Thermo Scientific, Massachusetts, U. S. A.), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 mM of each forward and reverse primer (ITS5 and ITS4 derived from White et al. (1990), IMP-*atpB* and IMP-*rbcL* derived from Janssens et al. (2006)), 0.05 u/μl Taq DNA polymerase (Thermo Scientific, Massachusetts, U. S. A.), and 4–8 ng/μl genomic DNA. Sequencing of amplicons of both regions, using the original amplification primers as the sequencing primers, was done by Macrogen (the Republic of Korea) and first Base (Singapore), respectively.

Data Matrix Composition and Phylogenetic Analyses—The chromatograms of the ITS forward and reverse sequences showed no evidence of polymorphism. For both loci forward and reverse sequences were therefore combined to create contigs in Geneious 6.1 (Biomatters Ltd, New Zealand). Sequence data matrices were initially aligned with ClustalW (as implemented in BioEdit version 7.0.5.2 (Hall 1999)) using default settings, and then manually adjusted in BioEdit. A partition homogeneity test implemented in PAUP* 4.0b10 (Swofford 2003) was used to determine whether the datasets were incongruent (Farris et al. 1995).

Maximum parsimony analysis (MP) of the separate datasets was conducted using PAUP*. Characters were equally weighted and character states were specified as unordered. Heuristic searches were conducted with tree-bisection-and-reconnection (TBR) branch swapping on 10,000 random addition replicates, with 10 trees held at each step. Support for individual clades was measured by non-parametric bootstrapping (MP-BS) (Felsenstein 1985), using 1,000 pseudoreplicates. For each pseudo-replicate, a heuristic search for the most parsimonious tree was performed by TBR branch swapping on 100 random addition starting trees, with 3 trees held at each step.

For Bayesian analysis, Modeltest 3.06 (Posada and Crandall 1998) was used to determine the best models of sequence evolution for each locus using the Akaike Information Criterion. Bayesian analyses of separate datasets were conducted with MrBayes 3b4 (Huelsenbeck and Ronquist 2001). Two simultaneously independent analyses were run for ten million generations, starting from different random trees. Every 500 generations, a tree was sampled from the chain, resulting in a total of 20,000 sampled trees. The first 25% of sampled trees were discarded as burn-in. Tracer v1.6 was used to check whether stationarity was reached among the post burn-in sample. Support for each clades was measured by posterior probability (PP). Data sets and consensus trees are deposited in TreeBASE (study number 16521).

Testing Monophyly of Taxonomic Classifications—We tested monophyly of taxa by visually inspecting topologies and assess whether taxa that belong to a previous classification of *Semeiocardium* form a monophyletic clade. To determine membership of previous classifications of *Semeiocardium* we refered to the list of species supplied by Utami (2009) and the diagnostic key of Yu et al. (2015).

Character Evolution—To test whether morphological characters used to define *Semeiocardium* are synapomorphies, we reconstructed the evolution of three floral characters across the genus using character optimization: 1) lateral united petals: connate or free, 2) number of ovary locules: four or five, and 3) outer lateral sepals: connate or free. The degree of fusion of the lateral united petals and the number of ovary locules were included because they were used by Utami (2009) to define *I.* subg. *Semeiocardium*. Furthermore, the degree of fusion of the outer lateral sepals was included, because this character was originally used to describe *Semeiocardium* as a distinct genus within Balsaminaceae (Bleeker 1858; Backer 1935; Grey-Wilson 1989a). Seed coat characteristics used by Utami (2009), and pollen characters used by Yu et al. (2015) were not included because of lack of sufficient data. Characters were scored using fresh material or, in case no fresh material was available, herbarium specimens or literature. We used Fitch parsimony (Fitch 1971) as a criterion for character optimization. To account for phylogenetic uncertainty, we traced character histories on 15,000 post burn-in trees from the Bayesian analysis using the 'Trace Character Over Trees' command in Mesquite 2.75 (Maddison and Maddison 2011).

Results

Phylogenetic Analyses—Visual inspection of topologies based on nuclear and chloroplast DNA sequences revealed several well-supported differences. The partition homogeneity test confirmed significant incongruence between the ITS and *atpB-rbcL* datasets ($p = 0.001$). For the remainder, nuclear and plastid datasets are analyzed separately. Topologies based on ITS sequences were better resolved and showed higher support than topologies based on *atpB-rbcL* sequences (Table 1; Fig. 2). In all consensus topologies, both Balsaminaceae and *Impatiens* were monophyletic (Fig. 2). Within *Impatiens*, the deepest split was between *I. omeiana* and the remainder of the genus (Fig. 2). According to the nuclear topology, the next split was between clade 1 containing mainly Southeast Asian species and clade A containing species from all distribution areas (Fig. 2). Clade 1 consists of the "connate lateral united petals clade" (CLUP clade) and a small sister clade comprising species with free lateral united petals: *I. damrongii*, *I.* aff. *augustini*, and *I. stenosepala* (Fig. 2). The CLUP clade contains most representatives of *I.* subg. *Semeiocardium* and many other Southeast Asian taxa: 43 out of a total of 75 Southeast Asian taxa sampled (Figs. 2, 3). Clade 2 comprises mainly Chinese taxa. Clade 3 consists of representatives from most *Impatiens* hotspots, and also included *I. santasukii* and *I. muscicola* of *I.* subg. *Semeiocardium* (Fig. 2).

In the plastid topology clades 1, 2, 3, and the CLUP clade was also monophyletic, confirming the non-monophyly of *I.* subg. *Semeiocardium*. However, clade 1 and 2 together (clade B), and clade 3 was sister to clade B (Fig. 2). Moreover, in clade 1, the CLUP clade was sister to *I. damrongii* + *I.* aff. *augustini*, and subsequently *I. stenosepala* (Fig. 2).

It was not possible to unambiguously determine whether species belong to *I.* sect. *Semeiocardium*. Several species are characterized by combinations of characters, which are typical for membership of several sections. We therefore were unable to test the monophyly of *I.* sect. *Semeiocardium*.

Table 1. Characteristics of the ITS and *atpB-rbcL* datasets.

Data	ITS	*atpB-rbcL*
Model of DNA evolution	GTR + I + G	GTR + G
Number of characters	861	1,114
Number of parsimony-informative characters (%)	526 (61.1%)	253 (22.7%)
MP Tree length	4,072	645
% of resolved nodes in Bayesian consensus tree	88.6%	50.6%
% Nodes supported by PP>0.95	65.1%	48.8%
% of resolved nodes in strict concensus tree based on Maximum Parsimony	81.3%	50.6%
% Nodes supported by BS>80	65.7%	25.9%
Consistency Index (CI)	0.29	0.58
Retention index (RI)	0.76	0.88

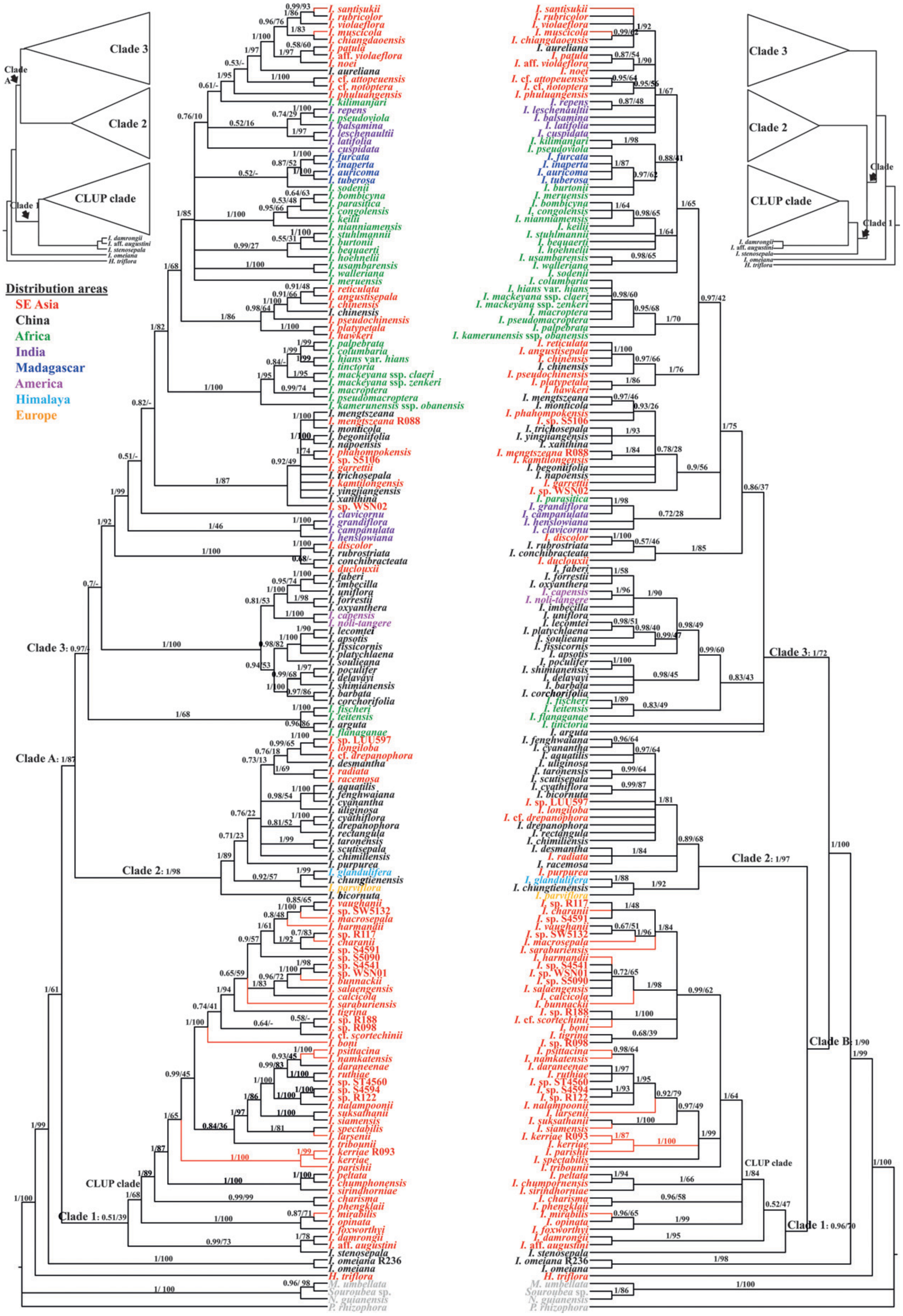

FIG. 2. Summary of the bayesian consensus tree based on nuclear ITS (left) and plastid *atpB-rbcL* (right) DNA sequence data. Support values for key nodes are indicated (PP/MP-BS). Branches in red lead to the members of *I.* subg. *Semeiocardium.* The color of taxon names is indicative of their distribution.

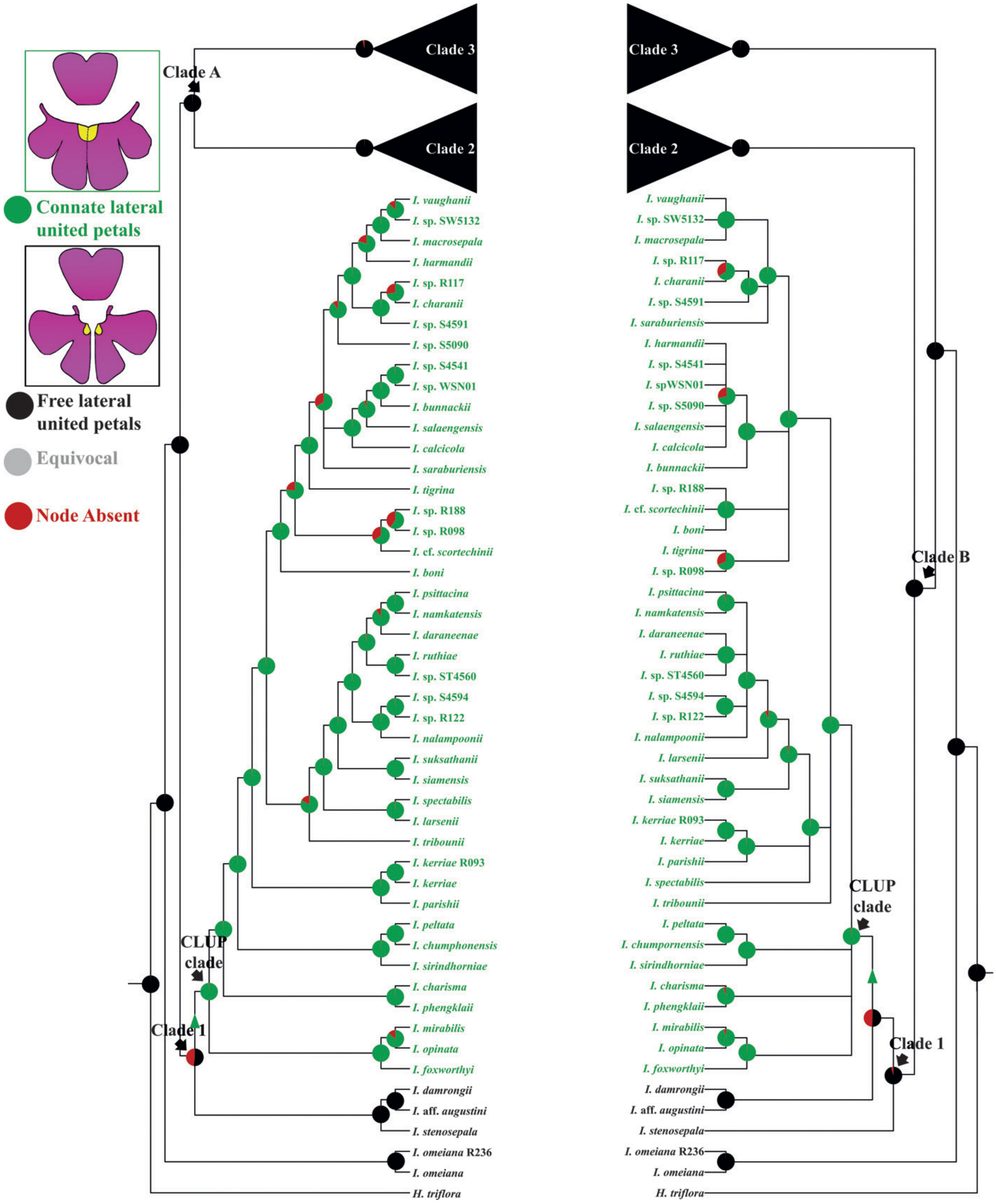

FIG. 3. Parsimony ancestral character state reconstructions of lateral united petals based on 15,000 post burn-in trees from the Bayesian inference plotted on the Bayesian consensus tree from the ITS (left) and *atpB-rbcL* (right) data. Taxa in green possess a four-locular ovary, whereas those in black (including all taxa that are part of clade 2 and 3) possess free lateral united petals. Circles at nodes represent pie charts indicating the percentage of Bayesian post burn-in trees with a particular reconstruction, equivocal reconstruction, or the node absent.

Character Evolution—Re-examination of the morphological characters for *I. santisukii* and *I. muscicola* revealed that their lateral united petals and outer lateral sepals are not connate but free, and that their ovary is five-locular. In both the nuclear and plastid topologies, connate lateral united petals evolved only once from free lateral united petals, and represent a synapomorphy for the CLUP clade. Reconstruction of the evolution of ovary locule number differs between nuclear and plastid topologies. According to the nuclear topology, 47% of the Bayesian trees sampled suggest a transition from a five-locular ovary in *Hydrocera*, to a four-locular ovary in *Impatiens* once and a single reversal (Fig. 4). For the plastid topology, a five-locular ovary is ancestral, with two transitions to a four-locular ovary (Fig. 4).

Connate outer lateral sepals are not a synapomorphy in either the nuclear or plastid topology respectively. Although connate outer lateral sepals are confined to a small group of species of the CLUP clade, shifts between connate and free lateral united petals have occurred six times in both topologies (Fig. 5).

Discussion

Both nuclear and plastid topologies show that *I.* subg. *Semeiocardium* is not monophyletic. Thirteen of the 15 included species are part of the CLUP clade together with 30 other representatives from Southeast Asia (Fig. 2). This clade is characterized by connate lateral united petals as a synapomorphy (Fig. 3). The two species of *I.* subg. *Semeiocardium* which are not part of this clade also do not possess connate lateral united petals.

Besides connate lateral united petals, species in the CLUP clade also possess a four-locular ovary. Indeed, this character association had already been suggested by Shimizu and Takao (1982) and Shimizu (1987). However, Yu et al. (2010) reported that some species with a four-locular ovary lack connation of the lateral united petals. These species belong to two lineages: the lineage towards *I. omeiana*, and the lineage(s) towards *I. damrongii*, *I.* aff. *augustini*, and *I. stenosepala*. The evolutionary pathways of the lateral united petals and the ovary in *Impatiens* preclude their joint use as diagnostic characters for infrageneric taxa. However, if connate lateral united petals are considered alone, they define a well-supported clade.

Impatiens santisukii and *I. muscicola* were included in *I.* subg. *Semeiocardium* on the basis of the typical diagnostic characters of this subgenus Utami (2009). In our phylogenetic analyses these two species do not form part of the CLUP clade, but instead group together with other pink, flat-flowered taxa distributed from tropical Africa to Southeast Asia. The phylogenetic position of *I. santisukii* and *I. muscicola* would imply independent origins of connate lateral united petals and a four-locular ovary. However, on re-examination of these characters for these two species using fresh material, we observed that their lateral united petals are free (cf. Shimizu 1970, 2000), and that they possess a five-locular ovary, similar to the other species of the clade to which they belong.

We detected several cases of well-supported incongruence between nuclear and plastid topologies (cf., Janssens et al. 2006). However, the incongruence did not affect our ability to firmly reject the monophyly of *I.* subg. *Semeiocardium*, as in both gene trees the subgenus was not monophyletic. Nevertheless, the incongruence did affect certain inferences of character evolution. Connate lateral united petals were a synapomorphy for the CLUP clade in both gene trees, and the phylogenetic trees confirmed in both cases Grey-Wilson's claim (1980a) that the evolutionary shift occurred from free to connate petals. Connate outer lateral sepals were used as a diagnostic character for the genus *Semeiocardium* (Bleeker 1858; Backer 1935). Grey-Wilson (1989a) suggested that this character state occurs throughout the genus *Impatiens*, and it was not used by Utami (2009) as diagnostic for *I.* subg. *Semeiocardium*. According to both gene trees connate outer lateral sepals are homoplasious. However, this character state does not occur as widely scattered across the genus as Grey-Wilson (1989a) assumed but it is only present in the CLUP clade, where shifts between free and connate sepals frequently occured. Further research is required to understand the functional significance of connate outer lateral sepals, for instance for interactions with specific pollinators. Inferences on the evolution of the number of ovary locules are most strongly affected by the gene tree incongruence. According to the plastid topology, a four-locular ovary evolved twice independently from a five-locular ovary. Reconstructions according to the set of Bayesian topologies based on nuclear DNA sequences are mostly ambiguous, although in a subset of topologies there is evidence for a scenario where the ancestral condition in *Impatiens* was a five-locular ovary, with a single shift to a four-locular ovary and a single reversal to a five-locular ovary. The main difference between reconstructions according to the nuclear and plastid topology respectively, is thus that either a four-locular ovary is not homologous (plastid), or a five-locular ovary is not homologous (nuclear). Considering that the floral morphology of *I. omeina* (four-locular) is very similar to that of some early diverging species of Clade 1 (four-locular), it appears more reasonable that the four-locular ovary is homologous. This would be consistent with the ITS topology. Further sampling of nuclear DNA, to reconstruct multiple independent gene trees, supplemented by a floral ontogenetic study on the four-locular species from the different clades, is required to test this hypothesis.

The recent infragereric classification of *Impatiens* by Yu et al. (2015) proposed a new *I.* sect. *Semeiocardium* within *I.* subg. *Impatiens*. Their study included only eight representatives of this section. We expanded the sampling from the Southeast Asian *Impatiens* hotspot, including many potential members of *I.* sect. *Semeiocardium*. To determine whether species are member of *I.* sect. *Semeiocardium*, we used the diagnostic key to the subgenera and sections of *Impatiens* provided by Yu et al. (2015). In several cases species did not key out to a particular section because they contained conflicting character states. For instance, *I. opinata* and *I. mirabilis* are characterized by a four-locular ovary and hammerhead-shaped capsule (suggestive of membership in *I.* sect. *Clavicarpa*), but also by many ovules per carpel (suggestive of *I.* sect. *Semeiocardium*). Moreover, the number of ovules per locule varies from one to many even within the same species for some *Impatiens* (Shimizu and Takao 1985). We therefore conclude that while the diagnostic key of Yu et al. (2015) may have been sufficient to key out a relatively limited number of species, it does not always work for other *Impatiens* species. This highlights the need for seeking further characters or character combinations to diagnose monophyletic clades. In our analysis the posession of connate lateral united petals was identified as a synapomorphy

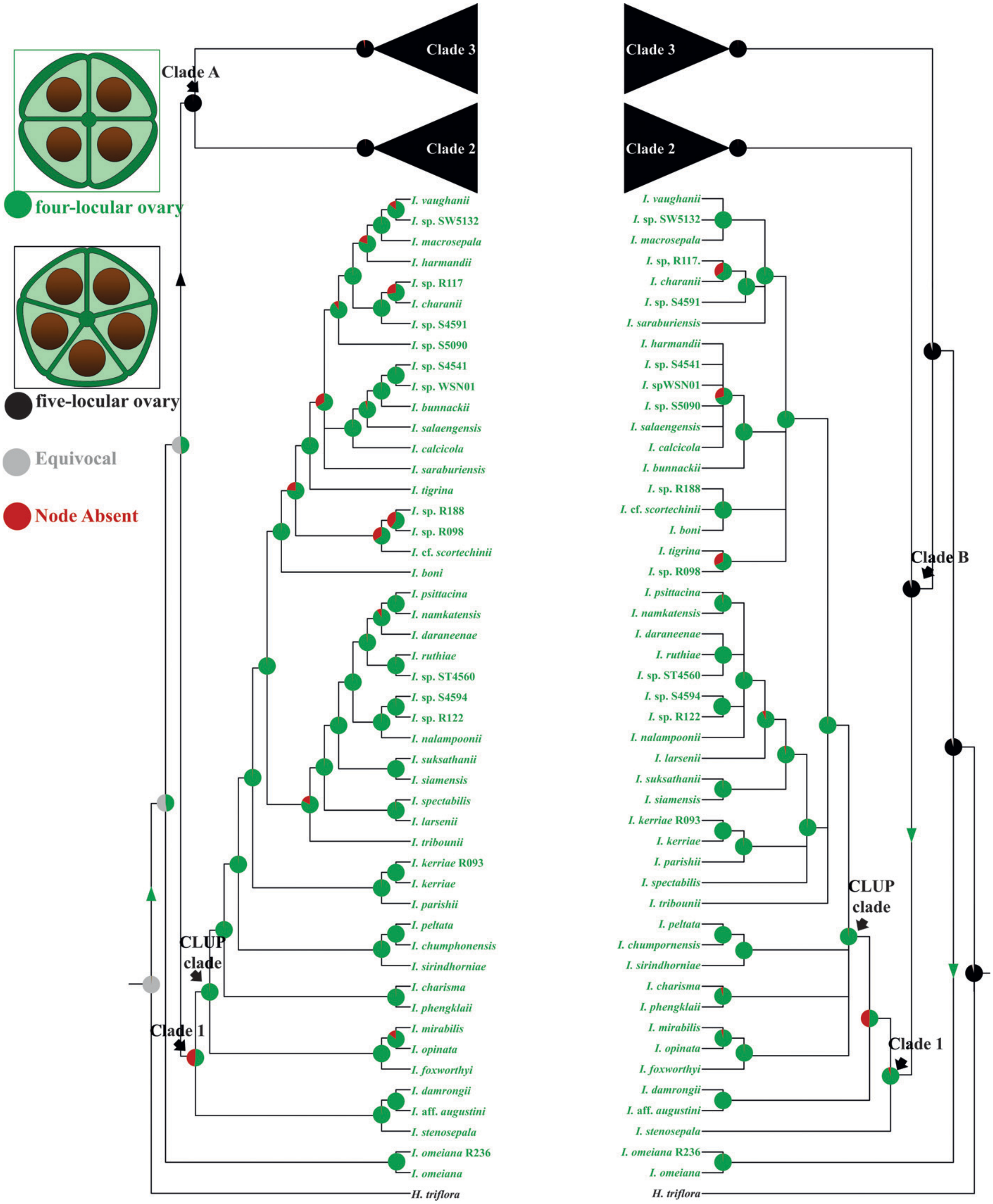

FIG. 4. Parsimony ancestral character state reconstructions of the number of ovary locules based on 15,000 post burn-in trees from the Bayesian inference plotted on the Bayesian consensus tree from the ITS (left) and *atpB-rbcL* (right) data. Taxa in green possess connate outer lateral sepals, whereas those in black (including all taxa that are part of clade 2 and 3) possess a five-loecular ovary. Circles at nodes represent pie charts indicating the percentage of Bayesian post burn-in trees with a particular reconstruction, equivocal reconstruction, or the node absent.

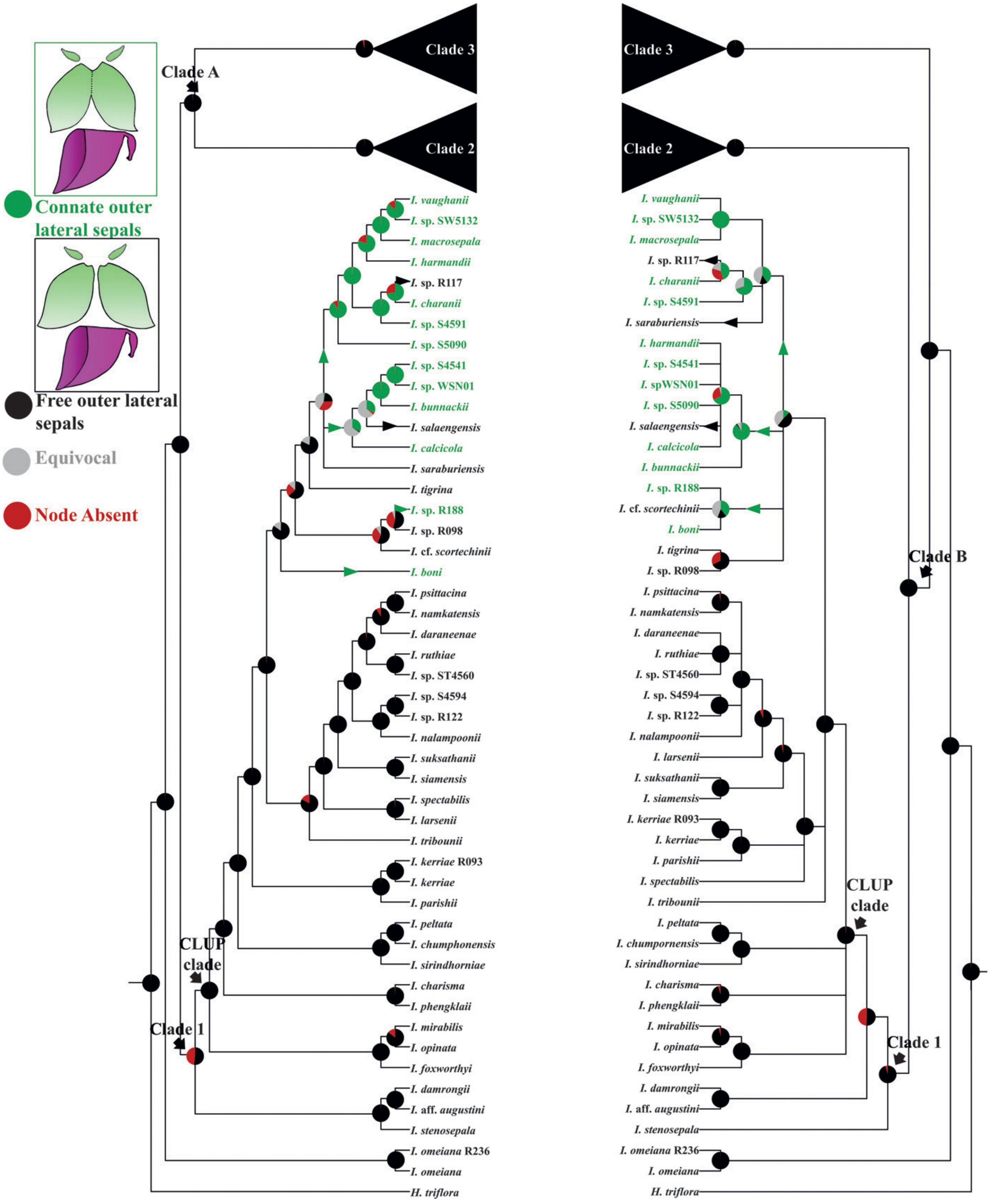

FIG. 5. Parsimony ancestral character state reconstructions of outer lateral sepals based on 15,000 post burn-in trees from the Bayesian inference plotted on the Bayesian consensus tree from the ITS (left) and *atpB-rbcL* (right) data. Taxa in green possess connate lateral united petals, whereas those in black (including all taxa that are part of clade 2 and 3) possess free outer lateral sepals. Circles at nodes represent pie charts indicating the percentage of Bayesian post burn-in trees with a particular reconstruction, equivocal reconstruction, or the node absent.

TABLE 2. List of *Impatiens* species with connate lateral united petals. Species marked with * were included in molecular analysis. Species marked with ^{U} were included in *I.* subg. *Semeiocardium* by Utami (2009). The distribution is indicated by country (PEN = Peninsular part of Thailand; C = Central)

	Species	Distribution
1	*I. adenioides* Suksathan & Keerat.	PEN Thailand
2	*I. angulata* S. X. Yu, Y. L. Chen & H. N. Qin	S China
3	*I. alboflava* Miq. ^{U}	Indonesia, W Sumatra
4	*I. alborosea* Tardieu	Vietnam
5	*I. arriensii* (Zoll.) T. Shimizu ^{U}	Indonesia, Madura
6	*I. boni* Hook. f.*$^{, U}$	NW Vietnam
7	*I. bunnackii* T. Shimizu (nom.nud.)*$^{, U}$	NE Thailand
8	*I. calcicola* Craib*	SW Thailand
9	*I. capillipes* Hook. f. & Thomson	SE Myanmar
10	*I. cardiophylla* Hook. f.	PEN Thailand, Cambodia
11	*I. charanii* T. Shimizu*$^{, U}$	SW Thailand
12	*I. charisma* Suksathan & Keerat.*	SW Thailand
13	*I. chumphonensis* T. Shimizu*	PEN Thailand
14	*I. crassisepala* Tard.	NE Laos
15	*I. cryptoneura* Hook. f.	Malaysia
16	*I. daraneenae* Suksathan & Triboun*	N Thailand
17	*I. dempoana* Hook. f.	S Sumatra
18	*I. doitungensis* Triboun & Sonsupab	N Thailand
19	*I. foxworthyi* M. R. Hend.*	PEN Thailand
20	*I. hainanensis* Y. L. Chen	S China
21	*I. harmandii* Hook. f.*$^{, U}$	NE Thailand
22	*I. hongsonensis* T. Shimizu ^{U}	N Thailand
23	*I. jiewhoei* Triboun & Suksathan	SW Thailand
24	*I. kanburiensis* T. Shimizu*$^{, U}$	SW Thailand
25	*I. kerriae* Craib*$^{, U}$	N Thailand, Vietnam
26	*I. lanessani* Hook. f.	S Cambodia
27	*I. larsenii* T. Shimizu*$^{, U}$	PEN Thailand
28	*I. laumonieri* T. Shimizu	Indonesia, Sumatra
29	*I. lobulifera* S. X. Yu, Y. L. Chen & H. N. Qin.	S China
30	*I. macrosepala* Hook. f.*$^{, U}$	PEN Thailand
31	*I. micromeris* Hook. f.	S Myanmar
32	*I. mirabilis* Hook. f.*$^{, U}$	PEN Thailand
33	*I. morsei* Hook. f.	S China
34	*I. musyana* Hook. f.	Vietnam
35	*I. nalampoonii* T. Shimizu*	N Thailand
34	*I. namkatensis* T. Shimizu*$^{, U}$	N Thailand
35	*I. obcordifolia* Tardieu	Vietnam
36	*I. obesa* Hook. f.	S China
37	*I. opinata* Craib*	PEN Thailand
38	*I. pachycaulon* M. F. Newman	C Laos
39	*I. parishii* Hook. f.*$^{, U}$	N Thailand, Myanmar
40	*I. peltata* Hook. f.*	SW Thailand
41	*I. phengklaii* T. Shimizu & Suksathan*	SW Thailand
42	*I. pingxiangensis* H. Y. Bi & S. X. Yu	S China
43	*I. polyneura* K. M. Liu	S China
44	*I. psittacina* Hook. f.*$^{, U}$	N Thailand, Myanmar
45	*I. putii* Craib*	SW and PEN Thailand
46	*I. pygmaea* Hook. f.	Vietnam
47	*I. ridleyi* Hook. f.	Malaysia
48	*I. rupestris* K. M. Liu & X. Z.Cai	S China
49	*I. ruthiae* Suksathan & Triboun*	NE Thailand
50	*I. salaengensis* T. Shimizu*	N Thailand
51	*I. saraburiensis* T. Shimizu (nom. nud.) *, ^{U}	SW Thailand
52	*I. sarawakensis* T. Shimizu	Malaysia, Indonesia
53	*I. scortechinii* Hook. f.*	PEN Thailand, Malaysia, Indonesia
54	*I. siamensis* T. Shimizu*$^{, U}$	SW and PEN Thailand
55	*I. sirindhorniae* Triboun & Suksathan*	PEN Thailand
56	*I. spectabilis* Triboun & Suksathan*	SW Thailand
57	*I. spireana* Hook. f.	Laos
58	*I. suksathanii* Ruchis. & Triboun*	SW Thailand
59	*I. tigrina* Suksathan & Triboun*	NE Thailand
60	*I. tribuana* Utami & Nurainas	Indonesia, West Sumatra
61	*I. tribounii* T. Shimizu & Suksathan*	SW Thailand
62	*I. vaughanii* Hook. f.*	PEN Thailand, Malaysia
63	*I. verruciferae* Hook. f. ^{U}	Vietnam
64	*I. winkleri* Hook. f.	Borneo
65	*I. zygosepala* Hook. f.	Cambodia

for a large clade to which many species belong which posess characters that are largely consistent with a subgroup of *I.* sect. *Semeiocardium* (i.e. they have a four-locular ovary and obconic capsules). As opposed to fruit, seed, or pollen characters, this floral morphological character can be readily identified from fresh material and herbarium material and therefore serves as an excellent diagnostic character. Based on the possession of connate lateral united petals, an additional 34 unsampled species are expexted to be part of the CLUP clade. The majority of these additional taxa are also distributed in Southeast Asia (Table 2; Fig. 1). However, connate lateral united petals cannot be used to delimit *I.* sect. *Semeiocardium*, as it excludes *I. stenosepala* and its allies. Further research is required to identify characters that unambiguously identify the clade to which *I. stenosepala* belongs, before new section delimitations can be applied.

The combination of molecular phylogenetics and morphological character state reconstruction has facilitated the delimitation of natural infrageneric lineages within the complex genus *Impatiens*. This study suggests that despite the fact that molecular studies demonstrated the artificial nature of previous *Impatiens* classifications, new monophyletic and clearly diagnosable lineages can be found via extended character state research. To improve the infrageneric classification of *Impatiens*, future studies should attempt to increase the taxon sampling, in combination with performing detailed morphological analyses to refine the infrageneric taxonomy of the genus *Impatiens*.

ACKNOWLEDGMENTS. We would like to acknowledge the Ex-situ Conservation of Native Highland Flora project, QSBG, to provide us with material. Moreover, we would like to thank Daniel C. Thomas, Peter van Welzen and Peter Hovenkamp from Naturalis Biodiversity Center for useful discussions about taxonomy. This work was supported by a Development and Promotion of Science and Technology talents project (DPST) Scholarship, The Institute for the Promotion of Teaching Science and Technology (IPST), Thailand.

LITERATURE CITED

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* 141: 399–436.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

Backer, C. A. 1935. *Semeiocardium* Zoll., a misinterpreted genus of Balsaminaceae. *The Gardens' Bulletin*. *Straits Settlement* 9: 70–72.

Bleeker, P. 1858. Lets over de Natuurlijke Geschiedenis van Madoera, H. Zollinger. *Natuurkundig Tijdschrift voor Nederlandsch-Indië* 17: 245.

Bremer, B., K. Bremer, N. Heidari, P. Erixon, R. G. Olmstead, A. A. Anderberg, M. Källersjö, and E. Barkhordarian. 2002. Phylogenetics of asterids based on 3 coding and 3 noncoding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* 24: 274–301.

Chen, Y. L., S. Akiyama, and H. Ohba. 2008. Balsaminaceae. Pp. 43–114 in *Flora of China* 12. eds. Z. Y. Wu, P. H. Raven, and D. Y. Hong. Beijing: Science Press and St. Louis: Missouri Botanical Garden Press.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.

Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for aspecific topology. *Systematic Zoology* 20: 406–416.

Grey-Wilson, C. 1980a. *Impatiens of Africa*. Rotterdam: Balkema.

Grey-Wilson, C. 1980b. *Hydrocera triflora*, its floral morphology and relationship with *Impatiens*. Studies in Balsaminaceae. *Kew Bulletin* 34: 221–227.

Grey-Wilson, C. 1989a. *Semeiocardium* Zoll.; Is It a Good Genus? Studies in Balsaminaceae: IX. *Kew Bulletin* 44: 107–113.

Grey-Wilson, C. 1989b. A Revision of Sumatran *Impatiens*: Studies in Balsaminaceae: VIII. *Kew Bulletin* 44: 67–106.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* 41: 95–98.

Hooker, J. D. 1874. *Impatiens*. Pp. 440–483 in *The Flora of British India*. 1, ed. J. D. Hooker. London: L. Reeve & Co.

Huelsenbeck, J. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Janssens, S. B., K. Geuten, Y. M. Yuan, P. Küpfer, and E. F. Smets. 2006. Phylogenetics of *Impatiens* and *Hydrocera* using plastid *atpB-rbcL* spacer sequences. *Systematic Botany* 31: 171–180.

Janssens, S. B., K. Geuten, T. Viaene, Y. M. Yuan, Y. Song, and E. F. Smets. 2007. Evolution of the *AP3/DEF* K-domain in *Impatiens* and its use for phylogenetic reconstruction. *Molecular Phylogenetics and Evolution* 43: 225–239.

Janssens, S. B., E. B. Knox, S. Huysmans, E. F. Smets, and V. S. F. T. Merckx. 2009. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. *Molecular Phylogenetics and Evolution* 52: 806–824.

Kress, W. J., R. A. D. Filipps, E. Farr, and A. Y. Y. Kyi. 2003. *A Checklist of the Trees, Shrubs, Herbs, and Climbers of Union of Myanmar.* Contributions from the Unites States National Herbarium, 45. National Museum of Natural History, Washington D.C., 590 pp.

Maddison, W. P. and D. R. Maddison. 2011. *Mesquite: A modular system for evolutionary analysis*. Version 2.75. http://mesquiteproject.org.

Nieuwland, J. A. and J. Lunnel. 1916. Enumerantur plantae Dakotae septentrionalis vasculares. *American Midland Naturalist* 4: 474.

Perrier de la Bâthie, H. 1927. Un nouveau genre de Balsaminees. *Bulletin de l'Académie Malgache* 10: 22.

Peter, A. 1928. Balsaminaceae. *Abhandlungen der Koniglichen Gesellschaft der Wissenschaften Gottingen* 13: 84.

Posada, D. and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Ramadevi, D. and L. L. Narayana. 1990. Morphology of the flower and fruit of *Hydrocera triflora* Wight and Arn. emend Venkat. and Dutt — an elucidation. *Proceedings of the Indiana Academy of Sciences* 100: 43–49. (Plant Science).

Rao, R. V. S., K. R. Ayyangar, and R. Sampathkumar. 1986. On the karyological characteristics of some members of Balsaminaceae. *Cytologia* 51: 251–260.

Shimizu, T. 1970. Contribution to the Flora of Southeast Asia. II. *Impatiens* of Thailand and Malaya. *The Southeast Asian Studies* 8: 187–217.

Shimizu, T. 1987. A note on the distribution of genus *Impatiens* (Balsaminaceae) in Southeast Asia. *Acta Phytotaxonomica et Geobotanica* 38: 53–56.

Shimizu, T. 2000. New species of Thai *Impatiens* (Balsaminaceae) 2. *Bulletin of the National Science Museum, Series B* 26: 35–42.

Shimizu, T. and S. Takao. 1982. Taxonomic significance of the inner structure of the ovary in the genus *Impatiens* (Balsaminaceae). *Botanical Magazine. (Shokubutsu-gakuzasshi). Tokyo* 95: 89–99.

Shimizu, T. and S. Takao. 1985. Taxonomic discussions on the four-carpellate species of *Impatiens* (Balsaminaceae). *Acta Phytotaxonomica et Geobotanica* 36: 97–106.

Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4 beta 10. Sunderland: Sinauer Associates.

Techaprasan, J., S. Klinbunga, C. Ngamriabsakul, and T. Jenjittikul. 2010. Genetic variation of *Kaempferia* (Zingiberaceae) in Thailand based on plastid DNA (*psbA-trnH* and *petA-psbJ*) sequences. *Genetics and Molecular Research* 9: 1957–1973.

Utami, N. 2009. The status of *Semeiocardium* Zoll. (Balsaminaceae). *Reinwardtia* 13: 21–23.

Warburg, O. and K. Reiche. 1895. Balsaminaceae. Pp. 383–392 in *Die naturlichen Pflanzenfamilien* 3, eds. A. Engler and K. Prantl. Leipzig: Engelmann.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR protocols: A guide to methods and applications*. eds. Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White.

Yu, S. X., X. R. Zhou, G. F. Xu, L. Meng, and H. Y. Bi. 2010. Floral development in *Impatiens chishuiensis* (Balsaminaceae): formation of two well-developed anterolateral sepals and four carpels. *Plant Systematics and Evolution* 286: 21–26.

Yu, S. X., S. B. Janssens, X. Y. Zhu, M. Lidén, T. G. Gao, and W. Wang. 2015. Phylogeny of *Impatiens* (Balsaminaceae): integrating molecular and morphological evidence into a new classification. *Cladistics*, doi: 10.1111/cla.12119.

Yuan, Y. M., Y. Song, K. Geuten, E. Rahelivololona, S. Wohlhauser, E. Fischer, E. Smets, and P. Küpfer. 2004. Phylogeny and biogeography of Balsaminaceae inferred from ITS sequence data. *Taxon* 53: 391–404.

APPENDIX 1. List of accessions included in this study. For each accession we include: species name, locality, voucher number, ITS GenBank accession number, and *atpB-rbcL* GenBank accession number.

Hydrocera triflora (L.) Wight & Arn., Sri Lanka, *Robyns 7260, 0249369* (L), AY348853, DQ147895. *I.* sp. ST4560, Loei, Thailand, *Suksathan & Triboun 4560* (QBG), KC905464, KC905601. *I. angustisepala* Tardieu, Bolaven, Southern Laos, *Suksathan & Wongnak 5471*(QBG), KC905465, KC905557. *I. apsotis* Hook. f., Sichuan, China, *Yuan CN2k2-159* (NEU), AY348744, DQ147810. *I. aquatilis* Hook. f., Yunnan, China, *Song CNY017* (NEU), AY348745, DQ147811. *I. arguta* Hook. f. & Thoms., Yunnan, China, *Yuan CN2k-74* (NEU), AY348746, DQ147812. *I.* cf. "attopeuensis", Bolaven, S Laos, *Suksathan & Wongnak 5471* (QBG), KC905471, KC905553. *I.* aff. *augustini*, Nan, Thailand, *Suksathan 5131* (QBG), KC905515, KC905618. *I. aureliana* Hook. f., Yunnan, China, *Yuan CN2k1-56* (NEU), AY348747, DQ147814. *I. auricoma* Baill., Comores origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE1* (NEU), AY348748, —. *I. auricoma* Baill., Comoros origin, cult. Bot. Gard. Marburg, *Janssens SJ001* (LV), —, DQ147815. *I. balsamina* L., Thailand, cult., *Ruchisansakun 102* (QBG), KC905466, —. *I. balsamina* L., Indian origin, cult. Kruidtuin Leuven, *Janssens SJ003* (LV), —, DQ147816. *I. barbata* Comber, Yunnan, China, *Yuan CN2k2-178* (NEU), AY348750, DQ147818. *I. begoniifolia* S. Akiyama & H. Ohba, Yunnan, China, *Yuan CN2k1-51* (NEU), AY348752, DQ147819. *I. bequaerti* De Wild., Congo origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE2* (NEU), AY348753, FJ826630. *I. bicornuta* Wall., Yunnan, China, *Yuan CN2k-55* (NEU), AY348754, DQ147821. *I. bombycina* W. Lobin & E. Fischer, African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE3* (NEU), AY348755, FJ826631. *I. boni* Hook. f., Ninh Binh, Vietnam, cultivated in Thailand, *Ruchisansakun 187* (QBG), KC905468, KC905595. *I bunnackii* T.Shimizu, Loei, Thailand, *Suksathan & Triboun 4554* (QBG), KC905469, KC905585. *I. burtonii* Hook. f., Kenya origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE4* (NEU), AY348757, —. *I. burtonii* Hook. f., Uganda, *Knox 2803* (LV), —, FJ826885. *I. calcicola* Craib, Petchaburi, Thailand, *Suksathan 4855* (QBG), KC905470, KC905587. *I. campanulata* Wight, South India origin, cult. by Ray Morgan, UK, *Ray Morgan s. n.* (NEU), AY348758, DQ147822. *I. capensis* Meerb., Quebec, Canada, *Küpfer s. n.* (NEU), AY348759, —. *I. capensis* Meerb., North American origin, cult. Holden arboretum, *Janssens SJ009* (LV), —, DQ147823. *I. charanii* T. Shimizu, Saraburi, Thailand, *Ruchisansakun 158* (QBG), KC905477, KC905580. *I. charisma* Suksathan & Keerat., Kanchanaburi, Thailand, *Ruchisansakun 154* (QBG), KC905478, KC905612. *I. chiangdaoensis* T. Shimizu, Chiang Mai, Thailand, *Ruchisansakun 142* (QBG), KC905476, KC905546. *I. chimiliensis* Comber, Yunnan, China, *Yuan CN2k-51* (NEU), AY348760, DQ147824. *I. chinensis* L., Phetchabun, Thailand, *Suksathan & Triboun 4546* (QBG), KC905479, KC905558. *I. chinensis* L., Yunnan, China, *Yuan CN2k1-49* (NEU), AY348761, DQ147825. *I. chumphonensis* T. Shimizu, Chumphorn, Thailand, *Suksathan & Triboun 4848* (QBG), KC905480, KC905610. *I. chungtienensis* Y. L. Chen, Yunnan, China, *Yuan CN2k2-204* (NEU), AY348762, DQ147826. *I. clavicornu* Turcz., South Indian origin, cult. by Ray Morgan, UK, *Ray Morgan s.n.* (LV), AY348804, DQ147827. *I. columbaria* J. J. Bos,Gabon origin, cult. Bot. Gard. Koblenz Univ, *Fischer NE6* (NEU), AY348764, —. *I. columbaria* J. J. Bos, African origin, cult. Nat. Bot. Gard. Meise, *FB/S2966* (BR), —, DQ147828. *I. conchibracteata* Y. L. Chen & Y. Q. Lu, Sichuan, China, *Hao 427* (NEU), AY348765, DQ147829. *I. congolensis* G. M. Schulze & R. Wilczek, Congo origin, Africa, cult. Bot. Gard., *Fischer NE7* (NEU), AY348766, DQ147830. *I. corchorifolia* Franch., Yunnan, China, *Chassot & Yuan 99-173* (NEU), AY348767, DQ147831. *I. cuspidata* Wight & Arn., South India origin, cult. by Ray Morgan, UK, *Ray Morgan s. n.* (NEU), AY348769, DQ147832. *I. cyanantha* Hook. f., Yunnan, China, *Yuan CN2k1-84* (NEU), AY348770, DQ147833. *I. cyathiflora* Hook. f., Yunnan, China, *Chassot & Yuan 99-29* (NEU), AY348771, DQ147834. *I. damrongii* T. Shimizu, Phitsanulok, Thailand, *Suksathan & Triboun 4544* (QBG), KC905482, KC905617. *I. daraneenae* Suksathan & Triboun, Chiang Mai, Thailand, *Suksathan 5148* (QBG), KC905483, KC905599. *I. delavayi* Franch.,Yunnan, China, *Chassot & Yuan 99-154* (NEU), AY348773, DQ147836. *I. desmantha* Hook. f., Yunnan, China,*Yuan CN2k2-30* (NEU), AY348774, DQ147837. *I. discolor* DC., Chiang Mai, Thailand, *Ruchisansakun 204* (QBG), KC905484, KC905568. *I.* cf. *drepanophora* Hook. f., Nan, Thailand,*Suksathan 4681* (QBG), KC905472, KC905572. *I. drepanophora* Hook. f., Yunnan, China, *Yuan CN2k1-41* (NEU), AY348776, DQ147838. *I. duclouxii* Hook. f., Chiang Mai, Thailand, *Suksathan 4691* (QBG), KC905485, KC905569. *I. faberi* Hook. f., Sichuan, China, *Song S007* (NEU), AY348778, DQ147841. *I. fenghwaiana* Y. L. Chen, Guangxi, China, *Yuan CN2k1-78* (NEU), AY348779, DQ147842. *I. fischeri* Warb., Kenya, *Odile Phaehler & Magali Schnell I-01* (NEU), AY348781, —. *I. fischeri* Warb., Kenya, *Menn. and Bar.-Kui. 284* (U), —, DQ147843. *I. fissicornis* Maxim., Shaanxi, China, *Wang SH-003* (NEU), AY348782, DQ147844. *I. flanaganae* Hemsl., Africa, *RBG Edinburgh 19860179* (E), AY348783, DQ147846. *I. forrestii* Hook. f. ex W. W. Smith, Yunnan, China, *Yuan CN2k-79* (NEU), AY348784, DQ147847. *I. foxworthyi* Henders., Thailand, *Ruchisansakun 240* (QBG), KC905616. *I. furcata* H.Perrier, African origin, cult. Bot. Gard. Koblenz Univ, *Fischer NE8* (NEU), AY348786, —. *I. furcata* H.Perrier, Madagascar, *Fischer EF8* (NEU), —, FJ826642. *I. garrettii* Craib, Chiang Mai, Thailand, *Suksathan 5186* (QBG), KC905487, KC905564. *I. glandulifera* Arn., Western Himalaya origin, cult. Bot. Gard. Neuchâtel, *Yuan JBE1* (NEU), AY348788, —. *I. glandulifera* Arn., Belgium, Leuven (introduced; Himalayan origin), *Janssens SJ002* (LV), —, DQ147848. *I. grandiflora* Herb.Madr. ex Wall., India, Cultivated in Thailand, *Ruchisansakun 229* (QBG), KC905489, KC905567. *I. harmandii* Hook. f., Thailand, *Suksathan 4573* (QBG), KC905462, KC905578. *I. hawkeri* W. Bull, Cultivated, Thailand, *Ruchisansakun 103* (QBG), KC905489, KC905560. *I. henslowiana* Arn., Sri Lanka origin, cult. Bot. Gard. Koblenz Univ, *Fischer NE10* (NEU), AY348790, FJ826645. *I. hians* Hook. f. var. hians,West African origin, cult. Bot. Gard. Berlin, *Schwerdtfeger 9492a* (B), HM454297, DQ147849. *I. hoehnelii* T. C. E. Fries, African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE9* (NEU), AY348792, —. *I. hoehnelii* T. C. E. Fries, Kenya, Knox 2846 (LV), —, FJ826647. *I.* sp. S5106, Thailand, *Suksathan 5106* (QBG), KC905490, KC905563. *I. imbecilla* Hook. f., Sichuan, China, *Hao 426* (NEU), AY348796, DQ147851. *I. inaperta* H. Perrier, Madagascar origin, cult. Bot. Gard. Neuchâtel, *Yuan JBE2* (NEU), AY348797, FJ826649. *I. kamerunensis* Warb. subsp. *obanensis* (Keay) Grey-Wilson, Ghana, *Jongkind 1926* (WAG), HQ176459, FJ826652. *I. kamtilongensis* Toppin, Mae Hong Son, Thailand, *Suksathan 4324* (QBG), KC905491, KC905565. *I. keilii* Gilg subsp. *keilii*, African origin, cult. Bot. Gard. Koblenz Univ, *Fischer NE11* (NEU), AY348798, —. *I. keilii* Gilg subsp. *keilii*, Tanzania, *Knox 3575* (LV), —, FJ826654. *I. kerriae* Craib, Chaing Mai, Thailand, *Ruchisansakun 093* (QBG), KC905492, KC905607. *I. kerriae* Craib, Chiangmai, Thailand, *Chassot 99-238* (NEU), AY348799, DQ147853. *I. kilimanjari* Oliver, Africa, *JMG 94613* (BR), AY348800, —. *I. kilimanjari* Oliver, Tanzania, *Knox 3652* (LV), —, FJ826656. *I. larsenii* T. Shimizu, Surat Thani, Thailand, *Suksathan & Triboun 4852* (QBG), KC905493, KC905593. *I. latifolia* L., India origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (NEU), AY348801, —. *I. latifolia* L., India origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (LV), —, DQ147854. *I. lecomtei* Hook. f., Yunnan, China, *Yuan CN2k2-202* (NEU), AY348802, DQ147855. *I. leschenaultii* Wall.,India Origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (NEU), AY348803, DQ147856. *I. longiloba* Craib, Chiang Mai, Thailand, *Ruchisansakun 140* (QBG), KC905494, KC905571. *I. mackeyana* Hook. f. subsp. *claeri* (N.Hall) Grey-Wilson,Gabon origin, cult. Nat Bot. Gard. Koblenz Univ., *Fischer EF5* (NEU), HM454291, FJ826657. *I. mackeyana* Hook. f. subsp. *zenkeri* (Warb.) Grey-Wilson, African origin, cult. Bot. Gard. Koblenz Univ., *Fischer EF21* (NEU), HM454292, DQ147857. *I. macroptera* Hook. f., Equatorial Guinea, *de Wilde 12014*(WAG), HM454301, FJ826658. *I. macrosepala* Hook. f., Phangnga, Thailand, *Ruchisansakun 190* (QBG), KC905495, KC905577. *I.* sp. WSN01, Thailand, *Wongnak s.n.* (QBG), KC905496, KC905584. *I. mengtszeana* Hook. f., Chiang Mai, Thailand, *Ruchisansakun 088* (QBG), KC905498, KC905561. *I. mengtszeana* Hook. f., Yunnan, China, *Yuan CN2k1-38* (NEU), AY348806, DQ147858. *I. meruensis* Gilg. subsp. *cruciata* (T.C.E. Fr.) Grey-Wilson, Tanzania origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (NEU), AY348807, FJ826661. *I. mirabilis* Hook. f., Trang, Thailand, *Suksathan 4095* (QBG), KC905499, KC905614. *I. monticola* Hook. f., Sichuan, China, *Hao 425* (NEU), AY348810, DQ147860. *I. muscicola* Craib, Chiang Mai, Thailand, *Ruchisansakun 186* (QBG), KC905500, KC905547. *I.* sp. R117, Thailand, *Ruchisansakun 117* (QBG), KC905501, KC905579. *I. nalampoonii* T. Shimizu, Lampang, Thailand, *Suksathan 4595*, KC905502, KC905604. *I. namkatensis* T. Shimizu, Mae Hong Son, Thailand, *Wongnak s.n.* (QBG 37080), KC905503, KC905598. *I. napoensis* Y. L. Chen, Yunnan, China, *Yuan CN2k1-61* (NEU), AY348811, DQ147861. *I. niamniamensis* Gilg, African origin, cult. Bot. Gard. Koblenz Univ, *Fischer NE13* (NEU), AY348812, —. *I. niamniamensis* Gilg, African origin, cult. Nat. Bot. Gard., *Meise FB/S2590* (BR), —, DQ147862. *I. noei* Craib, Saraburi, Thailand, *Maknoi s.n.* (QBG), KC905504, KC905548. *I. noli-tangere* L., Jilin, China, *Yuan CN2k1-86* (NEU), AY348813, —. *I. noli-tangere* L., Korea, *Janssens SJ008* (LV), —,FJ82664. *I.* cf. "notoptera", Sakon Nakhon Thailand, *Suksathan & Triboun 4578* (QBG), KC905473, KC905545. *I. omeiana* Hook. f., Sichuan, cult. Univ. California Bot. Gard. Berkeley, *2002.0214* (UC), HQ718765, DQ147864. *I. omeiana* Hook. f., China origin, cult. Chiang Mai, Thailand, *Ruchisansakun 236* (QBG), KC905505, KC905619. *I. opinata* Craib, Krabi, Thailand, *Ruchisansakun 242* (QBG), KC905506, KC905615. *I. oxyanthera* Hook. f., Sichuan, China, *Song S008* (NEU), AY348814, DQ147865. *I. palpebrata* Hook. f., Gabon, *Jongkind 5724* (WAG), HM454299, FJ826666. *I. parasitica* Bedd., India origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (NEU), AY348815, —. *I. parasitica* Bedd., India origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (LV), —, FJ826667. *I. parishii* Hook. f., Tak, Thailand, *Ruchisansakun 083* (QBG), KC905507, KC905608. *I. parviflora* DC., Poland, *Ronikier s.n.*(NEU), AY348816, DQ147866. *I. patula* Craib, Tak, Thailand, *Ruchisansakun 203* (QBG), KC905509, KC905549. *I. peltata* Hook. f., Phatthalung, Thailand, *Suksathan 4323* (QBG), KC905510, KC905609. *I. phahompokensis* T. Shimizu & Suksathan, Chiang Mai, Thailand, *Suksathan 4734* (QBG), KC905511, KC905562. *I.* sp. S5090, Thailand, *Suksathan 5090* (QBG), KC905512, KC905582. *I. phengklaii* T. Shimizu & Suksathan, Kanchanaburi, Thailand, *Ruchisansakun 155* (QBG), KC905513, KC905613. *I.* sp. S4541, Thailand, *Suksathan 4541* (QBG), KC905514, KC905583. *I.* sp. R098, Thailand, *Ruchisansakun 098* (QBG), KC905516, KC905596. *I. phuluangensis* T. Shimizu, Phitsanulok, Thailand, *Suksathan 4545* (QBG), KC905517, KC905554. *I. platychlaena* Hook. f., Sichuan, China, *Song S009* (NEU), AY348818, DQ147867. *I. platypetala* Lindl., Bali, Indonesian origin, cult. by Ray Morgen, U.K., *Ray Morgan s.n.* (NEU), AY348819, DQ147868. *I. poculifer* Hook. f., Yunnan, China, *Yuan CN2k2-209* (NEU), AY348820, DQ147870. *I.* sp. S4591, Thailand, *Suksathan 4591* (QBG), KC905518, KC905581. *I. pseudochinensis* T. Shimizu, Chaiyaphum, Thailand, *Ruchisansakun 218* (QBG), KC905519, KC905559. *I. pseudomacroptera* Grey-Wilson, Gabon, *de Wilde 10390* (WAG), HM454300, FJ826670. *I. pseudoviola* Gilg., central Africa, *RBG Edinburgh 19680124* (E), AY348822, DQ147871. *I. psittacina* Hook. f., Chiang Mai, Thailand, *Ruchisansakun 139* (QBG), KC905520, KC905597. *I. purpurea* Hand.-Mazz., Yunnan, China, *Song Y007* (NEU), AY348823, DQ147872. *I. racemosa* DC., Chaing Mai, Thailand, *Ruchisansakun 198* (QBG), KC905522, KC905574. *I. radiata* Hook. f., Chaing Mai, Thailand, *Ruchisansakun 133* (QBG), KC905523, KC905573. *I. rectangula* Hand.-Mazz., Yunnan, China, *Yuan CN2k1-26* (NEU), AY348825, DQ147874. *I. repens* Moon, Sri Lanka origin, cult. in Thailand, *Ruchisansakun 086* (QBG), KC905524, KC905555. *I. reticulata*, Trang, Thailand, *Suksathan & Triboun 4845* (QBG), KC905525, KC905556. *I. rubricolor* Tardieu, Bolaven, S Laos, *Suksathan & Wongnak 5473* (QBG), KC905474, KC905552. *I. rubrostriata* Hook. f., Yunnan, China, *Yuan CN2k1-44* (NEU), AY348828, DQ147876. *I. ruthiae* Suksathan & Triboun, Loei, Thailand, *Suksathan 4775* (QBG), KC905526, KC905600. *I. salaengensis* T. Shimizu, Thailand, *Ruchisansakun 148* (QBG), KC905527, KC905586. *I. santisukii* T. Shimizu, Chiang Mai, Thailand, *Ruchisansakun 106* (QBG), KC905528, KC905550. *I. saraburiensis* T.Shimizu, Thailand, *Ruchisansakun 163* (QBG), KC905529, KC905588. *I.* cf. *scortechinii*, Nakhon si Thammarat, Thailand, *Suksathan & Triboun 4846* (QBG), KC905475, KC905594. *I. scutisepala* Hook. f., Yunnan, China, *Yuan CN2k-56* (NEU), AY348830, DQ147878. *I. shimianensis* Ge Chen Zhang & L. B. Zhang, Sichuan, China, *L. B. Zhang & G. Zhang 4803* (CDBI, MO), HQ011920, HQ011921. *I.* sp. WSN02, Thailand, *Wongnak s.n.* (QBG), KC905530, KC905566. *I. siamensis* T. Shimizu., Trang, Thailand, *Suksathan & Triboun 4834* (QBG), KC905606. *I. sirindhorniae* Triboun & Suksathan, Krabi, Thailand, *Suksathan & Triboun 4853* (QBG), KC905531, KC905611. *I. sodenii* Engl. & Warb. ex Engl., Kenya origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE15* (NEU), AY348832, —. *I. sodenii* Engl. & Warb. ex Engl., Tanzania, *Knox 3318* (LV), —, FJ826675. *I. soulieana* Hook. f., Sichuan, China, *Yuan CN2k2-163* (NEU), AY348833, DQ147880. *I. spectabilis* Triboun & Suksathan, Kanchanaburi, Thailand, *Ruchisansakun 085* (QBG), KC905532, KC905592. *I.* sp. R188, N Vietnam, Cultivated in Thailand, *Ruchisansakun 188* (QBG), KC905533, KC905590. *I.* sp. LUU597, Vietnam, *LUU597* (QBG), KC905534, KC905570. *I. stenosepala* Pritz. ex Diels, Shaanxi, China, Wang Sh001 (NEU), AY348835, DQ147881. *I. stuhlmannii* Warb., African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE17* (NEU), AY348836, HE617198. *I. suksathanii* Ruchis. & Triboun, Thailand, *Ruchisansakun 210* (QBG), KC905508, KC905605. *I. taronensis*Hand.-Mazz., Yunnan, China, *Yuan CN2k-57* (NEU), AY348838, DQ147882. *I. teitensis* Grey-Wilson, African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE18* (NEU), AY348840, DQ147883. *I.* sp. S4594, Thailand, *Suksathan 4594* (QBG), KC905536, KC905602. *I.* sp. R122, Prae, Thailand, *Ruchisansakun 122* (QBG), KC905535, KC905603. *I. tigrina* Suksathan & Triboun, Loei, Thailand, *Ruchisansakun 157* (QBG), KC905537, KC905589. *I. tinctoria* A.Rich., African origin, cult. Nat. Bot. Gard. Meise, *2002–1249–61* (BR), AY348842, DQ147884. *I. tribounii* T. Shimizu & Suksathan, Kanchanaburi, Thailand, *Ruchisansakun 212* (QBG), KC905538, KC905591. *I. trichosepala* Y. L. Chen, Yunnan, China, *Yuan CN2k1-68* (NEU), AY348843, DQ147885. *I. tuberosa* H. Perrier, Madagascan origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE19* (NEU), AY348844, —. *I. tuberosa* H. Perrier, Madagascan origin, cult. Bot. Gard. Univ. Kopenhagen, *Janssens SJ005* (LV), —, DQ147886. *I. uliginosa* Franch., Yunnan, China, *Yuan CN2k2-173* (NEU), AY348845, DQ147887. *I. uniflora* Hayata, Taiwain, China, Zhengyu Jiang T1, AY348846, DQ147888. *I. usambarensis* Grey-Wilson, African origin, cult. Bot. Gard. Koblenz Univ., *Fischer NE20* (NEU), AY348847, DQ147889. *I. vaughanii* Hook. f., Satun, Thailand, *Suksathan & Triboun 4843* (QBG), KC905540, KC905575. *I. violaeflora* Hook. f., Chiang Mai, Thailand, *Ruchisansakun 104* (QBG), KC905541, KC905551. *I.* aff. *violaeflora*, Thailand, *Ruchisansakun 170* (QBG), KC905463, KC905544. *I. walleriana* Hook. f., Kenya, *Odile Phaehler & Magali Schnell I08* (NEU), AY348849, —. *I. walleriana* Hook. f., African origin, cult. Nat. Bot. Gard., Meise S3926 (BR), —, DQ147892. *I.* sp. SW5132, Thailand, *Suksathan & Wongnak 5132* (QBG), KC905543, KC905576. *I. xanthina* Comber, Yunnan, China, *Yuan CN2k1-15* (NEU), AY348850, DQ147893. *I. yingjiangensis* S. Akiyama & H. Ohba, Yunnan, China, *Yuan CN2k1-55* (NEU), AY348851, DQ147894. *Marcgravia umbellata* L., South American origin, cult. Nat. Bot. Gard. Meise, *FB/S3782* (BR), AY452669, DQ147897. *Norantea guianensis* Aubl.,sample from botanical Garden, origin unknown, Jard. Bot. Nat. Belgique *FB/S3779* (BR), AY348855, DQ147898. *Pelliciera rhizophorae* Planch. and Triana, Costa Rica, *Pennington et al. 586* (K), AY348856, DQ147899. *Souroubea* sp., Peru, BG Univ. *Utrecht 76GR00102* (U), AY348857, DQ147900.