

## Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) Using Chloroplast *atpB-rbcL* Spacer Sequences

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**ABSTRACT.** Balsaminaceae are a morphologically diverse family with ca. 1,000 representatives that are mainly distributed in the Old World tropics and subtropics. To understand the relationships of its members, we obtained chloroplast *atpB-rbcL* sequences from 86 species of Balsaminaceae and five outgroups. Phylogenetic reconstructions using parsimony and Bayesian approaches provide a well-resolved phylogeny in which the sister group relationship between *Impatiens* and *Hydrocera* is confirmed. The overall topology of *Impatiens* is strongly supported and is geographically structured. *Impatiens* likely originated in South China from which it colonized the adjacent regions and afterwards dispersed into North America, Africa, India, the Southeast Asian peninsula, and the Himalayan region.

Balsaminaceae are annual or perennial herbs with flowers that exhibit a remarkable diversity. The family consists of more than 1,000 species (Grey-Wilson 1980a; Clifton 2000), but only two genera are recognized. *Impatiens* is a very large genus of over 1,000 species, while *Hydrocera triflora* is the only member of the genus *Hydrocera*. Though the two genera are very similar, several features distinguish them. *Hydrocera* has five free sepals and five free petals, while *Impatiens* usually has three free sepals and always five petals, four of which are united into two lateral pairs. In addition, *Hydrocera* produces an indehiscent pseudo-berry (Grey-Wilson 1980b; Raghuvver et al. 1993), whereas *Impatiens* have an explosively dehiscent capsule. *Impatiens* is mainly distributed in montane areas in the Old World tropics and subtropics (Grey-Wilson 1980a; Yuan et al. 2004) and *Hydrocera triflora* has a distributional area confined to the lowlands of Indo-Malaysia (Grey-Wilson 1980b; Ramadevi and Narayana 1989). A high diversity of *Impatiens* species is found in tropical Africa, Madagascar, South India, the eastern Himalayas, Southeast Asia, and Southwest China (Grey-Wilson 1980a). The temperate regions in Europe, North America, and northern China are inhabited by only a few species. Indigenous *Impatiens* species are absent from South America and Australia.

Despite the diversity of floral morphological features within this genus, taxonomic studies on Balsaminaceae have encountered several difficulties, leaving numerous relationships within *Impatiens* unresolved (Hooker 1875; Warburg and Reiche 1895; Hooker 1905). Until now, only two molecular surveys have been carried out to unravel the relationships within the Balsaminaceae. Fujiihashi et al. (2002) published the first molecular phylogeny of *Impatiens*. However, the use of *Tropaeolum* as an outgroup and the limited taxon sampling (25 representatives) resulted in limited insights

in the intrageneric relationships of *Impatiens*. The most recent molecular intrageneric study on Balsaminaceae utilized Internal Transcribed Spacer (ITS) sequences and provided new phylogenetic insights in *Impatiens* (Yuan et al. 2004). In this study, we follow up the ITS study with chloroplast *atpB-rbcL* sequence data and propose a preliminary reconstruction of the intrageneric relationships in *Impatiens*.

### MATERIALS AND METHODS

**Taxon Sampling.** Representatives of both *Impatiens* and *Hydrocera* were included in our sampling. We chose our sampling to represent the whole geographic and taxonomic diversity of the family. Fresh material, silica dried specimens, and herbarium specimens of 86 *Impatiens* species are used in this molecular study. Recent molecular results confirmed a well supported clade of Tetrameristaceae and Marcgraviaceae with Balsaminaceae (Morton et al. 1996; Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Anderberg et al. 2002; Bremer et al. 2002; APG 2003; Geuten et al. 2004). Therefore, we used representatives of Marcgraviaceae (*Marcgravia umbellata*, *M. maguire*, *Norantea guianensis*, *Souroubea* sp.) and Tetrameristaceae (*Pelliciera rhizopora*) as the outgroups. The list of taxa with authorities, localities, voucher numbers and accession numbers is shown in Appendix 1.

**Molecular Protocols.** Total genomic DNA was extracted using a modified version of the hot CTAB protocol (Saghai-Marouf et al. 1984; Doyle and Doyle 1987). The lysis buffer was described in Chase and Hills (1991) with 3% PVP-40 that was added to bind more phenolic compounds. The aqueous phase was extracted three times with chloroform-isoamylalcohol (24/1 v/v). After an ethanol-salt precipitation (1/10 volume Natrium-Acetate 3M and 2 vol. ethanol 100%) and subsequent centrifugation, the pellet was washed two times and air-dried. The pellet was dissolved in 10mM TrisHCl (pH 8.5) and stored at 4°C (K. Geuten, in mss.)

Specific primers for the *atpB-rbcL* spacer in Balsaminaceae were designed: IMP-*atpB* (5'-ACATCTAGTACCGGACCAATGA-3') and IMP-*rbcL* (5'-AACACCAGCTTTGAATCCAA-3'). The temperature profile consisted of 2 min initial denaturation at 94°C and 30 cycles of 1 min denaturation at 94°C, 30s primer annealing at 51°C, and 30s extension at 72°C. Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems). Cycle sequencing reactions were performed as in Geuten et al. (2004).

**Data Matrices and Alignment.** *atpB-rbcL* sequences were initially aligned with CLUSTALX applying the default parameters

for gap opening and gap extension. Further adjustment of the preliminary aligned data matrix was performed manually using MacClade 4.05 (Maddison and Maddison 2002). For the parsimony-based analyses, we coded the indels in the *atpB-rbcL* spacer as separate characters, following the 'simple indel coding' method (Simmons and Ochoterena 2000). Non-informative or ambiguous gaps were not coded. Both indel coded and non-coded data matrices were analyzed using maximum parsimony. The ITS matrix of Yuan et al. (2004) was used for a combined analysis. The same regions as described in the ITS manuscript and 77 characters were removed from the combined data matrix. Species that were absent in the nuclear or the chloroplast data set were excluded to avoid phylogenetic incongruence caused by the presence of missing data. All sequences were submitted to GenBank (Appendix 1) and the data sets and the representative trees are deposited in TreeBASE (study accession S1378).

**Phylogenetic Analysis.** The *atpB-rbcL* dataset was analyzed both separately and in combination with ITS. A partition homogeneity test (implemented in PAUP\*4.0b10a) was used to determine whether the datasets were providing different signal in the combined analysis.

Maximum parsimony (MP) analyses were conducted using PAUP\* 4.0b10a (Swofford 2002). Heuristic searches were conducted with tree-bisection reconnection (TBR) branch swapping on 10,000 random addition replicates, with five trees held at each step. Characters were equally weighted and character states were specified to be unordered. Non-parametric bootstrap analysis (MP-BS) was carried out to calculate the relative support for individual clades found in the parsimony analysis (Felsenstein 1985). For each of the 500 bootstrap replicates, a heuristic search was conducted with identical settings as in the original heuristic analysis.

The best fitting substitution model for Bayesian analysis was selected using a series of likelihood ratio tests as implemented in ModelTest 3.06 (Posada and Crandall 1998). A Bayesian analysis was conducted with MrBayes 3b4 (Huelsenbeck and Ronquist 2001). Four chains (one cold, three heated), initiated from a random starting tree were run for 10 million generations. Every 500 generations, a tree was sampled from the chain for a total of 20,000 trees. Due to the burn-in, 10,000 sample points were discarded until stationarity was established among the chains. PAUP\* 4.0b10 was used to calculate a 50% majority-rule consensus tree and to report the posterior probabilities for each clade. Posterior probabilities have been shown to overestimate branch support, so they will be interpreted with caution (Suzuki et al. 2003).

**Character Evolution and Biogeographical Implementation.** Cytological and morphological characters such as pollen type, capsule shape and number of sepals were mapped together with geographic distribution on the *atpB-rbcL* tree using MacClade 4.05 (results not shown).

## RESULTS

***atpB-rbcL* Spacer.** The aligned data matrix contains 961 characters from which 277 (29%) are variable. Of the total variable positions, 154 (56%) are parsimony informative. A total of 46 indels were found, of which 21 (46%) were insertions and 25 deletions (54%). Only 25 gaps were found to be potentially informative and were included as separate characters.

Parsimony analysis of the non-gapped data matrix resulted in 6407 most parsimonious trees. The strict consensus tree is well resolved (Fig. 1), especially among the most early diversified branches. Bootstrap analysis shows relatively high support for many of the lineages. Although the use of the coded indels did not change the general topology, we noticed an overall increase of the bootstrap support for almost every lineage and a reduction in the number of shortest trees to

1531 (Fig. 1). The Bayesian consensus tree is well resolved and most of the lineages have support percentages of 95% or higher (Fig. 2).

Both Bayesian analysis and MP highly support the monophyly of the family (BS: 100 / BPP: 100). With respect to the outgroup chosen, our data confirm the sistergroup relationship between *Impatiens* and *Hydrocera* with very high support value (BS: 100 / BPP: 100). Clade 1 (BS: 98 / BPP: 100) contains only one species (*I. omeiana*) and is sister to all the other *Impatiens* species. The remaining representatives of *Impatiens* are divided into two large clades, which are both highly supported (clade I—BS: 95 / BPP: 100; clade II—BS: 92 / BPP: 100). The first large group comprises three lineages, two of which are moderately supported (clades 3, 4). Clade 4 (BS: 70 / BPP: 100) is sister to clade 3 and consists mainly of endemic South Chinese species. Clade 3 includes species that are mainly distributed in the Himalaya and the more temperate regions of Eurasia (BS: 82 / BPP: 95).

Clade II is a more heterogeneous collection of species with a distribution in Asia, Africa, and Madagascar. Most of the clades in this heterogeneous group are highly supported by both MP and Bayesian analysis, but the relationships among them are not well resolved. It is worth noting that almost every well-supported lineage in this second group has a specific area of distribution (Fig. 1). Clade 6 comprises mainly South Chinese species including two North American species. Bayesian analysis shows that this Chinese-American clade is sister to a small African lineage (clade 5), which is isolated from the other African species. However, the Bayesian support value for this relationship is low (BPP: 74), whereas the MP bootstrap value does not support this relationship.

Two other groups of South Chinese species are resolved in clade 7 (BS: 94 / BPP: 100) and clade 9 (BS: 97 / BPP: 100). Clade 9 is part of a large group (clade III) comprising mainly African species and species from Madagascar, Southeast Asia, and South India. Within clade III, two South Indian lineages show no affinity to each other (clade 8 and clade 15). Clade 8 comprises South Indian species, while clade 15 comprises mainly species from South India and Vietnam (BS: 84 / BPP: 100). Clade 15 includes also one species from Myanmar and Yunnan, China. The relationships within the latter group remain mostly unresolved. A West African clade (clade 10—BS: 97 / BPP: 100), a Central African clade (clade 13—BS: 94 / BPP: 100), an East African clade (clade 14—BS: 99 / BPP: 100) and a Southeast Asian clade (clade 11—BS: 79 / BPP: 100) are resolved within clade III as well. Madagascan species (clade 12) are resolved as a highly supported monophyletic group (BS: 87 / BPP: 100). Both Bayesian analyses and MP point out that the Madagascan clade might be sister to *I. meruensis* from Eastern Africa.

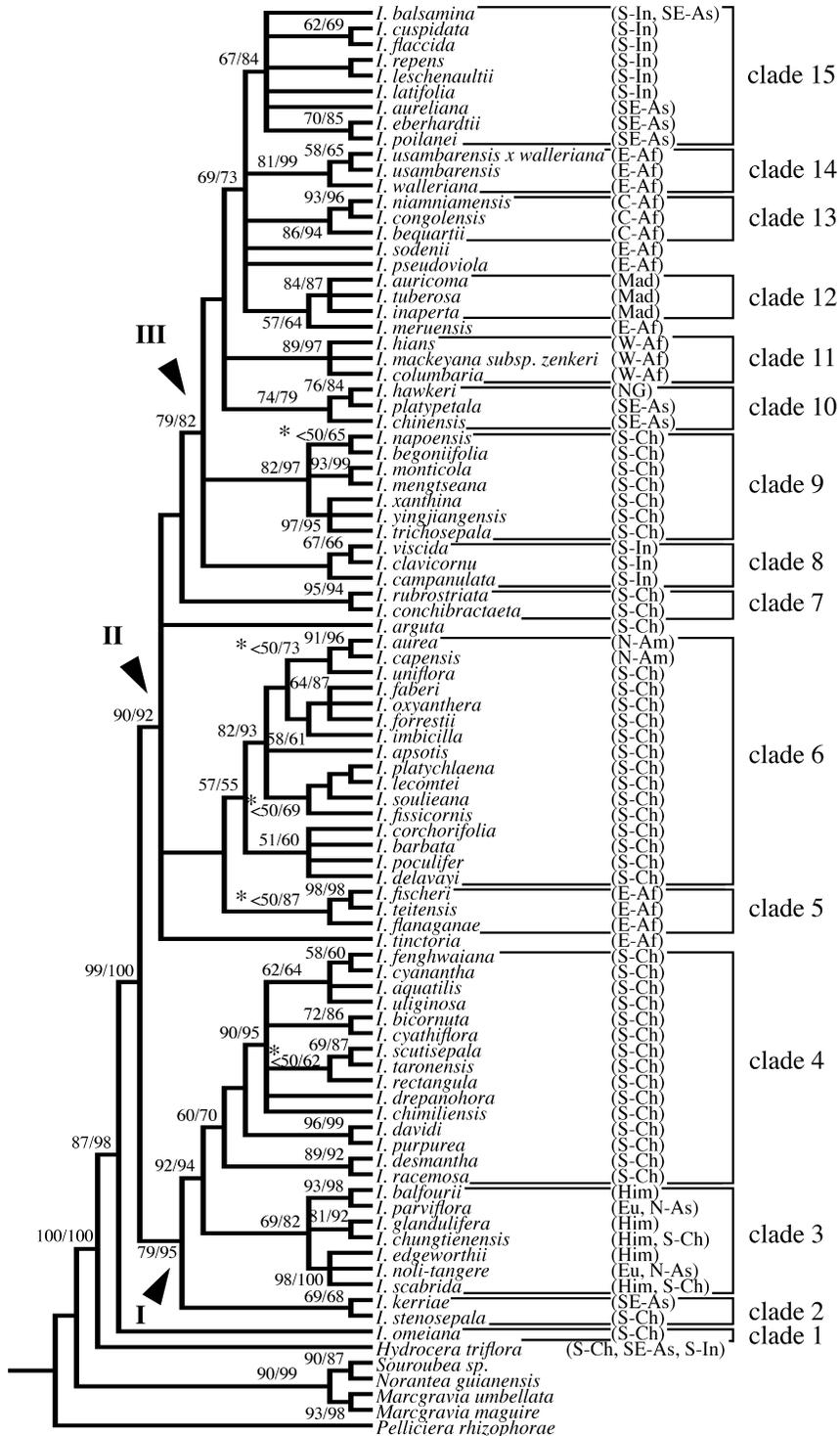


FIG. 1. Strict consensus tree from the parsimony analysis based on *atpB-rbcL* spacer data. Roman figures are assigned to deeper internal nodes that are highly supported, whereas specific clades are numbered 1 through 15. The first number on the branch always represents bootstrap support of the non-gap-coded data matrix and the second number always indicates bootstrap support of the gap-coded data matrix. Branches that collapse in the non-gap-coded consensus tree have been indicated by an asterisk. Distribution areas are shown after the species name (C-Af: Central Africa, E-Af: East Africa, Eu: Europe, Him: Himalaya, Mad: Madagascar, N-Am: North America, N-As: North Asia, NG: New Guinea, S-Ch: South China, SE-As: South East Asia, S-In: South India, W-Af: West Africa).

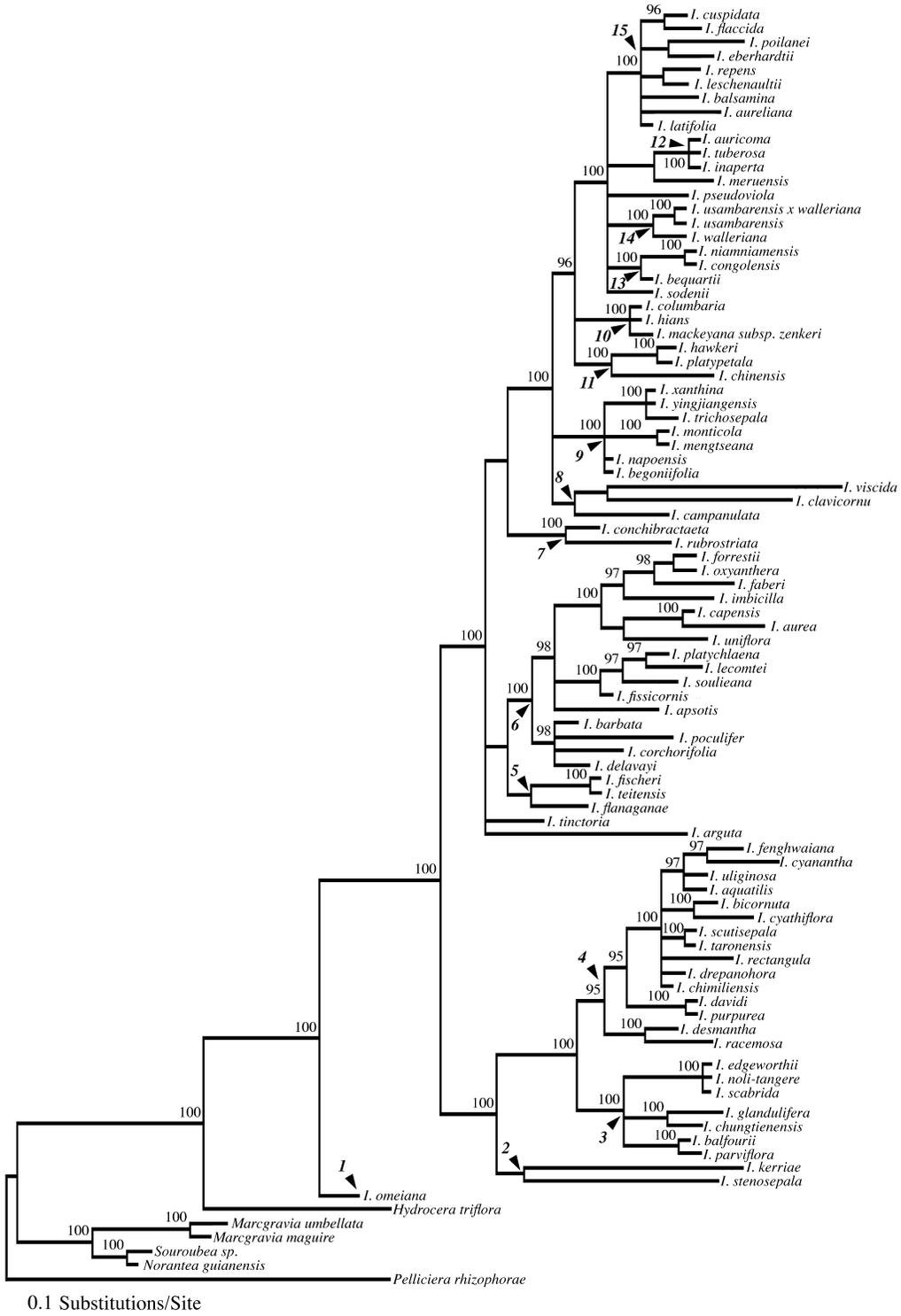


FIG. 2. Bayesian consensus phylogram based on the branch length of the complete *atpB-rbcL* dataset. Numbers on the branch indicate posterior probabilities higher than 95%.

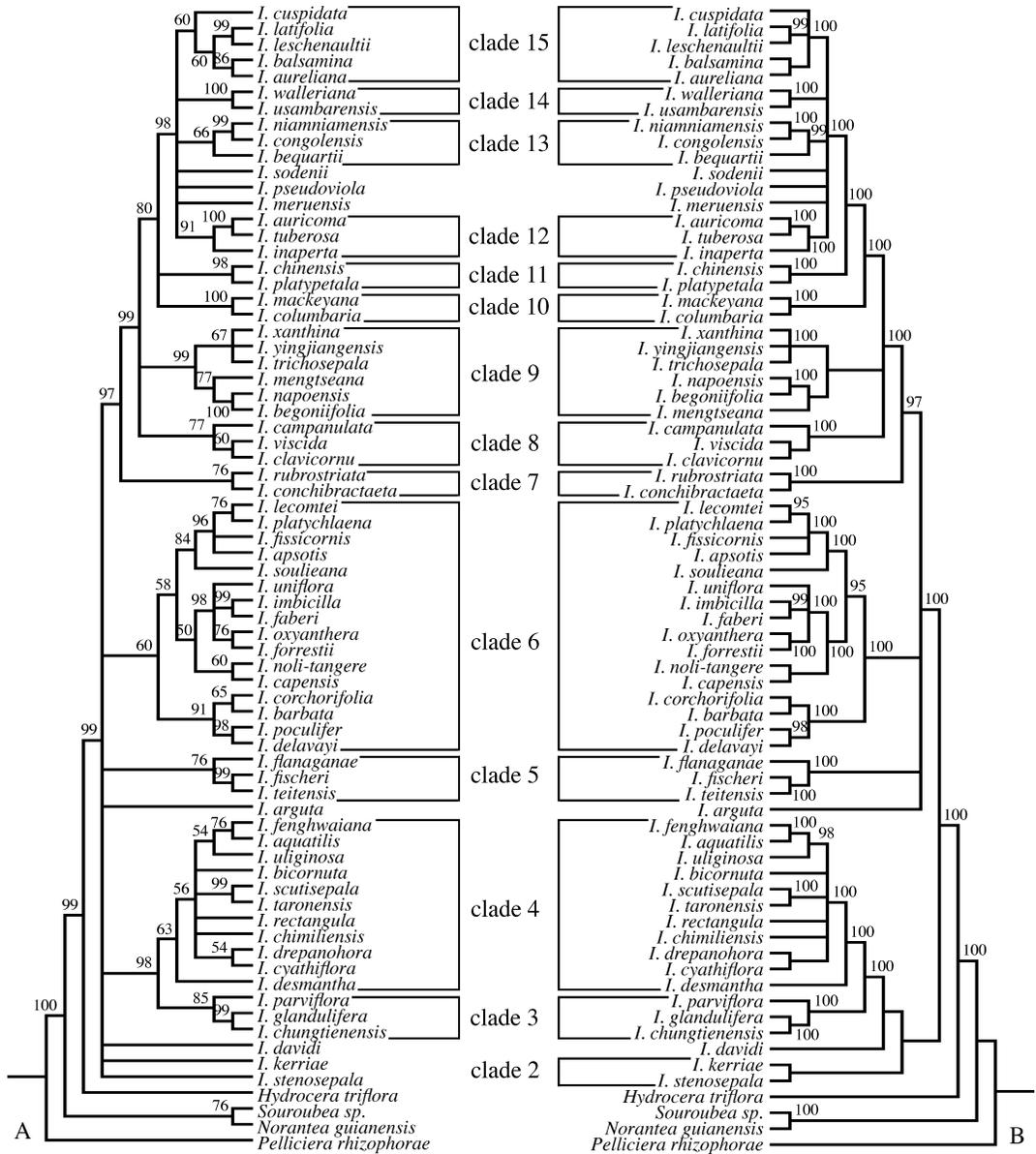


FIG. 3. Phylogenetic trees based on combined ITS and *atpB-rbcL* spacer data. A. Strict consensus tree from the parsimony analysis. Bootstrap values are present above the branches. B. Bayesian consensus cladogram. Numbers above the branch indicate posterior probabilities. Only posterior probabilities higher than 95% are shown.

However, this relationship is only moderately supported (BS: 64 / BPP: 92).

**Combined ITS and *atpB-rbcL*.** The combined data matrix contains 72 species and 1722 characters from which 610 (35 %) are variable. Of the total variable positions, 417 (68%) are parsimony informative. The partition homogeneity test found no significant difference between ITS and *atpB-rbcL* spacer partitions of the combined dataset ( $p > 0.05$ ). The parsimony consensus tree produced by combining the data sets (Fig. 3A) is mainly in agreement with the consensus tree produced

by the chloroplast analysis. However, bootstrap support values are remarkably lower for the majority of the branches. Additionally, the earliest diverged lineages of *Impatiens* are collapsed, showing *I. arguta*, *I. kerriae*, *I. stenosepala*, *I. davidi*, and the main *Impatiens* clade as one basal polytomy. Bayesian analysis of the combined data matrix resulted in greater resolution (Fig. 3B) than the parsimony bootstrap analysis of the combined data (Fig. 3A).

In comparing *atpB-rbcL* with the combined ITS and *atpB-rbcL* dataset, we notice a small number of incon-

gruent relationships between both phylogenies. One of the largest dissimilarities is found in the position of *I. noli-tangere*. According to the combined analysis (Fig. 3), *I. noli-tangere* is sister to *I. capensis* (clade 6), but with only chloroplast data (Figs. 1, 2) *I. noli-tangere* forms a well-supported clade with *I. edgeworthii* and *I. scabrida* (clade 6). A second incongruence is found in the position of *I. cyathiflora* (clade 4). The combined analysis shows a sistergroup relationship between *I. drepanophora* and *I. cyathiflora* (Fig. 3), whereas *I. cyathiflora* is sister to *I. bicornuta* in the chloroplast phylogeny (Figs. 1, 2). Another difference between the combined and chloroplast phylogenies is the position of *I. uniflora* and *I. imbicilla* (clade 6). In the chloroplast phylogeny (Figs. 1, 2), *I. uniflora* is sister to the North American lineage, while in the combined analysis (Fig. 3) *I. uniflora* is located in a trichotomy with *I. imbicilla* + *I. faberi* and *I. forrestii* + *I. oxyanthera*. In the combined analysis, *I. imbicilla* is sister to *I. faberi*, whereas chloroplast data group *I. imbicilla* as sister to a polytomy consisting of *I. faberi*, *I. oxyanthera*, and *I. forrestii*.

#### DISCUSSION

Phylogenetic analysis of chloroplast *atpB-rbcL* spacer data provides a well supported tree that we regard as a good hypothesis of evolutionary relationships in Balsaminaceae. Although the chloroplast and combined data phylogenies resemble each other closely, we noticed some minor differences, which are also present when the *atpB-rbcL* phylogeny is compared with only the nuclear one. According to Grey-Wilson (1980c) many *Impatiens* species originated through hybridization. Therefore, we assume that these minor dissimilarities are probably caused by the occurrence of reticulate evolution. However, since ITS is susceptible to segregation, concerted evolution, and recombination, which causes an ITS phylogeny sometimes to be inaccurate or to reflect an incomplete species tree (Alvarez and Wendel 2003), we must be cautious with our conclusions about hybridization in *Impatiens*.

We prefer to use the plastid phylogeny instead of the combined ITS/*atpB-rbcL* dataset for further discussion. The combined data hypothesis is less resolved with lower support values, which could be caused by the very high substitution rate of ITS1 and ITS2, even for the greater taxon sampling used in Yuan et al. (2004), resulting in the suppression of the phylogenetic signal of the *atpB-rbcL* dataset (Mansion and Struwe 2004; Schneeweiss et al. 2004).

**Chloroplast Phylogeny in Comparison to Global Intra-generic Classification.** Due to hybridization events, rapid speciation, and the occurrence of transitional character states (Grey-Wilson 1980a, c; Merlin and Grant 1985; Shimizu et al. 1996; Tsukaya 2004), normally important taxonomic characters seem to be rather randomly distributed throughout the whole genus.

Consequently, the use of morphological data to divide the genus *Impatiens* into natural groupings has caused many difficulties in the past. Warburg and Reiche (1895) presented the only global intrageneric classification. Due to its artificial nature, the established subgenera and sections of this early classification were hardly used in later morphological and molecular studies. In fact, even the authors recognized their classification as 'not natural'. Together with previously published molecular data (Yuan et al. 2004), this study confirms the artificiality of their attempt. For example, according to Warburg and Reiche's (1895) classification, *I. latifolia* (sect. *Enantiophyllon*, subg. *Caulimpatiens*), *I. balsamina* (sect. *Microcentron*, subg. *Caulimpatiens*) and *I. flaccida* (sect. *Macrocentron*, subg. *Caulimpatiens*) belong to three different sections, but our analysis clearly illustrates that each of these species are part of the same south Indian lineage (clade 15). Moreover, Warburg and Reiche (1895) suggested that *I. repens* and *I. glandulifera* belong to the same section (sect. *Microcentron*, subg. *Caulimpatiens*). According to our results, *I. glandulifera* is part of a more basal clade in *Impatiens* (clade 3), while *I. repens* is found in the more derived, south Indian clade together with *I. flaccida*, *I. balsamina*, and *I. latifolia* (clade 15).

**Phylogenetic Relationships within Balsaminaceae.** Although many earlier studies at family level or above showed that Balsaminaceae are part of the basal clade in Ericales, the use of only very few *Impatiens* species in those surveys could not provide a definite answer regarding the monophyly of the family (Savolainen et al. 2000; Soltis et al. 2000; Albach et al. 2001; Anderberg et al. 2002; Bremer et al. 2002; APG 2003; Geuten et al. 2004). Yuan et al. (2004) included adequate taxon sampling to test and affirm the monophyly of Balsaminaceae. This conclusion was strongly confirmed by our study (BS: 100 / BPP: 100).

Based on differences in floral morphology, anatomy, and habitat, many botanists regarded *Hydrocera* as a distinct genus in Balsaminaceae (Warburg and Reiche 1895; Venkateswarlu and Lakshinarayana 1957; Grey-Wilson 1980b; Ramadevi and Narayana 1989; Raghuveer et al. 1993). However, it could have been possible that *Hydrocera* was nested within the genus *Impatiens*. Yuan et al. (2004) recognized both genera as two distinct lineages, but the strict consensus of the ITS trees could not give a decisive answer regarding the phylogenetic relationship between the genera. Nevertheless, our results illustrate with very high support (BS: 100 / BPP: 100) that *Hydrocera* is sister to *Impatiens*.

**Phylogenetic Relationships within Impatiens, a Biogeographical Interpretation.** Although the *atpB-rbcL* dataset refines our knowledge of the phylogenetic relationships in *Impatiens*, it remains very difficult to find morphological synapomorphies for the different clades that we find in the *atpB-rbcL* tree. In contrast, many of

the clades can be easily associated with a specific area of distribution. It is therefore interesting to compare the available biogeographical data with our molecular phylogeny (Fig. 1).

Biogeographical optimization onto the chloroplast phylogeny illustrates that *Impatiens* probably originated in South China. The North American species form a strongly supported clade that is nested within a South Chinese lineage (clade 6). Previous studies already suggested that the North American species (*I. capensis* and *I. aurea*) are derived from an East Asian ancestor (Grey-Wilson 1980a; Yuan et al. 2004).

Yuan et al. (2004) concluded that the African species are not monophyletic. Both nuclear and chloroplast trees illustrate that the African continent has been colonized at least twice. One of the dispersals resulted in a small clade that is characterized by long-linear fruits and the presence of five sepals (clade 5), whereas the other group of African species has only three sepals and more fusiform capsules. However, due to the uncertain phylogenetic position of the African species *I. tinctoria* (five sepals with long-linear fruits), and the West African lineage (clade 10), it is not possible to conclude with certainty how many times the African continent has been colonized. New Guinean species are closely related to those of China, which is consistent with findings of Grey-Wilson (1980d).

The Madagascan species form a highly supported clade (clade 12), originated from an African ancestor (Fig. 1). Due to insufficient sampling, it is not possible to conclude with confidence that the invasion of Madagascar happened from a single colonization event or through multiple invasions. South India was colonized at least twice, certainly once by an East Asian ancestor (clade 8), another time most likely by an ancestor with African affinities (clade 15), confirming the assumption of Yuan et al. (2004).

**The Implication of the Molecular Phylogeny for Character Evolution.** One of the most important taxonomic characters in *Impatiens* is the shape of the dehiscent fruit capsule. Grey-Wilson (1980a) delimited a group of northern species with long-linear fruits, a group with broad fusiform fruits belonging to a group of southerly oriented species. Chloroplast spacer data do not provide evidence for the delimitation of these two lineages in *Impatiens*, as suggested by Grey-Wilson (1980a). Moreover, our analysis supports the results of Yuan et al. (2004), which showed that long-linear capsules are plesiomorphic whereas broadly fusiform capsules are a synapomorphy for a large clade consisting of mainly African, Madagascan, and Indian species (clade III). Grey-Wilson's correlation between fruit type and geographic distribution turned out to be correct.

Karyological data offer interesting insights into the molecular phylogeny of *Impatiens*. According to numerous karyological studies, the majority of species in

*Impatiens* possess one of the following haploid chromosome numbers  $n=7$ ,  $n=8$ ,  $n=9$ ,  $n=10$  (Song et al. 2003). Several different hypotheses have been suggested regarding the ancestral haploid chromosome number in *Impatiens*. Koshoo (1957) assumed that  $n=7$  or  $n=10$  were basic chromosome numbers in *Impatiens*. On the contrary, Rao et al. (1986) suggested  $n=8$  as ancestral number, whereas Jones and Smith (1966) and Akiyama et al. (1992) proposed  $n=7$  to be the basic chromosome number. Based on their molecular data, Yuan et al. (2004) suggested that  $n=10$  might be the ancestral chromosome number in *Impatiens*. Due to a lack of karyological data for some taxa it is not possible to provide a definite answer about the basic chromosome number in *Impatiens*. However, our phylogeny points out that  $n=10$  might indeed be ancestral, yet the possibility of  $n=9$  as ancestral chromosome number should not be ruled out.

Several botanists have repeatedly emphasized the extremely high degree of floral variation in *Impatiens* (Hooker 1875; Grey-Wilson 1980a; Akiyama and Ohba 2000; Yuan et al. 2004). Most likely, this high floral diversity is correlated with the evolution of an ancestral *Impatiens* flower with separate and equal perianth organs into the zygomorphic *Impatiens* flower (Grey-Wilson 1980a). One of the trends in this global developmental pattern is the loss of the upper pair of lateral sepals, resulting in a calyx with three sepals (Grey-Wilson 1980a). Although anterolateral sepals are generally absent in *Impatiens*, they are still present in some species as small, rudimentary organs. In others, they occur only in an early developmental stage and disappear later (P. Caris, in mss.). Eleven species in our sample are known to have five sepals in mature flowers and according to our results these species are scattered all over the molecular phylogeny. Regarding our molecular phylogeny and the floral ontogenetic study of P. Caris (in mss.), the occurrence of five sepals is plesiomorphic in *Impatiens*. Consequently, the loss of the anterolateral pair is not a synapomorphy for the genus.

Pollen characters are useful for the recognition of broad evolutionary trends in *Impatiens* (Grey-Wilson 1980d; Janssens et al. 2005). Comparing our molecular phylogeny with previous palynological studies (Huynh 1968; Grey-Wilson 1980d; Lu 1991), various trends in the evolutionary development of the pollen can be observed. Tricolpate pollen grains with a triangular, oblate shape and elongated colpi are the plesiomorphic pollen type in Balsaminaceae. This kind of pollen is observed in *Hydrocera triflora* and some endemic *Impatiens* on Mount Omei in China (Lu 1991; Janssens et al. 2005). Lu (1991) suggested that these Chinese species consisting of *Impatiens omeiana* and its 3-colpate, triangular shaped allies are closely related to *Hydrocera triflora*. Our molecular data confirm this

hypothesis since *I. omeiana* is resolved as sister to the remaining species in *Impatiens*. Furthermore, a trend from triangular 3-colpate pollen to rectangular 4-colpate pollen is observed in the major *Impatiens* clade that is sister to *I. omeiana*. However, in several lineages the rectangular 4-colpate pollen type reverses again to 3-colpate pollen (Huynh 1968; Grey-Wilson 1980d).

We believe that a more extensive sampling and additional data from faster evolving DNA sequences will be necessary to obtain an even better view on the evolution and the biogeography of more recently diverged taxa. Additionally, more extensive morphological studies are needed in order to better comprehend the subclades that are established by molecular data. It is obvious that a more thorough biogeographical survey with dating of the internal nodes will play an important role in the understanding of the evolution and the family.

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## APPENDIX 1

Accession numbers, voucher data and origin of plant material for taxa included in the DNA analyses.

*Impatiens apsotis* Hook.f.: China, Sichuan, *Yuan CN2k2–159* (NEU): DQ147810 *atpB-rbcL*, AY348744 ITS. *Impatiens aquatilis* Hook.f.: China, Yunnan, *Song CNY017* (NEU): DQ147811 *atpB-rbcL*, AY348745 ITS. *Impatiens arguta* Hook.f. and Thoms.: China, Yunnan, *Yuan CN2k–74* (NEU): DQ147812 *atpB-rbcL*, AY348746 ITS. *Impatiens aurea* Muhl.: North American origin, cult. Holden

- patiens kerriae* Craib: Thailand, Qingmai, *Chassot* 99–238 (NEU): DQ147853 *atpB-rbcL*, AY348799 ITS. *Impatiens latifolia* L.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147854 *atpB-rbcL*, AY348801 ITS. *Impatiens lecomtei* Hook.f.: China, Yunnan, *Yuan* CN2k2–202 (NEU): DQ147855 *atpB-rbcL*, AY348802 ITS. *Impatiens leschenaultii* Wall.: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147856 *atpB-rbcL*, AY348803 ITS. *Impatiens mackeyana* subsp. *zenkeri* Hook.f.: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer* NE21 (NEU): DQ147857 *atpB-rbcL*, AY348852 ITS. *Impatiens mengtseana* Hook.f.: China, Yunnan, *Yuan* CN2k1–38 (NEU): DQ147858 *atpB-rbcL*, AY348806 ITS. *Impatiens meruensis* Gilg.: Tanzanian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147859 *atpB-rbcL*, AY348807 ITS. *Impatiens monticola* Hook.f.: China, Sichuan, *Hao* 425 (NEU): DQ147860 *atpB-rbcL*, AY348810 ITS. *Impatiens napoensis* Y.L. Chen: China, Yunnan, *Yuan* CN2k1–61 (NEU): DQ147861 *atpB-rbcL*, AY348811 ITS. *Impatiens niarniamensis* Gilg.: African origin, cult. Nat. Bot. Gard. Meise, *FB/S2590* (BR): DQ147862 *atpB-rbcL*, AY348812 ITS. *Impatiens noli-tangere* L.: Temperate origin, cult. Kruidtuin Leuven, *Janssens* SJ007 (LV): DQ147863 *atpB-rbcL*, AY348813 ITS. *Impatiens omeiana* Hook.f.: China, Sichuan, cult. Univ. California Bot. Gard. Berkeley, 2002.0214 (UC): DQ147864 *atpB-rbcL*. *Impatiens oxyanthera* Hook.f.: China, Sichuan, *Song* S008 (NEU): DQ147865 *atpB-rbcL*, AY348814 ITS. *Impatiens parviflora* DC.: Belgium, Leuven, *Janssens* SJ004 (LV): DQ147866 *atpB-rbcL*, AY348816 ITS. *Impatiens platychlaena* Hook.f.: China, Sichuan, *Song* S009 (NEU): DQ147867 *atpB-rbcL*, AY348818 ITS. *Impatiens platypetala* Lindl.: Bali, Indonesian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147868 *atpB-rbcL*, AY348819 ITS. *Impatiens poilanei* Tardieu: Vietnam, Anam, *Song s.n.* (NEU): DQ147869 *atpB-rbcL*. *Impatiens poculifer* Hook.f.: China, Yunnan, *Yuan* CN2k2–209 (NEU): DQ147870 *atpB-rbcL*, AY348820 ITS. *Impatiens pseudoviola* Gilg.: African origin, cult. Roy. Bot. Gard. Edinburgh, 19680124 (E): DQ147871 *atpB-rbcL*, AY348822 ITS. *Impatiens purpurea* Hand.-Mazz.: China, Yunnan, *Song* Y007 (NEU): DQ147872 *atpB-rbcL*, AY348823 ITS. *Impatiens racemosa* DC.: China, Xizang, *De Haas* 2620 (U): DQ147873 *atpB-rbcL*. *Impatiens rectangula* Hand.-Mazz.: China, Yunnan, *Yuan* CN2k1–26 (NEU): DQ147874 *atpB-rbcL*, AY348825 ITS. *Impatiens repens* Moon: Indian origin, cult. Nat. Bot. Gard. Leiden, 17953 (L): DQ147875 *atpB-rbcL*. *Impatiens rubrostriata* Hook.f.: China, Yunnan, *Yuan* CN2k1–44 (NEU): DQ147876 *atpB-rbcL*, AY348828 ITS. *Impatiens scabrida* DC.: Himalayan origin, cult. Holden arboretum, 941314 (DBG): DQ147877 *atpB-rbcL*. *Impatiens scutisepala* Hook.f.: China, Yunnan, *Yuan* CN2k–56 (NEU): DQ147878 *atpB-rbcL*, AY348830 ITS. *Impatiens sodenii* Engl. and Warb. ex Engl.: African origin, cult. Roy. Bot. Gard. Edinburgh, 19660477 (E): DQ147879 *atpB-rbcL*, AY348832 ITS. *Impatiens soulieana* Hook.f.: China, Sichuan, *Yuan* CN2k2–163 (NEU): DQ147880 *atpB-rbcL*, AY348833 ITS. *Impatiens stenosepala* Pritz. ex Diels: China, Shaanxi, *Wang* SH-001 (NEU): DQ147881 *atpB-rbcL*, AY348835 ITS. *Impatiens taronensis* Hand.-Mazz.: China, Yunnan, *Yuan* CN2k–57 (NEU): DQ147882 *atpB-rbcL*, AY348838 ITS. *Impatiens teitensis* Grey-Wilson: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer* NE18 (NEU): DQ147883 *atpB-rbcL*, AY348840 ITS. *Impatiens tinctoria* A.Rich: African origin, cult. Nat. Bot. Gard. Meise, 2002–1249–61 (BR): DQ147884 *atpB-rbcL*. *Impatiens trichosepala* Y.L. Chen: China, Yunnan, *Yuan* CN2k1–68 (NEU): DQ147885 *atpB-rbcL*, AY348843 ITS. *Impatiens tuberosa* H. Perrier: Madagascan origin, cult. Bot. Gard. Univ. Copenhagen, *Janssens* SJ005 (LV): DQ147886 *atpB-rbcL*, AY348844 ITS. *Impatiens uliginosa* Franch.: China, Yunnan, *Yuan* CN2k2–173 (NEU): DQ147887 *atpB-rbcL*, AY348845 ITS. *Impatiens uniflora* Hayata: China, Taiwan, *Zhengyu* Jiang T1 (NEU): DQ147888 *atpB-rbcL*, AY348846 ITS. *Impatiens usambarensis* Grey-Wilson: African origin, cult. Bot. Gard. Koblenz Univ., *Fischer* NE20 (NEU): DQ147889 *atpB-rbcL*, AY348847 ITS. *Impatiens usambarensis* Grey-Wilson × *walleriana* Hook.f.: African origin, cult. Roy. Bot. Gard. Edinburgh, 19821569 (E): DQ147890 *atpB-rbcL*. *Impatiens viscida* Wight: South Indian origin, cult. by Ray Morgan, U.K., *Ray Morgan s.n.* (LV): DQ147891 *atpB-rbcL*, AY34768 ITS. *Impatiens walleriana* Hook.f.: African origin, cult. Nat. Bot. Gard. Meise, 53926 (BR): DQ147892 *atpB-rbcL*, AY348849 ITS. *Impatiens xanthina* Comber: China, Yunnan, *Yuan* CN2k1–15 (NEU): DQ147893 *atpB-rbcL*, AY348850 ITS. *Impatiens yingjiangensis* S.Akiyama and H.Ohba: China, Yunnan, *Yuan* CN2k1–55 (NEU): DQ147894 *atpB-rbcL*, AY348851 ITS. *Hydrocera triflora* Wight and Arn.: Sri Lanka, *Robyns* 7260, 0249369 (BR): DQ147895 *atpB-rbcL*, AY348853 ITS. *Marcgravia maguire* de Roon: French Guyana, cult. Nat. Bot. Gard. Meise, *FB/S3780* (BR): DQ147896 *atpB-rbcL*. *Marcgravia umbellata* L.: South American origin, cult. Nat. Bot. Gard. Meise, *FB/S3782* (BR): DQ147897 *atpB-rbcL*. *Norantea guianensis* Aubl.: South American origin, cult. Nat. Bot. Gard. Meise, *FB/S3779* (BR): DQ147898 *atpB-rbcL*, AY348855 ITS. *Pelliciera rhizophorae* Planch. and Triana: Costa Rica, *Pennington et al.* 586 (K): DQ147899 *atpB-rbcL*, AY348856 ITS. *Souroubea* sp.: Peru, 76GR00102 (U): DQ147900 *atpB-rbcL*, AY348857 ITS.