



Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change

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ABSTRACT

Impatiens comprises more than 1000 species and is one of the largest genera of flowering plants. The genus has a subcosmopolitan distribution, yet most of its evolutionary history is unknown. Diversification analyses, divergence time estimates and historical biogeography, illustrated that the extant species of *Impatiens* originated in Southwest China and started to diversify in the Early Miocene. Until the Early Pliocene, the net diversification rate within the genus was fairly slow. Since that time, however, approximately 80% of all *Impatiens* lineages have originated. This period of rapid diversification coincides with the global cooling of the Earth's climate and subsequent glacial oscillations. Without this accelerated diversification rate, *Impatiens* would only have contained 1/5th of its current number of species, thereby indicating the rapid radiation of the genus.

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1. Introduction

Global climate changes have played a crucial role in shaping species evolution either by increased rates of extinction, or by bursts of diversification. Earth's climate has changed dramatically altered during the past 65 million years and likewise several lines of evidence have been found that correlate these fluctuations with an increase in speciation rate (Bowie et al., 2004; Erkens et al., 2007; Gamble et al., 2008; Hughes and Eastwood, 2006; Jaramillo et al., 2006; Johnson et al., 2006; Merckx et al., 2008; Plana et al., 2004; Richardson et al., 2001). Nevertheless, it is still being debated when the majority of the current angiosperm diversity has originated during the course of evolution (Davies et al., 2004). For a long time, it was considered that the recent climatic oscillations of the Pleistocene and Pliocene were crucial in promoting general diversification among tropical and subtropical species (Aubréville, 1962; Crowe and Crowe, 1982; Grey-Wilson, 1980a; Haffer, 1969; Mayr and O'Hara, 1986; Moritz et al., 2000; Prance, 1973). This hypothesis of recent speciation in the Quaternary is also referred to as the 'refuge' hypothesis and assumes allopatric speciation in populations as they became isolated from each other due to glacial aridity and rainforest fragmentation. Despite possible molecular evidence for recent explosive diversification events in some tropical lineages (Harris et al., 2000 on *Aframomum*; Rich-

ardson et al., 2001 on *Inga*), several recent studies suggest that these fluctuations played a much smaller role than previously thought and additionally suggest that the massive divergence of tropical species probably started already in the Tertiary or even earlier (Bowie et al., 2004; Glor et al., 2001; Knapp and Mallet, 2003; Moritz et al., 2000; Pennington et al., 2004; Plana et al., 2004; Zink and Slowinsky, 1995).

The Pleistocene refuge hypothesis invokes the occurrence of a heterogeneous topography as cool and dry climate conditions forced rainforest taxa to withdraw into small refugial pockets (Haffer, 1969; Haffer and Prance, 2001; Mayr and O'Hara, 1986; Prance, 1973). In Africa for instance most of the postulated distribution areas of glacial forest refugia are assumed to be associated with more mountainous areas (Maley, 1987; Prance, 1982). In South America, the situation is more complex with several refuge areas being located in lowland Amazonia, yet also some uplifted areas are considered as possible refuges for the neotropical flora (Haffer, 1969, 1982; Prance, 1982). Moreover, endemic taxa are frequently associated with montane regions as geological and altitudinal differences create many habitat types (Grey-Wilson, 1980a; Plana et al., 2004). Investigating tropical or subtropical taxa which are often endemic to lowland or montane rainforests would make it possible to test the influence of glacial oscillations in light of the current species diversity in the tropics. In this aspect, balsams are of particular interest. *Impatiens* (Balsaminaceae) is among the most species-rich genera of flowering plants with over 1000 species and several newly described species every year (e.g. Janssens

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et al., in 2009; Pöcs, 2007). Diversity hotspots for the genus can be observed in tropical Africa, the Himalayan region, Madagascar, South India and Sri Lanka and Southeast Asia (Bhaskar and Razi, 1981; Chen, 2001; Grey-Wilson, 1980a, 1989; Rahelivololona et al., 2003; Shimizu, 1979; Toppin, 1920). Balsams are a characteristic element of tropical and subtropical montane forests above 500–800 m, even sometimes growing as high as 5000 m. Although there are notable exceptions, most species of *Impatiens* cannot endure persistent drought or extended exposure to direct sunlight (Fischer, 2004). As a result *Impatiens* species are typically confined to stream margins, waterside boulders, and wet and/or montane forests. For moisture-loving plants like *Impatiens*, upland rainforests are islands of suitable habitats in a vast landscape that is at least seasonally dry. By migrating along the mountain slopes with its moist habitat, *Impatiens* is able to minimize the effect of large climatic fluctuations. As a consequence, populations of *Impatiens* are easily isolated from other closely related lineages, which could result in an increase in the rate of species diversification.

The present paper investigates the origin and evolution of the Balsaminaceae with special emphasis on tempo and timing of diversification in the genus *Impatiens*. We assess the evolutionary relationships between the major clades of *Impatiens* and date their origin in order to gain insight into causes of speciation. In addition, we examine whether the global cooling at the Miocene–Pliocene boundary and the subsequent climatic fluctuations during Pliocene and Pleistocene might have triggered adaptive radiation or whether the vast number of *Impatiens* lineages had already been largely established in the Tertiary. Furthermore, we provide additional understanding in the importance of the refuge theory as a possible explanation for the present tropical biodiversity.

2. Materials and methods

2.1. Taxon sampling

Species of *Impatiens* were selected to represent the geographic and taxonomic diversity of the genus. In total 117 *Impatiens* species were included from which chloroplast (*atpB-rbcl*) and nuclear (introns 4 and 5 of *ImpDEF1* and *ImpDEF2*) sequences have been produced. Janssens et al. (2006) demonstrated that *Impatiens omeiana* is sister to the other sampled species of *Impatiens*. Therefore, this species was chosen as outgroup for phylogenetic analyses of the remaining samples. A list of taxa with authorities, localities, voucher information and GenBank Accession Nos. is listed in Tables 1 and 2.

2.2. Molecular protocols and phylogenetic analysis

Total genomic DNA was extracted using a modified version of the hot CTAB protocol (Janssens et al., 2006). Primers and temperature profiles used for the amplification of *atpB-rbcl*, *ImpDEF1* and *ImpDEF2* follow Janssens et al. (2006) and Janssens et al. (2007), respectively. Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems). Cycle sequencing reactions were performed as in Janssens et al. (2006).

Initial alignment of the DNA sequences was carried out with CLUSTALX, followed by manual adjustment using MacClade 4.05 (Maddison and Maddison, 2002). The chloroplast *atpB-rbcl* and the nuclear *ImpDEF1/ImpDEF2* datasets were analyzed separately and combined using a maximum likelihood (ML) based method with RaxML (Stamatakis et al., 2005). A partition homogeneity test (implemented in PAUP*4.0b10a; Swofford, 2002) was used to evaluate whether the data matrices provide different signal in the combined analyses. The most appropriate substitution model for each gene marker was determined using a series of likelihood ratio tests as implemented in ModelTest 3.06 (Posada and Crandall, 1998).

ML analyses were carried out on the CIPRES computer cluster (CyberInfrastructure for Phylogenetic REsearch) at the San Diego Supercomputing Center (www.phylo.org). Model parameters were computed with RAXML-VI HPC (Stamatakis, 2006). Two runs were performed, starting from a completely randomized tree, each with 100 inferences on the original alignment. Non-parametric bootstrapping (ML-BS) was carried out by calculating six times 167 replicates on the CIPRES computer cluster. Because a few nodes in the ML phylogram were collapsed and a fully resolved tree was necessary to calculate diversification rates and lineages-through-time plots, we set the branch length of these nodes collapsed to 0.000001, under the assumption that a lack of resolution indicated a rapid radiation (Harris et al., 2000; Hughes and Eastwood, 2006).

2.3. Molecular dating

When estimating the age of the most recently diversified lineages in *Impatiens*, we encountered several problems which made it impossible to immediately calibrate the obtained *Impatiens* phylogeny. (1) Lack of a suitable fossil record for *Impatiens* made it impossible to directly calibrate the *Impatiens* topology. Although there is one fossil pollen record available for the genus (*Impatiensiditis brevicolpus* – Pliocene; Song et al., 2004) the assignment of this fossil pollen to clades in *Impatiens* was impossible. (2) Lack of resolution with the chloroplast *atpB-rbcl* spacer for the most recently diversified lineages. This issue could be solved by using the fast-evolving introns 4 and 5 of *ImpDEF1* and *ImpDEF2*, which are very effective to elucidate relationships at inter- and intraspecific levels (Janssens et al., 2007). However, because of their high nucleotide and indel substitution rate, it was impossible to amplify and sequence these loci for other taxa than *Impatiens*. Due to the high amount of missing data for *ImpDEF1* and *ImpDEF2*, it was impossible to use these loci for the construction of the asterid phylogeny, which was applied estimate the age of the lineages towards *Impatiens*.

As a result, we followed a two-stage strategy to calculate the divergence times for the most diversified branches in *Impatiens*. By extending the *atpB-rbcl* dataset of *Impatiens* with 319 *atpB-rbcl* accessions of asterid taxa from GenBank, we were able to cover almost all major lineages of this large subclass (Cornales – Cornaceae and Hydrangeaceae; Ericales – Actinidiaceae, Ebenaceae, Ericaceae, Lecythidaceae, Marcgraviaceae, Primulaceae, Sapotaceae, Styracaceae and Tetrameristaceae; Garryales – Garryaceae; Aquifoliales – Aquifoliaceae and Helwingiaceae; Gentianales – Apocynaceae, Gentianaceae, Loganiaceae and Rubiaceae; Lamiales – Gesneriaceae, Globulariaceae, Lamiaceae, Lentibulariaceae, Orobanchaceae, Plantaginaceae and Scrophulariaceae; Solanales – Solanaceae; Apiales – Apiaceae and Araliaceae; Asterales – Asteraceae, Campanulaceae and Menyanthaceae; Dipsacales – Adoxaceae, Caprifoliaceae, Dipsacaceae and Valerianaceae). Two accessions from the rosoid clade were used as outgroup. This large-scale approach allowed us to integrate multiple fossil calibration points in order to minimize the bias produced by a single calibration point (Merckx et al., 2008). The asterid dataset contains 437 taxa and 3880 characters and was analyzed with RAXML using similar settings as for the combined chloroplast-nuclear dataset of *Impatiens*. ML bootstrapping of the asterid dataset was also carried out as described above. A χ^2 likelihood ratio test, used to assess rate heterogeneity among lineages, indicated that the substitution rates in *atpB-rbcl* are not clocklike. As a result, we applied a relaxed clock model using the Penalized Likelihood method (PL; Sanderson, 2002) as implemented the r8s software package. The rooted ML phylogram was used as input file for the dating analysis. The following calibration points were used for age estimation: (1) the crown group of *Diospyros*, (Ebenaceae) constrained to a minimum of 54.9 mya corresponding to the Eocene age estimate of the fossil *Austrodiospyros cryptostoma* (Basinger and Christophel, 1985); (2) minimum age

Table 1
Accession Nos., voucher data and origin of plant material for taxa included in the combined DNA analyses of *Impatiens*.

Taxon	Origin	Voucher	Accession No. <i>ImpDEF1</i>	Accession No. <i>ImpDEF2</i>	Accession No. <i>atpB-rbcL</i>
<i>Impatiens aquatilis</i> Hook.f.	China, Yunnan	Song CNY017 (NEU)	–	EF133611	DQ147811
<i>Impatiens arachnoides</i> H.Perrier	Madagascar	Janssens SJ010 (LV)	FJ826680	–	FJ826628
<i>Impatiens assurgens</i> Baker f.	Zambia	Dessein SD720 (BR)	FJ826681	FJ826735	FJ826629
<i>Impatiens aurea</i> Muhl.	North American origin, cult. Holden arboretum	Janssens SJ008 (LV)	EF133560	EF133613	DQ147813
<i>Impatiens aureliana</i> Hook.f.	China, Yunnan	Yuan CN2k1-56 (NEU)	EF133561	EF133614	DQ147814
<i>Impatiens auricoma</i> Baill.	Comoros origin, cult. Bot. Gard. Marburg	Janssens SJ001 (LV)	EF133562	EF133615	DQ147815
<i>Impatiens balfourii</i> Hook.f.	Himalayan origin, cult. Denver Bot. Gard.	33051 (DBG)	EF133564	EF133616	DQ147817
<i>Impatiens balsamina</i> L.	Indian origin, cult. Kruidtuin Leuven	Janssens SJ003 (LV)	EF133563	EF133617	DQ147816
<i>Impatiens bequaertii</i> De Wild.	Congo origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE1 (NEU)	FJ826682	FJ826736	FJ826630
<i>Impatiens begoniifolia</i> S. Akiyama and H. Ohba	China, Yunnan	Yuan CN2k1-51 (NEU)	EF133565	EF133619	DQ147819
<i>Impatiens bicaudata</i> H. Perrier	Madagascan origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133566	EF133618	–
<i>Impatiens bombycina</i> Lobin and E. Fischer	African origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE3 (NEU)	FJ826683	FJ826737	FJ826631
<i>Impatiens briartii</i> De Wild. and Th. Dur.	Zambia	Dessein 1018 (BR)	FJ826684	FJ826738	FJ826632
<i>Impatiens bururiensis</i> Grey-Wilson	Burundi	Reekmans 8110 (BR)	–	FJ826739	FJ826633
<i>Impatiens burtonii</i> Hook.f. var. burtonii	Uganda	Knox 2803 (LV)	FJ826685	FJ826740	FJ826685
<i>Impatiens campanulata</i> Wight	South Indian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133567	EF133620	DQ147822
<i>Impatiens capensis</i> Meerb.	North American origin, cult. Holden arboretum	Janssens SJ009 (LV)	EF133568	EF133621	DQ147823
<i>Impatiens catati</i> Baill.	Madagascar	Janssens SJ011 (LV)	FJ826686	–	FJ826634
<i>Impatiens cecili</i> N.E.Br.	Zimbabwe	Knox 4353 (LV)	FJ826687	FJ826741	FJ826635
<i>Impatiens chevalieri</i> Tardieu	Vietnam, Balat	Song and Puoong 2004–07 (NEU)	–	FJ826742	FJ826636
<i>Impatiens chinensis</i> L.	China, Yunnan	Yuan CN2k1-49 (NEU)	EF133569	EF133622	DQ147825
<i>Impatiens chungtienensis</i> Y.L. Chen	China, Yunnan	Yuan CN2k2-204 (NEU)	EF133570	EF133623	DQ147826
<i>Impatiens clavicornu</i> Turcz.	South Indian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133571	EF133624	DQ147827
<i>Impatiens columbaria</i> J.J. Bos	African origin, cult. Nat. Bot. Gard. Meise	FB/S2966 (BR)	EF133572	EF133625	DQ147828
<i>Impatiens conchibracteata</i> Y.L. Chen	China, Yunnan	Hao 427 (NEU)	EF133573	EF133626	DQ147829
<i>Impatiens confusa</i> Grey-Wilson ssp. longicornu Grey-Wilson.	Tanzania	Knox 3239 (LV)	FJ826688	FJ826743	FJ826637
<i>Impatiens congolensis</i> G.M. Schulze and R. Wilczek	African origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE7 (NEU)	EF133574	EF133627	DQ147830
<i>Impatiens cuspidata</i> Wight and Arn.	South Indian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133575	EF133628	DQ147832
<i>Impatiens cyanantha</i> Hook.f.	China, Yunnan	Yuan CN2k1-84 (NEU)	–	EF133629	DQ147833
<i>Impatiens davidi</i> Franch.	China, Fujian	Yuan CN2k-09 (NEU)	EF133576	EF133630	DQ147835
<i>Impatiens desmantha</i> Hook.f.	China, Yunnan	Yuan CN2k-30 (NEU)	EF133578	–	DQ147837
<i>Impatiens digitata</i> Warb. ssp. digitata	Tanzania	Knox 3653 (LV)	FJ826689	FJ826744	FJ826638
<i>Impatiens eberhardtii</i> Tardieu	Vietnam, Anam	Song s.n. (NEU)	EF133579	EF133632	DQ147839
<i>Impatiens edgeworthii</i> Hook.f.	Himalayan origin, cult. Univ. California Bot. Gard. Berkeley	89.2005 (UC)	EF133580	EF133633	DQ147840
<i>Impatiens engleri</i> Gilg ssp. engleri	Tanzania	Knox 3627 (LV)	FJ826690	FJ826745	FJ826639
<i>Impatiens eryaleia</i> Launert ssp. eryaleia	Tanzania	Knox 3262 (LV)	–	FJ826746	FJ826640
<i>Impatiens ethiopia</i> Grey-Wilson	Ethiopia	Jansen 5505 (WAG)	–	–	FJ826641
<i>Impatiens faberi</i> Hook.f.	China, Sichuan	Song S007 (NEU)	EF133581	EF133634	DQ147841
<i>Impatiens fenghwaiana</i> Y.L. Chen	China, Guangxi	Yuan CN2k-41 (NEU)	–	EF133635	DQ147842
<i>Impatiens fischeri</i> Warb.	Kenya	Menn. and Bar.-Kui. 284 (U)	FJ826691	–	DQ147843
<i>Impatiens flaccida</i> Arn.	South Indian origin, cult. Nat. Bot. Gard. Meise	FB/S3925 (BR)	EF133582	EF133636	DQ147845
<i>Impatiens flanaganae</i> Hemsl.	South-African origin, cult. Roy. Bot. Gard. Edinburgh	19860179 (E)	–	FJ826747	DQ147846
<i>Impatiens forrestii</i> Hook.f. ex W.W. Smith	China, Yunnan	Yuan CN2k-79 (NEU)	EF133583	EF133637	DQ147847
<i>Impatiens furcata</i> H. Perrier	Madagascar	Fischer EF8 (NEU)	FJ826692	FJ826748	FJ826642
<i>Impatiens glandulifera</i> Arn.	Belgium, Leuven (introduced; Himalayan origin)	Janssens SJ002 (LV)	EF133584	EF133638	DQ147848
<i>Impatiens gordonii</i> Horne	Seychelles	Kew 2398	FJ826693	FJ826749	FJ826643
<i>Impatiens hamata</i> Warb.	Tanzania	Knox 3558 (LV)	FJ826694	FJ826750	FJ826644
<i>Impatiens hawkeri</i> W. Bull	New Guinean origin	Janssens SJ006 (LV)	EF133586	EF133640	DQ147850
<i>Impatiens hians</i> Hook.f.	West African origin, cult. Bot. Gard. Berlin	Schwerdtfeger 9492a (B)	EF133585	EF133639	DQ147849
<i>Impatiens henslowiana</i> Arn.	Sri Lanka origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE10 (NEU)	FJ826695	FJ826751	FJ826645
<i>Impatiens hochstetteri</i> Warb. ssp. hochstetteri	Kenya	Knox 2633 (LV)	FJ826696	FJ826752	FJ826646
<i>Impatiens hochstetteri</i> Warb. ssp. hochstetteri	South Africa	Knox 4355 (LV)	FJ826697	FJ826753	–
<i>Impatiens hoehnelii</i> T.C.E. Fr.	Kenya	Knox 2846 (LV)	FJ826698	FJ826754	FJ826647
<i>Impatiens hydrogetonoides</i> Launert ssp. hydrogetonoides	Zambia	Dessein SD719 (BR)	FJ826699	FJ826755	FJ826648
<i>Impatiens imbecilla</i> Hook.f.	China, Sichuan	Hao 426 (NEU)	EF133587	EF133641	DQ147851
<i>Impatiens inaperta</i> H. Perrier	Madagascar	JBE2 (NEU)	FJ826700	–	FJ826649
<i>Impatiens ioides</i> G.M. Schulze	Tanzania	Knox 3571 (LV)	FJ826701	FJ826756	FJ826650
<i>Impatiens irvingii</i> Hook.f.	Gabon	Ngok Banak 2022 (WAG)	FJ826702	FJ826757	FJ826651
<i>Impatiens kamerunensis</i> Warb. ssp. kamerunensis	Cameroon	J.J.F.E. de Wilde 8638 (BR)	FJ826703	FJ826758	FJ826653
<i>Impatiens kamerunensis</i> Warb. ssp. obanensis (Keay) Grey-Wilson	Ghana	Jongkind 1926 (WAG)	FJ826704	FJ826759	FJ826652
<i>Impatiens keilii</i> Gilg ssp. keilii	Tanzania	Knox 3575 (LV)	FJ826705	FJ826760	FJ826654
<i>Impatiens keilii</i> Gilg ssp. pubescens Grey-Wilson	Tanzania	Knox 3587 (LV)	FJ826706	FJ826761	FJ826655
<i>Impatiens kerriae</i> Craib	Thailand, Qingmai	Chassot 99–238 (NEU)	–	EF133642	DQ147853

Table 1 (continued)

Taxon	Origin	Voucher	Accession No. <i>ImpDEF1</i>	Accession No. <i>ImpDEF2</i>	Accession No. <i>atpB-rbcL</i>
<i>Impatiens kilimanjari</i> Oliv.	Tanzania	Knox 3652 (LV)	FJ826707	–	FJ826656
<i>Impatiens latifolia</i> L.	South Indian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133588	EF133643	DQ147854
<i>Impatiens lukwangulensis</i> Grey-Wilson	Tanzania	Knox 3570 (LV)	EU723716	EU723723	EU723709
<i>Impatiens mackeyana</i> Hook.f. ssp. <i>claei</i> (N.Hallé) Grey-Wilson	Gabon origin, cult. Nat Bot. Gard. Koblenz Univ.	Fischer EF5 (NEU)	FJ826708	FJ826762	FJ826657
<i>Impatiens mackeyana</i> Hook.f. ssp. <i>zenkeri</i> (Warb.) Grey-Wilson	African origin, cult. Bot. Gard. Koblenz Univ.	Fischer EF21 (NEU)	FJ826709	–	DQ147857
<i>Impatiens macroptera</i> Hook.f.	Equatorial Guinea	de Wilde 12014 (WAG)	FJ826710	FJ826763	FJ826658
<i>Impatiens mannii</i> Hook.f.	East Congo	Geuvens Carvalho 4534 (BR)	FJ826711	FJ826764	FJ826659
<i>Impatiens mannii</i> Hook.f.	Cameroon	Merckx s.n. (LV)	FJ826712	FJ826765	FJ826660
<i>Impatiens mazumbaiensis</i> Grey-Wilson	Tanzania	Knox 3643 (LV)	EU723717	EU723724	EU723710
<i>Impatiens mengtseana</i> Hook.f.	China, Yunnan	Yuan CN2k1-38 (NEU)	EF133589	EF133644	DQ147858
<i>Impatiens meruensis</i> Gilg ssp. <i>cruciata</i> (T.C.E. Fr.) Grey-Wilson	Kenya	Ray Morgan s.n. (LV)	FJ826713	FJ826766	FJ826661
<i>Impatiens meruensis</i> Gilg ssp. <i>meruensis</i>	Tanzania	Knox 3328 (LV)	FJ826714	FJ826767	FJ826662
<i>Impatiens mildbraedii</i> Gilg telekii (T. C.E.Fr.) Grey-Wilson	Kenya	Knox 3882 (LV)	FJ826715	FJ826768	FJ826663
<i>Impatiens monticola</i> Hook.f.	China, Sichuan	Hao 425 (NEU)	EF133591	EF133646	DQ147860
<i>Impatiens nana</i> Engl.	Tanzania	Knox 2589 (LV)	EU723719	EU723726	EU723712
<i>Impatiens napoensis</i> Y.L. Chen	China, Yunnan	Yuan CN2k1-61 (NEU)	FJ826716	FJ826769	DQ147861
<i>Impatiens niarniamensis</i> Gilg	African origin, cult. Nat. Bot. Gard. Meise	FB/S2590 (BR)	FJ826717	FJ826770	DQ147862
<i>Impatiens noli-tangere</i> L.	Korea	Janssens SJ008	–	–	FJ826664
<i>Impatiens omeiana</i> Hook.f.	China, Sichuan, cult. Univ. California Bot. Gard. Berkeley	2002.0214 (UC)	EF133592	EF133647	DQ147864
<i>Impatiens pallide-rosea</i> Gilg ssp. <i>lupangaensis</i> (G.M. Schulze) Grey-Wilson	Tanzania	Knox 3572 (LV)	FJ826718	–	FJ826665
<i>Impatiens pallide-rosea</i> Gilg ssp. <i>pallide-rosea</i>	Tanzania	Knox 3552 (LV)	EU723720	–	EU723713
<i>Impatiens palpebrata</i> Hook.f.	Gabon	Jongkind 5724 (WAG)	FJ826719	FJ826772	FJ826666
<i>Impatiens parasitica</i> Bedd.	Indonesian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	FJ826720	FJ826771	FJ826667
<i>Impatiens parviflora</i> DC.	Belgium, Leuven (introduced; Asian origin)	Janssens SJ004 (LV)	EF133593	EF133648	DQ147866
<i>Impatiens percordata</i> Grey-Wilson ssp. <i>percordata</i>	Tanzania	Knox 4420 (LV)	FJ826721	FJ826773	FJ826668
<i>Impatiens platypetala</i> Lindl.	Bali, Indonesian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133594	EF133649	DQ147868
<i>Impatiens poilanei</i> Tardieu	Vietnam, Anam	Song s.n. (NEU)	EF133595	EF133650	DQ147869
<i>Impatiens pseudohamata</i> Grey-Wilson	Tanzania	Knox 3553 (LV)	FJ826722	FJ826774	FJ826669
<i>Impatiens pseudomacroptera</i> Grey-Wilson	Gabon	de Wilde 10390 (WAG)	FJ826723	FJ826775	FJ826670
<i>Impatiens pseudoviola</i> Gilg.	African origin, cult. Roy. Bot. Gard. Edinburgh	19680124 (E)	EF133597	–	DQ147871
<i>Impatiens pseudozombensis</i> Grey-Wilson	Tanzania	Knox 3229 (LV)	FJ826724	FJ826776	FJ826671
<i>Impatiens purpurea</i> Hand.-Mazz.	China, Yunnan	Song Y007 (NEU)	EF133598	EF133652	DQ147872
<i>Impatiens raphidothrix</i> Warb.	Tanzania	Knox 2592 (LV)	FJ826725	FJ826777	FJ826672
<i>Impatiens rectangula</i> Hand.-Mazz.	China, Yunnan	Yuan CN2k1-26 (NEU)	EF133599	EF133653	DQ147874
<i>Impatiens rubromaculata</i> Warb. ssp. <i>imigiensis</i> Grey-Wilson	Tanzania	Knox 3462 (LV)	FJ826726	–	FJ826673
<i>Impatiens rubromaculata</i> Warb. ssp. <i>rubromaculata</i>	Tanzania	Knox 3667 (LV)	EU723721	EU723727	EU723714
<i>Impatiens rubrostriata</i> Hook.f.	China, Yunnan	Yuan CN2k1-44 (NEU)	EF133600	EF133654	DQ147876
<i>Impatiens sakeriana</i> Hook.f.	Cameroon	Merckx VM125 (LV)	FJ826727	FJ826778	FJ826666
<i>Impatiens scabrida</i> DC.	Himalayan origin, cult. Holden arboretum	941314 (DBG)	EF133601	EF133655	DQ147877
<i>Impatiens serpens</i> Grey-Wilson	Tanzania	Knox 3551 (LV)	FJ826728	FJ826779	FJ826674
<i>Impatiens siculifer</i> Hook.f.	China, Yunnan	Yuan CN2 k-80 (NEU)	–	FJ826780	–
<i>Impatiens sodenii</i> Engl.	Tanzania	Knox 3318 (LV)	FJ826729	FJ826781	FJ826675
<i>Impatiens sylvicola</i> Burttt Davy and Greenway	Zimbabwe	Knox 4324 (LV)	EU723722	EU723728	EU723715
<i>Impatiens taronensis</i> Hand.-Mazz.	China, Yunnan	Yuan CN2 k-57 (NEU)	EF133602	EF133656	DQ147882
<i>Impatiens teitensis</i> Grey-Wilson	African origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE18	–	FJ826782	DQ147883
<i>Impatiens thamnoidea</i> G. M. Schulze	Tanzania	Knox 3576 (LV)	FJ826730	FJ826783	FJ826676
<i>Impatiens tinctoria</i> A. Rich ssp. <i>elegantissima</i> (Gilg) Grey-Wilson	Kenya	Knox 2639 (LV)	–	FJ826784	FJ826677
<i>Impatiens trichosepala</i> Y.L. Chen	China, Yunnan	Yuan CN2k1-68 (NEU)	EF133603	EF133657	DQ147885
<i>Impatiens tuberosa</i> H. Perrier	Madagascan origin, cult. Bot. Gard. Univ. Kopenhagen	Janssens SJ005 (LV)	EF133604	EF133658	DQ147886
<i>Impatiens tweedieae</i> E. A. Bruce	Kenya	Knox 2776 (LV)	FJ826731	FJ826785	–
<i>Impatiens ulugurensis</i> Warb.	Tanzania	Knox 3554 (LV)	FJ826732	FJ826786	FJ826678
<i>Impatiens uliginosa</i> Franch.	China, Yunnan	Yuan CN2k2-173 (NEU)	–	EF133659	DQ147887
<i>Impatiens uniflora</i> Hayata	China, Taiwan	Zhengyu Jiang T1 (NEU)	EF133605	EF133660	DQ147888
<i>Impatiens usambarensis</i> Grey-Wilson	African origin, cult. Bot. Gard. Koblenz Univ.	Fischer NE20 (NEU)	EF133606	EF133661	DQ147889
<i>Impatiens viscida</i> Wight	South Indian origin, cult. by Ray Morgan, UK	Ray Morgan s.n. (LV)	EF133608	EF133663	DQ147891
<i>Impatiens volkensii</i> Warb.	Tanzania	Knox 4117 (LV)	FJ826733	–	FJ826679
<i>Impatiens walleriana</i> Hook.f.	African origin, cult. Nat. Bot. Gard. Meise	S3926 (BR)	EF133609	EF133664	DQ147892
<i>Impatiens yingjiangensis</i> S.Akiyama and H.Ohba	China, Yunnan	Yuan CN2k1-55 (NEU)	FJ826734	FJ826787	DQ147894

of the crown group of Asclepiadoideae (Apocynaceae) constrained to 38 mya on the basis of the earliest fossil of this group from the Oligocene and Miocene (Muller, 1981); (3) a minimum age constraint of 87 mya to the Cornaceae based on the fossil fruits of *Hiro-*

noia fusiformis that were found in the Coniacian stratigraphic zone (Takahashi et al., 2002; Anderson et al., 2005); (4) minimum age constraint of 42 mya for *Sambucus* (Adoxaceae) based on the finding of fossil endocarps from the late Eocene to Pliocene found in

Europe (Reid and Chandler, 1926); (5) based on reports of fossil fruits of *Fraxinus* (Oleaceae), occurring in America from the Eocene onwards, a minimum age for this genus was constrained to 54.9 mya (Call and Dilcher, 1992); (6) minimum age of *Weigela* (Caprifoliaceae) constrained to 24.6 mya based on reports of distinctive winged seeds from the Miocene and Pliocene (Lancucka-Srodoniowa, 1967); (7) the crown node of Solanaceae is constrained to a minimum age of 54.9 mya, corresponding to the earliest Solanaceae fossil known from the Eocene (Collinson et al. (1993)); (8) recent estimation of divergence times based on *ndhF* and *rbcl* suggest that Asteraceae have originated in the mid Eocene (Kim et al., 2005). As a result, the crown node of this clade was constrained at a minimum age of 47 mya; (9) the lineage towards *Valeriana* (Valerianaceae) is constrained at a minimum age of 11.2 mya, corresponding with the report of fossil fruits from the late Miocene and Pliocene in Europe (Bell and Donoghue, 2005); (10) minimum age of *Ixora* (Rubiaceae) constrained to 5.3 mya based on the fossil collection of *I. casei* from the Marshall Islands (Leopold, 1969); (11) in addition, we assigned a fixed age of 128 mya to crown node of the asterids, a calibration point estimated by Bremer et al. (2004). In order to determine the impact of the different fossil calibration points on the overall dating estimation, we used the method of ‘fossil cross-validation’ developed by Near and Sanderson (2004). Their approach evaluates the difference between the fossil ages and the estimated molecular ages by re-evaluating the dating analysis with only a single calibration point. By calculating the summed square value (SS) between fossil constraint and molecular estimate, we noticed that calibration points 1 and 2 exhibited the highest scores (Fig. 1). Removal of these calibration points from our dataset resulted in a significant decline of the average squared deviation (s), whereas subsequent removal of other fossil constraints did not alter s (Fig. 2). Consequently, we removed fossil constraints 1 and 2, as they were determined as most inconsistent calibration points for the asterid dataset.

Confidence intervals for each node were obtained as described in the r8s manual. PHYLIP (Felsenstein, 1995) was used to generate 100 bootstrapped datasets of the original dataset. For each bootstrapped data matrix, we generated a phylogram with the RAxML procedure. Using the PL method in r8s 1.70, we recalculated divergence times for each of the phylograms using the same calibration points as above. These results were then used to estimate standard deviations for each node.

The obtained age estimate for the split between *I. omeiana* and the remainder of the genus was then applied as fixed calibration point to calculate the divergence events within *Impatiens*. The boundaries of the confidence interval for this node were taken into account in the secondary calibration approach of *Impatiens*. Hence we analyzed the *Impatiens* dataset for an age of 22.5, 22.5 + 5.6 and 22.5 – 5.6 million years ago. In addition to these indirectly obtained age estimates, we used the following biogeographic age estimates for cross-validation: The age of Mt Kilimanjaro, a recent volcanic mountain dated at 1.1 mya harbouring the endemic species *Impatiens digitata* ssp. *digitata* and *Impatiens kilimanjari* (Knox and Palmer, 1998).

To infer age estimates for the most diversified lineages in *Impatiens*, we used the combined chloroplast and nuclear data matrix. A likelihood ratio test showed that a clocklike evolution for the *ImpDEF1/ImpDEF2* dataset also had to be rejected. Consequently, we calculated the divergence times on the combined data using the penalized likelihood method (Sanderson, 2002) onto the rooted ML phylogram.

2.4. Ancestral area distribution

In order to reconstruct biogeographic ancestral areas, we used the likelihood reconstruction method (Pagel, 1999; Schluter

et al., 1997) implemented in Mesquite v1.12 (Maddison and Maddison, 2008). The geographic distribution of *Impatiens* has been coded using major geographic units: Africa, Madagascar, South-west China, the Himalayas, India and Sri Lanka, North America, Southeast Asia. A Markov *k*-state one-parameter model (Mk1), assuming a single rate of transition between two character states was applied for ML ancestral area reconstruction. In order to determine the best estimate of the reconstructed characters state at each node, a likelihood ratio test was used with a likelihood decision limit of 2.0. With log-likelihoods differing by 2 or more, the lowest negative log-likelihood was regarded as best value, whereas nodes with log-likelihoods differences lower than two were assumed to be ambiguous.

2.5. Speciation rates and timing of diversification in the genus *Impatiens*

One aspect of this study is to examine whether the timing of speciation in *Impatiens* is correlated with climate changes during the Pliocene–Pleistocene. By generating a lineages-through-time (LTT) plot for the PL chronogram derived from the combined *Impatiens* dataset, we obtain a schematic visualization of the net diversification rate of the genus *Impatiens*. The outline of the LTT plot is used to evaluate whether changes in diversification rate have occurred during the history of *Impatiens*. Only one individual per species was included in the LTT plot as the inclusion of individuals representing additional subspecies would inflate the speciation rate. Present sampling only contains about 11% of all known *Impatiens* species (113 out of 1000 species). As a result, we tested the consequence of an incomplete taxon sampling on the profile of the empirical LTT plot by generating simulated phylogenies with the software program Phylogen 1.1 (Rambaut, 2002). The following simulation was executed. A total of 1000 replicate phylogenies were generated, containing 1000 extant taxa of which 884 were randomly pruned from each tree. Each simulation was generated under the assumption of a constant birth–death rate ($b/d = 0$). Branch lengths of the resulting phylograms were rescaled with TreeEdit 1.0 (Rambaut and Charleston, 2002) to 22.5 million years, the estimated crown node age of *Impatiens*. We subsequently evaluated the empirical LTT curve by comparison with the mean LTT simulation and its 95% confidence interval.

In order to assess a quantitative method that could illustrate an overall change in diversification rate within the evolutionary history of *Impatiens*, the rate of speciation was calculated for each lineage ($[\ln(N) - \ln(N_0)]/T$; Baldwin and Sanderson, 1998). Additionally, we used the constant rate (CR) test of Pybus and Harvey (2000) to investigate a possible increase in overall diversification rate within *Impatiens*. The CR method as implemented in Genie 3.0 (Pybus and Harvey, 2000) follows an approach of γ -statistics in which the relative position of the nodes in the empirical phylogeny is compared to the position of those nodes that are assumed to be under a model of constant diversification. Positive γ -values ($\gamma > 0$) indicate an apparent acceleration of the overall diversification rate, assuming that nodes are closer to the tips than expected under a pure-birth–death model ($\gamma = 0$). On the contrary, negative γ -values ($\gamma < 0$) display a significant decrease in diversification rate. The null hypothesis of constant birth–death rate can be rejected when $\gamma < -1.645$ (one-tailed test). Because the γ -statistics is biased by extinction and incomplete taxon sampling, we simulated pure-birth topologies ($d/b = 0$) using Phylogen 1.1 (Rambaut, 2002). Analyses were carried out under the assumption that our *Impatiens* sampling ($n = 116$) only represents a small fraction (f) of the real diversity of the genus. We simulated 1000 pure-birth topologies with n/f tips, with the f -scores varying from 0.06 to 1. The resulting trees were then pruned to 117 taxa and for each of the pruned trees the γ -statistics was computed using Genie 3.0

Table 2GenBank accessions of *atpB-rbcl* spacer sequences of the asterid used in the large age estimation analysis.

Species	Accession No.	Family	Order	Superorder
<i>Kirengeshoma palmata</i>	EF437413	Hydrangeaceae	Cornales	Basal Asterids
<i>Cornus mas</i>	X83988	Cornaceae	Cornales	Basal Asterids
<i>Actinidia chinensis</i>	FJ866479	Actinidiaceae	Ericales	Basal Asterids
<i>Hydrocera triflora</i>	DQ147895	Balsaminaceae	Ericales	Basal Asterids
<i>Impatiens</i>	DQ147810–DQ147894	Balsaminaceae	Ericales	Basal Asterids
<i>Diospyros kaki</i>	X91004	Ebenaceae	Ericales	Basal Asterids
<i>Euclea pseudebenus</i>	FJ866480	Ebenaceae	Ericales	Basal Asterids
<i>Lissocarpa guianensis</i>	AF421094	Ebenaceae	Ericales	Basal Asterids
<i>Acrothamnus hookeri</i>	AY971370	Ericaceae	Ericales	Basal Asterids
<i>Acrothamnus maccraei</i>	AY971371	Ericaceae	Ericales	Basal Asterids
<i>Acrotiche ramiflora</i>	AY636036	Ericaceae	Ericales	Basal Asterids
<i>Andersonia sprengelioides</i>	AF155843	Ericaceae	Ericales	Basal Asterids
<i>Andromeda polifolia</i>	AF366584	Ericaceae	Ericales	Basal Asterids
<i>Androstoma empetrifolia</i>	AY372540	Ericaceae	Ericales	Basal Asterids
<i>Archeria comberi</i>	AF155840	Ericaceae	Ericales	Basal Asterids
<i>Archeria serpyllifolia</i>	AY971372	Ericaceae	Ericales	Basal Asterids
<i>Astroloma xerophyllum</i>	AY372554	Ericaceae	Ericales	Basal Asterids
<i>Brachyloma daphnoides</i>	AF155859	Ericaceae	Ericales	Basal Asterids
<i>Brachyloma preissii</i>	AY372555	Ericaceae	Ericales	Basal Asterids
<i>Bruckenthalia spicifolia</i>	AY520757	Ericaceae	Ericales	Basal Asterids
<i>Budawangia gnidioides</i>	AF155852	Ericaceae	Ericales	Basal Asterids
<i>Chamaedaphne calyculata</i>	AF366616	Ericaceae	Ericales	Basal Asterids
<i>Coleanthera myrtooides</i>	AY372556	Ericaceae	Ericales	Basal Asterids
<i>Conostephium preissii</i>	AY372557	Ericaceae	Ericales	Basal Asterids
<i>Cosmelia rubra</i>	AF155842	Ericaceae	Ericales	Basal Asterids
<i>Croninia kingiana</i>	AF208750	Ericaceae	Ericales	Basal Asterids
<i>Cyathodes platystoma</i>	AY372560	Ericaceae	Ericales	Basal Asterids
<i>Cyathodes pumila</i>	AY372558	Ericaceae	Ericales	Basal Asterids
<i>Cyathopsis floribunda</i>	AY636040	Ericaceae	Ericales	Basal Asterids
<i>Daboecia cabtabrica</i>	AY520758	Ericaceae	Ericales	Basal Asterids
<i>Diplycosia cinnamomifolia</i>	AF366587	Ericaceae	Ericales	Basal Asterids
<i>Dracophyllum longifolium</i>	AF155845	Ericaceae	Ericales	Basal Asterids
<i>Epacris obtusifolia</i>	AY636042	Ericaceae	Ericales	Basal Asterids
<i>Erica hispida</i>	AY520767	Ericaceae	Ericales	Basal Asterids
<i>Gaultheria tomentosa</i>	AF366611	Ericaceae	Ericales	Basal Asterids
<i>Harrimanella hypnoides</i>	AF155833	Ericaceae	Ericales	Basal Asterids
<i>Kalmia latifolia</i>	AB247970	Ericaceae	Ericales	Basal Asterids
<i>Leptecophylla tameiameia</i>	AY372569	Ericaceae	Ericales	Basal Asterids
<i>Leucopogon milliganii</i>	AY372575	Ericaceae	Ericales	Basal Asterids
<i>Leucothoe fontanesiana</i>	AF366612	Ericaceae	Ericales	Basal Asterids
<i>Lissanthe symandra</i>	AY636038	Ericaceae	Ericales	Basal Asterids
<i>Lyonia lucida</i>	AF155836	Ericaceae	Ericales	Basal Asterids
<i>Lysine ciliatum</i>	AF155848	Ericaceae	Ericales	Basal Asterids
<i>Melichrus urceolatus</i>	AY372595	Ericaceae	Ericales	Basal Asterids
<i>Melichrus urceolatus</i>	AY372595	Ericaceae	Ericales	Basal Asterids
<i>Monotoca elliptica</i>	AY005085	Ericaceae	Ericales	Basal Asterids
<i>Oligarrhena micrantha</i>	AF155854	Ericaceae	Ericales	Basal Asterids
<i>Pentachondra involucreta</i>	AY005087	Ericaceae	Ericales	Basal Asterids
<i>Pernettya tasmanica</i>	AF155835	Ericaceae	Ericales	Basal Asterids
<i>Planocarpa petiolaris</i>	AY372594	Ericaceae	Ericales	Basal Asterids
<i>Prionotes cerinthoides</i>	AF155838	Ericaceae	Ericales	Basal Asterids
<i>Rhododendron simsii</i>	AM296078	Ericaceae	Ericales	Basal Asterids
<i>Richea pandanifolia</i>	AF155844	Ericaceae	Ericales	Basal Asterids
<i>Rupicola sprengelioides</i>	AF155851	Ericaceae	Ericales	Basal Asterids
<i>Sphenotoma dracophylloides</i>	AF155846	Ericaceae	Ericales	Basal Asterids
<i>Styphelia tubiflora</i>	AY372591	Ericaceae	Ericales	Basal Asterids
<i>Trochocarpa laurina</i>	AY005092	Ericaceae	Ericales	Basal Asterids
<i>Woolisia pungens</i>	AF155847	Ericaceae	Ericales	Basal Asterids
<i>Zenobia pulverulenta</i>	AF366615	Ericaceae	Ericales	Basal Asterids
<i>Corytophora alta</i>	AF076769	Lecythidaceae	Ericales	Basal Asterids
<i>Eschweilera simiorum</i>	X91001	Lecythidaceae	Ericales	Basal Asterids
<i>Marcgravia maguirei</i>	DQ147896	Marcgraviaceae	Ericales	Basal Asterids
<i>Marcgravia umbellata</i>	DQ147897	Marcgraviaceae	Ericales	Basal Asterids
<i>Norantea guianensis</i>	DQ147898	Marcgraviaceae	Ericales	Basal Asterids
<i>Souroubea</i> sp.	DQ147900	Marcgraviaceae	Ericales	Basal Asterids
<i>Primula cuneifolia</i>	AB003575	Primulaceae	Ericales	Basal Asterids
<i>Chromolucuma rubriflora</i>	EF558591	Sapotaceae	Ericales	Basal Asterids
<i>Chrysophyllum imperiale</i>	EF558592	Sapotaceae	Ericales	Basal Asterids
<i>Eberhardtia aurata</i>	EF558594	Sapotaceae	Ericales	Basal Asterids
<i>Elaeolum glabrescens</i>	EF558593	Sapotaceae	Ericales	Basal Asterids
<i>Changiosyrax dolichocarpa</i>	DQ317984	Styracaceae	Ericales	Basal Asterids
<i>Pterostyrax psilophyllus</i>	DQ317980	Styracaceae	Ericales	Basal Asterids
<i>Sinojackia sarcocarpa</i>	DQ317979	Styracaceae	Ericales	Basal Asterids
<i>Pelliciera rhizophorea</i>	DQ147899	Tetrameristaceae s.l.	Ericales	Basal Asterids
<i>Aucuba japonica</i>	AB087779	Garryaceae	Garryales	Euasterids 1

(continued on next page)

Table 2 (continued)

Species	Accession No.	Family	Order	Superorder
<i>Garrya ovata</i>	AB087788	Garryaceae	Garryales	Euasterids 1
<i>Alstonia scholaris</i>	DQ359161	Apocynaceae	Gentianales	Euasterids 1
<i>Dischidia astephana</i>	DQ334576	Apocynaceae	Gentianales	Euasterids 1
<i>Gunessia pepo</i>	DQ334570	Apocynaceae	Gentianales	Euasterids 1
<i>Hoya kentiana</i>	DQ334564	Apocynaceae	Gentianales	Euasterids 1
<i>Madangia inflata</i>	DQ334583	Apocynaceae	Gentianales	Euasterids 1
<i>Marsdenia carvalhoi</i>	DQ334563	Apocynaceae	Gentianales	Euasterids 1
<i>Micholitzia obcordata</i>	DQ334601	Apocynaceae	Gentianales	Euasterids 1
<i>Gentiana oreodoxa</i>	DQ398621	Gentianaceae	Gentianales	Euasterids 1
<i>Mitrasacme pygmaea</i>	DQ131694	Loganiaceae	Gentianales	Euasterids 1
<i>Mitreola petiolata</i>	DQ131696	Loganiaceae	Gentianales	Euasterids 1
<i>Adina rubella</i>	DQ131698	Rubiaceae	Gentianales	Euasterids 1
<i>Alberta minor</i>	DQ131699	Rubiaceae	Gentianales	Euasterids 1
<i>Argostemma hookeri</i>	AJ234032	Rubiaceae	Gentianales	Euasterids 1
<i>Asperula cynanchica</i>	X81689	Rubiaceae	Gentianales	Euasterids 1
<i>Augusta longifolia</i>	DQ131703	Rubiaceae	Gentianales	Euasterids 1
<i>Aulacocalyx jasmiflora</i>	DQ131704	Rubiaceae	Gentianales	Euasterids 1
<i>Belonophora coriacea</i>	DQ131706	Rubiaceae	Gentianales	Euasterids 1
<i>Bertiera dewevrei</i>	DQ131707	Rubiaceae	Gentianales	Euasterids 1
<i>Cephalanthe occidentalis</i>	DQ131710	Rubiaceae	Gentianales	Euasterids 1
<i>Chiococca alba</i>	DQ131711	Rubiaceae	Gentianales	Euasterids 1
<i>Chomelia brasiliana</i>	DQ131712	Rubiaceae	Gentianales	Euasterids 1
<i>Cinchona pubescens</i>	AJ233990	Rubiaceae	Gentianales	Euasterids 1
<i>Coffea congensis</i>	AM412393	Rubiaceae	Gentianales	Euasterids 1
<i>Colletocema dewevrei</i>	DQ131713	Rubiaceae	Gentianales	Euasterids 1
<i>Commithca liebrechtis</i>	AJ233999	Rubiaceae	Gentianales	Euasterids 1
<i>Coptosapelta diffusa</i>	AJ233987	Rubiaceae	Gentianales	Euasterids 1
<i>Coryanthe mayumbensis</i>	DQ131715	Rubiaceae	Gentianales	Euasterids 1
<i>Cosmocalyx spectabilis</i>	DQ131716	Rubiaceae	Gentianales	Euasterids 1
<i>Coussarea hirticalyx</i>	DQ131717	Rubiaceae	Gentianales	Euasterids 1
<i>Crossopteryx febrifuga</i>	DQ131719	Rubiaceae	Gentianales	Euasterids 1
<i>Cruciata glabra</i>	X81097	Rubiaceae	Gentianales	Euasterids 1
<i>Cruckshansia hymenocodon</i>	AJ234004	Rubiaceae	Gentianales	Euasterids 1
<i>Cubanola domingensis</i>	DQ131720	Rubiaceae	Gentianales	Euasterids 1
<i>Dictyandra arborescens</i>	DQ131723	Rubiaceae	Gentianales	Euasterids 1
<i>Didymosalpinx lanciloba</i>	DQ131724	Rubiaceae	Gentianales	Euasterids 1
<i>Diplospora dubia</i>	DQ131725	Rubiaceae	Gentianales	Euasterids 1
<i>Duperrea pavettifolia</i>	DQ131726	Rubiaceae	Gentianales	Euasterids 1
<i>Ecpoma hierniana</i>	DQ131727	Rubiaceae	Gentianales	Euasterids 1
<i>Eosantho cubensis</i>	DQ131729	Rubiaceae	Gentianales	Euasterids 1
<i>Erithalis fruticosa</i>	DQ131730	Rubiaceae	Gentianales	Euasterids 1
<i>Euclinia longiflora</i>	DQ131731	Rubiaceae	Gentianales	Euasterids 1
<i>Exostema lineatum</i>	DQ131732	Rubiaceae	Gentianales	Euasterids 1
<i>Faramea porophylla</i>	AJ234008	Rubiaceae	Gentianales	Euasterids 1
<i>Fermelia buxifolia</i>	DQ131736	Rubiaceae	Gentianales	Euasterids 1
<i>Gaertnera</i> sp.	AJ234012	Rubiaceae	Gentianales	Euasterids 1
<i>Galium scabrum</i>	X76462	Rubiaceae	Gentianales	Euasterids 1
<i>Gardenia imperialis</i>	DQ131737	Rubiaceae	Gentianales	Euasterids 1
<i>Guettarda platypoda</i>	DQ131739	Rubiaceae	Gentianales	Euasterids 1
<i>Hayataella michelloides</i>	AB247242	Rubiaceae	Gentianales	Euasterids 1
<i>Hedyotis littoralis</i>	AJ234027	Rubiaceae	Gentianales	Euasterids 1
<i>Heinsia crinita</i>	DQ131740	Rubiaceae	Gentianales	Euasterids 1
<i>Hillia triflora</i>	AJ233993	Rubiaceae	Gentianales	Euasterids 1
<i>Hoffmannia refulgens</i>	X81684	Rubiaceae	Gentianales	Euasterids 1
<i>Hydnophytum formicarum</i>	X76480	Rubiaceae	Gentianales	Euasterids 1
<i>Isertia rosea</i>	DQ131743	Rubiaceae	Gentianales	Euasterids 1
<i>Ixora coccinea</i>	AM412400	Rubiaceae	Gentianales	Euasterids 1
<i>Ixora finlaysonia</i>	DQ131744	Rubiaceae	Gentianales	Euasterids 1
<i>Keetia multiflora</i>	DQ131745	Rubiaceae	Gentianales	Euasterids 1
<i>Kellogia galioides</i>	AY570768	Rubiaceae	Gentianales	Euasterids 1
<i>Ladenbergia</i> sp.	DQ131747	Rubiaceae	Gentianales	Euasterids 1
<i>Leptactina leopoldi-secundi</i>	DQ131748	Rubiaceae	Gentianales	Euasterids 1
<i>Luculia pinceana</i>	DQ131749	Rubiaceae	Gentianales	Euasterids 1
<i>Manettia cordifolia</i>	AJ234023	Rubiaceae	Gentianales	Euasterids 1
<i>Mitragyne inermis</i>	DQ131751	Rubiaceae	Gentianales	Euasterids 1
<i>Morelia senegalensis</i>	DQ131752	Rubiaceae	Gentianales	Euasterids 1
<i>Morinda lucida</i>	DQ131753	Rubiaceae	Gentianales	Euasterids 1
<i>Mycetia malayana</i>	AJ234033	Rubiaceae	Gentianales	Euasterids 1
<i>Myrmecoda platytyrea</i>	AB044151	Rubiaceae	Gentianales	Euasterids 1
<i>Myrmephytum beccarii</i>	AB044152	Rubiaceae	Gentianales	Euasterids 1
<i>Otiophora scabra</i>	DQ131756	Rubiaceae	Gentianales	Euasterids 1
<i>Paratriaina xerophila</i>	DQ131759	Rubiaceae	Gentianales	Euasterids 1
<i>Pausinystalia johimbe</i>	DQ131760	Rubiaceae	Gentianales	Euasterids 1
<i>Pavetta ternifolia</i>	DQ131761	Rubiaceae	Gentianales	Euasterids 1
<i>Pentas lanceolata</i>	AB247149	Rubiaceae	Gentianales	Euasterids 1
<i>Phialanthus stillans</i>	DQ131762	Rubiaceae	Gentianales	Euasterids 1

Table 2 (continued)

Species	Accession No.	Family	Order	Superorder
<i>Plocama pendula</i>	AJ234035	Rubiaceae	Gentianales	Euasterids 1
<i>Pomax umbellata</i>	DQ131767	Rubiaceae	Gentianales	Euasterids 1
<i>Portlandia grandiflora</i>	DQ131768	Rubiaceae	Gentianales	Euasterids 1
<i>Pouchetia africana</i>	DQ131770	Rubiaceae	Gentianales	Euasterids 1
<i>Pseudosabicea floribunda</i>	DQ131771	Rubiaceae	Gentianales	Euasterids 1
<i>Psilanthus leroyi</i>	AM412399	Rubiaceae	Gentianales	Euasterids 1
<i>Psychotria undata</i>	DQ131774	Rubiaceae	Gentianales	Euasterids 1
<i>Putoria calabrica</i>	X81672	Rubiaceae	Gentianales	Euasterids 1
<i>Remijia macronemia</i>	DQ131775	Rubiaceae	Gentianales	Euasterids 1
<i>Rothmannia</i> sp.	DQ131776	Rubiaceae	Gentianales	Euasterids 1
<i>Rubia tinctorum</i>	X76474	Rubiaceae	Gentianales	Euasterids 1
<i>Rudgea bremekampiana</i>	DQ131778	Rubiaceae	Gentianales	Euasterids 1
<i>Rutidea schlechteri</i>	DQ131779	Rubiaceae	Gentianales	Euasterids 1
<i>Sabicea diversifolia</i>	DQ131781	Rubiaceae	Gentianales	Euasterids 1
<i>Sacosperma paniculatum</i>	DQ131782	Rubiaceae	Gentianales	Euasterids 1
<i>Salzmannia axillaris</i>	DQ131784	Rubiaceae	Gentianales	Euasterids 1
<i>Sarcocephalus latifolius</i>	DQ131785	Rubiaceae	Gentianales	Euasterids 1
<i>Schumanniphyton magnificum</i>	DQ131786	Rubiaceae	Gentianales	Euasterids 1
<i>Serissa foetida</i>	AJ234034	Rubiaceae	Gentianales	Euasterids 1
<i>Spermacece assurgens</i>	X81679	Rubiaceae	Gentianales	Euasterids 1
<i>Stelechante cauliflora</i>	DQ131789	Rubiaceae	Gentianales	Euasterids 1
<i>Stipularia elliptica</i>	DQ131790	Rubiaceae	Gentianales	Euasterids 1
<i>Tricalysia elliotii</i>	DQ131791	Rubiaceae	Gentianales	Euasterids 1
<i>Trichostachys perfoliatum</i>	AF446995	Rubiaceae	Gentianales	Euasterids 1
<i>Urophyllum glabrum</i>	DQ131793	Rubiaceae	Gentianales	Euasterids 1
<i>Valantia muralis</i>	X76473	Rubiaceae	Gentianales	Euasterids 1
<i>Vangueria infausta</i>	DQ131794	Rubiaceae	Gentianales	Euasterids 1
<i>Vangueria infausta</i>	DQ131794	Rubiaceae	Gentianales	Euasterids 1
<i>Wendlandia</i> sp.	DQ131796	Rubiaceae	Gentianales	Euasterids 1
<i>Achimenes admirabilis</i>	AJ439982	Gesneriaceae	Lamiales	Euasterids 1
<i>Aeschynanthus longiflorus</i>	AJ490920	Gesneriaceae	Lamiales	Euasterids 1
<i>Alsobia dianthiflora</i>	AJ490924	Gesneriaceae	Lamiales	Euasterids 1
<i>Besleria melancholica</i>	AJ490923	Gesneriaceae	Lamiales	Euasterids 1
<i>Chirita longgangensis</i>	AJ490903	Gesneriaceae	Lamiales	Euasterids 1
<i>Chirita spadiceiformis</i>	AJ490904	Gesneriaceae	Lamiales	Euasterids 1
<i>Chrysothemis pulchella</i>	AY423115	Gesneriaceae	Lamiales	Euasterids 1
<i>Cobanantha calochlamys</i>	AJ490926	Gesneriaceae	Lamiales	Euasterids 1
<i>Codonantha serrulata</i>	AJ439981	Gesneriaceae	Lamiales	Euasterids 1
<i>Columnnea sanguinea</i>	AJ490927	Gesneriaceae	Lamiales	Euasterids 1
<i>Cyrtandra cupulata</i>	AJ490886	Gesneriaceae	Lamiales	Euasterids 1
<i>Didissandra frutescens</i>	U91313	Gesneriaceae	Lamiales	Euasterids 1
<i>Didymocarpus citrinus</i>	AJ490906	Gesneriaceae	Lamiales	Euasterids 1
<i>Didymocarpus cordatus</i>	AJ490907	Gesneriaceae	Lamiales	Euasterids 1
<i>Emarhendia bettiana</i>	AJ490908	Gesneriaceae	Lamiales	Euasterids 1
<i>Episcia cupreata</i>	AJ490928	Gesneriaceae	Lamiales	Euasterids 1
<i>Epithema taiwanese</i>	AY423117	Gesneriaceae	Lamiales	Euasterids 1
<i>Fieldia australis</i>	AY423112	Gesneriaceae	Lamiales	Euasterids 1
<i>Haberlea rhodopensis</i>	AJ490909	Gesneriaceae	Lamiales	Euasterids 1
<i>Henckelia albomarginata</i>	AJ490910	Gesneriaceae	Lamiales	Euasterids 1
<i>Jovellana punctata</i>	AY423109	Gesneriaceae	Lamiales	Euasterids 1
<i>Koellikeria erinoides</i>	AJ439983	Gesneriaceae	Lamiales	Euasterids 1
<i>Kohleria eriantha</i>	AY423114	Gesneriaceae	Lamiales	Euasterids 1
<i>Lenbrassia australiana</i>	AJ490921	Gesneriaceae	Lamiales	Euasterids 1
<i>Loxonia hirsutum</i>	AJ490891	Gesneriaceae	Lamiales	Euasterids 1
<i>Mitraria coccinea</i>	AY423113	Gesneriaceae	Lamiales	Euasterids 1
<i>Monophyllae horsfieldii</i>	U91315	Gesneriaceae	Lamiales	Euasterids 1
<i>Nautilocalyx melittifolius</i>	AJ439984	Gesneriaceae	Lamiales	Euasterids 1
<i>Nenatanthus villosus</i>	AJ439980	Gesneriaceae	Lamiales	Euasterids 1
<i>Paliavana sericiflora</i>	AJ439963	Gesneriaceae	Lamiales	Euasterids 1
<i>Paraboea capitata</i>	AJ490911	Gesneriaceae	Lamiales	Euasterids 1
<i>Petrocosmea nervosa</i>	AJ490912	Gesneriaceae	Lamiales	Euasterids 1
<i>Primulina tabacum</i>	AJ490913	Gesneriaceae	Lamiales	Euasterids 1
<i>Ramonda myconi</i>	AJ490914	Gesneriaceae	Lamiales	Euasterids 1
<i>Rhabdothamnops sinensis</i>	AJ490915	Gesneriaceae	Lamiales	Euasterids 1
<i>Rhynchoglossum obliquum</i>	AJ490898	Gesneriaceae	Lamiales	Euasterids 1
<i>Rhytiophyllum tomentosumbesleria</i>	AJ490930	Gesneriaceae	Lamiales	Euasterids 1
<i>Saintpaulia velutina</i>	AJ490916	Gesneriaceae	Lamiales	Euasterids 1
<i>Sarmiaeta scandens</i>	AJ490922	Gesneriaceae	Lamiales	Euasterids 1
<i>Sinningia bulbosa</i>	AJ439910	Gesneriaceae	Lamiales	Euasterids 1
<i>Stauranthera grandiflora</i>	AJ490900	Gesneriaceae	Lamiales	Euasterids 1
<i>Streptocarpus holstii</i>	AJ490917	Gesneriaceae	Lamiales	Euasterids 1
<i>Streptocarpus rexii</i>	AJ490918	Gesneriaceae	Lamiales	Euasterids 1
<i>Titanotrichum oldhamii</i>	AY423111	Gesneriaceae	Lamiales	Euasterids 1
<i>Trisepalum speciosum</i>	AJ490919	Gesneriaceae	Lamiales	Euasterids 1
<i>Vanhouttea gardneri</i>	AJ439974	Gesneriaceae	Lamiales	Euasterids 1
<i>Whytockia sasakii</i>	AY423116	Gesneriaceae	Lamiales	Euasterids 1

(continued on next page)

Table 2 (continued)

Species	Accession No.	Family	Order	Superorder
<i>Globularia salicina</i>	AY818898	Globulariaceae	Lamiales	Euasterids 1
<i>Phlomis crinita</i>	AY792758	Lamiaceae	Lamiales	Euasterids 1
<i>Utricularia gibba</i>	EF529719	Lentibulariaceae	Lamiales	Euasterids 1
<i>Fraxinus excelsior</i>	AY911655	Oleaceae	Lamiales	Euasterids 1
<i>Plantago media</i>	AY818903	Plantaginaceae	Lamiales	Euasterids 1
<i>Antirrhinum majus</i>	AJ490883	Scrophulariaceae	Lamiales	Euasterids 1
<i>Calceolaria arachnoidea</i>	AY423108	Scrophulariaceae	Lamiales	Euasterids 1
<i>Digitalis purpurea</i>	AY818897	Scrophulariaceae	Lamiales	Euasterids 1
<i>Gratiola aurea</i>	EF529725	Scrophulariaceae	Lamiales	Euasterids 1
<i>Paulownia tomentosa</i>	AY423104	Scrophulariaceae	Lamiales	Euasterids 1
<i>Scrophularia canina</i>	AY423105	Scrophulariaceae	Lamiales	Euasterids 1
<i>Tetranema mexicanum</i>	AJ490884	Scrophulariaceae	Lamiales	Euasterids 1
<i>Verbascum speciosum</i>	AJ490885	Scrophulariaceae	Lamiales	Euasterids 1
<i>Veronica incana</i>	AY818908	Scrophulariaceae	Lamiales	Euasterids 1
<i>Aureliana fasciculata</i>	AF397083	Solanaceae	Solanales	Euasterids 1
<i>Capsicum baccatum</i>	AF397101	Solanaceae	Solanales	Euasterids 1
<i>Datura stramonium</i>	AF397076	Solanaceae	Solanales	Euasterids 1
<i>Jaltomata auriculata</i>	AF397081	Solanaceae	Solanales	Euasterids 1
<i>Lycianthe lenta</i>	AF397093	Solanaceae	Solanales	Euasterids 1
<i>Lycopersicon pimpinellifolium</i>	AF397079	Solanaceae	Solanales	Euasterids 1
<i>Nicandra physalodes</i>	AJ490882	Solanaceae	Solanales	Euasterids 1
<i>Schizanthus x wisetonensis</i>	AY423103	Solanaceae	Solanales	Euasterids 1
<i>Solanum shanesii</i>	AF397088	Solanaceae	Solanales	Euasterids 1
<i>Tubocapsicum anomalum</i>	AF397082	Solanaceae	Solanales	Euasterids 1
<i>Wythania coagens</i>	AF397084	Solanaceae	Solanales	Euasterids 1
<i>Chaerophyllum nodosum</i>	DQ829733	Apiaceae	Apiales	Euasterids 2
<i>Myrrhis odorata</i>	DQ829738	Apiaceae	Apiales	Euasterids 2
<i>Oreomyrrhis azurellaceae</i>	DQ829705	Apiaceae	Apiales	Euasterids 2
<i>Osmorhiza longistylus</i>	DQ829737	Apiaceae	Apiales	Euasterids 2
<i>Aralia cachemirica</i>	AY753224	Araliaceae	Apiales	Euasterids 2
<i>Brassaiopsis shweliensis</i>	AY753233	Araliaceae	Apiales	Euasterids 2
<i>Eleutherococcus trifoliatus</i>	AY753235	Araliaceae	Apiales	Euasterids 2
<i>Fatsia japonica</i>	AY163545	Araliaceae	Apiales	Euasterids 2
<i>Harmsioplanax aculeata</i>	AY753251	Araliaceae	Apiales	Euasterids 2
<i>Hedera maderensis</i>	AY163533	Araliaceae	Apiales	Euasterids 2
<i>Heteropanax fragrans</i>	AY753236	Araliaceae	Apiales	Euasterids 2
<i>Macropanax dispermus</i>	AY753241	Araliaceae	Apiales	Euasterids 2
<i>Merillioanax cordifolius</i>	AY753243	Araliaceae	Apiales	Euasterids 2
<i>Panax assamicus</i>	AY753245	Araliaceae	Apiales	Euasterids 2
<i>Polyscias javanica</i>	AY753252	Araliaceae	Apiales	Euasterids 2
<i>Schleffera hypoleucoides</i>	AY753247	Araliaceae	Apiales	Euasterids 2
<i>Tupidanthus calyptratus</i>	AY753250	Araliaceae	Apiales	Euasterids 2
<i>Helwingia japonica</i>	X94941	Helwingiaceae	Aquifoliales	Euasterids 2
<i>Ilex discolor</i>	AF471629	Aquifoliaceae	Aquifoliales	Euasterids 2
<i>Achillea millefolium</i>	EU129084	Asteraceae	Asterales	Euasterids 2
<i>Bellis perennis</i>	X91000	Asteraceae	Asterales	Euasterids 2
<i>Ligularia franchetiana</i>	AB375447	Asteraceae	Asterales	Euasterids 2
<i>Apetahia margaretae</i>	DQ285286	Campanulaceae	Asterales	Euasterids 2
<i>Brighamia insignis</i>	DQ285256	Campanulaceae	Asterales	Euasterids 2
<i>Burmeistera crispiloba</i>	DQ285281	Campanulaceae	Asterales	Euasterids 2
<i>Centropon gutierrezii</i>	DQ285282	Campanulaceae	Asterales	Euasterids 2
<i>Clermontia fauriei</i>	DQ285259	Campanulaceae	Asterales	Euasterids 2
<i>Cyanea pilosa</i>	DQ285291	Campanulaceae	Asterales	Euasterids 2
<i>Delissea subcordata</i>	DQ285264	Campanulaceae	Asterales	Euasterids 2
<i>Isotoma axillaris</i>	DQ285283	Campanulaceae	Asterales	Euasterids 2
<i>Lobelia hypoleuca</i>	DQ285266	Campanulaceae	Asterales	Euasterids 2
<i>Nymphoides cordata</i>	EF529716	Menyanthaceae	Asterales	Euasterids 2
<i>Sclerotheca jayorum</i>	DQ285273	Campanulaceae	Asterales	Euasterids 2
<i>Trematolobelia macrostachys</i>	DQ285271	Campanulaceae	Asterales	Euasterids 2
<i>Adoxa moschatellina</i>	AF446990	Adoxaceae	Dipsacales	Euasterids 2
<i>Sambucus racemosa</i>	AF446988	Adoxaceae	Dipsacales	Euasterids 2
<i>Sinadoxa corydalisfolia</i>	AF446989	Adoxaceae	Dipsacales	Euasterids 2
<i>Tetradoxa omeiensis</i>	AF446991	Adoxaceae	Dipsacales	Euasterids 2
<i>Viburnum acerifolium</i>	AF446987	Adoxaceae	Dipsacales	Euasterids 2
<i>Leycesteria formosa</i>	EU265521	Caprifoliaceae	Dipsacales	Euasterids 2
<i>Lonicera sempervirens</i>	EU265563	Caprifoliaceae	Dipsacales	Euasterids 2
<i>Symphoricarpos orbiculatus</i>	AF446994	Caprifoliaceae	Dipsacales	Euasterids 2
<i>Triosteum perfoliatum</i>	AF446995	Caprifoliaceae	Dipsacales	Euasterids 2
<i>Triplostegia glandulifera</i>	AF447009	Caprifoliaceae	Dipsacales	Euasterids 2
<i>Diervilla sessilifolia</i>	AF446997	Diervillaceae	Dipsacales	Euasterids 2
<i>Weigela hortensis</i>	AF446998	Unplaced	Dipsacales	Euasterids 2
<i>Dipsacus mitis</i>	AF447007	Dipsacaceae	Dipsacales	Euasterids 2
<i>Knautia macedonica</i>	AY362501	Dipsacaceae	Dipsacales	Euasterids 2
<i>Scabiosa columbaria</i>	AF447008	Dipsacaceae	Dipsacales	Euasterids 2
<i>Heptacodium miconioides</i>	AF446996	Unplaced	Dipsacales	Euasterids 2
<i>Abelia x grandiflora</i>	AF446999	Linnaeaceae	Dipsacales	Euasterids 2

Table 2 (continued)

Species	Accession No.	Family	Order	Superorder
<i>Dipelta yunnanensis</i>	AF447000	Linnaeaceae	Dipsacales	Euasterids 2
<i>Kolkwitzia amabilis</i>	AF447002	Linnaeaceae	Dipsacales	Euasterids 2
<i>Linnaea borealis</i>	AF447001	Linnaeaceae	Dipsacales	Euasterids 2
<i>Zabelia biflora</i>	EU265530	Linnaeaceae	Dipsacales	Euasterids 2
<i>Acanthocalyx albus</i>	AF447003	Morinaceae	Dipsacales	Euasterids 2
<i>Cryptothladia chinensis</i>	AF447004	Morinaceae	Dipsacales	Euasterids 2
<i>Morina longifolia</i>	AF447005	Morinaceae	Dipsacales	Euasterids 2
<i>Centranthus ruber</i>	AF448572	Valerianaceae	Dipsacales	Euasterids 2
<i>Fedia graciliflora</i>	AF448575	Valerianaceae	Dipsacales	Euasterids 2
<i>Nardostachys jatamansii</i>	AF447010	Valerianaceae	Dipsacales	Euasterids 2
<i>Plectritis macrocera</i>	AF447015	Valerianaceae	Dipsacales	Euasterids 2
<i>Valeriana bractescens</i>	AF448580	Valerianaceae	Dipsacales	Euasterids 2
<i>Valeriana celtica</i>	AY362516	Valerianaceae	Dipsacales	Euasterids 2
<i>Valerianella locusta</i>	AF447014	Valerianaceae	Dipsacales	Euasterids 2
<i>Castanea crenata</i>	AB124938	Rosids	Rosids	Outgroup
<i>Vatairea fusca</i>	EF466253	Rosids	Rosids	Outgroup

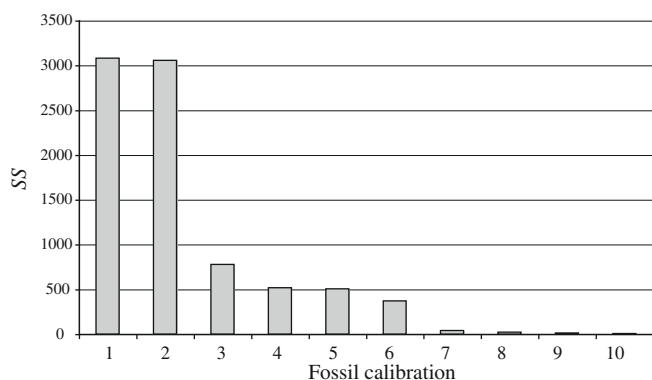


Fig. 1. Histogram of the summed square values (SS) of the deviations between estimated molecular and fossil ages for each calibration point.

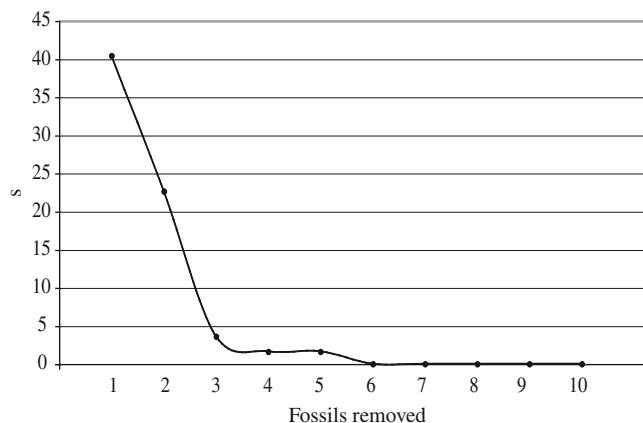


Fig. 2. Plot illustrating the effect of the removal of fossil calibration points on the average squared deviation (s).

(Pybus and Rambaut, 2002). In addition, we calculated the 95% confidence interval and the mean γ -value for each of the simulated datasets.

3. Results

3.1. Sequence characteristics and phylogenetic results

Sequence characteristics for all datasets are summarized in Table 3. Ambiguously aligned nucleotides were removed from both chloroplast and nuclear data matrices. Although some loci have not been amplified from a few species, their absence probably did not

alter the analyses when coded as missing data in the combined data matrix. A recent study demonstrated that highly incomplete taxa can be accurately placed in phylogenies as long as many characters were sampled throughout (Wiens, 2005).

The ML analysis of the enlarged chloroplast *Impatiens*-asterids dataset yielded a well-supported phylogenetic hypothesis in which the different orders are well defined and the relationship between the orders largely corroborates the study of Bremer et al. (2002). Nevertheless, we observed some discrepancies between their phylogeny and the present study, yet all of which are weakly supported in both studies. Whereas our results reveal a sistergroup relationship between Solanales and Gentianales (ML-BS: 56), and Asterales and Dipsacales (ML-BS: 73), the study of Bremer et al. (2002) shows a closer affinity between Solanales and Lamiales (maximum parsimony-BS: <50) on the one hand, and an unresolved polytomy with Dipsacales, Asterales and Apiales on the other. The phylogeny of Bremer et al. (2004) however, which is based on the molecular results of Bremer et al. (2002) indicates a sistergroup relationship between Dipsacales and Asterales.

Within the order Ericales, the balsaminoid clade is sister to the other Ericalean families (ML-BS: 100; e.g. Bremer et al., 2002; Schönenberger et al., 2005). In contrast to several molecular studies on Ericales and asterids, in which the mutual relationships between Balsaminaceae, Tetrameristaceae s.l. and Marcgraviaceae remained unresolved (APG, 2003; Savolainen et al., 2000; Schönenberger et al., 2005), the present study reveals a well supported sistergroup relationship between Tetrameristaceae s.l. (*Pelluciera*) and the Balsaminaceae–Marcgraviaceae clade (ML-BS: 98). Although many of the early diversified nodes in *Impatiens* are well resolved, the most recent nodes within the genus show limited resolution (Fig. 3). However, the enlarged asterid dataset was put together to resolve the basal relationships within the balsaminoid clade, which are subsequently used in the time divergence estimation procedure.

ML analysis of the nuclear *ImpDEF1/ImpDEF2* data matrix of *Impatiens* yielded highly congruent trees, supported by moderate bootstrap support (data not shown). Topologies based on *ImpDEF1/ImpDEF2* are slightly better resolved than those based on plastid *atpB-rbcL* sequences. The separately analyzed plastid and nuclear datasets of *Impatiens* corroborated the major clades recognized by Janssens et al. (2006) with no incongruent clades found between the two major data sets. Furthermore, the partition homogeneity test was not significant ($P > 0.05$), showing that chloroplast and nuclear partitions of the combined data were not in conflict. For the combined data matrix, the ML search yielded a well-resolved topology with strong support for most of the clades (Fig. 4). In comparison to the separate *ImpDEF1/ImpDEF2* and *atpB-rbcL* analyses, the combined phylogeny is better resolved and support values are generally higher.

Table 3
Sequence characteristics of the nuclear and chloroplast loci and the combined matrix.

Analyzed markers	Asterid dataset		<i>Impatiens</i> dataset	
	<i>atpB-rbcl</i>	<i>ImpDEF1/ImpDEF2</i>	<i>atpB-rbcl</i>	Combined
Analyzed characters	3822	5637	989	6626
Variable characters	908	804	248	1052
Parsimony informative characters	687	468	133	601

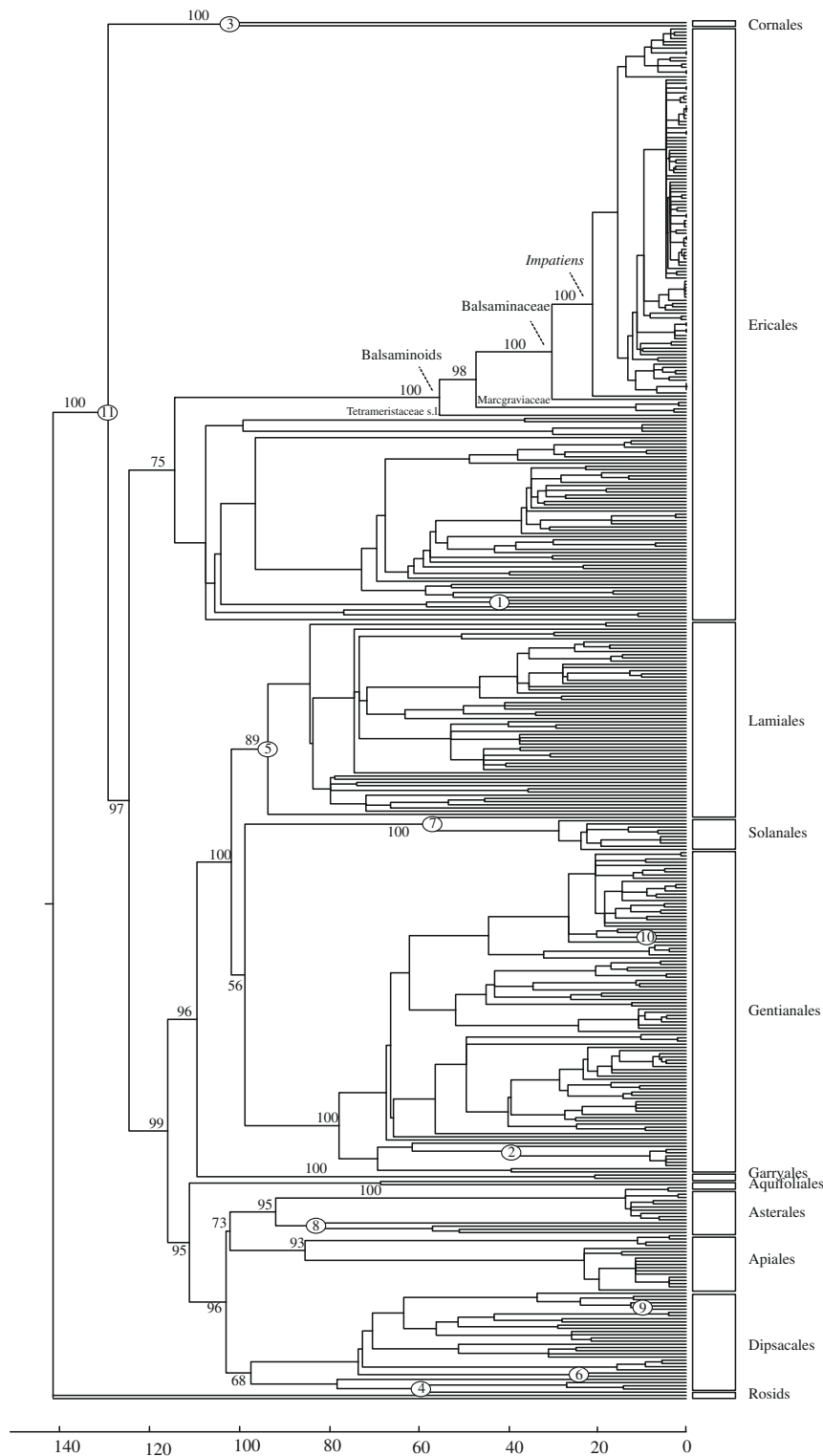


Fig. 3. ML phylogram of the asterid *atpB-rbcl* dataset. The considered calibration points (1–11) are indicated on the tree. ML bootstrap support values for the most basal nodes until the nodes at order level are highlighted on the branches. Support values at lower taxonomical level are not shown.

3.2. Divergence time estimates

Using the ‘fossil cross-validation’ method of [Near and Sander-son \(2004\)](#) we identified the Ebenaceae fossil *A. cryptostoma* and

the earliest known Asclepioid fossil as most inconsistent calibration points in our analysis. The node to which *A. cryptostoma* was assigned has been dated 11 million years younger than assumed by fossil data, whereas the crown node of the Asclepiodeae was

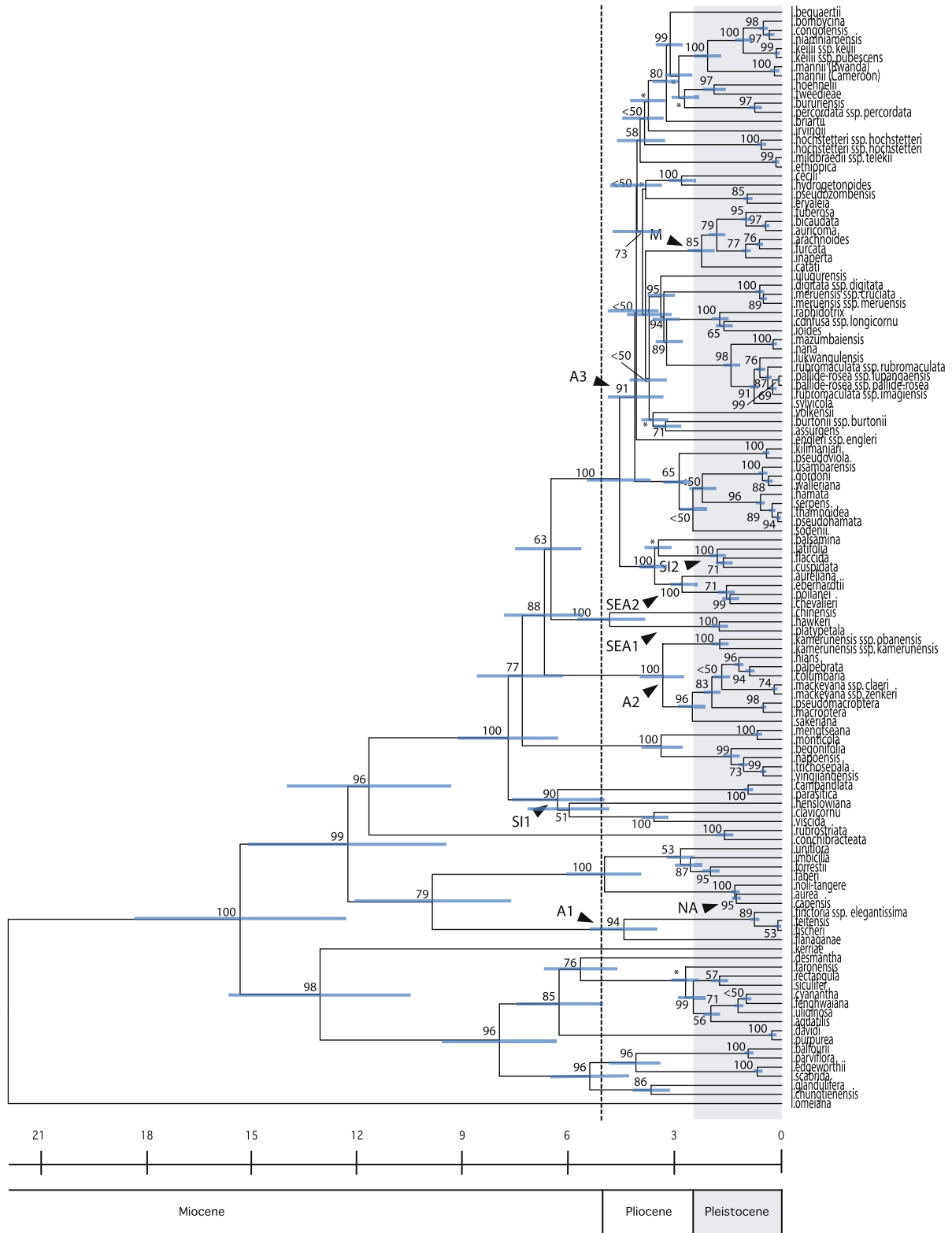


Fig. 4. ML phylogram based on the combined chloroplast and nuclear dataset of *Impatiens*. Asterisks indicate the presence of collapsed branches in strict consensus tree. Numbers on the branch represent ML bootstrap support values. Clades M (Madagascar), SEA1 (Southeast Asia), SEA2 (Southeast Asia), SI1 (South India), SI2 (South India), NA (North America), A1 (Africa), A2 (Africa) and A3 (Africa) are indicated with an arrow. Blue bars indicate age intervals.

Table 4
Estimated ages for the crown and stem groups of the asterid orders.

Order	Penalized likelihood age estimates (Mya)		Bremer et al. (2004) age estimates (Mya)	
	Stem	Crown	Stem	Crown
Cornales	128*	104 ± 13.1	128*	112
Ericales	123 ± 10.5	117 ± 9.2	127	114
Garryales	112 ± 9.3	20 ± 8.6	114	–
Lamiales	104 ± 8.2	95 ± 11.9	106	97
Gentianales	101 ± 7.9	79 ± 10.2	108	78
Solanales	101 ± 11.8	58 ± 9.1**	106	100
Aquifoliales	113 ± 9.8	62 ± 11.9***	121	113
Apiales	104 ± 11.2	87 ± 14.1	113	84
Dipsacales	105 ± 14.2	99 ± 15.4	111	101
Asterales	104 ± 12.1	94 ± 11.2	112	93

* The crown node of the asterid was fixed at 128 Mya.

** The Convolvulaceae were not included in our dating analysis, which explains the young age estimate for the crown group of the Solanales.

*** The Stemonuraceae and Cardiopteridaceae were not included in our dating analysis, which explains the young age estimate for the crown group of the Aquifoliales.

estimated approximately 15 million years younger than suggested by fossil data. However, when including both fossil calibration points in the overall dating analysis, the dating estimates of the *Impatiens* nodes were hardly altered. Nevertheless, both calibration points were removed from the final dating analysis.

Applying the divergence times of the selected asterid fossils and the previously estimated age of the asterid crown group by Bremer et al. (2004), we dated the split between Marcgraviaceae and Balsaminaceae at 48.2 mya (SD = ±9.3 mya). The divergence between *Impatiens* and *Hydrocera* is estimated at 30.7 mya (SD = ±8.6 mya), whereas the crown group of *Impatiens* is estimated at 22.5 mya (SD = ±5.6 mya). The age estimations of the asterid orders with their standard deviations are listed in Table 4. In order to infer the divergence times for the remaining lineages in *Impatiens*, we used the estimated crown age of *Impatiens* (22.5 my) as secondary calibration points for the ML phylogram of the combined nuclear-plastid dataset (Fig. 4). In addition, geological evidence was used to cross-validate the asterid-based divergence times.

3.3. Ancestral area distribution

Maximum likelihood-based ancestral area reconstruction illustrates that the vast majority of the current diversification of *Impatiens* has originated in Asia (Fig. 5 and Table 5). Southwest China was unambiguously reconstructed as the ancestral area for the earliest-diverging lineages. Although likelihood ratio values were in general significant for the *Impatiens* topology, we noticed that the root node, which connects *Impatiens* to its sistergroup *Hydrocera*, has an insignificant likelihood ratio value. Apparently, this insignificance occurs rather frequently in likelihood-based ancestral state reconstruction analyses as the amount of uncertainty related with reconstruction increases in time (Schluter et al., 1997).

Biogeographic reconstructions indicate that *Impatiens* dispersed into South India via two independent colonization events from Southwest China (clades SI1 and SI2), whereas the African continent was colonized from Southwest China in three independent dispersal events (clades A1, A2 and A3).

Similarly, our reconstructions show that only one speciation event from Southwest China accounts for the diversity of *Impatiens* in North America (clade NA). Furthermore, the Southeast Asian (clades SEA1 and SEA2) and Himalayan species originated from Southwest China. In contrast, the Malagasy clade (clade M) is derived from a single colonization event from Africa (Fig. 5 and Table 5).

3.4. Speciation rates and timing of diversification in *Impatiens*

The slope of the LTT plot obtained from the PL tree for *Impatiens* is slightly convex until 4.5 million years ago and then becomes steeper (Fig. 6). Simulated LTT curves for *Impatiens* diversity indicate a hypothesized steep slope near the root of the genus that gradually decreases towards the tips. Interestingly, between 15 million years ago and present-day, the empirical LTT plot is situated outside the 95% significance level of the simulated LTT plots.

In order to statistically test whether this observation truly indicates a late increase in net diversification rate, we evaluated our results with γ -statistics (Fig. 7). For our chronogram, we computed a negative γ -value ($\gamma = -6.27$), indicating that the process of lineage accumulation in *Impatiens* did not remain constant in time. Simulations carried out to consider the effect of incomplete sampling (113 species sampled from 1000 extant species), indicated that under a true value of 0, *Impatiens* would only contain 207 species with the 95% interval, ranging between 120 and 427 species to obtain a γ -value as extreme as -6.27 . As a result, we are able to reject the hypothesis that the obtained γ -value was the result of a poor sampling density.

Similar results are obtained when the diversification rate was calculated for every possible node and its accompanying lineage (Fig. 8). Between 22.5 and 5 mya, computed speciation rates are estimated between 0.03 and 0.56 species per million years. During the next 5 my, rates of species diversification rates fluctuate between 0.07 and 3.8 species per million years.

4. Discussion

4.1. Divergence time estimates in asterids

Table 4 lists the stem and crown group estimates for all asterid orders. Although the obtained divergence estimates generally corroborate the results of Bremer et al. (2004), the majority of the stem group estimates are slightly younger, whereas most of the crown group estimates are a little older. The largest differences in age estimate between our study and the study of Bremer et al. (2004) were observed for the stem node age of the orders in the euasterids 2 clade and the crown group age of the orders Solanales, Cornales and Aquifoliales. These larger dissimilarities are possibly due to the use of a single gene marker (*atpB-rbcL*) that was not applied in the multigene dating analysis on the asterids by Bremer et al. (2004). In addition, we were unable to sample all the earliest-diverging lineages of each order, resulting in much younger crown node estimates for some of the above-mentioned lineages. The use of a single gene for generating age estimates also caused

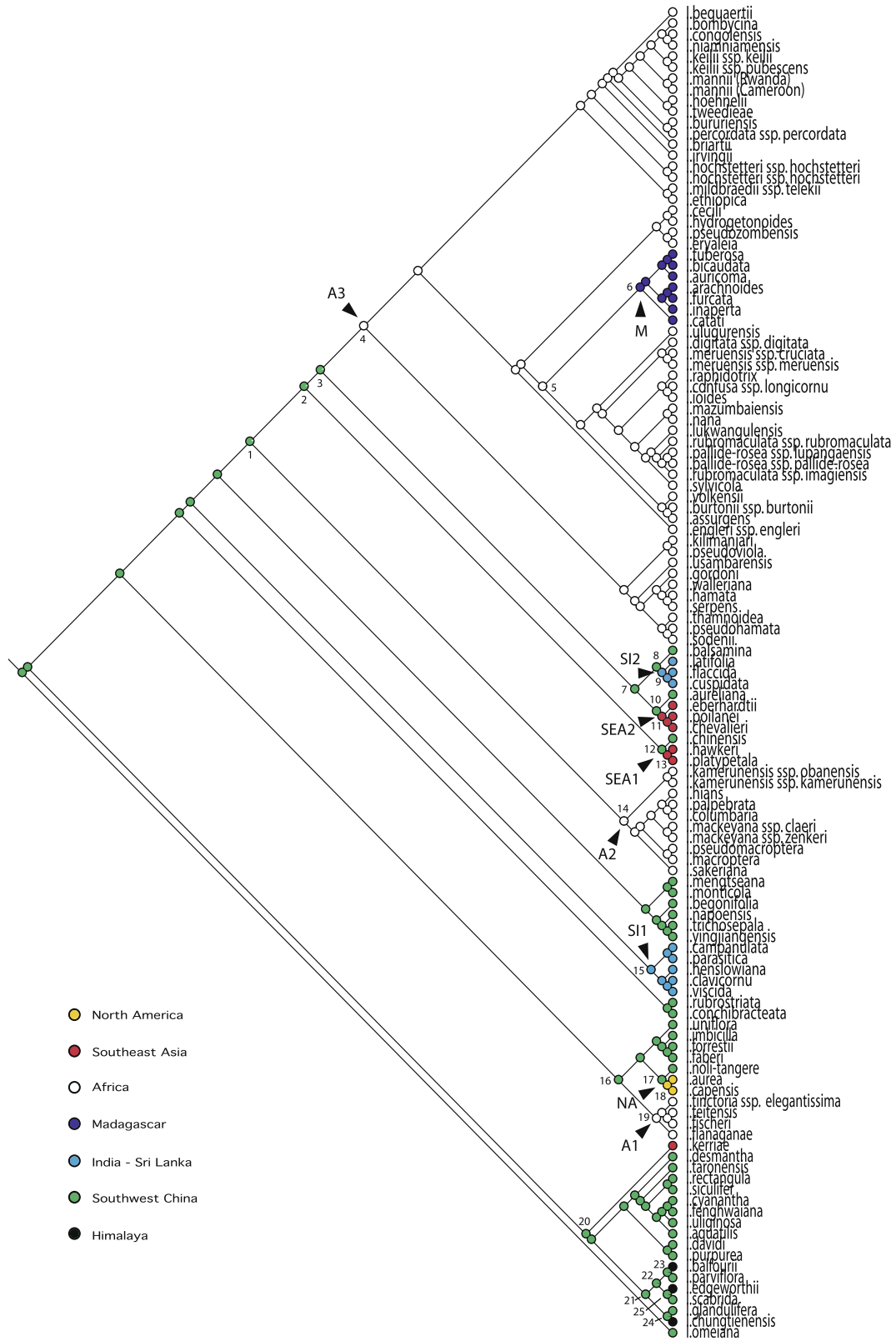


Fig. 5. ML topology illustrating the reconstruction of ancestral area distribution of *Impatiens* ancestors. Pie charts at each node indicate proportional likelihoods of the most common recent ancestor for each clade. Clades M (Madagascar), SEA1 (Southeast Asia), SEA2 (Southeast Asia), S11 (South India), S12 (South India), NA (North America), A1 (Africa), A2 (Africa) and A3 (Africa) are indicated with an arrow.

the bootstrap confidence intervals to be fairly large, indicating a certain inaccuracy of the *atpB-rbcl* dataset. However, the overall similarity in ordinal age estimates in Bremer et al. (2004) and the present study suggests that our chronogram presents a

plausible scenario for other nodes among the asterids (e.g. *Impatiens*).

Despite the slightly younger age estimate for most of the stem nodes of the asterid orders, the crown group of the Ericales, to

Table 5
Proportional likelihoods values for reconstruction of ancestral areas. Values in bold indicate best estimates. Node numbers correspond to those shown in Fig. 5.

NODE	Africa	Madagascar	Himalaya	Southwest China	North America	Southeast Asia	South India-Sri Lanka	Inferred ancestral area
1	6.5	<0.05	<0.05	93.3	<0.05	<0.05	<0.05	Southwest China
2	5.6	<0.05	<0.05	94.2	<0.05	0.1	<0.05	Southwest China
3	6.1	<0.05	<0.05	93.7	<0.05	0.1	<0.05	Southwest China
4	99.1	<0.05	<0.05	0.8	<0.05	<0.05	<0.05	Africa
5	99.0	0.8	<0.05	<0.05	<0.05	<0.05	<0.05	Africa
6	0.8	99.0	<0.05	<0.05	<0.05	<0.05	<0.05	Madagascar
7	0.4	<0.05	<0.05	99.1	<0.05	0.2	0.1	Southwest China
8	0.1	<0.05	<0.05	98.4	<0.05	1.3	<0.05	Southwest China
9	<0.05	<0.05	<0.05	0.8	<0.05	<0.05	99.1	South India-Sri Lanka
10	0.1	<0.05	<0.05	98.5	<0.05	<0.05	1.2	Southwest China
11	<0.05	<0.05	<0.05	0.8	<0.05	99.0	<0.05	Southeast Asia
12	1.9	<0.05	<0.05	95.2	<0.05	2.8	<0.05	Southwest China
13	<0.05	<0.05	<0.05	0.8	<0.05	99.1	<0.05	Southeast Asia
14	98.2	<0.05	<0.05	1.6	<0.05	<0.05	<0.05	Africa
15	<0.05	<0.05	<0.05	<0.05	<0.05	0.9	99.0	South India-Sri Lanka
16	0.9	<0.05	<0.05	99.0	<0.05	<0.05	<0.05	Southwest China
17	<0.05	<0.05	<0.05	98.4	1.5	<0.05	<0.05	Southwest China
18	<0.05	<0.05	<0.05	1.7	98.2	<0.05	<0.05	North America
19	99.1	<0.05	<0.05	0.8	<0.05	<0.05	<0.05	Africa
20	<0.05	<0.05	<0.05	99.1	<0.05	0.8	<0.05	Southwest China
21	<0.05	<0.05	1.3	98.6	<0.05	<0.05	<0.05	Southwest China
22	<0.05	<0.05	2.5	97.4	<0.05	<0.05	<0.05	Southwest China
23	<0.05	<0.05	3.7	96.2	<0.05	<0.05	<0.05	Southwest China
24	<0.05	<0.05	3.7	96.2	<0.05	<0.05	<0.05	Southwest China
25	<0.05	<0.05	2.6	97.3	<0.05	<0.05	<0.05	Southwest China

which *Impatiens* belongs, was dated three million years older (117 ± 10.5 my) than calculated by Bremer et al. (2004). In addition, the study of Bremer et al. (2004) estimated the crown group of the balsaminoid clade at approximately 64 million years, a date that corresponds with our results (58.9 my). The crown group age of the balsaminoids is also slightly older than the first known fossil of *Pelliciera rhizophorae* from the Late Eocene (54.9 mya; Graham, 1975; Rull, 1999). We decided not to apply this age estimate in the asterid dating analysis but to use the fossil pollen age of *Pelliciera* to cross-validate our obtained results. By applying the fossil age of *P. rhizophorae* to the node directly leading towards Balsaminaceae and Marcgraviaceae, the estimated age of *Impatiens* would have been strongly influenced by this one calibration point,

whereas all other fossil calibration points would have had no value in dating *Impatiens*.

4.2. Origin and evolution of the Balsaminaceae

Only recently, molecular evidence has demonstrated that Balsaminaceae are closely related to Marcgraviaceae, both being part of the balsaminoid clade in the Ericales (Bremer et al., 2002; Schönenberger et al., 2005). Despite their close affinity, these families have very different distribution areas. The Balsaminaceae have a worldwide distribution except for Australia and South America, whereas the Marcgraviaceae are restricted to tropical forests of South and Central America (Dressler, 2004; Fischer, 2004). It seems plausible that their most recent common ancestor was present by the start of the Tertiary (Grey-Wilson, 1980a). Although the split between Marcgraviaceae and Balsaminaceae is dated in this study in the Middle Eocene (48.1 mya), the ancestral stock of both families already existed during the Middle Paleocene (58.9 mya). This

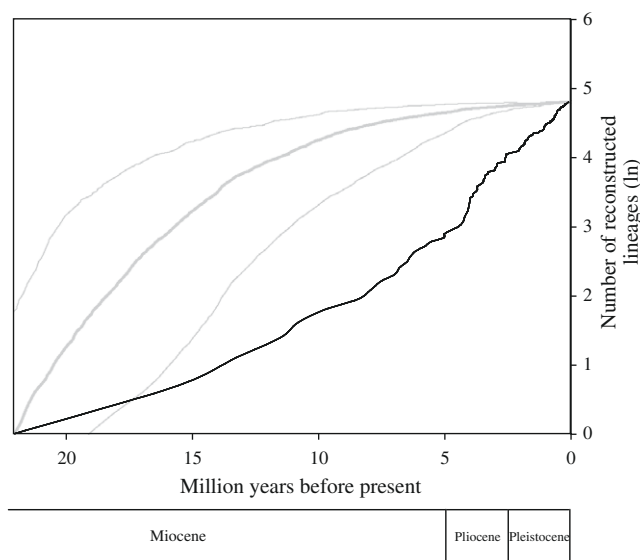


Fig. 6. Semilogarithmic lineage-through-time (LTT) plot for the *Impatiens* clade sister to *I. omeiana* are indicated by a black line. LTT plot from simulated phylogenies illustrating the effect of an incomplete sampling is shown by a grey line. The thin grey lines indicate the 95% interval while the thick grey line designates a mean value.

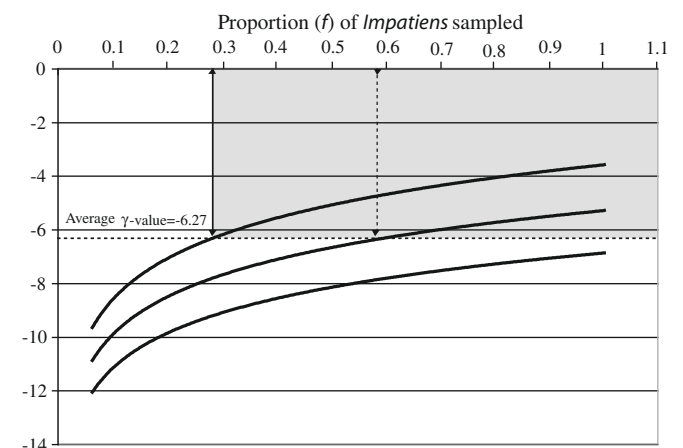


Fig. 7. Relationship between γ and the proportion of extant taxa in *Impatiens* (f). The thin grey lines indicate the 95% interval while the thick black line appoints the mean γ -value. The shaded area represents the range of f -values consistent with the observed γ at the 95% level.

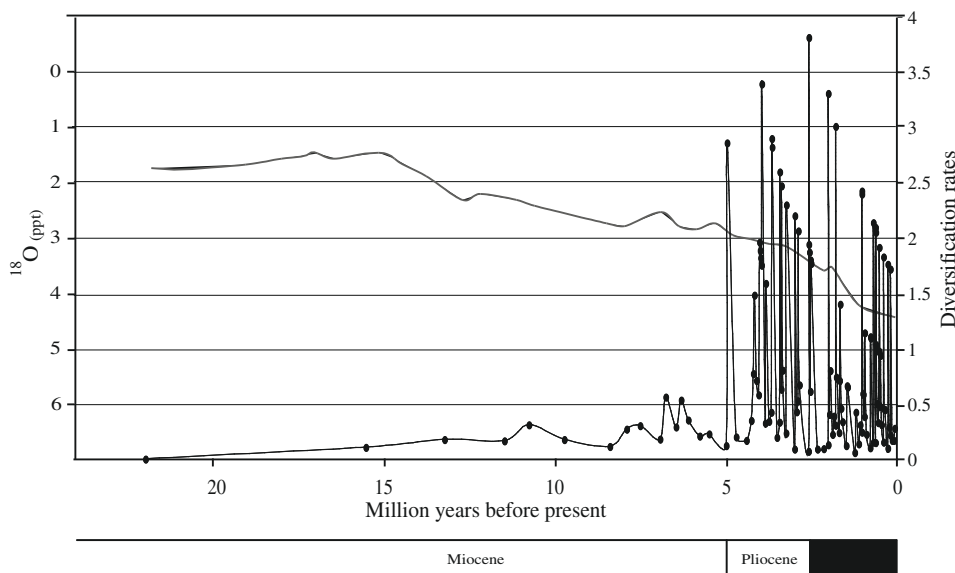


Fig. 8. Diversification rates calculated for the genus *Impatiens* (black line). Superimposed (grey line) is a time-averaged record of sea surface temperatures (Zachos et al., 2001).

confirms the hypothesis of Grey-Wilson (1980a) suggesting an origin for Balsaminaceae ca. 50 million years ago.

It lasted another 18 million years before the lineage leading to the extant species of *Impatiens* diverged from the lineage leading to the extant species of *Hydrocera* (30.7 mya, Late Eocene). Despite this early separation, the extant species of *Impatiens* have a coalescence point in the Early Miocene (22.5 mya; Fig. 3). It is unclear whether this phenomenon of no net diversification during a period of 8 million years is due to low rates of speciation or high rates of extinction because no fossil data are available for this group. A similar question applies to the sister group of *Impatiens*, the monospecific *Hydrocera triflora*, which has a restricted Indo-Malaysian distribution (Grey-Wilson, 1980b). Since the origin of this lineage in the Late Eocene, climatic conditions have changed significantly, resulting in several periods of drought and huge fluctuations in sea level (Berggren et al., 1995; Haq et al., 1987; Riggs, 1984). Due to its restriction to swampy lowland habitats (not occurring above 100 m altitude) and a less efficient dispersal mechanism than its sistergroup *Impatiens*, other *Hydrocera* lineages could have gone extinct during that time. Alternatively, speciation could be slowed down by other causes (e.g. fluctuating sea levels), resulting in only very low diversification. This remarkable situation in which lack of diversification occurred in the sister lineage of a very species-rich genus is analogous with the monospecific genus *Hillebrandia* and its species-rich sister genus *Begonia* (ca. 1400 species, Clement et al. 2004).

4.3. Diversification patterns in *Impatiens*: the effect of a global climate change

Despite our ability to reconstruct the basal phylogenetic relationships within *Impatiens* using only the chloroplast *atpB-rbcl* spacer data, most of the recently diversified lineages remained unresolved. By combining the rapidly evolving *ImpDEF1* and *ImpDEF2* markers with the chloroplast *atpB-rbcl* spacer, we were able to resolve many of the previously unresolved relationships in *Impatiens*. However, due to rapid evolution of the *ImpDEF* genes, we were obliged to use an approach of secondary calibration to assess the evolutionary history of the rapid diversification of *Impatiens*. Several studies indicate that secondary calibration can cause considerable problems in molecular dating (Graur and Mar-

tin, 2004; Hedges and Kumar, 2003, 2004). Nevertheless, we considered this a valid approach for our study because we used the same dating protocol, a similar phylogeny reconstruction method and complementary taxon sampling for both dating analyses. Furthermore, in order to reduce the problem of secondary calibration, we used additional geologic evidence together with the calibration points based on direct and indirect fossil data.

The present study indicates that diversification in *Impatiens* increased since the last 4.6–5 million years (Fig. 8). This period of exceptional diversification, during which 84% of all *Impatiens* lineages were established, can be linked with the global cooling that occurred during the Pliocene and Pleistocene (Fig. 6). In addition, when comparing the proportion of 207 *Impatiens* lineages ($b/d = 0$) equalling the calculated γ -value of -6.27 with the approximately 1000 currently described taxa, it is clear that the climatic conditions correlate well with the rapid diversification within the genus. This can also be seen in the fluctuating species diversification rates of the last 4.6 million years (between 0.09 and 4 species per million year; Fig. 8), which are probably related to the glacial–interglacial cycles that were initiated during this period (Zachos et al., 2001). Our study demonstrates that the current species richness of *Impatiens* is the result of a sudden diversification boost and did not originate via gradual accumulation of species over a long geological period. In addition the massive species diversity in *Impatiens* occurred through changing climatic conditions in the Pliocene and Pleistocene, and was not the outcome of an enhanced speciation event in the Tertiary or earlier. Consequently, the “refuge” hypothesis, in which rainforest species populations became isolated from each other during times of cool and dry climate conditions, may be a plausible model to explain the enormous diversity in *Impatiens*. The plants are characterized by explosively dehiscent capsules with seeds travelling for the most part less than a meter. In addition, most balsams are montane plants, often restricted to a small area, sometimes no more than a single mountain peak or a mountain range. The periodicity of glacial cycles during the Pleistocene could have resulted in several alternating episodes of contraction and expansion of the montane and lowland rainforests with vegetation belts slightly shifting down and upslope during these varying climatic conditions (Kebede et al. 2007). As a result, *Impatiens* would have been forced to migrate along with the rainforest belts in order to retain its required habitat type. With

its area of distribution being extremely fragmented during certain climatic episodes, many different *Impatiens* populations were probably isolated for several thousands of years. The continuous cycle of dispersal subsequent to fragmentation and isolation that has been induced by climatic fluctuation during the Pliocene and Pleistocene almost certainly contributed to the rapid radiation of *Impatiens*.

4.4. Biogeographic patterns in *Impatiens*

Biogeographic reconstruction indicates that the center of origin for *Impatiens* is Southwest China, from where the genus subsequently dispersed to Africa, India, the Himalayas, Southeast Asia, Central Asia and North America. These results are consistent with the fact that China contains 222 species or roughly 1/4th of all currently described *Impatiens* species, thereby largely surpassing South India and Sri Lanka, the Himalayas, Africa (Grey-Wilson, 2008). Of these Chinese species, the majority is endemic to the provinces of Sichuan and Yunnan in the Southwest of China. In addition, the only fossil bearing a relation to extant *Impatiens* was found in the East China Sea (*I. brevicolpus* – Santan Formation, Pliocene; Song et al., 2004).

In the past, several morphological similarities have been observed in species endemic to Africa and South India, suggesting a close affinity between these taxa and even a possible migration route connecting these two areas (Grey-Wilson, 1980a). Despite the resemblance between various African and South Indian balsams, the present study illustrates that the South Indian species originated from two independent dispersal events (clades SI1 and SI2), with Southwest China reconstructed as the area of origin for each clade. However, one of the two South Indian lineages (clade SI2) is closely related to one of the African subclades (clade A3), hence explaining the overall similarities between taxa from two clades. This African-affiliated lineage colonized South India between the Late Pliocene and the Early Pleistocene. In contrast, the other South Indian lineage (clade SI1) previously dispersed in the Late Miocene.

Based on the close resemblance of the North American lineage to some Chinese species, Grey-Wilson (1980a) suggested that the genus arrived only recently in the New World. Our data corroborate his interpretation, as the colonization of North America is estimated to have occurred in the Pleistocene between 1.32 and 1.27 million years ago (clade NA). Interestingly, this time estimate corresponds with a glacial maximum that lasted from 1.35 until 1.25 mya (Berger et al., 1999; Bintanja and Van de Wal, 2008; Raymo 1997). The North American clade is sister to *Impatiens nolitangere*, one of the few *Impatiens* species that is not endemic to a restricted geographic area but is a widespread species in temperate Eurasia, including Southwest China (Chen et al., in press). North America may have been colonized via the Bering land bridge in association with glacial–interglacial stages during the Pleistocene.

Because some Chinese species are distributed as far as the Himalayan chain, it has been considered that there is a strong biogeographic correlation between these two distribution areas (Grey-Wilson, 2008). Present data show that all species endemic to the Himalayas have a sister species which is distributed or even endemic to China. As many other hotspots for *Impatiens*, also the Himalayan region is characterized by a considerable number of species (ca. 120 spp.). All these Himalayan endemics appear to find their origin in (Southwest) China via several separate dispersal events, which occurred as early as the Late Miocene and not via a single colonization event as for example with the Malagasy or North American *Impatiens* species.

The sister species to the New Guinean endemic, *Impatiens hawkeri*, is located in the Southeast Asian Archipelago. In addition, the closest relative of these two species is distributed in South China

and the northern provinces of India, Myanmar and Vietnam close to the Chinese border. As a result, the most recent common ancestor of the New Guinean species dispersed from South China towards the Southeast Asian Archipelago from where New Guinea was eventually reached (clade SEA1). According to our results, the lineage towards *I. hawkeri* recently crossed the Wallace's Line from Sunda to Sahul Shelves, thereby arriving on New Guinea during the Early Pleistocene. This recent age estimate is in concordance with the difficulties that were encountered to delimit species of the existing lineages on the island (Grey-Wilson, 1980b). As a result, Grey-Wilson lumped all previously described Papuan *Impatiens* species in a single hugely variable species, which is subdivided in 15 groups. A population genetic approach will be required to further investigate the complex evolution of this group.

Ancestral area reconstruction clearly indicates that *Impatiens* colonized Africa on three separate occasions. Previous studies demonstrated that Africa was colonized more than once, but the exact number of dispersal events could not be determined (Janssens et al., 2006; Yuan et al., 2004). The most recent common ancestor of the three African clades is located in Southwest China. Although our initial work (Janssens et al., 2006) left open the possibility that India may have been colonized from Africa, this expanded study suggests that African *Impatiens* never recolonized Asia. The only colonization events of *Impatiens* from Africa were to the offshore islands of the Seychelles and Madagascar (and then to the Comoros) in the Indian Ocean. Although only 4% (7/±150) of the Malagasy *Impatiens* species were sampled for this study, all species are closely related, raising the possibility that most or all of this diversity was derived from a single colonization event (clade M). The initial colonization of Africa by *Impatiens* apparently occurred relatively early, as the oldest group arrived during the Late Miocene (clade A1). All species present in this old African clade are restricted to East and South Africa and characterized by disjunct distribution areas. Considering the older age of this lineage, the disjunct distribution areas of its representatives and the low rate of diversification, this clade may have experienced extinction in the intervening areas. The second dispersal of *Impatiens* to Africa occurred between the Late Miocene and Early Pliocene (clade A2). Representatives of this clade are endemic to West Africa. The third colonization of Africa occurred during the Late Miocene and Early Pliocene (clade A3). This lineage quickly diversified after reaching Africa, resulting in the largest diversification of *Impatiens* in Africa. Most *Impatiens* species in this clade are restricted to the montane forests and highlands of Central and East Africa. Additionally, only a few species are present in both Central and West Africa. Most species that occur in both Central and West Africa have a disjunct distribution, only occurring in mountainous regions of West Africa, and the eastern part of Congo and neighbouring mountainous regions, yet absent in the lowland forests of central Congo. One exception is *Impatiens niamniamensis*, which has a continuous distribution from Central to West Africa throughout the Congo River basin. Because the present Congolian lowland forests have been considerably affected by periods of glacial aridity, we assume that this species recolonized the Congo basin only recently. Species with disjunct distribution areas in Central and West Africa may have also been affected by ice age aridity. For example *Impatiens mannii* is inferred to have originated during the Pleistocene in the volcanic regions of Central Africa and may have migrated to West Africa, in which case it would have been more widespread throughout the tropical rainforests of central Africa. Climatic fluctuations related to glacial events reduced the central African rainforests (Coetzee, 1993; Plana, 2004). Only the more mountainous populations of *I. mannii* may have survived, having failed to recolonize the lowlands like *I. niamniamensis*. Alternatively, *I. mannii* may have reached West Africa via long-

distance dispersal. In either case, the morphological similarity between both western and central African populations of *I. mannii* indicates a recent isolation.

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