

Impatiens smetsiana, another example of convergent evolution of flower morphology in *Impatiens*

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Abstract

Background and aims – The genus *Impatiens* is known for its enormous convergent phenotypic adaptation, with similar floral traits having independently evolved in distantly related lineages. The large functional convergence of *Impatiens* flowers causes a high degree of homoplasy for several phenotypic characters resulting in increased difficulties to distinguish between species with a similar morphology that are only distantly related, however. As a result, some species remain under the radar, as they are confused with other well-known species. This was the case for a new *Impatiens* species from the Tchabal Mbabo Mountains in Cameroon – *Impatiens smetsiana* – that was initially mistaken for the morphologically similar species *I. erecticornis*, an endemic from Central East Africa.

Material and methods – A combined molecular-morphological approach was applied in which phylogenetics (ITS, *ImpDEF1*, and *ImpDEF2*), biogeography, and age estimation analyses were combined with morphological data on floral and vegetative structures.

Key results – In this study, we demonstrate the close affinity of the newly collected material with a group of Equatorial West African species, including *I. filicornu*, *I. nzabiana*, *I. oumina*, and *I. kamerunensis*. The present finding represents a clear case of convergent evolution in which two distantly related taxa independently converged on practically the same flower morphology.

Conclusion – Within *Impatiens*, several examples of floral homoplasy have been observed yet not in such a clear way. The convergent evolution of the flowers of *I. smetsiana* and *I. erecticornis* is undoubtedly closely correlated with an adaptation to a similar pollination syndrome.

Keywords

Adamawa, biogeography, Cameroon, convergent evolution, diversification, *Impatiens*, speciation

INTRODUCTION

Although Hooker made an initial revision of the Indian representatives of the genus *Impatiens* L. in 1859 (Hooker and Thomson 1859), it took him more than 40 years to recommence his study on Indian balsams. In fact, he spent the last 10 years of his life trying to unravel the complex diversity of the genus and came to the conclusion that *Impatiens* are ‘a terror to botanists, deceitful above all plants and desperately wicked’ (Hooker 1875, 1905; Huxley 1918; Grey-Wilson 1980). Already during his

first survey, Hooker points to the huge floral variety that is present within *Impatiens* stating that ‘it would be difficult to indicate another genus in the vegetable kingdom, presenting amongst its species so many and such different modifications of structure, and of which the species are so excessively prone to vary’ (Hooker and Thomson 1859). As a result of this floral variation, it has always been very difficult to divide the genus into natural groupings using solely morphological data (Hooker and Thomson 1859; Warburg and Reiche 1895; Hooker 1905). The genus *Impatiens* is known for its enormous

convergent phenotypic adaptation, with similar floral traits having independently evolved in distantly related lineages (Yuan et al. 2004; Janssens et al. 2006, 2009b; Yu et al. 2015; Vandeloos et al. 2019; Abrahamczyk et al. 2021; Ruchisansakun et al. 2021). The large functional convergence of *Impatiens* flowers causes a high degree of homoplasy for several phenotypic characters. In general, it is assumed that the majority of convergent traits are the result of an adaptation for particular ecological conditions. Moreover, morphological adaptation to pollinators is considered to be a widespread phenomenon in floral evolution as there is often a strong selection towards certain floral traits due to behaviour and morphology of the pollinator (Okuyama et al. 2008). Phylogenetic analyses have demonstrated that most of the phenotypic homoplasy in *Impatiens* is the result of convergent evolution (Yuan et al. 2004; Janssens et al. 2006, 2007, 2008).

Grey-Wilson (1980) highlights the occurrence of floral morphological similarities between species endemic to India, Africa, Madagascar, and Southeast Asia and assumes a clear affinity between those species. However, recent molecular analyses showed that the intrageneric relationships within *Impatiens* are strongly geographically structured, with each main clade restricted to a specific geographic area and hardly any amalgamation of geographically different taxa in a single clade (Yuan et al. 2004; Janssens et al. 2006, 2007, 2009a; Yu et al. 2015; Ruchisansakun et al. 2016). In addition, these studies revealed the enormous homoplastic nature of floral characters in *Impatiens*. Despite the clear evidence of convergent evolution in several different *Impatiens* lineages (e.g. red flowered species with bucciniform spurred sepals: *I. niamniamensis* Gilg (Central Africa), *I. parasitica* Bedd. (South India), and *I. humblotiana* Baill (Madagascar); pink flowered species with a filiform spur: *I. walleriana* Hook.f. (East Africa), *I. lyallii* Baker (Madagascar), *I. latifolia* L. (South India), and *I. hawkeri* W.Bull (Papua New Guinea)), this convergence occurs especially in certain floral traits (e.g. the spurred sepal, the lateral united petals, and the dorsal petal). Despite the overall similarity in flower morphology between two convergent *Impatiens* species, slight morphological differences between the species remain present. For the non-trained eye, however, it is often difficult to spot these small dissimilarities between different species, resulting in an underestimation of the real number of species for a certain lineage. For example, within *Impatiens*, several recently described species were originally identified as a morphologically similar but less closely related species (e.g. Janssens et al. 2009b, 2010, 2011). The present paper investigates the convergent relationship of a new *Impatiens* species with its morphological doppelgänger for which it was originally mistaken for. The new species is described and illustrated, and its evolutionary history is studied using biogeographic analyses and age estimation analyses.

MATERIAL AND METHODS

Morphological description and data analysis

Two accessions of the new species were collected by the second and last author in May 2009. The material was collected in northern Cameroon (Adamawa region). Descriptive terminology follows Grey-Wilson (1980) except for simple symmetrical plane shapes (SACDBT 1962).

In order to study the convergent evolution of *I. smetsiana* and *I. erecticornis* R.Wilczek & G.M.Schulze, we analysed the overall morphology of those two species with the closest allies of *I. smetsiana* using a Multiple Factor Analysis for mixed data (continuous and discrete) as implemented in the FactoMineR R package (Lê et al. 2008). The scores obtained for each individual in the first two dimensions were used to interpret the degree of phenotypical convergence between the new species and its morphological doppelgänger, *I. erecticornis*. Morphological traits used in the analysis are provided in Table 1.

Phylogenetic analyses

In order to determine the phylogenetic position of *I. smetsiana* among the African lineages, we added sequences of the new species to a combined nuclear *ImpDEF1/ImpDEF2* and nuclear ribosomal ITS data matrix. Based on earlier phylogenetic studies (Volkmar et al. 2014), *I. rubrostriata* Hook.f. and *I. conchibracteata* Y.L.Chen & Y.Q.Lu serve as outgroup species. Total genomic DNA was isolated from silica-dried leaf material using a modified CTAB protocol (Doyle and Doyle 1987), which is optimized for *Impatiens* by Janssens et al. (2006, 2009b). Primers and temperature profiles used for the amplification of *ImpDEF1/ImpDEF2*, and ITS follow Janssens et al. (2007, 2008) and White et al. (1990), respectively. Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems). Purified amplification products were sent to Macrogen, Inc. (Seoul, South Korea) for sequencing. Sequences obtained in this study were submitted to GenBank (Supplementary file 1).

Contiguous sequences were assembled using Geneious v.7.0.6 (Biomatters, New Zealand). Automatic alignments were carried out with MAFFT (Katoh et al. 2002) under an E-INS-i algorithm, a 100PAM/k=2 scoring matrix, a gap open penalty of 1.3 and an offset value of 0.123. Subsequent manual fine-tuning of the aligned dataset was done in Geneious v.7.0.6. Congruency between the different datasets was inferred using different methods. First, a series of incongruence length difference tests (ILD; Farris et al. 1995) were carried out with PAUP* v.4.0b10 (Swofford 2003) using the following parameters: simple taxon addition, TBR branch swapping, and heuristic searches of 1000 repartitions of the data. Despite the well-

known sensitivity of the ILD test (Barker and Lutzoni 2002), the results of this test were compared in light of the resolution and support values of the obtained nuclear and nuclear ribosomal topologies. As a result, possible conflict between data matrices was visually inspected, searching for conflicting relationships within each topology that are strongly supported (hard vs soft incongruence; Johnson and Soltis 1998).

The best-fit nucleotide substitution model for each plastid and nuclear dataset was selected using jModelTest v.2.1.4. (Posada 2008) out of 88 possible models under the Akaike information criterion (AIC). The GTR+I+G model was found as best fit for *ImpDEF1*, whereas the GTR+G model was calculated as best substitution model for *ImpDEF2* and ITS. As a result, we used a mixed-model approach to apply different evolutionary models on each DNA region of the combined dataset (Ronquist and Huelsenbeck 2003). Bayesian inference analyses were conducted with MrBayes v.3.1 (Huelsenbeck and Ronquist 2001) on four individual data partitions and a combined data matrix. Each analysis was run two times for 20 million generations. Trees were sampled every 5,000th generation. Chain convergence and ESS parameters were inspected with TRACER v.1.4 (Rambaut and Drummond 2007). Only nodes with Bayesian posterior probabilities (BPP) above 0.95 are considered as well supported (Suzuki et al. 2002). Maximum Likelihood analyses were conducted using the RAxML search algorithm (Stamatakis et al. 2005) as implemented in RAxML v.7.2.8 (Stamatakis et al. 2008) under the GTRGAMMA approximation of rate heterogeneity for each gene (Stamatakis 2006). Five hundred bootstrap trees were inferred using the RAxML Rapid bootstrap algorithm (ML-BS) to provide support values for the best-scoring ML tree.

Divergence time estimation

The molecular clock hypothesis was tested using a chi² likelihood ratio test (Felsenstein 1988) and demonstrated that the substitution rates in the combined dataset are not clock-like ($p < 0.001$ for all markers). BEAUti v.1.8.0 (Drummond and Rambaut 2007) was used to prepare the xml file prior to the dating analysis in BEAST v.1.8.0 (Drummond and Rambaut 2007). The latter software program applies the Bayesian methodology to compute divergence times. However, in order to surpass the zero likelihood issue in BEAST, we used a starting tree that was obtained by carrying out a ML analysis in RAxML v.7.2.8 (Stamatakis et al. 2008) under GTRGAMMA model with the rooted likelihood tree as input tree for a penalized likelihood (PL) analysis in the software program r8s (Sanderson 2003). Due to differing substitution models among the different gene markers used, a partitioned Bayesian MCMC analysis was performed under the Yule speciation model and a relaxed lognormal clock. Partitions were unlinked for the model of evolution. The analysis ran for 30 million generations and was sampled each 5,000th generation. Convergence of the chains and ESS parameter

evaluation (ESS > 200) was performed with TRACER v.1.6 (Rambaut and Drummond 2007). A maximum clade credibility tree using a posterior probability limit of 0.5 was calculated using TreeAnnotator v.1.8.0. (Drummond and Rambaut 2007). The previously computed age estimate for the most recent common ancestor of the Afro-Madagascan and Equatorial West African clades of Janssens et al. (2009a) was used to calibrate the ingroup of the current study. This calibration point was given a normal distribution with a mean value of 7.87 Ma and a standard deviation of 0.5.

RESULTS

Morphological data analysis

The first two components of the multivariate analysis explained 69% of the total variation in continuous traits. Dimension 1 correlated with upper lateral petal shape, leaf apex, flower colour, bract shape, and lateral sepal size. Dimension 2 with dorsal petal shape, lower lateral petal shape, inflorescence, and to a lesser extent leaf margin and petiole length. The multiple factor analysis for mixed data demonstrates that *Impatiens smetsiana* (Fig. 1A–B) and its morphological doppelgänger *I. erecticornis* (Fig. 1D) cluster well together (Fig. 2). This clustering is mainly the result of overall similarities in floral morphology between both species and to a lesser extent because of similarities in vegetative morphology. In addition to the morphological resemblance of *I. smetsiana* and *I. erecticornis*, also *I. kamerunensis* subsp. *obanensis* and *I. kamerunensis* subsp. *kamerunensis* aggregate well in the cluster analysis (Fig. 2).

Phylogenetic analyses

No significant incongruence between ITS and *ImpDEF1/ImpDEF2* ($p > 0.05$) was found using the partition homogeneity test. Visual examination of the two different partitions of the combined dataset corroborates this congruency analysis. ML and BI analysis of the combined dataset generated topologies with moderate to high support values (Fig. 3).

Impatiens smetsiana is part of a small clade consisting only of Equatorial West African species (Fig. 3). Phylogenetic analyses indicate that *I. smetsiana* is sister to a clade containing *I. oumina*, *I. nzabiana*, *I. kamerunensis* subsp. *kamerunensis*, and *I. kamerunensis* subsp. *obanensis* (ML: 65, BI: 0.91). *Impatiens filicornu* Hook.f. is sister to this entire clade (ML: 94, BI: 1.00). The clade formed by the novelty, and the above-mentioned *Impatiens* species [(((*I. nzabiana*, *I. oumina*) (*I. kamerunensis* subsp. *kamerunensis*, *I. kamerunensis* subsp. *obanensis*)) *I. smetsiana*) *I. filicornu*] is sister to the extant species of the Equatorial West African *Impatiens* clade, except *I. sakeriana*, which is the most early divergent species of this Equatorial West African lineage (Janssens et al. 2009a).

Table 1. Comparison of taxonomic useful characters of the species closely related to *I. smetsiana* and the morphologically similar *I. erecticornis*. Size indicates both measurements for length and width, respectively.

	<i>I. erecticornis</i>	<i>I. filicornu</i>	<i>I. smetsiana</i>	<i>I. kamerunensis</i> subsp. <i>kamerunensis</i>	<i>I. kamerunensis</i> subsp. <i>obanensis</i>	<i>I. nzabiana</i>
Height (cm)	40–150	35	40–60	40	50–60 (100)	40
Leaf arrangement	spirally	spirally	spirally	opposite-subopposite	spirally	spirally
Petiole length (cm)	1.2–7.5	2.5–6	2–6.3	0.5–4	0.5–4	1.2–2.5
Leaf size (cm)	5.4–14.3 × 2.4–7.0	3.2–8.5 × 1–5	7.5–11 × 3.7–4.5	2.8–10 × 1.6–3.8	4.3–14 × 2.2–7.0	3–5 × 0.9–1.3
Leaf shape	ovate	ovate-narrowly ovate (elliptic)	elliptic	ovate, oblong-narrowly ovate	ovate, oblong-narrowly ovate	narrowly ovate-rhombic to narrowly elliptic
Leaf apex	acuminate	acute to shortly acuminate	acuminate	acute to acuminate	acute to acuminate	acuminate
Leaf base	cuneate- attenuate	attenuate-rounded	attenuate	rounded-shortly attenuate	rounded-shortly attenuate	cuneate
Lateral veins	7–10 pairs	4–5(–6) pairs	5–7 pairs	4–9 pairs	4–9 pairs	6–8 pairs
Leaf margin	crenate	shallowly crenate	crenate	shallowly crenate to crenate-serrate	shallowly crenate to crenate-serrate	finely crenate-serrate
Fimbriae	present	present	absent	often present	often present	present
Inflorescence	3–6 flowered subumbellate raceme	4–9 flowered subumbellate raceme	6–many flowered subumbellate raceme	lax 6–many flowered axillary or pseudo-terminal raceme	lax 6–many flowered axillary or pseudo-terminal raceme	lax axillary racemes
Flower colour	pink	pink-purplish	pink	pink-purplish	pink-purplish	pink
Peduncle length (cm)	3–9.5	8–14	5.5–14	5.5–16	5.8–26	11–12
Bract size (mm)	5–8 × 4–6	3–6 × 3–4	5–6 × 4–5	2–4.5	2–4.5	1
Bract shape	ovate	ovate	ovate	ovate lanceolate to linear lanceolate	ovate lanceolate to linear lanceolate	linear lanceolate
Lateral sepal length (mm)	6–7	3–4	3.8–4.5	2.5–4	2.5–4	1.2
Lateral sepal shape	ovate	ovate	ovate	ovate lanceolate to linear lanceolate	ovate lanceolate to linear lanceolate	narrowly and obliquely navicular
Lower sepal length (mm)	9–11	5	8–9	3–6	4–9	7–8
Spur length (mm)	23–28	10–35	23–35	13–30	35–55	22.5–25
Spur shape	curved filiform	curved filiform	curved filiform	curved filiform	curved filiform	curved and flattened filiform
Dorsal petal size (mm)	6–8 × 4–6	4.4 × 4.0	10.5 × 9.5	3–13 × 3–10	3–13 × 3–10	5–6 × 7–8

Table 1. (continued) Comparison of taxonomic useful characters of the species closely related to *I. smetsiana* and the morphologically similar *I. erecticornis*. Size indicates both measurements for length and width, respectively.

	<i>I. erecticornis</i>	<i>I. filicornu</i>	<i>I. smetsiana</i>	<i>I. kamerunensis</i> subsp. <i>kamerunensis</i>	<i>I. kamerunensis</i> subsp. <i>obanensis</i>	<i>I. nzabiana</i>
Dorsal petal shape	cucullate	oblong	oblong-shallowly cucullate	oblong-suborbicular	oblong-suborbicular	suborbicular
Lateral united petals length (mm)	24–35	10–16	19–21	12–15	16–20	16.5–18.0
Upper lateral petal size (mm)	13–19 × 7–11	8 × 2–3	12–13 × 8–9	7–13 × 3–10	7–13 × 3–10	12.0–13.5 × 5
Upper lateral petal shape	oval-kidney shaped	narrowly oblong-elliptic	oval-kidney shaped	transversely and broadly oblong to ± kidney shaped	transversely and broadly oblong to ± kidney shaped	narrowly oblong
Lower lateral petal size (mm)	15–27 × 8–13	8–10 × 6–8	15.5–16.5 × 12.5–13	3–10 × 5.5–13	3–10 × 5.5–13	14.5–15.0 × 8.0–8.5
Lower lateral petal shape	asymmetrically obovate	suborbicular-oblong	asymmetrically obovate	suborbicular to obliquely oblong	suborbicular to obliquely oblong	suborbicular
Size ligulate appendage of lower lateral petal (mm)	not applicable	3–5 × 1–2	4–4.5 × 2.5–4.1	3–7 × 1–2.5	3–7 × 1–2.5	7.0–7.5 × 2.5–3.0
Fruit length (mm)	9–15	13–5 mm	unknown	9–17	9–17	6.5

Divergence time estimation

Within twenty million generations, stationarity among the different chains as well as ESS values over 200 were obtained. The BEAST maximum clade credibility tree analysis is depicted in Fig. 3. Divergence time for Equatorial West African lineage corroborates the study of Janssens et al. (2009a) and is estimated at 3.67 Ma (4.99–2.74 Ma (95% highest priority density (HPD))) (Fig. 3). Additionally, time divergence estimates of the Afro-Madagascan clade to which *I. erecticornis* belongs are in accordance with earlier dating analyses of Janssens et al. (2009a), having a mean age of 4.43 Ma (5.39–3.46 Ma (95% HPD)). The split between *I. erecticornis* and its sister species (*I. bequaertii*) is situated in the Pleistocene (2.32 Ma (3.25–1.24 Ma (95% HPD))), suggesting a Pleistocene origin. The stem node age of *I. smetsiana* is situated in the Pliocene at 2.61 Ma (3.64–1.73 Ma (95% HPD)).

DISCUSSION

Diagnostic characters and relationships

Examination of the general morphological characters of *I. smetsiana* shows that the novelty is morphologically very similar to *I. erecticornis* in general appearance: spirally arranged leaves with acuminate leaf apex and attenuate leaf base, subumbellate racemes, ovate bracts and lateral sepals, a curved filiform spur, oval-kidney shaped upper lateral petals and asymmetrically obovate lower lateral petals and equally sized lateral petals. *Impatiens smetsiana* differs from *I. erecticornis* in leaf shape (elliptic vs ovate), the absence of fimbriae at the base of the leaf margins, the number of secondary veins (5–7 vs 7–10), the number of flowers per inflorescence (> 6 vs 3–6), and the dorsal petal lacking a distinct crest. Despite the morphological resemblance, molecular analyses indicate that *I. erecticornis* is part of a distinct clade of Equatorial East African species that are only distantly related to the Equatorial West African lineage to which *I. smetsiana* belongs. The most recent common ancestor of both species is located in Southwest China around 7.87 Ma (Janssens et al. 2009a). To date, *I. erecticornis* is only found in eastern DR Congo and in western Rwanda (Albertine Rift) (Fischer 1997).

Furthermore, molecular phylogenetic analyses demonstrated the close affiliation of *I. smetsiana* with the morphologically more distinct species *I. filicornu*, *I. kamerunensis* subsp. *kamerunensis*, *I. kamerunensis* subsp. *obanensis*, *I. nzabiana*, and *I. oumina*. Although there are still some common morphological characteristics between the novelty and the first four species mentioned here, there is only limited morphological resemblance with *I. oumina* and *I. nzabiana* except for a simple or sparsely branched stem and spirally arranged leaves (Janssens et al. 2011). When comparing *I. smetsiana* with the two *I. kamerunensis* subspecies, it is clear that *I. kamerunensis*

subsp. *obanensis* is morphologically more similar to the new species than *I. kamerunensis* subsp. *kamerunensis*. Only the morphological character spur length of the new species falls in between that of the two subspecies of *I. kamerunensis*. In comparison to *I. kamerunensis* subsp. *kamerunensis*, the new species is significantly larger in overall size (on average between 50 and 60 cm in height), has spirally arranged leaves instead of an opposite-subopposite leaf arrangement, a larger lower sepal (up to 9 mm long) and larger lateral petals (19–21 mm), four characters it shares with *I. kamerunensis* subsp. *obanensis*.

In addition, *I. smetsiana* differs from *I. kamerunensis* s.l. in having a subumbellate raceme instead of a lax axillary or pseudo-terminal raceme, and a distinct shape of the united lateral petals. Although molecular analyses strongly support a sister group relationship of *I. smetsiana* with *I. nzabiana*, *I. oumina*, and *I. kamerunensis* s.l., the novelty has also several morphological characters in common with the more early branched off *I. filicornu*. *Impatiens filicornu* is similar to *I. smetsiana* by the presence of a simple or sparsely branched stem with spirally arranged leaves, subumbellate racemes, ovate bracts and lateral sepals,



Figure 1. A. Frontal view of *I. smetsiana* flower. B. Lateral view of *I. smetsiana* flower. C. Frontal view of *I. kamerunensis* subsp. *kamerunensis* flower. D. Frontal view of *I. erecticornis* flower. A–B: Dessein & Taedoumg 2971 (BR [BR0000034048368]); C: Dessein et al. 2673 (BR [BR0000005605668]); D: Fischer s.n. (BONN). Photos by: A–C: Steven Dessein, D: Eberhard Fischer.

and pink flowers with a whitish filiform spur. However, it can be easily distinguished from the new species by the presence of fimbriae at the leaf base, the smaller size of both plants and flowers, and the narrowly oblong-elliptic upper lateral petals and suborbicular-oblong lower lateral petals.

Convergent flower morphology in *Impatiens*: *I. smetsiana* vs *I. erecticornis*

Unravelling higher level taxonomic relationships in *Impatiens* based on morphological data has proven difficult due to the huge floral variation present in the genus (Hooker and Thomson 1859; Hooker 1875, 1905; Warburg and Reiche 1895). Recent molecular phylogenetic analyses identified large incongruence between molecular data and morphological characters in which similar flower types are randomly distributed throughout the phylogeny (Yuan et al. 2004; Janssens et al. 2006; Yu et al. 2015). The study of Janssens et al. (2006) demonstrated that the main *Impatiens* lineages are biogeographically delimited to one of the major hotspots for the genus. Interestingly, in each of these biogeographically delimited clades in Africa, Madagascar, South India, and Southeast Asia, there is a trend of convergent flower evolution with the independent development of the same major flower types: (1) flat flower type with a shallow lower sepal and long filiform spur, (2) funnel flower type with cucullate dorsal petal, deeply navicular lower sepal and short filiform spur, (3) funnel flower type with cucullate dorsal petal, large saccate lower sepal and bucciniform spur. In general, homoplasy of similar floral characters amongst biogeographically delimited lineages within *Impatiens* is fairly obvious to recognize, as the development of each of these flower types in each delineated clade is rather distinctive. The present finding is remarkable as it implies a rather pronounced case of convergent evolution in which two distantly related taxa (their MRCA was

probably a funnel shaped flower with a saccate spurred sepal; Janssens et al. 2008) independently converged into practically the same flower morphology. It is generally accepted that the convergent evolution of complex phenotypic traits such as the complete turnover of the flower morphology is the result of stochastic processes (Wood et al. 2005). Moreover, the majority of cases in which morphological homoplasy is clearly present appears to be the result of natural selection. As for *I. smetsiana* and *I. erecticornis*, the convergent evolution of their flowers is undoubtedly closely correlated with an adaptation to a similar pollination syndrome. The typical floral morphology of the two convergent *Impatiens* species investigated is exclusively psychophilous (butterfly-pollinated). The shape of the flower causes the pollinator to position itself on the often large lower lateral petals, directly given access to the long nectar spur through a narrow opening between the two pairs of lateral petals underneath the stamens. With its long proboscis, the butterfly can easily access the nectar, which is situated near the tip of the spur. Morphological homoplasy is often suggested to be the outcome of unique genetic changes. In such an extreme case of convergent evolution in which complex floral morphological traits evolved several times, one could assume that only a few major quantitative trait loci (QTLs) are involved. This would cause the number of pathways from phenotype 'A' to phenotype 'B' to be significantly limited and thus increases the possibility of complex morphological homoplasy to occur under a common genetic basis. However, because the dynamics on how genes regulate the eventual flower morphology in *Impatiens* is still unclear, it is difficult to say whether convergent evolution of complex morphological traits, as is the case for *I. smetsiana* and *I. erecticornis*, have a common genetic background.

Biogeographical understanding of the origin of *I. smetsiana*

Molecular data demonstrates that *I. smetsiana* is only very distantly related to its morphological doppelgänger *I. erecticornis* endemic to the Albertine Rift, but is in fact closely related to the *Impatiens* species of the Equatorial West African clade containing *I. kamerunensis* subsp. *kamerunensis*, *I. kamerunensis* subsp. *obanensis*, *I. nzabiana*, and *I. oumina*. Because *I. nzabiana* and *I. oumina* are strict endemics of the Massif du Chaillu (South Gabon) and their divergence time is estimated between 0.61 and 0.017 Ma, both species had most likely no influence on the divergence of *I. smetsiana* which happened approximately two million years earlier. As a result, we further refer to the Equatorial West African species *I. kamerunensis* subsp. *kamerunensis* and *I. kamerunensis* subsp. *obanensis* when discussing the biogeography and evolution of *I. smetsiana*. In contrast to *I. smetsiana*, which is putatively endemic to the Tchabal Mbabo Mountains in Central North Cameroon (Adamawa Region), *I. kamerunensis* subsp. *kamerunensis*

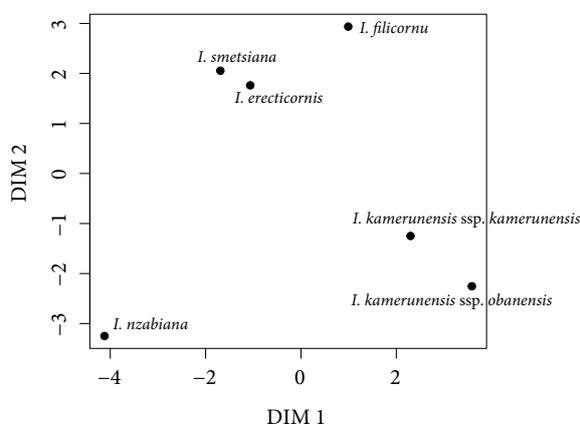


Figure 2. Multivariate plot based on variation in morphological characters (continuous and discrete) of Table 1 obtained by a Multiple Factor Analysis for mixed data.

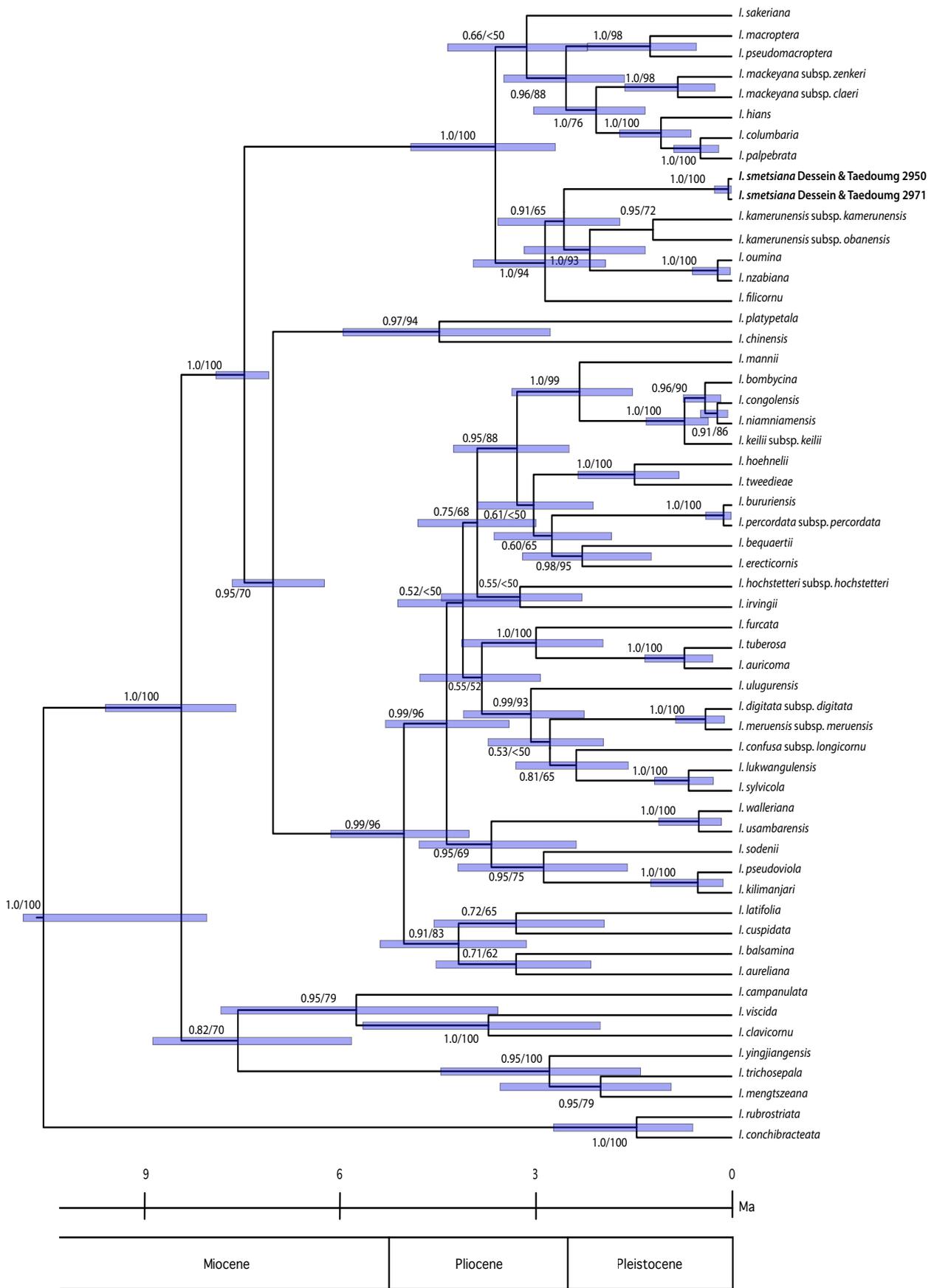


Figure 3. Maximum clade credibility inferred from combined ITS, *ImpDEF1*, and *ImpDEF2* as obtained from the BEAST software program. Numbers on branches represent Bayesian Posterior Probabilities and Maximum Likelihood Bootstrap Support, respectively. Blue bars indicate age intervals (95% HPD credibility).

and *I. kamerunensis* subsp. *obanensis* are more widely distributed and occur in Southwest Cameroon (Northwest, Southwest, West, and Littoral Region), southern Nigeria, Ghana, and Togo. Interestingly, the known distribution area of *I. smetsiana* does not overlap with that of *I. kamerunensis* subsp. *kamerunensis* and *I. kamerunensis* subsp. *obanensis*. The montane forests of Tchabal Mbabo to which *I. smetsiana* is endemic, are part of the Afromontane regional centre of endemism. According to Thomas and Thomas (1996), the Tchabal Mbabo forests are of a rare, dry type, which is completely

surrounded by deciduous woodland or shrubland. *Impatiens smetsiana* was found in a small creek on the north escarpment edge of these montane forests, close to the gallery forests that dissect the plateau grasslands, with a population of *I. mackeyana* subsp. *zenkeri* thriving downstream of the novelty. Most likely, the steep slopes of the Tchabal Mbabo montane escarpment forest created an orographic effect resulting in a suitable microclimate for *Impatiens* populations to grow in that area (Chapman 2004).

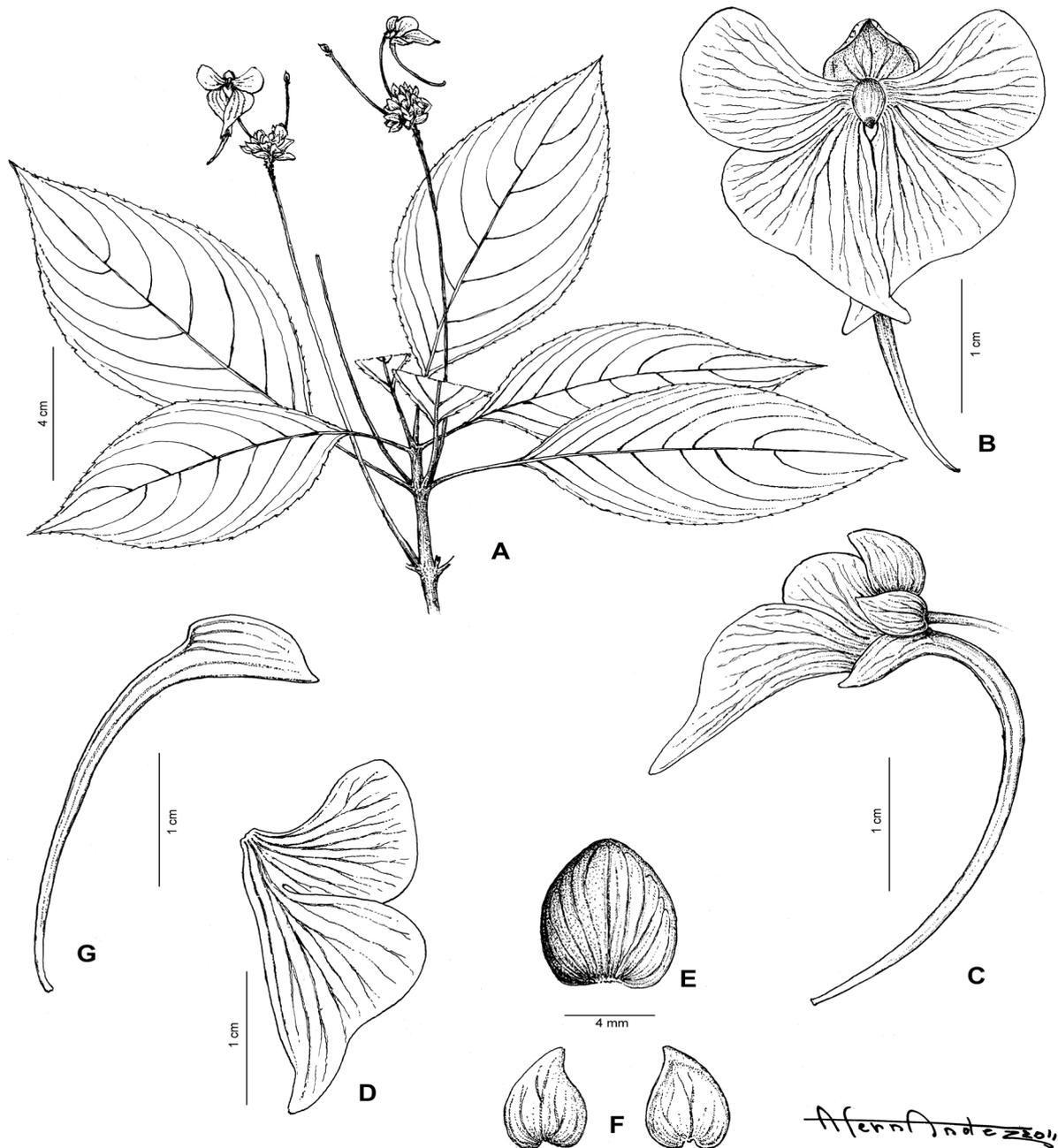


Figure 4. *Impatiens smetsiana*. A. Habit. B. Frontal view of flower. C. Lateral view of flower. D. United lateral petals. E. Dorsal petal. F. Lateral sepals. G. Lower sepal and spur. Drawn by Antonio Fernandez from *Dessein & Taedoumg* 2971 (BR [BR0000034048368]).

TAXONOMIC TREATMENT

Impatiens smetsiana S.B.Janssens, Taedoumg & Dessein, sp. nov.

urn:lsid:ipni.org:names:77300987-1

Figs 1, 4

Type. CAMEROON – Adamawa Region • Chabal Mbabo; 7°13'54"N, 12°5'57"E; 8 May 2009; Dessein S. & Taedoumg H. 2950; holotype: BR [BR0000024941891]; isotype: L [L.4446681], YA, WAG.

Diagnosis. *Impatiens smetsiana* belongs to the Equatorial West African *Impatiens* lineage. It differs from *I. kamerunensis*, *I. filicornu*, and *I. nzabiana* in having an elliptic leaf shape, no leaf fimbriae, an oblong-shallowly cucullate dorsal petal shape, and an asymmetric obovate lower lateral petal shape.

Description. Erect perennial herb up to 60 cm tall. **Stems** simple or branched, glabrous. **Leaves** spirally arranged; petiole 2–6.3 cm long; leaf blades 7.5–11 × 3.7–5.5 cm, ± elliptic, attenuate at the base, ± acuminate at the apex, glabrous; lateral veins 5–7 at each side of the midrib; leaf margins crenate without short filiform fimbriae at the base. **Flowers** in 6 to many flowered subumbellate racemes; pedicels 15–21 mm long, slender, glabrous; bracts green with red tips; ovate, 5–6 × 4–5 mm. **Lateral sepals** 2, green,

3.8–4.5 mm, ovate, glabrous. **Lower sepal** pink, 8–9 × 7–7.5 mm, narrowly and obliquely navicular, abruptly constricted into a 23–35 mm long curved filiform white spur. **Dorsal petal** pink, 10.5 × 9.5 mm, shallowly cucullate, with a shallow narrow dorsal crest. **Lateral united petals** bright pink with a small white spot at the base of the upper lateral petal of each lateral united pair and a deep pink spot at the base of the lower lateral petal of each pair, 19–21 mm long, with the upper petal of each pair equal in appearance compared to the lower one. **Upper petal** 12–13 × 8–9 mm, oval-kidney shaped; lower petal of each pair 15.5–16.5 × 12.5–13 mm, asymmetrically obovate, distally produced into 4.0–4.5 × 2.5–4.1 mm ligulate appendage. **Stamens** 5, alternating with the petals, connate to a ring. **Ovary** 5-locular, glabrous. **Fruits** fusiform. **Seeds** unknown.

Distribution. *Impatiens smetsiana* is endemic to Cameroon. Only known from Tchabal Mbabo (type location; Fig. 5).

Habitat and ecology. *Impatiens smetsiana* grows between 1900 and 2100 m, in gallery forest. Following species were collected together with the new *Impatiens*: *Campylospermum* sp., *Carapa* sp., *Cassipourea* sp., *Chassalia* sp., *Clematis* sp., *CreMASpora triflora*, *Echinops* sp., *Gladiolus* sp., *Hypoxis* sp., *Moraea schimperi*, *Multidentia dichrophylla*, *Pavetta* sp., *Pentanisia* sp., *Peperomia* sp., *Psychotria moseskemei*, *Psychotria peduncularis* var. *peduncularis*, *Psychotria psychotrioides*,

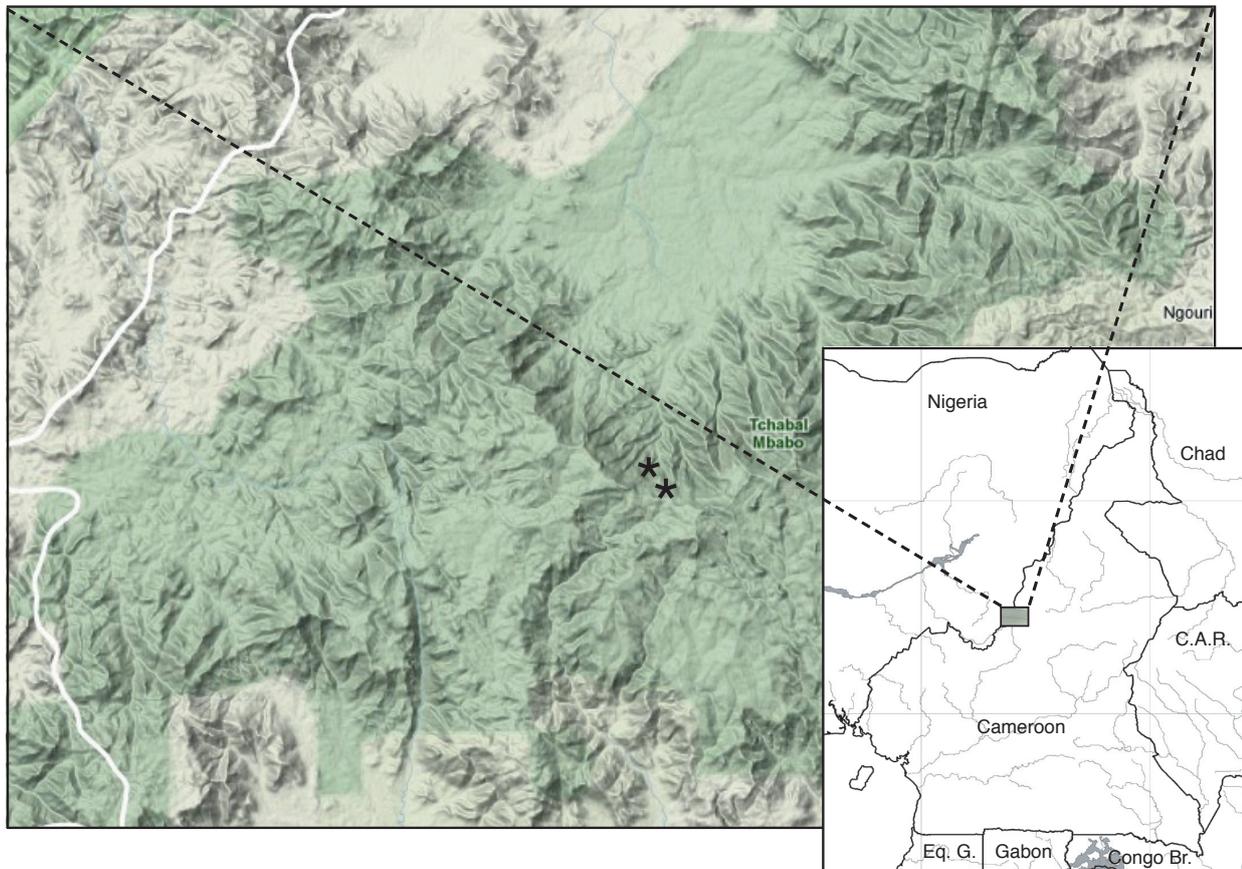


Figure 5. Distribution of *I. smetsiana* (black asterisks) in the Tchabal Mbabo region. Eq.G.: Equatorial Guinea; C.A.R.: Central African Republic; Congo Br.: Congo Brazzaville.

Psychotria succulenta, *Psydrax kraussioides*, *Rothmannia* sp., and *Spermacoce verticillata*.

Phenology. *Impatiens smetsiana* flowers in May (based on two records), fruiting occurs immediately after flowering.

Etymology. The species epithet *smetsiana* refers to the Belgian botanist, Erik F. Smets.

Preliminary IUCN conservation assessment. Vulnerable: VU D2. The species has been collected twice in a non-protected area in the Chabal Mbabo region. Human pressure in the region (mainly cattle breeding) is significant and results in the degradation and destruction of forested areas (Chapman 2004). Because of the restricted and fragmented distribution range of *I. smetsiana*, we would apply the category vulnerable under criterion D2. Human activities can make the species become critically endangered or even extinct within a rather short timeframe (IUCN 2012, 2019).

Additional material examined. CAMEROON – Adamawa Region • Chabal Mbabo; 7°14'14"N, 12°5'24"E; 9 May 2009; Dessein S. & Taedoumg H. 2971; BR [BR0000034048368].

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REFERENCES

- Abrahamczyk S, Jandová M, Líblová Z, Janssens SB, Dostálek T, Holstein N, Fischer E (2021) Pre- and postzygotic mechanisms preventing hybridization in co-occurring species of the *Impatiens purpureoviolacea* complex. *Ecology and Evolution* 11: 17485–17495. <https://doi.org/10.1002/ece3.8382>
- Barker FK, Lutzoni FM (2002) The utility of the incongruence length difference test. *Systematic Biology* 51: 625–637. <https://doi.org/10.1080/10635150290102302>
- Chapman HM (2004) Botanical survey of Tchabal Mbabo, Adamaoua Province, Cameroon. For: Transboundary Collaboration for Ecosystem Conservation: the Mountain Forests of Gashaka-Gumti National Park, Nigeria and Tchabal Mbabo, Cameroon; project number RAF/G43/A/1G/31. Bird Life International, 1–33.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214. <https://doi.org/10.1186/1471-2148-7-214>
- Farris JS, Källersjö M, Kluge AG, Bult C (1995) Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572. <https://doi.org/10.2307/2413663>
- Felsenstein J (1988) Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics* 22: 521–565. <https://doi.org/10.1146/annurev.ge.22.120188.002513>
- Fischer E (1997) Contributions to the Flora of Central Africa V: two new species of *Impatiens* (Balsaminaceae) from Eastern Zaire. *Bulletin du Jardin botanique national de Belgique / Bulletin van de National Plantentuin van België* 66: 63–71. <https://doi.org/10.2307/3668136>
- Grey-Wilson C (1980) *Impatiens* of Africa. Balkema, Rotterdam, 1–248.
- Hooker JD (1875) *Flora of British India*. L. Reeve, Kent, 1–740.
- Hooker JD (1905) An epitome of the British Indian species of *Impatiens*. *Records of the Botanical Survey of India* 4: 1–58.
- Hooker JD, Thomson T (1859) Praecursores ad Floram Indicam.-Balsamineae. *Journal of the Proceedings of the Linnean Society of London. Botany* 4: 106–157. <https://doi.org/10.1111/j.1095-8339.1859.tb01160.x>
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
- Huxley L (1918) *Life and Letters of Sir J.D. Hooker*. Volume II. John Murray, London, 1–569.
- IUCN (2012) IUCN Red List Categories and Criteria. Version 3.1. Second Edition. <https://www.iucnredlist.org/resources/categories-and-criteria> [accessed 02.01.2022]
- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. <https://www.iucnredlist.org/resources/redlistguidelines> [accessed 02.01.2022]
- Janssens SB, Fischer E, Stévant T (2010) New insights into the origin of two new epiphytic *Impatiens* species (Balsaminaceae) from West Central Africa based on molecular phylogenetic analyses. *Taxon* 59: 1508–1518. <https://doi.org/10.1002/tax.595015>
- Janssens SB, Dessein S, Smets E (2011) Portrayal of *Impatiens nzabiana* (Balsaminaceae): a morphological, molecular and biogeographic study of a new Gabonese species. *Systematic Botany* 36: 440–448. <https://doi.org/10.1600/036364411X569624>
- Janssens SB, Knox EB, Dessein S, Smets EF (2009a) *Impatiens msisimwanensis* (Balsaminaceae): description, pollen morphology and phylogenetic position of a new East African species. *South African Journal of Botany* 75: 104–109. <https://doi.org/10.1016/j.sajb.2008.08.003>
- Janssens SB, Viaene T, Huysmans S, Smets EF, Geuten KP (2008) Selection on length mutations after frameshift can explain the origin and retention of the AP3/DEF-like paralogs

- in *Impatiens*. *Journal of Molecular Evolution* 66: 424–435. <https://doi.org/10.1007/s00239-008-9085-5>
- Janssens SB, Knox EB, Huysmans S, Smets EF, Merckx VSFT (2009b) Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: result of a global climate change. *Molecular Phylogenetics and Evolution* 52: 806–824. <https://doi.org/10.1016/j.ympev.2009.04.013>
- Janssens S, Geuten K, Yuan Y-M, Song Y, Küpfer P, Smets E (2006) Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast *atpB-rbcL* spacer sequences. *Systematic Botany* 31: 171–180. <https://doi.org/10.1600/036364406775971796>
- Janssens S, Geuten K, Viaene T, Yuan Y-M, Song Y, Smets E (2007) Phylogenetic utility of the *AP3/DEF* K-domain and its molecular evolution in *Impatiens* (Balsaminaceae). *Molecular Phylogenetics and Evolution* 43: 225–239. <https://doi.org/10.1016/j.ympev.2006.11.016>
- Johnson LA, Soltis DE (1998) Assessing congruence: empirical examples from molecular data. In: Soltis PS, Soltis DE, Doyle JJ (Eds) *Molecular Systematics of Plants II: DNA Sequencing*: 297–348. Kluwer, Boston. https://doi.org/10.1007/978-1-4615-5419-6_11
- Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Lê S, Josse J, Husson F (2008) FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* 25(1): 1–18. <https://doi.org/10.18637/jss.v025.i01>
- Okuyama Y, Pellmyr O, Kato M (2008) Parallel floral adaptations to pollination by fungus gnats within the genus *Mitella* (Saxifragaceae). *Molecular Phylogenetics and Evolution* 46: 560–575. <https://doi.org/10.1016/j.ympev.2007.09.020>
- Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Rambaut A, Drummond AJ (2007) Tracer v.1.4.4. <http://beast.bio.ed.ac.uk/Tracer> [accessed 01.01.2019]
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ruchisansakun S, Mertens A, Janssens SB, Smets EF, van der Niet T (2021) Evolution of pollination syndromes and corolla symmetry in Balsaminaceae reconstructed using phylogenetic comparative analyses. *Annals of Botany* 127: 267–280. <https://doi.org/10.1093/aob/mcaa184>
- Ruchisansakun S, van der Niet T, Janssens SB, Triboun P, Techaprasan J, Jenjittikul T, Suksathan P (2016) Phylogenetic analyses of molecular data and reconstruction of morphological character evolution in Asian *Impatiens* section *Semeiocardium* (Balsaminaceae). *Systematic Botany* 40: 1063–1074. <https://doi.org/10.1600/036364415X690102>
- SACDBT (Systematics Association Committee for Descriptive Biological Terminology) (1962) II. Terminology of simple symmetrical plane shapes (Chart 1). *Taxon* 11: 145–148. <https://doi.org/10.2307/1216718>
- Sanderson MJ (2003) r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19 (2): 301–302. <https://doi.org/10.1093/bioinformatics/19.2.301>
- Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <https://doi.org/10.1093/bioinformatics/btl446>
- Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21: 456–463. <https://doi.org/10.1093/bioinformatics/bti191>
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771. <https://doi.org/10.1080/10635150802429642>
- Suzuki Y, Glazko GV, Nei M (2002) Over credibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences* 99: 16138–16143. <https://doi.org/10.1073/pnas.212646199>
- Swofford DL (2003) PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland.
- Thomas DHL, Thomas J (1996) Tchabal Mbabo Botanical Survey. Report for WWF, 1–33.
- Vandelook F, Janssens SB, Gijbels P, Fischer E, Van den Ende W, Honnay O, Abrahamczyk S (2019) Nectar traits differ between pollination syndromes in Balsaminaceae. *Annals of Botany* 124: 269–279. <https://doi.org/10.1093/aob/mcz072>
- Volkmar U, Smets EF, Lenz H, Janssens SB (2014) Intron evolution in a phylogenetic perspective: divergent trends in the two copies of the duplicated *def* gene in *Impatiens* L. (Balsaminaceae). *Journal of Systematics and Evolution* 52: 134–148. <https://doi.org/10.1111/jse.12070>
- Warburg O, Reiche K (1895) Balsaminaceae. In: Engler A, Prantl K (Eds) *Planzenfamilien*: 583–592. Dunckel and Humblott, Berlin.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis MA, Gelford DH, Sninsky JJ, White TJ (Eds) *PCR Protocols: a Guide to Methods and Applications*: 315–322. Academic Press, New York. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
- Wood TE, Burke JM, Rieseberg LH (2005) Parallel genotypic adaptation: when evolution repeats itself. *Genetica* 123: 157–170. <https://doi.org/10.1007/s10709-003-2738-9>
- Yu S-X, Janssens SB, Zhu X-Y, Lidén M, Gao T-G, Wang W (2015) Phylogeny of *Impatiens* (Balsaminaceae): integrating molecular and morphological evidence into a new classification. *Cladistics* 32: 179–197. <https://doi.org/10.1111/cla.12119>
- Yuan Y-M, Song Y, Geuten K, Rahelivololona E, Wohlhauser S, Fischer E, Smets E, Küpfer P (2004) Phylogeny and biogeography of Balsaminaceae inferred from ITS sequences. *Taxon* 53: 391–404. <https://doi.org/10.2307/4135617>

SUPPLEMENTARY FILE

Supplementary file 1

List of taxa, their localities, voucher information, and GenBank accession numbers (*ImpDEF1*, *ImpDEF2*, ITS) for the plant material used in this study.

Link: <https://doi.org/10.5091/plecevo.89701.suppl1>