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Source: Systematic Botany, 36(2) : 440-448

Published By: The American Society of Plant Taxonomists

URL: <https://doi.org/10.1600/036364411X569624>

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Portrayal of *Impatiens nzabiana* (Balsaminaceae): a Morphological, Molecular and Biogeographic Study of a New Gabonese Species

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Communicating Editor: Kenneth M. Cameron

Abstract—Recent fieldwork in Gabon yielded material of a previously unknown *Impatiens* species that resembles *Impatiens kamerunensis* and *I. oumina*. Molecular analyses based on *ImpDEF1*, *ImpDEF2* and ITS in combination with a thorough comparative morphological study confirmed the status of the newly collected material. It is phylogenetically positioned within the West-African *Impatiens* clade. *Impatiens nzabiana* can be distinguished from *I. kamerunensis* in having a narrowly lanceolate leaf shape, a 7–7.5 mm long appendage on the lower lateral petal and the presence of 3–8 mm long fimbriae at the leaf base. Molecular data strongly support a sister group relationship with *I. oumina*, whereas both *I. nzabiana* and *I. oumina* are sister to *I. kamerunensis*. *Impatiens oumina* is similar to *I. nzabiana* by the presence of long fimbriae near the base of the leaf and simple or sparsely branched stems, but can be easily distinguished from the new species by the narrowly ovate leaf shape, the smaller size of both the plants and the flowers, the subumbellate racemes, the pinkish-white corollas and the white spur sepals. By calculating age estimates and investigating the biogeography of *I. nzabiana* and its closest relatives, we have been able to hypothesize the evolutionary history of the new species.

Keywords—diversification, Gabon, *Impatiens*, Massif du Chaillu, phylogeny, pollen morphology, taxonomy.

Traversed by the Equator, Gabon is one of the most biodiversity rich countries in tropical Africa (Pomeroy 1993). Despite its enormous biodiversity richness, Gabon is among the least explored countries in Sub-Saharan Africa. Closed canopy forests are considered to cover about 80% of the country's surface, which is the highest percentage of forestation in any African nation. Estimates of the total number of plant species in Gabon are approximately 7,500 plant species (Sosef et al. 2006), a number that increases with each field expedition (e.g. Breteler 2001; Dauby et al. 2008; de Wilde and van Valkenburg 2005; Ntore et al. 2009; Stévant et al. 2010). In addition, an estimated 22% of the Gabonese plants are considered to be endemic to the country (Brenan 1978; Breteler 1989). The majority of the botanical biodiversity of Gabon is concentrated along the country's two major mountain chains (Monts de Cristal and Massif du Chaillu) and some smaller mountain ranges that are located more closely to the Atlantic Ocean (Doudou Mountains and Mayombé). The Monts de Cristal and the Massif du Chaillu are regarded as the geographic backbone of the country, resulting in a rugged landscape that reaches from the northwest to the south-central region of Gabon. The Massif du Chaillu is the largest elevated region in the country, with Mt Songo being the highest mountain in Gabon (1,022 m; Stévant and Leal 2008). The mountain range has been postulated to be a biological refugium during periods of drought (Anthony et al. 2007; Plana 2004; Sosef 1994).

Impatiens contains over 1,000 species that are often associated with possible refuge areas. Indeed, representatives of the genus prefer upland and montane evergreen forests, often restricted to a limited geographic region or even a single mountain peak. As a result, a high degree of local endemism is common within the genus resulting in the discovery of several new *Impatiens* species worldwide as inventory work progresses. In Africa, ten new species have been described since Grey-Wilson's (1980a) revision of the African *Impatiens* (Bos 1991; Cheek and Csiba 2002; Cheek and Fischer 1999; Fischer 1997; Fischer et al. 2003; Frimodt-Möller and Grey-Wilson 1999; Hallé and Louis 1989; Janssens et al. 2009b; Pócs 2007).

Of these recently discovered species, two are endemic to the evergreen rainforests of Gabon. Fieldwork in Gabon carried out by the first two authors in 2008 yielded material of an additional new *Impatiens* species. The present paper describes and illustrates this novelty, and additionally investigates its pollen morphology and phylogenetic position within the genus.

MATERIALS AND METHODS

Terminology—Descriptive terminology follows Grey-Wilson (1980a) except for simple symmetrical plane shapes (Anonymous 1962).

Pollen Morphological Study—Pollen grains of *I. nzabiana* were prepared for scanning electron microscopy (SEM) observation following the critical point drying method described by Janssens et al. (2005). Observations were carried out with a Jeol JSM-6400 microscope (25 kV). Size measurements of the pollen were determined from SEM-micrographs using Macnification v1.5.2 (www.orbicule.com). The terminology of pollen shape in polar view follows Reitsma (1970) while terms for shape classes in equatorial view were adopted from Erdtman (1971). Further pollen terminology follows Punt et al. (2007; <http://www.bio.uu.nl/palaeo/glossary/glos-int.htm>).

Molecular Phylogenetic Analyses—We used the most recent molecular phylogeny of the African *Impatiens* (Janssens et al. 2009a) in order to ascertain the position of *I. nzabiana* among the African lineages. Subsequently, the exact phylogenetic relationship of the new species was determined by reanalyzing the clade to which *I. nzabiana* belongs. Primers and temperature profiles used for the amplification of *atpB-rbcL*, *ImpDEF1/ImpDEF2* and ITS follow Janssens et al. (2006, 2007) and White et al. (1990), respectively. DNA extraction, PCR amplification, sequencing, sequence assembly and alignment were followed as described by Janssens et al. (2008). All sequences obtained were submitted to GenBank (Appendix 1) and the data sets and the representative trees are deposited in TreeBASE (study number S10796).

Nuclear *ImpDEF1/ImpDEF2*, and nuclear ribosomal ITS datasets were analyzed separately and in combination. A partition homogeneity test (implemented in PAUP* 4.0b10a) was used to evaluate whether the data matrices provided different signal in the combined analyses. Maximum parsimony (MP) analyses involved heuristic searches conducted with PAUP* 4.0b10 (Swofford 2002), using following settings: TBR branch swapping on 10,000 random addition replicates, with five trees held at each step, MulTrees option in effect and character states specified as unordered and unweighted. Nonparametric bootstrap analysis (MP-BS) was performed to assess nodal support (Felsenstein 1985). For each of the 500 bootstrap replicates, a heuristic search was conducted with identical settings as in the original heuristic analysis.

Prior to the maximum likelihood (ML) and Bayesian inference (BI) analyses, we estimated the best-fitting substitution model for *ImpDEF1*, *ImpDEF2* and ITS under the Akaike information criterion (AIC) as implemented in Modeltest 3.06 (Posada and Crandall 1998). Bayesian analyses were conducted with MrBayes 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Four chains (one cold, three heated), initiated from a random starting tree were run simultaneously for 10 million generations. Every 500 generations, a tree was sampled from the chain for a total of 20,000 trees. Due to the burn-in, 10,000 sample points were discarded until stationarity was established among the chains.

A ML based method was carried out using the RaxML algorithm of Stamatakis et al. (2005). Maximum likelihood 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. Nonparametric bootstrapping (ML-BS) was carried out using the Rapid Bootstrap algorithm on the CIPRES computer cluster.

Dating Estimation—To calculate the divergence time estimate of *I. nzabiana* and *I. oumina*, we used a previously computed age estimate of the West African lineage (3.45 million year ago) as a fixed calibration point (Janssens et al. 2009a). Confidence intervals were estimated by reanalyzing the dataset using either the available minimum or maximum age of the node towards the West African lineage as fixed calibration points (Janssens et al. 2009a). A likelihood ratio test showed that a clock like evolution for the combined dataset had to be rejected. Consequently, we calculated divergence time estimates using the penalized likelihood method (Sanderson 2002) implemented in the r8s software package (Sanderson 2004). The optimal rate-smoothing penalty parameter was determined by the statistical cross-validation method implemented in r8s.

RESULTS

Pollen Morphology of *Impatiens nzabiana*—Pollen of *I. nzabiana* is medium sized (P: 25.1 μm ; E: 27.2 μm) with a circular polar outline (Fig. 1A) and a spheroid shape (P/E: 0.93). Number of apertures is unknown. Apertures are simple and consist only of an ectocolpus (length 5.3 μm). No margo has been observed around the colpus. Pollen grains have a reticulate ornamentation (Fig. 1B), with lumina that range in size between 1.9 μm and 4.2 μm being on average 3.1 μm (measured according to the longest axis). The density of the lumina within *I. nzabiana* is 0.13 μm^{-2} . The muri are slender with a rounded top edge. Inside the lumina, few solitary granules are observed on the foot layer (Fig. 1B). Orbicules are absent on the inner locule wall.

Phylogenetic Position of *Impatiens nzabiana*—Ambiguously aligned nucleotides were excluded from the data matrices. Some loci could not be sequenced for a few species, yet these missing data had no noticeable influence on the combined matrix. With all markers combined, we obtained a dataset of 13 species and 1,673 analyzed characters of which 304 are variable and 120 parsimony informative. No incongruent relationships were found between ITS and *ImpDEF1/ImpDEF2*. This result was supported by the partition homogeneity test, which showed no significant difference between both partitions of the combined dataset ($p > 0.05$). Maximum parsimony, ML, and BI analyses of the combined dataset generated similar topologies with generally high support values.

Impatiens nzabiana belongs to a small clade of only West Central African species (Fig. 2). Species that are part of this clade belong mainly to the *I. hians*, *I. macroptera*, or *I. filicornu* species complexes, which are informal groups proposed by Grey-Wilson (1980a, b). Present results indicate that *I. nzabiana* is sister to *I. oumina* (MP-BS: 99, ML: 99, BI: 1.0). Together, they are sister to a clade containing *I. kamerunensis* subsp. *kamerunensis* and *I. kamerunensis* subsp. *obanensis*, which share

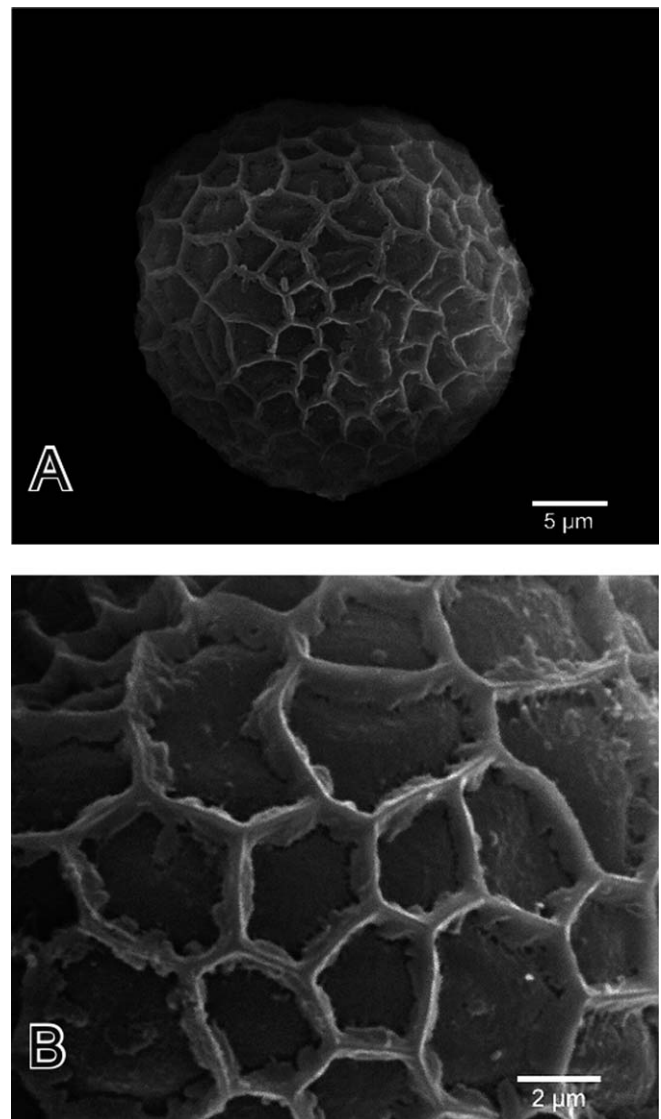


FIG. 1. SEM of *Impatiens nzabiana* pollen grains. A. Polar view of a reticulate pollen grain with subcircular outline. B. Detailed view of the reticulate sexine. The muri are slender with a rounded top edge.

some floral morphological similarities with our new species. The clade formed by the novelty, *I. oumina*, and the two subspecies of *I. kamerunensis* is sister to the extant species of the West-African *Impatiens* clade (see Janssens et al. 2009a).

Divergence Time Estimates—By applying the calculated divergence time estimates of the node towards the West African lineage as calibration point (Janssens et al. 2009a), the split between *I. nzabiana* and *I. oumina* was dated to have occurred at least 0.18 million years ago (mya). The confidence interval for this node has an upper boundary at 0.21 mya and a lower boundary at 0.15 mya (Fig. 2).

TAXONOMIC TREATMENT

Impatiens nzabiana S. B. Janssens & Dessein sp. nov.—TYPE: GABON. Ngounié Province: Mouyanama falls, *Dessein 2044* (holotype: BR; isotype: WAG, LBV).

Affinis *I. ouminae* N. Hallé sed ab illa differt primo adspectu habitu majori, inflorescentiis racemosis (contra inflorescentias

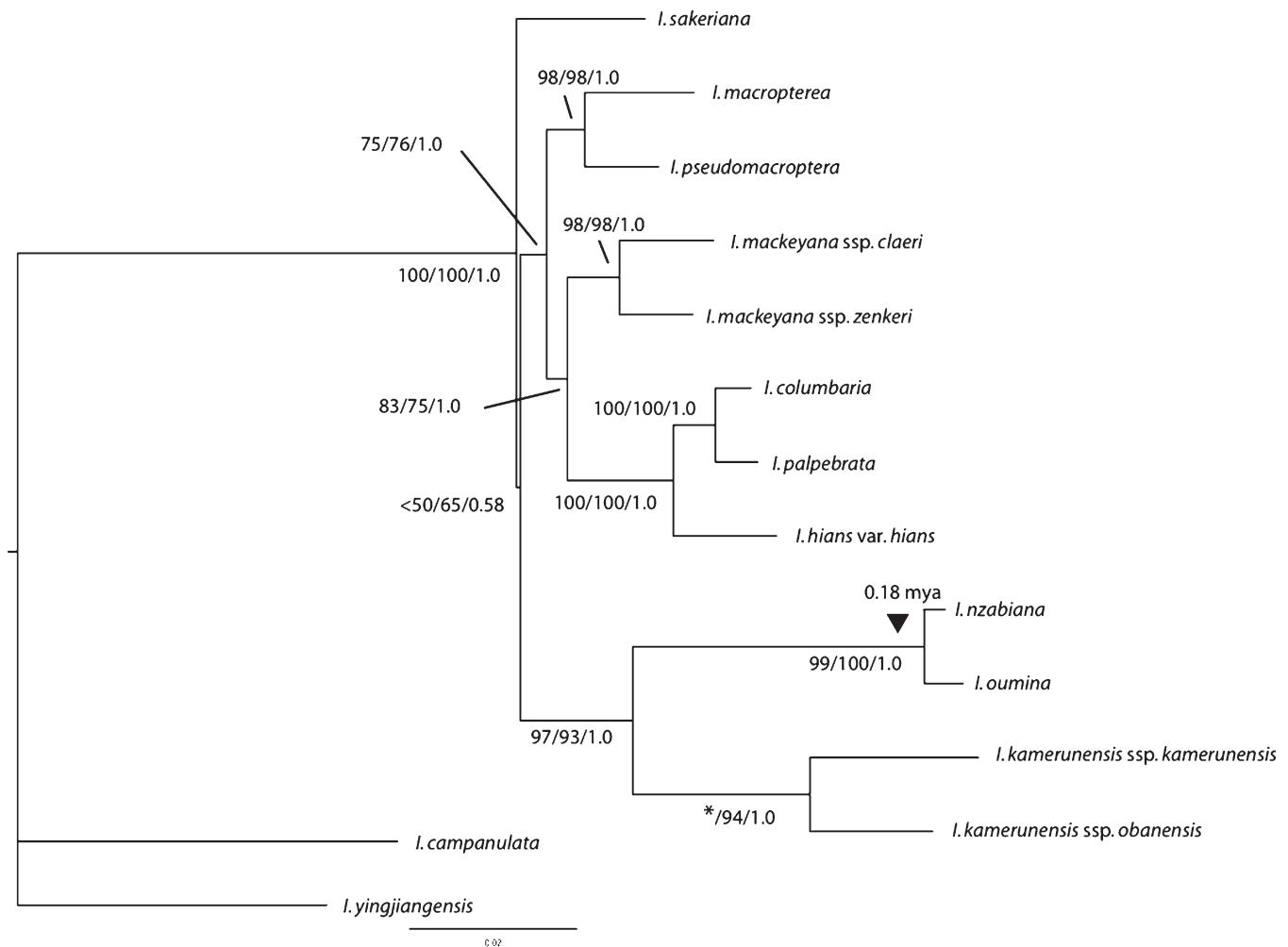


FIG. 2. Phylogram based on combined *ImpDEF1/ImpDEF2* and ITS data. Numbers on branches represent maximum parsimony bootstrap support, maximum likelihood bootstrap support and Bayesian posterior probabilities, respectively. The arrow indicates the age of the node investigated.

subumbelliformes), corollis majoribus atque floribus rubro-violaceis (contra corollas roseas et calcaria albida in *I. ouminae*).

Erect perennial herb to 40 cm tall. Stems simple or sparsely branched, glabrous. Leaves spirally arranged; petiole 1.2–2.5 cm long; leaf blades 3.0–5.0 × 0.9–1.3 cm, narrowly ovate-rhombic to narrowly elliptic, cuneate at the base, ± acuminate at the apex, sparsely pubescent above, glabrous below; lateral veins 6–8 at each side of the midrib; leaf margins finely crenate-denticulate with several short filiform fimbriae (2–3 on each side, 3–7 mm long) at the base. Flowers in rather lax axillary racemes, pink; pedicels 11.0–12.0 mm long, slender, glabrous; bracts linear-lanceolate, to 1 mm long. Lateral sepals 2, ca. 1.2 × 3.0 mm, narrowly lanceolate, glabrous. Lower sepal 7.0–8.0 × 0.5 mm, narrowly and obliquely navicular, abruptly constricted into a short 22.5–25.0 mm long curved and flattened filiform spur. Dorsal petal 5.0–6.0 × 7.0–8.0 mm, suborbicular, with a narrow dorsal crest terminating in a short acute point. Lateral united petals deep pink with a small white spot at the base of the upper lateral petal of each lateral united pair and a large white spot at the base of the lower lateral petal of each pair, 16.5–18.0 mm long, with the upper petal of each pair slightly smaller than the lower one; upper petal 12.0–13.5 × 5 mm, narrowly oblong; lower petal of each pair 14.5–15.0 × 8.0–8.5 mm, suborbicular, distally produced into 7.0–7.5 ×

2.5–3.0 mm ligulate appendage. Stamens 5, alternating with the petals, connate to a ring. Ovary 5-locular, glabrous. Fruits fusiform, 6.5 × 1.5 mm, seeds unknown. Figures 3, 4.

Phenology—*Impatiens nzabiana* flowers in February and May (based on three records).

Distribution—*Impatiens nzabiana* is endemic to Gabon. Only known from Mt. Songo in the Massif du Chaillu (type collection; Fig. 5).

Ecology—*Impatiens nzabiana* grows at ca. 675 m, in the spray of the Mouyanama Falls.

Etymology—The species epithet *nzabiana* refers to the Gabonese botanist, Thomas Nzabi, who contributed to the collection of the new species during our field expedition in Gabon.

Additional Specimens Examined—GABON. Ngounié Province: Massif du Chaillu, Mouyanama falls, 1°39'00.8" S 11°45'17.5" E, 29 May 1981, Breteler 7713 (WAG); Ngounié Province: Massif du Chaillu, Mouyanama falls 1°39'00.8" S 11°45'17.5" E, 3 Feb 1983, de Wilde et al. (WALKB) 328 (BR, WAG, LBV, MO).

IUCN Conservation Status—Although the species has been collected four times, it is only known from the type locality at the Mouyanama Falls in Ngounié Province. In 1947 Reverend Georges Klein installed a Protestant missionary station close to the falls, thereby enhancing the cultivation of crops in the surrounding area. Several years ago, however, the mission was closed and became uninhabited. Recently, it has been

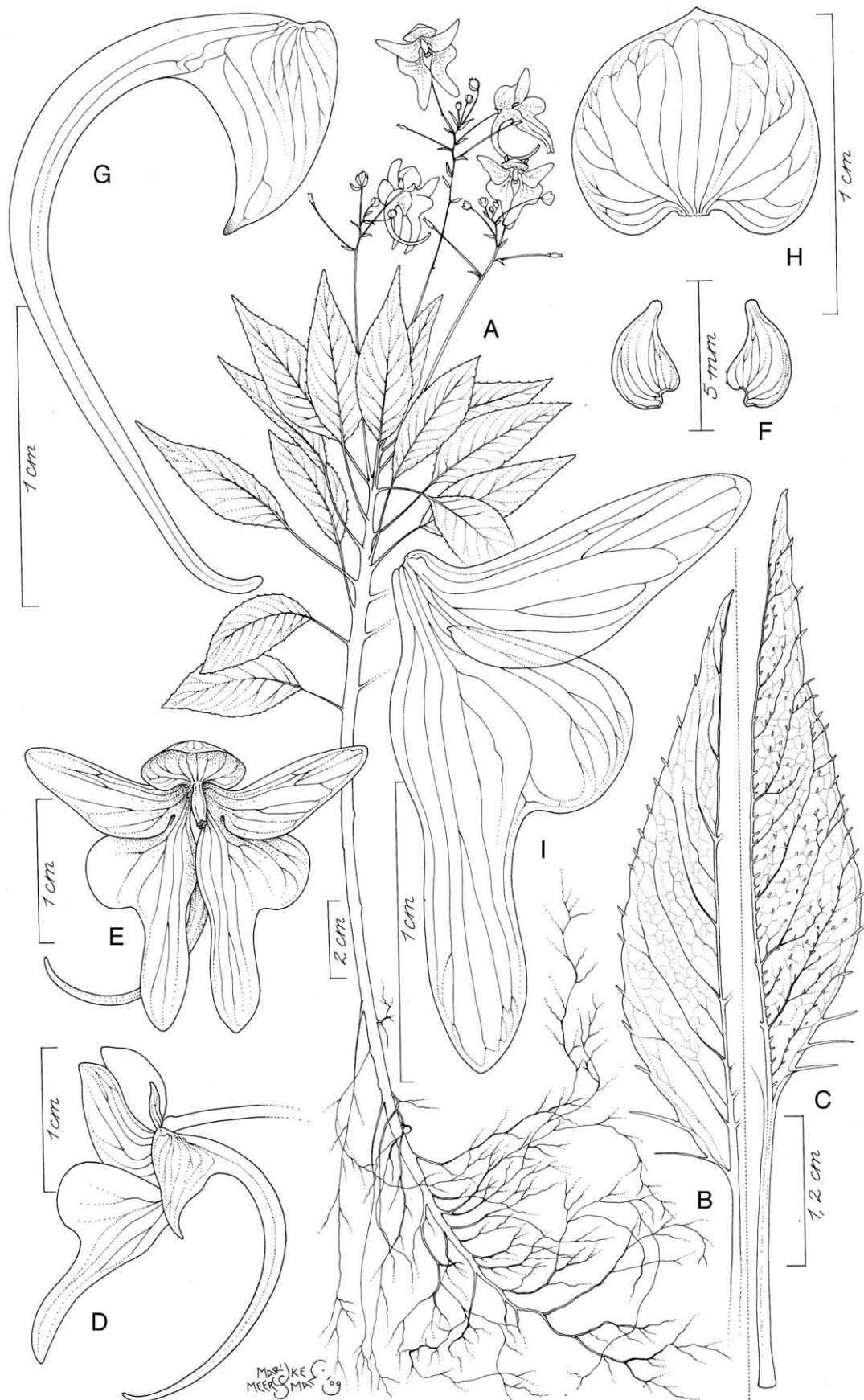


FIG. 3. *Impatiens nzabiana*. A. Habit. B. Abaxial leaf detail. C. Adaxial leaf detail. D. Lateral view of flower. E. Frontal view of flower. F. Lateral sepals. G. Lower sepal and spur. H. Dorsal petal. I. United lateral petals. Drawn by M. Meersman from Dessein et al. 2044.



FIG. 4. Morphology and habitat of *I. nzabiana* and allied species. A. Lateral view of flower of *I. nzabiana*. B. Frontal view of *I. nzabiana* flower. C. Mouyanama Falls. D. Frontal view of *I. oumina* flower. E. Frontal view of *I. kamerunensis* subsp. *kamerunensis* flower. F. Frontal view of *I. kamerunensis* subsp. *obanensis* flower. G. Lateral view of flower of *I. oumina*. H. lateral view on flower of *I. kamerunensis* subsp. *kamerunensis*. I. lateral view on flower of *I. kamerunensis* subsp. *obanensis*. (A., B. Dessein et al. 2044; D., G. Dessein et al. 2021; E., H. Dessein et al. 2549; F., I. Dessein et al. 2909).

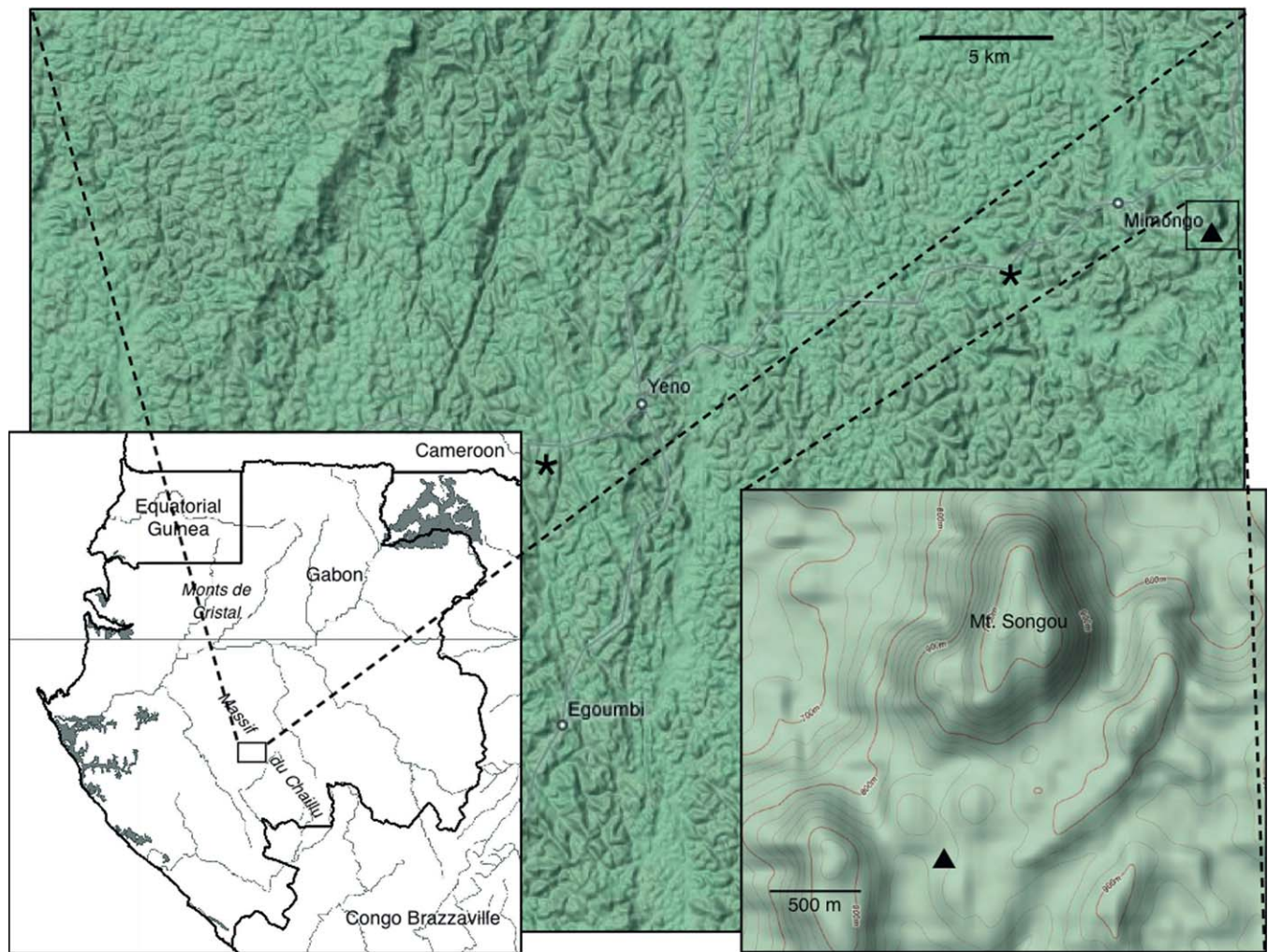


FIG. 5. Distribution map of *Impatiens oumina* (black asterisk) and *I. nzabiana* (black triangle).

decided that this location would become renovated to create a scientific research center to stimulate tourism in the area, thereby increasing possible threats to native vegetation. Due to the restricted distribution of *I. nzabiana*, we would apply the category of “vulnerable” under criterion D2. The latter is

considered a special criterion due to the restricted distribution area (only one locality) of the new species. As a result it is possible that the species could become critically endangered or even extinct within a very short period of time (IUCN 2001).

KEY TO THE CLOSELY RELATED SPECIES OF *IMPATIENS NZABIANA*

- | | |
|--|---|
| 1. Leaves opposite or subopposite | <i>I. kamerunensis</i> ssp. <i>kamerunensis</i> |
| 1. Leaves spirally arranged | 2 |
| 2. Leaf base slightly cordate to rounded | <i>I. oumina</i> |
| 2. Leaf base abruptly or gradually cuneate | 3 |
| 3. Spur 35–55 mm long | <i>I. kamerunensis</i> ssp. <i>obanensis</i> |
| 3. Spur 22.5–25 mm long | <i>I. nzabiana</i> |

DISCUSSION

Diagnostic Characters and Relationships—By examining the overall morphology of *I. nzabiana*, it is clear that the new species is similar to *I. kamerunensis* sensu Grey-Wilson in having an unbranched simple stem, a rather lax axillary raceme as its inflorescence type, a narrowly and obliquely navicular lower sepal, and a flattened filiform spur. *Impatiens nzabiana* differs from *I. kamerunensis* sensu Grey-Wilson in having a

narrowly lanceolate leaf shape, a 7–7.5 mm appendage at the lower lateral petal, the presence of 3–8 mm long fimbriae at the leaf base, and the presence of slender muri with a rounded top edge. *Impatiens kamerunensis* sensu Grey-Wilson consists of the two formerly recognized species *I. kamerunensis* s. s. and *I. obanensis* (Hutchinson and Dalziel 1954; Grey-Wilson 1980a) which he defined as subspecies *I. kamerunensis* subsp. *kamerunensis* and subsp. *obanensis*. Although Grey-Wilson is aware that typical specimens of each subspecies look different,

he states that the differences are mainly quantitative and that some specimens are difficult to place. When comparing *I. nzabiana* with either of the two *I. kamerunensis* subspecies, it can be noticed that the new species is characterized by a mixture of morphological characters found in the subspecies. Compared to *I. kamerunensis* subsp. *obanensis*, the new species is smaller in overall size (rarely exceeding 40 cm in height), and has a shorter spur (22.5–25 mm), two characters it shares with *I. kamerunensis* subsp. *kamerunensis*. However, *I. nzabiana* differs from this last taxon in having spirally arranged leaves and enlarged lateral united petals (16.5–18 mm), two characters which are similar in *I. kamerunensis* subsp. *obanensis*. *Impatiens kamerunensis* sensu Grey-Wilson is known from localities in Cameroon, Nigeria, Bioko, Ghana, and Togo but is absent in Gabon. Despite the gross morphological similarities between *I. nzabiana* and *I. kamerunensis*, the latter is not sister to *I. nzabiana*. Molecular data strongly support a sister group relationship with *I. oumina*, a species endemic to the Massif du Chaillu. Together, *I. oumina* and *I. nzabiana* are sister to *I. kamerunensis*. Comparative morphological studies often considered *I. oumina* related to *I. filicornu* or *I. palpebrata*, yet it always remained difficult to taxonomically place the species within the genus (Grey-Wilson 1980a). *Impatiens oumina* is similar to *I. nzabiana* by the presence of long fimbriae near the base of the leaf and simple or sparsely branched stems, but can be easily distinguished from the new species by the narrowly ovate leaf shape, the smaller size of both the plants and the flowers, the subumbellate racemes, the pinkish-white corollas and the white spur sepals. Although Grey-Wilson (1980a) considered *I. kamerunensis* as closely allied to *I. filicornu*, the link with *I. oumina* has never been made, possibly because the material Grey-Wilson investigated at that period was very scarce and in bad shape (*Le Testu* 2328, collected in 1916, and *Le Testu* 5225, collected in 1925).

In 1981, F. Breteler collected *I. nzabiana* for the first time at the type locality. Unfortunately the species was misidentified as *I. gongolana*. Two years later, an expedition under supervision of J. de Wilde (WALKB-series) collected the species again and identified it as *I. gongolana*. After closely examining *I. gongolana*, it is clear that this species does, indeed, show some morphological similarities with *I. nzabiana*, but it is unquestionably a different species. Both are characterized by differences in general shape of the lateral united petals, the lower sepal, and the spur.

Biogeographical and Ecological Interpretation—*Impatiens* shows a wide range of floral variation, which causes enormous difficulties in demarcating natural groupings at the subgeneric level (Janssens et al. 2006). As a result, several traditional taxonomists have designed artificial classification systems for *Impatiens* in the past (Hooker 1875; Hooker 1905; Warburg and Reiche 1895). Recent molecular studies on the genus, however, revealed a large incongruence between molecular data and morphological characters (Janssens et al. 2006, 2009a). According to these results, biogeographic history can be used to explain the phylogenetic clustering of morphologically different species. Our former studies, however, focused at an intraspecific level, and revealed biogeographic clustering only of major lineages. Here we illustrate that biogeographic clustering also occurs at lower phylogenetic levels.

Molecular phylogenetic analyses show that *I. nzabiana* is most closely related to *I. oumina*. Although both species occur in the same overall distribution area of the Massif du

Chaillu, there appears to be no distributional overlap between *I. oumina* and *I. nzabiana*. The latter species is restricted to its type locality at the Mouyanama Falls, situated roughly five km west of the village Mimongo. *Impatiens oumina*, in contrast, has a larger distribution in the Massif du Chaillu located between Mimongo, and Kembélé, two villages which are situated in the center of the massif at roughly 20 km distance from each other (the distribution area of the rare *I. oumina* species is based on the few collections of the species that were made in the past). Although it is possible that *I. oumina* is also present west of Mimongo and the Mouyanama Falls, it has to be noted that *Impatiens* gradually becomes more rare when going towards the rain shadow side of the Massif du Chaillu located at the west side of the central mountain range. At this side of the massif, only widespread *Impatiens* species are found (e.g. *I. mannii*, *I. niarniamensis* and *I. mackeyana* ssp. *clairii*), whereas rare *Impatiens* species, which are thought to be an indication for ancient forest refugia are absent.

Interestingly, *I. nzabiana* was found in the close vicinity of *I. issemei*, *I. niarniamensis* and *I. sp. nov.* “chailluensis,” whereas the recollected *I. oumina* was found in a small creek together with *I. niarniamensis* and *I. pseudomacroptera*. No hybrids between either of these species, or between *I. nzabiana* and *I. oumina* were observed in the field. Based on the present distribution data of both sister species we can state that *I. nzabiana* and *I. oumina* have adjacent distribution areas without overlap. The allo-parapatric distribution of the two species might correspond to the predicted consequences of secondary contact between previously isolated populations (Avisé et al. 1987). With the current data, however, it is difficult to unravel the evolutionary process that resulted in the diversification of *I. nzabiana* and *I. oumina*.

Remarkably the diversification of *I. oumina* and *I. nzabiana* is estimated at 0.18 million years ago. This age corresponds to the Illinois-Riss high latitude glacial event, which lasted from 130,000 until 200,000 yr ago. The sudden decrease in overall temperature and accumulation of water along the poles during this glaciation event probably resulted in an increased aridification on the African continent (DeMenocal 1995). Although the Massif du Chaillu is considered to receive a higher amount of precipitation in comparison to its neighboring lowland areas today (2,000–2,300 mm), the area was probably more susceptible to drought in the past than such places as the Monts de Cristal in the north of Gabon, for example, due to its more inland location. Hence it is likely that the increased global aridification during the ice ages interfered with the water balance of this region, resulting in habitat conditions that were less suitable for *Impatiens* to grow in some particular areas of the massif. Nevertheless, the number of rare *Impatiens* species in the massif compared to neighboring areas might indicate its role as refugium during less favorable climate conditions. The Chaillu Massif is characterized by a variable topography of plateaus, valleys and steep slopes. One of these steep sloped mountains is Mt. Songo, the mountain where we collected *I. nzabiana*. The orographic effect of this mountain on its surrounding area results in a regional hotspot from which several new species have been described in the last few years (Janssens et al. 2010; Ntoré et al. 2009; Stévant and Leal 2008). Due to the high altitude of Mt. Songo (1,022 m, highest peak of Gabon), the surrounding area was presumably cooler and mistier than more open areas of the massif. The presence of such microhabitats on Mt. Songo probably help to explain its wealth of biodiversity, and we plan to

initiate thorough population genetic studies in order to unravel the complex evolution of the African *Impatiens* species.

ACKNOWLEDGMENTS. The CENAREST and the Herbar National du Gabon (IPHAMETRA) are acknowledged for permission to conduct research in Gabon under the project of "Sud Expert Plantes," a project under French Ministry of Foreign Affairs. We also thank the curator of WAG for making their collections available. Yves Issembe, Thomas Nzabi and Olivier Lachenaud are acknowledged for their help during the field expedition to Gabon and Marijke Meersman for the botanical drawing. Research grants of the K. U. Leuven (OT/05/35), the Fund for Scientific Research-Flanders (FWO Belgium) (G.0104.01) and the Central Africa Regional Program of the Environment (CARPE) financially supported this study. SBJ holds a Postdoctoral research grant from FWO.

LITERATURE CITED

- Anonymous. 1962. Systematics association committee for descriptive biological terminology. II. Terminology of simple symmetric plane shapes (chart 1). *Taxon* 11: 145–156.
- Anthony, N. M., M. Johnson-Bawe, K. Jeffery, S. L. Clifford, K. A. Abernethy, C. E. Tutin, S. A. Lahm, L. J. T. White, J. F. Utley, E. J. Wickings, and M. W. Bruford. 2007. The role of Pleistocene refugia and rivers in shaping gorilla genetic diversity in central Africa. *Proceedings of the National Academy of Sciences USA* 104: 20432–20436.
- Avise, J. C., J. Arnold, R. Martin Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18: 489–522.
- Bos, J. J. 1991. Novitates gabonensis 4. Another new species of *Impatiens* (Balsaminaceae) from Gabon. *Adansonia* 3–4: 240–241.
- Brenan, J. P. M. 1978. Some aspects of the phytogeography of tropical Africa. *Annals of the Missouri Botanical Garden* 65: 437–478.
- Breteler, F. J. 1989. Gabon. Pp. 198–202 in *Floristic inventory of tropical countries*, eds. D. G. Campbell, and H. D. Hammond. New York: New York Botanical Garden.
- Breteler, F. J. 2001. Novitates Gabonenses: 40. A new species of *Strombosopsis* (Olacaceae) from Gabon. *Adansonia* 23: 303–306.
- Cheek, M. and L. Csiba. 2002. A new epiphytic species of *Impatiens* (Balsaminaceae) from western Cameroon. *Kew Bulletin* 57: 669–674.
- Cheek, M. and E. Fischer. 1999. A tuberous and epiphytic new species of *Impatiens* (Balsaminaceae) from southwest Cameroon. *Kew Bulletin* 57: 669–674.
- Dauby, G., I. Parmentier, and T. Stévant. 2008. A new species of *Afrothlismia* (Burmanniaceae) from Gabon. *Nordic Journal of Botany* 25: 268–271.
- DeMenocal, P. B. 1995. Plio-Pleistocene African climate. *Science* 270: 53–59.
- De Wilde, J. J. F. E. and J. L. C. H. van Valkenburg. 2005. *Begonia sosefiana* (Begoniaceae): a new species in section *Loasibegonia* from Gabon. *Blumea* 50: 467–471.
- Erdtman, G. 1971. *Pollen morphology and plant taxonomy. Angiosperms. Corrected reprint of 1952 edition*. New York: Hafner Publishing Company.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791.
- Fischer, E. 1997. Contributions to the Flora of Central Africa V: Two new species of *Impatiens* (Balsaminaceae) from eastern Zaïre. *Bulletin du Jardin Botanique National de Belgique* 66: 63–71.
- Fischer, E., J.-B. Dhetchuvi, and C. Ntaganda. 2003. A new species of *Impatiens* (Balsaminaceae) from Nyungwe Forest, Rwanda. *Systematics and Geography of Plants* 73: 91–95.
- Frimodt-Möller, C. and C. Grey-Wilson. 1999. Two new taxa of *Impatiens* (Balsaminaceae) from the Udzungwa Mountains, Tanzania. *Kew Bulletin* 54: 185–189.
- Grey-Wilson, C. 1980a. *Impatiens of Africa*. Rotterdam: Balkema.
- Grey-Wilson, C. 1980b. Hybridisation in African *Impatiens*. Studies in Balsaminaceae. *Kew Bulletin* 34: 221–227.
- Hallé, N. and A. M. Louis. 1989. Un nouvel *Impatiens* (Balsaminaceae) du Gabon. *Adansonia* 11: 11–15.
- Hooker, J. D. 1875. *Flora of British India*. Kent: L. Reeve.
- Hooker, J. D. 1905. An epitome of the British Indian species of *Impatiens*. *Records of the Botanical Survey of India* 4: 1–58.
- Huelsenbeck, J. and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- Hutchinson, J. and J. M. Dalziel. 1954. Balsaminaceae. Pp. 160–162 in *Flora of West Tropical Africa, 2nd*, eds. F. N. Hepper. London.
- IUCN. 2001. *IUCN red list categories and criteria: version 3.1*. Gland, Switzerland: IUCN.
- Janssens, S. B., E. Fischer, and T. Stévant. 2010. New insights on the origin of epiphytic *Impatiens* (Balsaminaceae) in West Equatorial Africa based on molecular phylogenetic and biogeographical analysis. *Taxon* 59: 1508–1518.
- Janssens, S. B., E. B. Knox, S. Huysmans, E. Smets, and V. S. F. T. Merckx. 2009a. Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. *Molecular Phylogenetics and Evolution* 52: 806–824.
- Janssens, S. B., E. B. Knox, S. Dessein, and E. Smets. 2009b. *Impatiens msimwanensis* (Balsaminaceae): Description, pollen morphology and phylogenetic position of a new East African species. *South African Journal of Botany* 75: 104–109.
- Janssens, S. B., T. Viaene, S. Huysmans, E. F. Smets, and K. P. Geuten. 2008. Selection on length mutations after frameshift can explain the origin and retention of the AP3/DEF-like paralogues in *Impatiens*. *Journal of Molecular Evolution* 66: 424–435.
- Janssens, S., K. Geuten, Y.-M. Yuan, Y. Song, P. K pfer, and E. Smets. 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast *atpB-rbcL* spacer sequences. *Systematic Botany* 31: 171–180.
- Janssens, S. B., K. Geuten, T. Viaene, Y.-M. Yuan, Y. Song, and E. Smets. 2007. Phylogenetic utility of the AP3/DEF K-domain and its molecular evolution in *Impatiens* (Balsaminaceae). *Molecular Phylogenetics and Evolution* 43: 225–239.
- Janssens, S., F. Lens, S. Dressler, K. Geuten, E. Smets, and S. Vinckier. 2005. Palynological variation in balsaminoid ericales. II Balsaminaceae, Tetrameristaceae, Pellicieraceae and general conclusions. *Annals of Botany* 96: 1061–1073.
- Ntore, S., O. Lachenaud, S. B. Janssens, and S. Dessein. 2009. Four new *Pauridiantha* (Rubiaceae) species reflect the richness of Gabon's rainforests. *Belgian Journal of Botany* 142: 177–193.
- Plana, V. 2004. Mechanisms and tempo of evolution in the African Guineo-Congolian rainforest. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 359: 1585–1594.
- P cs, T. 2007. A new species of *Impatiens* (Balsaminaceae) from the Nguru Mountains (Tanzania). *Acta Botanica Hungarica* 49: 377–383.
- Pomeroy, D. 1993. Centers of high biodiversity in Africa. *Conservation Biology* 7: 901–907.
- Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Punt, W., S. Blackmore, S. Nilsson, and A. Le Thomas. 2007. *Glossary of pollen and spores terminology. LPP Contributions Series nr 1*. Utrecht: Laboratory of Palaeobotany and Palynology Foundation, Review of Palaeobotany and Palynology.
- Reitsma, T. 1970. Suggestions towards a unifications of descriptive terminology of angiosperm pollen grains. *Review of Palaeobotany and Palynology* 10: 39–60.
- Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.
- Sanderson, M. J. 2004. R8s version 1.70, User's manual. Davis, California: Distributed by the author.
- Sosef, M. S. M. 1994. *Refuge begonias: Taxonomy, phylogeny and historical biogeography of Begonia sect. Loasibegonia and sect. Scutobegonia in relation to glacial rain forest refuges in Africa*. Ph. D. dissertation. Wageningen, Netherlands: Wageningen Agricultural University.
- Sosef, M. S. M., J. J. Wieringa, C. C. H. Jongkind, G. Achoundong, Y. Azizet Issembe, D. Bedigian, R. G. van den Berg, F. J. Breteler, M. Cheek, J. Degreef, R. Faden, R. E. Gereau, P. Goldblatt, L. J. G. van der Maesen, L. Ngok Banak, R. Niangadouma, T. Nzabi, B. Nziengui, Z. S. Rogers, T. St vant, C. M. Taylor, J. L. C. H. van Valkenburg, G. Walters, and J. J. F. E. de Wilde. 2006. Check-list des plantes vasculaires du Gabon / Checklist of Gabonese vascular plants. *Scripta Botanica Belgica* 35: 1–438.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Stamatakis, A., T. Ludwig, and H. Meier. 2005. Raxml-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21: 456–463.
- St vant, T. and M. E. Leal. 2008. The biodiversity of Bouvala. *Internal report for the Central African Program for the Environment*. Gabon, Libreville: Missouri Botanical Garden.
- St vant, T., V. Cawoy, T. Damen, and V. Droissart. 2010. Taxonomy of Atlantic Central African orchids 1. A new species of *Angraecum*

- sect. *Pectinaria* (Orchidaceae) from Gabon and Equatorial Guinea. *Systematic Botany* 35: 252-256.
- Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4. Sunderland: Sinauer Associates.
- Warburg, O. and K. Reiche. 1895. Balsaminaceae. Pp. 583–592 in *Planzenfamilien*, eds. A. Engler and K. Prantl. Berlin: Dunckel and Humblott.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. Pp. 315–322 in *PCR Protocols: a guide to methods and applications*, Eds. M. A. Innis, D. H. Gelford, J. J. Sninsky and T. J. White. New York: Academic Press.
- APPENDIX 1. List of species names, voucher information and GenBank accession numbers of plant material used in this study. Information is listed as follows: taxon name, locality, voucher number, GenBank accession (ITS, *ImpDEF1*, *ImpDEF2*).—: information not applicable.
- Impatiens campanulata* Wight, African origin, cult. Nat. Bot. Gard. Meise, FB/S2966 (BR), AY348758, EF133567, EF133620. *I. columbaria* J. J. Bos, African origin, cult. Nat. Bot. Gard. Meise, FB/S2966 (BR), HM454298, EF133572, EF133625. *I. hians* Hook. f. var. *hians*, West African origin, cult. Bot. Gard. Berlin, Schwerdtfeger 9492a (B), HM454297 EF133585, EF133639. *I. kamerunensis* Warb. ssp. *kamerunensis*, Cameroon, J. J. F. E. de Wilde 8638 (BR),—, FJ826703, FJ826758. *I. kamerunensis* Warb. ssp. *obanensis* (Keay) Grey-Wilson, Ghana, Jongkind 1926 (WAG), HQ176459, FJ826704, FJ826759. *I. mackeyana* Hook. f. ssp. *clari* (N. Hallé) Grey-Wilson, Gabon origin, cult. Nat Bot. Gard. Koblenz Univ., Fischer EF5 (NEU), HM454291, FJ826708, FJ826762. *I. mackeyana* Hook. f. ssp. *zenkeri* (Warb.) Grey-Wilson, African origin, cult. Bot. Gard. Koblenz Univ., Fischer EF21 (NEU), HM454292, FJ826709, —. *I. macroptera* Hook. f., Equatorial Guinea, de Wilde 12014 (WAG), HM454301, FJ826710, FJ826763. *I. nzabiana* S. B. Janssens & Dessein, Gabon, Dessein 2044, HQ176461, —, —. *I. oumina* N. Hallé, Gabon, Dessein 2021, HQ176460, —, —. *I. palpebrata* Hook. f., Gabon, Jongkind 5724 (WAG), HM454299, FJ826719, FJ826772, *I. pseudomacroptera* Grey-Wilson, Gabon, de Wilde 10390 (WAG), HM454300, FJ826723, FJ826775. *I. sakeriana* Hook. f., Cameroon, Merckx VM125 (LV), HQ176462, FJ826727, FJ826778. *I. yingjiangensis* S. Akiyama & H. Ohba, China, Yunnan, Yuan CN2k1-55 (NEU), AY348851, FJ826734, FJ826787.