

# Morphology, molecular phylogenetics and biogeography of *Impatiens akomensis* (Balsaminaceae), a new species from Cameroon

Steven B. Janssens<sup>1,\*</sup>, Bonaventure Sonké<sup>2,3,4</sup>, Olivier Lachenaud<sup>1</sup>,  
Benny Lemaire<sup>5</sup>, Murielle Simo-Droissart<sup>2</sup> & Erik Smets<sup>5,6</sup>

<sup>1</sup>Botanic Garden Meise, Nieuwelaan 38, BE-1860 Meise, Belgium

<sup>2</sup>Plant Systematic and Ecology Laboratory, Higher Teacher Training College, University of Yaoundé I, P.O. Box 047 Yaoundé, Cameroon

<sup>3</sup>Service d'Evolution Biologique et Ecologie, CP 160/12, Université Libre de Bruxelles, 50 Avenue F. Roosevelt, BE-1050 Bruxelles, Belgium

<sup>4</sup>Missouri Botanical Garden, Africa & Madagascar Department, P.O. Box 299, 63166–0299 St. Louis, Missouri, U.S.A.

<sup>5</sup>Plant Conservation and Population Biology, KU Leuven, BE-3000 Leuven, Belgium

<sup>6</sup>Netherlands Centre for Biodiversity Naturalis, Leiden University, PO Box 9517, 2300 RA Leiden, The Netherlands

\*Author for correspondence: [steven.janssens@br.fgov.be](mailto:steven.janssens@br.fgov.be)

**Background and aims** – A recent field expedition in the Campo-Ma'an/Akom II region in Cameroon resulted in the collection of a previously unknown *Impatiens* species, which resembles *Impatiens macroptera* and *Impatiens pseudomacroptera*. This study aims to describe and infer the evolutionary history of this novelty by using phylogenetics, biogeographic inference and dating estimation analyses.

**Methods** – The new species was subject to a palynological, molecular phylogenetic and biogeographical analysis. In addition, a thorough morphological comparison with its closely related species was conducted.

**Key results** – Morphological comparison and molecular phylogenetics corroborate its status as a new species, which is given the name *Impatiens akomensis*. Molecular data, based on *ImpDEF1*, *ImpDEF2* and ITS indicate that *Impatiens akomensis* is most closely related to the Cameroonian *I. macroptera*, whereas the lineage towards *I. macroptera* and the novelty is sister to the Gabonese *I. pseudomacroptera*. *Impatiens akomensis* differs from *I. macroptera* and *I. pseudomacroptera* in having very strongly asymmetric white flowers, much thicker leaves, a narrowly elliptic leaf shape and a serrulate leaf margin, smaller lateral united petals (23.1–24.2 mm) and larger bracts (12–20 mm). The new species qualifies for endangered status under IUCN criteria B2 and probably B1.

**Conclusions** – Dating analyses in combination with biogeographic inference, and ecological and environmental interpretation of the novelty and its closely related species allows us to infer the putative evolutionary history of *Impatiens akomensis*.

**Key words** – Cameroon, diversification, *Impatiens*, new species, taxonomy.

## INTRODUCTION

Located in the Lower Guinea subcentre of endemism, the Campo-Ma'an/Akom II region belongs to one of the most important biodiversity hotspots worldwide (White 1979, Davis et al. 1994). The Campo-Ma'an/Akom II area harbours c. 2,300 species of higher plants of which 23% are restricted to the Lower Guinea subcentre of endemism (roughly from the base level of the Niger River to the base level of the Congo River) and 6% are endemic to Cameroon (Tchouto et al. 2006). Furthermore, according to Tchouto et al. (2006), 29 species only occur in the Campo-Ma'an/Akom II region. New endemic species from this region are regularly described (Sonké et al. 2007, 2008, Lachenaud & Séné 2010, 2012, Sonké et al. 2012, Verstraete et al. 2013). In addition,

ecological studies comparing tree biodiversity in species-rich forests of Gabon and Cameroon show that the Campo-Ma'an/Akom II region has the highest level of tree biodiversity compared to the Massif du Chaillu (Gabon), the Monts de Cristal (Gabon), and the Ngovayang Massif (Cameroon), which are known for their high diversity and degree of endemism (Droissart et al. 2011, Gonmadje et al. 2011). Furthermore, Parmentier et al. (2011) demonstrated that the Campo-Ma'an/Akom II region (including the Lolodorf-Bipindi area) is the most biodiverse region in southern Cameroon (their study was based on alpha diversity estimates of trees). Atlantic montane areas in Gabon and Cameroon (e.g. Ngovayang Massif, Campo-Ma'an/Akom II region, Monts de Cristal, Doudou Mts., Massif du Chaillu, Mayombé) are often con-

sidered as putative forest refuges during past Pleistocene climate fluctuations (Maley 1987, Sosef 1994, Plana 2004). According to Maley (1987), during climatically unfavorable episodes, persistent and extensive stratiform cloud layers provided Atlantic coastal montane forests with sufficient humidity under the form of a prevalent and fine precipitation.

Recent studies on *Impatiens* (balsams, Janssens et al. 2009a, 2009b, 2010, 2011) indicate the large importance of past climate fluctuations on the diversification of this large genus. The current species richness of *Impatiens* (> 1,000 spp.) is the result of a sudden diversification boost, correlated with changing climate conditions of the Pliocene and Pleistocene. The periodicity of glacial cycles likely resulted in several alternating episodes of contraction and expansion of the upland and montane rainforests, which are the preferential habitat of *Impatiens* species. In fact balsams are often restricted to a limited geographic region or even a single mountain peak.

In May 2009, fieldwork in South Cameroon yielded material of a new *Impatiens* species that resembles the Cameroonian species *I. macroptera* Hook.f. and the Gabonese species *I. pseudomacroptera* Grey-Wilson (fig. 1). The novelty was found in a small creek in the mountainous eastern part of the Campo-Ma'an/Akom II area, one of the putative Pleistocene forest refuges of West-Central Africa (fig. 2).

The present paper documents and illustrates the new species, named *I. akomensis*, and additionally provides pollen morphological characters for this new species. In addition, the evolutionary history of the novelty is inferred using phylogenetics, biogeographic inference and dating estimation analyses.

## MATERIAL AND METHODS

### Taxonomic study

Material of *I. akomensis* was collected by O. Lachenaud, B. Lemaire, M. Simo-Droissart and B. Sonké, in May 2009 (voucher *Lachenaud* 679). Additionally, herbarium specimens of the new species and its close relatives were examined at BR, BRLU, K, LBV, P, WAG and YA (abbreviations after Thiers 2013). Measurements, colours and other details given in the descriptions are based on living material, spirit and herbarium specimens, and data derived from photographic illustrations and field notes. Botanical descriptive terminology follows Grey-Wilson (1980a) except for simple symmetrical plane shapes (Systematics Association Committee for Descriptive Terminology 1962). Phytogeographical considerations follow White (1979).

### Pollen morphological study

Pollen grains of *I. akomensis* (voucher *Lachenaud* 679) were prepared for scanning electron microscopy (SEM; Jeol JSM-6400 microscope). Observations followed the critical point drying method described by Janssens et al. (2005). Magnification v1.5.2 ([www.orbicule.com](http://www.orbicule.com)) was used for size measurements of the pollen. Terms for shape classes in equatorial view follow Erdtman (1971). Terminology of pollen shape in

polar view follows Reitsma (1970). Further pollen terminology is adopted from Punt et al. (2007).

### Molecular phylogenetic analyses

The most recent molecular phylogeny of the African *Impatiens* (Janssens et al. 2009b) was used to determine the overall phylogenetic position of *I. akomensis* (voucher *Lachenaud* 679). The clade to which the new species belongs was reanalysed using ITS, *ImpDEF1* and *ImpDEF2*. DNA extraction, PCR amplification, sequencing, sequence assembly and alignment were followed as described by Janssens et al. (2006). 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. Voucher information and GenBank accession numbers of species used in this study are listed in table 1. Alignment of ITS and *ImpDEF1/ImpDEF2* are added as supplementary data (electronic appendix 1).

Nuclear ribosomal ITS and nuclear *ImpDEF1/ImpDEF2* datasets were analyzed both separately and combined. In order to evaluate whether the data matrices provided different signal in the combined analyses, a partition homogeneity test (implemented in PAUP\* 4.0b10a) was conducted (Swofford 2002).

Model selection for the Bayesian inference analyses was conducted with ModelTest 3.06 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC). The GTR+G model was selected for ITS, *ImpDEF1* and *ImpDEF2*. Bayesian analyses were carried out with MrBayes 3.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Four chains (one cold, three heated), initiated from a random starting tree were run simultaneously for 10 million generations. Every 1,000 generations, a tree was sampled from the chain for a total of 10,000 trees. Due to the burn-in, 50% of the sample points were discarded. Convergence of the chains was examined with TRACER 1.4 (Rambaut & Drummond 2007). This resulted in an effective sampling size (ESS) parameter exceeding 100, which assumes a sufficient sampling and acceptable mixing.

Maximum Likelihood analyses were carried out using the RaxML search algorithm (Stamatakis et al. 2005) under the GTRGAMMA approximation of rate heterogeneity for each gene (Stamatakis 2006). Two hundred bootstrap trees were inferred using the RaxML Rapid bootstrap algorithm (ML-BS) to provide support values for the best-scoring ML tree.

### Dating estimation

A  $\chi^2$  likelihood ratio test demonstrated that a strict molecular clock for the combined dataset had to be rejected ( $\chi^2 = 76.12$ ;  $df = 15$ ;  $P = 2.4 \times 10^{-5}$ ). As a result, age estimates were obtained by applying a relaxed clock model using the penalized likelihood (PL; Sanderson 2002) method as 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. A previously computed age estimate of the West-Central African clade ( $3.45 \pm 0.72$  million year ago) was used as fixed calibration point (Janssens et al. 2009b) to calculate the age esti-

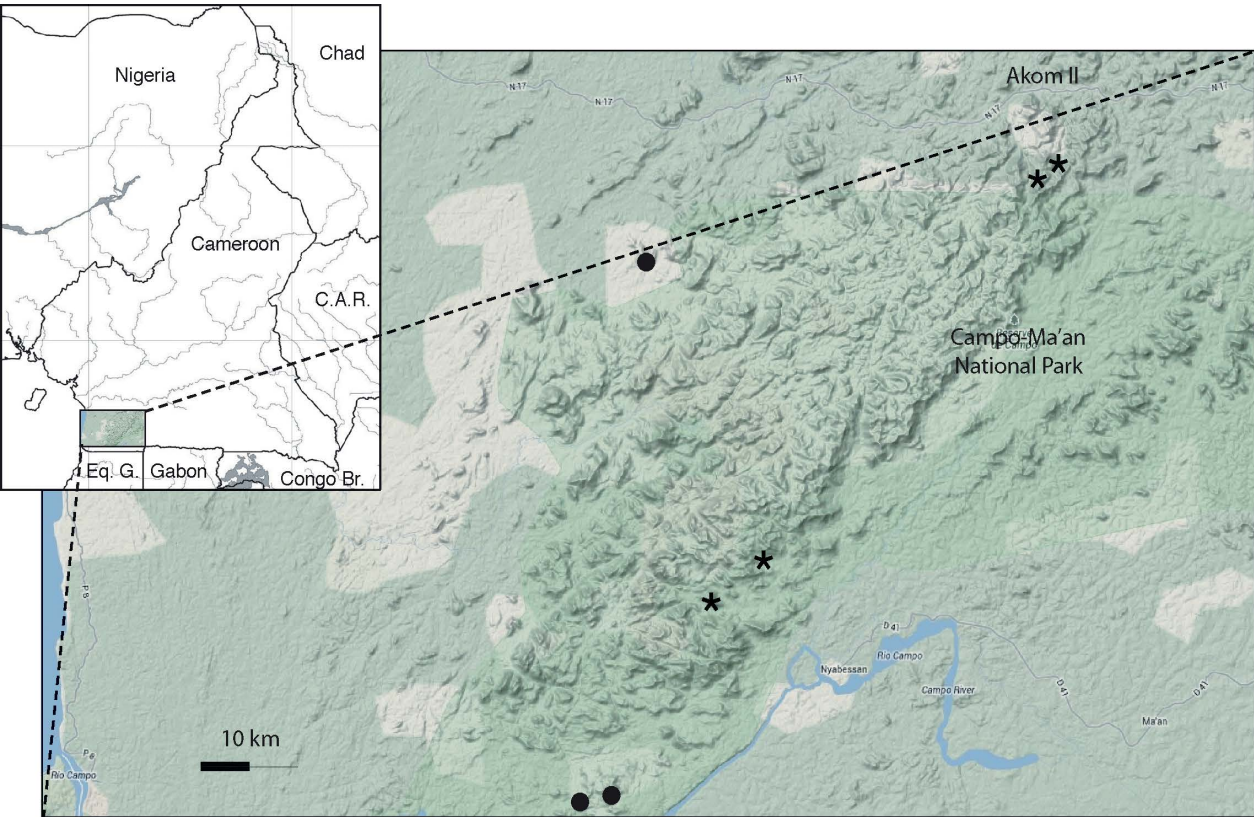




**Figure 1** – Morphology and habitat of *Impatiens akomensis* and allied species: A, habitat and habit of *I. akomensis*; B, view on the inflorescence of *I. akomensis*; C, frontal view of *I. akomensis* flower; D, lateral view of flower of *I. akomensis*; E, lateral view of *I. pseudomacroptera* flower; F, lateral view of *I. macroptera* flower. A–D from Lachenaud et al. 679, E from Dessein et al. 2023, F from Merckx VM107.

**Table 1 – List of species names, voucher information and GenBank accession numbers of plant material used in this study.**

Taxon	Origin	Voucher	ITS	ImpDEF1	ImpDEF2
<i>Impatiens akomensis</i>	Cameroon	<i>Lachenaud</i> 679	KP324829	-	-
<i>I. campanulata</i> Wight	South Indian origin, cult. By Ray Morgan, UK	<i>Ray Morgan</i> s.n. (LV)	AY348758	EF133567	EF133620
<i>I. columbaria</i> J.J.Bos	African origin, cult. Nat. Bot. Gard. Meise	<i>Billiet</i> S2966 (BR)	HM454298	EF133572	EF133625
<i>I. hians</i> Hook.f. var. <i>bipindensis</i> (Gilg) Grey-Wilson	Gabon	<i>Dessein</i> 1179 (BR)	HM454296	HM454289	HM45290
<i>I. hians</i> Hook.f. var. <i>hians</i>	West African origin, cult. Bot. Gard. Berlin	<i>Schwerdtfeger</i> 9492a (B)	HM454297	EF133585	EF133639
<i>I. kamerunensis</i> Warb. subsp. <i>obanensis</i> (Keay) Grey-Wilson	Ghana	<i>Jongkind</i> 1926 (WAG)	HQ176459	FJ826704	FJ826759
<i>I. mackeyana</i> Hook.f. subsp. <i>claei</i> (N.Hallé) Grey-Wilson	Gabon origin, cult. Bot. Gard. Koblenz Univ.	<i>Fischer</i> EF5 (NEU)	HM454291	FJ826708	FJ826762
<i>I. mackeyana</i> Hook.f. subsp. <i>zenkeri</i> (Warb.) Grey-Wilson	African origin, cult. Bot. Gard. Koblenz Univ.	<i>Fischer</i> EF21 (NEU)	HM454292	FJ826709	EU373295
<i>I. macroptera</i> Hook.f.	Equatorial Guinea	<i>de Wilde</i> 12014 (WAG)	HM454301	FJ826710	FJ826763
<i>I. palpebrata</i> Hook.f.	Gabon	<i>Jongkind</i> 5724 (WAG)	HM454299	FJ826719	FJ826772
<i>I. pseudomacroptera</i> Grey-Wilson	Gabon	<i>de Wilde</i> 10390 (WAG)	HM454300	FJ826723	FJ826775
<i>I. sakeriana</i> Hook.f.	Cameroon	<i>Merckx</i> VM125 (LV)	HQ176462	FJ826727	FJ826778
<i>I. yingjiangensis</i> S.Akiyama & H.Ohba	China, Yunnan	<i>Yuan</i> CN2k1-55 (NEU)	AY348851	FJ826734	FJ826787



**Figure 2 – Distribution of *Impatiens akomensis* (black asterisks) and *I. macroptera* (black dots) in Campo-Ma'an/Akom II region. Eq.G.: Equatorial Guinea; C.A.R.: Central African Republic; Congo Br.: Congo Brazzaville.**



mates within the lineage containing *I. akomensis*. Confidence intervals were calculated by reanalyzing the dataset using the available minimum and maximum age of the node towards the West-Central African lineage as fixed calibration points (Janssens et al. 2009b).

## RESULTS

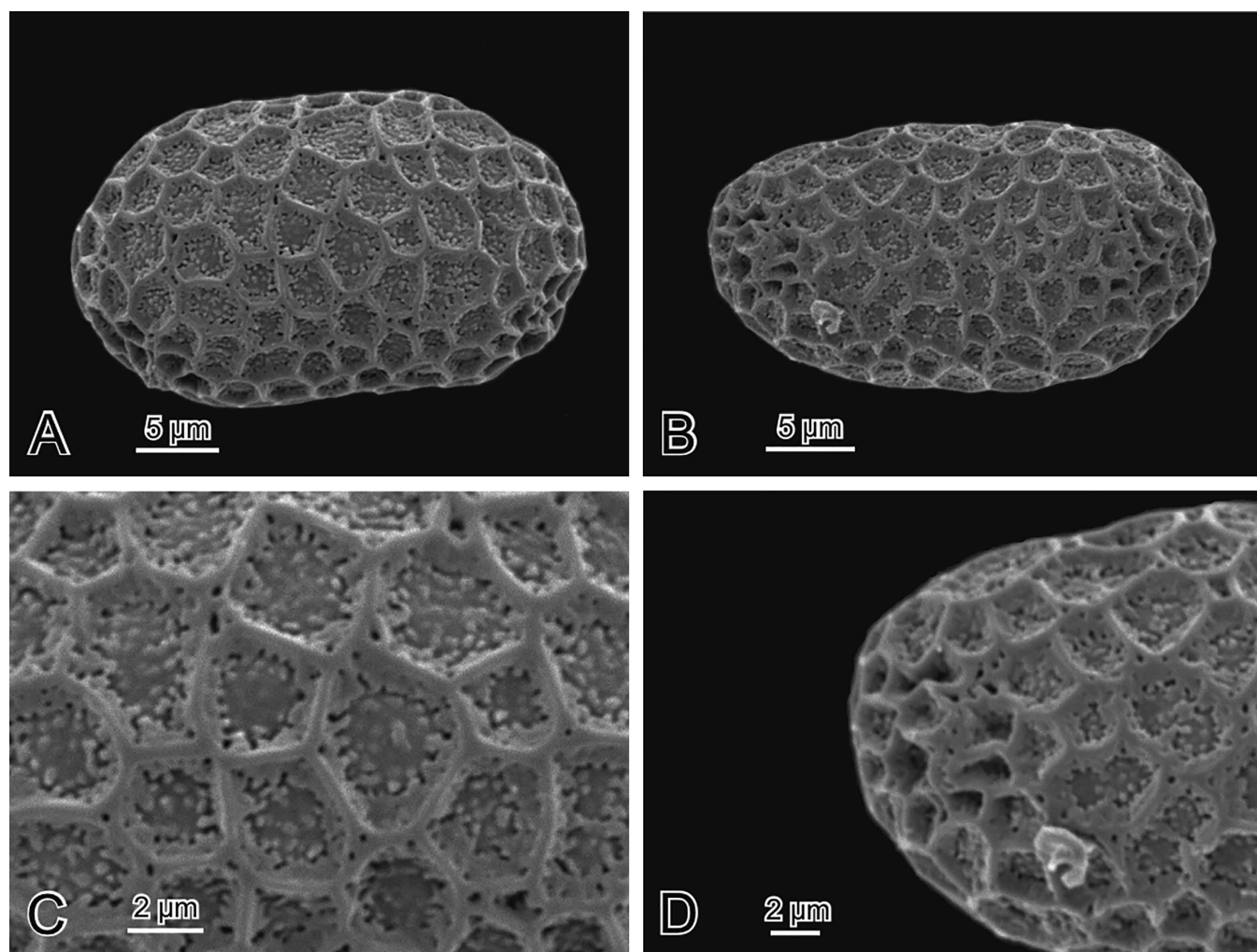
### Pollen morphology of *Impatiens akomensis*

Pollen grains are medium sized (P: [ $P_{\min}$  : 18.0  $\mu\text{m}$ ;  $P_{\text{mean}}$  : 18.5  $\mu\text{m}$ ;  $P_{\max}$  : 19.2  $\mu\text{m}$ ]; E: [ $E_{\min}$  : 33.1  $\mu\text{m}$ ;  $E_{\text{mean}}$  : 34.3  $\mu\text{m}$ ;  $E_{\max}$  : 35.5  $\mu\text{m}$ ]) and have an oblate shape (P/E: 0.53; fig. 3B). They have an elliptic amb (fig. 3A) with four simple apertures only consisting of an ectocolpus (length 6.1  $\mu\text{m}$ ). No margo has been observed around the colpus (fig. 3D). Pollen grains are characterized by a reticulate sexine ornamentation (fig. 3B–C). Lumina are irregular in shape and range in size between 1.8  $\mu\text{m}$  and 4.5  $\mu\text{m}$  being on average 3.2  $\mu\text{m}$  (measured according to the longest axis). The density of the lumina

is 0.35  $\mu\text{m}^{-2}$ . Muri are variable in thickness, always having a rounded top edge. Inside the lumina, very few solitary granules are observed on the foot layer (fig. 3C). Orbicules are absent on the inner locule wall.

### Phylogenetic position of *Impatiens akomensis*

Although we were unable to sequence the *ImpDEF1* and *ImpDEF2* loci in *I. akomensis*, the partition homogeneity test showed no significant incongruence between ITS and *ImpDEF1/ImpDEF2* ( $P = 0.001$ ) indicating that the missing data did probably not significantly influence the analysis of the combined matrix. In addition, visual inspection did not detect hard incongruences between the topologies resulting from the phylogenetic analysis of each ITS and *ImpDEF1/ImpDEF2* regions separately (electronic appendices 2 & 3) and as a result both datasets were combined. Statistics for the aligned sequence data are given in table 2. ML analysis of the combined dataset generated a topology with generally high support values.



**Figure 3** – SEM of *Impatiens akomensis* pollen grains: A, polar view of a reticulate pollen grain with subcircular outline; B, equatorial view of an oblate pollen grain; C, detailed view of the reticulate sexine with irregular shaped lumina and muri with a rounded top edge; D, detailed view of the colpus without margin.

Table 2 – Statistics for the aligned data matrix.

	ITS	ImpDEF1/ImpDEF2
Number of taxa	13	11
Aligned matrix length	653	1040
Number of variable characters	76	138

*Impatiens akomensis* is part of a small clade of West-Central African species (fig. 4). This clades includes the *I. hians*, *I. macroptera* and *I. filicornu* species complexes, which are informal groups proposed by Grey-Wilson (1980a, 1980b). The combined phylogeny shows that *I. akomensis* is sister to *I. macroptera* (ML support: 73, BI: 0.95). In addition, the *I. akomensis* – *I. macroptera* clade is sister to *I. pseudomacroptera* (ML support: 81, BI: 1.0). The lineage formed by *I. akomensis*, *I. macroptera* and *I. pseudomacroptera* is sister to the clade containing *I. columbaria*, *I. palpebrata*,

the two subspecies of *I. mackeyana* and the two varieties of *I. hians* (see Janssens et al. 2009b, 2010, 2011).

Divergence time estimates

With the calculated dating estimate of the West Central African lineage as calibration point (Janssens et al. 2009b), we calculated the split between *I. akomensis* and *I. macroptera* at 2.20 million years ago (ma), with a confidence interval from 2.77 ma to 1.73 ma (fig. 4). The diversification estimate of the node between sister lineages *I. pseudomacroptera* and *I. akomensis*/*I. macroptera* is dated at 2.28 ma, with a confidence interval from 2.67 ma to 1.79 ma (fig. 4).

DISCUSSION

Taxonomic affinity

As stated in the introduction, the closest relatives of *I. akomensis* are *I. macroptera* and *I. pseudomacroptera*. All three species share a long-pedunculate inflorescence, a broadly

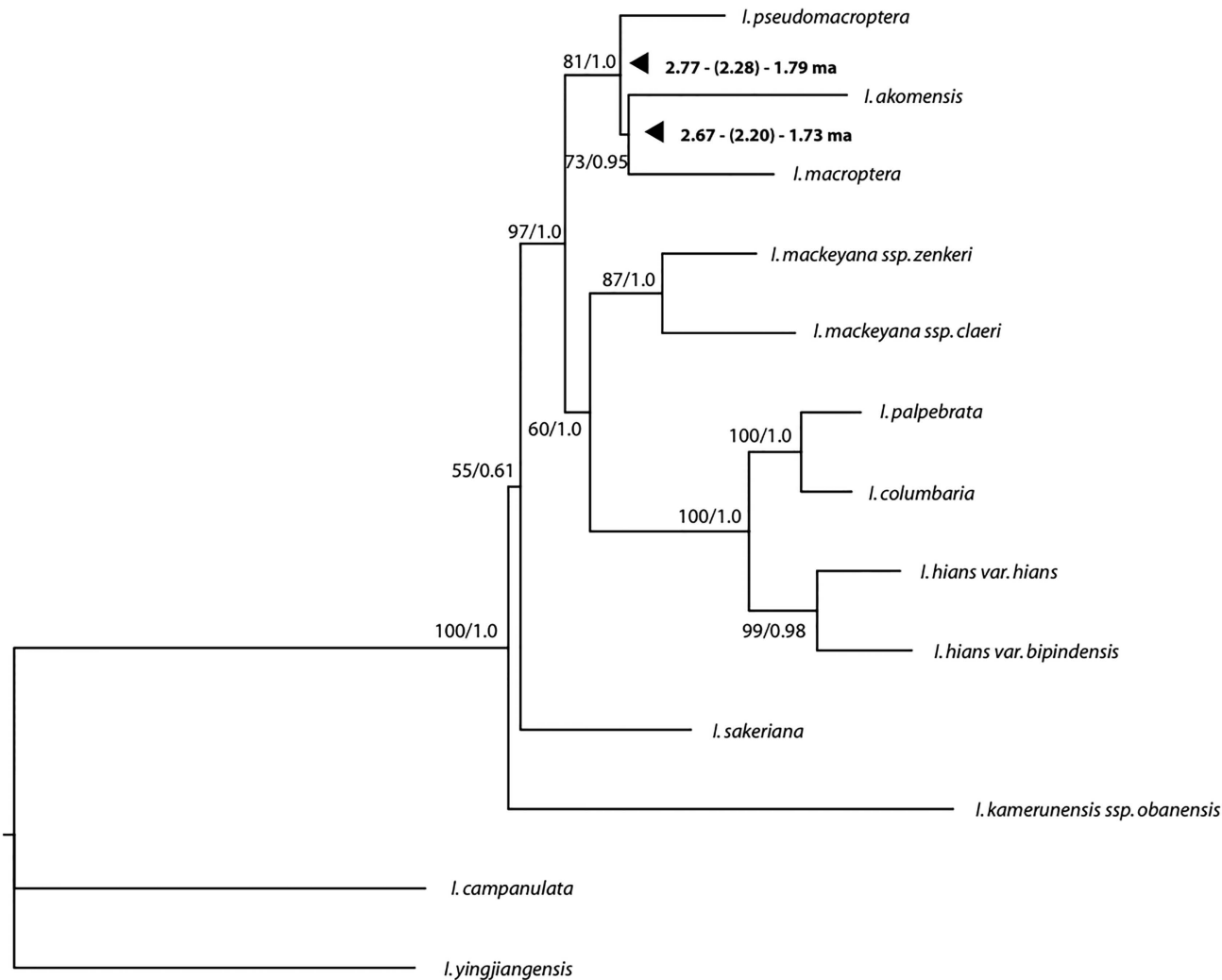


Figure 4 – Phylogram based on combined *ImpDEF1*/*ImpDEF2* and ITS data. Numbers on branches represent Maximum Likelihood Bootstrap Support and Bayesian Posterior Probabilities, respectively. The arrow indicates the age of the nodes investigated with additional confidence intervals [(max (mean) min)].

**Table 3 – Comparison of taxonomic useful characters, geographic distribution, altitudinal ranges and habitat of *Impatiens akomensis*, *I. pseudomacroptera* and *I. macroptera*.**

Species	<i>I. macroptera</i>	<i>I. akomensis</i>	<i>I. pseudomacroptera</i>
Size	20–40(–50) cm	20–40 cm	<b>100–180 cm</b>
Petiole length	4.5–9 cm	1.8–7.2 cm	1.5–5 cm
Leaf size	8–18(–19.5) × 4.4–9 cm	13.0–14.5 × 3.5–5.5 cm	9.5–22.0 × 2.8–8.5 cm
Leaf shape	broadly ovate	<b>narrowly elliptic</b>	oblanceolate to oblanceolate-ovate
Leaf apex	acuminate-acute	acuminate	apiculate
Leaf base	abruptly cuneate	cuneate	gradually cuneate
Leaf margin	conspicuously crenate	<b>inconspicuously serrulate</b>	conspicuously crenate
Flowers	pink, faintly asymmetrical	<b>white, strongly asymmetrical</b>	violet, faintly asymmetrical
Peduncle	1.5–20 cm	12.2–15.2 cm	8.5–16 cm
Bracts	<b>4–5 mm</b>	12–20 mm	8–16 mm
Bract shape	ovate-oblong to linear-lanceolate	elliptic	elliptic
Lateral sepal size	<b>4–5 × 3 mm</b>	6.0 × 9.0 mm	8–10 × 3–6 mm
Lateral sepal shape	ovate-oblong, acute	acuminate	ovate, acuminate
Lower sepal	16–18 × 11–13 mm	10.0–18.0 × 20.0–24.0 mm	16–21 × 12–20 mm
Spur length	6–9 mm	7–8 mm	<b>11–13 mm</b>
Spur orientation	<b>overlap spur-lower sepal</b>	no overlap spur-lower sepal	no overlap spur-lower sepal
Dorsal petal	10–11 × 14 mm	12.0–13.5 × 7–8 mm	10–12 × 8–10 mm
Lateral united petals	28–44 mm	<b>23–24 mm</b>	29–32 mm
Upper lateral petal	14–15 × 6–8 mm	14.0 × 10.0 mm	10–15 × 11–13 mm
Lower lateral petal	26–33 × 12–16 mm	24.0 × 11.4 mm	20–22 × 17–20 mm
Fruit	14–18 × 3–4 mm	14 mm	18–21 × 4–7 mm
Equatorial axis length	/	33.05–35.48 µm	35.09 µm
Pollen aperture length	/	5.76–6.65 µm	6.09 µm
Distribution	Cameroon - Equatorial Guinea	Cameroon	Gabon

saccate lower sepal abruptly constricted into a short incurved spur and a cucullate dorsal petal (table 3). *I. akomensis* most obviously differs from its two relatives in having very strongly asymmetric white flowers (fig. 1C–D), and leaf blades which are more narrowly elliptic, much thicker (in life) and inconspicuously serrulate on the margin. In *I. macroptera* and *I. pseudomacroptera* the flowers are pink to violet and only slightly asymmetric (the apices of lower petals being not shifted to the right as in *I. akomensis*), and the leaf blades are broader, relatively thin, and conspicuously crenate on the margin. Furthermore, *I. akomensis* has smaller lateral united petals (23.1–24.2 mm) and tends to have larger bracts (12–20 mm) than its two relatives.

*Impatiens akomensis* also combines some characters of *I. macroptera* with others of *I. pseudomacroptera* (table 3). It resembles *I. macroptera* in being a relatively small plant (20–40 cm), and in having acuminate leaves and a short spur (7–8 mm), while with *I. pseudomacroptera* it shares an el-

liptic bract shape, and no overlap of the incurved spur with its lower sepal.

*Impatiens pseudomacroptera* has long been included in *I. macroptera* (e.g. Hallé 1962) until Grey-Wilson (1980a) separated the two species. In addition to morphological differences, *I. macroptera* and *I. pseudomacroptera* also differ in their distribution, with *I. macroptera* known from Cameroon and Equatorial Guinea and *I. pseudomacroptera* only known from Gabon. Although *I. macroptera* and *I. pseudomacroptera* share a large number of morphological characters, they are not true sister species. Molecular analyses clearly indicate a sister group relationship of *I. akomensis* with *I. macroptera*, which are sister to *I. pseudomacroptera*. Based on known collections, *I. akomensis* and *I. macroptera* have never been collected together so far, thereby indicating that there is no apparent overlap in distribution range for both species. However, despite being limited to the montane area of the Campo-Ma'an/Akom II region in South Came-

room, *I. akomensis* is rather well distributed throughout the eastern part of the Campo-Ma'an/Akom II region with the two currently known most distant populations at least sixty km away from each other (fig. 2). In contrast, the smallest distance between a population of *I. macroptera* and *I. akomensis* is only 25 km.

The first known collection of *I. akomensis* (Tchouto & Elad 2824) was made in 2000, close to the current type locality in the Akom II area. Two years later, another collection of *I. akomensis* (Elad, Tchouto, Ekwadi & Nnanga 1553) was made further south near Ebianemeyong in the Campo-Ma'an National Park. Unfortunately both specimens were misidentified as *I. filicornu* C.B. Clarke ex Hook.f., a species known for its rather variable leaf blade shape and spur length. After close examination of the two herbarium sheets, it is clear that these specimens have some morphological characters in common with *I. filicornu*, but unquestionably belong to *I. akomensis*. *Impatiens filicornu* differs from *I. akomensis* in having smaller leaf blades ( $3.2\text{--}8.5 \times 1.5$  cm), an attenuate to rounded blade base, a crenate blade margin, smaller ovate bracts (3–4 mm in length), pink to purplish flowers, ovate lateral sepals, a small lower sepal (5 mm) and smaller lateral united petals (10–16 mm). The spur of *I. filicornu* is curved filiform in shape and long in size (10–35 mm). Interestingly, *I. filicornu* specimens from Nigeria and Cameroon tend to have longer spurs and narrower, lanceolate or elliptical leaf laminae than specimens from the southern part of the distribution area (Grey-Wilson 1980a).

### Divergence mode and evolutionary history of *I. akomensis*

The Campo-Ma'an/Akom II area where the new species *I. akomensis* is found is known to be an important center of biodiversity on the African continent. The area probably functioned as Pleistocene refugium during glacial and interglacial fluctuations (Tchouto et al. 2006, 2009, Sosef 1994, 1996, Droissart et al. 2011). Besides the novelty described here, the Campo-Ma'an/Akom II area is characterized by the occurrence of many more endemic species in its rich forest species diversity. These so-called Pleistocene refuges are of great importance for the evolution of *Impatiens* as its species have a rather limited dispersal ability. Balsams have typical capsular fruits which are explosively dehiscent, thereby ejecting the small but rather heavy seeds and dispersing them across usually less than a meter. As a result, secondary agents such as streams or small rivers are essential for long-distance dispersal. *Impatiens akomensis* was found both in the North and the South of the Campo-Ma'an/Akom II refuge area (Efoulan and Ebianemeyong, respectively) which indicates that it had the time to disperse within the boundaries of the putative forest refuge, a result which is confirmed by the dating analysis that estimated the divergence age of *I. akomensis* from its sister species at 2.2 ma. Molecular phylogenetic analyses show that *I. akomensis* is most closely related to *I. macroptera*. The morphological differences between both taxa are prominent and thorough investigation of herbarium specimens did not reveal any intermediates. Interestingly, both sister species appear in the same overall distribution area of the Campo-Ma'an uphill region, however

they are not known to occur sympatrically in the same area (fig. 2). In contrast to the limited distribution of *I. akomensis*, *I. macroptera* is also present in montane forests of Equatorial Guinea (Rio Muni) and in Northwest and Central Cameroon. Although *I. akomensis*, as far as is known, only grows near small streams, *I. macroptera* can also occur in submontane forests away from water (O. Lachenaud, Botanic Garden Meise, Belgium, pers. obs.; Grey-Wilson 1980a).

During fieldwork, a species of the insect suborder Heteroptera was observed in a flower of *I. akomensis* (fig. 1B–C) whereas none of the putative pollinators of *I. macroptera* (large solitary forest bees, Grey-Wilson 1980a) were found. It remains speculative however, whether this Heteroptera species might be a pollinator of *I. akomensis* as no pollen was observed onto the smooth body of the insect.

According to Johnson (2007), sympatric speciation can almost be excluded as a possibility of pollinator-driven speciation. He states that it would be unlikely for a flower color mutant in a specific population to attract a totally different set of pollinators in comparison to the other individuals of the population, thereby indicating that a pollinator shift would likely occur after for example a substrate-induced or other ecological adaptation. Around the period of divergence, the most recent common ancestor of *I. akomensis* and *I. macroptera* most likely occurred in the vicinity of the distribution area where both species currently occur. A possible hypothesis about the divergence of *I. akomensis* from its sister species could be that it was induced by a slight difference in habitat preference and subsequent differentiation in flower coloration and shape. Despite the possibility that *I. akomensis* probably did not diverge fully allopatrically, one has to be very cautious with making assumptions about the speciation mode. Although several ecological, genetic, biogeographic and phylogenetic results could give an indication about the putative speciation mode of a species or species complex, it remains very difficult to produce an indisputable and incontestable answer as it is hardly impossible to take all evolutionary, ecological and genetic elements into account. A more thorough sampling of *I. macroptera* and *I. akomensis* in Cameroon and subsequent population genetic study could be useful to address more clearly the speciation mode that gave rise to these two species.

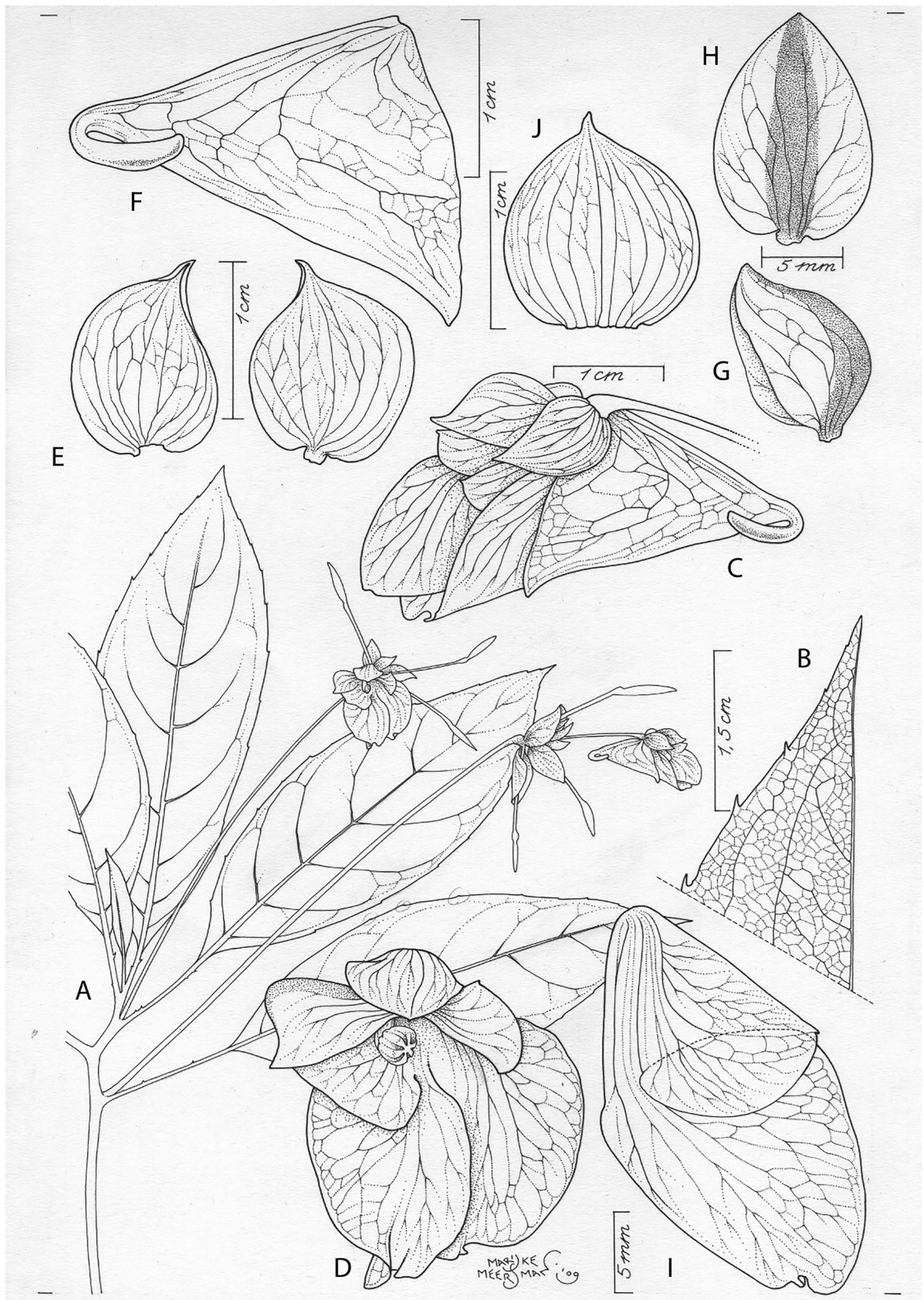
### TAXONOMY

***Impatiens akomensis* S.B.Janssens, Sonké & O.Lachenaud sp. nov.**

Inflorescentiis longe pedunculatis, sepalo inferiore amplio saccato et breviter calcarato, petaloque superiore cucullato *I. macropterae* Hook.f. et *I. pseudomacropterae* Grey-Wilson valde affinis; sed ab ambabus conspicue differt floribus albis (nec roseo-violaceis) et valde asymmetricis, foliis angustiore ellipticis, valde crassioribus et margine serrulatis (nec crenatis), petalisque lateralibus brevioribus. – Type: Cameroon, South Region (“Province”), Efoulan south of Akom II, 2°45'15.54"N 10°32'27.48"E, 10 May 2009, Lachenaud, Lemaire, Simo & Sonké 679 (holo-: BR; iso-: WAG, YA).

**Description** – Erect perennial herb, 20 to 40 cm tall. **Stems** simple or sparsely branched, glabrous. **Leaves** spirally ar-





**Figure 5** – *Impatiens akomensis*: A, habit; B, abaxial leaf detail; C, lateral view of flower; D, frontal view of flower; E, lateral sepals; F, lower sepal and spur; G, lateral view of dorsal petal; H, frontal view of dorsal petal; I, united lateral petals. Drawn by M. Meersman from Lachenaud 679.

ranged; petiole 1.8–7.2 cm long; blades 13.0–14.5 × 3.5–5.5 cm, narrowly elliptic, cuneate at the base, acuminate at the apex, very thick (in life) and shiny above, glabrous on both sides; 6–7 strongly ascending lateral veins at each side of the midrib; leaf margins finely serrulate with several short filiform fimbriae at the base. Inflorescence a subumbellate axillary raceme; peduncle 12.2–15.2 cm long, glabrous; pedicels 12.2–15.2 mm long, glabrous; bracts elliptic, 1.2–2.0 × 1.4–1.6 cm, glabrous. **Flowers** white (spotted yellow and red in throat), strongly asymmetrical (the mouth always shifted to the left), entirely glabrous. Lateral sepals 2, c. 6.0 × 9.0 mm, lanceolate. Lower sepal white, saccate, 20.0–24.0 × 10.0–18.0 mm, gradually constricted into a green 7.0–8.0 mm long curved spur. Dorsal petal 9.0–13.5 × 14.0 mm, cucullate, with a narrow dorsal crest. Lateral united petals white with small yellow and red spots at the base of the upper lateral petal of each lateral united pair, 24.0 mm long, with the upper petal of each pair smaller than the lower one; upper petal 10.0 × 14.0 mm, ovate-oblong; lower petal of each pair 24.0 × 11.4 mm, ovate-suborbicular, the apex shifted to the right, with a slight emargination distally along the inner margin. Stamens 5, alternating with the petals, connate to a ring. Ovary 5-locular, glabrous. **Fruits** fusiform, 1.4 cm in length, seeds unknown. Fig. 5.

**Additional specimens examined** – Cameroon, South Region (‘Province’), Campo-Ma’an area, Ebianemeyong, 2°27’37”N 10°17’46”E, 24 May 2002, *Elad, Tchouto, Ekwadi & Nnanga* 1553 (KRI n.v., SCA n.v., WAG); South Region (‘Province’), Campo Ma’an area, Efoulan, Ongongo and Nkolomekok hills in Akom II area, 2°44’56”N 10°32’27”E, 24 Apr. 2000, *Tchouto & Elad* 2824 (KRI, WAG, YA); Cameroon, South Region (‘Province’), Campo-Ma’an area, Ebianemeyong, 2°29’2.04”N 10°20’40.8”E, s.d., *Droissart* 1823 (BRLU, YA).

**Phenology** – *Impatiens akomensis* flowers in April and May (based on four records). However, a large majority of the plants encountered in the type locality in May 2009 were sterile, which suggests that flowering is either uncommon or very scattered through the year.

**Distribution** – *Impatiens akomensis* is known from the Lower Guinea subcenter of endemism (White 1979), and is restricted to the Campo Ma’an/Akom II area of South Cameroon (fig. 2).

**Ecology** – *Impatiens akomensis* grows on wet rocks along shaded forest creeks, between 400 and 600 m elevation; it is locally abundant and gregarious in this habitat. The area from which *I. akomensis* is known, supports a closed-canopy evergreen forest with many epiphytes and a rich herb layer, classified by Letouzey (1985) as Biafran evergreen forests rich in Caesalpiniaceae.

**Etymology** – The species epithet *akomensis* refers to the Akom II region where the species was discovered.

**Conservation Status** – IUCN Red List Category: Endangered (EN B2ab(iii)). A preliminary risk of extinction assessment was made using the IUCN red list categories and criteria (IUCN 2001). Due to the rather restricted and fragmented distribution range of *I. akomensis*, the species qualifies for “vulnerable” under criterion D2. Indeed, human activities in the region could make the species become critically en-

dangered or even extinct within a very short period of time (IUCN 2001). *Impatiens akomensis* is highly localized, as it is known only from three localities several kilometres apart in the Campo-Ma’an/Akom II region. Despite the fact that the species is highly conspicuous in flower, and the fact that several lengthy visits have been made to this region over recent years, *Impatiens akomensis* has been collected only three times in three different locations in the Campo-Ma’an/Akom II region (the two localities from the Akom II area even only represent a single location for the IUCN), it has not been collected elsewhere so far. It is to be hoped that more plants and new sites for *Impatiens akomensis* will be located in the future. The extent of occurrence (EOO) of *Impatiens akomensis* was not estimated, but its area of occupancy (AOO) is estimated to be 12 km<sup>2</sup> (which falls within the limits for endangered status under the criterion B2). This species is known from four subpopulations that represent a total of three “locations” according to the IUCN (which falls within the limits for endangered status under the criterion B2).

Although this region is nationally and internationally recognized for its great biological importance (Tchouto 2004), the Campo-Ma’an rainforest suffers intense human pressure leading to degradation of several patches of lowland and coastal rainforest, there is thus a continuous loss of the species habitat. *Impatiens akomensis* qualifies therefore for endangered status under criteria B2 and probably B1.

## SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>) and consist of: (1) aligned sequences of the combined ITS and *ImpDEF1/ImpDEF2* dataset; (2) phylogram based on the *ImpDEF1/ImpDEF2* dataset; and (3) phylogram based on the ITS dataset.

## ACKNOWLEDGEMENTS

We thank the curators of BR, BRLU, K, LBV, P, WAG and YA for making their collections available. Steven Dessein, Jeannette Sonké and Hermann Taedoumg are acknowledged for their help during the field expedition to Cameroon and Marijke Meersman for the botanical drawing. Sam Proost is acknowledged for his help during the initial phase of the project, Vincent Merckx for the use of his photograph and Tariq Stévant for his input on the IUCN status of the species. This study was financed by research grants of the K.U.Leuven (OT/05/35) and the Fund for Scientific Research-Flanders (FWO Belgium)(G.0104.01). SBJ had a Postdoctoral research grant from FWO during the course of this study. OL was a research fellow of the F.R.S.-F.N.R.S. (National Fund for Scientific Research) during the course of this study. Both the F.R.S.-F.N.R.S. and the F.F.R.S.A. (Fondation pour Favoriser les Recherches Scientifiques en Afrique) supported our research in Cameroon. The American Orchid society is gratefully acknowledged for supporting the Ph.D. research of MSD in Cameroon. BL was supported by the Institute for the Promotion of Innovation by Science and Technology in Flan-

ders (IWT Vlaanderen n° 71488). BS's visits to Belgium and France in 2013 during which this paper was finalized were funded by the "Institut de Recherche pour le Développement (IRD)", under 'PEERS – ISMOBIAC' project. Thanks are due to Dr. Pierre Couteron (Institut de Recherche pour le Développement IRD - AMAP), to Prof Jean-Louis Doucet (Université de Liège, Gembloux Agro-Bio Tech, Unité de Gestion des Ressources forestières et des Milieux naturels, Laboratoire de Foresterie des Régions tropicales et subtropicales) and Dr. Tariq Stévant (Herbarium et Bibliothèque de Botanique africaine, Université Libre de Bruxelles), for their assistance.

## REFERENCES

- Davis S.D., Heywood V.H., Hamilton A.C. (1994) Centres of plant diversity: a guide and strategy for their conservation. Vol. 1: Europe, Africa, South West Asia and the Middle East. Gland, WWF & IUCN.
- Droissart V., Sonké B., Hardy O.J., Simo M., Taedoumg H., Nguembou K.C., Stévant T. (2011) Do plant families with contrasting functional traits show similar patterns of endemism? A case study with Central African Orchidaceae and Rubiaceae. *Biodiversity and Conservation* 20: 1507–1531. <http://dx.doi.org/10.1007/s10531-011-0042-z>
- Erdtman G. (1971) Pollen morphology and plant taxonomy. Angiosperms. Corrected reprint of 1952 edition. New York, Hafner Publishing Company.
- Gonmadje C.F., Doumenge C., McKey D., Tchouto M.G.P., Sunderland T.C.H., Balinga M.P.B., Sonké B. (2011) Tree diversity and conservation value of Ngovayang's lowland forests, Cameroon. *Biodiversity and Conservation* 20: 2627–2648. <http://dx.doi.org/10.1007/s10531-011-0095-z>
- 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. <http://dx.doi.org/10.2307/4119063>
- Hallé N. (1962) *Flore du Gabon*. Vol. 4: Melianthaceae, Balsaminaceae, Rhamnaceae. Series 4. Paris, Muséum National d'Histoire Naturelle.
- Huelsenbeck J., Ronquist F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755. <http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- IUCN (2001) IUCN Red List categories and criteria: version 3.1. IUCN Species Survival Commission. Gland, IUCN.
- Janssens S., Lens F., Dressler S., Geuten K., Smets E., Vinckier S. (2005) Palynological variation in balsaminoid ericales. II. Balsaminaceae, Tetrameristaceae, Pellicieraceae and general conclusions. *Annals of Botany* 96: 1061–1073. <http://dx.doi.org/10.1093/aob/mci257>
- 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. <http://dx.doi.org/10.1600/036364406775971796>
- Janssens S.B., Geuten K.P., 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. <http://dx.doi.org/10.1016/j.ympev.2006.11.016>
- Janssens S.B., Viaene T., Huysmans S., Smets E.F., Geuten K.P. (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. <http://dx.doi.org/10.1007/s00239-008-9085-5>
- Janssens S.B., Knox E.B., Dessein S., Smets E. (2009a) *Impatiens msisimwanensis* (Balsaminaceae): description, pollen morphology and phylogenetic position of a new East African species. *South African Journal of Botany* 75: 104–109. <http://dx.doi.org/10.1016/j.sajb.2008.08.003>
- Janssens S.B., Knox E.B., Huysmans S., Smets E., Merckx V.S.F.T. (2009b) Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: Result of a global climate change. *Molecular Phylogenetics and Evolution* 52: 806–824. <http://dx.doi.org/10.1016/j.ympev.2009.04.013>
- Janssens S.B., Fischer E., Stévant T. (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., 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. <http://dx.doi.org/10.1600/036364411X569624>
- Johnson S.D. (2007) Pollinator-driven speciation in plants. In: Harder L.D., Barret, C.H. (eds) *Ecology and evolution of flowers*: 295–310. New York, Oxford University Press.
- Lachenaud O., Séné O. (2010) Un nouveau *Psychotria* (Rubiaceae) du sud Cameroun. *Plant Ecology and Evolution* 143: 105–108. <http://dx.doi.org/10.5091/plecevo.2010.371>
- Lachenaud O., Séné O. (2012) Un nouveau *Psychotria* (Rubiaceae) rhéophyte du Sud Cameroun. *Plant Ecology and Evolution* 145: 423–427. <http://dx.doi.org/10.5091/plecevo.2012.714>
- Letouzey R. (1985) Notice de la carte phytogéographique du Cameroun au 1:500000. Toulouse, Institut de la Carte Internationale de la Végétation.
- Maley J. (1987) Fragmentation de la forêt dense humide africaine et extension des biotopes montagnards du quaternaire récent: nouvelles données polliniques et chronologiques. Implications paléoclimatiques et biogéographiques. *Paleoecology of Africa* 18: 307–334.
- Parmentier I., Harrigan R.J., Buermann W., Mitchard E.T.A., Saatchi S., Malhi Y., Bongers F., Hawthorne W.D., Leal M.E., Lewis S.L., Nusbaumer L., Sheil D., Sosef M.S.M., Affum-Baffoe K., Bakayoko A., Chuyong G.B., Chatelain C., Comiskey J.A., Dauby G., Doucet J.-L., Fauset S., Gautier L., Gillet J.-F., Kenfack D., Kouame F.N., Kouassi E.K., Kouka L.A., Parren M.P.E., Peh K.S.-H., Reitsma J.M., Senterre B., Sonké B., Sunderland T.C.H., Swaine M.D., Tchouto M.G.P., Thomas D., Van Valkenburg J.L.C.H., Hardy O.J. (2011) Predicting alpha diversity of African rain forests: models based on climate and satellite-derived data do not perform better than a purely spatial model. *Journal of Biogeography* 38: 1163–1174. <http://dx.doi.org/10.1111/j.1365-2699.2010.02467.x>
- Plana V. (2004) Mechanisms and tempo of evolution in the African Guineo-Congolian rain forest. *Philosophical Transactions of the Royal Society London B* 359: 1585–1594. <http://dx.doi.org/10.1098/rstb.2004.1535>
- Posada D., Crandall K.A. (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818. <http://dx.doi.org/10.1093/bioinformatics/14.9.817>
- Punt W., Blackmore S., Nilsson S., Le Thomas A. (2007) Glossary of pollen and spores terminology. LPP Contributions Series nr 1. Utrecht, Laboratory of Palaeobotany and Palynology Foundation. Available at <http://www.pollen.mtu.edu/glos-gtx/glos-int.htm> [accessed 20 Apr. 2015].



- Rambaut A., Drummond A.J. (2007) Tracer v1.4. Available at <http://beast.bio.ed.ac.uk/Tracer> [accessed 20 Apr. 2015].
- Reitsma T. (1970) Suggestions towards a unifications of descriptive terminology of angiosperm pollen grains. *Review of Palaeobotany and Palynology* 10: 39–60. [http://dx.doi.org/10.1016/0034-6667\(70\)90021-7](http://dx.doi.org/10.1016/0034-6667(70)90021-7)
- Ronquist F., Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. <http://dx.doi.org/10.1093/bioinformatics/btg180>
- 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. Available at <http://loco.biosci.arizona.edu/r8s/r8s1.7.manual.pdf> [accessed 20 Apr. 2015].
- Sonké B., Djuikouo M.-N., Robbrecht E. (2007) *Calycosiphonia pentemara* sp. nov. (afrotropical Rubiaceae) from the 'Lower Guinea' area. *Nordic Journal of Botany* 25: 275–280. <http://dx.doi.org/10.1111/j.0107-055X.2007.00141.x>
- Sonké B., Dessein D., Taedoumg H., Groeninckx I., Robbrecht E. (2008) A new species of *Colletocema* (Rubiaceae) from southern Cameroon with a discussion of relationships among basal Rubioideae. *Blumea* 53: 533–547. <http://dx.doi.org/10.3767/000651908X607495>
- Sonké B., Taedoumg H., Robbrecht E. (2012) A reconsideration of the Lower Guinean species of *Sericanthe* (Rubiaceae, Coffeeae), with four new species from Cameroon and Gabon. *Botanical Journal of the Linnean Society* 169: 530–554. <http://dx.doi.org/10.1111/j.1095-8339.2012.01254.x>
- 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. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.
- Sosef M.S.M. (1996) Begonias and African rain forest refuges general aspects and recent progress. In: Van der Maesen L.J.G., Van Der Burgt X.M., Van Medenbach de Rooy J.M. (eds) *The biodiversity of African plants. Proceedings XIV<sup>th</sup> AETFAT Congress 22–27 August 1994*, Wageningen, The Netherlands: 602–611. Dordrecht, Kluwer Academic Publishers. [http://dx.doi.org/10.1007/978-94-009-0285-5\\_73](http://dx.doi.org/10.1007/978-94-009-0285-5_73)
- Stamatakis A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690. <http://dx.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. <http://dx.doi.org/10.1093/bioinformatics/bti191>
- Swofford D.L. (2002) PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), Version 4. Sunderland, Sinauer Associates.
- Systematics Association Committee for Descriptive Terminology (1962) II. Terminology of simple symmetrical plane shapes (Chart 1). *Taxon* 11: 145–156. <http://dx.doi.org/10.2307/1216718>
- Tchouto M.G.P. (2004) Plant diversity in a central African rain forest: implications for biodiversity conservation in Cameroon. PhD thesis, Departement of Plant Sciences, Biosystematics Group, Wageningen, The Netherlands.
- Tchouto M.G.P., Yemefack M., De Boer W.F., De Wilde J.J.F.E., Cleef A.M. (2006) Biodiversity hotspots and conservation priorities in the Campo-Ma'an rainforests, Cameroon. *Biodiversity and Conservation* 15: 1219–1252. <http://dx.doi.org/10.1007/s10531-005-0768-6>
- Tchouto M.G.P., de Wilde J.J.F.E., de Boer W.F., van der Maesen L.J.G., Cleef A.M. (2009) Bio-indicator species and Central African rain forest refuges in the Campo-Ma'an area, Cameroon. *Systematics and Biodiversity* 7: 21–31. <http://dx.doi.org/10.1017/S1477200008002892>
- Thiers B. (2013) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. Available at <http://sweetgum.nybg.org/ih/> [accessed 20 Oct. 2013].
- Verstraete B., Lachenaud O., Smets E., Dessein S., Sonké B. (2013) Taxonomy and phylogenetics of *Cuviera* (Rubiaceae–Vanguerieae) and reinstatement of the genus *Globulostylis* with the description of three new species. *Botanical Journal of the Linnean Society* 173: 407–441. <http://dx.doi.org/10.1111/boj.12062>
- White F. (1979) The Guineo-Congolian Region and its relationship to other phytochoria. *Bulletin du Jardin botanique national de Belgique* 49: 11–55. <http://dx.doi.org/10.2307/3667815>
- White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelford D.H., Sninsky J.J., White T.J. (eds) *PCR protocols: a guide to methods and applications*: 315–322. New York, Academic Press. <http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1>

Manuscript received 17 Jan. 2015; accepted in revised version 20 Apr. 2015.

Communicating Editor: Myriam Heuertz.