

# *Impatiens msisimwanensis* (Balsaminaceae): Description, pollen morphology and phylogenetic position of a new East African species

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## Abstract

*Impatiens msisimwanensis* S.B. Janssens and E.B. Knox is described as a new species from the Udzungwa Mountains in Tanzania. A comparative morphological study illustrates the affinity between the newly discovered species, *I. nana* and *I. sylvicola*. Using a molecular approach we assessed the phylogenetic position of *I. msisimwanensis* among the East African *Impatiens* species, thereby confirming its status as a new species. In addition, the pollen morphology of *I. msisimwanensis* was thoroughly examined.

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## 1. Introduction

*Impatiens* is one of the largest angiosperm genera comprising over one thousand recognized species with several new species being described each year (e.g. Chen, 2000; Shimizu, 2000; Fischer and Rahelivololona, 2002, 2004; Jin and Ding, 2002; Fischer et al., 2003b; Huang et al., 2004). In Africa alone, nine new species have been described since Grey-Wilson's revision of the African *Impatiens* was published in 1980, bringing the number of African species to 118 (Hallé and Louis, 1989; Bos, 1991; Fischer, 1997; Cheek and Fischer, 1999; Frimodt-Møller and Grey-Wilson, 1999; Cheek and Csiba, 2002; Fischer et al., 2003a; Pócs, 2007). Tropical Africa is one of the diversity hotspots for balsams and is home to one ninth of all known species. *Impatiens* species are a common element of the upland and montane evergreen forests, which have an insular geographic distribution in Africa, being generally surrounded by dry and unsuitable habitats. Therefore, most African balsams are restricted to a limited geographic region or

even a single mountain peak, resulting in a high degree of local endemism. This high frequency of very localized species and subspecies probably results from a combination of a poor long distance dispersal capacity, the plasticity of floral morphological characters and the occurrence of large climatic oscillations during Pliocene and Pleistocene (Grey-Wilson, 1980).

Fieldwork in central Tanzania by the second author in 1996 yielded material of a new species which is described and illustrated here. In addition, detailed pollen morphological observations and inference of its phylogenetic position are included.

## 2. Material and methods

### 2.1. Material studied

Morogoro Region: Kilombero District; Udzungwa National Park, 7.733 S 36.567 E, 22 October 1996, Knox 3461 (BR, IND; Figs. 1 and 2).

### 2.2. Terminology

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

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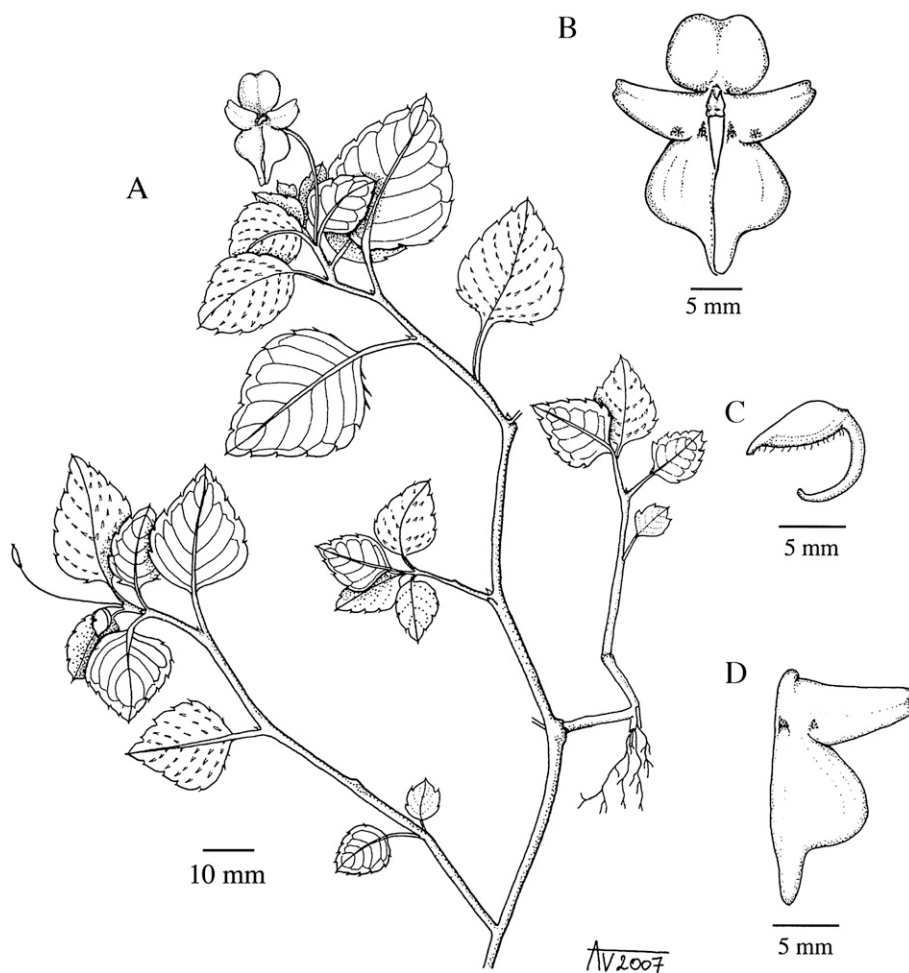


Fig. 1. *Impatiens msisimwanensis*. (A) Habit; (B) Flower; (C) Lower sepal and spur; (D) United lateral petals. Drawn by A. Vandepierre from Knox 3461.

### 2.3. Pollen morphological study

Pollen observations were made following the critical point drying method described by Janssens et al. (2005), with a Jeol JSM-6400 microscope (25 kV). Size measurements of pollen were determined from SEM-micrographs using Carnoy 2.0 (Schols et al., 2002).

### 2.4. Molecular study

In order to find out the phylogenetic position of *Impatiens msisimwanensis*, we used a recent molecular study of the African *Impatiens* (Janssens, 2008). Subsequently, the exact sister group relationship of the new species is then determined by reanalyzing the clade to which *I. msisimwanensis* and its sister species belong.

Methods used for DNA extraction, PCR amplification, sequencing, alignment and data analyses were carried out as described by Janssens et al. (2006, 2007). Taxa that were used to determine the phylogenetic position of *I. msisimwanensis* are listed in Table 1 with inclusion of localities, voucher information and GenBank accession numbers.

## 3. Results

### 3.1. Description of *I. msisimwanensis*

*Impatiens msisimwanensis* S.B. Janssens & E.B. Knox sp. nov. affinis *I. nanae* Engl. & Warb. ex Engl. et *I. sylvicolae* Burt Davy & Greenway sed ab illa differt caulibus glabris, lamina foliarum rhombeis–ovatis subtus glabris atque calcari brevior.

TYPE.—Tanzania: Udzungwa National Park, South Msisimwana Ridge, Knox 3461 (BR!; holo-; IND!, K: iso-).

Decumbent herb up to 20 cm tall; stems green, weakly branched, rooting at the lower nodes, up to 40 cm long, glabrous. Leaves deep green above, very light green below, spirally arranged; petiole 4–12 mm long; lamina 14–27 × 9–23 mm, broadly ovate–rhombic, base broadly cuneate, apex ± acute, midrib and lateral veins above sparsely pubescent, glabrous below; lateral veins 4 to 6 at each side of the midrib, impressed above; margin crenate–denticulate; base with short filiform fimbriae at the base. Flowers solitary, axillary; pedicel up to 25–27 mm long, slender, glabrous; bracts inconspicuous to 1 mm long, subulate. Lateral sepals green, ca. 4.0 × 0.4 mm, linear, sparsely beset with articulate hairs. Lower sepal deep pink with white spur, 6–8 × 5–7 mm, navicular, sparsely pubescent above, abruptly

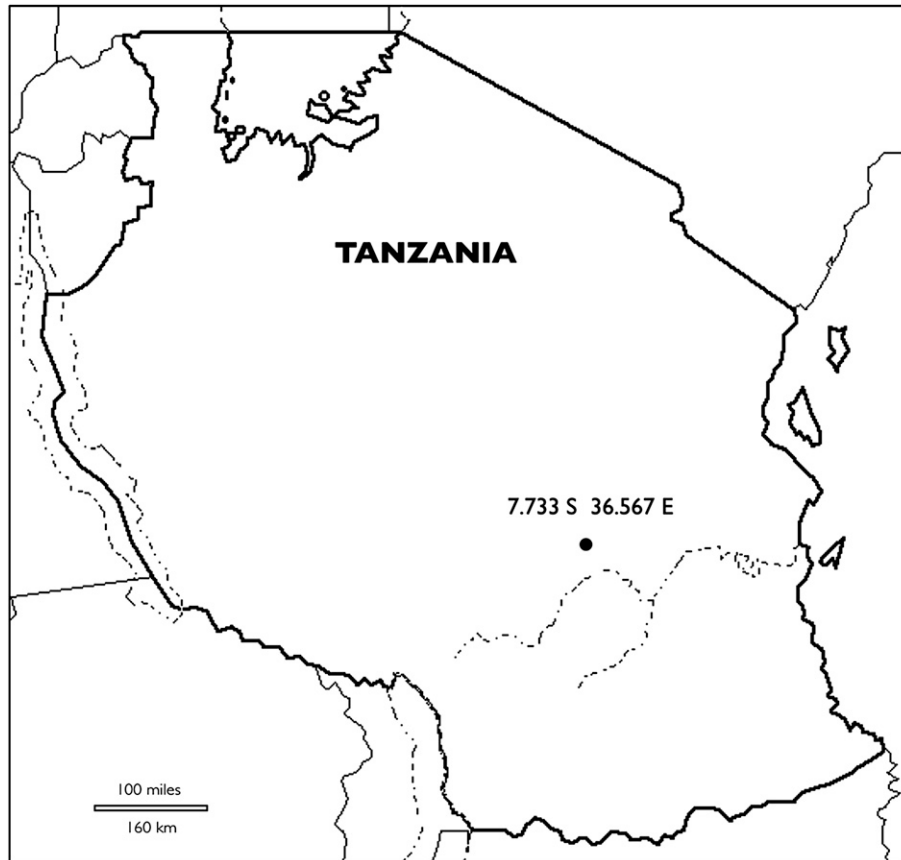


Fig. 2. Locality of the single known collection of *Impatiens msisimwanensis*.

constricted into a short 9–11 mm long curved filiform spur. Dorsal petal deep pink, 7–8×9–10 mm, suborbicular, emarginate, with a narrow dorsal crest terminating in a short acute point, pubescent on the crest. Lateral united petals deep pink with a dark magenta spot at the base of the upper lateral petal of each lateral united pair, 15–21 mm long, with the upper petal of each pair slightly smaller than the lower one; upper petal 8–9×4 mm, oblong; lower petal of each pair 8–9×7 mm, suborbicular, distally drawn out into 4–5×2.0–2.5 mm appendage. Stamens 5, alternating with the petals, connate to a ring. Ovary 5–locular, glabrous. Fruit and seeds unknown.

### 3.2. Phenology

*Impatiens msisimwanensis* flowers in October (based on one record).

### 3.3. Distribution and habitat

*Impatiens msisimwanensis* is endemic to Tanzania, growing in the upland forested area of the Udzungwa Mountains at 1800 m (Fig. 2).

Table 1  
Accession numbers, voucher data and origin of plant material for analysed taxa

Taxon	Origin	Voucher	Accession number <i>atpB-rbcL</i>	Accession number <i>ImpDEF1</i>	Accession number <i>ImpDEF2</i>
<i>Impatiens lukwangulensis</i> Grey-Wilson	Tanzania	Knox 3570 (LV)	EU723709	EU723716	EU723723
<i>Impatiens mazumbaiensis</i> Grey-Wilson	Tanzania	Knox 3643 (LV)	EU723710	EU723717	EU723724
<i>Impatiens msisimwanensis</i> S.B. Janssens & E.B. Knox	Tanzania	Knox 3461 (BR)	EU723711	EU723718	EU723725
<i>Impatiens nana</i> Engl.	Tanzania	Knox 2589 (LV)	EU723712	EU723719	EU723726
<i>Impatiens pallide-rosea</i> Gilg	Tanzania	Knox 3552 (LV)	EU723713	EU723720	–
<i>Impatiens rubromaculata</i> Warb.	Tanzania	Knox 3667 (LV)	EU723714	EU723721	EU723727
<i>Impatiens sylvicola</i> Burt & Davy Greenway	Zimbabwe	Knox 4324 (LV)	EU723715	EU723722	EU723728

### 3.4. Etymology

The specific epithet refers to the first collection site of the species (Msisimwana ridge).

### 3.5. Pollen morphology of *I. msisimwanensis*

Pollen of *I. msisimwanensis* is medium sized (P: 29.1  $\mu\text{m}$ ; E: 28.9  $\mu\text{m}$ ), and is spheroidal in shape (Fig. 3A). The polar outline of the 3-aperturate pollen grains is subcircular (Fig. 3B). Apertures are simple, consisting only of an ectocolpus (length 7.3  $\mu\text{m}$ ; width 2.5  $\mu\text{m}$ ; Fig. 3C). Ectocolpi have acute ends. Pollen grains have a reticulate ornamentation (Fig. 3D), with lumina ranging in size from 2.0  $\mu\text{m}$  to 5.5  $\mu\text{m}$  and being on average 3.9  $\mu\text{m}$  (measured according to the longest axis). The muri are sharply crested in shape. Inside the lumina, granules can be observed on the foot layer (Fig. 3D). Orbicules are absent on the inner locule wall.

### 3.6. Phylogenetic position of *I. msisimwanensis*

Although some loci could not be amplified for a few species, these missing data had no apparent influence in the combined matrix. Ambiguously aligned nucleotides were removed from both chloroplast and nuclear data matrices. The original dataset that is used to locate the clade to which *I. msisimwanensis* belongs, consists of 53 African species (data not shown; Janssens, 2008). While the nuclear *ImpDEF1/ImpDEF2* part of this large African dataset contains 5637 analyzed characters

(818 variable and 478 parsimony informative characters), the chloroplast *atpB-rbcL* part of this large dataset contains 989 analyzed characters (224 variable and 122 parsimony informative characters). The small African subclade to which *I. msisimwanensis* belongs, and which is reanalyzed for this study, includes 7 species. The nuclear part of this small subclade consists of 1177 analyzed characters (48 variable and 22 parsimony informative characters), whereas the chloroplast section contains 708 analyzed characters (13 variable and 5 parsimony informative characters).

When analyzing the small African subclade with Bayesian and Maximum Parsimony methods, we observed that both nuclear *ImpDEF1/ImpDEF2* and chloroplast *atpB-rbcL* data matrices yielded highly congruent trees for both methods. Although *ImpDEF1/ImpDEF2* topologies were much better resolved than the *atpB-rbcL* based topologies, no incongruent relationships were found between these two datasets. Additionally the partition homogeneity test found no significant difference between both partitions of the combined dataset ( $P > 0.05$ ). When evaluating the combined data matrix, Bayesian analysis yielded a highly supported, well-resolved topology. Maximum parsimony analysis resulted in a similar topology with moderately to highly supported branches. The combined dataset was used for further discussions.

*Impatiens msisimwanensis* is part of a small clade of East African species, most of which belong to the *I. rubromaculata* aggregate of Grey-Wilson (1980). Moreover, the majority of its members are restricted to the Eastern Arc Mountains in Tanzania. The clade to which the new species belongs is

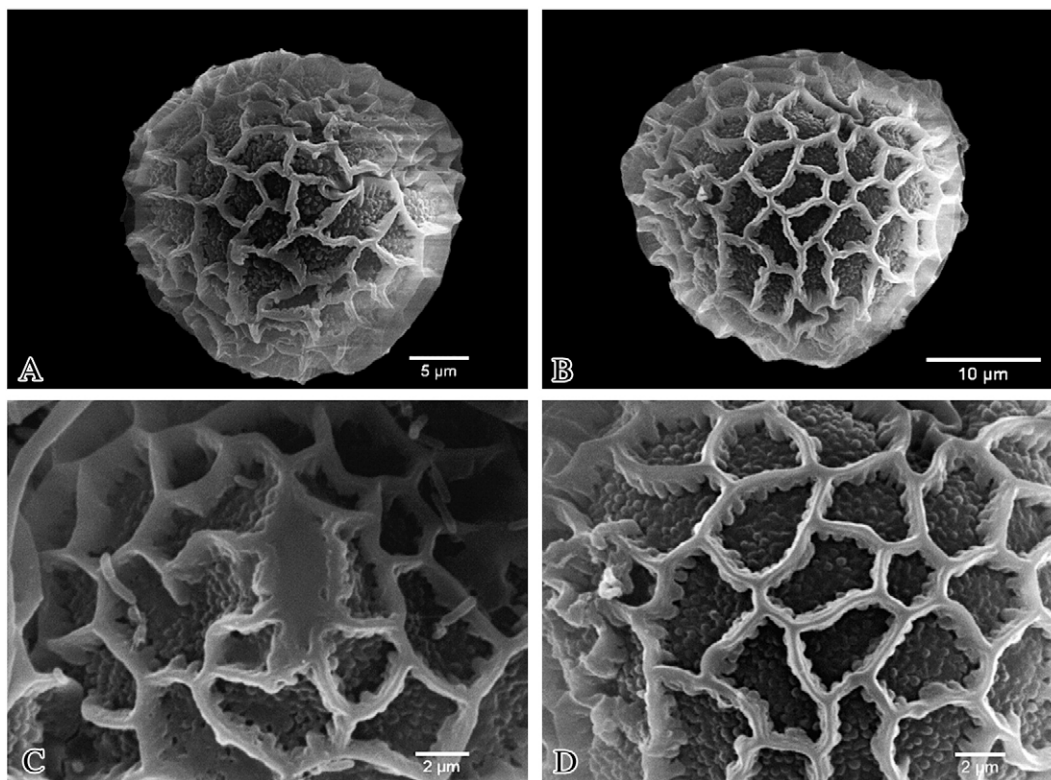


Fig. 3. SEM of *Impatiens msisimwanensis* pollen grains. (A) Equatorial view; (B) Polar view of a reticulate 3-colpate pollen grain with subcircular outline; (C) Detail of short colpus; (D) Detailed view of the reticulate sexine. The muri are sharply crested.

considered to be more closely related to the Madagascan representatives than to other African *Impatiens* species.

Within the small East African subclade that has been phylogenetically reanalyzed in order to avoid interference from more distantly related species, we observed that *I. msisimwanensis* is sister to a clade containing *I. sylvicola*, *I. lukwangulensis* Grey-Wilson, *I. rubromaculata* Warb. and *I. pallide-rosea* Gilg. In addition, a clade comprising *I. nana* and *I. mazumbaiensis* Grey-Wilson is sister to *I. msisimwanensis* and the above-mentioned clade (Fig. 4).

#### 4. Discussion

The morphology of *I. msisimwanensis* is similar to that of *I. nana* and *I. sylvicola* (Table 2). Soon after Engler and Warburg (1894) named *I. nana* from material collected in the Usambara Mountains of northeastern Tanzania (note that the holotype is Holst 224, not 324 as previously reported; Grey-Wilson 1980, 1982), Warburg (1895) described material from nearby Kilimanjaro (*I. papilionacea* Warb.), Taveta (Kenya; *I. tawetensis* Warb.), and a different site in the Usambaras (*I. trichochila* Warb.), and Gilg (1909) named yet another form from the Usambaras (*I. trichantha* Gilg). The variation in *I. nana* has been revealed by many subsequent collections from these and other sites, and these other early names are now regarded as synonyms (Grey-Wilson, 1980). *I. msisimwanensis* differs from *I. nana* in leaf shape, pubescence of the stem and the leaves, spur length, general shape of the lateral united petals, and number of pollen apertures (Table 2). *I. nana* is mainly distributed in northeastern Tanzania at elevations between 400 and 3000 m, but it does extend south to the Udzungwa Mountains where it is found in different habitats, generally at lower elevations than that of *I. msisimwanensis*. In contrast, *I. msisimwanensis* grows sympatrically with another member of this clade, *I. rubromaculata* subsp. *imagiensis* Grey-Wilson, which is readily distinguished from the new species by differences in shape and size of the lateral petals and the spur.

*I. msisimwanensis* can also be easily distinguished from *I. sylvicola* by differences in the shape and margin of the leaves, pubescence of the stem, and general shape of the lateral sepals and the length of the united lateral petals (Table 2). In contrast to *I. nana*, *I. sylvicola* does not have an overlapping distribution with *I. msisimwanensis*, as it only occurs south of Lake Malawi.

The molecular phylogenetic evidence (Fig. 4) places *I. msisimwanensis* within a morphologically coherent clade comprised of species in Grey-Wilson's (1980) *I. rubromaculata* aggregate. Neither of the morphologically most similar species

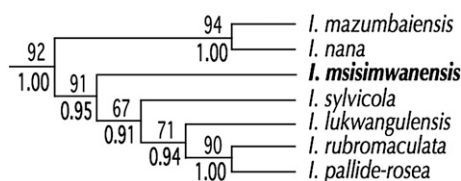


Fig. 4. Phylogenetic hypothesis based on combined *ImpDEF1/ImpDEF2* and *atpB-rbcL* data. Numbers above branches represent bootstrap support while numbers below branches indicate posterior probabilities.

Table 2

Comparison of taxonomically useful characters in *Impatiens msisimwanensis*, *I. nana* and *I. sylvicola*

	<i>I. msisimwanensis</i>	<i>I. nana</i>	<i>I. sylvicola</i>
Habit	Decumbent	Erect	Scrambling or erect
Stem pubescence	Glabrous	Densely pubescent at the apices	Sparsely to densely pubescent
Leaf shape	Ovate–rhombic	Ovate to elliptic, narrowly elliptic or narrowly obovate	Broadly ovate to ovate, (narrowly) elliptic
Leaf margin	Crenate–denticulate	Serrate to crenate–denticulate	Crenate to crenate–serrate
Leaf pubescence	Pubescent on veins adaxially; glabrous abaxially	Sparsely pubescent adaxially, more densely pubescent abaxially	Sparsely pubescent adaxially and abaxially
Pedicel length	25–27 mm	22–52 mm	24–32 mm
Lateral sepals Shape	Linear	Narrowly ovate to narrowly elliptic	Narrowly ovate–triangular
Lower sepal length	6–8 mm	7–10 mm	9–11 (–13) mm
Spur length	9–11 mm	14–20 (–25) mm	11–15 (–18) mm
Dorsal petal size	7–8×9–10 mm	8–11×12–16 mm	7–11×8–13 mm
Lateral united petals length	17–18 mm	15–21 mm	8–14 mm
Upper petal size	8–9×4 mm	7–9×9–12 mm	9–11×6–8 mm
Upper petal shape	Oblong	Ovate or suborbicular to oblong	Broadly and obliquely obovate or oblong
Lower petal size	8–9×7 mm	6–9×7–10 mm	8–10×5–7 mm
Lower petal shape	Suborbicular	Transversely oblong or suborbicular	Suborbicular or broadly obovate
Pollen apertures	3	4	Unknown

(*I. nana* and *I. sylvicola*) is the immediate sister species of *I. msisimwanensis*. This could be due to convergence in floral morphology, but more likely reflects the common ancestral form from which other species in this clade have diverged more rapidly. Intraspecific sampling across the geographic and altitudinal range of these species is needed to reconstruct the evolutionary and biogeographic history of this interesting group.

The present collection together with two other recently described new taxa of *Impatiens* from the same region (Frimodt-Möller and Grey-Wilson, 1999) highlight the importance of the Udzungwa Mountains as a significant evolutionary region for the speciation and distribution of *Impatiens* in the Eastern Arc Mountains and their surrounding regions.

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