

# A NEW VARIETY OF *IMPATIENS PLATYPETALA* (BALSAMINACEAE) IN WEST JAVA, INDONESIA AND ITS RELATIONSHIP USING INTERNAL TRANSCRIBED SPACER

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*Impatiens platypetala* Lindl. (Balsaminaceae) with a small and white flower variant was recently discovered in West Java, Indonesia. This variant was known for some time but rarely found and collected in the field, thus there is little information on this flora. This specimen resembles *I. platypetala* based on morphological characters. Phylogenetic analysis using Internal Transcribed Spacer (ITS) was performed to check its relationship with other *I. platypetala* accessions. The ITS result showed that the small white flower *Impatiens* was nested in *I. platypetala* clade, thus this is only a variant of *I. platypetala*. Therefore, a new variety, *I. platypetala* var. *minialba* Utami has been proposed here. A detailed description, notes on habitat and distribution, phenology, conservation status, and photographs of the small and white flower variety are provided.

Keywords: Balsaminaceae, *Impatiens platypetala*, new variety, white and small flowers, West Java

## INTRODUCTION

*Impatiens* L. (Balsaminaceae) consists of 850–1000 species (Grey-Wilson 1980, Utami & Wiriadinata 2002, Song 2006, Janssens et al. 2012, Utami 2013 & 2014). Most *Impatiens* species are important ornamental plants with economic values and as a source for medicines. The *Impatiens* species are concentrated in five hotspots such as southeast Asia and south western China, eastern to central Himalayas, southern India, tropical Africa and Madagascar (Grey-Wilson 1980). This genus is represented on almost all major Malesian islands, such as Sumatra, Java, Borneo, Sulawesi, the Philippines, and New Guinea (Grey-Wilson 1989). In Indonesia, there are approximately 52 species of *Impatiens* with high species diversity and endemism in Sumatra and Java (Grey-Wilson 1989, Shimizu & Utami 1997, Utami & Shimizu 1998, Utami & Wiriadinata 2010, Utami 2005, 2006, 2009, 2011, 2012a, 2012b, 2013, 2014, 2020 & 2022, Utami & Ardiyani 2015, Utami & Tihurua 2021, Utami et al. 2022).

In Java, there are eight known species of *Impatiens* of which five are endemic to the

island: *Impatiens arriensii* (Zoll.) T. Shimizu, *I. chonoceras* Hassk., *I. javensis* (Blume) Steud., *I. microceras* Backer, and *I. zollingeri* Kuntze, though unfortunately, *I. microceras* has been reported extinct in the wild (Backer & Bakhuizen van den Brink 1963, Utami 2014). Recently, *I. arriensii* was also reported from Vietnam (Lê 2003, POWO 2022) and Thailand (GBIF 2022a). However, as far as we collected and observed, *I. arriensii* is endemic to Madura Island, East Java, Indonesia (Utami 2000). *Impatiens javensis* is reported to occur in Padang, Sumatra, based on the type specimen of *I. sumatrana* Miq. (a synonym of *I. javensis*) (Anonymous s.n., barcode: U 0000714, stored U; GBIF 2022b). A few years ago, Utami found an additional, very small population of *I. javensis* in Padang. The other three *Impatiens* species in Java: *I. balsamina* L., *I. platypetala* Lindl., and *I. walleriana* Hook.f., are widely cultivated as ornamental plants.

*Impatiens platypetala* is distributed in Sumatra, Java, Borneo, Sulawesi, Maluku, Papua New Guinea, the Philippines (Hooker 1909), and

Malaya (POWO 2023). This species has widely been known as an ornamental plant because of its broad and flat flowers which are brightly coloured i.e., purple to pink, yellow to orange. According to Backer and Bakhuizen van den Brink (1963), *I. platypetala* consists of three subspecies, namely *I. platypetala* ssp. *platypetala* (flower is large with different shades of purple color), ssp. *aurantiaca* (Teysm. ex Kds) Steen. (flower is large with salmon-orange color), and ssp. *nematoceras* (Miq.) Steen. (flower is small with pink to purple color). Backer and Bakhuizen van den Brink (1963) also mentioned that *I. platypetala* ssp. *platypetala* with plain white flower and sometime without spur (f. *ecalcarata* Bakh.) is *I. platypetala* var. *nivea* Bakh. ex Steen. The ssp. *aurantiaca* is from Sulawesi and is occasionally cultivated as ornamental plants in Java (Figure 2).

In October 2021, a botanical survey in Cipaniis, West Java, Indonesia, was conducted. We collected a specimen of *Impatiens* with small and white flowers that grew on river banks, that appeared to be the white variant of *I. platypetala*. This specimen matches with another white specimen of *I. platypetala*, *Harry Wiriadinata* HW14274 (BO), collected from Mt. Ciremai National Park in Kuningan, West Java (near Cipaniis) on 19 June 2014. The small- and white-flowered *I. platypetala* is known only from Java and is rare in the wild. Therefore, little information is available regarding this variant. The increase of land conversion in Java is expected to cause the extinction of the wild small- and white-flowered variant, just as *I. microceras* became extinct in the wild due to land use change (Utami 2014).

To gain insights into the relationships within *Impatiens*, we conducted a phylogenetic analysis. Previous research has investigated the phylogeny of *Impatiens* using various markers, including *rbcL* and *trnL-trnF* spacer (Fujihashi et al. 2002), Internal Transcribed Spacers (ITS) (Yuan et al. 2004, Utami & Ardiyani 2015), and the intergenic spacer (IGS) between *atpB* and *rbcL* (Janssens et al. 2006, Utami & Ardiyani 2015). In this study, we focused on utilizing ITS to examine the phylogenetic relationship between the white- and small-variant of *I. platypetala* and other variants, as well as, other species.

## MATERIALS AND METHODS

*Impatiens* explorations were conducted in Cipaniis and Bogor, West Java, Indonesia in 2021, as well as Mt. Singgalang, Sumatra, Indonesia in 2022, with the purpose of collecting DNA samples. DNA of the nuclear region of the ITS of 3 accessions of *I. platypetala* [two from Mt. Singgalang (purplish pink to purple and large flowers) and one from Kuningan (small and white flowers)] and 1 accession of *Impatiens* sp. from Bogor were sequenced. We used *Hydrocera triflora* (L.) Wight & Arn. (Balsaminaceae), *Norantea guianensis* Aubl. (Marcgraviaceae), and *Souroubea* sp. (Marcgraviaceae) as the outgroups based on the publication of Yuan et al. (2004) and Janssens et al. (2006). DNA sequences from another 16 species of *Impatiens* were obtained from the NCBI GenBank (Table 1). We selected these species which were placed in Clades 10–15 based on Utami & Ardiyani (2015) where *I. platypetala* was placed. We did not select other Javan species because we only aimed to see the relationship of small and white variant with other *I. platypetala* accessions. These made a total of 23 accessions for the analysis. All new sequences have been deposited in the NCBI GenBank database.

Total DNA was isolated from the silica-dried leaves following the Geneaid Plant Genomic DNA Purification Mini Kit (Geneaid Biotech Ltd.) protocol. Before the isolation process, we homogenised the tissue into fine powder using a modified reciprocal chainsaw (Alexander et al. 2007). Nuclear DNA sequences of the ITS were amplified using GoTaq<sup>®</sup> PCR Core Systems (Promega). The polymerase chain reaction (PCR) was performed in 12.5  $\mu$ L of reaction mixture containing 10 ng DNA template, 7.5  $\mu$ L GoTaq Promega, 2  $\mu$ M of forward primer ITS5p (5'-GGAAGGAGAAGTCGTAACAAGG-3') and 2  $\mu$ M of reverse primer ITS8p (5'-CACGCTTCTCCAGACTACA-3'). We follow Yuan et al. (2004) cycling conditions. The PCR profile was as follows: a 30 cycle-reaction with denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute and extension at 72 °C for 1.5 minutes, in addition to an initial denaturation at 94 °C for 3 minutes and final extension at 72 °C for 5 minutes. We sent the samples for purification of the PCR product as well as sequencing to 1<sup>st</sup> BASE using ABI PRISM 3730  $\times$  1 genetic analyser (www.base-asia.com).

**Table 1** *Impatiens* and outgroups used in this study

No.	Species	Locality	Voucher	Accession Number (ITS)
1	<i>Impatiens auricomma</i>	Madagascar	Fischer NE1	AY348748
2	<i>Impatiens balsamina</i>	Southern India, South East Asia	Yuan CN2k1-06 (NEU)	AY348749
3	<i>Impatiens balsamina</i> *	Bogor, West Java	NU 2153 (BO)	OQ719829*
4	<i>Impatiens beccarii</i>	Solok, West Sumatra	NU 2051 (BO)	KJ472434
5	<i>Impatiens chinensis</i>	East Asia	Yuan, CN2k1-49 (NEU)	AY348761
6	<i>Impatiens congolensis</i>	Central Africa	Fischer NE7	AY348766
7	<i>Impatiens hians</i>	West Africa	Geuten s.n.	AY348791
8	<i>Impatiens kilimanjari</i>	Africa	JMG 94613 (BR)	AY348800
9	<i>Impatiens niamniamensis</i>	Central Africa	Fischer NE13	AY348812
10	<i>Impatiens</i> sp.	South Sulawesi	NU 2053 (BO)	KJ472446
11	<i>Impatiens platypetala</i>	South Sulawesi	NU 2057 (BO)	KJ472447
12	<i>Impatiens</i> sp.	Takolekayu, Central Sulawesi	MA 155 (BO)	KJ472448
13	<i>Impatiens platypetala</i>	Bali	Ray Morgan s.n.	AY348819
14	<i>Impatiens platypetala</i> *	Mt. Singgalang, West Sumatra	IPGPD 1574 (BO)	OQ719720*
15	<i>Impatiens platypetala</i> *	Mt. Singgalang, West Sumatra	IPGPD 1575 (BO)	OQ719723*
16	<i>Impatiens platypetala</i> *	Kuningan, West Java	NU 2151 (BO)	OQ719799*
17	<i>Impatiens</i> sp.*	Bogor, West Java	NU 2152 (BO)	OQ719828*
18	<i>Impatiens tuberosa</i>	Madagascar	Fischer NE19	AY348844
19	<i>Impatiens usambarensis</i>	East Africa	Fischer NE20	AY348847
20	<i>Impatiens walleriana</i>	East Africa	Fischer NE21	AY348852
21	<i>Hydrocera triflora</i>	Sri Lanka	Robyns 7260, 0249369 (L)	AY348853
22	<i>Norantea guianensis</i>	-	FB/S3779 (BR)	AY348855
23	<i>Souroubea</i> sp.	-	76GR00102 (U)	AY348857

\* indicates newly sequenced taxa

Newly generated forward and reverse DNA sequences were made into contig and checked using the ChromasPro version 1.5 (Technelysium) program. Manual checking by eyes were done based on chromatograms. The consensus sequences were then exported to FASTA format for subsequent analysis. We added sequences from GenBank to the data matrix, which included representatives of *Impatiens* and the outgroups (*H. triflora*, *N. guianensis*, and *Souroubea* sp.). The data matrix was finally realigned using MUSCLE (Edgar 2004) as implemented in MEGA X (Kumar et al. 2018). Minor manual adjustment was done to the automatic alignment result.

Phylogenetic analyses were performed using MEGA X (Kumar et al. 2018) with maximum likelihood (ML). Also, clade support was estimated using bootstrap values which were considered as having strong support when more than 50%. The BLAST was done in the NCBI GenBank to verify the closest entity to our DNA

sequences. Before performing ML analysis, model of evolution was searched using Find Best Model in MEGA X. This was continued by ML analyses using the best model found. Bootstrap was applied using 1,000 replicates.

## RESULTS

The data set contained 23 sequences with the length to 750 bp. Models with the lowest Bayesian Information Criterion (BIC) scores are considered to describe the best substitution pattern (Nei & Kumar 2000, Kumar et al. 2018). Best model of evolution analysis resulted in Kimura 2-parameter with Gamma distribution (+G). Models with the lowest BIC scores are considered to describe the best substitution pattern K2: Kimura 2-parameter, Gamma distributed. Evolutionary analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. A

discrete Gamma distribution was used to model evolutionary rate differences among sites.

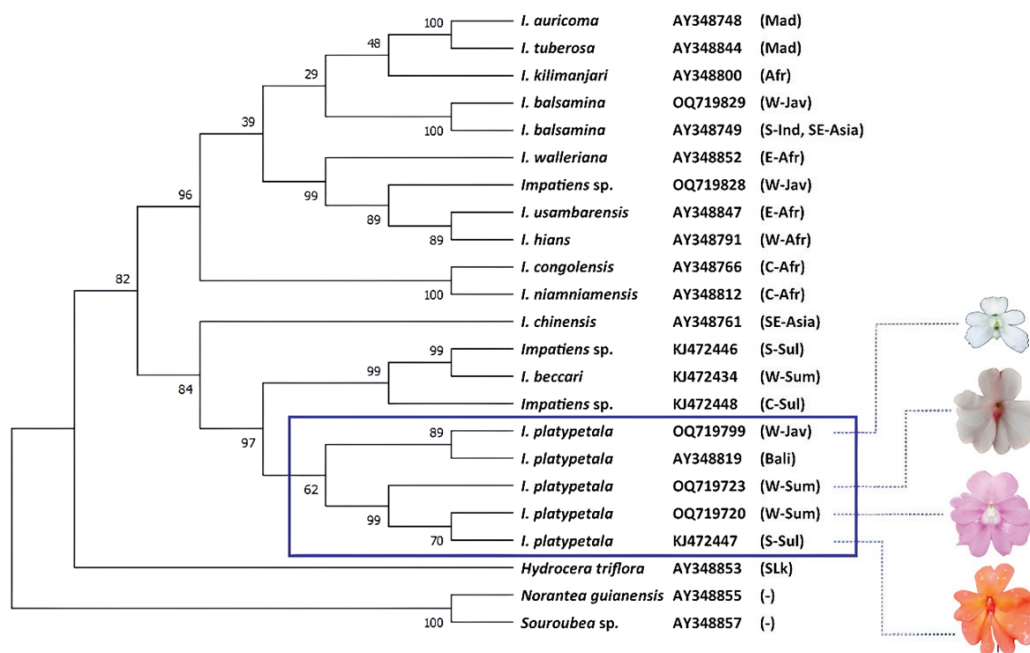
The result of phylogenetic analysis using the ITS data can be seen in Figure 1. *Impatiens platypetala* formed a clade with BS 62 which consisted all *I. platypetala* in this study, i.e., *I. platypetala* OQ719799 from Kuningan, West Java which have small and white flower, *I. platypetala* AY348819 from Bali taken from the GenBank with no information on the flower colour, *I. platypetala* OQ719723 from Mt. Singgalang, West Sumatra which have very light purple (or purplish-white) flower with purple spot, *I. platypetala* OQ719720 from Mt. Singgalang, West Sumatra with purplish pink flower, and *I. platypetala* KJ472447 with yellowish orange flower from South Sulawesi.

### DISCUSSION

*Impatiens platypetala* was first described by Lindley (1846) with a line drawing (Figure 2A). This species, mainly for purple to pink and yellow to orange flowers (Figure 2), is characterized by broad-petaled flowers (4–5 × 5.5–6.5 cm) and verticillate leaves. *Impatiens platypetala* can be distinguished from other Javan species by

several characters such as broad-petaled flower, long spur, and verticillate leaves. The small and white variant also possessed these characters however different in size and colour. A white-flowered variant from Java has smaller flowers (3–3.5 × 2.5–3.5 cm). Fruit size of purple to pink- and yellow to orange-flowered variants are 1.6–2 × 0.4–0.6 cm, whereas that of white-flowered variant is c. 1 × 0.3 cm. The species is perennial, tuberous, glabrous, with leaves broad oblong-lanceolate whorled and subtly serrate along margin. Unifloral peduncles are shorter than the spur and filiform (Lindley 1846).

The molecular study showed that the colour of the flower character was mixed up in the *I. platypetala* clade. The clade with BS 62 consisted of *I. platypetala* with small and white flowers (from West Java), very light purple or pink flowers (from West Sumatra), and yellow flowers (from Sulawesi). *Impatiens platypetala* OQ719799 from Kuningan, West Java had a sister *I. platypetala* AY348819 which was taken from GenBank. This molecular result convinced that the *Impatiens* collected from Kuningan, West Java with small and white flower was included in *I. platypetala*. Thus, a new variety, *I. platypetala* var. *minialba* Utami has been proposed here. A detailed description, notes on habitat and



**Figure 1** Maximum likelihood tree using the Internal Transcribed Spacer; bootstrap support values of 1000 replicates (%) are shown at the nodes. Blue box indicates a clade of *I. platypetala*



distribution, phenology, conservation status, and photographs of the species of small and white flower variant are provided.

### Taxonomic Treatment

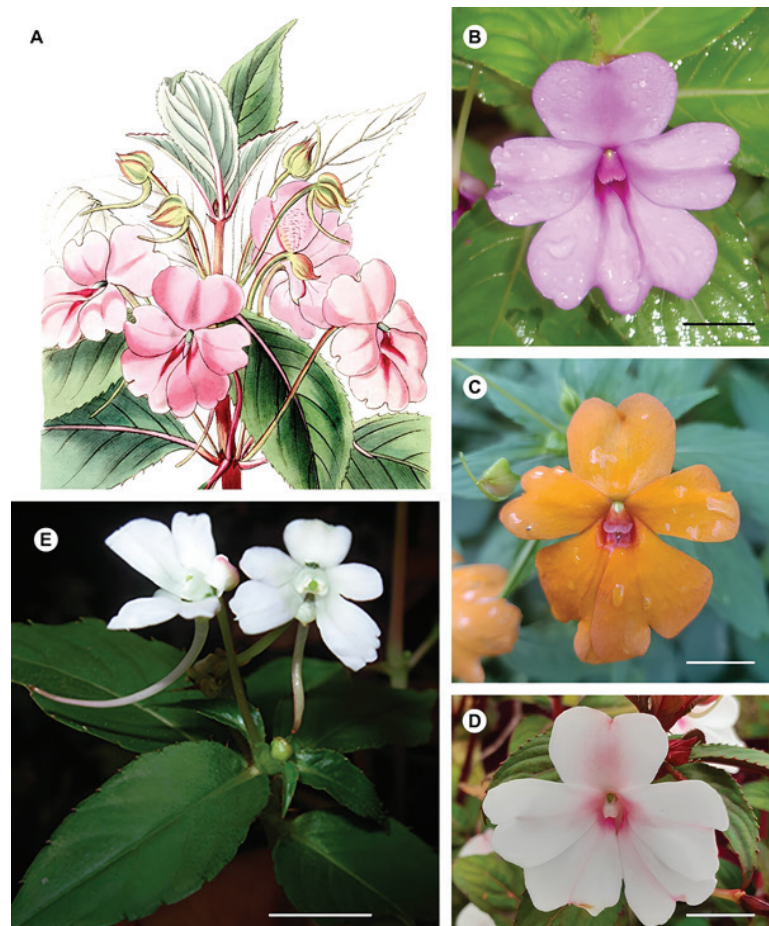
#### *Impatiens platypetala* var. *minialba* Utami var. nov. (Figure 2E)

Type: Indonesia, Java, West Java, Kuningan, Cipaniis, along river side, 6°48'39.0"S; 108°27'15.0"E, 451 m asl, 21 Oct. 2021, Nanda Utami NU2151 (holotype BO).

**Description.** Perennial herb up to 20–25 cm tall; stems erect. Leaves opposite, higher ones in whorls of 3–5, congested towards the apices of the stems; petiole c. 0.5 cm long; lamina ovate to elliptic, 3–3.5 × 2.5–3.5 cm, base acute, margin serrate, apex acute, upper surface pubescent,

lower surface glabrous, lateral veins 3–5 pairs. Flowers 2–3.5 × 1–1.5 cm, white. Pedicel c. 2.5 cm long. Sepals: lateral sepals 2, ovate, c. 0.5 × 0.2 cm; lower sepal deeply navicular, c. 0.5 × 0.3 cm, constricted into a curved spur of 3–4.5 cm long. Petals: dorsal petal cucullate, c. 0.3 × 0.2 cm wide when flattened, with a keel-like crest; lateral united petals free, c. 0.8 × 0.5 cm, upper part of each pair ovate, c. 0.8 × 0.5 cm, lower part of each pair ovate, c. 0.5 × 0.3 cm. Stamens white. Ovary 5 carpels, glabrous. Fruit a capsule, c. 1 × 0.3 cm. Seeds unknown.

**Habitat and distribution.** So far, *I. platypetala* with a small and white flower was only found in West Java, particularly in Cipaniis and Mt. Ciremai National Park. This plant grows in pine forest along streams and rivers in lowland up to 451 m altitude.



**Figure 2** Flower variants of *Impatiens platypetala*: A – Drawing with purplish pink flower; B – Purplish pink flower from Java; C – Yellowish orange flower from Sulawesi; D – very light purple (to purplish white) flower with purple spot from Sumatra; E – Small and white flower from Java. Scale bars = 2 cm. Photos: A from modification of Lindley (1846); B and E taken by Nanda Utami; C by Laode Alhamd; D by I Putu Gede P. Damayanto

**Phenology.** *Impatiens platypetala* var. *minialba* was collected with flowers in June and October.

**Etymology.** The name of this variety refers to the morphological character of small, white flowers.

**Conservation status.** As no other population data are available, the preliminary conservation status of Data Deficient (DD) seems appropriate (IUCN 2012, 2022). The small- and white-flowered variant is only found in West Java and likely more threatened.

**Specimens examined.** INDONESIA. Java, West Java, Kuningan, Cipaniis, along river side, 6°48'39.0"S; 108°27'15.0"E, 451 m asl, 21 Oct. 2021, Nanda Utami NU2151 (BO); West Java, Kuningan, Mt. Ciremai National Park, 19 Jun. 2014, Harry Wiradinata HW14274 (BO).

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