

# A new species of *Viola* (Violaceae) from Jiangxi Province, China

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## Abstract

*Viola ministipulata*, a newly discovered species of Violaceae from the Sanqingshan mountain in Jiangxi Province, China, is described and illustrated here. The identification is based on comprehensive morphological and molecular evidences. While the new species shares similarities with *V. fukienensis* W. Becker in terms of leaf color and stoloniferous habit, it can be differentiated by its smaller leaves and flowers, distinct petal coloration, stamens and lanceolate appendages of equal length, and comparatively smaller and prematurely withered stipules. Additionally, the sepals of *V. ministipulata* feature a distinctive basal appendage.

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**Keywords:** Morphology, phylogeny, sect. *Viola*, subsect.

*Rostratae*

## Introduction

*Viola* L. is the largest genus within the Violaceae, comprising approximately 668 species (Clausen 1929, Huang et al. 2023). Marcussen et al. (2022) have further classified this genus into two subgenera, 31 sections, and 20 subsections. There are 6 sections and 10 subsections in China, including sect. *Chamaemelum*, sect. *Danxiaviola*, sect. *Himalayum*, sect. *Melanium* (subsect. *Bracteolatae*), sect. *Plagiostigma* (subsect. *Australasiaticae*, subsect. *Bilobatae*, subsect. *Bulbosae*, subsect. *Diffusae*, subsect. *Formosanae*, subsect. *Patellares*, subsect. *Stolonosae*) and sect. *Viola* (subsect. *Rostratae*, subsect. *Viola*) (Marcussen et al. 2022). Within *V.* subsect. *Rostratae* (Kupffer) W. Becker, there are 14 species recognized in the *Flora Rerpublicae Popularis Sinicae* (FRPS) (Wang 1991). This subsection is distinguished by capsules that are trigonous, erect at maturity, and have an explosive dispersal mechanism.

*Viola* is widely distributed in temperate regions of both hemispheres and at high elevations in the mountain systems of the subtropics, and China stands out as one of taxonomic and morphological diversity (Marcussen et al. 2022; Ballard et al. 1998, Wahlert et al. 2014). Despite this significance, comprehensive data on the diversity of *Viola* in China are still lacking. Nevertheless, ongoing efforts have led to the continuous discovery of new species (Zhou and Xing 2007, Chen and Yang 2008, 2009, Zhou et al. 2008a, Dong et al. 2009; Ning et al. 2012, Huang et al. 2021, Li et al. 2022, Huang et al. 2023). During our field surveys in southern China in 2022, a putative new species belongs to *V.* subsect. *Rostratae* was collected. After careful morphological and molecular comparison, the new species is described and illustrated it here.

## Materials and methods

Field investigation and observations were conducted during the flowering and fruiting periods of the putative new species. Leaf materials of the putative new species and related species were collected and stored with silica gel in zip-lock plastic bags until use for comparisons and taxonomical treatment. Morphological characters of the putative new species were observed and measured based on fresh and dry specimens using a micrometer and a stereomicroscope. Voucher specimens were deposited in the Herbarium of Sun Yat-sen University (SYS).

Total DNA was extracted with a modified CTAB method (Doyle and Doyle 1987). Regions of the partial internal transcribed spacer 1, the 5.8S ribosomal RNA gene and partial internal transcribed spacer 2 were amplified using the previously reported primers ITS1, ITS4 (White et al. 1990). PCR amplifications were performed following Fan et al. (2015). The sequences of the new species, and related species downloaded from NCBI, were aligned using MEGA ver. 6.0 (Tamura et al. 2013) with ClusterW and subsequently manually adjusted. Phylogenetic constructions were based on maximum likelihood (ML), and was run by Iqtree ver. 2.0.3 (Minh 2020), selecting as best-fit model SYM+ FQ + I with 2000 bootstraps (BS).

## Results

The length of aligned sequences is 870 bps. The new species is a sister species of *Viola fukienensis* on the ML tree (BS=97%, ML), belonging to *V.* subsect. *Rostratae* (BS=99%). Additionally, *V. kosanensis*, *V. arcuminata* and *V. ovato-oblonga* form a monophyletic group (BS=76%), *V. kiangsiensis* form a monophyletic group with *V. fukienensis* (BS=97%), while *V. pendulicarpa* is confirmed to belong to subsect. *Rostratae*.

## Discussion

*Viola ministipulata* (Fig. 2, 3) has previously been misidentified as *V. fukienensis* in China, it is widely distributed throughout the Huaiyu Mountains of southeastern China. The key distinctions between *V. ministipulata* and *V. fukienensis* include significantly smaller flowers (10 × 9 mm versus 14 × 8 mm) and leaves (1.5 – 3.7 cm long versus 1.7 – 4.5 cm long), stamens and lanceolate appendages equal length (instead of ovate-triangular), and smaller, prematurely withered stipules (2 – 6 mm versus 6 – 15 mm).

*Viola fukienensis* from Yongtai (Fujian Province) and *V. kiangsiensis* from Wugongshan (Jiangxi Province), originating from their type localities respectively, form a monophyletic group on the phylogenetic tree. Despite this close relationship, these two species still have conspicuous morphological differences. For example, the abaxial surface of the leaves of the former is purple, while that of the latter is green; the appendages of the sepals of the former are shorter than those of the latter, and the flower color of the former is whiter than that of the latter (Fig. 4). In addition, conserved evolution of ITS sequences is observed in some cases. For example, *V. stewardiana* and *V. grypoceras* with large morphological differences formed a monophyletic group (Gong et al. 2010). Considering both morphological and molecular evidences, further population genetics evidence will be necessary to determine the status of these two species.

We searched Gushan in Fuzhou, Fujian Province, the type locality of *Viola kosanensis*, in May 2023 and found the only species in subsect. *Rostratae* that matched the original description of *V. kosanensis* in terms

of its characteristics. However, this species sometimes produced aerial stems, which *V. kosanensis* did not have according to the original description and type specimen. Marc (2020) reported that some violets had the trait of having erect or sprawling flower stems in spring (from March to May) and dying back in winter (from December to February). And they had a perennial rhizome ending in a cluster of leaves in winter. The type specimen of *V. kosanensis* did not have aerial stems, possibly because it was collected in January, which was winter time. We compared the basal leaves of our specimens with those of the type specimen of *V. kosanensis* and found a high similarity in morphology (see Fig. 5E, F, Table 1), thus our specimens belong to *V. kosanensis*.

The type specimen of *V. fukienensis* also did not have aerial stems in spring when it was collected by Becker. Additionally, the mature plants of *V. kosanensis* have aerial stem and pinnatifid stipules

which resembled *Viola acuminata* instead of *V. fukienensis* (versus stolon stem, stipules lanceolate, margin long fimbriate, abaxial leaves purple) (Fig. 4, 5). This suggested *V. kosanensis* and *V. fukienensis* were different species. The phylogenetic tree also supported this conclusion, as *V. kosanensis* and *V. fukienensis* were distantly related, and *V. kosanensis* and *V. acuminata* formed one clade (see Fig. 1). Thus we confirmed that *V. kosanensis* and *V. fukienensis* should not be synonymized. Furthermore, phylogenetic tree showed that *V. kosanensis*, *V. acuminata* and *V. ovato-oblonga* clustered together, and they had some certain morphological similarities, but there were also conspicuous differences (Table 1).

Considering these observations and findings, *Viola fukienensis* is a good species. The taxonomic status of *V. kosanensis* as a distinct species or a synonym of *V. acuminata* or *V. ovato-oblonga* needs further investigation.

## Taxonomic treatment

*Viola ministipulata* J. M. Dai, Yan S. Huang & Q. Fan, *sp. nov.* (Fig. 2, 3)

### Diagnosis

A species is similar to *Viola fukienensis* W. Becker, but differs in smaller stipules (2 – 6 mm long vs. 6 – 15 mm long), different leaf shape (vs. ovate-triangular to reniform vs. ovate-triangular), conspicuous lateral veins (vs. sunken lateral veins), petals gradually changing from light purple to dark purple (vs. light purple), and lanceolate nectaries of 2 anterior stamens (vs. ovate-triangular).

### Type

China, Jiangxi: Yushan county, Sanqingshan Mountain, in rocky crevices, 28deg54'N, 118deg33'E, 1410 m a.s.l., 03 Mar. 2023, Q. Fan 20017 (holotype: SYS; isotype: SYS).

### Etymology

The epithet refers to the small stipules (2 – 6 mm) of this species.

### Vernacular name

We propose a Chinese name, Xiǎo Tuō Yè Jīn Cài (小托叶堇菜), to reflect the small stipules of the new species.

### Description

Perennial herb, 4 – 8 cm high, erect or obliquely ascending rhizomes, aerial stems absent, rare stolons. Leaves numerous at growing tip of rhizome at anthesis, forming rosette-shaped clusters; stipules light green to brown, lanceolate and smallish, base adnate to the petiole, 2 – 6 × 0.5 mm, margin short fimbriate; petiole 1.9 – 7.8 cm long, glabrous, very narrowly winged in upper part; leaf blade 1.5 – 3.7 × 1.1 – 3.0 cm, cordate, ovate-triangular to reniform, deeply cordate at base, apex obtuse or shortly acuminate, margin distinctly crenate with 13 – 16 teeth, abaxially purple to green, glabrous, adaxially dark green to light green, and with conspicuous marginal veins. Chasmogamous flowers 1 – 4 arising from the terminal rosette; peduncle

4.0 – 5.5 cm long, exceeding length of leaves, purple-green, glabrous, with 2 opposite bracteoles generally slightly above the middle, although they may be lower on cleistogamous flowers produced later in the season; bracteoles 3.5 – 4 mm long, narrowly lanceolate, with fimbriae at base; sepals ovate-lanceolate, apex acute, 3.3 – 6 mm without the appendage; sepal appendage truncated or acute, ca. 1 mm long. Petals light purple to dark purple, upper petals and lateral petals have yellow to green patch at base, the upper two are usually truncated or acute, 5.5 × 2.5 mm, apex obtuse; lateral petals oblong, 6.5 × 2.5 mm, with straight to slightly clavate hairs at base; anterior petals with purple guidelines, ca. 8 mm including spur; spur with rounded end, saccate, 3 mm long and 2.2 mm in diameter. Stamens 5, with appendages 2.2 – 2.5 × 0.9 – 1.3 mm; nectaries of 2 anterior stamens as long as stamens, lanceolate, 2.3 × 0.5 mm. Cleistogamous flowers ca. 6 mm long; peduncles purple, 4-6 cm long; bracteoles linear-lanceolate, ciliate, ca. 4.5 mm long, apex acuminate. Sepals light purple, glabrous, lanceolate, 0.5–1.5 × 5 – 7 mm, with entire margin, acuminate apex; Petal 5, one of them is larger than others, linear-lanceolate, white, 1–1.5 mm long; stamens 5, 1–1.5 mm, terminal appendages and nectar spurs inconspicuous. Ovary green, and style yellow, 3.2 mm high; style narrower and geniculate at base, clavate; stigma enlarged and papillose-hairy at apex, shortly beaked in front. Capsules oblong to trigonous, purple, glabrous. Seeds yellow, ovoid, glabrous, 1.5 – 2 mm long; elaiosomes conspicuous.

### Phenology

Chasmogamous flowers from March to April and December, cleistogamous flowers from May to November, and fruits from May to December.

### Distribution and habitat

*Viola ministipulata* is currently known from Sanqingshan Mountain and Huaiyushan Mountain, Yushan County, Jiangxi Province, China Here, the species is usually growing on rocky crevices on slopes or in ravines at altitudes of 800 – 1410 m a.s.l.

### Conservation status

During our investigation, several populations of *Viola ministipulata* were found with more than 5000 mature individuals in a total area of about 200 km<sup>2</sup>. Most of them are located in the Sanqingshan UNESCO Global Geopark and Huaiyushan National Forest Park, which are well protected and we observed no active threat or continuing decline in population size. Thus, the species should be considered as least concern (LC).

### Additional specimen examined (paratype)

China, Jiangxi: Yushan County, Huaiyu Mountain, 28°53'N, 117°58', 833 m a.s.l., 19 July 2022, H. W. Wang s.n. (SYS).

**Table 1 Morphological differences between the species *Viola ministipulata* and the related species.**

	<i>V. ministipulata</i>
Leaf shape	cordate, ovate-triangular to reniform, deeply cordate at base, apex obtuse or shortly acuminate
stipules	light green to brown, lanceolate and smallish, base adnate to the petiole, 2 – 6 mm long, margin short fimbriate
Rhizome	erect or obliquely ascending rhizomes
nectaries	nectaries of 2 anterior stamens as long as stamens, lanceolate, 2.3 mm.
Capsules	oblong to trigonous, purple, glabrous
seeds	yellow, ovoid, glabrous,

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