# *Viola pendulipedunculata* (Violaceae), a new species from Guangdong Province, China

## Abstract

*Viola pendulipedunculata* (Violaceae), a new species from Baiwan Nature Reserve of Guangdong

Province in China, is described and illustrated. The new species is most similar to *V. nanlingensis* morphologically, but it can be easily distinguished by its narrowly oblong anterior petals (vs spatulate anterior petals), curved and drooping peduncles (vs erect peduncles), and seed with obvious elaiosomes (vs inconspicuous elaiosomes) and the whole plant. Our phylogenetic analysis, based on ITS sequences, confirms that the new species belongs to *V*. sect. *Diffusae,* and mostly related to *V. yunnanensis* in the phylogeny.

Keywords:*Viola* sect. *Plagiostigma* subsect. *Diffusae*, southern China, karst landscapes

## Introduction

*Viola* L. is a large and diverse genus of Violaceae, comprising 664 existing species of herbs, subshrubs, and shrubs, distributed in temperate and subtropical regions of the world. *Viola* species have various morphological, ecological, and economic values, such as ornamental, medicinal, edible, and dyeing plants (Marcussen et al. 2022).

*Viola* sect. *Plagiostigma* subsect. *Diffusae* W. Becker is a group of about 20 species distributed in the southeastern Asia (Marcussen et al. 2022). This subsection is characterized by having perennial rhizomes, rosette apical leaves, pale pink flowers with short appendages and rounded spurs, and seeds without obvious elaiosomes. Subsect. *Diffusae* is one of the most morphologically diverse and taxonomically complex groups within genus Viola. Previous studies have revealed that subsect. *Diffusae* is polyphyletic and has multiple origins from different lineages of sect. *Plagiostigma*, and 9 new species of China have been published in this subsection in recent years, i.e., *V. changii* J. S. Zhou & F. W. Xing, *V. nanlingensis* J. S. Zhou & F. W. Xing, *V. jinggangshanensis* Z. L. Ning & J. P. Liao, *V. guangzhouensis* A. Q. Dong, J. S. Zhou & F. W. Xing, *V. huizhouensis* Yan S. Huang & Q. Fan, *V. qingruii* Yan S. Huang & Q. Fan, *V. heyuanensis* Yan S. Huang, Q. L. Ye & Q. Fan, *V. chaozhouensis* Yan S. Huang, J. H. Ding & Q. Fan, and *V. longissima* Yan S. Huang & Q. Fan (Zhou & Xing 2004, Zhou et al. 2008, Ning et al. 2012, Dong et al. 2014, Huang et al 2019, Huang et al. 2023), thus, the diversity of *Diffusae* species in China has been underestimated.

In this study, we report a new species of *Viola* in subsect. *Diffusae* from Guangdong, which was discovered during a field survey in 2023. The new species differs from other *Diffusae* species by having larger flowers, curved and drooping peduncles, and seed with obvious elaiosome.

We describe and illustrate the new species based on morphological observations and compare it with its closely related species. We also provide ITS sequences to reconstruct the phylogeny. Furthermore, we discuss the conservation status of this new species.

## Material and methods

Field investigations and observations were conducted during the flowering and fruiting periods of the putative new species. Leaf material of the putative new species and their related species, was collected and stored with silica gel in zip–lock plastic bags until use for comparisons and taxonomical treatment. Morphological data for description 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 the modified CTAB method (Doyle and Doyle 1987). The regions of partial internal transcribed spacer 1, 5.8S ribosomal RNA gene and partial internal transcribed spacer 2 were amplified using previously–reported primers ITS1, ITS4 (White et al. 1990). PCR amplifications were performed following Fan et al. (2015). The sequences of the species and related ones, downloaded from NCBI, were aligned using MEGA 6.0 (Tamura et al. 2013) with ClusterW and subsequently manually adjusted. Phylogenetic constructions were based on carried out with Maximum Likelihood (ML), and ML was run by Iqtree 2.0.3 (Minh 2020), selecting best–fit model GTR+I+G with 2000 bootstraps.

Main quantitative characteristics of the putative new species, *V. nanlingensis* and *V. yunnanensis* were statistically analysed using IBM SPSS version 22.0 (Bühl et al. 2014).

## Results

## Taxonomic treatment

***Viola pendulipedunculata* Yan S. Huang & Q. Fan, sp. nov. (Fig. 1, 2)**

***Diagnosis***

A species most similar to *Viola nanlingensis,* but differing in narrowly oblong anterior petals (vs. spatulate anterior petals), curved and drooping peduncles (vs. erect peduncles), and seed with obvious elaiosomes (vs. inconspicuous elaiosomes).

**Type:** China. Guangdong: Qingyuan City, Qingxin District, Baiwan Nature Reserve, in rocky crevices, 24.22°N, 112.80'E, 384 m a.s.l., 25 April 2023, Y. S. Huang 23042501 (holotype: SYS; isotype: SYS).

***Etymology***

The specific epithet refers to its curved and drooping peduncles during fruiting period.

***Vernacular name***

We propose a Chinese name, Bái Wān Jǐn Cài (白湾堇菜), to reﬂect the type locality of the new species.

***Description***

Annual or rarely perennial, stoloniferous herbs with basal leaves rosulate, ca 5-10 cm tall. Rhizome erect or obliquely erect, rather slender, pale white, with dense remains of stipules and petioles. Lateral stolons rare, with an apical rosette of leaves, usually producing adventitious roots. Stipules adnate to petioles about 1/3 at base, linear–lanceolate, 5–9 mm, acute at apex, with margins pinnatifid, glabrous. Petiole 4.0–7.5 cm, with wings narrow, sparsely pubescent along the margin. Leaf blade ovate or ovate–oblong, 1.5 – 2.9 × 3.0 – 4.9 cm, glabrous or sparsely pubescent along veins and margin; margin finely crenate; base wedge-shaped to truncated; apex obtuse. Flowers ca 2.5 cm in diam.; peduncle slender, 10–15 cm long, glabrous, with two opposite bracteoles above middle, usually bend and droop during fruiting period; bracteoles lanceolate, densely pubescent along the margin, ca. 8 mm long, apex acuminate or obtuse. Sepals green, with white spots, pubescent along the margin, lanceolate, 2.5 × 5 – 6 mm, with entire margin, acuminate apex, and truncate or rounded base, with extremely short semicircular appendages. Petals purple, anterior ones with apparent violet lines, posterior and lateral ones with a yellow to green patch at base; posterior petals, narrowly ovate, 1.35 cm long, glabrous, with entire margin and obtuse apex; lateral petals with straight to slightly clavate hairs at the base, oblong, 1.4 cm long, with entire margin and obtuse or erose apex; anterior petal narrowly oblong, with a short saccate spur at base, base puberulent, including spur 1.3 cm long, with entire margin and acuminate apex. Stamens 5, unequal, puberulent; anther thecae 1.8 – 2.2 mm long, with terminal appendages ca 1.5 mm long; posterior appendages (nectar spurs) of the two anterior stamens ca 1.5 mm long, triangular. Ovary ovoid to ellipsoid, ca. 1.5 mm, glabrous; style clavate, ca 1.6 mm long, conspicuous geniculate at base; stigma with thickened lateral margins, shortly beaked at apex. Capsule puberulent, light green to brown at maturity, ovoid to oblong, ca 9 mm long. Seeds brown, oblong, with obvious elaiosomes, ca 1 mm long.

***Phenology***

Chasmogamous flowers from April to May, cleistogamous flowers from May to September, and fruits from April to September.

***Distribution and habitat***

*Viola pendulipedunculata* is currently known only from the type locality, Baiwan Nature Reserve, Guangdong Province, China. The species grows on rocks and in rocky crevices of Karst landscape at altitudes of 300–500 m a.s.l.

***Conservation status***

Due to no subpopulations with more than 250 mature individuals and only be found in the type locality, *Viola pendulipedunculata* should be considered as Endangered (EN).

## Phylogeny

The aligned length of our ITS sequences was 725 bps in total. The putative new species belongs to the monophyletic *Viola* subsect. *Diffusae* (BS = 91%, Fig. 3), and forms a sister group with *V. yunnanensis* (BS = 83%)*.*

## Table 1 **Morphological differences between the species *Viola pendulipedunculata*, *V. yunnanensis* and *V. nanlingensis***

|  |  |  |  |
| --- | --- | --- | --- |
| characters | *Viola pendulipedunculata* | *V. yunnanensis* | *V. nanlingensis* |
| Leaf shape | narrowly-oblong to narrowly-triangular, base truncate or cuneate, apex acute, long-decurrent on petioles, glabrous or puberulous along margin | oblong or oblong-ovate, broadly cordate at base, apex acute, never decurrent on petioles, both surfaces densely gray-white puberulous | ovate to elliptic, broadly cordate at base, apex obtuse, narrowly long-decurrent on petioles, glabrous or puberulous along margin |
| Anterior petal | oblong-lanceolate | oblong-lanceolate | oblong spatulate |
| Rhizome | obliquely ascending or erect, with short internodes | elongate, obliquely ascending or procumbent and stemlike, slender | short, with numerous white rootlets and fibrous roots |
| peduncles | glabrous, usually bend and droop during fruiting period | puberulous, erect, curved at the end, lifted when capsule dehisces | glabrous, erect, curved at the end, lifted when capsule dehisces |
| seed | ovate, with tubercles on the surface and obvious elaiosome | globose, without tubercles, elaiosome absent | ovate, with tubercles on the surface and inconspicuous elaiosome |

## Table 2 Quantitative characteristics and significant difference analysis of the *species Viola pendulipedunculata, V. yunnanensis* and *V. nanlingensis*

|  |  |  |  |
| --- | --- | --- | --- |
| characters | *Viola pendulipedunculata* | *V. yunnanensis* | *V. nanlingensis* |
| *Lp* | 22.3±10.4 | 29.0±10.4 | 52.7±13.4 |
| *W* | 13.0±4.40 | 23.3±5.40 | 25.2±3.50 |
| *L* | 28.9±5.33 | 44.4±9.06 | 38.0±5.83 |
| *L*/*W* | 1.88±0.505 | 1.91±0.110 | 1.52±0.225 |
| *D* | 6.35±1.45 | 1.60±0.173 | 2.78±0.808 |

Note: *LP* = length of petiole; *W* = lamina width; *L* = lamina length; *D* = rhizome diameter. Independent-Sample Kruskal-Wallis Test was used and characters of rhizomes and basal leaves were measured for each species, all quantitative characteristics representing significant difference at the 0.1% nominal level.

## Discussion

In the ML tree, the new species and *V. yunnanensis* form a sister group, despite their different morphological traits, such as the leaf base of *V. pendulipedunculata* being conspicuously decurrent to the petiole, while that of *V. yunnanensis* being inconspicuous; and *V*. *pendulipedunculata* having purple flowers (vs. white flowers). Morphologically, the new species is similar to *V. nanlingensis*, such as nearly glabrous, thin leathery leaves and larger flowers. However, it could be easily distinguished from *V. nanlingensis* by its narrowly oblong anterior petals (vs spatulate anterior petals), curved and drooping peduncles (vs erect peduncles), and seeds with obvious elaiosome (vs inconspicuous elaiosomes). Furthermore, *V. pendulipedunculata* was distantly related to *V. nanlingensis* in the phylogenetic tree (Fig. 3).

The new species described here are distinct from most other *Diffusae* species by the curved and drooping peduncles (vs erect peduncles) and developed elaiosomes (vs inconspicuous elaiosomes). As an adaptation to obligate myrmecochory and a consequence of predator pressure (Beattle& Lyous. 1975, Marcussen et al. 2022), these morphological traits were usually observed in the subsect. *Viola*, i.e., *Viola odorata*. Other *Viola* species use a combination of explosive seed dispersal and partial ant exploitation, though the species of subsect. *Diffusae* are mainly ejecting seeds, i.e., *V. qingrui* (Huang et al. 2023). The unique traits of the new species might indicate adaptive evolution to the karst landscape.

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