

Veronicastrum wulingense (Plantaginaceae), a new species from Southwestern Hubei, China

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Abstract

Background: *Veronicastrum*, perennial herbs, the traditional herbs treated ascites diseases that caused by schistosomiasis in China, are mainly distributed in East Asia, and only *Veronicastrum virginicum* (L.) Farw. is disjunctively distributed in eastern North America. The south area of China (including Taiwan Island) is the richest in *Veronicastrum* species. During a field investigation of plant resources in Pingbaying National Forest Park, Southwestern Hubei, China, an unknown flowering population of *Veronicastrum* was found and collected on the thick humus layers adjacent to rocks under broad-leaved forests by walkways.

Results: This new species is similar to *V. axillare* and *V. rhombifolium*, but can be distinctly differs by these diagnostic characteristics of peduncles up to 7 cm, flowers clustered densely in rachis apex and steadily ca. 2–3 cm long, and pedicels up to 2.5 mm, which are unique in the all known *Veronicastrum* species of axillary inflorescences.

Conclusions: This new species *Veronicastrum wulingense* is supported by morphological and molecular evidence, and its plastid genome is reported in this study.

Background

Veronicastrum Heist. ex Fabr. is a genus composed of perennial herbs in the family Plantaginaceae in Lamiales (The Angiosperm Phylogeny Group 2016; Li et al. 2021). It comprises about 20 species worldwide, which mainly distributed in the East Asia (Hong et al. 1998), while only *Veronicastrum virginicum* (L.) Farw. is disjunctively distributed in the eastern North America. Morphologically, *Veronicastrum* species are very similar to the genus *Veronica* L., but can be distinguished from *Veronica* by equally 5-lobed sepals, inside of corolla being densely hairy, reticulated ellipsoid seeds, hairy filaments which are usually longer than corolla (Yamazaki 1957; Albach and Chase 2001), and different pollen (Hong 1984). Many species of this genus are important sources of some medicines and can be used to treat ascites diseases caused by schistosomiasis as traditional herbs in China (Chin and Hong 1979).

Previously, *Veronicastrum* species were placed in the genus *Veronica* in the tribe Veroniceae in Scrophulariaceae sensu lato, and later it was segregated from *Veronica* as a new genus by von Wettstein (1891) according to the significant morphological difference between them. However, these two genera, though separate, were still considered to be closely related (Albach and Chase 2001). The taxonomic position of *Veronicastrum* in Lamiales has changed considerably with the wide application of molecular systematics since its original circumscription. A series of molecular phylogenetic studies have revealed that the traditional delimitation of Scrophulariaceae was a complex polyphyly rather than monophyly, and found the Veroniceae was closely related to *Plantago* L. in almost all molecular analyses (Olmstead and Reeves 1995; Freeman and Scogin 1999; Olmstead et al. 2001; Albach et al. 2001; Xiao et al. 2020). Therefore, the Veroniceae clade was transferred to Plantaginaceae in the classifications of the

Angiosperm Phylogeny Group (APG) (The Angiosperm Phylogeny Group 2003) and gradually accepted by the taxonomic domain (Albach et al. 2005; Tank et al. 2006).

In East Asia, the genus *Veronicastrum* was divided into four sections in *Flora Reipublicae Popularis Sinicae*: Sect. *Cdlorhabdos* (Chin and Hong, 1979), Sect. *Plagiostachys*, Sect. *Pterocaulon*, and Sect. *Verouicastrum* (Yamazaki, 1957). In recent years, two new species of *V. loshanense* (Chen and Chou, 2008) and *V. nogerchii* (Ueharai et al, 2013) were found and published from Taiwan Island (China) and Chiba Prefecture (Japan), respectively. Until now, a total of 19 *Veronicastrum* species are known in the world according to The World Checklist of Vascular Plants (WCVP) (<https://wcsp.science.kew.org/>) (Govaerts et al. 2021).

During a field investigation of plant resources in Pingbaying National Forest Park, Xianfeng County, Southwestern Hubei, China, in June, 2021, an interesting flowering population of *Veronicastrum* was found and collected on the thick humus layers adjacent to rocks under broad-leaved forests by walkways, whose flowers densely clustered at rachis apex and steadily ca. 2–3 cm long, and whole plants showed purple-red when young and covered by densely short curly hairs. Our identification based on morphological characters using the available literature and herbarium specimens did not match with any of the known species of *Veronicastrum*. Phylogenetic analysis also supported the status of the collected species as a separate and new taxon. We thus describe it as a new species with illustration and name it *Veronicastrum wulingense*.

Methods

Morphological and taxonomic analyses

The morphological description of *Veronicastrum wulingense* was sourced from the observation and measurement of living plants, photographs taken during fieldwork, and designated type specimens. The whole living plants and inflorescences and dissected flowers were photographed carefully. The comparison of morphological features was implemented between the new species and other related *Veronicastrum* species based on *Flora of China*, *Flora of Taiwan*, related literature, and specimen examination from the virtual herbarium from China and abroad. Also, 14 *Veronicastrum* species materials from different places and *Pseudolysimachion spicatum* (L.) Opiz in China were collected, photographed, and specimens prepared. The color photo plate and hand drawing illustration of the new species were shown in Figs. 1 and 2, respectively. The morphological comparison of diagnostic characteristics between *V. wulingense* and the two most similar *Veronicastrum* species are provided (Table 1). The voucher specimens of the new species were deposited in the herbarium of Wuhan Botanical Garden (HIB), Chinese Academy of Sciences.

Table 1

Morphological comparison of the diagnostic characteristics between *Veronicastrum wulingense* and two similar *Veronicastrum* species.

Species/Characters	<i>Veronicastrum wulingense</i>	<i>Veronicastrum axillare</i>	<i>Veronicastrum rhombifolium</i>
Plant habit	Arching and rooting apically	Arching and rooting apically	Usually erect, rarely trailing and rooting
Stem	Terete, not angular, with densely short curly hairs	basally terete, angular, glabrous, rarely sparsely hairy on angles	Angular, with multicellular hirsute hairs
Petiole	Densely short curly hairs	Glabrous	Multicellular hirsute hairs
Leaf	Ovate to ovate-lanceolate, 3–12 × 2–6 cm, blade abaxially purple-red, sparse white pubescent to nearly glabrous	Ovate to ovate-lanceolate, 5–12 × 3–6 cm, glabrous	Rhomboid, rarely rhomboid-ovate, 4–6 × 1.5–3 cm, multicellular hispid hairs, sometimes abaxially purple
Serration	Undate, irregular, cuspidate to long apiculate dentate, apex upward, or crenate dentate	Obliquely deltoid dentate, apex acuminate	Serrate to crenate
Inflorescence	Axillary, 4–9 cm, peduncle 1–7 cm, rachis densely short curly hairs, usually surrounded by several small leafy involucre bracts in middle-upper part, flower cluster dense in peduncle apex, steadily ca. 2–3 cm	Axillary, rarely terminal, 1–3 cm, rachis glabrous, peduncle short to nearly lacking	Axillary, sometimes surrounded by several leafy involucre bracts and appearing terminal, 1–3 cm, rachis multicellular hispid hairs, peduncle nearly lacking to obviously stretched
Flower	Purple-red, pedicellate to subsessile (0–2.5 mm), with densely short curly hairs, corolla 5.5–7 mm	White, purple, or purple-red, sessile, corolla 4–5 mm	Purple, red, or white, sessile, corolla 5–6 mm
Corolla lobes	Ovate-triangular to narrowly triangular, 2/5–1/2 of corolla length	Ca. 2 mm, narrowly triangular	Subulate-triangular, 1/3–1/2 of corolla length

Specimens examined of *Veronicastrum* species

Veronicastrum axillare (Sieb. et Zucc.) Yamazaki, China: Zhejiang, Tianmu Mountain, August-31-1959, Zhejiang Plant Resource Survey Team, 29414 (PE, 01474974).

Veronicastrum stenostachyum (Hemsl.) Yamazaki, China: Sichuan, Emei Mountain, August-24-1980, Z.Y. Wu, et al., 0–22 (PE, 01493492).

Veronicastrum rhombifolium (Hand.-Mazz.) Tsoong, China: Sichuan, Mao County, July-30-1930, F.Z. Wang, 21941 (PE, 01475814).

Taxon sampling and DNA extraction, sequencing

In this study, 15 samples (12 taxa including *Veronicastrum wulingense* and *Pseudolysimachion spicatum* were newly sequenced to construct a phylogenetic tree to reveal the position of the new taxon in *Veronicastrum* (the voucher information of the 15 samples is listed in Table S1). Total genomic DNA was extracted from dry leaf materials that were kept in silica gels for preservation (Chase and Hills 1991) using a modified procedure of CTAB (cetyltrimethylammonium bromide) (Doyle and Doyle 1987; Li et al. 2013). The purified genomic DNA was fragmented to construct short-insert libraries for sequencing based on the Illumina paired-end technology platform (HiSeq-PE150 strategy) in the Novo gene Company (Beijing, China), and 6-GB reads of genome skimming data were obtained.

Plastid genome assembly, annotation

Initially, the 16 plastid genomes were assembled using GetOrganelle v1.7.2 with appropriate parameters (Jin et al. 2020). The Bandage software was used to visualize the final assembly graphs to evaluate the completeness and accuracy of the assembled plastid genomes (Wick et al. 2015). Next, the assembled plastid genomes were annotated and two inverted repeat (IR) regions were found using PGA software with *Amborella trichopoda* (Accession: AJ506156.2) Baill. and *Veronicastrum axillare* (Accession: NC_056895.1) as the references (Qu et al. 2019). We then checked the annotated genes and protein-coding regions and corrected detected errors manually using Geneious-v10.2.3 (Kearse et al. 2012). The information of plastid genome features of *V. wulingense* was also analyzed in the software Geneious. The circular plastid genome map of *V. wulingense* was drawn and visualized in OGDRAW online software (<https://chlorobox.mpimp-golm.mpg.de/OGDraw.html>) (accessed on 24 December 2021) (Greiner et al. 2019).

Sequence alignment and phylogenetic analysis

The phylogenetic position of *Veronicastrum wulingense* in the genus *Veronicastrum* was analyzed based on the maximum likelihood (ML) method that was performed by the IQ-TREE program with 1000 bootstrap replications in the software PhyloSuite-v1.2.2 (Nguyen et al. 2015; Zhang et al. 2020; Minh et al. 2013). To maximize the use of currently available and limited molecular DNA data of *Veronicastrum* species from our collection and NCBI online database, the concatenated molecular markers of *matK*, *rbcL*, and *trnH-psbA* were selected to reconstruct phylogenetic tree in the subfamily Plantagoideae including 15 newly sequenced samples (Table S1), 18 available plastid genomes from the NCBI database (Table S2), and three concatenated sequences (nine molecular markers of foreign species) (Table S3), of which outgroups were *Callitriche palustris* L. and *Hippuris vulgaris* L. The molecular markers of the 33 plastid genomes were extracted using Geneious-v10.2.3 (Kearse et al. 2012). Firstly, the 36

sequences of different molecular markers were aligned using the program MAFFT-v7.409 (Kato and Standley 2013), respectively. Next, three different dataset matrix of 38 sequences were concatenated using the program Concatenate Sequence in PhyloSuite-v1.2.2. Finally, The Partitionfinder 2 program was used to find the best-fit partition model of the two matrix sequences according to the Akaike information criterion (AIC) for ML analysis (Lanfear et al. 2017).

Results

Taxonomic treatment

Veronicastrum wulingense G.W. Hu & Q.F. Wang sp. nov. (Figs. 1 and 2)

Type. CHINA. Hubei, Enshi Tujia and Miao Autonomous Prefecture, Xianfeng county, Pingbaying township, Pingbaying National Forest Park, Sidong Gorge, elev. 1324 m, 23 Jun, 2021, S.X. Ding, H. Jiang, F.M. Mutie, G.W. Hu PBY-346 (holotype: HIB!, isotypes: HIB!).

Diagnosis. *Veronicastrum wulingense* belongs to the Sect. *Plagiostachys* species (Chin and Hong, 1979). Morphologically, it is similar to *V. axillare* and *V. rhombifolium*, but can be distinctly differs by these diagnostic characteristics of peduncles up to 7 cm, flowers clustered densely in rachis apex and steadily ca. 2–3 cm long, and pedicels up to 2.5 mm (Table 1), which are unique in the all known *Veronicastrum* species of axillary inflorescences.

Description. Herbs, perennial. Rhizomes short, horizontal. Stems densely short curly hairs, up to 90 cm long, terete, without angles, basally branched, arching and rooting apically. Leaves alternate, petioles short, often purple-brown, no more than 5 mm long, with short curly hairs. Leaf blade thick papery to leathery, ovate to ovate-lanceolate, 3–12 × 2–6 cm; base rounded, rarely broadly cuneate, apex acute to shortly acuminate; margin serrations undulate, irregular, cuspidate to long apiculate dentate, apex upward, or crenate. Leaf blades abaxially purple-red, sparse white pubescent to nearly glabrous on both surfaces, densely on veins; veins clear, lateral veins 4–6 pairs, concave on adaxial surface and convex on abaxial surface. Spicate inflorescences axillary, 4–9 cm long; peduncles 1–7 cm long, usually surrounded by several small leafy involucre bracts in middle-upper part; flowers pedicellate to subsessile (0–2.5 mm long), densely clustered at rachis apex, steadily ca. 2–3 cm long; peduncles, rachis, and pedicels densely short curly hairs. Calyxes deeply 5-lobed, one adaxial lobe smaller, bracts and calyx lobes linear lanceolate to long subulate, conspicuously shorter than corolla, densely short ciliate. Corollas purple to purple-red, 5.5–7 mm long; corolla tubes tubular, straight, inner surface crinite; corollas equally 4-lobed, lobes all straight, actinomorphic, ovate-triangular to narrowly triangular, 2/5–1/2 of corolla length. Stamens 2, slightly to conspicuously exserted, exceeding corolla by ca. 2–3 mm long, crinite at lower middle part; anthers orange-yellow, oblong, 1–1.5 mm long; anther locules connivent, not confluent; ovaries glabrous, stigmas small and slightly dilated, styles 5–7 mm long. Capsules ovoid-globose, 3–3.5 mm long, 2-grooved, 4-valved; style, bracts, and calyx persistent. Seeds numerous per capsule, small, oblong, seed coat reticulate. Fl. Jun–Jul, Fr. Aug–Oct.

Distribution and habitat. The new species *Veronicastrum wulingense* is currently known only from Sidong Gorge of Pingbaying National Forest Park in the Northcentral of Wulingshan Region, Southwestern Hubei, China—its type locality. All our collections were made on the thick humus layers adjacent to rocks under broad-leaved forests by walkways, at elevations of 1200–1400 m above sea level..

Etymology. The specific epithet '*wulingense*' refers to Wulingshan Region, where the new species is distributed. The Chinese name is 'Wu Ling Fu Shui Cao ()'.

Phenology. Based on our field surveys, the new species was observed flowering from June to July, and fruiting from August to October.

Conservation Significance. *Veronicastrum wulingense* is an endemic species currently only known from the type locality in China. Its distribution area is very narrow and only known few populations are known. Therefore, we recommended that this species be treated as a protected plant in China, and protect its habitat well.

The plastid genome features of *Veronicastrum wulingense*

The complete plastid genome of *Veronicastrum wulingense* is 152,370 bp in length and portrays a circular and quadripartite structure, typical of most angiosperms, including a large single-copy (LSC) region (87,034 bp), a small single-copy (SSC) region (18,492 bp), and a pair of inverted repeat (IR) regions (25,980 bp) that separate the LSC and SSC regions (Fig. 3). The GC content is 38.3% in the whole plastid genome, while that in the LSC, SSC, and IR regions are 36.5%, 32.3%, and 43.3%, respectively, which is relatively higher than the reported *Veronica persica* Poir. and *Veronica nakaiana* Ohwi. (related genus of *Veronicastrum*) in LSC and SSC regions (Choi et al. 2016). The GC content in IR regions is significantly higher than that in LSC and SSC, which may be due to the tRNA and rRNA genes that occupy a higher proportion of the regions and have a relatively higher GC content. A total of 132 functional genes were annotated in the plastid genome of *V. wulingense* and can be divided into four categories and subdivided into 18 groups (Table 2), which include 87 protein-coding genes (PCGs), 37 tRNA genes, and 8 rRNA genes (duplicated in two IR regions: 7 PCGs, 7 tRNA genes, and 4 rRNA genes). Also, 18 genes with introns were annotated, including 12 PCGs (*clpP* and *ycf3* genes contain two introns) and six tRNA genes. Additionally, the start codon of *rps19* gene transcription mutated and is non-canonical "GTG" instead of the most common "ATG".

Table 2

List of the annotated genes in the chloroplast genomes of *Veronicastrum wulingense*.

Category	Groups of Genes	Name of Genes
Self-replication	Ribosomal RNA	<i>rrn4.5^c, rrn5^c, rrn16^c, rrn23^c</i>
	Transfer RNA	<i>trnA-UGC^{a,c}, trnC-GCA, trnD-GUC, trnE-UUC, trnF-GAA, trnG-GCC, trnG-UCC^a, trnH-GUG, trnI-CAU^c, trnI-GAU^{a,c}, trnK-UUU^a, trnL-CAA^c, trnL-UAA^a, trnL-UAG, trnM-CAU, trnM-CAU, trnN-GUU^c, trnP-UGG, trnQ-UUG, trnR-UCU, trnR-ACG^c, trnS-UGA, trnS-GCU, trnS-GGA, trnT-GGU, trnT-UGU, trnV-UAC^a, trnV-GAC^c, trnW-CCA, trnY-GUA</i>
	Small subunit of ribosome	<i>rps2, rps3, rps4, rps7^c, rps8, rps11, rps12^{a,c}, rps14, rps15, rps16^a, rps18, rps19</i>
	Large subunit of ribosome	<i>rpl2^{a,c}, rpl14, rpl16^a, rpl20, rpl22, rpl23^c, rpl32, rpl33, rpl36</i>
	RNA polymerase subunits	<i>rpoA, rpoB, rpoC1^a, rpoC2</i>
Photosynthesis	Photosystem I	<i>psaA, psaB, psaC, psal, psaJ</i>
	Photosystem II	<i>psbA, psbB, psbC, psbD, psbE, psbF, psbH, psbl, psbJ, psbK, psbL, psbM, psbN, psbT, psbZ</i>
	Subunits of cytochrome	<i>petA, petB^a, petD^a, petG, petL, petN</i>
	ATP synthase	<i>atpA, atpB, atpE, atpF^a, atpH, atpI</i>
	NADH-dehydrogenase	<i>ndhA^a, ndhB^{a,c}, ndhC, ndhD, ndhE, ndhF, ndhG, ndhH, ndhI, ndhJ, ndhK</i>
Other genes	Rubisco large subunit	<i>rbcl</i>
	Translational initiation factor	<i>infA</i>
	Maturase K	<i>matK</i>
	Envelope membrane protein	<i>cemA</i>
	Acetyl-CoA carboxylase	<i>accD</i>

Note: ^a, genes with one intron; ^b, genes with two introns; ^c, two gene copied in IR regions.

Category	Groups of Genes	Name of Genes
	Proteolysis	<i>clpP</i> ^b
	Cytochrome c biogenesis	<i>ccsA</i>
Unknown	Conserved open reading frames	<i>ycf1, ycf2</i> ^c , <i>ycf3</i> ^b , <i>ycf4, ycf15</i> ^c
Note: ^a , genes with one intron; ^b , genes with two introns; ^c , two gene copied in IR regions.		

The phylogenetic position of *Veronicastrum wulingense*

The phylogenetic relationships of *Veronicastrum wulingense* were revealed in the genus *Veronicastrum* with high bootstrap value in the ML phylogenetic tree based on the 38 concatenated sequences of molecular markers (Fig. 4). The results displayed that the sister clade of *V. wulingense* is *V. rhombifolium* in the phylogeny. However, *V. axillare* is a separate clade and distant from *V. wulingense*, which is also similar to *V. wulingense* morphologically. Besides, the five *Veronicastrum* species of *Veronicastrum caulopterum* (Hance) Yamazaki, *Veronicastrum tagawae* (Ohwi) Yamazaki, *Veronicastrum longispicatum* (Merr.) Yamazaki, *Veronicastrum latifolium* (Hemsl.) Yamazaki, and *V. stenostachyum* showed unclearly and crossedly interspecific relationships, Also, it is found that the clade of *Pseudolysimachion spicatum* is nested in the clades of genus *Veronica* and verified *Veronica* is a paraphyly genus (Chen et al. 2020).

Discussion

Veronicastrum wulingense, a new species of Plantaginaceae from Wulingshan Region in Southwestern Hubei is reported in this study and is the first discovered new species of the genus *Veronicastrum* in the mainland region of China. This new species belongs to the Sect. *Plagiostachys* species (Chin and Hong, 1979) and is similar to *V. axillare* and *V. rhombifolium* in morphological features. Also, the significantly diagnostic characteristic that corolla lobes are 2/5–1/2 of the corolla length in Sect. *Plagiostachys* is just observed in *V. axillare* and *V. rhombifolium*. Between the former two, the characteristic of stems arching and rooting apically is nearly found in *V. axillare*, although the similar traits of stems trailing and rooting and leaf blades abaxially purple-red were also found in *V. rhombifolium*, it is rare and only known one specimen (F.Z. Wang, 21941 (PE, 01475814)) from Mao County in West Sichuan up to know, which suggests some morphological relationships with *V. wulingense*. However, these diagnostic characteristics, peduncles up to 7 cm, flowers clustered densely in rachis apex and steadily ca. 2–3 cm long, and pedicels up to 2.5 mm are unique in all *Veronicastrum* species of axillary inflorescences.

As for the reason why *Veronicastrum wulingense* is closely related to *V. rhombifolium* while distantly related to *V. axillare* in the phylogeny, *V. rhombifolium* and *V. axillare* may be two parallel evolution clades from the common ancestor and distributed in two separate regions. We speculate that *V. rhombifolium*

was only distributed in the surrounding area of Ya'an in Sichuan previously, however, it went across the Sichuan Basin in an opportunity and spread to the Wulingshan Region of Southwestern Hubei that is a similar habitat to the surrounding area of Ya'an at elevation and living environment. Consequently, the clade of *V. rhombifolium* in Wulingshan Region gradually evolved into the new species of *V. wulingense* in the long geographical isolation and separate evolution, which evolves the trait of stems trailing and rooting the same as *V. axillare* to adjust the local environment. Geographically, this new species is distributed in the Wulingshan Region in Central China, which is the central junction of floras in the East, West, South, and North of China, and has very high plant diversity and endemism (Zheng, 1983). *V. rhombifolium* and *V. axillare* are distributed in the distant West Sichuan (Southwest China) and the broad areas east of the Wulingshan Region (including East China, Taiwan Island, and Japan), respectively. For *V. rhombifolium*, *V. wulingense*, and *V. axillare*, they are distributed in three separate geographical units from west to east and have no geographic overlap, and reasonably divided three independent species.

As a whole, undoubtedly, the new taxon can be evidently distinguished from the whole *Veronicastrum* species morphologically. Herein, we treat it as a new *Veronicastrum* species based on morphological and molecular evidence.

Abbreviations

HIB

Wuhan Botanical Garden, Chinese Academy of Sciences, Herbarium

PE

Institute of Botany, the Chinese Academy of Sciences, Herbarium

ML

Maximum Likelihood

LSC

Large Single-Copy

SSC

Small Single-Copy

IR

Inverted Repeat

PCG

Protein-Coding Gene.

Declarations

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Authors' contributions

SXD, GWH, HJ, and ENW first discovered and collected the new species in the field survey. SXD, HJ, and JR conducted the molecular experiment. SXD and JT analyzed the data. SXD drafted the manuscript. GWH and QFW designed and supervised the study. GWH provided the funding. FMM and SXD reviewed the manuscript. All authors have read and agreed with the submission of this manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of Competing Interest

All the authors declare that there is no conflict of interest.

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Supplementary data

Supplementary data for this article are supplied online at xxxxxxxx

References

1. Albach DC, Chase MW (2001) Paraphyly of *Veronica* (Veroniceae; Scrophulariaceae): Evidence from the Internal Transcribed Spacer (ITS) Sequences of Nuclear Ribosomal DNA. *J Plant Res* 114(1):9–18. <https://doi.org/10.1007/pl00013971>
2. Albach DC, Meudt HM, Oxelman B (2005) Piecing together the "new" Plantaginaceae. *Am J Bot* 92(2):297–315. <https://doi.org/10.3732/ajb.92.2.297>
3. Albach DC, Soltis PS, Soltis DE, Olmstead RG (2001) Phylogenetic analysis of asterids based on sequences of four genes. *Ann Mo Bot Gard* 88(2):163–212. <https://doi.org/10.2307/2666224>
4. Chase MW, Hills HH (1991) Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40(2):215–220. <https://doi.org/10.2307/1222975>
5. Chen TT, Chou FS (2008) A new Taiwan species *Veronicastrum loshanense*. *Bot Stud* 49:281–285. <https://doi.org/10.18942/apg.KJ00008580360>
6. Chen ZD, Lu AM, Liu B, Ye JF (2020) Tree of life for Chinese vascular plants. Science Press, Beijing
7. Chin TL, Hong DY (1979) Scrophulariaceae. In Tsong PC, Yang HP (ed) *Flora Reipublicae Popularis Sinicae*, Vol 67(2). Science Press, Beijing, p 227–250
8. Choi KS, Chung MG, Park S (2016) The complete chloroplast genome sequences of three Veroniceae species (Plantaginaceae): comparative analysis and highly divergent regions. *Front Plant Sci* 7:355. <https://doi.org/10.3389/fpls.2016.00355>
9. Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15
10. Freeman CE, Scogin R (1999) Potential utility of chloroplast trnL (UAA) gene intron sequences for inferring phylogeny in Scrophulariaceae. *Aliso* 18(2):141–159. <https://doi.org/10.5642/aliso.19991802.16>
11. Greiner S, Lehwark P, Bock R (2019) OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic Acids Res* 47(W1):W59–W64. <https://doi.org/10.1093/nar/gkz238>
12. Govaerts R, Lughadha EN, Black N, Turner R, Paton A (2021) The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity. *Sci Data* 8(1):215. doi:10.1038/s41597-021-00997-6
13. Tank DC, Beardsley PM, Kelchner SA, Olmstead RG (2006) Review of the systematics of Scrophulariaceae s.l. and their current disposition. *Aust Syst Bot* 19(4):289–307. <https://doi.org/10.1071/sb05009>
14. Hong DY (1984) Taxonomy and evolution of the Veroniceae (Scrophulariaceae) with special reference to palynology. *Opera Bot* 75:1–60
15. Hong DY, Yang HB, Jin CL, Jin CL, Noel HH (1998) Scrophulariaceae. In: Wu ZY, Raven PH (eds) *Flora of China*, vol 18. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, pp 57–61
16. Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ (2020) GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. *Genome Biol* 21(1):241.

<https://doi.org/10.1186/s13059-020-02154-5>

17. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30(4):772–780. <https://doi.org/10.1093/molbev/mst010>
18. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C et al (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
19. Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Mol Biol Evol* 34(3):772–773. <https://doi.org/10.1093/molbev/msw260>
20. Li HT, Luo Y, Gan L, Ma PF, Gao LM, Yang JB, Cai J, Gitzendanner MA, Fritsch PW, Zhang T et al (2021) Plastid phylogenomic insights into relationships of all flowering plant families. *BMC Biol* 19(1):232. <https://doi.org/10.1186/s12915-021-01166-2>
21. Li J, Wang S, Yu J, Wang L, Zhou S (2013) A modified CTAB protocol for plant DNA extraction. *Chin Bull Bot* 48(1):72–78. <https://doi.org/10.3724/sp.J.1259.2013.00072>
22. Minh BQ, Nguyen MA, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* 30(5):1188–1195. <https://doi.org/10.1093/molbev/mst024>
23. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32(1):268–274. <https://doi.org/10.1093/molbev/msu300>
24. Olmstead RG, Pamphilis CW, Wolfe AD, Young ND, Elisons WJ, Reeves PA (2001) Disintegration of the Scrophulariaceae. *Am J Bot* 88(2):348–361. <https://doi.org/10.2307/2657024>
25. Olmstead RG, Reeves PA (1995) Evidence for the Polyphyly of the Scrophulariaceae based on chloroplast *rbcL* and *ndhF* sequences. *Ann Mo Bot Gard* 82:172–193. <https://doi.org/10.2307/2399876>
26. Qu XJ, Moore MJ, Li DZ, Yi TS (2019) PGA: a software package for rapid, accurate, and flexible batch annotation of plastomes. *Plant Methods* 15:50. <https://doi.org/10.1186/s13007-019-0435-7>
27. The Angiosperm Phylogeny Group (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants APG II. *Bot J Linn Soc* 141:399–436. <https://doi.org/10.1046/j.1095-8339.2003.t01-1-00158.x>
28. The Angiosperm Phylogeny Group (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot J Linn Soc* 181:1–20. <https://doi.org/10.1111/boj.12385>
29. Uehara K, Saiki K, Ando T (2013) *Veronicastrum noguchii* (sect. *Plagiostachys*, Plantaginaceae), a new species from Japan. *Acta Phytotax Geobot* 63(2):63–69
30. von Wettstein R (1891) Scrophulariaceae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien*, IV, 3b. Engelmann, Leipzig, pp 39–107

31. Wick RR, Schultz MB, Zobel J, Holt KE (2015) Bandage: interactive visualization of de novo genome assemblies. *Bioinformatics* 31(20):3350–3352. <https://doi.org/10.1093/bioinformatics/btv383>
32. Xiao J, Wang X, Li C, Liu H, Chen F (2020) Phylogeny of *Rehmannia* and related genera based on chloroplast genome. *Mol Plant Breed* 18(8):2527–2533. <https://doi.org/10.13271/j.mpb.018.002527>
33. Yamazaki T (1957) Taxonomical and phylogenetic studies of Scrophulariaceae-Veronicae with special reference to *Veronica* and *Veronicastrum* in eastern Asia. *J Fac Sci Univ Tokyo Sect 3 Bot* 7:91–162
34. Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol Ecol Resour* 20(1):348–355. <https://doi.org/10.1111/1755-0998.13096>
35. Zheng Z (1983) A study on the floristic characteristics and plant distribution in Hubei Province. *Wuhan Bot Res* 1(2):165–166

Figures

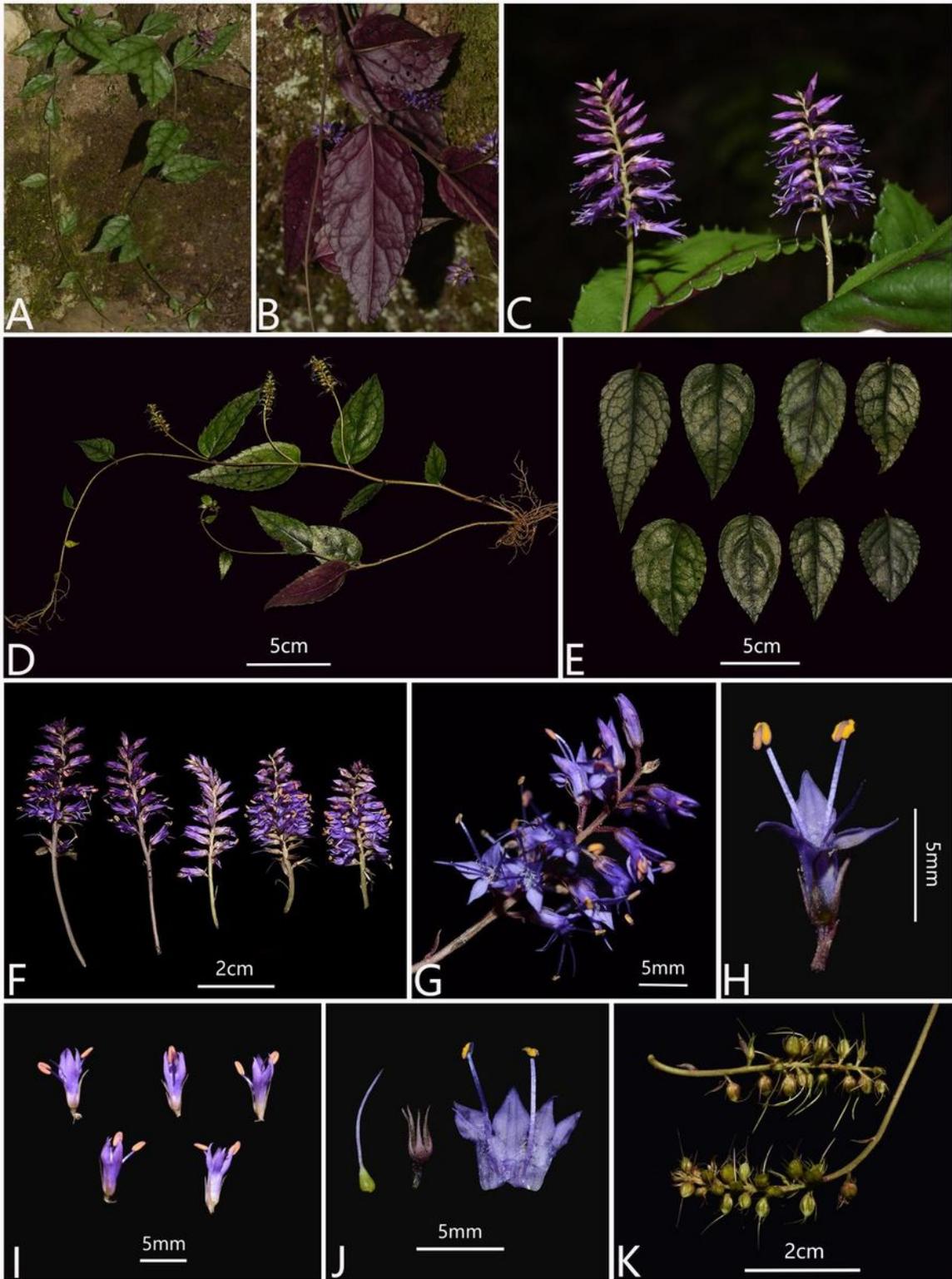


Figure 1

Veronicastrum wulingense (type locality). **A–B** habit; **C** leaf sawtooth; **D** individual; **E** leaves; **F–G** inflorescences; **H–I** flowers; **J** anatomical flower; **K** infructescences. Photographed by S.X. Ding.

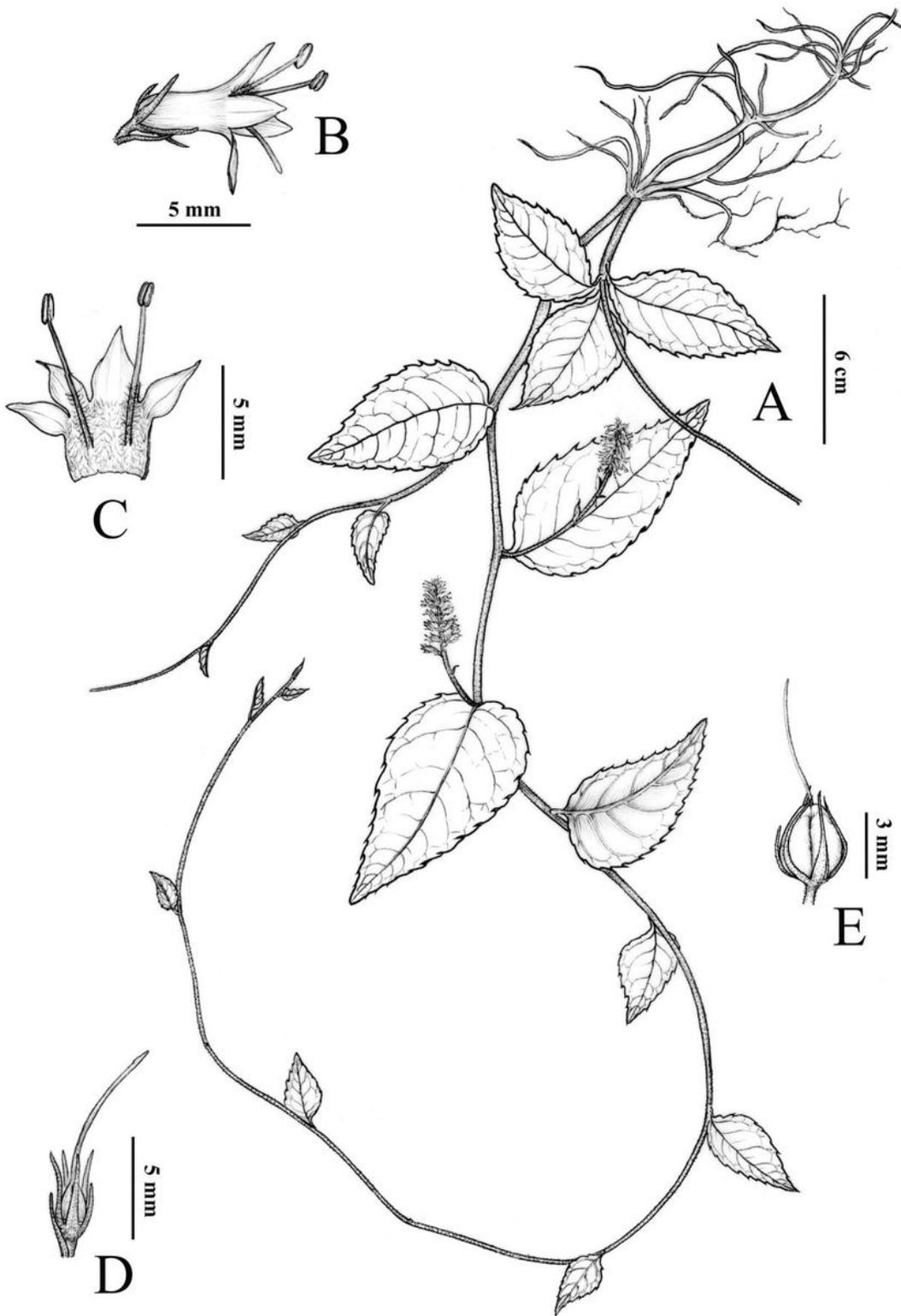


Figure 2

Illustration of *Veronicastrum wulingense*. **A** habit; **B** flower; **C** dissected corolla and stamens; **D** calyx and pistil; **E** capsule with persistent sepals. Drawn by J. Tian.

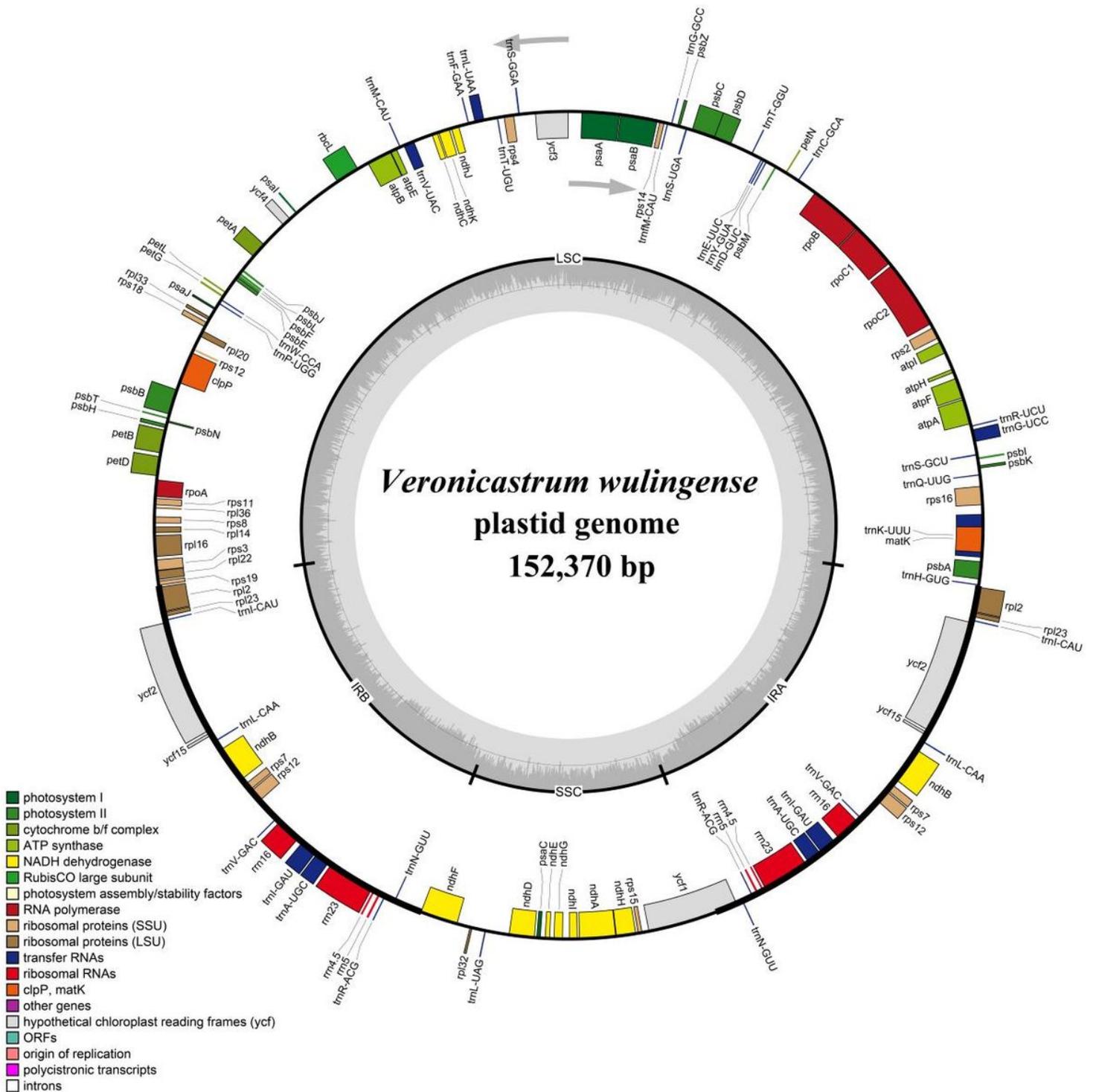


Figure 3

Circular plastid genome map of *Veronicastrum wulingense*. Genes drawn within the circle are transcribed clockwise, while those drawn outside are transcribed counterclockwise. Genes of different functional groups are colored by different colors. The darker gray in the inner circle corresponds to the DNA GC content, while the lighter gray corresponds to the DNA AT content.

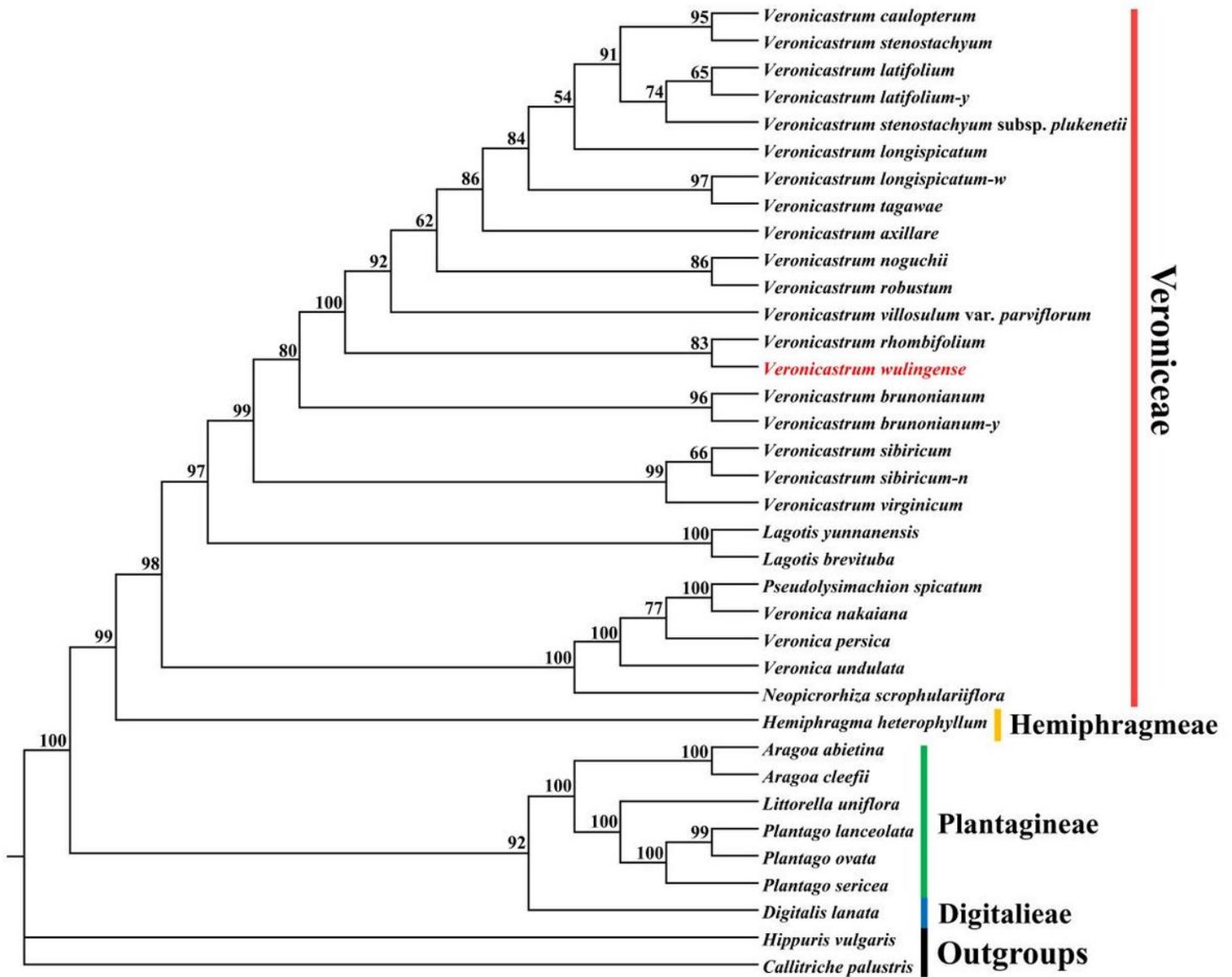


Figure 4

The phylogenetic position of *V. wulingense* in the genus *Veronicastrum* based on the maximum likelihood (ML) tree using 31 concatenated matrix sequences from three plastid markers.

Supplementary Files

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