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Two new stoloniferous species of *Viola* (Violaceae) from China

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Two new stoloniferous species of *Viola* (Violaceae) from southern China are described and illustrated. *Viola nitida* is recognizably different from *V. fargesii* as the plant is evergreen and glabrous throughout, the leaf blade adaxially nitid, base cuneate or shallowly cordate, and margin serrate. *Viola maoershanensis* is different from *V. diffusa* as the leaf blade is serrate, base cordate and not decurrent to petiole, the petiole wingless, the flowers larger, the petals bluish violet or pinkish white, and the lower petal obtuse at the apex. The chromosome numbers of the two new species were counted as $2n = 24$ (*V. nitida*) and $2n = 44$ (*V. maoershanensis*). The taxonomic positions of the two species are discussed. *Botanical Journal of the Linnean Society*, 2009, **159**, 349–356.

ADDITIONAL KEYWORDS: chromosome number – taxonomy – violets.

INTRODUCTION

Viola L. (Violaceae) is a genus of 525–600 species mainly distributed in the temperate regions of the Northern Hemisphere (Ballard, Sytsma & Kowal, 1999). This genus is richly represented in China, which constitutes a most important part of one of the primary centres of morphological and taxonomic diversity of this genus, i.e. the centre of the Himalayas and mountainous Asia (Ballard *et al.*, 1999). In the *Flora Reipublicae Popularis Sinicae* (volume 51), 109 species were recorded as native in China (Wang, 1991). This number has turned out to be far from precise, for at least 10 violet species have been described on the basis of the type material from China that appear to have been overlooked or ignored by Wang (Xing & Qin, 2004). New species are still found in China (Huang, 2003; Chen & Yang, 2005a, b, 2008).

In the course of preparing an account of Violaceae for the *Flora of China*, some stoloniferous fruiting specimens of *Viola* from Guangxi, Guizhou and Hunan provinces, e.g. *Guangfu Exped. 768* (PE, IBSC), *Z.B.Jian 50852* (PE, KUN, HGAS) and *X.G.Li 202890* (IBSC), caught our attention. These specimens had been previously identified as *V. davidii* Franch., but upon a careful examination we found they represent a quite different species. Fruiting and flowering specimens of this species were collected from Guizhou and Guangxi by the first author in 2004 and 2005, and were confirmed to represent a hitherto undescribed species. During the expedition to Guangxi in 2005, the first author also collected another stoloniferous violet, which, upon careful comparison, was found to represent yet another new species. Both new species are described here, and their taxonomic positions are discussed.

MATERIAL AND METHODS

Living material was transplanted and cultivated in the greenhouse at the Institute of Botany, Chinese Academy of Sciences, Beijing. For chromosome study, actively growing root tips were pretreated with 0.002 M 8-hydroxyquinoline solution at 4 °C for 4–5 h, then transferred into Carnoy's fixative solution for 12 h. They were macerated with 1 M HCl at 60 °C for 10 s. Meristematic tissue was placed on to a glass

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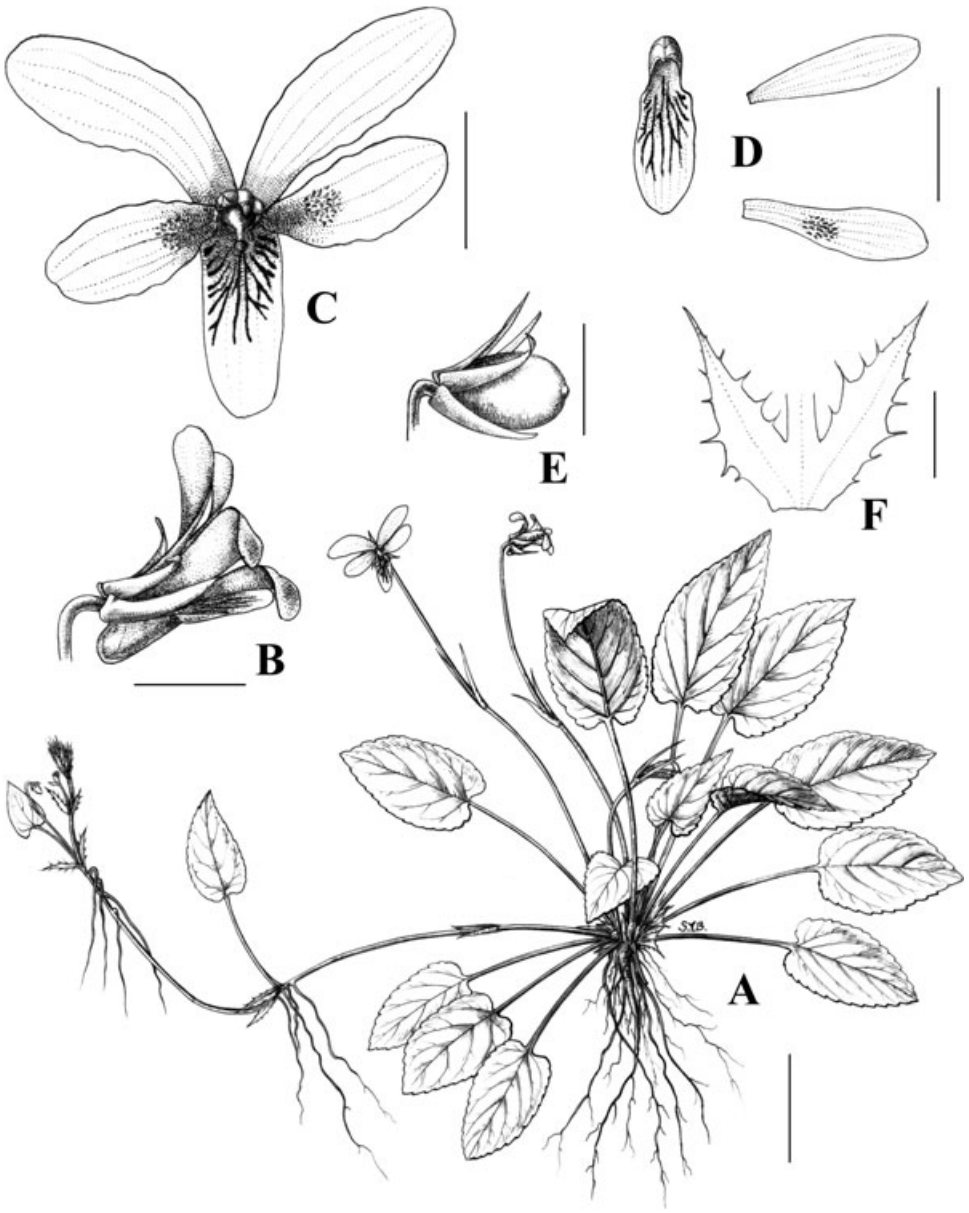


Figure 1. *Viola nitida* Y.S.Chen & Q.E.Yang (*Y.S.Chen 5026*). A, habit. B, flower in lateral view. C, flower in front view. D, petals; E, fruit and sepals. F, stipules. Scale bar = 2 cm (A), 5 mm (B–F).

slide, stained with 10% aceto-orcein for 1 h and squashed. Voucher specimens for this work are the type material (*Y.S.Chen 5026, Y.S.Chen 5029*) of the two new species, which are deposited in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE).

For morphological comparisons, herbarium specimens of relevant species kept at HGAS, IBSC, KUN, PE and SZ were examined.

Chromosome numbers

The chromosome number of *Viola nitida* was $2n = 24$ (Fig. 11) and that of *V. maoershanensis* $2n = 44$ (Fig. 12). The chromosomes in both species were rather small, not allowing a karyotype analysis to be made.

Description of new species

Viola nitida Y.S.Chen & Q.E.Yang, sp. nov. (Figs 1, 3–6).

Diagnosis: Violae fargesii H.Boissieu similis, sed planta sempervirente omnino glabra, laminis supra nitidis basi cuneatis vel non profunde cordatis margine serratis, sepalis capsulisque minoribus differt.

Type: China. Guangxi: Xing'an County, Mt Maoershan, streamside, altitude 300–700 m, 19.iii.2005 (fl), *Y.S.Chen 5026* (holotype, PE).

Description: Viola nitida is similar to *V. fargesii* H.Boissieu, but differs in the plant being evergreen and glabrous throughout, the leaf blades glossy above and cuneate or shallowly cordate at the base, with serrate margins, and the sepals and capsules smaller.

HERB perennial, evergreen, to 15 cm high, glabrous throughout. RHIZOME stout, densely noded, 2–4 cm, 5–6 mm thick, usually bearing stolons. STOLONS equally and distantly leaved, rooting at nodes or internodes. LEAVES nearly basal or alternate on stolons; blade ovate or oblong–ovate, 1.3–4(–6) × 1.2–3.2 cm (those on stolons rather small), margin shallowly serrate, apex obtuse or acute, base truncate, cuneate or shallowly cordate, glabrous on both sides, adaxially nitid; petiole (1–)2.5–4(–10) cm long, wingless; stipules green, 1/5–1/4 adnate to petiole at base, triangular, 15–20 × 3–4 mm, apex acuminate, margin sparsely fringed; stipules of the leaves on stolons oblong–lanceolate to triangular, 6–9 × 1–2 mm, margin sparsely fringed. FLOWERS white; pedicels glabrous, 2-bracteolate above middle, exceeding leaves at anthesis, shorter than leaves at fruiting; bracteoles opposite, linear, 6–9 mm; sepals lanceolate, *c.* 5 × *c.* 1 mm, apex acuminate, basal auricles short, *c.* 0.5 mm long, apex obtuse; upper and lateral petals oblong, 8–9 × *c.* 3 mm, apex rounded; lateral petals bearded; lower petal pinkish-purple striate, *c.* 7 mm long, including spur, apex rounded, spur *c.* 1 mm long; styles clavate, base slightly geniculate, thickened upwards, apex slightly flat, conspicuously margined on lateral sides, shortly beaked in front, with a stigma hole open upwards at tip of beak. CAPSULES trigonous and ellipsoid, 5–7 mm long, borne on erect peduncles, ejecting seeds explosively when mature. SEEDS ovoid, *c.* 1 mm long, *c.* 0.6 mm in diameter.

Distribution and habitat: Endemic to southern China (northern Guangxi, southern Guizhou and southwestern Hunan), growing in sandy soil or rocky crevices along streamside at altitudes of 300–1100 m.

Etymology: The epithet '*nitida*' of the new species refers to the shiny adaxial surface of the leaf blade.

Additional specimens examined: China. Guangxi: Longsheng County, Sanmen, Hongmaochong, 950 m, 5.vii.1955, *Guangfu Exped. 768* (PE, IBSC); Longsheng County, Pingshui, Hongtan, 29.xi.1954, *Longsheng Exped. 50147* (IBSC); Jinxiu County, Mt Dayaoshan, Dingbulong, 5.viii.1958, *Y. K. Li 400912* (IBSC); Xing'an County, Mt Maoershan, 1100 m, 22.xi.1956, *S.L.Yu 900364* (IBSC); Quanzhou County, Shanchuan, Sandujiang, 19.vi.1937, *J.X.Zhong 82059* (IBSC); Mengshan County, Gupu, 17.xii.1948, *J.X.Zhong 85040* (IBSC); Guizhou: Guiyang, Huaxi, 1060 m, 9.iv.1963, *Guizhou Institute of Biology 231* (HGAS); Guizhou: Guiyang, Huaxi, 1060 m, 15.vi.2004, *Y.S.Chen 4183* (PE); Leishan County, Qiaowai, 1000 m, 30.iv.1955, *Q.H.Chen 3234* (HGAS); Leishan County, Pingzhuang, 940 m, 28.vi.1965, *Z.B.Jian 50852* (PE, KUN, HGAS); Congjiang County, Guanghui, Mt Taiyangshan, 7.viii.1959, *Qinan Exped. 3330* (PE, HGAS, SZ); Hunan: Qianyang County, Mt Bamianshan, Xiapingjiang. 8.v.1959, *X. G. Li 202890* (IBSC).

Viola maoershanensis Y.S.Chen & Q.E.Yang, sp. nov. (Figs 2, 7–10).

Diagnosis: Violae diffusae Ging. similis, sed foliorum laminis margine serratis basi truncatis vel non profunde cordatis haud in petiolum decurrentibus, petiolis exalatis, floribus majoribus, petalis viridulo-violaceis vel subroseo-albis, petalo inferiore apice obtuso differt.

Type: China. Guangxi: Xing'an County, Mt Maoershan, Chaoran Valley, 650 m, 19.iii.2005 (fl), *Y.S.Chen 5029* (holotype, PE).

Description: Viola maoershanensis is similar to *V. diffusa*, but differs in the leaf blades being serrate, truncate or shallowly cordate at the base and not decurrent to the petiole, the petioles wingless, the flowers larger, the petals bluish-violet or pinkish-white and the lower petal obtuse at the apex.

HERB perennial, acaulescent. RHIZOME erect or obliquely ascending, densely noded, 1–2 cm, 3–5 mm in diameter; stolons ligneous, elongated, slender, sparsely puberulous when young, later glabrescent, rooting at nodes and producing rosettes of leaves and flowers at the apex. LEAVES nearly basal or alternate on stolons; blade oblong–ovate or triangular–ovate, 2–7 × 1–3 cm, margin obtusely serrate, apex obtusely acute, base truncate or shallowly cordate, pubescent on both sides, adaxially green, abaxially pale green; petiole pubescent, 2–7.5 cm long, wingless; stipules green, 1/4–1/3 adnate to petiole at base, triangular, 10–12 × *c.* 1.5 mm, apex acuminate, margin sparsely fringed. FLOWERS bluish-violet or pinkish-white; pedicels sparsely puberulous or subglabrous, 2-bracteolate above the middle, exceeding leaves at anthesis, shorter than leaves at fruiting; bracteoles opposite or subopposite, pubescent, linear, 7–10 mm long; sepals sparsely puberulous or glabrous, lanceolate, 5–7 × *c.* 1 mm, apex acuminate; basal auricles *c.* 1 mm long at anthesis, *c.* 2 mm long at fruiting, apex acute or dentate, margin usually ciliate; petals oblong–obovate, upper and lateral ones

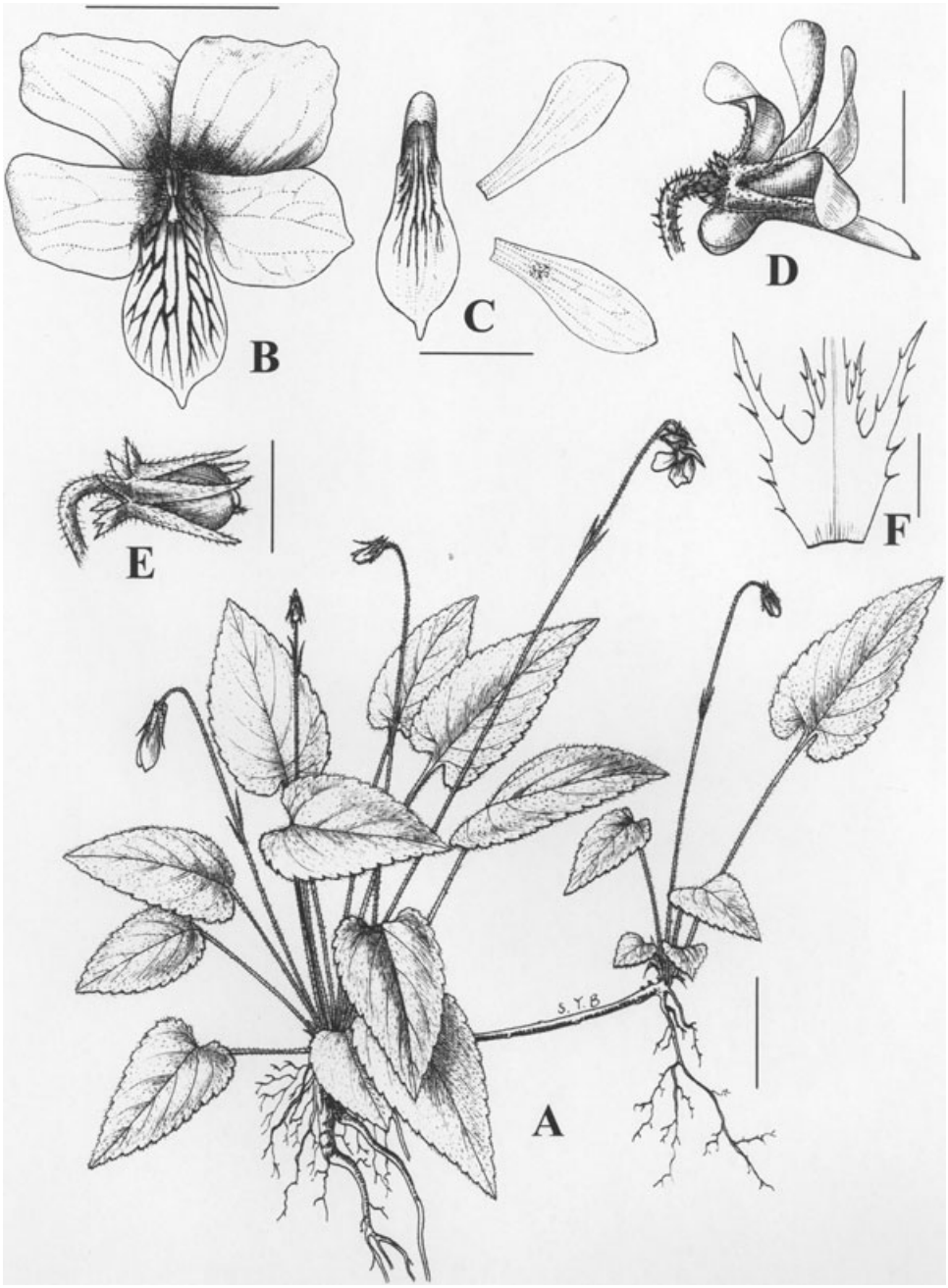
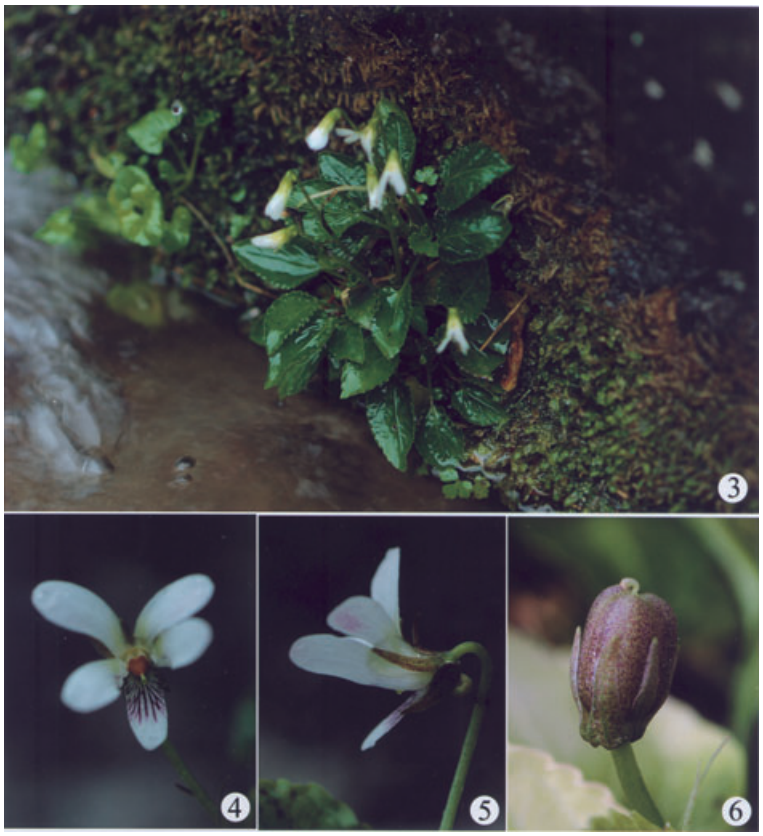


Figure 2. *Viola maoershanensis* Y.S.Chen & Q.E.Yang (*Y.S.Chen 5029*). A, habit. B, flower in front view. C, petals. D, flower in lateral view. E, fruit and sepals. F, stipules. Scale bar = 2 cm (A), 5 mm (B–F).

9–10 × 3–3.2 mm, apex rounded, lateral petals sparsely bearded, lower petal striate, 8–9 mm long, including spur, apex obtuse, spur *c.* 1 mm long, *c.* 2 mm in diameter; styles clavate, slightly geniculate, gradually thickened in upper part; stigmas thickly margined on lateral sides, shortly beaked in front. CAPSULES trigonous and ellipsoid, 5–6 mm long, glabrous. SEEDS ovoid, *c.* 1 mm long, *c.* 0.6 mm in diameter.

Distribution and habitat: This species is known only from its type locality, growing under bamboo forests in a mountain valley at an altitude of 650 m.



Figures 3–6. *Viola nitida* (photographed by Y.S.Chen). Fig. 3, habit. Fig. 4, flower in front view. Fig. 5, flower in side view. Fig. 6, fruit.

Etymology: The new species is named after its type locality, Mt Maoershan, Guangxi, China.

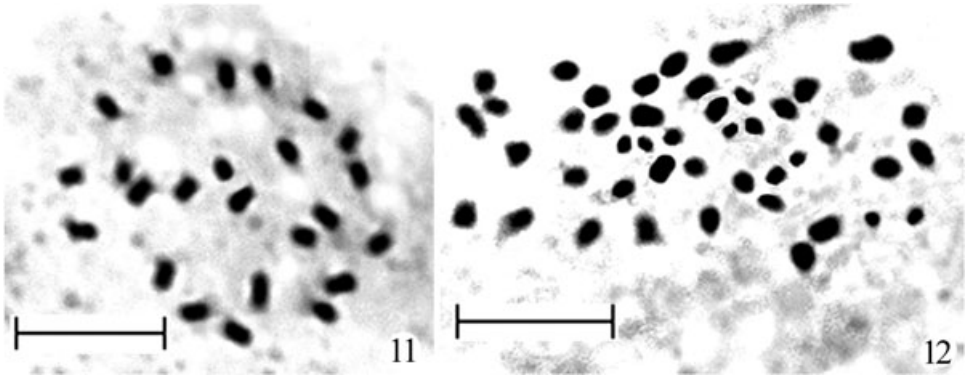
DISCUSSION

Viola nitida is distributed in Guangxi, Guizhou and Hunan, whereas *V. maoershanensis* is only known from its type locality. It is noteworthy that in Guangxi these two species were found to occur on the same mountain, i.e. Mt Maoershan, Xing'an County, but they grow in quite different habitats: *V. nitida* grows in sandy soil or rocky crevices of open places along streamsides, whereas *V. maoershanensis* grows under bamboo forests in a mountain valley. No intermediate forms between the two species were found on this mountain. The different chromosome numbers of the two species ($2n = 24$ in *V. nitida* vs. $2n = 44$ in *V. maoershanensis*) also suggests that they are two well differentiated species.

Viola nitida is a widely distributed species of Chinese violet. The most probable reason why it remained undescribed until now is because it had long been misidentified as *V. davidii*. *Viola nitida* is similar to *V. davidii* in that the plant is glabrous, the leaves evergreen, the leaf blade orbicular or suborbicular, the rhizome usually densely noded and the lateral petals bearded. It differs, however, as the rhizome is usually stout, the leaf blade abaxially light green, adaxially shiny, margin serrate, base cuneate or shallowly cordate, apex obtuse or acute, and the stipules green. The two species usually grow in different habitats: *V. nitida* often grows along streamsides at altitudes of 300–1100 m, whereas *V. davidii* often grows in montane forests or forest margins at altitudes of 1200–2800 m.

Taking into consideration the existence of the slender, elongated stolons with scattered leaves, the rootlets emitting from nodes and internodes of stolons, and the lower petal rounded at the apex, *V. nitida*, together with its closest ally, *V. fargesii*, belongs to *Viola* series *Australasiaticae* Okamoto. The species in this series were originally placed in the *Serpentes* group established by Becker (1923, 1925), who did not explicitly designate a formal taxonomic rank for it. This group, which has been treated as a section (Wang, 1991), subsection (Chang, 1949; Okamoto & Ueda, 1986; Okamoto, 1987; Yokota *et al.*, 1988) or series (Okamoto, Okada & Ueda, 1993), is characterized by

Figures 7–10. *Viola maoershanensis* (photographed by Y.S.Chen). Fig. 7, habit. Fig. 8, flower in front view. Fig. 9, flower in side view. Fig. 10, fruit.



Figures 11–12. Photomicrographs of chromosomes in two new violet species. Fig. 11, *Viola nitida* (*Y.S.Chen 6026*, $2n = 24$). Fig. 12, *Viola maoershanensis* (*Y.S.Chen 5029*, $2n = 44$). Scale bar = 5 μm.

an acaulescent stoloniferous habit (Okamoto *et al.*, 1993). *Viola fargesii*, a species described from type material collected from Chengkou County, Sichuan, China, was originally placed in the *Diffusae* group by Becker (1923, 1925). This species had long been overlooked or ignored by subsequent students, and did not appear in *Flora Reipublicae Popularis Sinicae* (volume 51). Our recent revision has shown that *V. principis* H. Boissieu, a widely distributed species in China, should be reduced to the synonymy of *V. fargesii* (Chen, 2006). From the characters of *V. fargesii*, such as the slender and elongated stolons with scattered leaves, the rootlets emitting from nodes and internodes of stolons, and the lower petal rounded at the apex, this species may

find a better position in *Viola* series *Australasiaticae* than in the *Diffusae* group. In fact, *V. principis* was correctly placed together with *V. davidii* in the *Serpentes* group by Chang (1949) and Wang (1991), who treated this group, respectively, as a subsection or a section. Judging from some of its characters, such as the glabrous, trigonous and ellipsoid capsule, the erect peduncles of mature fruits, and the clavate and apically margined styles, *V. fargseii* should belong to *Viola* series *Australasiaticae*.

Cytologically, *Viola* series *Australasiaticae* is little known, with few species in this series having chromosome number reports (Okamoto *et al.*, 1993). The chromosome numbers in this series are $2n = 22$, 44 or 46 (Okamoto *et al.*, 1993). The count $2n = 24$ reported in *V. nitida* represents a new chromosome number in this series. The chromosome number of *V. fargesii* is also $2n = 24$ (Chen, 2006), strongly supporting the close affinity of these two species.

Becker (1923) originally included only three species, *V. diffusa*, *V. nagasawai* Makino & Hayata and *V. fargesii* in his *Diffusae* group. This group is characterized by having thick and usually ligneous stolons producing new rosettes of leaves and flowers at the apex, the rootlets emitting only from nodes of stolons, the stipules fringed, the flowers shortly spurred, and the lower petal acute or obtuse at the apex. As mentioned above, *V. fargesii* may be better placed in *Viola* series *Australasiaticae*. From their morphological characters, both *V. lucens* W.Becker and *V. maoershanensis* should also belong to the *Diffusae* group. This group is distributed from Japan, China and the Himalaya to New Guinea, with all the four species occurring in China. *Viola diffusa* shows a great variation in chromosome number. The material of *V. diffusa* subsp. *diffusa* from both Japan and Taiwan was reported to have $2n = 26$ (Miyaji, 1929; Wang, 1989), while the material of *V. diffusa* subsp. *tenuis* (Benth.) W.Becker from Taiwan was reported to have $n = 37$ (Wang, 1989), and that from New Guinea $2n = 72$ (Borgmann, 1964). *Viola nagasawai* from Taiwan was reported to have $2n = 48$ (Wang, 1989). The present study has shown that the chromosome number of *V. maoershanensis* is $2n = 44$ (Fig. 12). The chromosome number of *V. lucens* is $2n = 26$ (Chen, 2006), the same as that of *V. diffusa* subsp. *diffusa*. Therefore, the *Diffusae* group has the chromosome numbers of $2n = 26$, 44, 48, 72 or 74, suggesting a complicated origin of this group.

Although *V. maoershanensis* has $2n = 44$, a number also occurring in *Viola* series *Australasiaticae*, this acaulescent species with ligneous stolons producing rosettes of leaves and flowers at the apex, the fringed stipules and shortly spurred flowers, seems to be better placed in the *Diffusae* group. The occurrence of the number $2n = 44$ in the two groups may suggest their close affinity. In fact, series *Australasiaticae* and the *Diffusae* group share several morphological characters: the acaulescent and usually stoloniferous habit; the stipules usually 1/5–1/3 adnate to petiole; the lower petal spur usually short; the style clavate, apex flat and margined on lateral sides; the capsules trigonous and the peduncles of mature fruits erect. Both groups have also a similar geographical distribution range, i.e. subtropical to tropical Asia. The *Diffusae* group differs from the *Australasiaticae* group mainly by having thick and usually ligneous stolons producing new rosettes of leaves and flowers at the apex, rootlets emitting only from nodes of stolons, and lower petal acute or obtuse at the apex. The *Australa-*

KEY TO *V. NITIDA*, *V. MAOERSHANENSIS* AND THEIR RELATED SPECIES

1. Stolons thick and usually ligneous, producing new rosettes of leaves and flowers at the apex; rootlets emitting only from nodes of stolons; lower petal acute or obtuse at the apex.
2. Leaf blade oblong–ovate to triangular–ovate; margin serrate; lower petal obtuse at the apex; spur subequaling sepal appendage……***V. maoershanensis***
2. Leaf blade ovate or ovate–oblong, margin crenulate, lower petal the shortest, apex acute; spur longer than sepal appendage.
3. Leaf base conspicuously decurrent to petiole……***V. diffusa***
3. Leaf base not decurrent to petiole; petiole nearly wingless.
4. Bracteoles linear, 1–2 mm broad; petiole usually as long as blade……***V. lucens***
4. Bracteoles lanceolate, 5–7 mm broad; petiole much longer than blade……***V. nagasawai***
1. Stolons slender and elongated, with scattered leaves; rootlets emitting from nodes and internodes of stolons; lower petal rounded at apex.
5. Plants usually densely pubescent; leaves withered in winter; petiole usually longer than leaf blade…***V. fargesii***
5. Plant glabrous; leaves evergreen in winter.
6. Rhizome usually slender, leaf blade abaxially glaucous, adaxially deep green, margin distinctly crenate, base cordate or truncate, apex obtuse, stipules brown……***V. davidii***
6. Rhizome usually stout, leaves blade abaxially light green, adaxially nitid, margin serrate, base cuneate or shallowly cordate, apex obtuse or acute, stipules green……***V. nitida***

siaticae group has slender and elongated stolons with scattered leaves, rootlets emitting from nodes and internodes of stolons and lower petal rounded at the apex. The true relationship of the two groups needs further study.

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