

Newly discovered native orchids of Taiwan (XIV)

Yung-I LEE¹, Sheng-Kun YU², Pei-Chun LIAO³, Kuo-Hsiung WANG⁴, Tsan-Piao LIN^{5,*}

1. Biology Department, National Museum of Natural Science, 1 Kuan-Chien Rd, Taichung 40453, Taiwan.

2. 5 Fl., No. 37, Ln. 134, Sec. 3, Xinyi Rd., Taipei 106, Taiwan.

3. Department of Life Science, National Taiwan Normal University, 88 Ting-Chow Rd., Sec. 4, Taipei 116, Taiwan.

4. Institute of Ecology and Evolutionary Biology, National Taiwan University, 1 Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.

5. Institute of Plant Biology, National Taiwan University, 1 Roosevelt Rd., Sec. 4, Taipei 106, Taiwan.

*Corresponding author's tel: +886-2-33662537; Email: tpl@ntu.edu.tw

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ABSTRACT: This report presents one new hybrid orchid of Taiwan, i.e., *Gastrochilus ×hsuehshanensis* which resulted from hybridization between *G. formosanus* and *G. rantabunensis*. Also, the existence of both *Cyrtosia javanica* and *Cypripedium macranthos* in Taiwan is questionable, and these were excluded from the Taiwanese orchid flora.

KEY WORDS: Gastrochilus × hsuehshanensis; Cyrtosia taiwanica; Cypripedium taiwanalpinum; DNA phylogeny, Orchidaceae, Taiwan.

The present paper is a continuation of efforts to update the orchid flora of Taiwan. A complete list of native orchids of Taiwan and their type information were recently published (Lin *et al.*, 2016). However, the emergence of newly discovered orchids from different locations has continued due to ongoing orchid hunting activities. A recent field trip resulted in the discovery of one new hybrid orchid in Taiwan. Moreover, names of many orchids need to be reviewed to ensure that living plants correctly fit the original descriptions. In this report, the names, *Cypripedium macranthos* and *Cyrtosia javanica*, used in Taiwan for a long time, were found not to agree with live plants; therefore, we need to replace them with new names.

New hybrid of Gastrochilus

This Gastrochilus hybrid was originally discovered by Chin-Chi Chen (陳金琪) and Han-Hui Lee (李漢輝) about 10 years ago. It was observed growing on the same trunk of a broadleaf tree with the parental species separated by a distance of only 1-2 m. The known population is small, with fewer than 10 individuals. The type specimen Gastrochilus × hsuehshanensis exhibits features similar to either G. formosanus or G. rantabunensis. We observed another individual on a nearby trunk bearing flowers that slightly differed from those of the type specimen. The flower has a cup-shaped lip with a rounded bottom which is more similar to that of G. rantabunensis (see Fig. 1A). This indicates that the existing population is derived not only from direct hybridization between these two parental species. This hybrid was briefly reported by Chung and Hsu (2016, p. 46) without giving it a scientific name.

Exclusion of *Cyrtosia javanica* and *Cypripedium macranthos* from the orchid flora of Taiwan

Cyrtosia javanica Blume was reported from Taiwan

by Leou and Chung in 1995. However, the plant morphology of the Taiwanese plant differs from that of *Cyr. javanica* published by C.L. Blume (1825). Taiwanese plants are usually only 5 cm above ground, in contrast to being \geq 20 cm above ground of *Cyr. javanica* (Blume 1835, Averyanov 2011). This confusion with identification was probably caused by similar flower gross morphologies. Checking several earlier publications, we summarize differences between these two species (Table 1).

The column of Cyr. javanica is curved and 3-lobed on the apical part (Blume 1825, 1835, Fig. 2). This differs from that of Cyr. taiwanica which shows a straight column and only a small column wing. The lip morphology of Cyr. javanica is quite confusing according to different authors. Blume (1825) showed a large ovate callus on the basal part of the lip disc (Fig. 2-2), and in 1835, he showed 2 large round thickenings on the apical part of the lip, while Comber (2001) depicted 2 thickened calli near the base, and J.J. Smith (1922-1923) demonstrated large ovate calli on the lip disc. J.J. Smith's figure is more or less similar to the Taiwanese plant, except for the very much thickened and raised sides of the lip. Our conclusion is that Cyr. taiwanica has a very different vegetative part from that of Cyr. javanica, and the lip feature also differs compared to Blume's publications (1825, 1835). It is interesting to note that a similar lip to the Taiwanese plant was observed in Cyr. plurialata Seidenf. (1995) which was misidentified as Galeola javanica by Seidenf. in 1978. The original specimen C.S. Leou 5830 used by Leou and Chung (1995) was not found. Herein, we designate L.H. Yang 197 as the type specimen.

Since the introduction of *Cypripedium macranthos* Swartz, a widely distributed species in temperate Asia, to Taiwan's flora in 1912, nobody has challenged the correctness of this name in describing the Taiwanese





Fig. 1. A-F. Photos of *Gastrochilus ×hsuehshanensis* T.P. Lin & S.K. Yu. A & B. Individuals of *G. ×hsuehshanensis* in their native habitat. The flower of B is used for comparison in C-F. C. Comparison between *G. formosanus* (right) and *G. ×hsuehshanensis* (left).
D. Flower comparison between *G. formosanus* (left) and *G. ×hsuehshanensis* (right). E. Flower from above view; *G. rantabunensis* (left) and *G. × hsuehshanensis* (right). F. Flower from side view; *G. rantabunensis* (left) and *G. ×hsuehshanensis* (right). G & H. *Cyrtosia taiwanica* K.H. Wang & T.P. Lin in its native habitat; G. Flowers, H. Fruits. I & J. *Cypripedium taiwanalpinum* Y.I. Lee, P.C. Liao & T.P. Lin at the type location. Photo of *Cyr. taiwanica* was taken by Mr. Kuo-Chu Yueh. Photo of *Cyp. taiwanalpinum* was taken by Chien-Fan Chen, Taiwan Forestry Research Institute, in 2012. Scale bar: C. 1 cm, D, F. 5 mm.



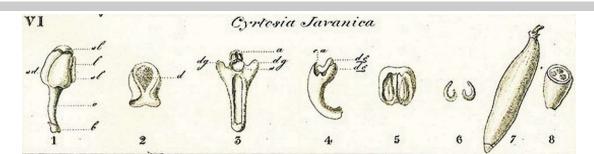


Fig. 2. Line-drawing of *Cyrtosia javanica* Blume cropped from pl. 6 of Blume (1825). 1. Flower; 2. Lip; 3. Column, frontal view; 4. Column, side view; 5. Anther, view from below; 6. Pollinia; 7. Fruit; 8. Fruit, cross-section.

Table 1	. Features	which can b	e used to	differentiate	Cyrtosia	taiwanica from Cyl	r. javanica
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Feature	Cyrtosia taiwanica	Cyrtosia javanica					
Rhizome	<3 cm long	To 12 cm long (Holttum 1953)					
Stem color Pale reddish-pink		Yellowish or pinkish-yellow (Áveryanov 2011)					
Stem	5–8 cm long	20–25 cm, slender (Blume 1835), Syntype of <i>C. javanica</i> (L0061304)					
Inflorescence	1 inflorescence per plant, inflorescence not branched	Several inflorescences per plant, 1–3 branches per inflorescence (Blume 1835)					
Flower color	Sepal pale reddish-brown, petal and lip bright-yellow	Pale-yellow (J.B. Comber 2001), yellowish to yellowish- orange (Averyanov 2011)					
Lip base and raised sides	Not thickened, yellow	A red thickening on either sides (Holttum 1953)					
Lip disc	A large, thick callus on the lip disc	A large, thick callus on the basal disc (Blume 1825); 2 large round calli on the apical part (Blume 1835)					
Column	Straight	Curved (Blume 1825, 1835, Holttum 1953)					
Column wings	Slightly dilated	Strongly dilated, 3-lobed (Blume 1825, 1835, Reichenbach 1862)					
Petals	Bright-yellow	White with yellowish tips (Holttum 1953)					
Fruit	Erect	Pendulous (Blume 1825, 1835; Reichenbach 1862)					

counterpart. The first author has studied Asian Cypripedium spp. and recently found that our plant is not conspecific with Cyp. macranthos of China and Japan. Indeed, Cyp. macranthos is similar in gross morphology to Cyp. taiwanalpinum, and the differences are easily overlooked. The most significant feature of Cyp. macranthos is a series of erect projections forming an ornamental border around the lip orifice that is completely lacking in Cyp. taiwanalpinum (Fig. 3, Table 2). In fact, we checked figures from Siberia (Curtis's Botanical Magazine 1829. Pl. 2938), China (Flora of China @ efloras.org), and Japan (Nakajima 2012), and all of them showed distinct, erect, scalloped projections at the entrance of the orifice. This feature also occurs in several varieties: Cyp. macranthos var. albiflorum Makino, Cyp. macranthos var. maximum Nakai, and Cyp. macranthos var. speciosum (Rolfe) Koidz. (Nakajima, 2012).

In addition, we also studied the molecular phylogenetic relationships among *Cyp. macranthos*, *Cyp. taiwanalpinum* and several species of *Cypripedium* using DNA sequences.

Molecular evidence supporting *Cyp. taiwanalpinum* being independent from *Cyp. macranthos*

We used three chloroplast (cp)DNA markers (see Supplemental table), including intron *rpl22* and the intergenic spacers, *atpl-atpH* and *psbA-trnH*, to reconstruct



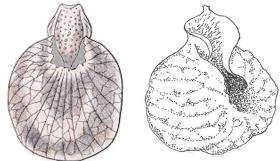


Fig. 3. Comparison of the lip morphology between *Cypripedium taiwanalpinum* (upper right, taken by Chien-Fan Chen) and *Cyp. macranthos* (upper left, taken by Y.I. Lee). Line drawing of *Cyp. macranthos* (lower left) was taken from Curtis's Bot. Mag. 56: t. 2938 (1829). The mouth is contracted and crenate with white margins (left) which is absent from *Cyp. taiwanianum* (right).



-JHKR2

N229

- TJ963

TJ962

C. calcicola

C. fasciolatun

0.99 JJ961

TN228

Taiwan

C franchetii

JR204

RS01

C macranthos

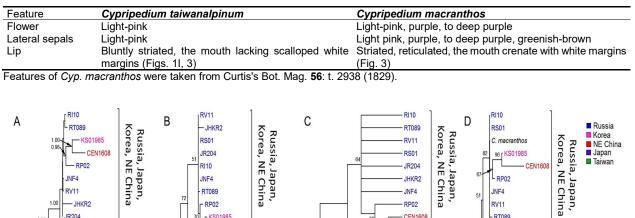
1.00

0.99

0.99

C. shanxiense

0.005



RP02

CEN1608

KS01985

TJ961

- TJ962

TN228

TN229

TJ963

C. franchetii

C. calcicola

C. fasciolatum

C. shanxiense

C. macrantho:

laiwan

Table 2. Comparison between Cypripedium taiwanalpinum and Cyp. macranthos.

RP02

macranth

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68 | TJ961

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C. calcico

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Taiwan

C franches

Fig. 4. Phylogenetic relationships among Cypripedium macranthos, Cyp. calcicola, Cyp. franchetii, Cyp. fasciolatum, and Cyp. shanxiense reconstructed using A. Bayesian inference (BI), B. Maximum-likelihood (ML), C. Maximum-parsimony (MP) and D. Neighbor-joining (NJ) methods. The number next to the node represents the posterior probability (BI) and bootstrap support (ML, MP, and NJ). Samples of Cyp. macranthos collected from Russia, Korea, Northeast China, and Japan, and Cyp. taiwanalpinum from Taiwan are denoted in blue, pink, red, purple, and green colors, respectively. Phylogenetic trees reconstructed by the four methods reveal a consistent inference of polyphyletic relationship of samples of Cyp. macranthos from Russia, Korea, NE China, and Japan, and samples of Cyp. taiwanalpinum from Taiwan.

phylogenetic relationships among Cyp. macranthos, Cyp. calcicola, Cyp. franchetii, Cyp. fasciolatum, Cyp. shanxiense, and Cyp. taiwanalpinum using Bayesian inference (BI), maximum-likelihood (ML), maximumparsimony (MP), and Neighbor-joining (NJ) approaches. Samples of Cyp. taiwanalpinum of Taiwan and samples of Cyp. macranthos were collected from populations of Taiwan, Russia, Japan, Korea, and NE China. The optimal substitution model used for the BI and ML analyses was HKY+ G based on the Akaike information criterion (AIC) determined using MEGA 7 (Kumar et al., 2016). The BI analysis was conducted with MrBayes 3.2.2 (Ronquist et al., 2012), with two runs of four Markov chain Monte Carlo (MCMC) algorithms and a random starting tree, each for 10 million generations with every 1000 generations sampled; the ML tree was created with PhyML 3.0 (Guindon et al., 2010), and branching support was assessed using aLRT SH-like nonparametric bootstrap resampling; the MP tree was created using TNT 1.5 (Goloboff et al., 2008) with default settings and 1000 bootstraps; the NJ analysis was conducted using MEGA 7 (Kumar et al., 2016) with 95% partial deletion for gaps, the maximum composite 342

likelihood model for nucleotide substitutions, and 1000 bootstraps for assessing branching support.

RV11

-RT089

JR204 IHKR2

> TN229 93

TJ961

TJ962

TJ963

C. shanxiense

0.005

C. calcicola

C. fasciolatum

TN228

laiwan

C francheti

The phylogenetic trees inferred by different methods revealed a consistent evolutionary relationship: sequences of Cyp. macranthos collected from NE China, Korea, Japan, and Russia were grouped with the published sequence in GenBank (accession no. NC 024421.1), while the sample of Cyp. taiwanalpinum of Taiwan grouped with Cyp. calcicola, Cyp. franchetii, and Cyp. fasciolatum but not with those from other regions (Fig. 4). In other words, the molecular evidence did not support Taiwan's population being monophyletic with Cyp. macranthos in other regions. Therefore, if Taiwan's population is to be regarded as Cyp. macranthos, at least the taxonomic status of Cyp. calcicola, Cyp. franchetii, and Cyp. fasciolatum must be reevaluated. In conclusion, the most reasonable taxonomic treatment is to separate Taiwan's population from Cyp. macranthos according to evidence of morphology, geographical distribution, and genetic data. Based on morphological features (Fig. 3) and molecular data (Fig. 4), we confirmed Cyp. taiwanalpinum was independent from Cyp. Macranthos.



TAXONOMIC TREATMENT

Gastrochilus ×hsuehshanensis T.P. Lin & S.K. Yu, hybrid nov.

Figs. 1A-F,5 雪山松蘭

Type: Taiwan: Taichung City, 1800 m, Mar. 12, 2019, *Sheng-Kun Yu s.n.*, (holo. TAI288270).

Plant small, ca. 3 cm long. Stem evident, similar to that of G. formosanus (Fig. 1 C), branched, rooting throughout, prostrate or pendulous. Leaves crowded, more or less similar to G. rantabunensis, distichous, elliptic, to 8×6 mm, fleshy, acute, green, often with reddish-brown dots, articulated, with a persistent sheath. Inflorescence usu. shorter than 1 cm, peduncles bearing 2 or 3 flowers. Floral bracts triangular, small. Ovary and pedicel 5 mm long. Flowers greenish, with whitish lip, minutely punctate on both surfaces; upper sepals elliptic, 3.7×2.7 mm, obtuse; lateral ones slightly oblique, $4.4 \times$ 2.3 mm; petals elliptic-orbicular, 3.5×2.5 mm, rounded, sometimes slightly ciliate which is similar to that of G. rantabunensis (Fig. 1B). Lip with a slightly compressed semicircular sac and a broad terminal limb; sac cup-like, with a round orifice, 3.5×3.5 mm, greenish-yellow, punctate on both sides, slightly tapering at bottom similar to that of G. formosanus; limb broadly semiorbicular, 3.5×6.2 mm, whitish, relatively dense hairs on upper surface, with denser hairs on greenish center (Fig. 1B, E); hairs white, shorter, and less dense than those of G. rantabunensis (Fig. 1E, F) but longer than those of G. formosanus (Fig. 1D). Column stout, with purplish blotches. Rostellum bifid; rostellar arms cylindrical, curved, rounded at apex. Anther-cap ovate, yellow; pollinia 2, globose, yellow, notched, attached to a horseshoe-shaped viscidium through a narrow translucent stipe.

Flowering time: March.

Distribution: Endemic. **Taiwan**: Found in forests only in one locality in Taichung City at an elevation of 1800 m.

Etymology: The specific epithet as well as vernacular names in Chinese is derived from the type locality Hsuehshan or Mt. Hsueh, Taichung City, Taiwan.

Cyrtosia taiwanica K.H. Wang & T.P. Lin, sp. nov. Figs. 1G, H, 2 肉果蘭

Type: Taiwan: Nantou: Chitou, between Chitou and Shanlinchi, June 6, 2011, *L.H. Yang 197* (TAIE28272).

Cyrtosia javanica auct. non Blume, Bijdr. Fl. Ned. Ind. 396, pl. 6. 1825: Leou & Chung in Q. J. Exp. For. Natl. Taiwan Univ. **9(4)**: 20, f. 1, 2. 1995; Su, Fl Taiwan 2^{nd} ed. **5**: 835. pl. 354. 2000. Taiwan: Nantou: Chitou, in artificial bamboo forest. *C.S. Leou* 5830 (not found).

Mycoheterotrophic. Flowering plants ca. 4–8 cm tall above ground. **Roots** fascicled, tuberous, clavate, $5-8 \times 0.8-1.2$ cm. **Stems** very short, reddish, covered with imbricate scales. Scales elliptic-ovate, apex acute,

concave, glabrous, yellowish-brown, turning darkbrown with age. Inflorescence terminal, 2-3 cm long, with 5-8 flowers. Floral bracts subtriangular. Pedicel and ovary pubescent. Flowers succulent, moderately opening, ca. 2 cm across; sepals thick, mealy-pubescent outside, pale reddish-brown on both surfaces, margins incurved; upper one elliptic, 16×8 mm, slightly concave; lateral ones broadly ovate or elliptic, 16×9.5 mm, slightly concave; petals oblong, bright-yellow, 15×7 mm, thinner than sepals, slightly grooved on back. Lip oblong in outline, 15×9 mm in natural state, glabrous, bright-yellow, centrally grooved underneath; lateral lobes inflexed and hovering above column but not meeting, rugose inside; midlobe semicircular, front edge incurved, emarginate at apex; disc highly thickened along midrib, whitish on apical half, yellow or greenishyellow on basal half, with a depression in center, also decorated with 2 small erose-margined keels in center. Column white but tinged yellowish on apical part, clavate, 8.5 mm long, dilated near apical part, shortly hirsute, hairs denser on basal half, with 2 small lateral wings at apex, apex margin serrulate. Rostellum recurved. Anther-cap yellow, deltoid from side view, short-papillose on upper surface; pollinia 2, obliquely oblong in outline, strongly folding and highly compressed. Fruits 5 cm long, spindle-like, yellowishbrown, more or less 3-ridged.

Flowering time: May-June.

Distribution: Endemic. **Taiwan**: Found in bamboo forests in central Taiwan at an elevation of ca. 1200 m.

Cypripedium taiwanalpinum Y.I. Lee, P.C. Liao, & T.P. Lin, *sp. nov.*

Figs. 11, J, 3, 6 奇萊喜普鞋蘭

Type: Taiwan: Nantou Co., Mt. Hohuan East Peak, 3394 m, June 4, 2012, *C.F. Chen 3522* (TAIF 390875, fig. 6).

Cypripedium macranthos auct. non Swartz, Kongl. Vetensk. Acad. Nya Handl. **21:** 251. 1800: Hayata, Icon. Pl. Formos. **2**: 136. 1912; Liu & Su, Fl. Taiwan **5**: 951, pl. 1575. 1978; Su, Fl. Taiwan **5**: 834, pl. 352. 2000. *Type*: E. Siberia. Specimen: Japan: May 1876, *J. Bisset 371* (K000077918).

Flowering plants 20–30 cm tall. Rhizomes stout, branching. Roots dense. **Stems** erect, 10–20 cm long, with a few tubular sheaths at base. **Leaves** 2–5, alternate, sessile, closely arranged when flowering, elliptic or ovate-lanceolate, $5-11 \times 3-4.6$ cm, apex acute, base shortly sheathed, plicate, entire, sparsely hirsute on both surfaces. **Peduncle** 4–9 cm long, densely hirsute. Floral bracts leafy, enclosing ovary, ovate, ca. $5-8 \times 2.5-3$ cm, sparsely ciliate. Pedicel and ovary ca. 2.7-3 cm long, glabrous. **Flowers** solitary, white to pale-pink, with red stripes and spots, ca. 5-7 cm in diam.; upper sepal ovate to orbicular, $3-3.3 \times 1.7-2.5$ cm, shortly bifid at apex; petals ovate-lanceolate, ca. $3.5-4.2 \times 1.4-1.7$ cm, apex acute, spotted with red, sparsely hirsute near base. **Lip**



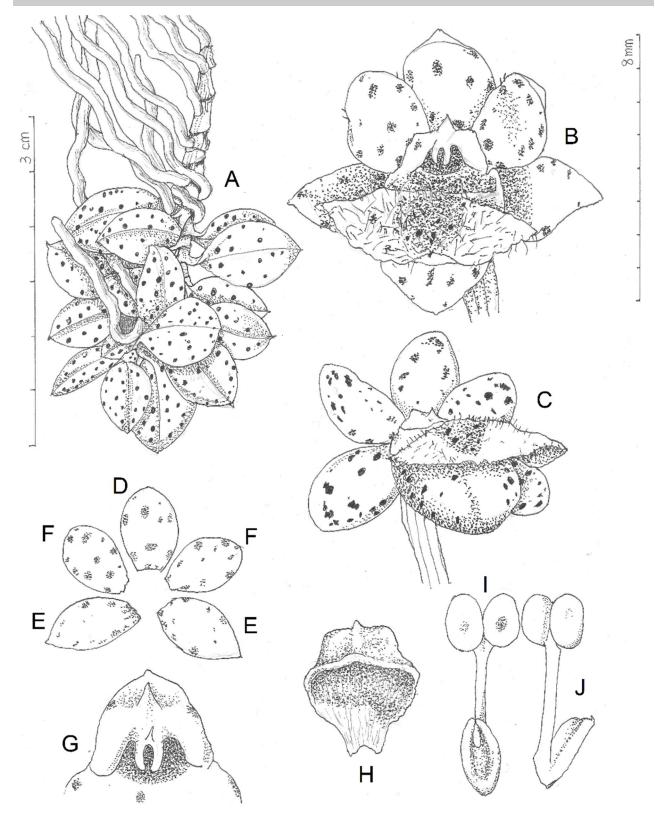


Fig. 5. Line drawing of the *Gastrochilus* ×*hsuehshanensis*. A. Plant; B. Flower, frontal view; C. Flower from another individual; D. Upper sepal; E. Lateral sepal; F. Petal; G. Column apex, frontal view; H. Anther-cap, view from below; I. Pollinarium, view from below; J. Pollinarium, view from side.





Fig. 5. Type specimen of *Cypripedium taiwanalpinum* Y.I. Lee, P.C. Liao, & T.P. Lin.

pouch-shaped or slightly compressed-globose, $2.6-3 \times 2$ cm, 1.5-1.8 cm tall, pinkish or yellowish, with red or purple spots and stripes, or white only, with a deep depression on top and forming an orifice inside, entrance of orifice not decorated with erect scalloped projections, dense brown hairs on rear surface near base of sac, more or less parallel veins radiating from a common depression at center of lip surface; orifice ca. 1 cm across. Column ca. 13 mm long, apex with 2 wing-like appendages, with a pair of fertile lateral stamens and a large thick sterile staminode. Each fertile anther globose, fleshy, 3 mm across, located at base of wing-like appendage, with 2 naked loculi, each loculus with 2 equal pollen masses, pollen masses powdery and glutinous; staminode whitish or pale-green, elliptic, $9 \times$ 6-7 mm, short-stalked, 1.2 mm wide near base, grooved on upper surface. Stigma thick, 3-lobed, 8 mm long, rounded at apex, lying just below staminode, not protruding beyond staminode.

Flowering time: April-July.

Distribution: Endemic. Taiwan: Grows in alpine grasslands and on steep cliffs at elevations of 2000–3900 m.

Etymology: The specific epithet is derived from the alpine mountains of Taiwan.

Note: Albino flowers have also been found in Taiwan. Some specimens, identified as *Cyp. taiwanianum nom*.

nud. should be *Cyp. segawae* Masamune with yellow flowers. However, the flower color is not easy to discriminate in old specimen.

Specimens examined: Taiwan: Taitung, Hsiangyang, May 6, 1936, K. Segawa s.n. (KPM, photo G00975 in PoT); Taiwan: Mt. Nanhuta, 3690 m, July 19, 1940, Masamune 3154 (TAI32538); Taiwan: Taichung Co., Mt. Nanhuta, 3400 m, July 6, 2005, S.W. Chung 7858 (TAIF231508); Taiwan: Hualien Co., Hsiulin Hsiang, Mt. Nanhuta, 3500 m, July 4, 2005, J.J. Xu and J.S. Dong 0734 (TNM10532); Taiwan: Taosai Peak, July 27, 2017, S.W. Chung 9511 (TAIF 512977 no image).

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Supplemental table: Sequences used in the phylogenetic analysis.

Species	Taxa code		- Source		
Species		psbA-trnH trnL-trnF atpl-atpH			
Cypripedium macranthos	RI10	MN057735	MN057750	MN057765	This study
Cypripedium macranthos	RP02	MN057736	MN057751	MN057766	This study
Cypripedium macranthos	RT089	MN057737	MN057752	MN057767	This study
Cypripedium macranthos	RV11	MN057738	MN057753	MN057768	This study
Cypripedium macranthos	RS01	MN057739	MN057754	MN057769	This study
Cypripedium macranthos	CEN1608	MN057740	MN057755	MN057770	This study
Cypripedium macranthos	JR204	MN057741	MN057756	MN057771	This study
Cypripedium macranthos	JHKR2	MN057742	MN057757	MN057772	This study
Cypripedium macranthos	JNF4	MN057743	MN057758	MN057773	This study
Cypripedium macranthos	KS01985	MN057744	MN057759	MN057774	This study
Cypripedium macranthos		KF262112.1	JF797026.1	JF797148.1	NCBI
Cypripedium taiwanalpinum	TN228	MN057745	MN057760	MN057775	This study
Cypripedium taiwanalpinum	TN229	MN057746	MN057761	MN057776	This study
Cypripedium taiwanalpinum	TJ961	MN057747	MN057762	MN057777	This study
Cypripedium taiwanalpinum	TJ962	MN057748	MN057763	MN057778	This study
Cypripedium taiwanalpinum	TJ963	MN057749	MN057764	MN057779	This study
Cypripedium franchetii		JQ923466.1	JF797024.1	JF797146.1	NCBI
Cypripedium calcicola		JF796977.1	JF797029.1	JF797137.1	NCBI
Cypripedium fasciolatum		JQ923461.1	JF797023.1	JF797145.1	NCBI
Cypripedium shanxiense		JF796975.1	JF797018.1	JF797136.1	NCBI