



Taxonomic revision of the formerly monotypic orchid genus *Dactylostalix*

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Abstract

The Calypsoinae orchid genus *Dactylostalix*, previously considered a monotypic genus endemic to Japan and the Russian Far East (the Kuril Islands and Sakhalin Island), is now redefined to encompass two species. This reclassification is based not only on the examination of type specimens and literature but also on molecular data. While *Pergamena uniflora* has long been regarded as a synonym of *Dactylostalix ringens*, it is distinguishable by its shorter scape, smaller flower, less spotted tepals, drooping sepals and lateral petals, labellum with smaller, narrowly triangular to ovate lateral lobes, more distinct keels on the adaxial surface of the lip, and a slender column with a smaller stigma and weakly developed clinandrium. We propose the new combination *Dactylostalix uniflora*, recognizing it as a distinct species within the genus *Dactylostalix*. Phylogenetic analysis utilizing genome-wide markers has also demonstrated that the two species are genetically distinct. Our findings, obtained through the integration of morphological data and molecular phylogenetics, indicate that *D. uniflora* represents a distinct evolutionary lineage from *D. ringens*. Examination of type specimens has led us to conclude that *Calypso japonica*, *Dactylostalix maculosa*, and *Dactylostalix ringens* f. *punctatus* are junior synonyms of *D. ringens*. Additionally, we designate the lectotypes for *P. uniflora* (= *D. uniflora*), *C. japonica*, and *D. maculosa*.

Key words: emended description, genetics, morphology, new combination, taxonomy, typification

Introduction

The epidendroid subtribe Calypsoinae is a small, largely temperate subtribe that includes 13 genera and ca. 80 species (Chase *et al.* 2015; Freudenstein *et al.* 2017). Due to their unique lifestyle, members of Calypsoinae have garnered significant attention for elucidating character evolution, such as shifts from autotrophy to mycoheterotrophy (Barrett *et al.* 2010; Suetsugu *et al.* 2021a, 2022; Suetsugu & Matsubayashi 2021; Yagame *et al.* 2021). The genus *Dactylostalix* Reichenbach (1878: 74) is currently recognized as a monotypic genus and is found only in Japan and the Russian Far East (the Kuril Islands and Sakhalin Island) (Freudenstein 1994; Freudenstein *et al.* 2005). The genus is characterized by the solitary flower, pollinarium with four superposed pollinia and a viscidium with a rudimentary stipe, the absence of a corm/pseudobulb, and a flat and non-plicate leaf within the subtribe (Freudenstein 1994; Freudenstein *et al.* 2005).

However, during recent field explorations, we observed several populations of *Dactylostalix* exhibiting a shorter scape, a smaller flower, and less spotted tepals, prompting us to investigate the possibility of these unusual

morphologies representing a distinct lineage from *D. ringens* Reichenbach (1878: 74; Fig. 1–2). Upon examining its morphology and comparing it to previously documented species, we have determined that these plants are congruent with *Pergamena uniflora* Finet (1900: 263; Fig. 3–4). While *P. uniflora* is currently considered a synonym of *D. ringens* (Freudenstein 1994; Freudenstein *et al.* 2005), it arguably constitutes a distinct lineage within the genus *Dactylostalix*, due to its isolated morphology from *D. ringens*. Therefore, we propose the combination *D. uniflora* (Finet) Suetsugu, rendering *Dactylostalix* no longer monotypic. As the protologue of *P. uniflora* lacks sufficient morphological detail (Finet 1900), we provide an emended description of *P. uniflora* based on the type specimens supplemented with newly collected specimens. Molecular data obtained by genome-wide markers (i.e., MIG-seq data) reveal a significant genetic divergence between *D. ringens* and *D. uniflora*, corroborating the presence of corresponding genetic distance to the morphological differences.

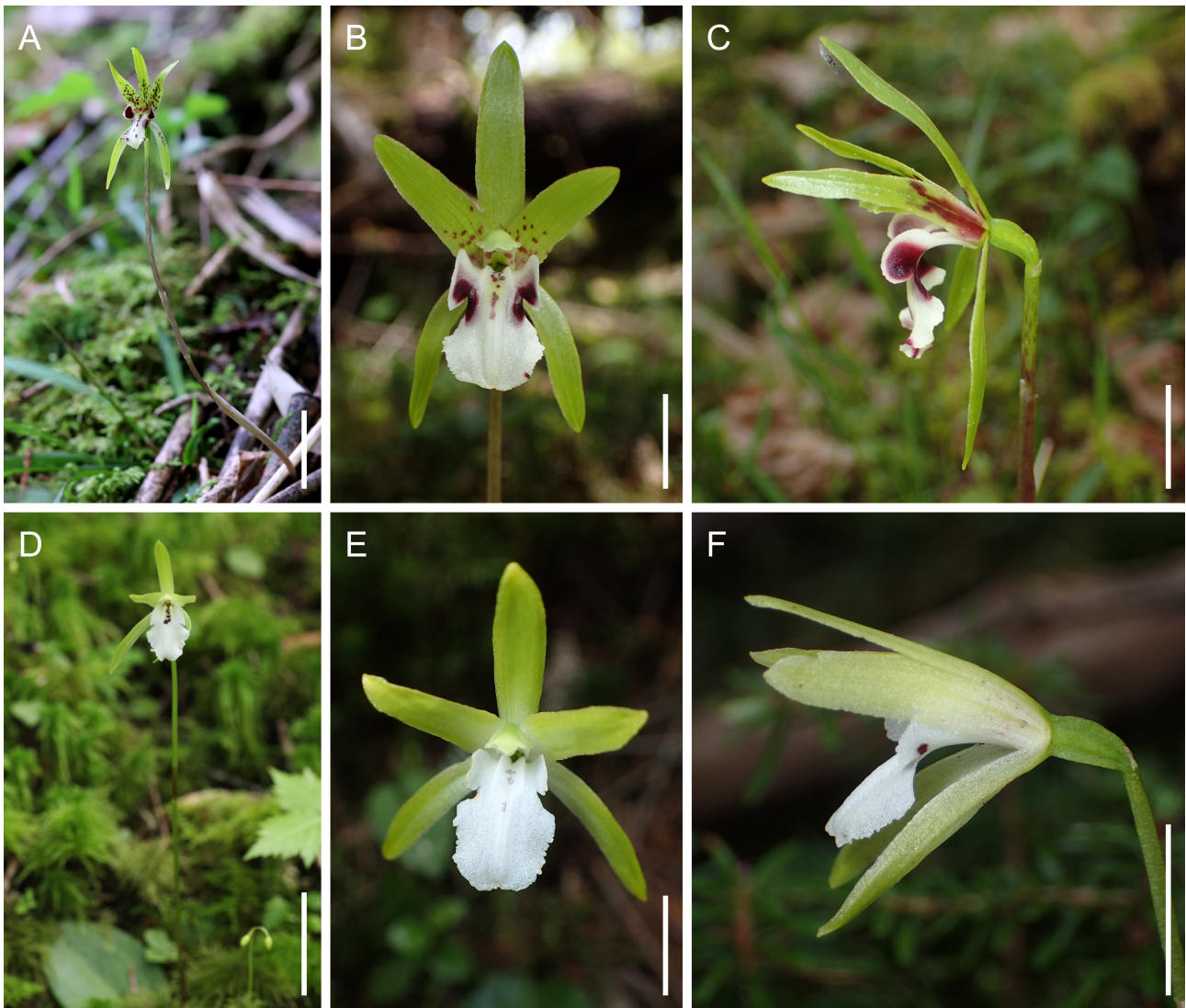


FIGURE 1. Morphological comparison of *Dactylostalix ringens* (A–C) and *D. uniflora* (D–F). (A) Flowering plant in Ena-shi, Gifu Pref. (B–C) Flower in Mt. Fuji, Fujinomiya-shi, Shizuoka Pref. (D) Flowering plant in the Akaishi Mountains, Yamanashi Pref. (E–F) Flower in Mt. Fuji, Fujinomiya-shi, Shizuoka Pref. Scale bars: 30 mm (A, D) and 10 mm (B–C & E–F).

Materials and methods

Morphological observations

We compared the morphological characteristics of *D. ringens* and *D. uniflora* using herbarium specimens housed in K, KYO, LE, P, SAPS, TI, TNS, and W and living plants collected during fieldwork between 2012 and 2022 (Table S1). These characteristics were evaluated using a stereomicroscope and measured with a digital caliper. Additionally,

we analyzed morphological differences through literature review and online digitized plant collections such as JSTOR Global Plants (<http://plants.jstor.org/>). Each newly collected sample from each population during our field survey was represented by at least one voucher specimen deposited in KYO (Table S1). The herbarium acronyms correspond to those listed in the Index Herbariorum (Thiers 2023).

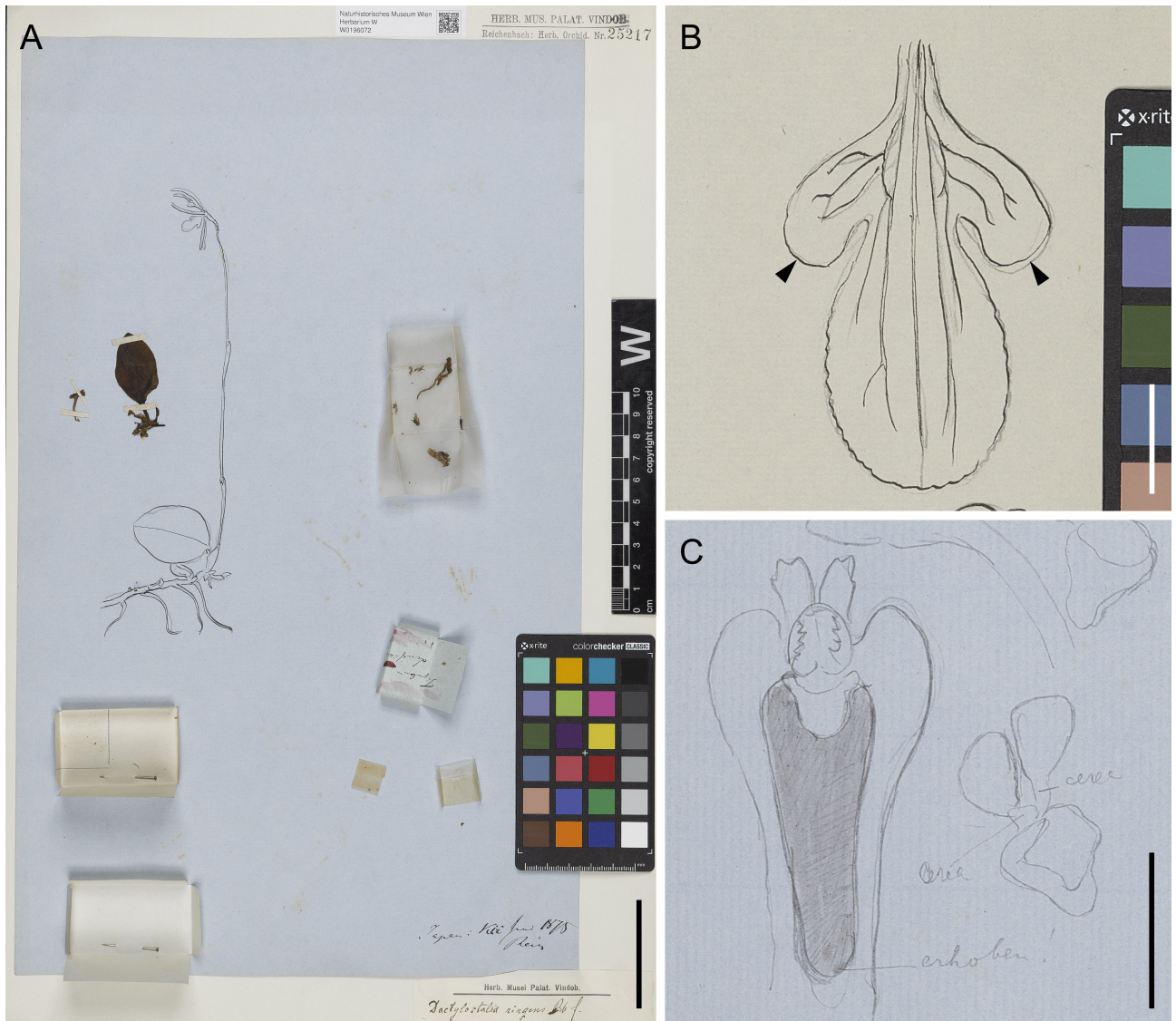


FIGURE 2. Holotype of *Dactylostalix ringens* (W0196072). (A) Habit and its line drawing. (B) Line drawing of the labellum. (C) Line drawing of the upper part of the column (left) and pollinarium (right). Black arrows indicate semi-orbicular side lobes. Scale bars: 50 mm (A) and 5 mm (B–C).

MIG-seq-based high-throughput genomic analysis

For multiplexed inter-simple sequence repeat genotyping (MIG)-seq analysis, we used 11 *D. uniflora* individuals and 14 *D. ringens* individuals collected from various locations in Japan (Table S1). Genomic DNA was extracted from silica-dried leaves using the CTAB method (Doyle & Doyle 1990). A MIG-seq library for these samples was prepared according to the method described by Suyama *et al.* (2022) and sequenced using an MiSeq system (Illumina, San Diego, CA, USA) with an MiSeq Reagent Kit v3 (150 cycles). The raw reads of the MIG-seq data were deposited in the DDBJ Sequence Read Archive under the BioProject accession number PRJDB17995.

After removal of primer sequences and low-quality reads (Suetsugu *et al.* 2021b), we obtained 2845217 reads (113809 ± 6088 reads per sample) from 3256786 raw reads (130271 ± 7120 reads per sample). We used the Stacks 2.65 pipeline for *de novo* single nucleotide polymorphism (SNP) discovery (Rochette *et al.* 2019) with the following parameters: minimum depth of coverage required to create a stack (m) = 3, maximum distance allowed between stacks (M) = 2, and number of mismatches allowed between sample loci while building the catalog (n) = 2. We filtered

out SNPs with high heterozygosity ($H_o \geq 0.6$), those with fewer than three minor alleles, and retained only those SNPs present in three or more samples. In total, 3489 loci and 6062 SNPs were used for subsequent analyses. We constructed a maximum-likelihood (ML) phylogeny based on SNPs using RAXML v. 8.2.10 (Stamatakis 2014) and a GTR substitution model with Lewis' ascertainment bias correction, with 1000 bootstrap replicates. Additionally, we used SplitsTree4 4.14 (Huson & Bryant 2006) to construct a Neighbor-Net network based on an uncorrected p distance matrix.

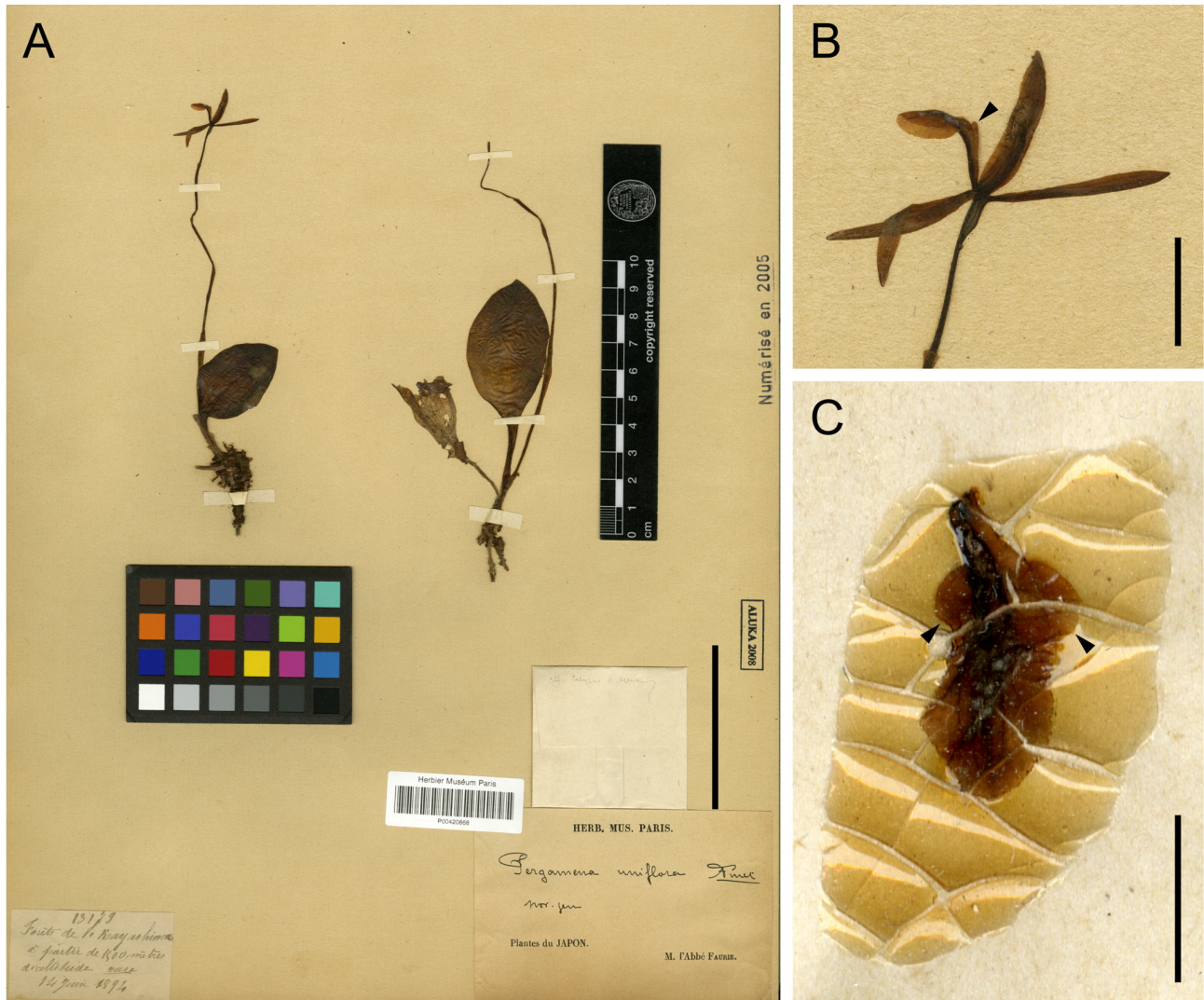


FIGURE 3. Lectotype of *Pergamena uniflora* designated in the present study (P00420868). (A) Habit. (B) Flower, lateral view. (C) Labellum. Black arrows indicate small ovate side lobes. Scale bars: 50 mm (A), 10 mm (B), and 5 mm (C).

Taxonomic treatment

Dactylostalix Reichenbach (1878:74) Type:—*Dactylostalix ringens* Reichenbach (1878: 74).

Rhizome creeping, pale brown. Scape arising from the rhizome, erect, glabrous, pale green, bearing one flower at the apex. Leaf solitary; leaf blade elliptic to ovate, pale green, obtuse at the apex, rounded or obtuse at the base; petiole broadly winged. Bract pale green. Ovary and pedicel pale green. Dorsal sepal oblanceolate to oblong, yellowish-green with or without reddish-purple spots around the base, apex obtuse or acute. Lateral sepals obliquely oblong to falcate, yellowish-green with or without reddish-purple spots around the base, apex obtuse or acute. Lateral petals oblanceolate to oblong, yellowish-green with or without reddish-purple spots around the base, apex obtuse or acute. Labellum protruding, reflexed at the apex, more or less 3-lobed, disc with 2 parallel keels; lateral lobes narrowly triangular to ovate, entirely white or white with a small reddish-purple spot at the apex of each lobe, or slightly obliquely

semiorbicular, white with a large reddish-purple spot at the apex of each lobe; posterior portion of midlobe ovate to cordate, entirely white or white with randomly arranged small reddish-purple spots, or white with randomly arranged small reddish-purple spots and 2 larger reddish-purple dots at the base, margin slightly undulate and irregularly crose-denticulate. Column curved, semi-cylindrical with column wings at the lateral part of the apex and 1 appendage at the central part of the apex; rostellum well-developed; stigma located just below the rostellum. Anther hemispheric; pollinia 4, in 2 pairs, obovoid; viscidium rhomboid. Capsule obovoid.



FIGURE 4. Line drawing of *Pergamena uniflora* based on P00420868. (A) Habit. (B) Dorsal sepal. (C) Lateral sepal. (D) Lateral petal. (E) Column, labellum, and ovary. (F) Labellum. (G) Apical part of the column, ventral view. (H) Apical part of the column, oblique ventral view. (I) Anther cap, lateral view. (J) Anther cap, ventral view. (K) Pollinarium. Scale bars: 20 mm (A), 10 mm (B–E), 5 mm (F–H), and 2.5 mm (I–K). Reproduced from Finet (1900).

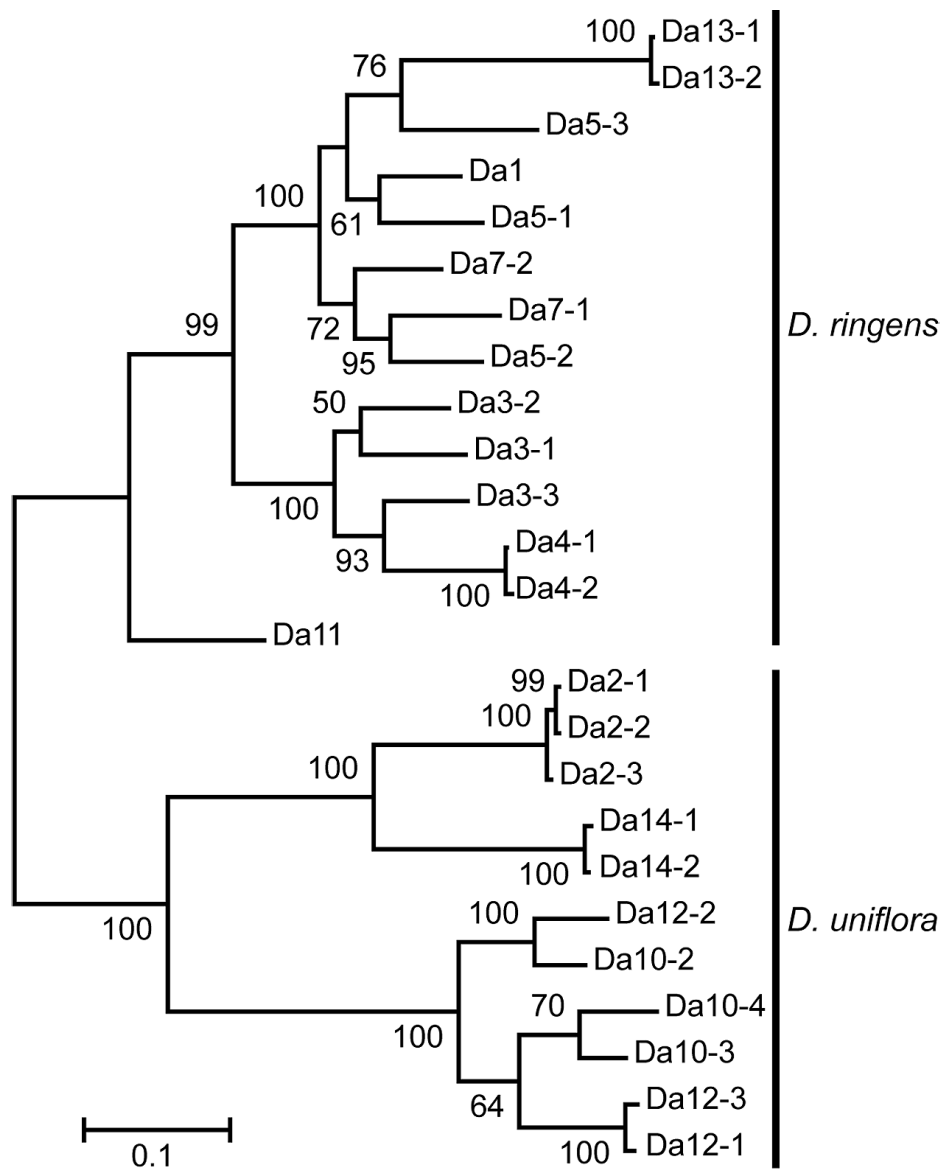


FIGURE 5. Phylogenetic tree of *Dactylosteinia ringens* and *D. uniflora* reconstructed from MIG-seq data. Nodes supported by bootstrap values < 50% are not shown. Branch length represents the average number of substitutions per site.

Morphology:—The genus *Dactylosteinia* is distinguished by its single flower, pollinarium featuring four stacked pollinia and a viscidium with a rudimentary stipe, the lack of a corm or pseudobulb, and a flat, non-plicate leaf within the subtribe Calypsoinae (Freudenstein 1994; Freudenstein *et al.* 2005). The genus *Dactylosteinia* encompasses two species (*D. ringens* and *D. uniflora*). *Dactylosteinia uniflora* can be differentiated from *D. ringens* by its shorter scape, smaller flower, less spotted tepals, drooping sepals and lateral petals, a labellum with smaller, narrowly triangular to ovate lateral lobes, more pronounced keels on the adaxial surface of the lip, and a slender column with a less developed clinandrium.

Distribution:—The genus *Dactylosteinia* is endemic to Japan and the Russian Far East (the Kuril Islands and Sakhalin Island) (Freudenstein 1994; Freudenstein *et al.* 2005). *Dactylosteinia ringens* is found over wider climate ranges, while *D. uniflora* is limited to the subarctic zone (high elevation areas in Honshu and Hokkaido). Even within sympatric populations, *D. ringens* tends to be found at lower elevations than *D. uniflora* populations. For instance, in Mt. Fuji, *Dactylosteinia ringens* is found over a broader range of elevations (800–2200 m) while *D. uniflora* is typically found in the subalpine zone (2200–2400 m). However, further quantitative assessments are needed to accurately determine the distribution of both species.

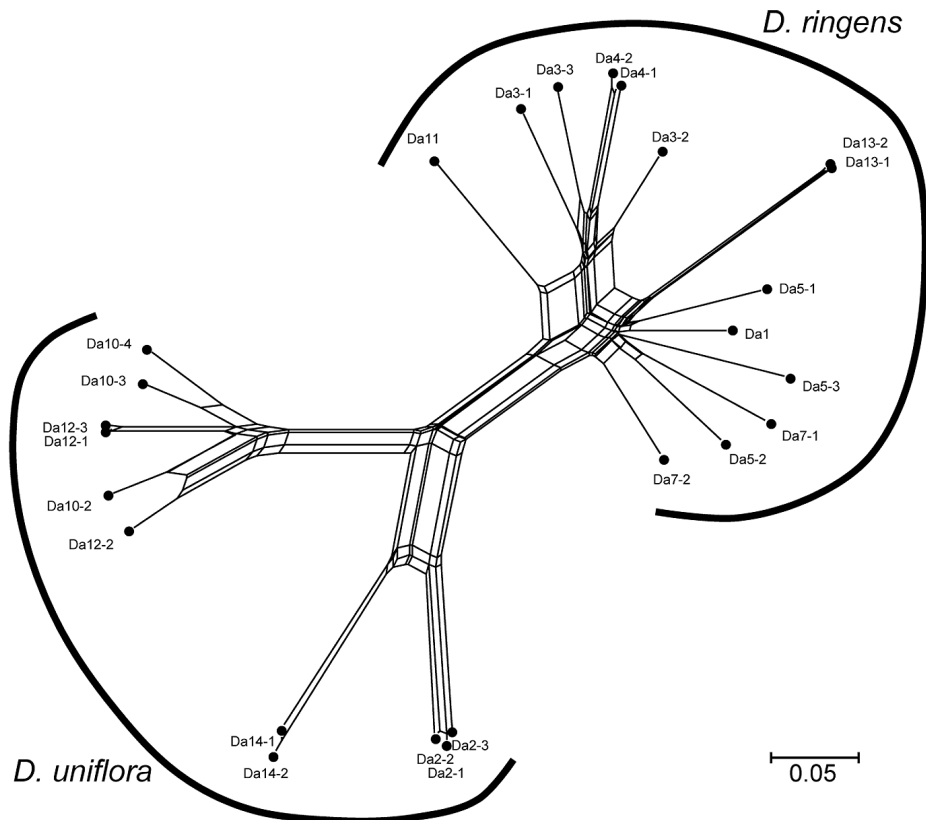


FIGURE 6. Neighbor-Net network of *Dactylostalix ringens* and *D. uniflora* reconstructed based on uncorrected p distance calculated from 6062 SNPs.

Phylogeny:—The maximum likelihood phylogenetic analysis revealed that *D. uniflora* forms a distinct clade from *D. ringens*, supported by a 100% bootstrap value (Fig. 5). Additionally, Neighbor-Net phylogenetic analysis indicated that *D. ringens* and *D. uniflora* represent at least two distinct genetic clusters (Fig. 6). This analysis also suggests some genetic differentiation within *D. uniflora*; however, morphologically, no clear differences were found between these two subgroups of *D. uniflora* based on our current dataset. Therefore, we conclude that it is appropriate to classify these as two distinct species, *D. ringens* and *D. uniflora*. Given that *D. uniflora* is distributed in high-altitude zones, these populations are likely prone to rapid differentiation.

Importantly, there was complete alignment between the morphological identification performed before obtaining genetic results and the genetic data. Thus, the diagnostic characteristics we employ are sufficiently robust to enable clear differentiation, ensuring that intraspecific variation does not compromise the classifications.

Dactylostalix ringens Reichenbach (1878: 74) (Figs. 1A–C, 2, 7B–C & 9–11).

Type:—JAPAN. **Kinki District**—Kii (= Wakayama Pref.: Mt. Koya?), June 1875, *Rein s.n.* (holotype: W0196072!).

= *Dactylostalix maculosa* Miyabe & Kudo (1915: 3). Type:—JAPAN. **Hokkaido District**—Oshima Prov.: Kamiiso-gun, Moheji-mura (= Hokkaido Pref.: Hokuto-shi, Moheji), 16 May 1906, *S. Ninoue s.n.* (lectotype: SAPS036659!), designated here.

= *Dactylostalix ringens* Reichenbach (1878: 74) var. *punctata* Miyabe & Tatewaki (1937: 49) ≡ *Dactylostalix ringens* Reichenbach (1878: 74) f. *punctata* (Miyabe & Tatewaki 1937: 49) Yonekura (2011: 239). Type:—JAPAN. **Hokkaido District**—Kushiro Prov.: Kamioboro (= Hokkaido Pref.: Akkeshi-gun, Akkeshi-cho, Kamioboro), 14 June 1937, *M. Tatewaki s.n.* (holotype: SAPS036661!).

= *Calypso japonica* Maximovich in Komarov (1901: 533) ≡ *Calypso bulbosa* (Linnaeus 1753: 951) Oakes (1842: 28) var. *japonica* (Maximovich in Komarov 1901: 533) Makino (1905: 14). Type:—JAPAN. **Chubu District**—Fujijama (= Shizuoka Pref.: Fujinomiya-shi, Mt. Fuji), 1894, *Tschonoski* (= *Chonosuke Sukawa*) *s.n.* (lectotype: the third individual from the right in the upper row of LE01012254!), designated here.

Rhizome creeping, pale brown. Scape arising from the rhizome, erect, (10–)15–26 cm long, 1.5–2.5 mm in diameter at the middle, glabrous, pale green, bearing one flower at the apex. Leaf solitary; leaf blade elliptic to ovate, 37–78 mm long, 20–46 mm wide, pale green, occasionally with reddish-purple spots, obtuse at the apex, rounded or obtuse at the base; petiole broadly winged, 10–28 mm long. Bract 2.1–3.2 mm long, pale green. Ovary and pedicel 6.5–10.4 mm long, pale green. Dorsal sepal oblanceolate to oblong, 18–30 × 3.3–7.5 mm, yellowish-green with reddish-purple spots around the base, apex obtuse or acute. Lateral sepals obliquely oblong to falcate, 16–28 × 2.6–5.7 mm, yellowish-green with reddish-purple spots around the base, apex obtuse or acute. Lateral petals oblanceolate to oblong, 16–27 × 3.2–7.2 mm, yellowish-green with reddish-purple spots around the base, apex obtuse or acute. Labellum protruding, distinctly reflexed at the apex, 3-lobed, 12–19 × 9.5–13.9 mm, disc with 2 low keels from base to basal 1/3; lateral lobes slightly obliquely semiorbicular, 5.0–9.3 × 2.8–4.0 mm, white with a large reddish-purple spot at the apex of each lobe; posterior portion of midlobe ovate to cordate, 7.8–13.2 × 9.5–13.9 mm, white with randomly arranged small reddish-purple spots and 2 larger reddish-purple dots at the base, margin slightly undulate and irregularly crenate-denticulate. Column curved, 9.7–13.2 × 3.4–7.0 mm at the widest part (a little above the middle) and 1.9–3.5 mm wide at the base, semi-cylindrical with column wings at the lateral part of the apex and 1 appendage at the central part of the apex; rostellum well-developed; stigma located just below the rostellum, 2.0–4.5 × 1.8–4.2 mm. Anther hemispheric, 1.3–2.0 mm in diameter; pollinia 4, in 2 pairs, obovoid, 1.3–2.0 × 0.8–1.1 mm; viscidium rhomboid, 0.6–1.0 mm long. Capsule obovoid, 2.2–2.9 cm long.



FIGURE 7. Original materials of *Calypso japonica* (LE01012254). (A) Habit. (B) Flower, front view. (C) Flower, lateral view. A black arrow in (A) indicates a lectotype designated here, while black arrows in (B) and (C) indicate semi-orbicular side lobes. The individual shown in (B) was designated as a lectotype in the present study. Scale bars: 50 mm (A) and 10 mm (B–C).



FIGURE 8. Original materials of *Calypso japonica* (LE01012252 & LE01012253). (A) Habit (Upper: LE01012252 & Lower: LE01012253). (B) Flower, front view (LE01012252). (C) Flower, lateral view (LE01012252). Black arrows indicate small, narrowly triangular side lobes. Scale bars: 50 mm (A) and 10 mm (B–C).

Additional specimens examined:—**RUSSIA. Kuril Islands**—Etorofu Island: Mt. Ooyama, 28 July 1931, *Ohwi s.n.* (SAPS). **JAPAN. Hokkaido District**—Hokkaido Pref.: Akkeshi-gun, Akkeshi-cho, Kamioboro, 14 June 1937, *Tatewaki 28156* (SAPS); loc. cit., 14 June 1937, *Tatewaki 28156* (SAPS); loc. cit., 14 June 1937, *Tatewaki 28156* (SAPS); loc. cit., 14 June 1937, *Tatewaki 28155* (SAPS); loc. cit., 14 June 1937, *Tatewaki 28155* (SAPS); loc. cit., 26 June 2023, *Shutoh Dall* (KYO); Samani-gun, Samani-cho, Fuyushima, Mt. Apoi, 15–19 July 1929, *Koizumi 19274* (TNS); loc. cit., date unknown, *Tsushima s.n.* (TI); loc. cit., 28 June 1974, *Kumata s.n.* (SAPS); loc. cit., 29 May 1927, *Tatewaki 9117* (SAPS); loc. cit., 12 June 1952, *Tatewaki & Tsujii s.n.* (SAPS); loc. cit., 12 June 1977, *Hara 4761d* (SAPS); Saru-gun, Hidaka-cho, 5 June 1952, *Samejima & Nishimura s.n.* (SAPS); Hakodate-shi, Mt. Hakodate, 27 May 1982, *Ito 5878* (SAPS); Hokuto-shi, Moheji, 25 May 1907, *Ninoue s.n.* (SAPS); Hiyama-gun, Assabu-cho, 24 May 1955, *Tatewaki, Igarashi & Watanabe s.n.* (SAPS); Nishi-gun, Otobe-cho, Asahitai, Takemori, 16 May 2000, *Takahashi 27041* (SAPS); Hiyama-gun, Assabu-cho, 10 May 2012, *Suetsugu Da4* (KYO); loc. cit., 21 October 2021, *Sugawahara Da3* (KYO). **Tohoku District**—Aomori Pref.: Mutsu-shi, Ohata-cho, Mt. Osorezan, June 1912, *Kinashi s.n.* (KYO); Higashitsugaru-gun, Hiranai-cho, Karibasawa, Mt. Eboshi-dake, 10 June 1928, *Kudo s.n.* (KYO); Mutsu-shi, Kawauchi-cho, Shishihata, Kawauchigawa Valley, 23 May 1996, *Koga 12243* (KYO); Nishitsugaru-gun, Hukaura-machi, Juniko, 19 June 1932, *Muramatu s.n.* (TI). Iwate Pref.: Kamaishi-shi, Kasshi-cho, Mt. Goyosan, 8 June 1952, *Kawasaki s.n.* (TNS). Akita Pref.: Odate-shi, Hinai-machi, 7 June 1931, *Matsuda s.n.* (TNS); Oga-shi, Oganakakokuyuchinai, Mt. Kenashi, 30 May 2004, *Horii 35073* (KYO). Fukushima Pref.: Mt. Azuma, July 1903, *Nakahara s.n.* (TI); Minamiaizu-gun, Hinoemata-mura, Mt. Magohyoe-yama, 24 July 1966, *Okuda 5370* (TNS). **Kanto District**—Tochigi Pref.: Shimotsuke-shi, 20 May 1915, *Numajiri s.n.* (KYO); Nikkou-shi, Mt.

Nyohozan, 20 July 1902, *Takeda s.n.* (SAPS); Nikkou-shi, near Shizu, 25 July 1902, *Takeda s.n.* (SAPS); loc. cit., 26 July 1902, *Takeda s.n.* (SAPS); Nikko-shi, Chugushi, Shizu, 3 July 1931, *Maekawa 4300* (TNS); Nikko-shi, Chugushi, Shizu, Mt. Nantai, 7 February 1931, *Tsuyama s.n.* (TI); Nikko-shi, Yumoto, 16 June 1878, *collector unknown s.n.* (TI); Nikko-shi, Yumoto, edge of Lake Yunoko, 2 July 1947, *Teramoto s.n.* (TI); Nikkou-shi, Konsei-ridge, date unknown, *Takeda s.n.* (SAPS). Gunma Pref.: Tone-gun, Katashina-mura, Tokura, 19 June 1951, *Mizushima 1200* (TI); Tone-gun, Katashina-mura, Tokura, Hatomachi-toge, 5-11 June 1948, *Kawasaki 4905* (TNS); loc. cit., 3 July 1941, *Hara s.n.* (TI); Tone-gun, Katashina-mura, Tokura, north of Mt. Shibutsu, 15 July 1929, *Hara s.n.* (TI); Agatsuma-gun, Tsumagoi-mura, Tashiro, Mt. Azumaya, 1 July 1934, *Hara s.n.* (TI); Agatsuma-gun, Nakanojo-cho, Iriyama, 22 June 2023, *Matsui, Ichikura & Ichikura Da13* (KYO). Saitama Pref.: Chichibu-gun, Yokoze-machi, Mt. Bukozan, 8 June 1941, *Shimizu s.n.* (TNS); Chichibu-shi, Nakatsugawa Mt. Ryokami, 19 May 1956, *Hara s.n.* (TI); Chichibu-shi, Nakatsugawa, Jumonji-toge, 8 June 1930, *Narita s.n.* (TI). **Chubu District**—Toyama Pref.: Nakaniikawa-gun, Tateyama-machi, Ashikuraji, Bunazaka, 9 June 1935, *Hashimoto s.n.* (TI); loc. cit., 7 June 1936, *Hashimoto 1384* (TI). Yamanashi Pref.: Minamitsuru-gun, Nishikatsura-cho, Shimokurechi, Mitsutoge, 10 June 1934, *Hiyama 4999* (TNS); Minamitsuru-gun, Narusawa-mura, Fujifunatsuguchi mountain trail, 19 May 1957, *Kanai s.n.* (TI); Minamitsuru-gun, Fujikawaguchiko-machi, Shoji, Aokigahara, northern foot of Mt. Fuji, 13 June 1965, *Kawano, Ohashi & Ihara s.n.* (TI); Minamiarupusu-shi, Asuyashiashikura, en route from Momonoki hot spring to Yashagami-toge, 4 June 1967, *Konta 6070* (TNS); Hokuto-shi, Kobuchisawa-cho, Mt. Yatsugatake, 9 July 1917, *Yamada s.n.* (KYO); loc. cit., 18 June 1955, *Murata & Horikawa 1076* (KYO); Nirasaki-shi, Maruno-machi, Shimotsuburai, Mt. Hohou, 15 July 1952, *Ito s.n.* (TNS). Nagano Pref.: Minamisaku-gun, Kitaaiki-mura, Mt. Ogura, 17 June 1977, *Murata 3875* (TI); Kitasaku-gun, Karuizawa-machi, 15 June 1958, *Koyama n.21182* (TNS); Minamisaku-gun, Kawakami-mura, en route from Senjogahara to Jumonji-toge, 8 August 1958, *Hotta 10225* (KYO); Shimotakai-gun, Yamanouchi-machi, Hirao, Mt. Yakebitai, 13 July 1980, *Midorikawa s.n.* (TI); Minamisaku-gun, Sakuho-cho, Yachiho Highland, 10 July 1974, *Mimoro, Tsugaru & Nishiyama 1846* (KYO); Chino-shi, Minotoguchi, Mt. Yatsugatake, 6 June 1990, *Midorikawa 906011* (TI); Chino-shi, Mt. Yatsugatake, July 1923, *Oka-Hubo s.n.* (TNS); Suwa-gun, Hara-mura, Mt. Akadake, 25 June 1936, *Ishizuka 67* (TI); Chiisagata-gun, Nagawa-machi, Daimon, July 1926, *Abe s.n.* (TNS); Ina-shi, Hasekurogouchi, Mt. Senjogatake, 11 July 1965, *Iwatsuki, Fukuoka & Naruhashi 197* (KYO); Ina-shi, Hasekurogouchi, Mt. Nyukasa, the point where the Oguro and Minamisawa Rivers converge, 21 June 1954, *Kanai s.n.* (TI); Shimoina-gun, Oshika-mura, Kashio, Mt. Takamori, 9 June 1957, *Asano 10643* (TI); Azumino-shi, Misatoogura, Kurosawa Waterfall, 28 May 1995, *Koga 11805* (KYO); Azumino-shi, Hotakaariake, Mt. Tsubakurodake, 25 June 1914, *Yazawa s.n.* (TNS); Matsumoto-shi, Azumi, Kamikochi, between Okumatashiro and Yokowo, 29 June 1968, *Sugiyama 11162* (TI). Gifu Pref.: Nakatsugawa-shi, Misaka, Fujimidai, 10 June 1965, *Asano 9331* (TI); Nakatsugawa-shi, Misaka, 15 May 2022, *Iwahori Da5* (KYO); loc. cit., 5 June 2022, *Iwahori Da6* (KYO); Takayama-shi, Takane-machi, Nomugi, Nomugi-toge, 13 June 1958, *Kanai s.n.* (TI); Nakatsugawa-shi, Nakatsugawa, Mt. Ena, 19 July 1961, *Hashimoto s.n.* (TI); Takayama-shi, Nyukawa-cho, Iwaidani, Mt. Norikura, 5 July 1936, *Koizumi s.n.* (KYO); loc. cit., 2 July 1966, *Okuyama s.n.* (TNS); Gero-shi, Hagiwara-cho, Sakurabora, Mt. Gozenyama, 23 May 1971, *Murata, Okamoto & Takahashi 31* (KYO). Shizuoka Pref.: Fujinomiya-shi, Awakura, Mt. Fuji, 28 June 2022, *Sato Da8-1* (KYO); Fujinomiya-shi, Mt. Fuji, 1864, *Tschonoski (=Sukawa) s.n.* (K); Fujinomiya-shi, Awakura, Mt. Fuji, 8 June 2022, *Sato Da7* (KYO); Shizuoka-shi, Aoi-ku, Tashiro, Mt. Chausudake, 7 July 1967, *Konta, Tsuno & Nagao Konta 6180* (TNS). Aichi Pref.: Kitashitara-gun, Shitara-cho, Tsugu Honsawa, Mennoki Touge, 18 May 1952, *Torii 12439* (KYO); Kitashitara-gun, Shitara-cho, Damine, Dandobenzaiten, 26 May 1951, *Torii 12375* (KYO). **Kinki District**—Mie Pref.: Mie-gun, Komono-cho, Komono, Mt. Gozaisho, 10 May 1958, *Iwatsuki & Kitagawa s.n.* (KYO). Hyogo Pref.: Mikatagun, Kami-cho, Muraokaku Yamada, Mt. Mikawayama, 5 May 1978, *Hashimoto 11101* (KYO); Shiso-shi, Chikusacho, Nishigouchi, Chikusa Kogen, 21 May 2000, *Kobayashi 34499* (KYO). Nara Pref.: Yoshino-gun, Tenkawa-mura, Dorogawa, Mt. Sanjogadake, 27 May 1955, *Murata & Iwatsuki 98* (KYO); Kamikitayama-mura, Mt. Odaigahara, 22 May 1968, *Hotta 16906* (KYO); Yoshino-gun, Tenkawa-mura, Dorogawa, 26 May 1955, *Murata & Iwatsuki 532* (KYO). Wakayama Pref.: Ito-gun, Koya-cho, Mt. Koya, May 1921, *Nakajima s.n.* (KYO); loc. cit., 1921, *Nakajima s.n.* (TI). **Shikoku District**—Tokushima Pref.: Mima-gun, Tsurugi-cho, Ichiu, Mt. Marusasa, 5 June 1984, *Yamazaki s.n.* (TI); Miyoshi-shi, Higashiyasugeoi, Mt. Tsurugi, 5 May 1961, *Tabata s.n.* (KYO). Ehime Pref.: Niihama-shi, Besshiyama, Mt. Higashiakaishi, 30 May 1957, *Murata & Shimizu 1846* (KYO); Kita-gun, Uchiko-cho, Nakagawa, Odamiyama Valley, 17 May 1925, *Yamashita s.n.* (KYO). Kochi Pref.: Agawa-gun, Niyodogawa-cho, Nanokawa, 21 May 1888, *unknown collector s.n.* (TI); loc. cit., 4 April 1890, *Watanabe s.n.* (TI).

Vernacular name:—Ichi-yo-ran (in Japanese).

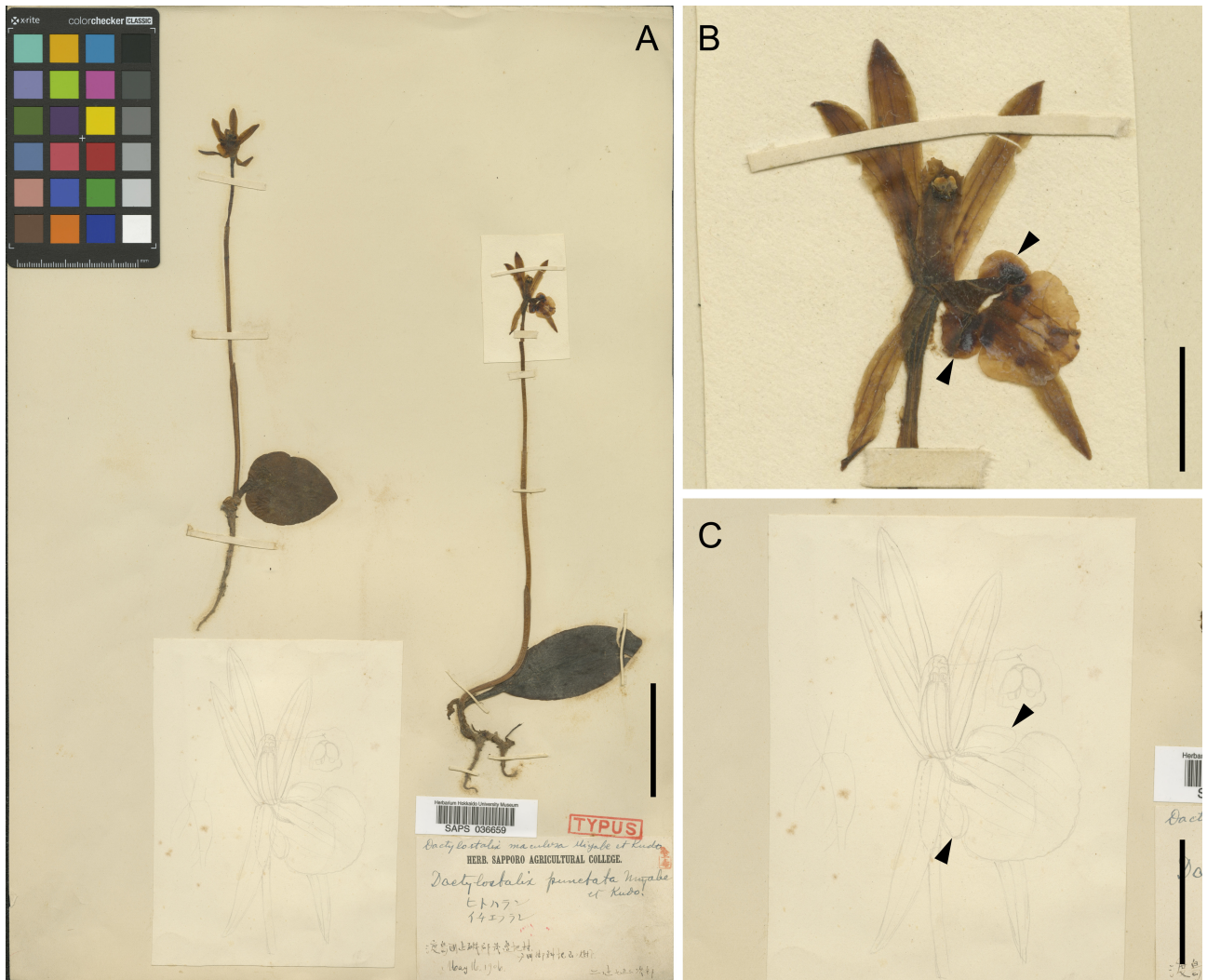


FIGURE 9. Lectotype of *Dactylostalix maculosa* designated in the present study (SAPS036659). (A) Habit. (B) Flower, front view. (C) Line drawing of the flower. Black arrows indicate semiorbicular side lobes. Scale bars: 50 mm (A) and 10 mm (B–C).

Taxonomic notes:—*Dactylostalix ringens* was described based on specimens from Kii (now Wakayama and southwestern Mie Prefectures) collected in June 1875 by Johannes Justus Rein (Reichenbach 1878). Given Rein’s travels in June 1875 (Rein 1881), the most likely location is Mt. Koya, Wakayama Prefecture. The plants currently treated as *D. ringens* (a common type within the *D. ringens* species complex) have been found predominantly around this type locality, encompassing not only Mt. Koya but also other areas in the Kinki District, including Wakayama and southwestern Mie Prefectures. Despite the poor preservation of its holotype specimen, line drawings based on this type specimen provide detailed lip morphology, crucial for identifying *Dactylostalix* species. The semiorbicular lateral lobes align well with typical *D. ringens* individuals (Fig. 2). Since *D. ringens* was described as having ligulate and rounded lateral lobes (Reichenbach 1878), *D. ringens* was undoubtedly described based on the entity we now recognize as *D. ringens*.

Calypso japonica was described based on specimens from three localities (Mt. Fuji, Shizuoka Prefecture; Hakone, Kanagawa Prefecture; and Nambu, Iwate Prefecture) collected in 1864–1866 by Sugawa Chonosuke (Komarov 1901). Reviewing the original materials, we found *C. japonica* was described based on a mixture of *D. ringens* and *D. uniflora* (Figs. 5–6). Specifically, individuals from Nambu (LE01012252) share characteristics with *D. uniflora*, such as small, narrowly triangular lateral lobes (Fig. 5). In contrast, most individuals from Mt. Fuji (LE01012254) align with *D. ringens* due to large, semiorbicular lateral lobes (Fig. 6). The individual from Hakone (LE01012253) was indeterminate due to late collection and subsequent difficulty in floral morphology assessment. Given the brevity of Maximovich’s description in Komarov (1901: 533), it is unclear whether *D. ringens* or *D. uniflora* was the focus. However, since *C. japonica* has been synonymized with *D. ringens* in subsequent studies (Freudenstein 1994; Freudenstein *et al.* 2005; Inoue 2016), we designate the *D. ringens* collected at Mt. Fuji as the lectotype to stabilize the nomenclatural position of

C. japonica. Notably, LE01012254 likely comprises a few individuals of *D. uniflora* (e.g., the second individual from the left). This supposition is justifiable, as our field surveys confirmed their co-occurrence in Mt. Fuji. To eliminate ambiguity, we have designated a single *D. ringens* plant (the third individual from the right in the upper row in Fig. 6A) as the lectotype of *C. japonica*.



FIGURE 10. Holotype of *Dactyloctenium ringens* var. *punctatum* (SAPS036661). (A) Habit. (B) Flower, front view. (C) Flower, lateral view. Black arrows indicate semiorbicular side lobes. Scale bars: 50 mm (A) and 10 mm (B–C).

Dactyloctenium maculosa was described based on specimens from Moheji, Hokkaido Prefecture, collected on 16 May 1906 and 25 May 1907 by Shimejiro Ninoue. Miyabe & Kudo (1915) noted that *D. maculosa* differs from *D. uniflora* by having a broader stem and a larger flower with a roundish labellum featuring conspicuous purple dots. However, these characteristics as outlined by Miyabe and Kudo (1915) align with those of *D. ringens*. *D. maculosa* was described based on two specimens collected on different dates. Given that (i) the sheet SAPS036659 was already marked as a type specimen and (ii) the key diagnostic characteristic (large, semiorbicular lateral lobes) was clearly identifiable on the sheet, we designate SAPS036659 as the lectotype and SAPS036660 as the isolectotype of *D. maculosa* (Fig. 7). The relative prevalence of *P. uniflora* in Hokkaido likely led to the erroneous assumption by Kudo that *D. ringens* and *P. uniflora* were identical, subsequently leading to the description of *D. maculosa* as a new species.

Dactyloctenium ringens f. *punctatum* was described based on a specimen from Kamioboro, Hokkaido Prefecture, collected on 14 June 1937 by Misao Tatewaki. *D. ringens* f. *punctatum* is distinguished from *D. ringens* f. *ringens* solely by the presence of purple-brown spots on the leaves. Since there are no significant differences in labellum and column morphology, which are crucial for identifying *Dactyloctenium* species, we have concluded that *D. ringens* f. *punctatum* is synonymous with *D. ringens*. Given that Miyabe & Kudo (1915) had originally named what is now recognized as *D. ringens* as *D. maculosa*, it may seem somewhat perplexing that, in Miyabe & Tatewaki (1937), *D. ringens* f. *punctatum*

was correctly identified as a variant of *D. ringens*. A likely explanation for this discrepancy is that, although Miyabe was the lead author in both publications, it was Kudo and Tatewaki who spearheaded the research and wrote the papers, respectively. It has been suggested that, possibly due to the rules of Hokkaido University at that time, Kudo and Tatewaki often listed Miyabe's name as the primary author in many papers, a practice contrary to the current norm of including the supervisor as the last author (Shutoh Kohtaro, personal communication). Therefore, it can be inferred that there was a difference in the understanding of *D. ringens* between Kudo and Tatewaki, with the latter accurately recognizing *D. ringens* f. *punctata* as a variant of *D. ringens*.

Preliminary conservation status:—So far, more than 1000 mature individuals of *D. ringens* have been observed. This species mainly thrives in densely forested regions, which are often situated within protected areas or forest parks. This species mainly thrives in densely forested regions, which are often situated within protected areas or forest parks. In accordance with the IUCN criteria (IUCN 2019), the conservation status of *D. ringens* is evaluated as “Least Concern”.



FIGURE 11. *Dactylostalix ringens* (Katsumi Iwahori Da5, KYO). (A) Habit. (B) Flower, lateral view. (C) Column, labellum, and ovary. (D) Dorsal sepal. (E) Lateral petal. (F) Lateral sepal. (G) Labellum. (H) Flatten labellum. (I) Column. (J) Pollinarium on the apical part of the column. (K) Anther cap and pollinarium. Scale bars: 30 mm (A) and 5 mm (B–K).

Dactylostalix uniflora (Finet) Suetsugu, *comb. nov.* (Figs. 1D–F, 3–4, 8 & 12)

Basionym: *Pergamena uniflora* Finet (1900: 263)

Type:—JAPAN. **Tohoku District**—Forêts de l'Hayashinie, à partir de 1500 mètres (= Iwate Pref.: Miyako-shi, Mt. Hayachine), 14 June 1894, *Faurie U.J.*, 13179 (lectotype: P00420868!; isolectotypes: P00420869!, K000943526! & KYO00090434!), designated here.

Rhizome creeping, pale brown. Scape arising from the rhizome, erect, 8–22 cm long, 1.0–1.5 mm in diameter at the middle, glabrous, pale green, bearing one flower at the apex. Leaf solitary; leaf blade elliptic to ovate, 35–60 × 15–29 mm, pale green, obtuse at the apex, rounded or obtuse at the base; petiole broadly winged, 8–21 mm long. Bract 1.4–2.5 mm long, pale green. Ovary and pedicel 6.0–9.2 mm long, pale green. Dorsal sepal oblanceolate to oblong, 15–27 × 2.1–4.0 mm, yellowish-green, apex obtuse or acute. Lateral sepals obliquely oblong to falcate, 13–25 × 1.5–3.1 mm, yellowish-green, apex obtuse or acute. Lateral petals oblanceolate to oblong, 13–25 × 2.2–3.7 mm, yellowish-green, apex obtuse or acute. Labellum protruding, slightly reflexed at the apex, indistinctly 3-lobed, 11.6–21 × 4.0–9.7 mm, disc with 2 distinct keels from the base to the middle, slightly divergent apically; lateral lobes narrowly triangular to ovate, 3.0–6.8 × 0.4–2.0 mm, entirely white or white with a small reddish-purple spot at the apex of each lobe; posterior portion of midlobe ovate, 5.5–8.6 × 4.9–8.2 mm, entirely white or white with randomly arranged small reddish-purple spots, margin slightly irregularly erose-denticulate. Column slightly curved, 8.5–15 × 2.7–4 mm at the widest part (a little below the top) and 1.7–3.2 mm wide at the base, semi-cylindrical with column wings at the lateral part of the apex and 1 appendage at the central part of the apex; rostellum well-developed; stigma located just below the rostellum, 0.9–3.5 × 0.8–3.2 mm. Anther hemispheric, 0.9–1.4 mm in diameter; pollinia 4, in 2 pairs, obovoid, 0.8–1.1 × 0.6–0.9 mm; viscidium rhomboid, 0.6–0.9 mm long. Capsule obovoid, 1.9–2.5 cm long.

Additional specimens examined:—RUSSIA. Kuril Islands—Shikotan Island: Shikotan-mura, Chiboi, 10 August 1931, *Ono s.n.* (SAPS); loc. cit., 3 August 1934, *Tatewaki 20876* (SAPS); loc. cit., 8 July 1934, *Ono s.n.* (TNS); loc. cit., 10 August 1931, *Ono 32* (TI); Shikotan-mura, 1937, *unknown collector s.n.* (TNS). **JAPAN. Hokkaido District**—Hokkaido Pref.: Nemuro-shi, Ochiishi-higashi, 7 July 2021, *Shutoh & Yano Da10* (KYO); loc. cit., 7 July 2021, *Shutoh & Yano 4851* (SAPS); loc. cit., 18 July 2023, *Shutoh Da12* (KYO); Nemuro-shi, Ochiishi, 2 July 1927, *Kondo s.n.* (TI); Ashoro-gun, Ashoro-cho, Moashoro, Mt. Meakan, 19 July 1981, *Takita 510* (KYO); Kitami-shi, Oketo-cho, August 1930, *Okamoto s.n.* (SAPS); Kato-gun, Kamishihoro-cho, Kamiotofuke, date unknown, *Amikawa & Konno s.n.* (SAPS); Kawakami-gun, Kawakami-cho, Mts. Taisetsu, 26 July 1937, *Hara s.n.* (TI); Hidaka-gun, Shinhidaka-cho, Shizunaitakami, Mt. Petegari, 16 July 1976, *Samejima & Sato 76, 0158* (SAPS); Kamikawa-gun, Higashikawa-cho, Mt. Asahidake, Mts. Taisetsu, 29 July 1917, *Koizumi 77970* (TNS); Kamikawa-gun, Higashikawa-cho, Mts. Taisetsu, 18 July 1941, *Okuyama s.n.* (TNS); Saru-gun, Hidaka-cho, Nukabira, 11 July 1952, *Nishimura s.n.* (SAPS); Sorachi-gun, Minamifurano-cho, Kanayama, Mt. Yubari, 12–14 July 1934, *Ohwi 4986* (KYO); Nakagawa-gun, Otoineppu-mura, Monomanai, 12 July 1930, *Terho s.n.* (SAPS); along the Ishikari River, 30 July 1952, *Tatewaki & Takahashi 43251* (SAPS). **Tohoku District**—Iwate Pref.: Miyako-shi, Mt. Hayachine, 9 July 1905, *Miura s.n.* (SAPS); loc. cit., 15 July 1968, *Enomoto 4666* (SAPS); loc. cit., 15 July 1968, *Enomoto 4667* (SAPS); loc. cit., 15 July 1968, *Enomoto 4668* (SAPS); Tono-shi, Tsukimoushi-cho, Kamitsukimoushi, Mt. Hayachine, 17 July 1936, *Muroi s.n.* (KYO); Miyako-shi, Mt. Hayachine, 9 July 1905, *Miura s.n.* (SAPS); loc. cit., 27 June 1913, *Tamaki s.n.* (TNS); loc. cit., 7 July 1933, *Toba 90* (TI); loc. cit., 27 June 1913, *Hayakawa s.n.* (TI); Hanamaki-shi, Mt. Keito, Mts. Hayachine, 16 July 1926, *Okada s.n.* (TNS); Nambu, 1865–1866, *Tschonoski (=Sukawa) s.n.* (LE). Akita Pref.: Oga-shi, Funagawaminatohonzanmonzen, Mt. Hon, 18 June 1933, *Muramatu s.n.* (TI). Fukushima Pref.: Minamiaizu-gun, Hinoemata-mura, Numayama-toge, date unknown, *Hayata 16491* (SAPS). **Kanto District**—Gunma Pref.: Agatsuma-gun, Tsumagoi-mura, Hoshimata, Manza-toge, 7–8 July 1929, *Koizumi 55271* (TNS); Agatsuma-gun, Nakanojo-cho, Iriyama, 22 June 2023, *Matsui, Ichikura & Ichikura* (KYO). Saitama Pref.: Chichibu-shi, Nakatsugawa, Jumonji-toge, 17 June 1950, *Shimizu s.n.* (TNS). **Chubu District**—Niigata Pref.: Minamiuonuma-gun, Yuzawa-cho, Mikuni, Mt. Naeba, 15 July 1927, *Ohwi s.n.* (KYO). Toyama Pref.: Toyama-shi, Arimine, en route from Magawa River to Tarobeidaira, 22 July 1936, *Hisauchi 1608* (TNS). Yamanashi Pref.: Kitatsuru-gun, Kosuge-mura, en route from Daibosatsu-toge to Mt. Koganesawayama, 16 July 1962, *Murata 27030* (KYO); Fujiyoshida-shi, Kamiyoshida, Mt. Fuji, 7 July 1977, *Inoue & Sugiyama s.n.* (TI); Kofu-shi, Kurobera-cho, Mt. Kinpu, 2 July 1948, *Shimizu s.n.* (TNS). Nagano Pref.: Minamisaku-gun, Kawakami-mura, Jumonji-toge, 10 July 1960, *Soma s.n.* (TNS); Shimotakai-gun, Yamanouchi-machi, Hirao, Mt. Akaishi, 14 July 1933, *Katumata 18657* (TI); loc. cit., 10 July 1971, *Tateishi s.n.* (TI); Chino-shi, Mt. Yatsugatake, 13 July 1930, *Asuyama s.n.* (TNS); Chino-shi, Karasawa-toge, Mt. Yatsugatake, 9 August 1938, *Watari s.n.* (TI); loc. cit., 9 July 1938, *Hara s.n.* (TI); loc. cit., 9 July 1938, *Hara s.n.* (TI); Ina-shi, Hasekurogochi, Mt. Senjogatake, 31 July 1949, *Furusawa s.n.* (TI); Shimoina-gun, Oshika-mura, Kashio, Mt. Hontani, 25 July 1949,

Furusawa s.n. (TI); Shimoina-gun, Oshika-mura, Sanpukutoge, 30 July 1927, *Saito s.n.* (TI); Kiso-gun, Agematsumachi, Mt. Komagatake, 13 July 1948, *Satomi s.n.* (TNS); loc. cit., 20 July 1924, *Muramatu s.n.* (TI); Azumino-shi, Hotakaariake, Mt. Ariake, 21 July 1921, *Koizumi 1885* (TNS). Shizuoka Pref.: Fujinomiya-shi, Awakura, Mt. Fuji, 22 September 2021, *Sato Da2* (KYO); loc. cit., 28 June 2022, *Sato Da9-1* (KYO); loc. cit., 28 June 2022, *Sato Da9-2* (KYO); loc. cit., 28 June 2022, *Sato Da9-4* (KYO); loc. cit., 28 June 2022, *Sato Da9-5* (KYO); Shizuoka-shi, Aoi-ku, Tashiro, Mt. Hijiridake, July 1921, *Sawada s.n.* (TI).

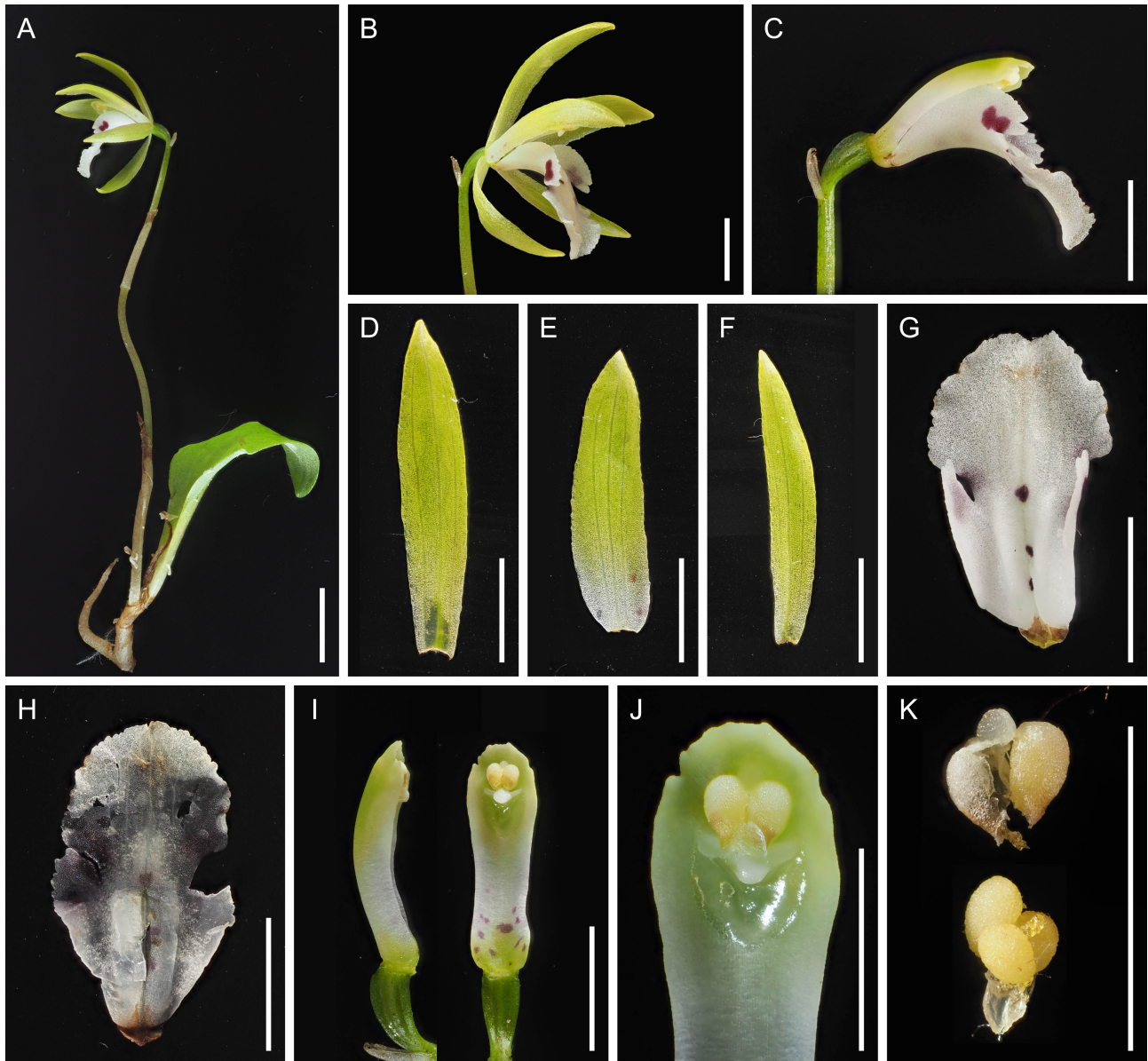


FIGURE 12. *Dactylostalix uniflora* (Masayuki Sato Da2, KYO). (A) Habit. (B) Flower, lateral view. (C) Column, labellum, and ovary. (D) Dorsal sepal. (E) Lateral petal. (F) Lateral sepal. (G) Labellum. (H) Flatten labellum. (I) Column. (J) Pollinarium on the apical part of the column. (K) Anther cap and pollinarium. Scale bars: 30 mm (A) and 5 mm (B–K).

Vernacular name:—Takane-ichi-yo-ran, nov. (in Japanese). The term “ichi-yo-ran” originates from the Japanese name for *D. ringens*, indicating an orchid species with a single leaf per individual. *Dactylostalix uniflora* prefers cooler environments compared to *D. ringens*, frequently found at higher elevations. Hence, the prefix “Takane”, which translates to high peaks or mountaintops, has been appended to the vernacular name of *D. ringens*.

Taxonomic notes:—*Pergamena uniflora* was described based on specimens from Mt. Hayachine, Iwate Prefecture, collected on 14 June 1894 by Urbanus Jean Faurie (Finet 1900). Although Finet (1900) described the labellum as free and unlobed, both line drawings in Finet (1900) and the original materials of *P. uniflora* show small but noticeable lateral lobes (Figs. 3–4). Although it differs from *D. ringens* in several morphological aspects, including smaller lateral lobes and more distinct keels on the adaxial surface of the lip, these differences do not justify separating

it into a separate genus. Thus, we propose the combination *D. uniflora* (Finet) Suetsugu, as it is considered a distinct species within the genus *Dactylosteinia*. We designate P00420868 as the lectotype and P00420869 & K000943526 as isolectotypes of *P. uniflora* (= *D. uniflora*), as P00420868 is most illustrative of the lateral lobe shape, a key diagnostic characteristic, and the line drawings in Finet (1900) were based on this specimen. Notably, no *D. ringens* specimens were found in Mt. Hayachine, the type locality of *D. uniflora*, according to our herbarium survey.

Morphological notes:—*Dactylosteinia uniflora* is morphologically similar to *D. ringens*, but can be easily distinguished by its smaller lateral lobe (obliquely narrowly triangular to ovate, 3.0–6.8 × 0.4–2.0 mm, entirely white or white with a small reddish-purple spot, versus semiorbicular, 5.0–9.3 × 2.8–4.0 mm, white with a large reddish-purple spot in *D. ringens*). Notably, *D. uniflora* exhibits variations in the size and shape of the lateral lobe, ranging from nearly absent to somewhat developed. However, even those relatively developed lobes are smaller than the semiorbicular lobes found in *D. ringens*. Additionally, *D. uniflora* is distinguishable from *D. ringens* by its shorter scape, smaller flower, less spotted tepals, drooping sepals and lateral petals, labellum with smaller lateral lobes, more distinct keels on the adaxial surface of the lip, and a slender column with a smaller stigma and weakly developed clinandrium.

Preliminary conservation status:—Our field and herbarium surveys indicate that *D. uniflora* is much less common than its closely related species *D. ringens*. Each population often contains fewer than 20 *D. uniflora* individuals, and even in the population (Mt. Fuji) that likely supports the largest number of individuals, the population size is estimated to be approximately 50. Therefore, we consider the conservation status of *D. uniflora* to be “Vulnerable” according to the IUCN criteria (IUCN 2019) under the D1 criteria, as it is estimated to have fewer than 1000 mature individuals.

Acknowledgements

The authors would like to express their gratitude to Katsumi Iwahori, Masayuki Sato, Yasutomi Sugawahara, Masayuki Matsui, Yukio Ichikura, Ayako Ichikura, Kohtaro Shutoh, Tomoko Kobayashi, Azuma Hino, Susumu Aoyagi, Norikazu Hashiba, Kunihiro Kojima for providing specimens or photographs for this study. We also thank Hidetoshi Nagamasu (Kyoto University) and Kohtaro Shutoh (Hokkaido University) for valuable discussions on *Dactylosteinia* taxonomy. We are also thankful to Kazuma Takizawa, Kazuki Inui, Ren Sato, Hidehito Okada, and Yukito Asano for their technical support. We appreciate the curators of K, KYO, LE, P, SAPS, TI, TNS, and W for granting access to herbaria and collection databases. We also thank Florent Martos (Muséum national d'Histoire naturelle) and Hiromitsu Ishikawa (Hokkaido University) for providing the high-resolution scanning images of the specimens deposited in P and SAPS, respectively. This study was supported financially by PRESTO (JPMJPR21D6, KS) from the Japan Science and Technology Agency and by the Environment Research and Technology Development Fund (#4-2001, KS and YS) from the Ministry of Environment, Japan, and MEXT Promotion of Distinctive Joint Research Center Program (JPMXP0622716984 and JPMXP0723833155, KS).

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