Bull. Natl. Mus. Nat. Sci., Ser. B, 43(3), pp. 79–86, August 22, 2017

Pogonia subalpina (Orchidaceae): a new species from Japan

Tomohisa Yukawa* and Yumi Yamashita

Tsukuba Botanical Garden, National Museum of Nature and Science,
Amakubo 4–1–1, Tsukuba, Ibaraki 305–0005, Japan
*E-mail: yukawa@kahaku.go.jp

(Received 11 May 2017; accepted 28 June 2017)

Abstract *Pogonia subalpina* T. Yukawa & Y. Yamashita (Orchidaceae) is newly described from marshy, subalpine grasslands of central and northern parts in Honshu, Japan. This species is similar to *Pogonia japonica* Rchb. f. and *P. minor* (Makino) Makino, but can be distinguished in flower by its short hairy crests on the disk of the labellum mid-lobe, by its glabrous abaxial surface of the labellum, and by its single, broad, purplish pink band along the mid-vein of the petals. Nucleotide sequence divergences among the three entities in the nuclear ribosomal DNA ITS and the plastid *trnK* 5' intron regions warrant independent species status of the new entity.

Key words: Japan, new species, Orchidaceae, *Pogonia*, taxonomy.

Introduction

The genus *Pogonia* Juss. is a small orchid genus in which four species, *i.e.*, *P. japonica* Rchb. f., *P. minor* (Makino) Makino, *P. ophioglossoides* (L.) Ker Gawl., and *P. yunnanensis* Finet, are currently accepted (Pridgeon *et al.*, 2003). *P. ophioglossoides* is widespread throughout eastern North America and the rest are distributed in continental East Asia and Japan. This genus thus represents a significant case of the eastern North American-Asian biogeographic disjunction (Chen, 1983). Cameron and Chase (1999) conducted a phylogenetic analysis of *P. japonica*, *P. minor* and *P. ophioglossoides* and also calibrated divergence time among them using the molecular clock hypothesis. They demonstrated that the disjunction between *P. ophioglossoides* in eastern North America with *P. japonica* and *P. minor* in eastern Asia is best explained by speciation following a northwestward migration via Bering land bridges in the Tertiary.

In Japan, distribution of two species, *P. japonica* and *P. minor* has been generally recognised (Yukawa, 2015). However, another entity that does not match morphologically with either of them was tentatively designated a Japanese name "Miyama-tokiso" (Takahashi, 1987). In this study we evaluate the taxonomic status of this neglected entity.

Materials and Methods

Observation and measurement of morphological characters was based on living plants, dried herbarium specimens, and spirit-preserved specimens listed in the section "Other specimens examined".

Materials used in the DNA analysis are shown in Table 1. DNA was extracted from fresh or dried leaves. Nucleotide sequences were determined by amplifying the internal transcribed spacer (ITS) regions of the 18S–26S nuclear ribosomal (nr) DNA and a region of the plastid genome encompassing ca.600 base pairs of the initial 5' region of *trnK* 5' intron via the polymerase chain reaction (PCR) from a total DNA

Table 1. Materials used for DNA sequencing. All voucher specimens are deposited at TNS

Species	Locality	Voucher
Pogonia subalpina (new entity)	Japan, Honshu. Miyagi Pref.	Y. Mikanagi s. n.
	Japan, Honshu. Niigata Pref.	C. Tsutsumi, T. Kuhara & M. Sato CT1079
	Japan, Honshu. Nagano Pref.	T. Yukawa 16-14
Pogonia japonica	Japan, Honshu. Kyoto Pref.	J. Nagasawa s. n.
	Japan, Hokkaido	T. Asanuma 2267
	Japan, Honshu. Niigata Pref.	C. Tsutsumi, T. Kuhara & M. Sato s. n.
Pogonia minor	Japan, Honshu. Nagano Pref.	T. Hashimoto s. n.

Table 2. Diagnostic characters among *Pogonia subalpina*, *P. japonica* and *P. minor*

Character	*P. subalpina* (new entity)	*P. japonica*	*P.minor*
Flowering shoot height (cm)	9–16	10–35	8–20
Leaf width (mm)	10–20	7–12	4–12
Flower posture	lateral	lateral	upright
Sepal openness	patent	patent	semi-closed
Purplish pink band along petal mid-vein	present	absent	absent
Petal margins	nearly entire	erose-dentate	entire
Labellum length (mm)	15–18	15–22	10–11.5
Labellum abaxial surface	glabrous	puberulent	puberulent
Labellum midlobe outline	ligulate	elliptic-obovate	ligulate
Labellum midlobe hairy crest length	less than 0.8 mm	2–3 mm	1 mm
Rostellum	developed	developed	absent

extract. Experimental methods follow those described in Topik *et al.* (2005) and Yukawa *et al.* (2005) in which the primer information of the sequenced regions is also shown. Voucher specimens were deposited at TNS.

The ITS and *trnK* 5' intron data sets were aligned using MUSCLE implemented in MEGA 7.0 (Kumar *et al.*, 2016) and modified manually to minimize the number of gaps. All insertions/deletions were coded as missing data in the phylogenetic analysis of ITS. *Cleistesiopsis bifaria* (Fernald) Pansarin, *C. divaricata* (L.) Pansarin & F. Barras and *Isotria verticillata* (Muhl. ex Willd.) Raf. were chosen as outgroup taxa on the basis of the results of previous phylogenetic analyses of *Pogonia* and allied genera (Cameron and Chase, 1999; Cameron, 2009). The evolutionary history was inferred by using a model-based Bayesian approach and the Maximum Likelihood method. A Bayesian search was performed using MeBayes 3.2 (Ronquist *et al.*, 2012) and using the program Kakusan 4 (Tanabe, 2011) to select the 'best-fit' model of evolution under the Akaike information criterion. Two separate runs were carried out using the GTR + I + Γ model in each analysis. The Markov chains were run for 1 million generations and the first 10 thousand runs were burned in. Maximum likelihood (ML) analysis was conducted using MEGA 7.0 based on the Tamura-Nei model (Tamura and Nei, 1993). The initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. Bootstrap analysis (Felsenstein, 1985) with 1000 replicates was used for ML analysis to assess the relative strength of support for all branches. The ML trees were visualized with MEGA 7.0.

Results and Discussion

Morphological characters

Characters discriminating the new entity from the closely related *Pogonia japonica* and *P. minor* are shown in Table 2. Among these, the most notable diagnostic character of this entity is its shorter hairy crests of the labellum mid-lobe

Table 3. Variable nucleotide sites and insertions/deletions found in nrITS and *trnK* 5' intron sequence alignment among *Pogonia subalpina*, *P. japonica* and *P. minor*

Genetic region	Site no.	*P. subalpina* (new entity)	*P. japonica*	*P. minor*
nrITS	18	T	T	C
	210	G	A	G
	289	G	T	G
	350	G	T	G
	547	T	G	G
trnK 5' intron	209	A	—	—
	214-18	TAACA	---	---
	228	C	T	T
	232	T	C	C
	235-41	---	ATTTTTA	---
	242-47	TACATA	TACATA	---
	248-53	---	TTTTTA	---
	254-58	TACAT	TACAT	---
	584	A	T	T
	587-90	TATG	ATCA	ATCA

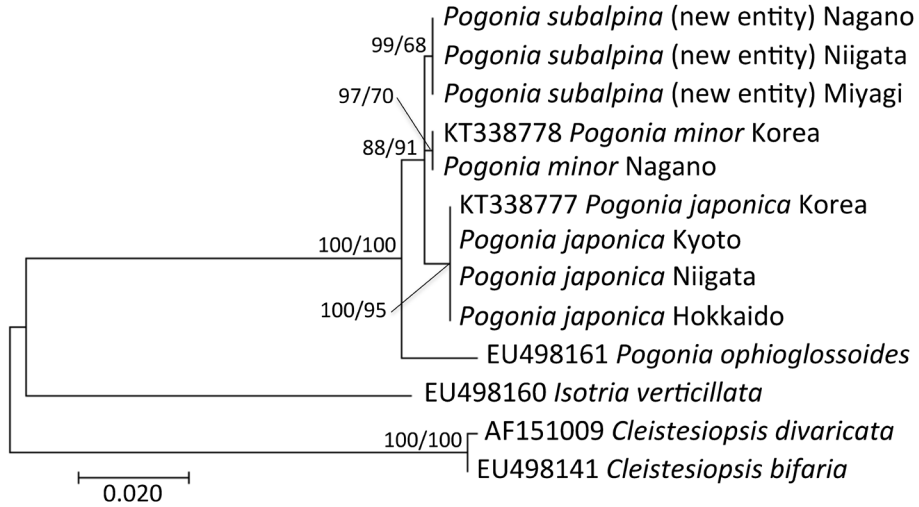

Fig. 1. Phylogenetic relationships of *Pogonia* inferred from nrITS sequences. The tree is rooted by outgroup taxa *Cleistesiopsis bifaria*, *C. divaricata* and *Isotria verticillata*. The tree was calculated using the maximum likelihood method and the topology was the same as the Bayesian inference analysis. Numbers at the nodes indicate Bayesian posterior probabilities (PP) and bootstrap percentages (BS) shown as PP/BS.

than those of the other species. A broad, purplish pink band along the mid-vein of the petals is another unique feature of this entity. The height of the flowering shoot overlaps among the three taxa, but the new entity is shorter than the others in most individuals. Flower size of this entity is generally intermediate between the two others. Besides, *P. ophioglossoides*, a North American congener, has an entire labellum and *P. yunnanensis* from southwestern part of China does not develop hairs on the labellum.

Macromolecular characters

In both ITS and *trnK* 5' intron regions, sequences were identical for three samples of the new entity collected at different sites. In ITS, the new entity was well demarcated from *Pogonia japonica* and *P. minor* by four and two substitu-

tions, respectively. In ca. 600 base pairs of the initial 5' region of *trnK* 5' intron, the new entity was discriminated from both *P. japonica* and *P. minor* by seven substitutions as well as four insertions/deletions (Table 3). Accumulations of synapomorphic nucleotide changes in rather stable genetic regions, lack of polymorphic sites in the nuclear genome, and divergence among the three taxa in the plastid genome did not indicate a hybrid origin of the new entity between *P. japonica* and *P. minor*. Phylogenetic analysis of ITS sequences further supported independent status of the new entity, although the order of cladogenesis was not resolved in the current data set (Fig. 1).

Taking account of both morphological and macromolecular evidences, we hereby describe the new entity as a separate species.

Taxonomic Treatment

Pogonia subalpina T. Yukawa & Y. Yamashita, sp. nov.

TYPE: JAPAN, Honshu, Nagano Pref., Hakuba-mura, Goryu, alt. 1600 m, 10 July 2016, *T. Yukawa 16-14* (TNS - holotype).

This species is similar to *Pogonia japonica* Rchb. f. and *P. minor* (Makino) Makino, but is separable by its short hairy crests on the disk of the labellum mid-lobe, by its glabrous abaxial surface of the labellum, and by its single, broad, purplish pink band along the mid-vein of the petals.

Plant terrestrial, 9–16 cm tall. **Rhizome** cylindric, slender. **Roots** 4–6, fascicled, fibrous, cylindric, pubescent, up to 9.5 cm long. **Stem** erect, 2-ridged, with several basal sheaths, green, dark purple-red at base, 6–11 cm long. **Leaf** 1, patent-suberect, lanceolate to oblanceolate, acute or obtuse, fleshy, coriaceous, base contracted and amplexicaul, green, lucid, 24–70 mm long, 10–20 mm wide. **Floral bract** positioned 3.5–6 cm above leaf, suberect, lanceolate, apex obtuse, fleshy, coriaceous, persistent, green, lucid, 11–40 mm long, 2–9 mm wide. **Flower** solitary, terminal. **Pedicellate ovary** clavate, 6-sulcate, purplish green-green, 10–22 mm long, 2 mm in diameter. **Dorsal sepal** linear-oblanceolate, subacute-obtuse, entire, patent, pale pink, 17–20 mm long, 4–4.5 mm wide. **Lateral sepals** linear-oblanceolate, subacute-obtuse, entire, slightly oblique, patent, pale pink, 17–19.5 mm long, 4 mm wide. **Petals** oblanceolate, obtuse, nearly entire, clasping, pale pink with a broad, purplish pink band along mid-vein, 16–19 mm long, 6–7 mm wide. **Labellum** weakly recurved, 3-lobed above middle, disk with a wide, longitudinal ridge extending from base and becoming continuous with crests on mid-lobe, adaxial surface sparsely puberulent, with a pair of globular ridges at base, pale to dark pink with cream yellow to cream ridge and hairs, abaxial surface glabrous, 15–18 mm long, 6–6.5 mm wide when flattened; lateral lobes oblique triangular, erect, apical margin irregularly incised, 2 mm long; mid-lobe, narrowly triangular-ligulate, obtuse, with hairy crests of 0.8 mm or less in length, margin irregularly incised-entire, 4.5–6 mm long, 2–3 mm wide. **Column** clavate, erect, adaxially papillose, 11 mm long, 2.5 mm in diameter; clinandrium erose at margin; operculum rectangular, 2 mm long, 2.5 mm wide; pollinia 2, granular-farinaceous, without accessory organs; rostellum short, broad, protruding above stigma. Figs. 2, 3, 4.

Japanese name: Miyama-tokiso.

Flowering period: June to August.

Distribution: JAPAN: Honshu (Tohoku and Chubu districts).

Ecology: The plants usually inhabit open, marshy, subalpine grasslands and wet cliffs from 1400 to 1750 m a.s.l., as shown in Fig. 4. Exceptionally, a few populations establish below the subalpine zone around the border of Fukushima and Niigata Prefectures where the habitats are characterized by tuff deposits. *Pogonia japonica* always grows in wet bogs in warm-temperate to subarctic habitats dominated by Sphagnum mosses, and *P. minor* unexceptionally grows in mesic grasslands in warm- to cool-temperate regions. Evidently, these three species occupy

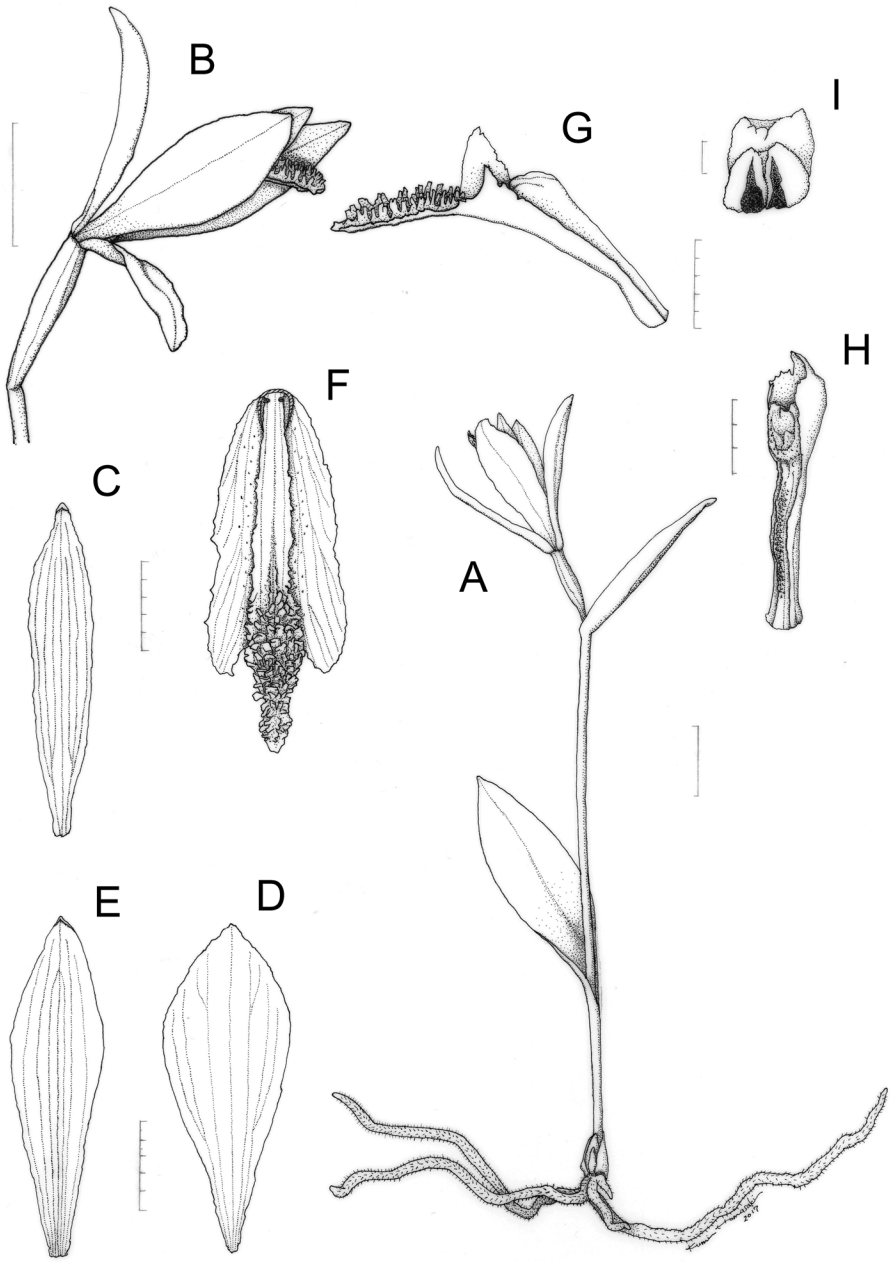

Fig. 2. *Pogonia subalpina* T. Yukawa & Y. Yamashita. A. Habit. B. Flower, side view; C. Lateral sepal; D. Petal; E. Dorsal sepal; F. Labellum; G. Column and labellum, side view; H. Column; I. Operculum. Drawn from *T. Yukawa 16-14* (holotype, TNS) by K. Hamasaki. Scale bars = 1 cm (A, B), 5 mm (C-G), 3 mm (H), or 1 mm (I).

different niches and are therefore ecologically isolated from each other.

Other specimens examined: JAPAN, Honshu: Akita Pref. Senboku-shi, Tazawako-machi, July 1992, *S. Mitsuhashi s.n.* (TNS8504799). Iwate Pref. Ichinoseki-shi, Mt. Kurikomayama, 2 Aug. 1935, *Chyoji Suzuki 101638* (TNS). Miyagi Pref. Katta-gun, Shichikashuku-machi, alt. 1550 m, 14

Fig. 3. Flower close-ups of three *Pogonia* species in Japan (photo courtesy of Toshiyuki Yamashita). A. *P. subalpina* T. Yukawa & Y. Yamashita; B. *P. japonica* Rchb.f.; C. *P. minor* (Makino) Makino.

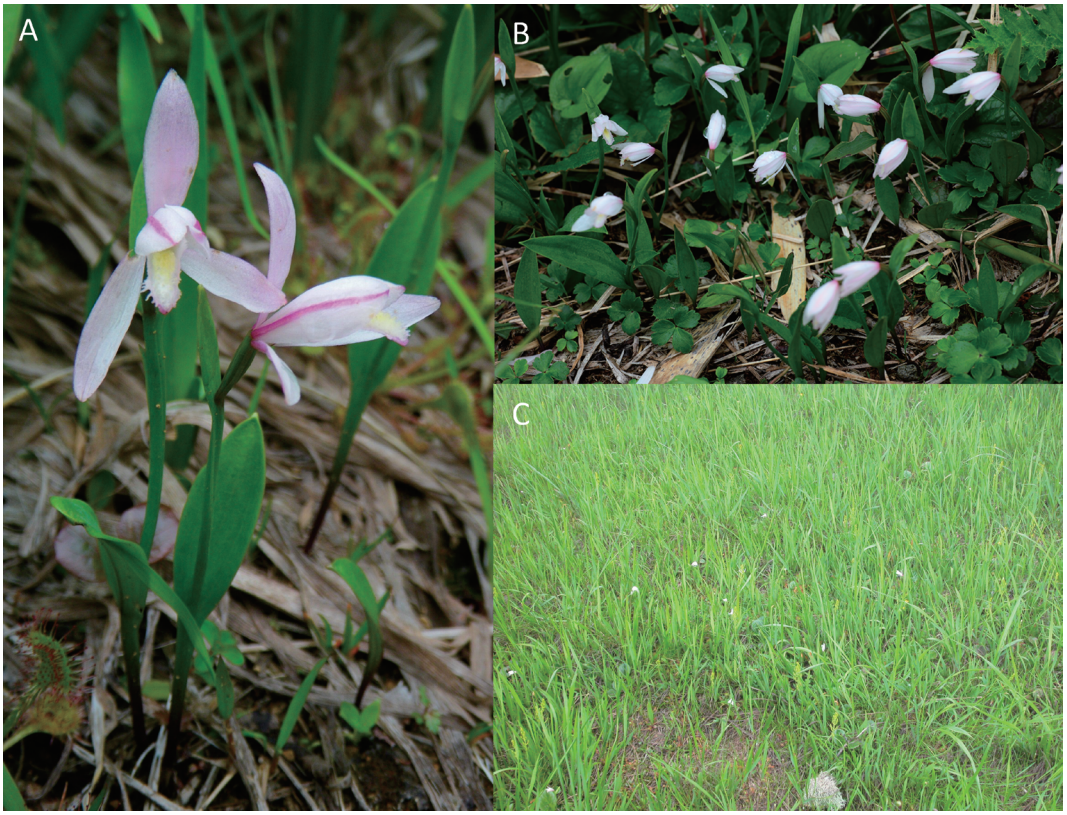

Fig. 4. *Pogonia subalpina* T. Yukawa & Y. Yamashita in the habitats. A. Flowering plant at the type locality (photo courtesy of Hayato Tsuboi); B. Population in a snowbed, subalpine grassland, Yamagata Prefecture (photo courtesy of Toshiyuki Yamashita); C. Population in a marshy, subalpine grassland, Niigata Prefecture (photo courtesy of Taiga Kuhara).

July 2005, *Y. Mikanagi s.n.* (TNS). Yamagata Pref., Tsuruoka-shi, Mt. Gassan, July 1888, *T. Nagasawa s.n.* (TNS54312). Fukushima Pref. Minamiaizu-gun, Hinoemata-mura, Mt. Aizukomagatake, 26 Aug. 1934, *H. Koidzumi 93962* (TNS788253); Minamiaizu-gun, Hinoemata-mura, Mt. Aizukomagatake, 26 Aug. 1934, *H. Koidzumi 93963* (TNS788255). Niigata Pref. Minamiuonuma-gun, Yuzawa-machi, Mt. Naebayama, alt. 1680 m, 15 July 2006, *C. Tsutsumi*, *T. Kuhara & M. Sato CT1079* (TNS 766274, 8505060); Uonuma-shi, Mt. Asakusadake, 28 July 1971, *Joju Haginiwa JH003081* (TNS953081); Kitauonuma-gun, Irihirose-mura, Mt. Asakusadake, 10 Aug. 1961, *Satoshi Saito 818* (FKSE40094); Kitauonuma-gun, Irihirose-mura, Ooshirakawa-Mt. Sumondake, alt. 1350 m, 7 Aug. 1981, *Y. Kadota s.n.* (TNS715904); Kitauonuma-gun, Yunotani-mura, 24-25 July 1974, *Satoshi Saito 39571* (FKSE44340); Nakauonuma-gun, Tsunan-machi, Komatsubara bog, Mt. Naebayama, *H. Koidzumi 24215* (TNS788254); Nakauonuma-gun, Tsunan-machi, Komatsubara bog, Mt. Naebayama, 9 July 1969, *Takajirou Kusumi s.n.* (TNS257698); Nakauonuma-gun, Tsunan-machi, Mt. Naebayama, 12 July 1958, *Kengo Soma s.n.* (TNS01064261); Nakauonuma-gun, Tsunan-machi, Mt. Naebayama, 21 July 1930, *H. Koidzumi 24126* (TNS788249); Nakauonuma-gun, Tsunan-machi, Mt. Naebayama, 21 July 1930, *H. Koidzumi 24127* (TNS788260). Nagano Pref. Kitaazumi-gun, Hakuba-mura, Mt. Happodake, 1923, *H. Koidzumi 48755* (TNS788265); Kitaazumi-gun, Hakuba-mura, Mt. Happodake, 1923, *H. Koidzumi 48756* (TNS788248); Kitaazumi-gun, Hakuba-mura, Mt. Happo, 14 July 1923, *H. Koidzumi 5521* (TNS); Kitaazumi-gun, Hakuba-mura, Mt. Hakuba-happo-yama, 24 July 1954, *Nobuaki Ookawa s.n*. (TNS629153). Gunma Pref. Tone-gun, Minakami-machi, Mt. Ichinokurayama, 6 Aug. 1934, *H. Koidzumi 92009* (TNS788256); Tone-gun, Minakami-machi, Mt. Mantaroyama, 9 Aug. 1934, *H. Koidzumi 92225* (TNS788263); Tone-gun, Minakami-machi, Mt. Mantaroyama, 9 Aug. 1934, *H. Koidzumi 92226* (TNS788271).

Photographic records: JAPAN, Honshu: Yamagata Pref. Nishimurayama-gun, Nishikawa-machi, Mt. Gassan, alt. 1440 m, 31 July 2011, photographed by Toshiyuki Yamashita. Miyagi Pref. Shiroishi-shi, Mt. Fubosan, alt. 1300 m, 9 July 2011, photographed by Toshiyuki Yamashita. Fukushima Pref. Minamiaizu-gun, Shimogomachi, Mt. Nagareishiyama, alt. 1755 m, 14 July 2012, photographed by Toshiyuki Yamashita; Nishiaizu-gun, Nishiaizu-machi, Koboiwa Rock, alt. 340 m, June 2016, photographed by Toshiyuki Yamashita. Niigata Pref. Gosen-shi, Kotsuradani, alt. ca. 250 m, 1 June 2013, photographed by Naoyuki Shimizu. Gifu Pref., Ohno-gun, Shirakawa-mura, Mt. Sanpoiwadake, alt. 1600 m, July 2011, photographed by Hiroshi Nakayama.

Acknowledgments

We would like to thank Taiga Kuhara, Yuki Mikanagi, Shunkei Mitsuhashi, Hiroshi Nakayama, Masaji Sato, Kazuhiro Sawa, Naoyuki Shimizu, Hayato Tsuboi, Chie Tsutsumi, Mihoko Watanabe, and Toshiyuki Yamashita for sharing observations and providing photographs, Hakuba-goryu Alpine Botanical Garden for permission to collect samples, Stephan Gale for critical reading of the manuscript, and Kumi Hamasaki for preparing the illustrations. We are grateful to the director of FKSE for allowing us to examine specimens. This study is partly supported by JSPS KAKENHI Grant Number JP15H04417 and by a National Museum of Nature and Science research grant entitled "Integrated analysis of natural history collections for conservation of highly endangered species" for T. Yukawa.

References

Cameron, K. M. 2009. On the value of nuclear and mitochondrial gene sequences for reconstructing the phylogeny of vanilloid orchids (Vanilloideae, Orchidaceae). Annals of Botany 104: 377–385.

Cameron, K. M. and Chase, M. W. 1999. Phylogenetic relationships of Pogoniinae (Vanilloideae, Orchidaceae): an herbaceous example of the eastern North

America-eastern Asia phytogeographic disjunction. Journal of Plant Research 112: 317–329.

Chen S.-C. 1984. A comparison of orchid floras of temperate North America and eastern Asia. Annals of the Missouri Botanical Garden 70: 713–723.

Chen, X., Gale, S. W. and Cribb, P. J. 2009. *Pogonia*. In: Wu, Z.-Y., Raven, P. H. and Hong, D.-Y. (eds.), Flora of China 25: 172–173. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis.

Felsenstein, J. 1985. Confidence limits in phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874.

Pridgeon, A. M., Cribb, P. J., Chase, M. W. and Rasmussen, F. N. 2003. Genera Orchidacearum: Vol. 3. 294–297. Oxford University Press, New York.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M. A. and Huelsenbeck, J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Takahashi, K. 1987. Nihon-yaseiran-kafu [Wild orchids of Japan]. Vol. 4. Mainichishinbunsha, Tokyo (in Japanese).

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Tanabe, A. S. 2011. Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analysis of multilocus sequence data. Molecular Ecology Resources 11: 914–921.

Topik, H., Yukawa, T. and Ito, M. 2005. Molecular phylogenetics of subtribe Aeridinae (Orchidaceae): insights from plastid *matK* and nuclear ribosomal ITS sequences. Journal of Plant Research 118: 271–284.

Yukawa, T. 2015. Orchidaceae. In: Ohashi, H., Kadota, Y., Murata, J., Yonekura, K., and Kihara, H. (eds.) Wild Flowers of Japan. Vol. 1. pp. 178–231, pl. 125–164. Heibonsha, Tokyo (in Japanese).

Yukawa, T., Kita, K., Handa, T., Topik, H. and Ito, M. 2005. Molecular phylogenetics of *Phalaenopsis* (Orchidaceae) and allied genera: Re-evaluation of generic concepts. Acta Phytotaxnomica Geobotanica 56: 141–161.