A new natural hybrid *Goodyera ×kazumae* (Orchidaceae) from Tomakomai City, Hokkaido Prefecture, Japan

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ABSTRACT: Several individuals of an unidentified *Goodyera* taxon were discovered in Tomakomai City, Hokkaido Prefecture, Japan. Detailed morphological analysis suggests that this taxon is a natural hybrid between *Goodyera schlechtendaliana* and *Goodyera henryi*, based on characteristics such as leaf margins, texture, venation patterns, rachis length, hair morphology on the ovary, and the shapes of the lip apex and rostellum. Supporting this, molecular data from genome-wide markers using next-generation sequencing (MIG-seq data) indicate that *G. ×kazumae* is a hybrid between *G. schlechtendaliana* and *G. henryi*. Neighbor-Net phylogenetic analysis shows *G. ×kazumae* occupies an intermediate position between the putative parent species. STRUCTURE analysis further confirms that *G. ×kazumae* comprises genetic components from both species. Therefore, we conclude that this taxon is a natural hybrid based on comprehensive morphological and molecular evidence.

KEY WORDS: DNA barcoding, *Goodyera henryi*, *Goodyera schlechtendaliana*, hybridization, MIG-seq.

INTRODUCTION

The genus *Goodyera* R.Br. (Orchidaceae, Orchidoideae, Cranichideae) comprises approximately 70 species, widely distributed across continents and regions including Africa, Europe, the Western Indian Ocean Islands, Asia, the southwestern Pacific islands, northeastern Australia, North America, and Mesoamerica (Govaerts *et al.*, 2022). Species of *Goodyera* can be terrestrial, lithophytic, or epiphytic, typically thriving in shaded environments, on mossy rocks, or along the moist tracks of perennial mountain streams (Pridgeon *et al.*, 2003). Key characteristics of this genus include creeping rhizomes, evergreen foliage often marked with white or golden venation on the upper surface, and flowers characterized by saccate lips, a single stigmatic lobe, and two sectile pollinia attached to a viscidium (Pridgeon *et al.*, 2003). A concave dorsal sepal forms a hood over the column along with the petals, while the lateral sepals are generally connivant. The lip comprises a concave-saccate hypochile and a sessile epichile (Guan *et al.*, 2014; Suetsugu and Hayakawa, 2019).

Explicit species delimitation is crucial for testing evolutionary theories and formulating conservation strategies. However, species identification within the genus *Goodyera* is often challenging, particularly for closely related species, due to convergent morphological features (Kallunki, 1976, 1981; Hu *et al.*, 2016; Suetsugu *et al.*, 2019, 2021a). Although molecular techniques have proven invaluable for investigating phylogenetic relationships within the genus, the resolution provided by traditional Sanger sequencing of regions such as ITS is sometimes insufficient for accurate species identification in *Goodyera* (Hu *et al.*, 2016; Suetsugu *et al.*, 2021a). A promising approach to distinguish closely related species involves the use of high-throughput sequencing. For instance, multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) has recently been recognized as a powerful tool for detecting reproductive isolation and hybridization, even among recently diverged species, including closely related *Goodyera* species (Tamaki *et al.*, 2017; Yoichi *et al.*, 2018; Hirano *et al.*, 2019; Suetsugu *et al.*, 2021a,b, 2022, 2024).

During a recent botanical survey in Tomakomai City, Hokkaido Prefecture, Japan, an unidentified taxon of *Goodyera* was collected. Intriguingly, these plants exhibited intermediate morphological characteristics between *Goodyera schlechtendaliana* Rchb.f. and *Goodyera henryi* Rolfe (Fig. 1), suggesting that this unknown taxon might be a natural hybrid of the two. This study aimed to determine the identity of the unknown *Goodyera* taxon and its relationship with *G. schlechtendaliana* and *G. henryi*, based on both MIG-seq data and plant morphology. From the combined morphological and molecular results, we concluded that the unknown taxon is indeed a natural hybrid between *G. schlechtendaliana* and *G. henryi*. We hereby describe the taxon as *Goodyera ×kazumae* Suetsugu, named in honor of Kazuma Sakakibara, who collected the type specimens.

Table 1. Morphological comparison among **Goodyera schlechtendaliana**, **G. ×kazumae** and **G. henryi** in Tomakomai City, Hokkaido Prefecture, Japan.

characters	G. schlechtendaliana	G. ×kazumae	G. henryi
leaf margin	hardly undulate	undulate	undulate
leaf texture	coriaceous	papyraceous	papyraceous
leaf coloration	dark green	light green	light green
lateral vein	conspicuous	conspicuous (occasionally inconspicuous)	inconspicuous
reticulate pattern	thick	narrow (occasionally inconspicuous)	none
inflorescence length	ca. 15 cm	5–7 cm	3–6 cm
rachis length	less than 1/2 of inflorescence	more than 1/2 of inflorescence	more than 1/2 of inflorescence
arrangement of flowers on rachis	laxly arranged	densely arranged	densely arranged
hair on peduncle and ovary	0.3–0.5 mm, clavate	ca. 0.1 mm, subulate	none
flower color	white	light reddish pink	light reddish pink
shape of lip apex	strongly recurved	strongly recurved	slightly recurved
shape of lateral sepal	lanceolate, strongly recurved and twisted	ovate-lanceolate, strongly recurved and twisted	ovate, slightly recurved and twisted
shape of lateral petal	rhombic-oblanceolate to oblong-oblanceolate, apex of hood strongly recurved	rhombic-oblanceolate to oblong-oblanceolate, apex of hood recurved	rhombic-oblanceolate, apex of hood slightly recurved
rostellum shape	narrowly triangular, 1/2 as long as column, apex acuminate	narrowly triangular, 1/2 as long as column, apex flattened and wedge-shaped	oblong to rectangular, 2/5 as long as column, apex flattened and wedge-shaped

MATERIAL AND METHODS

Field sampling

Four individuals of *Goodyera ×kazumae* were collected in Tomakomai City, Hokkaido Prefecture, Japan. Additionally, 16 individuals of *G. henryi* and 15 individuals of *G. schlechtendaliana* (including four individuals of *G. henryi* and three individuals of *G. schlechtendaliana* that co-occur with *G. ×kazumae*) were collected across Japan for comparative study. Recently, *G. crassifolia*, which is morphologically similar to *G. schlechtendaliana*, has been described in southwestern Korea and Japan (Oh *et al.*, 2022). However, this species differs from *G. schlechtendaliana* by having erect and weakly oblique lateral sepals, lateral column appendages, and one-to-three rostella (Oh *et al.*, 2022; Suetsugu *et al.*, 2022). We confirmed that our samples did not include any *G. crassifolia* specimens based on these morphological diagnostic characters. Leaves for DNA analysis were immediately desiccated using silica gel and stored at room temperature until DNA extraction. At least one voucher specimen from each population was deposited in the herbaria KYO, SPMN, and TNS (Table S1), following the Index Herbariorum (Thiers, 2023).

Morphological observation

We compared the morphological characters of *G. ×kazumae*, *G. schlechtendaliana*, and *G. henryi* using the above samples. Additional investigation of morphological variation for *G. schlechtendaliana* and *G. henryi* was conducted by reviewing literature and examining herbarium specimens from other localities stored in KYO, TI, and TNS. Morphological characters were observed visually and under a stereomicroscope and measured with a digital caliper.

MIG-seq analysis

Genomic DNA was extracted from silica-dried leaves using the CTAB method. A MIG-seq library for 35 *Goodyera* samples was prepared following the protocol described by Suyama *et al.* (2022). The library was sequenced on an Illumina MiSeq Sequencer using a MiSeq Reagent Kit v3 (150 cycle, Illumina). The raw MIG-seq data of 26 *Goodyera* samples had previously been deposited in the DDBJ Sequence Read Archive (DRA) under accession numbers DRA011506, DRA011700, and DRA016960 (Suetsugu *et al.*, 2021a,b, 2024). The remaining raw MIG-seq data have been deposited at DRA under BioProject accession number PRJDB18201.

After removing primer regions and low-quality sequencing reads, a total of 4,507,773 high-quality reads (averaging $127,159 \pm 7,633$ per sample) were obtained from 5,048,786 raw reads (averaging $144,251 \pm 8,504$ per sample). The Stacks 2.66 pipeline was used for *de novo* SNP discovery (Rochette *et al.*, 2019) with the following settings: minimum depth of coverage to create a stack (m) = 3, maximum distance allowed between stacks (M) = 2, and the number of mismatches allowed between sample loci when building the catalog (n) = 2. Only SNPs retained by 20 or more samples were extracted, and loci with high heterozygosity (Ho $\geq$ 0.6) or minor allele counts less than four were filtered out. PLINK 1.90 was used to remove SNPs in linkage disequilibrium with parameters: –make-bed –indep-pairwise 50 10 0.1 –allow-extra-chr. A total of 63 SNPs from 57 loci were used for subsequent

analyses.

To examine the origin of *G. ×kazumae*, a Neighbor-Net network was constructed using SplitsTree4 4.14 (Huson and Bryant, 2006) based on the uncorrelated *P* distance matrix derived from the SNP data. Population structure was further investigated using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), performing 30 independent runs with a burn-in period of 100,000 steps followed by 100,000 additional steps under the admixture model. The optimal number of clusters (K = 1–10) was determined using the Delta K method (Evanno *et al.*, 2005) in Structure Harvester 0.7 (Earl and vonHoldt, 2012). Results were visualized using CLUMPAK 1.1 (Cluster Markov Packager Across K) (Kopelman *et al.*, 2015).

RESULTS AND DISCUSSION

Detailed morphological examination revealed that the unknown *Goodyera* taxon likely exhibits a combination of traits from its putative parent species, *G. schlechtendaliana* and *G. henryi* (Figs. 1–2). Generally, *G. ×kazumae* has light green, papyraceous leaves with narrower (and occasionally obscure) white reticulate venation and undulate margins (Fig. 1). In contrast, *G. schlechtendaliana* displays dark green, coriaceous leaves with thicker white reticulate venation and almost entire margins. Meanwhile, *G. henryi* exhibits light green, papyraceous leaves without reticulate venation but with undulate margins (Lee *et al.*, 2010; Suetsugu and Hayakawa, 2019; Suetsugu *et al.*, 2021b). Additionally, *G. ×kazumae* can be distinguished from *G. schlechtendaliana* and *G. henryi* by the hair morphology on the peduncle and ovary (0.1 mm, subulate in *G. ×kazumae*, 0.3–0.5 mm, clavate in *G. schlechtendaliana*, and none in *G. henryi*). Overall, *G. ×kazumae* typically displays uniformity in the aforementioned key morphological traits, which are intermediate characteristics or those of either putative parent, supporting its hybrid origin (Table 1). We also note that some variability in the degree of conspicuousness of leaf venation (Fig. 1B, K) persists, likely influenced by the hybridization process (Lee *et al.*, 2012). Further descriptions and illustrations of *G. schlechtendaliana* and *G. henryi* (often referred to as *G. maximowicziana*) can be found in existing literature (Lee *et al.*, 2010; So and Lee, 2017; Suetsugu and Hayakawa, 2019; Suetsugu *et al.*, 2021b, 2024).

We also note that *G. ×kazumae* shares some morphological characteristics with *G. ×tamnaensis*, such as the white reticulation on the leaves and puberulent hairs on the pedicel and ovary (Lee *et al.*, 2010, 2012; Suetsugu *et al.*, 2021b). The similarity between *G. ×kazumae* and *G. ×tamnaensis* likely stems from their common parent, *G. schlechtendaliana* (Lee *et al.*, 2010, 2012; Suetsugu *et al.*, 2021b). Additionally, this similarity is amplified by the fact that the other parental species are closely related (*G. henryi* in *G. ×kazumae* and *G. similis* (= *G. velutina*) in *G. ×tamnaensis*) (Hu *et al.*, 2016; So and Lee, 2017; Suetsugu *et al.*, 2019, 2024). However, *G. ×kazumae* differs from *G. ×tamnaensis* in having light green non-velutinous leaves (vs. dark green velutinous leaves in *G. ×tamnaensis*), an inflorescence length of less than 10 cm (vs. 10–20 cm in *G. ×tamnaensis*), and a rachis constituting more than half of the inflorescence length (vs. less than half in *G. ×tamnaensis*) (Lee *et al.*, 2010, 2012; Suetsugu *et al.*, 2021b).

Molecular data further support the hybrid status of *G. ×kazumae*. The STRUCTURE analysis at K = 2 (the largest delta K for our data) indicated that *G. schlechtendaliana* and *G. henryi* were differentiated into two clusters, with *G. ×kazumae* showing genetic components of both species (Fig. 3). Similarly, the Neighbor-Net (SplitsTree) phylogenetic analysis showed that *G. schlechtendaliana* and *G. henryi* represent two distinct genetic clusters, with *G. ×kazumae* occupying an intermediate position between them (Fig. 4). Therefore, both the STRUCTURE and Neighbor-Net network analyses support a hybrid origin for *G. ×kazumae*.

Research has demonstrated that hybridization can lead to significant evolutionary outcomes, including increased genetic diversity and the emergence of new species or ecotypes (Lee *et al.*, 2010; Nakahama *et al.*, 2019). Given that *G. schlechtendaliana* and *G. henryi* rely on pollinators rather than being autogamous or apogamous (Suetsugu *et al.*, 2022, 2024), it is likely that natural hybridization has occurred through shared pollinators. Although the specific role of breeding systems and hybridization in the evolution of the *Goodyera* genus remains under-explored, emerging evidence suggests that hybridization plays a pivotal role in its evolutionary dynamics (Lee *et al.*, 2010, 2012; Hu *et al.*, 2016; So and Lee, 2017; Suetsugu *et al.*, 2019, 2024).

Notably, the hybrid between *G. schlechtendaliana* and *G. velutina* is known as *G. ×tamnaensis* (Lee *et al.*, 2010; Suetsugu *et al.*, 2021b), while the hybrid between *G. velutina* and *G. henryi* is identified as *G. ×maximo-velutina* (So and Lee, 2017; Suetsugu *et al.*, 2019). The hybrid between *G. schlechtendaliana* and *G. henryi* is now recorded as *G. ×kazumae*. Therefore, it has been revealed that these three parental species (*G. schlechtendaliana*, *G. velutina*, and *G. henryi*) produce hybrid species in all combinations when they co-occur. This phenomenon raises intriguing questions, such as why these species frequently hybridize without forming complete hybrid swarms. In a broader context, it remains unknown to what extent hybridization influences the diversification of this genus. Further comprehensive investigations are necessary to enhance our understanding of the impact of hybridization on the evolutionary dynamics of this genus.
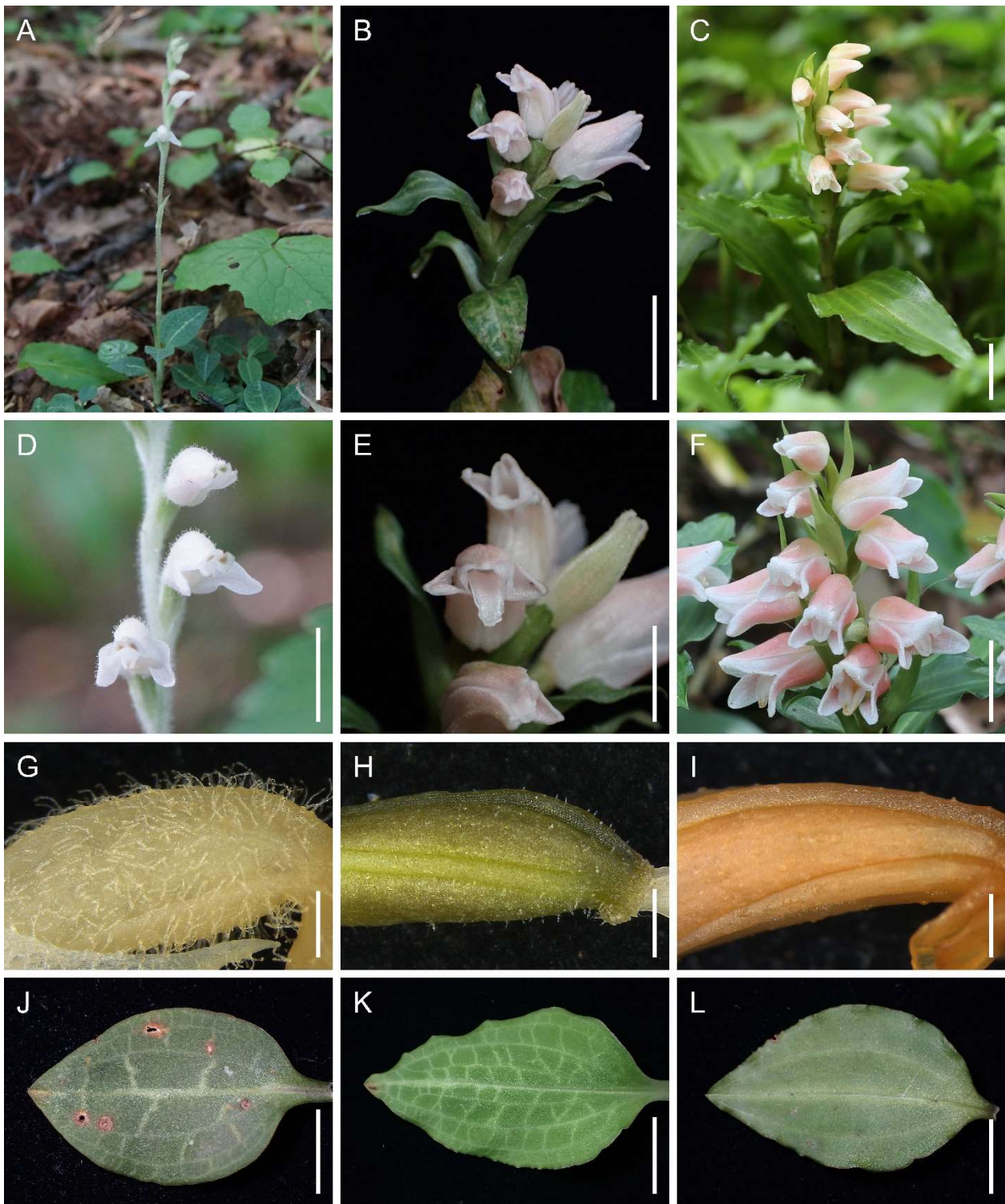
Fig. 1. Comparison among *Goodyera schlechtendaliana*, *G. ×kazumae*, and *G. henryi*. (A, D, G, J) *G. schlechtendaliana.* (B, E, H, K) *G. ×kazumae.* (C, F, I, L) *G. henryi.* **A–C.** Habit. **D–F.** Close-up of inflorescence. **G–I.** Ovary. **J–L.** Leaf. Scale bars: 3 cm (A), 1 cm (B–D, F–I), and 5 mm (E).
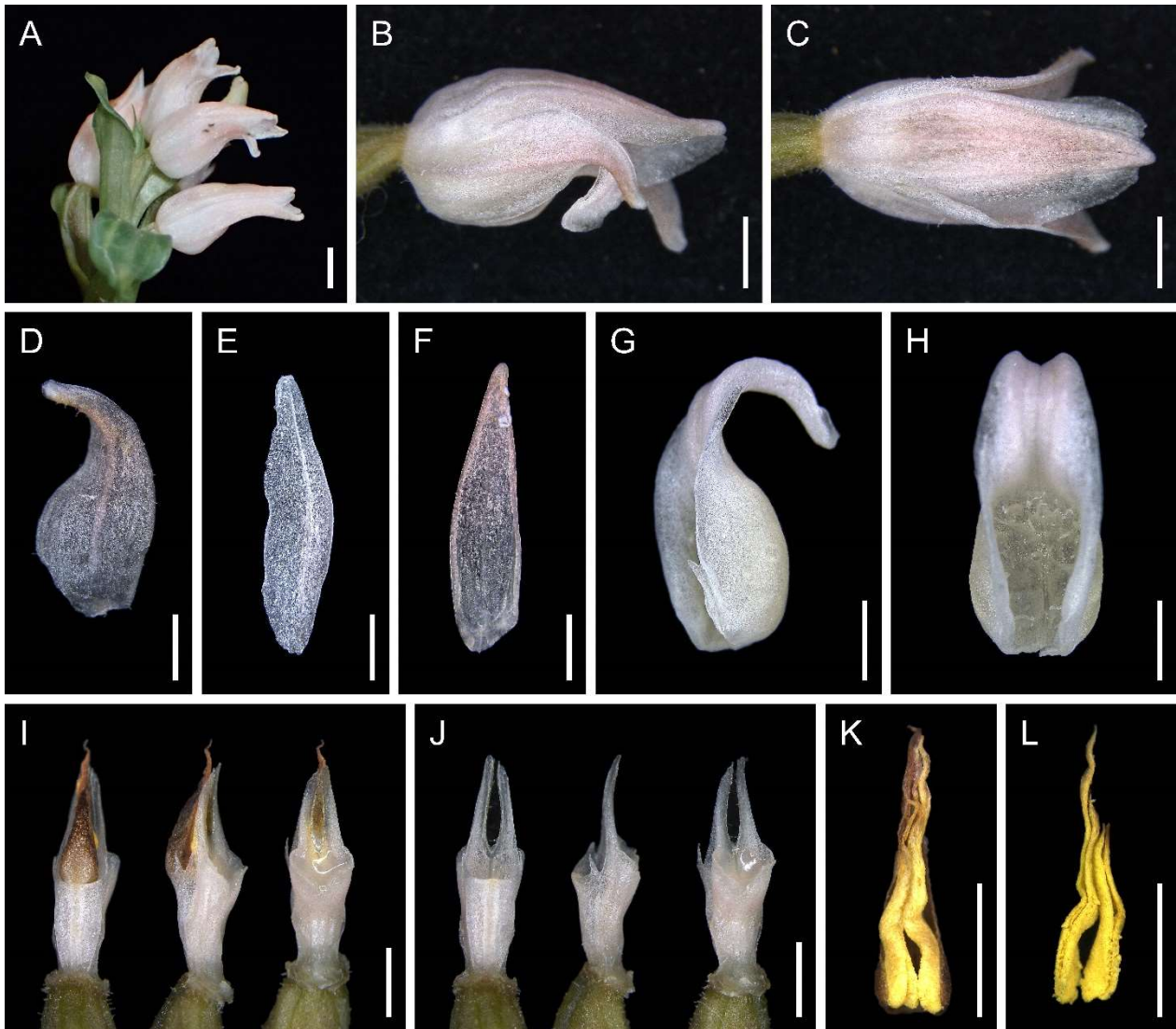
Fig. 2. Holotype of **G. ×kazumae** (*Kazuma Sakakibara G183*). **A.** Inflorescence. **B.** Flower, lateral view. **C.** Flower, dorsal view. **D.** Lateral sepal. **E.** Petal. **F.** Dorsal sepal. **G.** Labellum, lateral view. **H.** Labellum, adaxial view. **I.** Column and pollinarium, dorsal view (left), lateral view (middle), and ventral view (right). **J.** Column, dorsal view (left), lateral view (middle), and ventral view (right). **K.** Anther cap and pollinarium. **L.** Pollinarium. Scale bars: 2 mm.
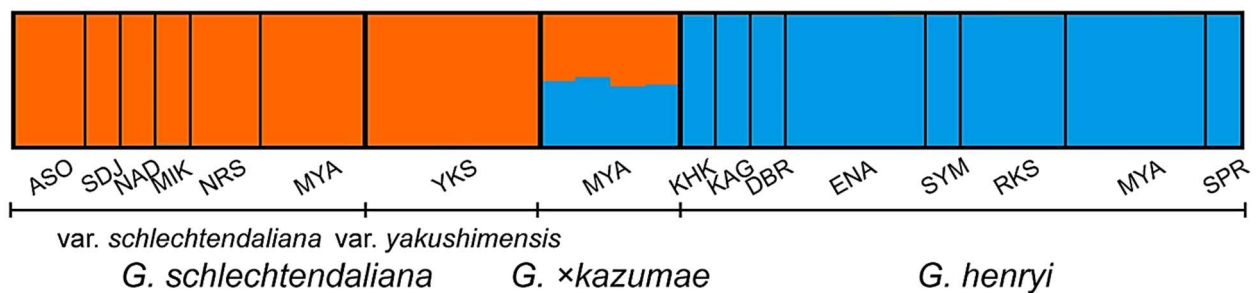

Fig. 3. Population structure derived from 35 **Goodyera** samples inferred by the STRUCTURE algorithm. $K = 2$ has the largest delta K for our data, indicating that $K = 2$ was optimal. Species and populations are separated by broad and narrow vertical black lines, respectively.
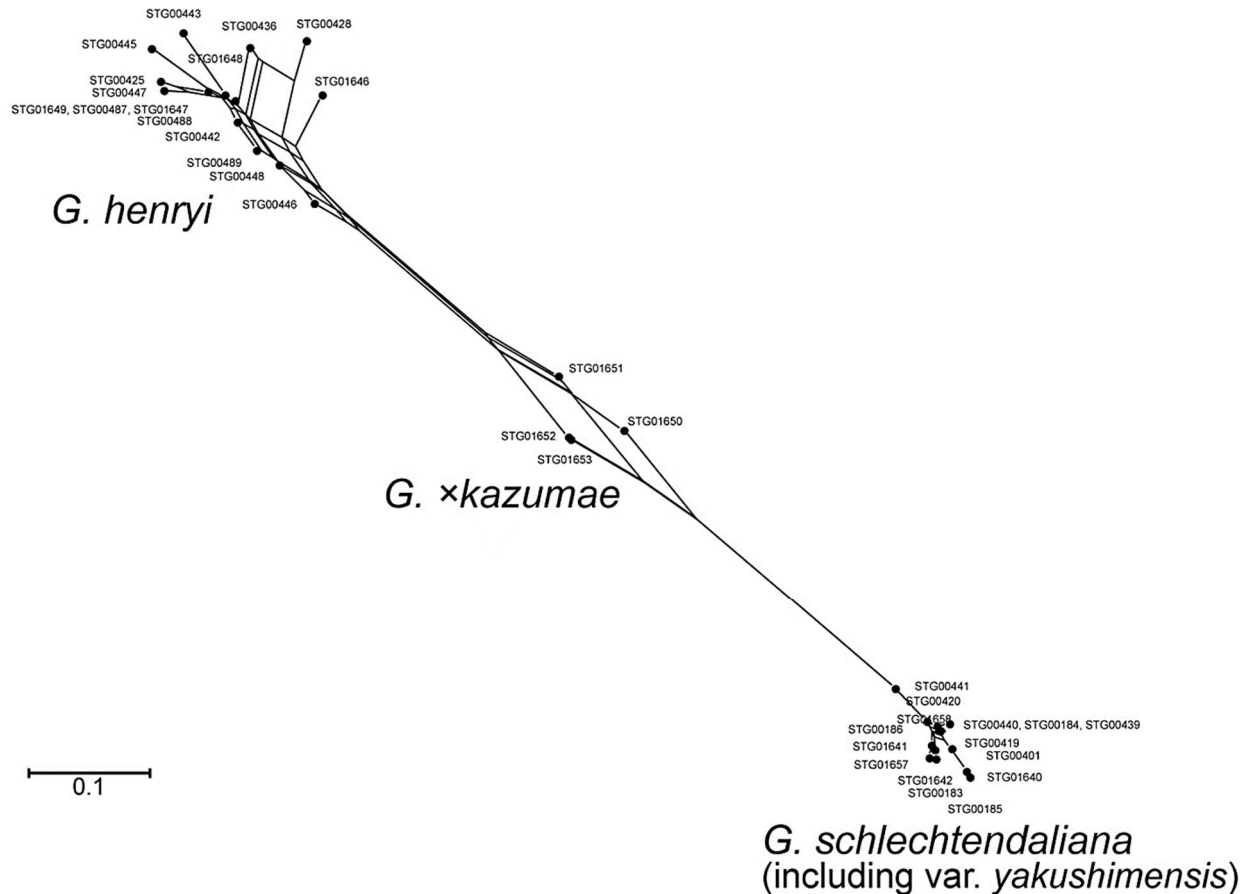
Fig. 4. Neighbor-Net network derived from 35 *Goodyera* samples based on uncorrected *P* distance calculated by 63 SNPs.

TAXONOMIC TREATMENT

Goodyera* ×*kazumae Suetsugu, *sp. nov.*

Figs. 1B, E, H, K & 2

Type: JAPAN. Hokkaido Prefecture, Tomakomai City, Maruyama, 10 August 2022, *Kazuma Sakakibara G183* (holotype: KYO, herbarium specimen and liquid-preserved material, labeled as the same specimen).

Diagnosis: *Goodyera* ×*kazumae* is similar to *G. henryi* but differs in having narrow white reticulation on the leaves (vs. no reticulation in *G. henryi*), subulate hair ca. 0.1 mm long on the peduncle and ovary (vs. glabrous in *G. henryi*), and a strongly recurved lip and lateral sepal apex (vs. not strongly recurved in *G. henryi*).

Terrestrial herbs, 6–12 cm tall. Rhizome slender, few-noded. Roots fleshy, yellowish-brown, with minute root hairs. Stems erect, terete, ca 5.5–11.0 cm long, 5–7-leaved. Leaves alternate, widely spaced or somewhat clustered toward apex; lamina ovate to lanceolate-ovate, 1.1–4.3 × 0.5–2.7 cm, length/width ratio 1.4–2.0, papyraceous, dorsally pale green with narrow (and occasionally obscure) white reticulation, margins undulate, apex acute; petiole-like base and tubular sheath ca. 1 cm long. Inflorescence green, 5–7 cm, pubescent, with 2–4 sterile bracts; rachis more than 1/2 of inflorescence, densely arranged, 5-10-flowered, secund; floral bracts lanceolate, 8.5–20.4 × 3.9–5.3 mm. Ovary and pedicel cylindric-fusiform, ca. 10 mm, pale green; hair on ovary and pedicel ca. 0.1 mm, subulate. Flowers resupinate, weakly open. Sepals free, sub-similar, light reddish-pink, sparsely pubescent on the outer surface, 1-veined; dorsal sepal narrowly elliptic-lanceolate, cymbiform, 8.1–10.5 × 2.4–2.9 mm, subacute at apex, forming a hood with petals; lateral sepals obliquely ovate-lanceolate, 7.5–10.5 × 3.2–4.0 mm, strongly recurved at 2/3 of its entire length from the base, apex obtuse. Petals rhombic-oblanceolate to oblong-oblanceolate, 8.4–10.3 × 2.4–2.9 mm, hood recurved at apex, white tinged with pink, 1-veined. Lip ovate-lanceolate, 7.7–10.5 mm long; hypochile concave-saccate, papillose inside; epichile ligulate, apex strongly recurved. Column ca. 6.5 mm long, clawed at base; rostellum narrowly triangular, 1/2 as long as column, apex flattened and wedge-shaped; stigma orbicular, surrounded by a hood-like rim, rim prominent at the base; anther cap ovate, ca. 4.2 × 1.2 mm; pollinia 2, oblanceolate attached to a narrow ellipsoid viscidium. Fruit not seen.

Distribution and habitat: *Goodyera* ×*kazumae* is currently known only from the type locality. Dozens of *G. ×kazumae* individuals occur in the understory of a cool-

temperate forest dominated by *Betula platyphylla* Sukaczev var. *japonica* (Miq.) H.Hara, *Alnus hirsuta* (Spach) Fisch. ex Rupr. var. *hirsuta* (Betulaceae), *Cornus controversa* Hemsl. (Cornaceae), *Quercus crispula* Blume (Fagaceae), *Magnolia obovata* Thunb. (Magnoliaceae), *Picea jezoensis* (Siebold et Zucc.) Carrière (Pinaceae), *Acer amoenum* Carrière, *Acer pictum* Thunb. subsp. *mayrii* (Schwer.) H.Ohashi (Sapindaceae), *Ulmus davidiana* Planch. var. *japonica* (Rehder) Nakai, and *Ulmus laciniata* (Trautv.) Mayr ex Schwapp. (Ulmaceae). In the understory, some shrubs and herbs, including *Pachysandra terminalis* Siebold et Zucc. (Buxaceae), *Hydrangea petiolaris* Siebold et Zucc., *Hydrangea* sp. (Hydrangeaceae), *Disporum smilacinum* A.Gray (Colchicaceae), *Dryopteris expansa* (C.Presl) Fraser-Jenk. et Jermy subsp. *assimilis* (S.Walker) Tzvelev (Dryopteridaceae), *Fraxinus* sp. (Oleaceae), *Viola kusanoana* Makino, *Viola grypoceras* A.Gray, *Viola selkirkii* Pursh ex Goldie f. *variegata* (Nakai) F.Maek. (Violaceae), as well as its parental taxa *G. schlechtendaliana* and *G. henryi*, are scattered.

Vernacular name: Shinonome-shusu-ran (in Japanese). It is a thoughtfully derived name, where "shinonome" refers to the appearance of the sky just before dawn when it begins to lighten but is not yet red, indicating the early stage of dawn. This name contrasts with the Japanese name of *G. henryi*, Akebono-shusu-ran, since "akebono" refers to the redness that spreads across the sky at dawn. The name "Shinonome-shusu-ran" aptly reflects the slightly lighter (whitish) hue of *G. ×kazumae* compared to *G. henryi*. The vernacular name was given by Kazuma Sakakibara, the discoverer of *G. ×kazumae*.

ACKNOWLEDGMENTS

LITERATURE CITED

Earl, D.A., vonHoldt, B.M. 2012 STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. **4(2)**: 359–361.

Evanno, G., Regnaut, S., Goudet, J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14(8)**: 2611–2620.

Govaerts, R., Bernet, P., Kratochvil, K., Gerlach, G., Carr, G., Alrich, P., Pridgeon, A., Pfahl, J., Campacci, M., Holland Baptista D., Tigges, H., Shaw, J., Cribb, P., George, A.S., Kreuz, K., Wood, J. 2022 World Checklist of Orchidaceae. Royal Botanic Gardens, Kew, UK. http://wcsp.science.kew.org/

Guan, Q.X., Chen, G.Z., Li, M.H., Chen, S.P. 2014 *Goodyera malipoensis* (Cranichideae, Orchidaceae), a new species from China: Evidence from morphological and molecular analyses. Phytotaxa **186(1)**: 51–60.

Hirano, T., Saito, T., Tsunamoto, Y., Koseki, J., Prozorova, L., Do, V.T., Matsuoka, K., Nakai, K., Suyama, Y., Chiba, S. 2019 Role of ancient lakes in genetic and phenotypic diversification of freshwater snails. Mol. Ecol. **28(23)**: 5032–5051.

Hu, C., Tian, H., Li, H., Hu, A., Xing, F., Bhattacharjee, A., Hsu, T., Kumar, P., Chung, S. 2016 Phylogenetic analysis of a 'jewel orchid' genus *Goodyera* (Orchidaceae) based on DNA sequence data from nuclear and plastid regions. Plos One **11(2)**: e0150366.

Huson, D.H., Bryant, D. 2006 Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. **23(2)**: 254–267.

Kallunki, J.A. 1976 Population studies in *Goodyera* (Orchidaceae) with emphasis on the hybrid origin of *G. tesselata*. Brittonia **28(1)**: 53–75.

Kallunki, J.A. 1981 Reproductive biology of mixed-species populations of *Goodyera* (Orchidaceae) in northern Michigan. Brittonia **33(2)**: 137–155.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I. 2015 CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. **15(5)**: 1179–1191.

Lee, C.S., Kim, S.-C., Yeau, S.H., Lee, N.S. 2012 Nuclear and cpDNA sequences demonstrate spontaneous hybridization between *Goodyera schlechtendaliana* Rchb. f. and *G. velutina* Maxim. (Orchidaceae) in Jeju Island, Korea. Syst. Bot. **37(2)**: 356–364.

Lee, C.S., Yeau, S.H., Lee, K.S., Lee, N.S. 2010 A new taxon of *Goodyera* (Orchidaceae): *G. ×tamnaensis*. Korean J. Pl. Taxon. **40(4)**: 251–254.

Nakahama, N., Suetsugu, K., Ito, A., Hino, M., Yukawa, T., Isagi, Y. 2019 Natural hybridization patterns between widespread *Calanthe discolor* (Orchidaceae) and insular *Calanthe izu-insularis* on the oceanic Izu Islands. Bot. J. Linn. **190(4)**: 436–449.

Oh, S.-H., Suh, H.-J., Seo, S.-W., Chung, K.-S., Yukawa, T. 2022 A new species of *Goodyera* (Orchidaceae: Orchidoideae) from Korea and Japan. J. Pl. Biol. **65(5)**: 357–363.

Pridgeon, A.M., Cribb, P.J., Chase, M.W., Rasmussen, F.N. 2003 Genera Orchidacearum 3. Orchidoideae (part 2), Vanilloideae. Oxford: Oxford University Press.

Pritchard, J.K., Stephens, M., Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics **155(2)**: 945–959.

Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M. 2019 Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. **28(21)**: 4737–4754.

So, J.-H., Lee, N.-S. 2017 The origin of new natural hybrid, *Goodyera ×maximo-velutina* (Orchidaceae) from Jeju Island, Korea. Phytotaxa **317(1)**: 61–68.

Suetsugu, K., Hayakawa, H. 2019 A new variety of *Goodyera schlechtendaliana* (Orchidaceae) from Yakushima and Okinawa, Japan. Acta Phytotax. Geobot. **70**: 49–55.

Suetsugu, K., Hirota, S.K., Nakato, N., Suyama, Y., Serizawa, S. 2022 Morphological, ecological, and molecular phylogenetic approaches reveal species boundaries and evolutionary history of *Goodyera crassifolia* (Orchidaceae, Orchidoideae) and its closely related taxa. PhytoKeys **212**: 111–134.

Suetsugu, K., Hirota, S.K., Shitara, T., Ishida, K., Nakato, N., Hayakawa, H., Suyama, Y. 2024 The absence of bumblebees on an oceanic island blurs the species boundary of two closely related orchids. New Phytol. **241**: 1321–1333.

Suetsugu, K., Hirota, S.K., Suyama, Y. 2021a A new natural hybrid, *Goodyera ×tanakae* (Orchidaceae) from Japan with a discussion on the taxonomic identities of *G. foliosa*, *G. sonoharae*, *G. velutina*, *G. ×maximo-velutina* and *G. henryi*, based on morphological and molecular data. Taiwania **66(3)**: 277–286.

Suetsugu, K., Hirota, S.K., Suyama, Y. 2021b First record of *Goodyera × tamnaensis* (Orchidaceae) from Boso Peninsula, Chiba Prefecture, Japan, based on morphological and molecular data. Taiwania **66(1)**: 113–120.

Suetsugu, K., Shitara, T., Nakato, N., Ishida, K., Hayakawa, H. 2019 First record of *Goodyera ×maximo-velutina* (Orchidaceae) from Kozu Island, Japan. Taiwania **64(4)**: 347–352.

Suyama, Y., Hirota, S.K., Matsuo, A., Tsunamoto, Y., Mitsuyuki, C., Shimura, A., Okano, K. 2022 Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny. Ecol. Res. **37(1)**: 171–181.

Tamaki, I., Yoichi, W., Matsuki, Y., Suyama, Y., Mizuno, M. 2017 Inconsistency between morphological traits and ancestry of individuals in the hybrid zone between two *Rhododendron japonoheptamerum* varieties revealed by a genotyping-by-sequencing approach. Tree Genet. Genomes **13(1)**: 1–10.

Thiers, B. 2023 Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium.

Yoichi, W., Kawamata, I., Matsuki, Y., Suyama, Y., Uehara, K., Ito, M. 2018 Phylogeographic analysis suggests two origins for the riparian azalea *Rhododendron indicum* (L.) Sweet. Heredity **121(6)**: 594–604.

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