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Taxonomic Status of *Lecanorchis suginoana* (Tuyama) Seriz. (Orchidaceae)

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Abstract *Lecanorchis suginoana* (Tuyama) Seriz. has been interpreted as a variety of *L. japonica* Blume, a variety of *L. kiusiana* Tuyama, or an independent species. To re-evaluate the taxonomic status of this entity, macromolecular, morphological, and ecological characters were investigated. Divergence of nucleotide sequences supported independent status of three close relatives, viz., *L. suginoana*, *L. kiusiana*, and *L. japonica*. Furthermore, the results of molecular phylogenetic analysis did not support infraspecific placement of *L. suginoana* in *L. japonica*. Moreover, flower colour, qualitative characters of labellum and column, quantitative characters of perianth lobes and column, flowering time, and associated vegetation types clearly distinguish *L. suginoana* from *L. kiusiana*. These differences corroborated the independent species status of *L. suginoana* rather than the infraspecific position of *L. kiusiana*.

Key words: Japan, *Lecanorchis*, molecular analysis, morphological analysis, Orchidaceae, taxonomy.

Introduction

The genus *Lecanorchis* Blume is a terrestrial, achlorophyllous orchid genus in which about 25 species are distributed throughout temperate to tropical regions in East to Southeast Asia and extending to New Guinea (WCSP, 2019). This group represents holomycoheterotrophy in their nutritional mode (Motomura *et al.*, 2010) and depends upon carbon nutrition from particular groups of ectomycorrhizal fungi belonging to Basidiomycota (Okayama *et al.*, 2012).

Lecanorchis japonica Blume var. *suginoana* Tuyama is one of the northernmost distributed members of the genus (Yukawa, 2015a, 2015b; Suetsugu *et al.*, 2018). In the original description, Tuyama (1982) pointed out close morphological affinities of this entity to *L. japonica* var. *japonica* and emphasized similarity of the shape of apical part of the column between them. In his revisionary work of the genus, Hashimoto (1990) proposed a new combination, *L. kiusiana* Tuyama var. *suginoana* (Tuyama) T.Hashim. He demonstrated common characters between this entity and *L. kiusiana* var. *kiusiana*, i.e., rather small plant, lip with marginal papilla and conspicuous branchlets of disc-hairs, ovary with an apparent ring-like excrescence below the calyculus, and rather short fruit. Subsequently, Serizawa (2005) proposed to recognize it as an independent species *L. suginoana* (Tuyama) Seriz. He emphasized that this entity can be distinguished from *L. kiusiana* var. *kiusiana* by several characters such as light yellow hairs on the disc of the labellum, earlier flowering time, and nearly truncate apical part of the column. However, these conflicting taxonomic views have not been tested so far. In this study we re-evaluate the taxonomic status of this entity by use of morphological, macromolecular, and ecological characters.

Materials and Methods

From the outset, we tentatively treated the three taxa as independent species, viz., *Lecanorchis suginoana*, *L. kiusiana*, and *L. japonica* (Fig. 1). Materials used in the DNA analysis are listed in Table 1. Besides, in the course of this study, populations of *L. suginoana* were found in Tochigi Prefecture, eastern part of Honshu, Japan and Ehime Prefecture, Shikoku, Japan (see Taxonomic Treatment). They are the first records of distribution in these areas. Total DNA of plants was extracted from fresh or silica-gel-dried samples with the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions. Nucleotide sequences were determined by amplifying the internal transcribed spacer (ITS) regions of the 18S–26S nuclear ribosomal DNA via the polymerase chain reaction (PCR) from a total DNA extract. Experimental methods follow those described in Topik *et al.* (2005) and Yukawa *et al.* (2005) in which the primer information of the sequenced region is also shown. Voucher specimens were deposited at the Department of Botany, National Museum of Nature and Science (TNS).

The ITS sequences were aligned using MUSCLE implemented in MEGA 7.0.14 (Kumar *et*

Fig. 1. Flowering individuals of *Lecanorchis suginoana* (A), *L. kiusiana* (B) and *L. japonica* (C).

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

Species	Locality	Voucher
Lecanorchis flavicans	Japan, Kagoshima Pref., Amami-oshima Is.	T. Yukawa 09-21
Lecanorchis japonica	Japan, Kagoshima Pref., Amami-oshima Is	H. Yamashita *s. n.*
	Japan, Shikoku, Ehime Pref., Uwajima-shi	S. Hyodo 17864
	Japan, Honshu, Gifu Pref., Mizunami-shi	H. Nakagawa *s. n.*
	Japan, Honshu, Tochigi Pref., Mashiko-machi	M. Takashima *s. n.*
	Japan, Honshu, Miyagi Pref., Shiroishi-shi	M. Takashima *s. n.*
	Japan, Honshu, Fukui Pref., Echizen-cho	T. Wakasugi & H. Enomoto 51886
Lecanorchis kiusiana	Japan, Shikoku, Ehime Pref., Uwajima-shi	S. Hyodo 17862
	Japan, Honshu, Shizuoka Pref., Kakegawa-shi	M. Takashima *s. n.*
	Japan, Honshu, Tochigi Pref., Mashiko-machi	M. Takashima *s. n.*
	Korea, Jeju Is.	S. Matsumoto 03-171
Lecanorchis suginoana	Japan, Honshu, Shizuoka Pref., Kakegawa-shi	T. Yukawa 14-10
	Japan, Honshu, Ibaraki Pref., Mito-shi	H. Uchiyama *s. n.*
	Japan, Honshu, Tochigi Pref., Yaita-shi	M. Takashima *s. n.*

al., 2016). All insertions/deletions were coded as missing data in the phylogenetic analysis of ITS. *Lecanorchis flavicans* Fukuy. was chosen as an outgroup taxon on the basis of the results of phylogenetic analysis of the whole genus (unpublished). The evolutionary history was inferred by using a model based Bayesian approach and the Maximum Likelihood (ML) method. A Bayesian search was performed using MrBayes 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 $GTR+I+\Gamma$ model in each analysis. The Markov chains were run for 1 million generations and the first 10 thousand runs were burned in. ML analysis was conducted using MEGA and the Kimura 2-parameter model (Kimura, 1980) was chosen as the best fitting model for our dataset. Bootstrap analysis (Felsenstein, 1985) with 1000 replicates was used to assess the relative robustness of support for all branches.

Since the results of molecular phylogenetic analysis demonstrated that *Lecanorchis suginoana* occupied a sister position of *L. kiusiana* and was distantly related to *L. japonica* (see Results and Discussion), morphological analyses were confined to the former two taxa. Observation of morphological characters was based on living plants, dried herbarium specimens, and spirit-preserved specimens listed in the section "Specimens examined". Detailed measurements of quantitative morphological characters were carried out from the flowers collected in Yaita and Kakegawa populations for *L. suginoana*, and Mashiko and Kakegawa populations for *L. kiusiana*. Yaita and Mashiko are situated in the eastern part of Honshu, Japan and Kakegawa is situated in the central part of Honshu, Japan. A single flower was taken from each individual and 63 samples were collected in total. Ten characters, viz., length of dorsal sepal, length of lateral sepal, length of petal, width of petal, length of labellum, width of labellum, length of column, and width of column, were measured for each sample. Student's t-test was performed to evaluate differences of these characters between the two taxa. Moreover, we conducted canonical discriminant analysis and cluster analysis (Ward's method) of the two taxa by using the same data set of morphological characters. All statistical analyses were performed using Bell Curve for Excel ver. 3.00 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

We further carried out detailed phenological surveys of *Lecanorchis suginoana* and *L. kiusiana* in the four populations used for morphological analyses (Yaita and Kakegawa populations for *L. suginoana*; Mashiko and Kakegawa populations for *L. kiusiana*). Five characters, viz., number of individuals, number of buds, number of flowers in full bloom, and number of wilted flowers, were recorded for the plants in each quadrat. In Yaita (*L. suginoana* habitat) and Mashiko (*L. kiusiana* habitat), ca. 47 km apart each other, 11 consecutive surveys were conducted in May and June 2012. In Kakegawa, the two taxa formed populations less than 20 m apart. Surveys were conducted in 17 and 30 May 2015. Furthermore, we recorded vegetation in association with *L. suginoana* and *L. kiusiana* at the above-mentioned four habitats. Methods for recording vegetation composition followed Braun-Blanquet (1964).

Results and Discussion

Macromolecular characters

In ca. 760 base pairs of ITS, three samples of *Lecanorchis suginoana* from different localities had identical sequences. Four samples of *L. kiusiana* had identical ITS sequences and two nucleotide substitutions discriminated these two taxa. In Kakegawa, populations of *L. suginoana* and *L. kiusiana* are less than 20 m apart. It is likely that reproductive isolation between these two taxa occurs because no trace of hybridization was detected in the nuclear nucleotide sequences investigated for these nearly sympatric populations. Two ITS phylotypes demarcated by a single substitution were recognized in seven samples of *L. japonica*. Both *L. suginoana* and *L.*

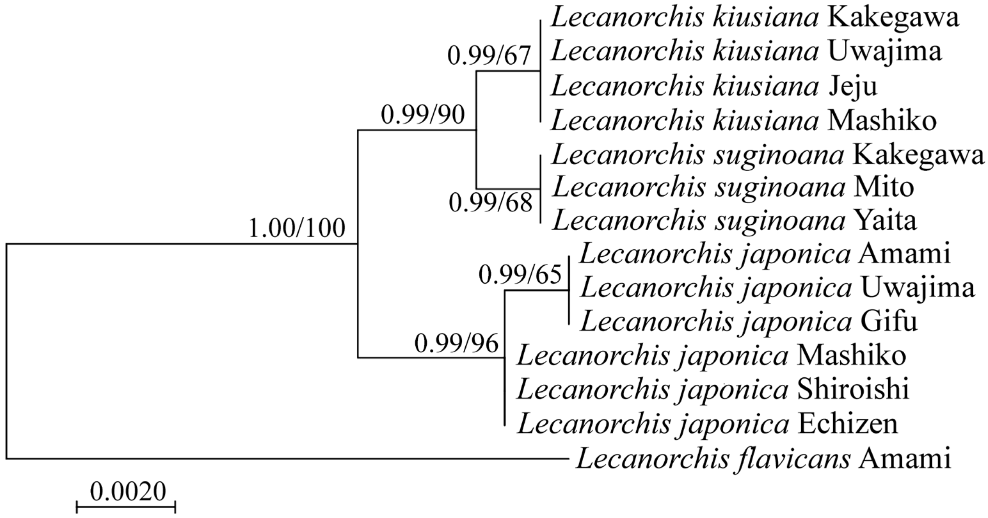

Fig. 2. Phylogenetic relationships of *Lecanorchis suginoana*, *L. kiusiana* and *L. japonica* inferred from nuclear ribosomal ITS sequences. The tree is rooted by an outgroup taxon *L. flavicans*. The tree was constructed using the maximum likelihood method and the topology was the same as the Bayesian inference analysis. Numbers at the nodes indicate Bayesian posterior probabilities (PP) and bootstrap percentages (BS) shown as PP/BS.

kiusiana were separated from *L. japonica* by five or six substitutions. Phylogenetic analysis of ITS showed that *L. suginoana* and *L. kiusiana* make a sister group relationship and these two taxa and *L. japonica* further form a clade (Fig. 2). These results coincide with Okayama *et al.* (2012) and demonstrate an independent taxonomic status of *L. suginoana*. Further, the phylogenetic relationships did not support an infraspecific placement of *L. suginoana* in *L. japonica* as proposed by Tuyama (1982).

Morphological characters

Our observations revealed that *Lecanorchis suginoana* is distinguishable from *L. kiusiana* by the following combination of qualitative morphological characters: yellowish-brown sepals and petals, yellow labellum hair; scattered, long, unicellular branchlets on labellum hair; weakly recurved labellum apex; and truncate column apex (Fig. 3, Table 2). These diagnostic characters were consistent among all the examined individuals and previous descriptions of the two taxa (e.g., Tuyama, 1982; Sugino, 1985; Hashimoto, 1990; Serizawa, 2005) except for *L. suginoana* f. *flava* Seriz., an anthocyanin defective flower form, having bright yellow sepals and petals.

Besides, *Lecanorchis suginoana* and *L. kiusiana* shared papillose labellum margins and unicellular branchlets on labellum hair, while these characters were not evident in *L. japonica* and its close relatives. Consequently, they are synapomorphies of the former two taxa and endorse their sister relationship revealed by the molecular phylogenetic analysis.

Regarding quantitative morphological characters, Student's t-test showed that length of dorsal sepal, length of lateral sepal, length of petal, width of petal, length of labellum, width of labellum, length of column, and width of labellum were significantly different between *Lecanorchis suginoana* and *L. kiusiana* ($p < 0.01$; Table 2). These differences indicated that *L. suginoana* has longer perianth lobes, wider petal, narrower labellum, longer column, and apically wider column than *L. kiusiana*. Results of the canonical discriminant analysis and the cluster analysis by using quantitative morphological characters also clearly separated these two taxa (Figs. 4, 5).

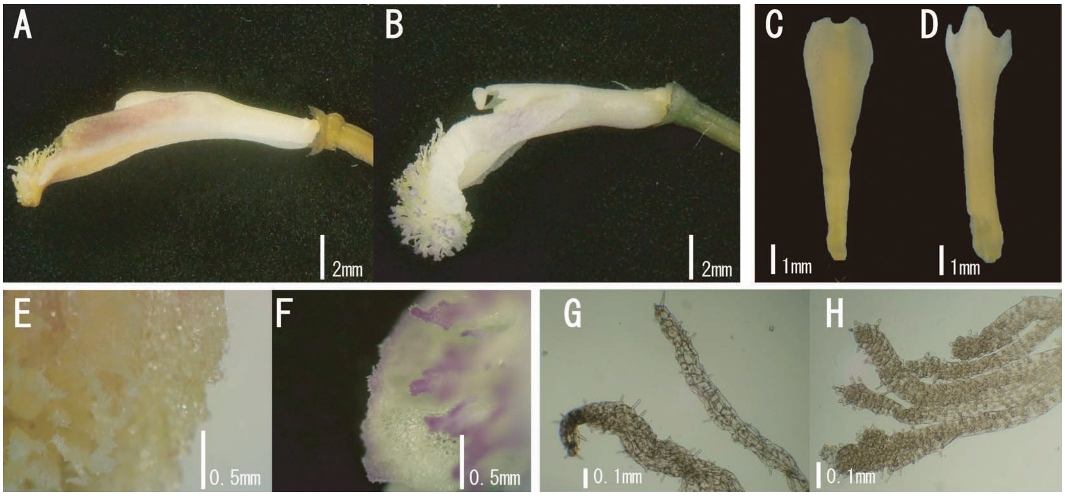

Fig. 3. Morphological comparison of *Lecanorchis suginoana* and *L. kiusiana*. Lateral view of labellum and column, *L. suginoana* (A) and *L. kiusiana* (B). Dorsal view of column, *L. suginoana* (C) and *L. kiusiana* (D). Marginal part of labellum, *L. suginoana* (E) and *L. kiusiana* (F). Labellum hair of *L. suginoana* (G) and *L. kiusiana* (H).

Table 2. Comparison of morphological characters in *Lecanorchis suginoana* and *L. kiusiana*

Character	*L. suginoana*	*L. kiusiana*
Colour of petals and sepals	yellowish brown	pale dull yellow
Shape of column apex	truncate	3 lobed
Colour of labellum hair	yellow	reddish purple
Length of branchlets on labellum hair (μm)	50–70	10–30
Density of branchlets on labellum hair	lax in mid to apical part	dense in apical part
Lateral view of labellum	weakly recurved	strongly recurved
Length of dorsal sepal: mean ± SD (mm)	15.8 ± 0.64* (N = 33)	14.3 ± 1.12* (N = 29)
Width of dorsal sepal: mean ± SD (mm)	3.14 ± 0.25 (N = 33)	3.32 ± 0.50 (N = 29)
Length of lateral sepal: mean ± SD (mm)	15.0 ± 0.57* (N = 34)	13.9 ± 0.90* (N = 29)
Width of lateral sepal: mean ± SD (mm)	2.88 ± 0.16 (N = 34)	2.81 ± 0.29 (N = 29)
Length of petal: mean ± SD (mm)	15.4 ± 0.55* (N = 34)	13.7 ± 0.93* (N = 29)
Width of petal: mean ± SD (mm)	3.76 ± 0.24* (N = 34)	3.54 ± 0.34* (N = 28)
Length of labellum: mean ± SD (mm)	13.9 ± 0.48* (N = 34)	13.4 ± 0.53* (N = 29)
Width of labellum: mean ± SD (mm)	6.49 ± 0.37* (N = 34)	8.37 ± 0.66* (N = 29)
Length of column: mean ± SD (mm)	9.27 ± 0.22* (N = 34)	8.46 ± 0.42* (N = 29)
Width of column: mean ± SD (mm)	2.17 ± 0.10* (N = 34)	1.96 ± 0.20* (N = 28)

* denotes significance at the 1% level.

Ecological characters

Figure 6 shows that flowering of *Lecanorchis suginoana* at Yaita population started on 1 June and reached the peak on 8 June. The last flowers of the season were observed on 18 June. Flowering of *L. kiusiana* at Mashiko population (47 km southward from Yaita) started on 8 June and reached the peak on 18 June. The last flowers of the season were observed on 24 June. This observation showed that the flowering period of *L. suginoana* was about one week earlier than that of *L. kiusiana* in the eastern part of Honshu, Japan, although about 10 days overlap period existed. In contiguous populations of these two taxa in Kakegawa, central part of Honshu, Japan, flowering of *L. suginoana* passed its peak on 22 May, while *L. kiusiana* just started flowering at the same date. On 30 May, flowering of *L. suginoana* already ended, while *L. kiusiana* just passed its peak (Table 3). These observations

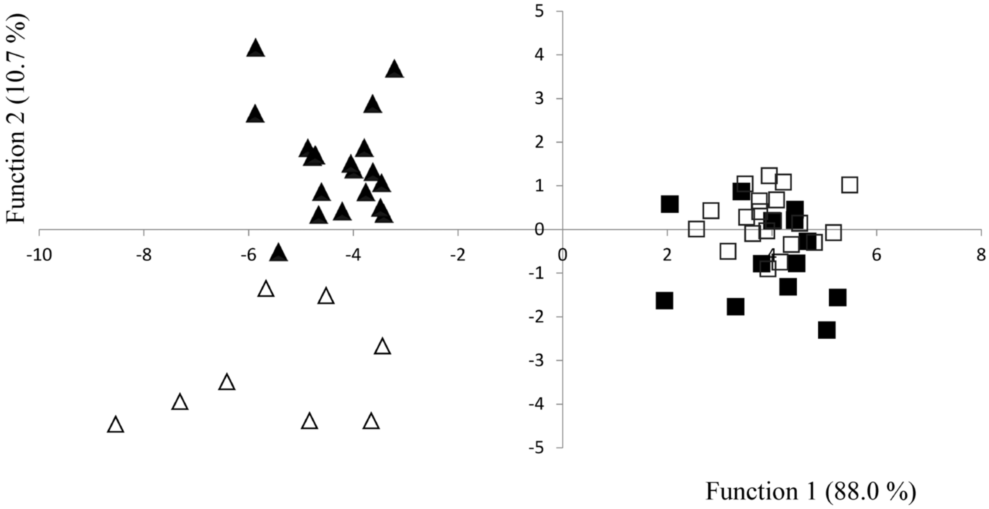

Fig. 4. Canonical discriminant analysis of 10 quantitative morphological characters of *Lecanorchis suginoana* and *L. kiusiana* flowers with the following four predefined groups: □ *L. suginoana*, Yaita population; ■ *L. suginoana*, Kakegawa population; △ *L. kiusiana*, Mashiko population; ▲ *L. kiusiana*, Kakegawa population. Percentages of total variance explained by the functions are given in parentheses. The analysis showed that the two taxa can be discriminated in 100% hit ratio.

demonstrated that earlier flowering of *L. suginoana* was likely to be a stable character.

Habitat differentiation between *Lecanorchis suginoana* and *L. kiusiana* is also noticeable (Fig. 7). Table 4 showed that two *L. suginoana* populations are associated with warm-temperate deciduous forest dominated by *Quercus serrata* Murray in the tree layer. In contrast, two *L. kiusiana* populations are in association with warm-temperate evergreen forest dominated by *Castanopsis sieboldii* (Makino) Hatus. ex T.Yamaz. et Mashiba subsp. *sieboldii* or *C. cuspidata* (Thunb.) Schottky in the tree layer. A similar habitat differentiation between these two *Lecanorchis* taxa from the standpoint of associated vegetation types was recorded by Sugino and Suzuki (1987).

Okayama *et al.* (2012) investigated mycorrhizal fungal diversity in 10 *Lecanorchis* taxa and found that they are symbiotic with several ectomycorrhizal fungi in Basidiomycota such as *Lactarius* (Russulaceae), *Russula* (Russulaceae), Atheliaceae, and *Sebacina* (Sebacinaceae). Since these fungal groups also associate with Fagaceae trees such as *Quercus* and *Castanopsis* and form ectomycorrhizae, tripartite symbioses likely establish among these organisms. Obviously, existence of appropriate fungal partners at habitats is indispensable for sustaining the life of *Lecanorchis* that completely depends upon nutrition to its symbiotic fungi. Okayama *et al.* (2012) found that different sets of fungi dominantly colonized with *L. suginoana* and *L. kiusiana*. They showed that the former species dominantly associated with *Lactarius* sp.1 and the latter mainly associated with *Lactarius* sp. 2. These findings indicate that habitat differentiation between the two *Lecanorchis* taxa is caused by different mycobiont communities between *Quercus* and *Castanopsis* forests, although sampling density in that study was not sufficient to substantiate this hypothesis.

Conclusions

Divergence of ITS sequences supported the independent status of *Lecanorchis suginoana*, *L. kiusiana*, and *L. japonica*. Moreover, the results of molecular phylogenetic analysis demonstrated that *L. suginoana* occupied a sister position of *L. kiusiana* and was distantly related to *L. japonica*.

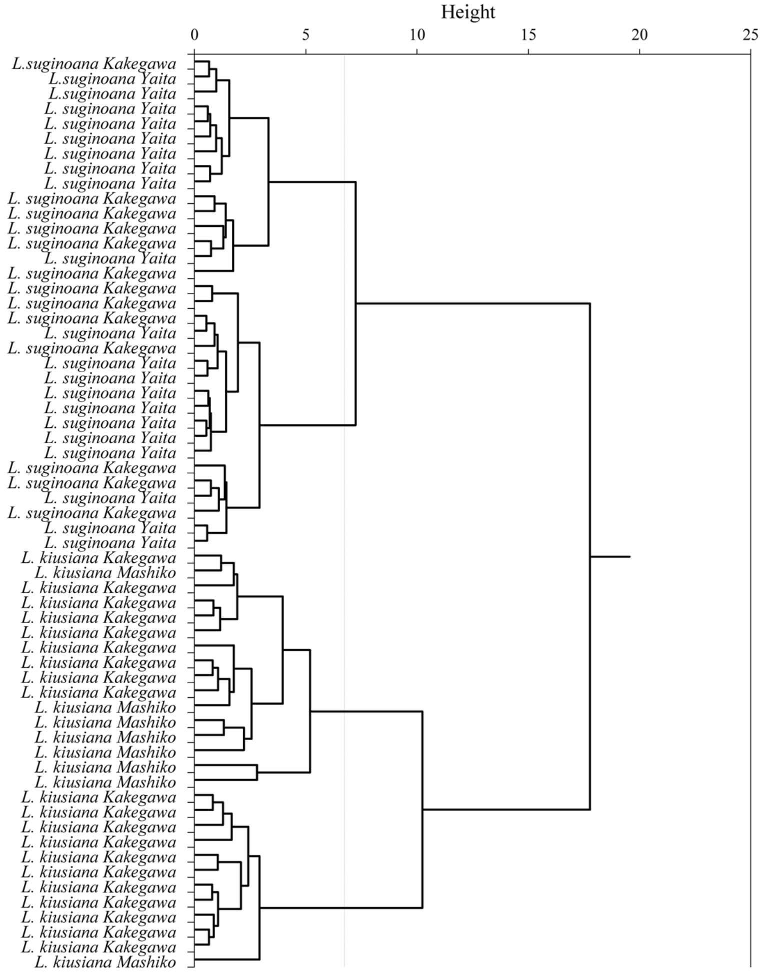

Fig. 5. Tree diagram generated by the cluster analysis of 10 quantitative morphological characters of *Lecanorchis suginoana* and *L. kiusiana* flowers using Ward's method. Height indicates the Euclidean distance. The results showed that the basal branching clusters correspond to the two taxa.

Thus, the infraspecific placement of *L. suginoana* in *L. japonica* was not supported. Moreover, flower colour, qualitative characters of labellum and column, quantitative characters of perianth lobes and column, flowering time, and associated vegetation types clearly distinguish *L. suginoana* from *L. kiusiana*. These differences corroborated the independent species status of *L. suginoana* rather than the infraspecific position of *L. kiusiana*.

Taxonomic Treatment

Lecanorchis suginoana (Tuyama) Seriz., Bunrui 5: 38 (2005)

[Figs. 1, 3]

Basionym: *Lecanorchis japonica* Blume var. *suginoana* Tuyama, J. Jpn. Bot. 57: 211 (1982). TYPE: JAPAN, Honshu, Shizuoka Pref., Haruno-cho, Sugi, 8 June 1980, *T. Tuyama s. n.* (TI).

Lecanorchis kiusiana Tuyama var. *suginoana* (Tuyama) T.Hashim., Ann. Tsukuba Bot. Gard. 9: 18 (1990).

Japanese name: Enshu-muyouran.

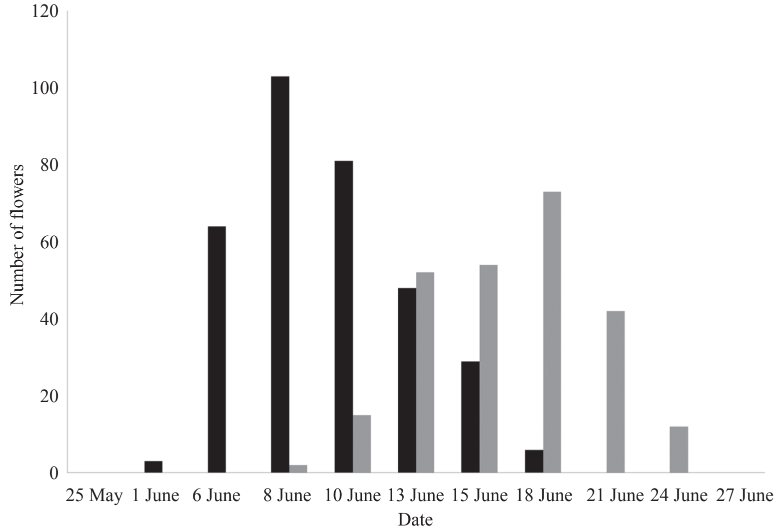

Fig. 6. Flowering period of *Lecanorchis suginoana* (Yaita population) and *L. kiusiana* (Mashiko population). These two populations are ca. 47 km apart and represent similar meteorological conditions. Black bars and gray ones indicate the number of flowers in full bloom of *L. suginoana* and *L. kiusiana* respectively.

Table 3. Flowering stage of *Lecanorchis suginoana* and *L. kiusiana* at two adjacent habitats in Kakegawa, central part of Honshu, Japan

Taxon		Date	
		22 May 2015	30 May 2015
L. suginoana	Number of buds	0	0
	Number of flowers in full bloom	59	0
	Number of wilted flowers	90	149
	Total number of flowers	149	149
	Number of individuals	35	35
L. kiusiana	Number of buds	132	8
	Number of flowers in full bloom	19	52
	Number of wilted flowers	0	80
	Total number of flowers	151	140
	Number of individuals	35	38

Flowering period: May to June.

Distribution: JAPAN: Honshu (Niigata Pref., Tochigi Pref., Ibaraki Pref., Saitama Pref., Tokyo Metro., Shizuoka Pref., Aichi Pref., Gifu Pref., Shiga Pref., Nara Pref., Kyoto Pref., Osaka Pref.); Shikoku (Ehime Pref., Kochi Pref.); Kyushu (Miyazaki Pref.); TAIWAN. Records of distribution were reviewed in Suetsugu *et al.* (2018).

Specimens examined: JAPAN. Honshu: Tochigi Pref. Nasushiobara-shi, Shimo-ohnuki, alt. 270 m, 11 June 2018, *T. Noguchi 180611-01* (TNS1311685); Yaita-shi, Yamada, alt. 255 m, 10 June 2010, *T. Ito s. n.* (TOCH167982); Yaita-shi, Yamada, 5 June 2012, *M. Takashima s. n.* (TNS8505115); Yaita-shi, Arai, alt. 260 m, 10 June 2010, *T. Ito s. n.* (TOCH167983); Yaita-shi, Higashi-izumi, alt. 260 m, 8 June 2012, *N. Hoshi & M. Takashima s. n.* (TOCH192869). Ibaraki Pref. Takahagi-shi, Daitakahagi, 6 June 1990, *M. Suzuki 21838* (INM); Hitachi-ohmiya-shi, Mt. Torinoko-san, alt. 430 m, 11 June 2005, *H. Ogura*, *Y. Maruyama & A. Narishima s. n.* (TOCH160932); Hitachi-ohmiya-shi, Torinoko,

Fig. 7. Habitats of *Lecanorchis suginoana* and *L. kiusiana*. A: *L. suginoana*, Yaita population. B: *L. suginoana*, Kakegawa population. C: *L. kiusiana*, Mashiko population. D: *L. kiusiana*, Kakegawa population.

alt. 450 m, 16 June 2013, *H. Uchiyama & H. Fujita s. n.* (TNS01184364); Mito-shi, Kawada-cho, alt. 30 m, 31 May 2013, *H. Uchiyama s. n.* (TNS8505120, 01184365). Shizuoka Pref. Shuchi-gun, Haruno-cho, Sugi, 28 June 1981, *T. Tuyama s. n.* (TI1326096, TI1328095); Shuchi-gun, Haruno-cho, Sugi, 15 June 1986 (TNS8504021); Shuchi-gun, Haruno-cho, 15 June 1986, *K. Kanda & Y. Hanei s. n.* (TNS8504481); Shuchi-gun, Haruno-cho, Sugi, 15 June 1986, *Y. Hanei s. n.* (TNS9504068, 9504069); Shimada-shi, Shitoro, alt. 170 m, 22 May 2015, *M. Takashima & M. Ohta s. n.* (TNS1311686); Kakegawa-shi, Higashiyama, alt. 210 m, 22 May 2015, *M. Takashima & M. Ohta s. n.* (TNS1311687, 1311688). Gifu Pref. Tajimi-shi, Higashimachi, 24 May 2013, *C. Tsubaki*, *K. Nomura & R. Nomura s. n.* (GPM-B918390); Gifu-shi, Tsubakibora, 30 May 2005, *R. Naruse s. n.* (GPM-B756065); Gifu-shi, Hora, 21 May 2004, *H. Minoura s. n.* (GPM-B756066); Mizunami-shi, Inazu-machi, Kozato, alt. 250 m, 12 June 2006, *M. Murase & S. Nagata s. n.* (GPM-B756993). Aichi Pref. Kota-cho, 26 March 1992, *K. Inami s. n.* (TNS8504035); Okazaki-shi, Kamisobumi-cho, alt. 120-150 m, 25 May 2005, *S. Fujii s. n.* (TNS772431); Nagoya-shi, Chikusa-ku, Higashiyama, 5 April 1984, *Y. Sawa 1411* (TNS01013786). Kyushu: Miyazaki Pref. Koyu-gun, Kawaminami-cho, Ohuchi, alt. 270 m, 1 June 2004, *T. Miamitani s. n.* (TNS9519671).

f. ***flava*** Seriz., Bunrui 5: 38 (2005).

TYPE: JAPAN, Honshu, Aichi Pref., Seto-shi, Kaisho-cho, alt. ca. 200 m, 29 May 2000, *S. Serizawa 76752* (AICH).

Specimens examined: JAPAN, Honshu: Shizuoka Pref. Kakegawa-shi, Higashiyama, alt. 255 m, 18 June 2014, *T. Yukawa 14-10* (TNS8505619). Gifu Pref. Mizunami-shi, Hiyo-

Table 4. Vegetation associated with *Lecanorchis suginoana* and *L. kiusiana* populations

Taxon		*L. suginoana*		*L. kiusiana*	
Locality		Yaita	Kakegawa 1	Mashiko	Kakegawa 2
Slope aspect		—	SE	SW	S
Slope degree		0°	20°	25°	10°
Canopy height		14 m	15 m	20 m	17 m
Canopy coverage (%)		85%	90%	95%	90%
Subtree layer coverage (%)		30%	35%	15%	15%
Shrub layer coverage (%)		0%	25%	10%	0%
Herb layer coverage (%)		30%	15%	3%	1%
Altitude		200 m	210 m	200 m	210 m
Quadrat size		15 m × 15 m	15 m × 10 m	20 m × 20 m	15 m × 15 m
	Taxon	Dominance・Sociability		Dominance・Sociability	
Camopy layer 8 m–	*Castanopsis sieboldii* (Makino) Hatus. ex T.Yamaz. et Mashiba subsp. *sieboldii*	・	・	5・5	・
	Castanopsis cuspidata (Thunb.) Schottky	・	・	・	4・4
	Quercus serrata Murray	4・4	4・4	・	2・2
	Cryptomeria japonica (L. f.) D.Don	・	・	・	2・1
	Clethra barbinervis Siebold et Zucc.	2・1	3・3	・	・
	Cerasus jamasakura (Siebod ex Koidz.) H. Ohba	・	2・2	・	・
	Carpinus laxiflora (Siebold et Zucc.) Blume	・	2・1	・	・
	Quercus sessilifolia Blume	・	・	1・1	・
	Quercus salicifolia Murray	・	・	1・1	・
	Ilex rotunda Thunb.	・	・	1・1	・
	Trachelospermum asiaticum (Siebold et Zucc.) Nakai var. *asiaticum*	+	・	・	・
Subtree layer 4–8 m	*Eurya japonica* Thunb. var. *japonica*	・	3・3	1・1	・
	Clethra barbinervis Siebold et Zucc.	2・1	2・2	・	・
	Machilus japonica Thunb.	・	・	・	2・2
	Lyonia ovalifolia (Wall.) Drude var. *elliptica* (Siebold et Zucc.) Hand.-Mazz.	2・1	・	・	・
	Cryptomeria japonica (L.f.) D.Don	2・1	・	・	・
	Alnus firma Siebold et Zucc.	・	1・1	・	・
	Castanopsis sieboldii (Makino) Hatus. ex T.Yamaz. et Mashiba subsp. *sieboldii*	・	・	1・1	・
	Quercus glauca Thunb. var. *glauca*	・	・	1・1	1・1
	Camellia japonica L. var. *japonica*	・	・	1・1	
	Cleyera japonica Thunb.	・	・	1・1	1・1
	Ilex integra Thunb.	・	・	2・2	1・1
	Ilex chinensis Sims	・	・	・	1・1
Shrub layer 1–4 m	*Eurya japonica* Thunb. var. *japonica*	・	2・2	1・2	・
	Cleyera japonica Thunb.	・	・	2・2	・
	Hydrangea hirta (Thunb.) Siebold et Zucc.	・	1・2	・	・
	Pieris japonica (Thunb.) D. Don ex G. Don	・	2・2	・	・
	Camellia japonica L. var. *japonica*	・	・	1・2	・
	Pleioblastus chino (Franch. et Sav.) Makino	・	1・2	・	・
	Lindera umbellata Thunb.	・	1・2	・	・
	Quercus glauca Thunb. var. *glauca*	・	・	1・1	・
	Quercus salicina Blume	・	・	+	・
	Quercus salicifolia Murray	・	・	1・1	・
	Padus grayana (Maxim.) C. K. Schneid.	・	1・1	・	・
	Wisteria floribunda (Wild.) DC.	+・2	・	・	・
	Ilex integra Thunb.	・	・	+	・
	Callicarpa mollis Siebold et Zucc.	・	・	+	・
Herb layer 0–1 m	*Rhododendron kaempferi* Planch. var. *kaempferi*	3・3	2・2	・	・
	Lecanorchis kiusiana Tuyama	・	・	1・2	1・2
	Lecanorchis suginoana (Tuyama) Seriz.	1・2	1・2	・	・
	Dryopteris erythrosora (D. C. Eaton) Kuntze	・	1・2	+	・
	Disporum smilacinum A. Gray	・	1・2	+	・
	Hydrangea hirta (Thunb.) Siebold et Zucc.	・	1・2	・	・
	Sasa ramosa (Makino) Makino	1・2	・	・	・
	Helonias orientalis (Thunb.) N. Tanaka	・	1・2	・	・
	Aucuba japonica Thunb. var. *japonica*	+・2	・	+	・
	Trachelospermum asiaticum (Siebold et Zucc.) Nakai var. *asiaticum*	・	・	+・2	・
	Pseudosasa japonica (Siebold et Zucc. ex Steud.) Makino ex Nakai	・	・	+・2	・
	Pleioblastus chino (Franch. et Sav.) Makino	・	+・2	・	・
	Toxicodendron tricocarpum (Miq.) Kuntze.	+	+	+	・
	Ilex crenata Thunb.	・	+	+	+
	Pertya glabrescens Sch. Bip. ex Nakai	・	+	+	・
	Cymbidium goeringii (Rchb. f.) Rchb.f. var. *goeringii*	・	+	+	・
	Platanthera minor (Miq.) Rchb.f.	・	+	・	+

Other companions. Running No. 1: *Cryptomeria japonica* (L.f) D.Don +, *Chamaecyparis obtusa* (Siebold et Zucc.) Endl. +, *Quercus serrata* Murray +, *Marus toringo* (Siebold) Siebold ex de Vriese +, *Tripterospermum japonicum* (Siebold et Zucc.) Maxim. +, *Hydrangea paniculata* Siebold +, *Smilax nipponica* Miq. +, No. 2: *Osmunda japonica* Thunb. +, *Ardisia crenata* Sims +, No. 3: *Illicium anisatum* L. var. *anisatum* +, *Quercus glauca* Murray +, *Quercus sessilifolia* Blume +, *Quercus myrsinifolia* Blume +, *Castanopsis sieboldii* (Makino) Hatus. ex T.Yamaz. et Mashiba subsp. *sieboldii* +, *Laurocerasus spinulosa* (Siebold et Zucc.) C.K.Schneid. +, *Camellia japonica* L. var. *japonica* +, *Eurya japonica* Thunb. var. *japonica* +, *Cleyera japonica* Thunb. +, *Ardisia japonica* (Thunb.) Blume var. *japonica* +, *Ilex rotunda* Thunb. +, *Viburnum erosum* Thunb. +, *Osmanthus heterophyllus* (G.Don.) P.S.Green +, *Ophiopogon japonicus* (Thunb.) Ker Gawl. var. *umbrosus* Maxim. +, No. 4: *Ilex pedunculosa* Miq. +, *Callicarpa japonica* Thunb. +, *Callicarpa mollis* Siebold et Zucc. +.

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