

A new variety of the mycoheterotrophic orchid *Lecanorchis thalassica* from Xieng Khouang Province, Laos

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ABSTRACT: Here, we describe a new variety of the mycoheterotrophic genus *Lecanorchis* (Orchidaceae), namely, *L. thalassica* T.P. Lin var. *laoensis* Suetsugu & T.C. Hsu, which was discovered during a botanical survey in Xieng Khouang Province, Laos. The new variety resembles *Lecanorchis thalassica* var. *thalassica* from Taiwan, owing to its relatively large tepals, tri-lobed lip, loosely flowered rachis, and obtuse sepals and petals, but is distinguished by a lip midlobe that is only centrally hairy and by additional projections below the column wings. An illustration and rDNA internal transcribed spacer (ITS) region (i.e., DNA barcode) sequence of the new variety from Laos are provided.

KEY WORDS: DNA barcoding, Laos, Lecanorchis, Mycoheterotroph, New locality, Orchidaceae.

INTRODUCTION

The mycoheterotrophic genus Lecanorchis Blume (Orchidaceae) comprises ca. 30 species that are widely distributed, with taxa reported from many countries including China, India, Indonesia, Japan, Korea, Laos, Malaysia, New Guinea, the Pacific islands, the Thailand, Philippines, Taiwan, and Vietnam (Seidenfaden 1978; Lin 1987; Hashimoto 1989, 1990; Pearce & Cribb 1999; Szlachetko & Mytnik 2000; Pridgeon et al. 2003; Averyanov 2005, 2011, 2013; Suddee & Pedersen 2011; Suetsugu & Fukunaga 2016; Suetsugu et al. 2018a,c,e). The genus can be distinguished from other orchid genera by numerous long, thick, horizontal roots that extend from a short rhizome, a cup-like structure (i.e., calyculus) that is located between the base of the perianth and apex of the ovary, and an elongate column that possesses a small wing on either side of the anther (Seidenfaden 1978; Hashimoto 1990; Szlachetko & Rutkowski 2000). However, the precise identification of Lecanorchis species is often hindered by the morphological similarity and brief flowering periods of Lecanorchis taxa (Suetsugu et al. 2018b; Suetsugu et al. 2016; Suetsugu et al. 2017b,d) and, in herbarium specimens, by the absence of important diagnostic characters since Lecanorchis flowers are easily lost or damaged during preservation (Suetsugu et al. 2016; Suetsugu et al. 2017a; Suetsugu et al. 2017b; Suetsugu et al. 2018d; Suetsugu

et al. 2018e).

Owing to the difficulty of identifying *Lecanorchis* specimens, the species diversity of the genus is likely to be underestimated (Suetsugu *et al.* 2016, 2018d). In fact, during a recent botanical survey in Xieng Khouang Province, Laos, we collected a flowering specimen of a previously undescribed *Lecanorchis* taxon. After careful examination, we designated the specimen as a new variety of *Lecanorchis thalassica* (as "*L. thalassicus*") T.P. Lin, which, until now, had only been reported from Taiwan (Lin 1987; Su 2000; Chen *et al.* 2009). Here we provide a description, illustration, and rDNA internal transcribed spacer (ITS) region (i.e., DNA barcode) sequence of the new variety from Laos.

MATERIALS AND METHODS

Morphological observation

The morphologies of the unidentified *Lecanorchis* specimen and previously described *Lecanorchis* species were compared by reviewing the literature, conducting field sampling, and examining both digitized plant specimens from online databases, such as JSTOR Global Plants (http://plants.jstor.org/), and specimens from multiple herbaria (KAG, KANA, KPM, KYO, MBK, NTUF, OSA, TAI, TAIF, TI, and TNS; abbreviations follow Index Herbariorum; Thiers 2017, http://sweetgum.nybg.org/science/ih/).

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Taxon	Location	Collection date Collection No.		GeneBank
				No.
L. thalassica var. laoensis	LAOS. Xieng khouang Province, Phonsavan District	4 May, 2018	T. Nishioka KS257 (FOF)	LC421224
L. thalassica	Taiwan. Nantou county	14 May, 2009	<i>T. C. Hsu 2201</i> (TAIF)	LC421225
L. japonica	JAPAN. Kagoshima Pref., Amami Island	21 Apr., 2018	H. Morita M24-001-KS258 (TNS)	LC421226

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Table 1. Lecanorchis spp.	Included in the current molecular analysi	s.

DNA barcoding

Flowers were collected from the unidentified Lecanorchis specimen and from two putative close relatives, L. japonica Blume and L. thalassica, and desiccated in the field using silica gel (Table 1). DNA was then isolated from the silica-dried materials using the CTAB method, and the rDNA internal transcribed spacer (ITS) region was PCR amplified in an iCycler (BioRad, Japan) using 10-µL reaction mixtures that contained 2 µL extracted DNA, 0.05 µL TaKaRa Ex Taq Hot Start Version (Takara Bio, Japan), 10 µM of each primer (AB101 and AB102; Douzery et al. 1993), 0.25 μ M of each dNTP, and 1 μ L 10× buffer, with the following conditions: initial denaturation at 94 °C for 5 min; followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; followed by a final elongation at 72 °C for 7 min. Finally, the amplified PCR products were purified using EconoSpin columns (Gene Design, Inc., Japan) and sent to Eurofins Genomics (Ebersberg, Germany) to be sequenced using the same primers used for PCR amplification.

RESULTS AND DISCUSSION

Morphological analysis revealed that the unidentified Lecanorchis specimen from Laos was similar to L. purpurea Masam., L. malaccensis Ridl., L. sarawakensis Suetsugu & Naiki, and L. thalassica in having relatively large flowers (tepals ca. 20 mm long) with tri-lobed lips, but the specimen could be easily distinguished from L. purpurea, L. malaccensis, and L. sarawakensis by its much longer rachis (>5 cm vs. <1.5 cm). The unidentified Lecanorchis specimen was also similar to L. japonica (especially L. japonica var. hokurikuensis (Masam.) T. Hashim. and L. japonica var. kiiensis (Murata) T. Hashim.) in having additional projections below column wing but could be distinguished from L. japonica and its varieties by its tepal apices (obtuse or slightly retuse vs. acute), lip and column adnation (ca. half of lip length vs. ca. one third of lip length), and floral condition (hardly open vs. widely open). These morphological characteristics of Laos specimens more closely matched the description of L. thalassica. In addition, the ITS sequences of the unidentified specimen and L. thalassica were identical, whereas those of the unidentified specimen and L. thalassica and L. japonica were not (i.e., two substitutions). The finding supports that the unidentified specimen from Laos are more closely related to L.

thalassica, while *L. thalassica* and *L. japonica* are likely to be independent species.

Interestingly, the unidentified specimen could still be distinguished from *L. thalassica* from Taiwan by a lip midlobe that was only centrally hairy (vs. almost completely hairy; Fig. 2-I) and by additional projections below column wing (vs. without projections; Fig. 2-J). However, considering that most of the specimen's characters, including tepal shape and the color and size of floral organs, were similar to those of *L. thalassica* from Taiwan, we describe the unidentified specimen from Laos as a new variety of *L. thalassica*, namely, *L. thalassica* var. *laoensis* Suetsugu & T.C. Hsu.

TAXONOMIC TREATMENTS

Lecanorchis thalassica T.P. Lin var. laoensis Suetsugu & T.C. Hsu, var. nov. Figs. 1 & 2

Type: LAOS, Xieng Khouang Province, evergreen forest, 19°41'17.1"N 103°08'22.7"E, ca. 1100m elevation, 4 May 2018, *T. Nishioka KS257* (holotype FOF!, dried plant on a herbarium sheet and liquid-preserved material in a bottle, labelled as the same specimen).

Diagnosis: Lecanorchis thalassica var. laoensis is similar to *L. thalassica* var. *Thalassica*, owing to its relatively large tepals, tri-lobed lip, loosely-flowered rachis, and obtuse sepals and petals, but is distinguished by a less hairy lip midlobe (< half surface area) and additional projections below its column wings.

Terrestrial, mycoheterotrophic herb. Rhizome Jshaped at the bottom; ascending part elongate, somewhat crooked, with several branches; descending part short, 2-3 cm long, simple, with radiate numerous roots. Roots of dried specimen ca. 2.5 mm in diameter, horizontally elongate, ca. 30 cm long. Stem branched at the base, erect; light yellowish to pinkish brown when young; blackened and hardened after flowering; 16-22 cm high, with several sheaths. Sheaths glabrous, triangular, acuminate, 8 to 15 mm long. Inflorescence terminal, loosely 3-6-flowered, flower-bearing part >5 cm long. Floral bracts triangular, acuminate, 6-7 mm long. Pedicellate ovary ascending, slightly muricate, 20-32 mm long, with a calyculus at the top. Calyculus ca. 1 mm high, with an irregularly denticulate margin. Flower basally surrounded by the calyculus, hardly open, somewhat nodding, pale brownish yellow. Dorsal sepal oblanceolate, ca. 17 mm long, 4.3 mm wide at the widest part and 0.9 mm wide at the base, apex obtuse. Lateral sepals obliquely oblong to oblanceolate, ca. 16.7 mm





Fig 2. *Lecanorchis thalassica* var. *laoensis* (drawn from the holotype). A. Habit. B. Fruits. C. Flower and pedicellate ovary. D-E. Lip and column. F. Dorsal sepal. G. Lateral petal. H. Lateral sepal. I. Flattened lip. J. Column. K. Hairs at the anterior part of cluster on lip. L. Midlobe edge of lip. M. Anther cap. Scale bars: A. 3 cm. B. 1 cm. C-H. 3 mm. I-M.1 mm. Drawn by Kumi Hamasaki.





Fig 1. Lecanorchis thalassica var. laoensis from Xieng Khouang Province, Laos. A. Habit. B. Flower.

long, 3.8 mm wide at the widest part and 1.3 mm wide at the base, apex obtuse. Petals obliquely oblanceolate, ca. 17.3 mm long, 4.9 mm wide and 0.6 mm wide at the base, apex obtuse or slightly retuse. Lip tri-lobed, adnate to ca. half its length from the base, 16.5–17.0 mm long, ca. 7.9 mm wide when spread, ca. 3.0 mm wide in the natural form; disc densely hairy; hairs lemon yellow, ca. 2 mm long, simple or rarely branched; lateral lobes slightly exceeding the column, abaxially purple, with fine serrations along the margins; midlobe transversely rectangular or depressedly obovate, with fine serrations along the margins, ca. 3.9 mm long, ca. 5.4 mm wide when spread. Column white, glabrous except for stigmalobes, ca. 11 mm long, ca. 2.5 mm wide at the widest part, fused with lip more than 2/3 of its length, with trapezoid wings at the top; column-wings with very conspicuous conical incision. Anther cap white, glabrous, ca. 1.7 mm wide. Fruit black, fusiform, erect or nearly erect, 28-39 mm together with the pedicel and the persistent calyculus.

Distribution: Lecanorchis thalassica var. laoensis is currently only known from a single population of ca. 10 flowering individuals in an evergreen broadleaved forest dominated by *Castanopsis* and *Lithocarpus* species in Xieng Khouang Province, Laos.

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