

Reappraisal of *Kitigorchis* (Orchidaceae)

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Abstract. *Kitigorchis*, a monotypic genus supposed to be endemic in the central part of Japan, was recently found in the Central Mountain Range of Taiwan. Further investigation of herbarium specimens and descriptions revealed that *Kitigorchis itoana* is conspecific with *Oreorchis indica*, previously recorded from the western Himalaya to southwestern China. This pattern of geographic distribution represents another example of disjunct distribution between Japan, Taiwan and the Himalayan region within the Eastern Asiatic Kingdom. Phylogenetic analyses using DNA sequence data from the internal transcribed spacer (ITS) region of the 18S-26S nuclear ribosomal DNA, *matK*, *trnT-L* intergenic spacer, *trnL* intron, *trnL-F* intergenic spacer, and *rpl* 16 intron showed that the monotypic *Kitigorchis* makes *Oreorchis* paraphyletic.

Keywords: Disjunct distribution; Japan; *Kitigorchis itoana*; Orchidaceae; *Oreorchis indica*; Phylogeny; Taiwan; Taxonomy.

Introduction

Maekawa (1971) established the orchid genus *Kitigorchis* on the basis of *Kitigorchis itoana* F. Maek. (Figure 1) together with *Oreorchis foliosa* (Lindl.) Lindl., *Oreorchis erythrochrysa* Hand.-Mazz., and *Tainia shimadai* Hayata. He separated *Kitigorchis* from *Oreorchis* by (1) the clustered, multi-branched rhizomes, (2) the distinct mentum, (3) the poorly developed calli on the lip, (4) the rigid leaf blade, and (5) the prominent nerves on the leaf. Subsequently, Hashimoto and Kanda (1981), Satomi (1982), Masamune (1984), Hashimoto (1987), Hashimoto et al. (1991), Imai (1997), Inoue and Ikegami (1997), and Environmental Agency of Japan (2000) followed Maekawa's treatment.

In their revisionary work of *Oreorchis* Lindl., Pearce and Cribb (1997) discussed the status of *Kitigorchis* and concluded that *Kitigorchis* is monotypic, comprising only *K. itoana*. They remarked that the clustered, multi-branched rhizomes of *K. itoana* are quite different from the subterranean organs of *Oreorchis*, but they did not recognize the other features used to separate these genera by Maekawa

(1971) as significant. They thus rejected Maekawa's transfer to *Kitigorchis* of the three taxa that do not form multi-branched rhizomes. They speculated that *Kitigorchis* is a genus intermediate between *Corallorhiza* R. Br. and *Oreorchis*. Since Maekawa's (1971) description of *K. itoana* was devoid of Latin diagnosis, it was not validly published. Throughout this paper, we provisionally use this invalid name because the development of the subject can be recognized correctly by use of this name and *K. itoana* has been used widely at least among literature in Japan.

In 2000 S.W. Chung and his associates found a terrestrial orchid in Taiwan that T. Yukawa confirmed to be *Kitigorchis itoana* (Figure 2). Previously, *K. itoana* was thought to be endemic in coniferous forests in a very narrow range in the central part of Japan on Mt. Yatsugatake, Mt. Komagatake, Mt. Karakitake, and Mt. Fujisan. This peculiar disjunct distribution led us to reexamine *Kitigorchis*.

Based on the results of a global phylogenetic analysis of the tribe Calypsoeae Dressler using *matK*, the maturase-encoding gene located in an intron of the plastid gene *trnK* (Yukawa, unpublished), *Kitigorchis* forms a clade with *Oreorchis* and *Corallorhiza*. *Cremastra* Lindl. is the sister group to the three genera and can be used as the outgroup for further analyses. As mentioned above, the generic status of *Kitigorchis* was based primarily on the

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single character, the clustered, multi-branched rhizomes. This character, however, may represent a plesiomorphic character in tribe Calypsoeae because *Corallorhiza* and *Cremastra* also have it. On the other hand, we did not find any morphological synapomorphies to unite *Oreorchis*

and *Corallorhiza*. It was apparent that phylogenetic analyses using macromolecular characters would be necessary to determine the generic status of *Kitigorchis*. In this paper we report on both morphological and macromolecular characters of *K. itoana* and allied taxa to clarify their taxonomic status and the phylogenetic affinity of the species.

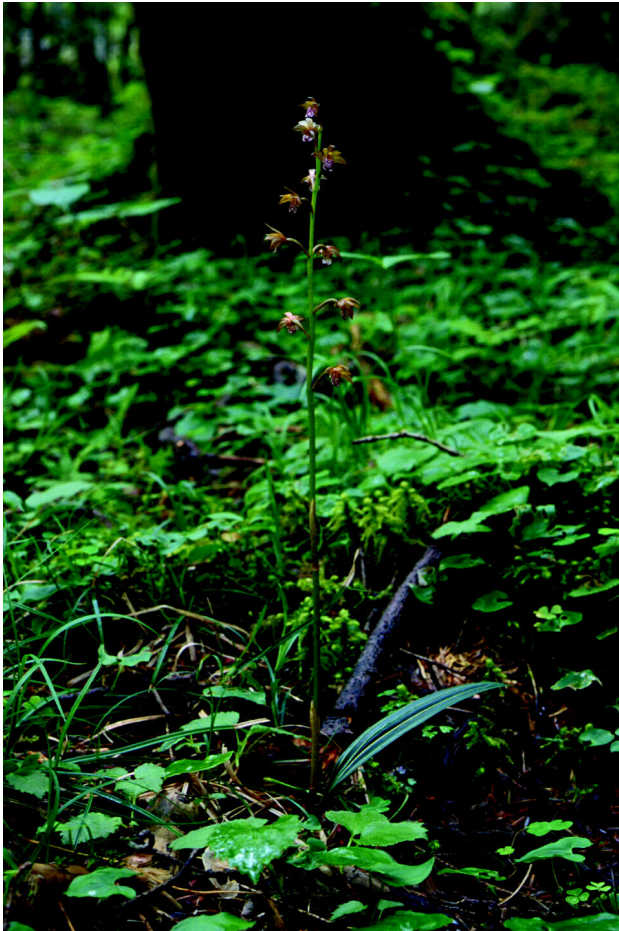


Figure 1. Flowering plant of *Kitigorchis itoana* F. Maek. (identified as *Oreorchis indica* (Lindl.) Hook. f. in this study) from Mt. Yatsugatake, Honshu, Japan. Photograph taken by Hiroshi Nakayama.

Materials and Methods

Materials collected for morphological and macromolecular studies are summarized in Table 1. Herbarium specimens studied are indicated in the taxonomic treatment. Fresh materials were preserved in 60% ethanol for morphological observations.

The molecular experimental methods are described in Yukawa et al. (1993, 1996). The internal transcribed spacer (ITS) region of the 18S-26S nuclear ribosomal DNA (including 5.8S ribosomal DNA and parts of 18S and 26S ribosomal DNA), *matK* of the plastid (including parts of *trnK* introns), *trnT-L* intergenic spacer of the plastid, *trnL* intron of the plastid, *trnL-F* intergenic spacer of the plastid, and *rpL16* intron of the plastid were amplified via the polymerase chain reaction (PCR) from a total DNA extract. We used the primers described in Douzery et al. (1999) for ITS; Yukawa et al. (1999) for *matK*; Taberlet et al. (1991) for *trnT-L* intergenic spacer, *trnL* intron, and *trnL-F* intergenic spacer; and Jordan et al. (1996) and Inoue and Yukawa (2002) for *rpL16* intron. DNA sequences were aligned manually, and gaps were treated as missing characters. The aligned data file is available from the first author upon request.

We initially conducted molecular phylogenetic analyses using two data sets. One was derived from ITS which represents the nuclear DNA sequences; the other comprising the five plastid DNA regions as mentioned above. Furthermore, the two data sets were combined to conduct analyses with more phylogenetic signals. Parsimony and distance analyses were conducted with PAUP* Version 4.0b10 (Swofford, 2002). The branch and bound search option was used to perform Fitch parsimony analyses (Fitch, 1971). Branch lengths for trees were

Table 1. Materials examined in morphological and macromolecular analyses.

Species	Country of origin	Voucher
<i>Corallorhiza trifida</i> Châtel.	Russia, Commander Isls.	<i>Berkutenko s. n.</i> (TNS ¹)
<i>Corallorhiza wisteriana</i> Conrad	USA, Florida	<i>Carlswald s. n.</i> (TNS)
<i>Cremastra aphylla</i> Yukawa	Japan, Honshu	<i>Yukawa 98-71</i> (TNS)
<i>Cremastra unguiculata</i> (Finet) Finet	Japan, Honshu	<i>Kurashige s. n.</i> (TNS)
<i>Cremastra variabilis</i> Blume	Japan, Kyushu	<i>Tanaka s. n.</i> (TNS)
<i>Kitigorchis itoana</i> F. Maek.	Taiwan	<i>Chung 3161</i> (TAIF)
<i>Kitigorchis itoana</i> F. Maek.	Japan, Honshu	<i>Yukawa 01-1</i> (TNS)
<i>Oreorchis erythrochrysea</i> Hand.-Mazz.	China, Yunnan	<i>Luo & Sun 766</i> (PE)
<i>Oreorchis fargesii</i> Finet	China, Hunan	<i>Luo 735</i> (PE)
<i>Oreorchis patens</i> (Lindl.) Lindl.	Japan, Honshu	<i>Kita s. n.</i> (TNS)

¹Herbarium acronyms follow Holmgren P.K., N.H. Holmgren, and L.C. Barnett (eds.). 1990. Index Herbariorum. Part I. The Herbaria of The World. Ed. 8. The New York Botanical Garden, Bronx, 693 pp.

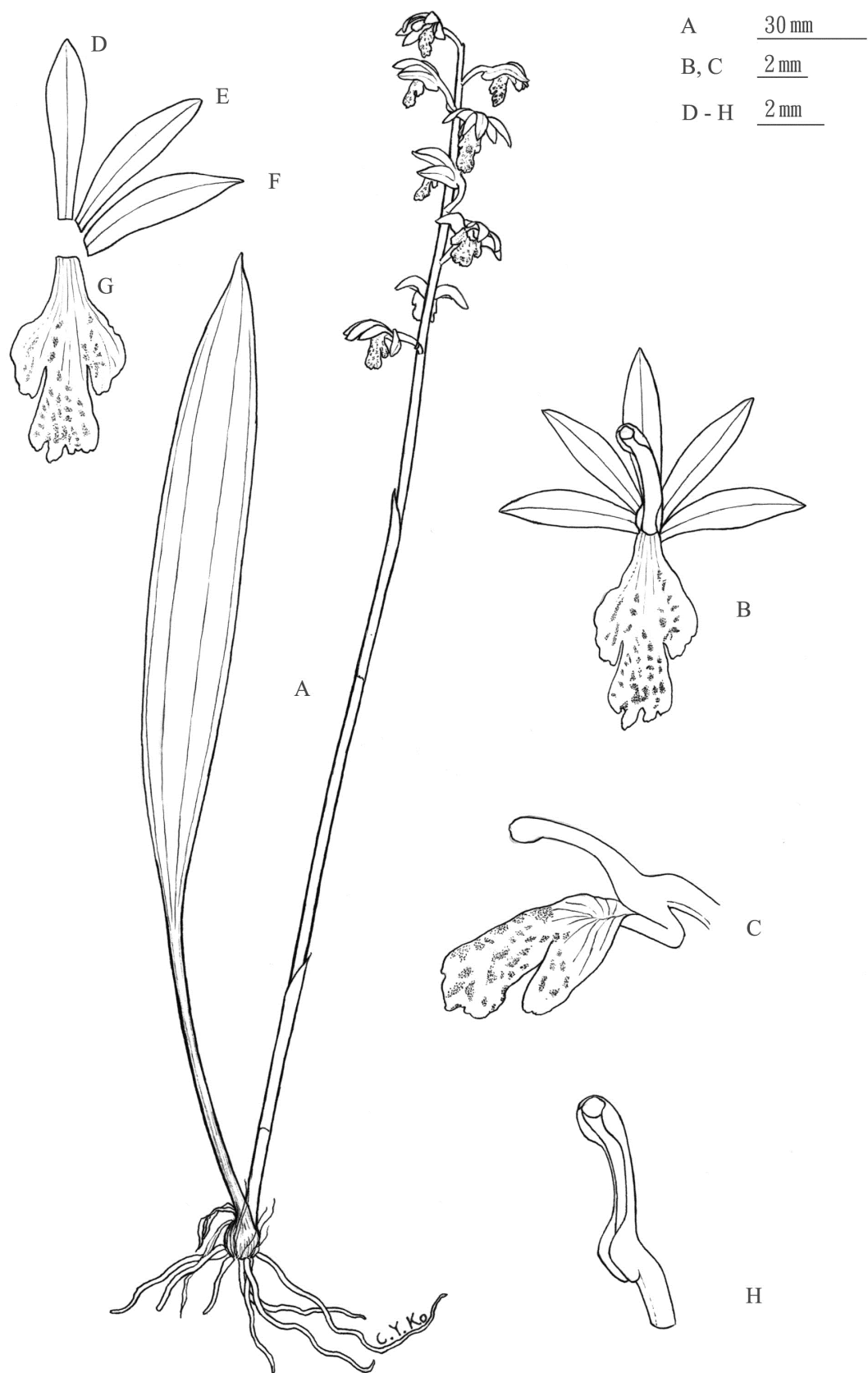


Figure 2. *Kitigorchis itoana* F. Maek. (identified as *Oreorchis indica* (Lindl.) Hook. f. in this study). A, Habit; B, Flower, front view; C, Column and labellum, side view; D, Dorsal sepal; E, Petal; F, Lateral sepal; G, Labellum; H, Column and column foot. Drawn from *Chung 3161* collected from Mt. Hohuanshan, Taiwan.

calculated using the DELTRAN optimization (Swofford and Maddison, 1987). Distance trees were obtained using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura two-parameter correction (Kimura, 1980). To assess the relative robustness for branches, the bootstrap method (Felsenstein, 1985) was used with 10,000 replicates.

Results and Discussion

Identification

Comparison of Japanese and Taiwanese plants of *K. itoana* did not show significant differences except for larger vegetative parts (leaves: to 19 cm long \times 2.7 cm wide in Japanese material, to 30 cm long \times 2.5 cm wide in Taiwanese material; inflorescence: to 30 cm in Japanese material, to 50 cm long in Taiwanese material) and more distinct lateral lobes of the labellum in plants from Taiwan (Figure 2). We suspected that the discovery of this significant, disjunct population might indicate a wider distribution of the species. We therefore examined specimens and descriptions of other genera in tribe Calypsoeae and found that *Oreorchis indica* (Lindl.) Hook. f., a species reported from the western Himalaya to southwestern China, is conspecific with *K. itoana*. Descriptions and illustrations of *O. indica* [owing to misidentification, illustrated as *Oreorchis foliosa* (Lindl.) Lindl. in King and Pantling (1898), Benerji and Pradhan (1984), and Deva and Naitani (1986); see Pearce and Cribb (1997)] coincide with *K. itoana* from Japan and Taiwan. Consequently, the distribution pattern of *K. itoana* represents another example of disjunct distribution between Japan, Taiwan and the Himalayan region within the Eastern Asiatic Kingdom (Wu and Wu, 1998).

The nomenclatural confusion between *O. indica* and *O. foliosa* was described in detail by Pearce and Cribb (1997) who treated the two taxa as varieties of a single species, *O. foliosa* var. *foliosa* and *O. foliosa* var. *indica* (Lindl.) N. Pearce and P. J. Cribb. The two taxa are easily distinguished by the presence or absence of a mentum and the raised lamellae between the lateral lobes of the labellum. Since these variations are not continuous and reflect different reproductive strategies, we consider the two taxa to be different species.

Tainia gokanzanensis Masamune, another species similar to *K. itoana*, is incompletely known (Turner, 1992) because the type specimen could not be located. Interestingly, the type was collected from Mt. Hohuanshan, the same mountain where *K. itoana* was found in Taiwan. When we examined the original description of *T. gokanzanensis* meticulously, we concluded that *T. gokanzanensis* from Taiwan is conspecific with *K. itoana*.

Reinvestigation of morphological characters of *K. itoana* supported Pearce and Cribb's (1997) view in which they recognized the clustered, multi-branched rhizomes as the sole diagnostic character of the genus. We also did not find synapomorphic characters for *K. itoana*, *Oreorchis foliosa*, *Oreorchis erythrochrysa*, and *Tainia shimadai* to validate Maekawa's (1971) original circumscription of *Kitigorchis*.

DNA Analyses

Initially, we determined the genetic divergence between the Japanese and Taiwanese plants of *K. itoana* using ITS sequences. Since the two sequences were identical, the Japanese material was used for phylogenetic analyses.

The results of the phylogenetic analyses derived from the nuclear and plastid data sets were consistent (results not shown). In such cases, analyses of combined data sets provide more resolution and internal support for relationships than do the individual data sets (e. g. Olmstead and Sweere, 1995). A combined data set was therefore used for further analyses.

The parsimony analysis using the combined data set resulted in a single most parsimonious (MP) tree of 483 steps (Figure 3), which showed a sister group relationship between *K. itoana* and *O. erythrochrysea* (100% bootstrap support). This clade further formed a clade with *Corallorhiza* and the remaining species of *Oreorchis*, albeit with moderate bootstrap support (68%). The tree had a consistency index (CI) of 0.94 (0.86 excluding uninformative characters) and a retention index (RI) of 0.89. The

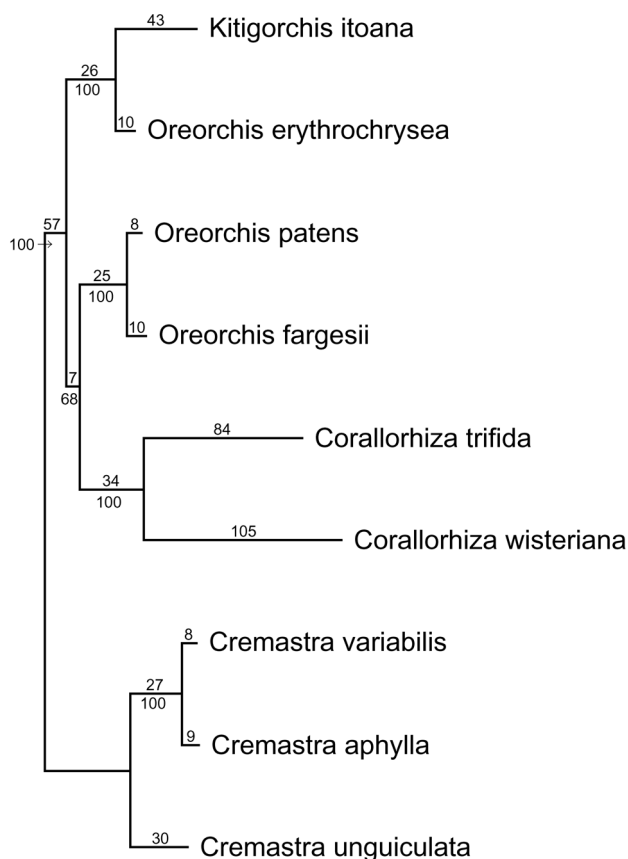


Figure 3. A single most-parsimonious Fitch tree based upon ITS, *matK*, *trnT-L* intergenic spacer, *trnL* intron, *trnL-F* intergenic spacer, and *rpl* 16 intron sequences: length=483, consistency index=0.94 (0.86 excluding uninformative characters), retention index of 0.89. Numbers above internodes correspond to branch length (DELTRAN optimisation). Numbers below internodes indicate bootstrap values from 10,000 replicates of Fitch parsimony analysis.

neighbor-joining (NJ) topology (Figure 4) was identical for the relationship between *K. itoana* and *O. erythrochrysea* (100% bootstrap support). In the NJ tree, this clade further made a clade with the rest of the species of *Oreorchis* (52% bootstrap support). Sixteen percent of the MP bootstrap replications showed this relationship.

Both the MP and NJ results definitely showed a sister group relationship between *K. itoana* and *O. erythrochrysea* (100% bootstrap support in both analyses). The relationships between *K. itoana*-*O. erythrochrysea* clade and the remaining species of *Oreorchis* and *Corallorhiza* are not clear. Scattered sampling in *Oreorchis* and *Corallorhiza* and long branches in *Corallorhiza* probably resulted in the low resolution. Ambiguous relationships among the 3 clades, namely, *K. itoana*-*O. erythrochrysea* clade, *O. patens*-*O. fargesii* clade, and *C. trifida*-*C. wisteriana* clade, did not provide a conclusive taxonomic arrangement of these genera. At the least, the current circumscription of *Kitigorchis* makes *Oreorchis* paraphyletic. If *O. erythrochrysea* is transferred to *Kitigorchis*, all three genera, *Kitigorchis*, *Oreorchis*, and *Corallorhiza*, exhibit monophyly. We did not, however, find any synapomorphic morphological characters found only in *K. itoana* and *O. erythrochrysea*. Another option is to unite the three genera into the single genus *Corallorhiza*, the earliest name among the three genera. Inclusion of more taxa from both *Oreorchis* and *Corallorhiza* in further phylogenetic analyses is desirable to determine the taxonomy of this group. Our provisional conclusion, based on our data, is to treat *K. itoana* as conspecific with *Oreorchis indica*, since doing otherwise would make *Oreorchis* paraphyletic.

Taxonomic Treatment

Oreorchis indica (Lindl.) Hook. f., Fl. Brit. Ind. 5: 709. 1890. *Corallorhiza indica* Lindl., J. Linn. Soc. Bot. 3: 26. 1859. *Oreorchis foliosa* (Lindl.) Lindl. var. *indica* (Lindl.) N. Pearce & P. J. Cribb, Edinburgh J. Bot. 54: 307. 1997.—TYPE: INDIA. Western Himalaya, Simla, on Hattu, 5 August 1847, T. Thomson 1724 (holotype: K-LINDL).

Tainia gokanzanensis Masam. in Humbert, Notul. Syst. (Paris) 6: 38. 1937, syn. nov.—TYPE: TAIWAN. Taityusyu, Mt. Gôkanzan (Hohuanshan), alt. ca. 3,300m, G. Masamune s. n. (holotype: TAI, not located).

Kitigorchis itoana F. Maek., The Wild Orchids of Japan in Colour. 300. 1971, nom. nud.

Habitat and Ecology. Terrestrial in coniferous forest and open meadows. Elevation 1,950–4,500 m. Flowering observed from June to August.

Distribution. Himalayan India, Nepal, Bhutan, China (Xizang, Yunnan, and Sichuan), Taiwan, Japan (central part of Honshu).

Specimens examined. JAPAN: Honshu, Mt. Yatsugatake, alt. 1,950 m, 24 Jun 2001, T. Yukawa 01-1 (TNS); Honshu, Mt. Fujisan, 2,000 m, 19 Jul 1984, F. Konta 15375 (TNS). TAIWAN: Hualien Hsien, Hsiulin Hsiang,

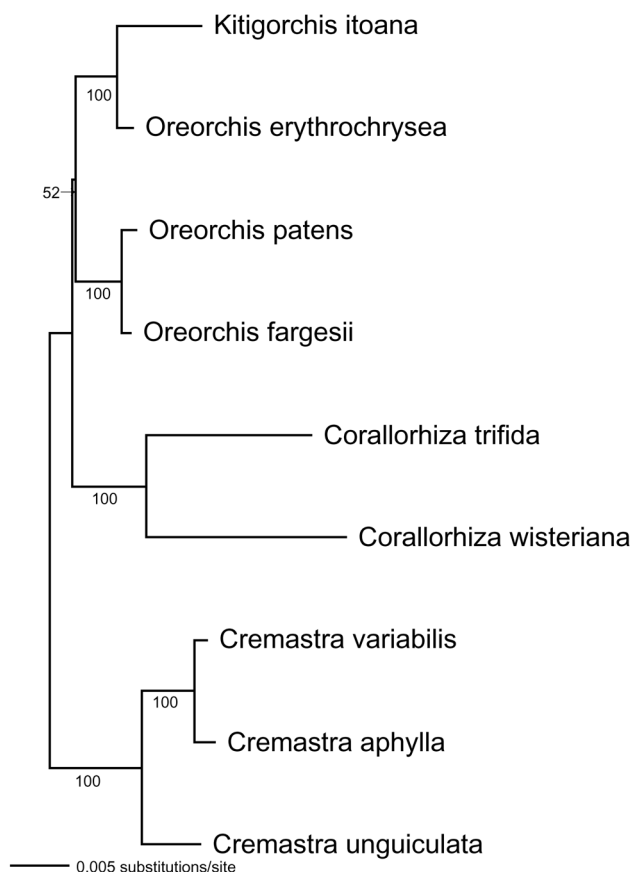


Figure 4. Neighbor joining (NJ) tree based upon ITS, *matK*, *trnT-L* intergenic spacer, *trnL* intron, *trnL-F* intergenic spacer, and *rpl* 16 intron sequences. Numbers below internodes indicate bootstrap values from 10,000 replicates of NJ analysis

Mt. Hohuanshan, behind High Altitude Experimental Station, Taiwan Endemic Species Research Institute, *Abies* forest, along a dry brook, ca. 3,000 m, N 24° 29' 42" E 121° 16' 37", 8 Jun 2000, flowering, S. W. Chung 3161 (TAIF); same loc., 1 Dec 2000, sterile, C. I. Peng 18199 (HAST). CHINA: Tibet, 3,400 m, 10 Jun 1956, Unknown collector 308 (PE). BHUTAN: Chamsa-Yabu Thang, 3,200–3,700 m, 15 May 1967, H. Kanai et al. s. n. (TI); Nala-Tzatogang, 3,200–3,400 m, 26 May 1967, H. Kanai et al. s. n. (TI).

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琥珀蘭屬植物（蘭科）之再評議

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琥珀蘭屬 (*Kitigorchis*) 以往被視為特產於日本中部山區的單型屬蘭科植物，最近在台灣的中央山脈合歡山區發現其分布。作者等進一步研閱標本及文獻描述，發現琥珀蘭 (*Kitigorchis itoana*) 與分布於喜馬拉雅至中國西南的印度山蘭 (*Oreorchis indica*) 為同種植物。印度山蘭如此之植物地理分布為東亞植物區 (Eastern Asiatic Kingdom) 日本、台灣及西瑪拉雅間斷分布的又一例子。利用 18S-26S 核糖體 DNA, *matK*, *trnT-L* 基因間區序列、*trnL* 內插子、*trnL-F* 基因間區序列和 *rpl16* 內插子的 DNA 序列進行親緣關係分析，顯示琥珀蘭屬與山蘭屬形成並系關係 (paraphyletic)。

關鍵詞：間斷分布；日本；琥珀蘭；蘭科；印度山蘭；親緣；台灣；系統分類。