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NOTE

New Distribution Record of *Begonia grandis* (Begoniaceae, section *Diploclinium*) from Taiwan, with subspecies assignment based on morphology and molecular phylogeny

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ABSTRACT: *Begonia grandis* Dry. (Begoniaceae), previously recognized as endemic to continental China, was recently discovered from southern Taiwan. Close morphological observations and molecular phylogenetic analysis elucidated that the species in Taiwan belongs to subspecies *grandis*. *Begonia grandis* has a long history in cultivation especially in China and Japan but not in Taiwan. The collection locality in Taiwan had no trace of human activity. Therefore, *B. grandis* is considered to be native to Taiwan. The new finding in Taiwan marks the southernmost distribution record of the species. Unfortunately, the discovered population was destroyed by a landslide due to a typhoon in 2009. Further field survey in neighboring areas is needed to elucidate the current status of *B. grandis* in Taiwan.

KEY WORDS: Begoniaceae, *Begonia grandis*, ITS, new record, sect. *Diploclinium*, Taiwan, taxonomy

INTRODUCTION

The genus *Begonia* L. (Begoniaceae) comprises more than 1,500 species of herbs, shrubs, and lianas that are widely distributed in the tropics and subtropics of the world (Kiew, 2005; Tebbitt, 2005; Chung *et al.*, 2014). *Begonia* sect. *Diploclinium* (Lindl.) A.DC. includes more than 100 species distributed widely in Asia from India and Sri Lanka to the Himalayas, Indo-China, China, Taiwan, the Malesian region, and Fiji (Doorenbos *et al.*, 1998; Rubite, 2012; Rubite *et al.*, 2013). A recent molecular phylogenetic study revealed that the section is polyphyletic, grouped mainly by plesiomorphic characters and lacking synapomorphies (Thomas *et al.*, 2011), and the section awaits comprehensive taxonomic revision (Rubite, 2012; Rubite *et al.*, 2013). *Begonia grandis* Dry. is the type species of sect. *Diploclinium* (Doorenbos *et al.*, 1998). It is a deciduous herb with tubers and upright stems to 100 cm tall and characterized by tubercles in the leaf axils (Doorenbos *et al.*, 1998; Gu *et al.*, 2007; Ching-I Peng, personal observation). *Begonia grandis*, occurring naturally west from Tibet to Zhejiang and south from Yunnan to Liaoning of China, is one of the most widely distributed species in the genus and is the most northerly distributed species (Li *et al.*, 2014). The species grows in evergreen broad-leaved forests on montane slopes and stream sides and rocky cliffs in wet and shaded environments (Gu *et al.*, 2007). *Begonia grandis* is polymorphous and the latest taxonomic treatment recognized three subspecies (Gu *et al.*, 2007; Table 1); *B. grandis* subsp. *grandis* and *B. grandis* subsp. *sinensis* (A.DC.) Irmsch. are widely distributed in the species' distribution range, whereas *B. grandis* subsp. *holostyla* Irmsch. is endemic to Yunnan province.

During a field expedition in a lowland forest in Maolin district of Kaohsiung city, southern Taiwan on 14 March 2008, Yi-Fu Wang and Wei-Min Lin collected four tubers of begonia plants from a wet rocky slope beside bamboo vegetation near the halfway point of a road leading to Chingjenku Waterfall (22°52' N, 120°39' E; Fig. 1). They were brought to Dr. Cecilia Koo Botanic Conservation Center (KBCC) in southern Taiwan for cultivation. One of the four tubers grew up into a flowering plant in 2009 and turned out to be *B. austrotaiwanensis* Y.K.Chen & C.-I Peng (sect. *Platycentrum*), a deciduous species occurring in southern Taiwan. Interestingly, the other three tubers grew up, yielded flowers in 2011, and turned out to be *B. grandis* that was previously not documented for Taiwan.

In this paper, we report the new distribution record of *B. grandis* in Taiwan, together with detailed

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Table 1. Diagnostic characters and altitudinal distribution range of the three subspecies of *Begonia grandis*, following Gu *et al.* (2007).

	Subspecies *grandis*	Subspecies *sinensis*	Subspecies *holostyla*
Stem	Stout	Weak	Weak
Leaf blade	Ovate to broadly ovate	Elliptic- to triangular-ovate	Triangular-ovate
Abaxial side of leaf blade	Red, at least on veins	Pale, occasionally reddish	—
Style	Connate at base	Connate at base	Free, not branched
Stigma	U-shaped, spirally tortuous	U-shaped, spirally tortuous	Capitate or reniform
Filament	Connate at ≥ 2 mm above base	Connate at < 2 mm above base	Connate at < 1 mm above base
Altitudinal range (m asl.)	100–1,100	300–3,400	2,200–2,800

morphological features diagnostic for the subspecies assignment. We also conducted a molecular phylogenetic analysis to confirm the subspecies assignment. In addition, we comment on its conservation status in Taiwan.

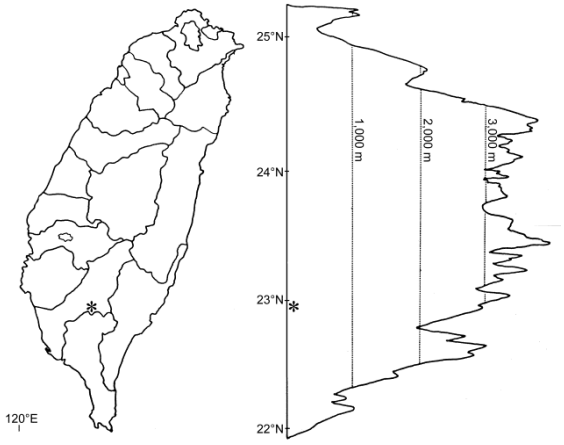


Fig. 1. The locality of *Begonia grandis* (indicated by asterisks) in Maolin district of Kaohsiung city, southern Taiwan.

METHOD

Morphological study

Propagules of *Begonia grandis* from southern Taiwan were cultivated in the experimental greenhouse of Biodiversity Research Center, Academia Sinica. Fully grown plants (*Ching-I Peng 23576*, HAST) were used for morphological observations.

Molecular phylogenetic analysis

Molecular phylogenetic analysis was conducted based on the internal transcribed spacer (ITS) region (including ITS1 and ITS2 and the 5.8S rRNA gene) of nuclear ribosomal DNA (nrDNA), which was proved to be useful for resolving phylogeny of closely related *Begonia* species (Nakamura *et al.*, 2013; Ding *et al.*, 2014). The analysis incorporated one sample of *B. grandis* from Taiwan (*Ching-I Peng 23576*, HAST; GenBank accession number, AB972939) and ITS sequences of Chinese samples obtained from GenBank: two samples of *B. grandis* subsp. *grandis* (AF485089 with two ribotypes and GU176064), one sample of *B. grandis* subsp. *sinensis* (GU176067), and one sample of *B. grandis* subsp. *holostyla* (AF485088 with two ribotypes). For the Taiwanese sample, methods of DNA extraction, polymerase chain reaction (PCR) amplification of ITS, and DNA sequencing followed Nakamura *et al.* (2014). DNA sequences were aligned using ClustalX ver. 1.8 (Thompson *et al.*, 1997) and then manually adjusted. Our ongoing study on Asian *Begonia* phylogeny indicated that *B. grandis* is very distantly related to other congeners (Peng *et al.*, unpublished data), suggesting that selection of different outgroup taxa may affect ingroup topology. We used a Bayesian method implemented in BEAST ver. 1.7.5 program (Drummond *et al.*, 2005, 2012), which can estimate the root of a phylogeny without using an outgroup by enforcing the (relaxed) molecular clock constraint (Drummond and Rambaut, 2007). HKY substitution model was employed as the best-fit model for the ITS data, as estimated using KAKUSAN4 (Tanabe, 2011). The molecular clock hypothesis was rejected for the ITS data ($P < 0.0001$) based on the likelihood ratio test (Felsenstein, 1988; Swofford, 2002). Therefore, a relaxed-clock uncorrelated lognormal distribution model was used for rate variation among lineages (Drummond *et al.*, 2006). The Speciation Birth–Death tree prior was employed for the branching rates. The unweighted pair-group method of arithmetic averages (UPGMA) was used to construct a starting tree. Default priors were used for the remaining parameters. MCMC chains were run for 10 million generations and sampled every 1,000 generations. Convergence of all parameters was checked using Tracer ver. 1.5.0 (Drummond and Rambaut, 2007) and the first 10% of the sampled trees were discarded as burn-in. The effective sample sizes of parameters in the log file were 430–9,001 after the burn-in, indicating satisfactory sampling of the posterior distributions of each parameter. A maximum clade credibility tree was

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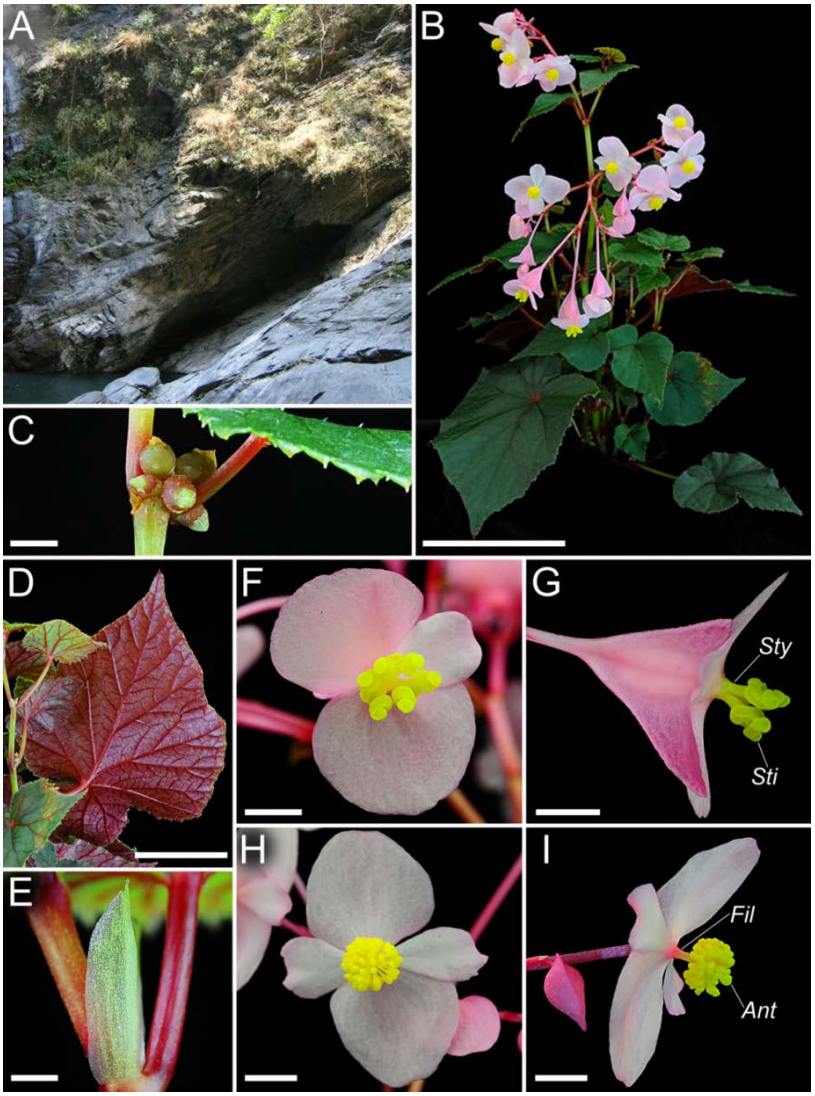


Fig. 2. *Begonia grandis* from southern Taiwan. A: Habitat. B: Habit. C: Tubercle. D: Leaf, abaxial side. E: Stipule. F: Pistillate flower, ventral view. G: Pistillate flower, lateral view. H: Staminate flower, ventral view. I: Staminate flower, lateral view. Abbreviations: Ant, anther; Fil, filament; Sti, stigma; Sty, style. Scale bar: B = 10 cm; C = 5 mm; D = 5 cm; E = 2 mm; F–I = 5 mm. The photo of habitat (A) does not show the exact collection locality but a similar environment 200–300 m away from the collection locality, which was not photographed before being destroyed by Typhoon Morakot in 2009. All plant photos are from Ching-I Peng 23576 (HAST) grown in the experimental greenhouse of Academia Sinica.

estimated with a burn-in of 10 % of the sampled trees and a posterior probability (*PP*) limit of 0.5 by TreeAnnotator ver. 1.5.4 (Drummond and Rambaut, 2007), and visualized with FigTree ver. 1.4.2 (Drummond and Rambaut, 2007).

RESULTS

Morphological features

Begonia grandis from Taiwan had stout stems, ovate to broadly ovate leaf blade, red abaxial side of leaf blade, styles connate at base, U-shaped and spirally tortuous stigmas, and filaments connate at ≥ 2 mm above base (Fig. 2). Based on these morphological features, the sample was identifiable as *B. grandis* subsp. *grandis*, following Gu *et al.* (2007).

Molecular phylogeny based on ITS

The aligned length of the ITS sequences was 739 bp. No indels were observed except for length difference in mononucleotide repeats. The Bayesian maximum clade credibility tree is shown (Fig. 3). Only clades supported by *PP* ≥ 0.90 are considered below. The phylogeny was

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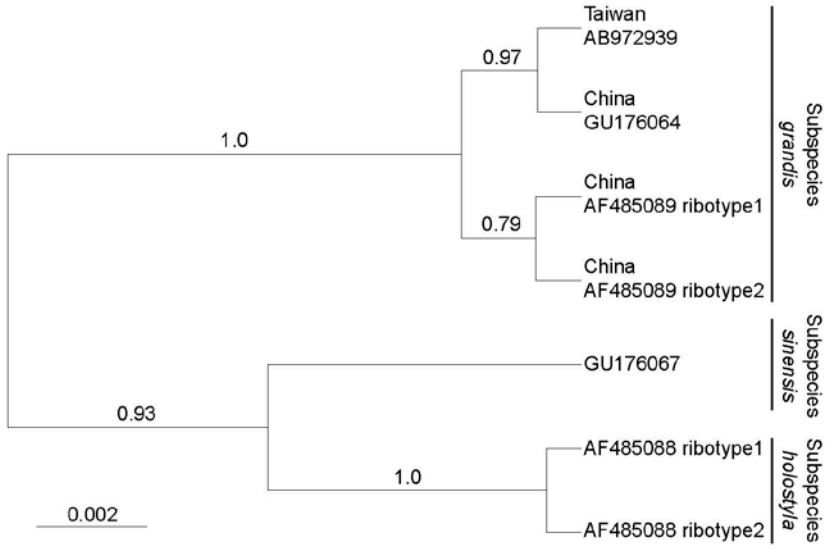


Fig. 3. Bayesian majority-rule consensus tree of three subspecies of *Begonia grandis* based on ITS sequences. The numerals on branches are Bayesian posterior probabilities (*PP*). Scale bar shows the number of expected substitutions per site.

rooted to separate two large clades: one comprised the Taiwanese sample and the Chinese samples of *B. grandis* subsp. *grandis* (*PP* = 1.0) and the other included *B. grandis* subsp. *sinensis* and *B. grandis* subsp. holostyla (*PP* = 0.93). Within the latter clade, the two ribotypes of *B. grandis* subsp. *holostyla* formed a subclade (*PP* = 1.0) and were separated from *B. grandis* subsp. *sinensis*.

DISCUSSION

Eighteen taxa of *Begonia* were previously known to be indigenous to Taiwan (Chen, 1993; Peng *et al.*, 2005; Peng and Ku, 2009). The present study documents yet another species, *B. grandis*, to this *Begonia*-rich island. The detailed morphological observations and molecular phylogenetic analysis elucidated that the species in Taiwan is assignable to subsp. *grandis* of this wide-ranging species. Considering that seven *Begonia* species were added (Peng *et al.*, 2005; Peng and Ku, 2009; present study) after the publication of the second edition of Flora of Taiwan (Chen, 1993) and that many parts of this mountainous island are difficult to access, it is possible that further field surveys may uncover new *Begonia* species and/or new distribution records.

The new finding of *Begonia grandis* in Taiwan marks the southernmost distribution record of the species and the first report of the species outside Asian Continent. The species may have migrated from the Asian Continent to Taiwan via long-distance dispersal crossing the sea, or alternatively, via land bridges repeatedly formed during the Pleistocene glaciation (Liu, 1988; Nakamura *et al.*, 2009), as was suggested for multiple plant lineages (Wang *et al.*, 2004; Chiang and Schaal, 2006; Wei *et al.*, 2010; Nakamura *et al.*, 2012).

Begonia grandis has a long history in cultivation due to its high value as an ornamental, medicinal, edible, and cultural plant especially in China and Japan (Li *et al.*, 2014) but the species is not known to be grown in any parts of Taiwan. We collected the species from the lowland forest in southern end of the Central Mountain Range of Taiwan and not around resident houses, temples, or other artificial constructions. Therefore, *B. grandis* is considered to be native and not naturalized in Taiwan. The species was not previously discovered probably because of its rarity and deciduous habit.

Concerning the conservation status in Taiwan, *B. grandis* is known only from the cited collection locality. The number of plants was not investigated when collected because in the beginning it was misidentified as *B. austrotaiwanensis* commonly found in that area. The discovered population was destroyed by a landslide due to Typhoon Morakot in August, 2009. Thereafter, *B. grandis* has not been re-collected from that locality. Further field survey is needed to elucidate the current condition and to determine IUCN Red List category (IUCN, 2010, 2012) of *B. grandis* in Taiwan. Our living collection kept in the greenhouse can be utilized for *ex situ* conservation and restoration of the wild population.

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