Phytotaxa 145 (1): 27–37 (2013)
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.145.1.3

Begonia tandangii (Begoniaceae, section *Baryandra*), a new species from Luzon Island, the Philippines

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Abstract

We describe *Begonia tandangii*, a new species of *Begonia* sect. *Baryandra* from the Sierra Madre Mountain Range of Luzon Island, the Philippines. *Begonia tandangii* has a close resemblance to *B. fenicis* in gross morphology, differing in having leaf margin sparsely fringed with minute hairs (vs. glabrous or with minute hairs only on teeth) and capsules with broadly-ovate outline and an acuminate apex (vs. capsules with broadly-obovate outline and a rounded to truncate apex). Phylogenetic analyses of Philippines species of sect. *Baryandra* based on ITS sequences revealed that *B. tandangii* was clearly separated from *B. fenicis*. *Begonia tandangii* is currently known only from the type locality in a coastal forest of Baler, Aurora Province, which is in the neighborhood of Aurora Memorial National Park.

Key words: *Begonia*, Begoniaceae, ITS phylogeny, Philippines, sect. *Baryandra*, sect. *Diploclinium*, Sierra Madre Mountain Range

Introduction

The genus *Begonia* Linnaeus (1753: 1056), (Begoniaceae, e.g., Doorenbos *et al.* 1998) comprises more than 1,500 species (Kiew 2005, Tebbitt 2005). The Philippines, where more than 100 species are recorded (Golding & Wasshausen 2002), is one of the centers of *Begonia* species diversity in the world (Rubite 2012). Philippine begonias are assignable to three sections, namely, sect. *Baryandra* A. de Candolle (1859: 122), sect. *Petermannia* (Klotzsch 1855: 74) A. de Candolle (1859: 128), and sect. *Platycentrum* (Klotzsch 1855: 123) A. de Candolle (1859: 134) (Rubite 2012, Rubite *et al*. 2013). *Begonia* sect. *Baryandra* includes ca. 50 species, having its center of diversity in the Philippines but also with a few species in Borneo and New Guinea (Rubite *et al*. 2013). The section, comprising species previously included in sect. *Diploclinium* (Lindley ex. R. Wight 1852: 9) A. de Candolle (1859: 129), has recently been revised (Hughes 2008, Rubite & Madulid 2009, Hughes *et al*. 2010, 2011, Rubite 2012, Rubite *et al*. 2013). However, further field survey in the Philippines may discover new species because *Begonia* species generally have narrow distribution ranges and the Philippines has not been botanically fully explored (Rubite & Madulid 2009).

The Sierra Madre is a chain of mountains in the eastern coast of north and central Luzon Island (14°–19° N; Fig. 1), where the largest contiguous forest in the Philippines is found. In the south-central part of the mountain range, we discovered an unknown *Begonia* which resembles *B. fenicis* Merrill (1908: 421) of sect. *Baryandra* in gross morphology, green (neither purple-brown nor purplish-red) and non-peltate leaves, and five-tepalled pistillate flowers. *Begonia fenicis* has been reported from islets north of Luzon Island but not from Luzon Island (Merrill 1908, Hatusima 1975, Chen 1993). Basing on detailed morphological and molecular phylogenetic analyses, we confirmed that the unknown *Begonia* is a new species of sect. *Baryandra*, which is named *Begonia tandangii* C.-I Peng & R.Rubite (below).

Accepted by Manuel Belgrano: 6 Oct. 2013; published: 11 Nov. 2013

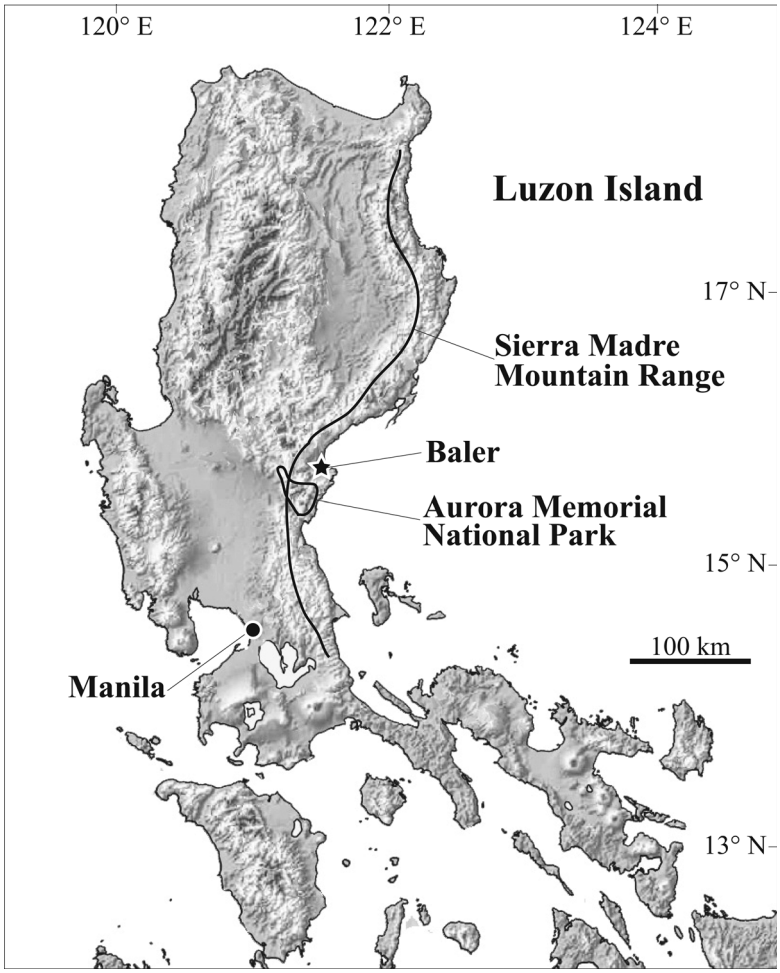

FIGURE 1. Map of Luzon Island indicating Baler of Aurora Province, the type locality of *Begonia tandangii*.

Materials and Methods

Morphological study

Four plants of *Begonia tandangii* (*Ching-I Peng 23400*, HAST) were studied. For comparison, specimens of *B. fenicis* in PNH and HAST were examined (Appendix 1); these specimens covered the entire range of *B. fenicis* and included an isolectotype (*E. Fénix, Bur. Sci. 3619*, PNH) and isosyntypes (*E.A. Mearns, Bur. Sci. 3207*, PNH; *E. Fénix, Bur. Sci. 3893*, PNH) (Merrill 1912 [1911]).

Molecular analyses

Total genomic DNA was extracted from four plants of *B. tandangii* (*Ching-I Peng 23400*, HAST) using fresh leaves following the method of Murray & Thompson (1980). ITS region (including ITS1 and ITS2 spacer regions and the 5.8S rRNA gene) was amplified using PCR. PCR was performed in 25 µl total volume with the following reagents: about 10 ng of genomic DNA, 1 unit of Taq DNA polymerase master mix (Ampliqon, Rødovre, Denmark), 0.4 µM of each primer, and 2% DMSO. The primers ITS1 and ITS4 (White *et al.* 1990) were used, with the PCR cycle condition 95°C for 5 min, 1 cycle of 97°C for 2 min, 50°C for 1 min, 72°C for 1 min, 25 cycles of 95°C for 1 min, 50°C for 2 min, 72°C for 3 min, and 72°C for 10 min. The PCR fragments were used as templates for cycle sequencing reactions with the same primers used in the PCR, and direct sequencing was performed on an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The four samples had identical ITS sequence, and thus one accession was used for *B. tandangii* in the following analyses. The sequence was deposited in the DDBJ (DNA Data Bank of Japan) databases (since 1983).

To test the phylogenetic distinction of *B. tandangii*, we incorporated 21 out of 55 species of sect. *Baryandra* (including *B. fenicis*) in phylogenetic analyses: 19 from the Philippines and 2 from Borneo (Appendix 2). ITS data for these species were reported in a preceding molecular study of sect. *Baryandra* (Rubite *et al*. 2013). For outgroups, three species of sect. *Reichenheimea* (Klotzsch 1855: 54) A. de Candolle (1864: 385) were used (Appendix 2), following the result of Rubite *et al*. (2013). DNA sequences were aligned using ClustalX ver. 1.8 (Thompson *et al*. 1997) and then manually adjusted. Phylogenetic analyses were based on a Bayesian approach using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) and a maximum parsimony (MP) criterion using PAUP* ver. 4.0b10 (Swofford 2002).

In the Bayesian phylogenetic analysis, the substitution model for the ITS data was selected using KAKUSAN4 (Tanabe 2011) based on Bayesian information criterion (BIC). Two separate runs of Metropolis coupled Markov chain Monte Carlo (MCMCMC) analyses were performed, each with a random starting tree and four chains (one cold and three heated). The MCMCMC length was three million generations, and the chain was sampled every one hundredth generation from the cold chain. The mixing and convergence of the MCMC chains of the two runs was assessed by inspection of the trace plots of parameters using Tracer ver. 1.5.0 (Drummond & Rambaut 2007); the first 3000 sample trees (10% of the total 30,000 sample trees) were discarded as burn-in. After the burn-in, the effective sample sizes (ESS) of all parameters were > 200, indicating that the analyses sampled the posterior distributions of each parameter satisfactorily, and the values of Average Standard Deviation of Split Frequency (ASDSF) were below 0.005. The 50% majority rule consensus tree and Bayesian posterior probabilities (*PP*) of all the post-burn-in trees was generated using TreeView ver. 1.6.6 (Page 1996).

In the MP phylogenetic analysis, indels were treated as missing data. The characters were treated as unordered, and the character transformations were equally weighted. The branch collapse option was set to collapse at a minimum length of zero. A heuristic parsimony search was performed with 200 replicates of random additions of sequences with ACCTRAN character optimization, tree bisection–reconnection (TBR) branch swapping, and MULTREES and STEEPEST DESCENT options on. Statistical support for each clade was assessed by bootstrap analysis (Felsenstein 1985). Ten thousand replicates of heuristic searches, with the TBR branch swapping switched on and MULTREES options off, were performed to calculate bootstrap percentages (*BP*).

Chromosome Cytology

Somatic chromosome morphology was studied for two plants of *B. tandangii* (*Ching-I Peng 23400*, HAST) and three plants of *B. fenicis* (the Philippines, Lutao Island, Niutoushan, *Koh Nakamura 20101493*; Taiwan, Lanyu Island, Zhonghenggonglu, *Koh Nakamura 20101366*; Japan, Iriomote Island, Hinai River, *Koh Nakamura 20100209*, HAST). The procedures of pretreatment, fixation and staining for chromosome observations followed Peng *et al.* (2012).

Results

Morphological study

Detailed morphological comparison between *Begonia tandangii* and *B. fenicis* found salient characters to distinguish them. In *Begonia tandangii*, leaf margin was sparsely fringed with minute hairs less than 1 mm long; capsules were broadly ovate in outline, with an acuminate apex (Fig. 2 & 3). On the other hand, in *B. fenicis*, leaf margin was glabrous or had minute hairs only on teeth; capsules were broadly obovate in outline, with a rounded to truncate apex (Fig. 4). In other features, these two species were not clearly distinguished because character states/values in *B. tandangii* were within variation ranges of those in *B. fenicis*.

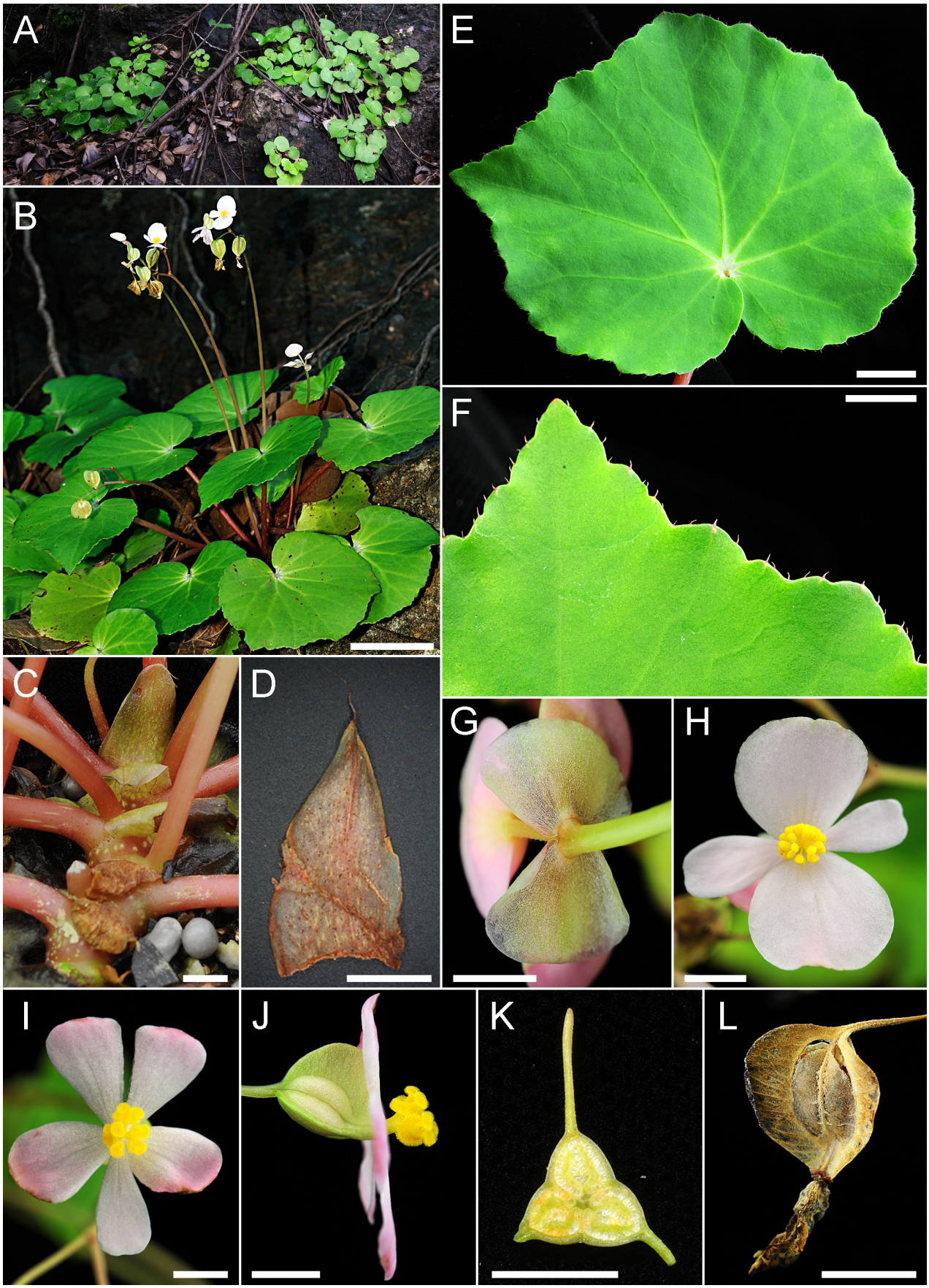

FIGURE 2. *Begonia tandangii*. A. Habitat. B. Habit. C. Rhizome. D. Stipule. E. Leaf, adaxial view. F. Leaf margin sparsely fringed with minute hairs. G. Bracts. H. Staminate flower. I. Pistillate flower, ventral view. J. Pistillate flower, lateral view. K. Transverse section of developing capsule. L. Capsule with persistent tepals. Scale bars are 5 cm for B, 5 mm for C–D and F–L, 10 mm for E. [All photos from *Ching-I Peng 23400* (HAST)]

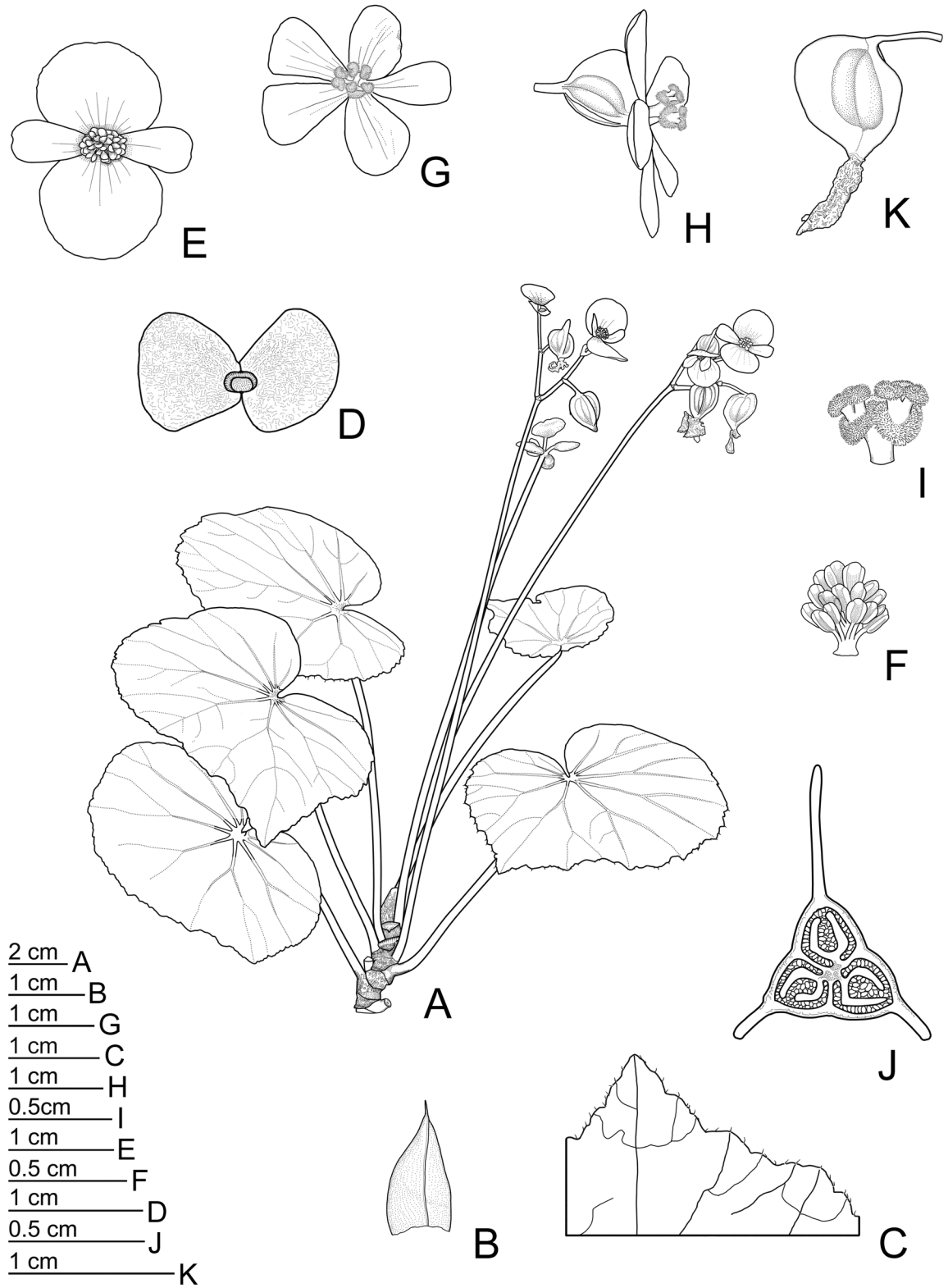

FIGURE 3. *Begonia tandangii*. A. Habit. B. Stipule. C. Leaf margin sparsely fringed with minute hairs. D. Bracts. E. Staminate flower. F. Stamens. G. Pistillate flower, ventral view. H. Pistillate flower, lateral view. I. Styles and stigmas. J. Transverse section of developing capsule. K. Capsule with persistent tepals. All drawn from *Ching-I Peng 23400* (HAST) by Chien-Yu Ke.

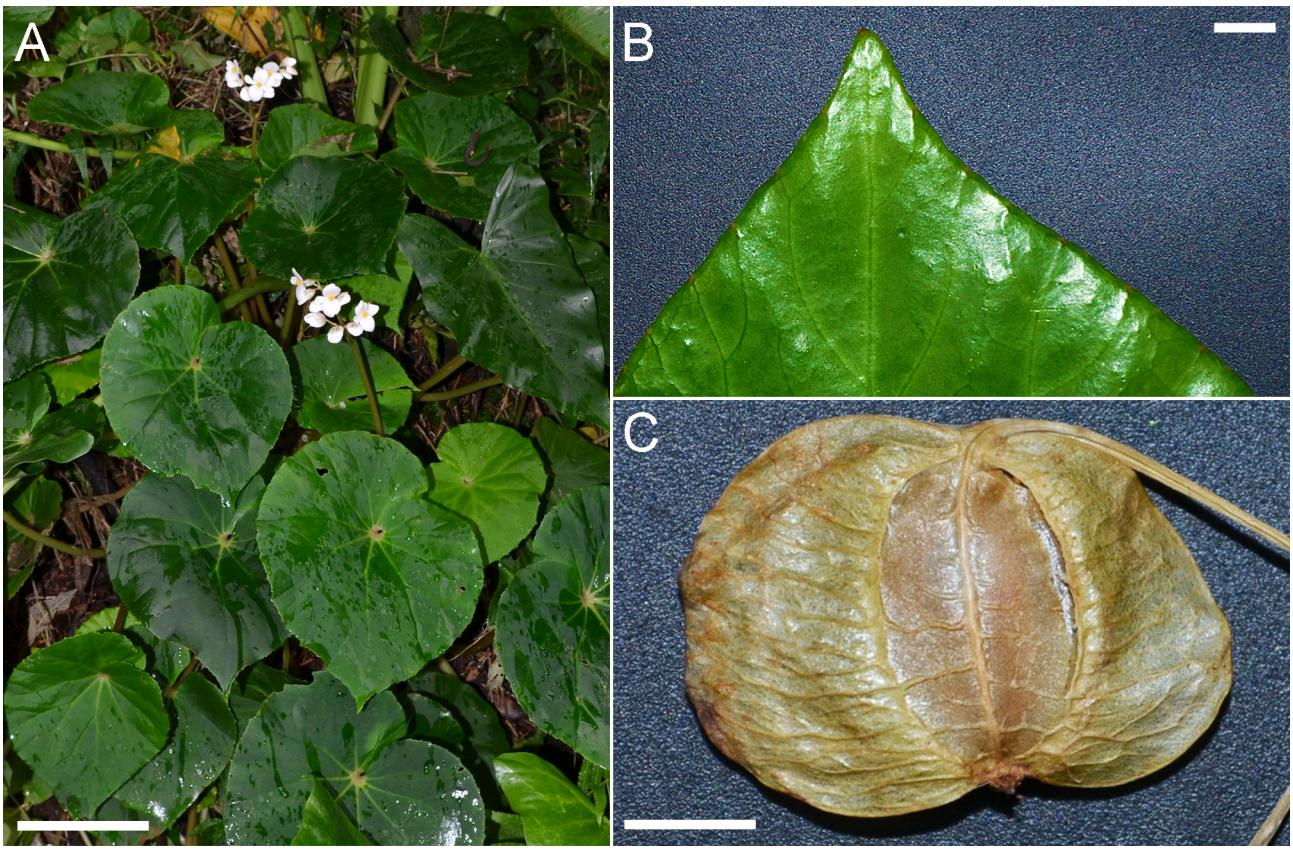

FIGURE 4. *Begonia fenicis*. A. Habit. B. Glabrous leaf margin. C. Capsule. Scale bars are 5 cm for A, 5 mm for B and C. [A from *Koh Nakamura 11969* (HAST); B from *Koh Nakamura 20101477* (HAST); C from *Koh Nakamura 11754* (HAST)]

Phylogenetic relationships based on ITS

The aligned length of the ITS data was 842 bp. 275 nucleotide substitutions were found in 192 variable sites and 130 sites were parsimony informative among them. In the Bayesian analysis, the BIC selected the GTR+G substitution model. The 50% majority rule consensus tree with mean branch length of all the post-burn-in trees is depicted (Fig 5). The MP analysis yielded 7 equally parsimonious trees of 761 steps with a consistency index (CI) = 0.656, a retention index (RI) = 0.712, and a rescaled consistency index (RC) = 0.467. The topology of the MP strict consensus tree (not shown) was the same as that of the Bayesian tree, except the three nodes which collapsed in the MP tree (*BP* < 50). *BP*s are plotted on the Bayesian tree. *Begonia tandangii* resided in a well-supported clade (*PP* = 0.97 / *BP* = 97.9) with *B. chloroneura* P.Wilkie & Sands (1999: 132), *B. fenicis*, *B. hernandioides* Merrill (1912 [1911]: 392) and *B. tayabensis* Merrill (1918: 38). In this clade, *B. tandangii* and *B. fenicis* were clearly separated because *B. fenicis* formed a terminal clade with *B. hernandioides* (*PP* = 0.97 / *BP* = 71.9), which clade was connected with *B. chloroneura* (*PP* = 0.99 / *BP* = 78.3).

Chromosome Cytology

Somatic chromosomes at metaphase of *Begonia tandangii* were determined to be $2n = 28$ in the two plants (Fig. 6A), and those of *B. fenicis* were also determined to be $2n = 28$ in the three plants (Fig. 6B). The 28 chromosomes gradually varied in length from ca. 0.9 to 1.3 µm in *B. tandangii*, and from ca. 1.1 to 1.7 µm in *B. fenicis*. The centromere positions could not be determined due to the small size of the chromosomes. This result differs from a previous report of the chromosome number of *B. fenicis* ($2n = 26$; Oginuma & Peng 2002). Our study revealed that *B. tandangii* and *B. fenicis* had the same chromosome number and were karyologically indistinguishable.

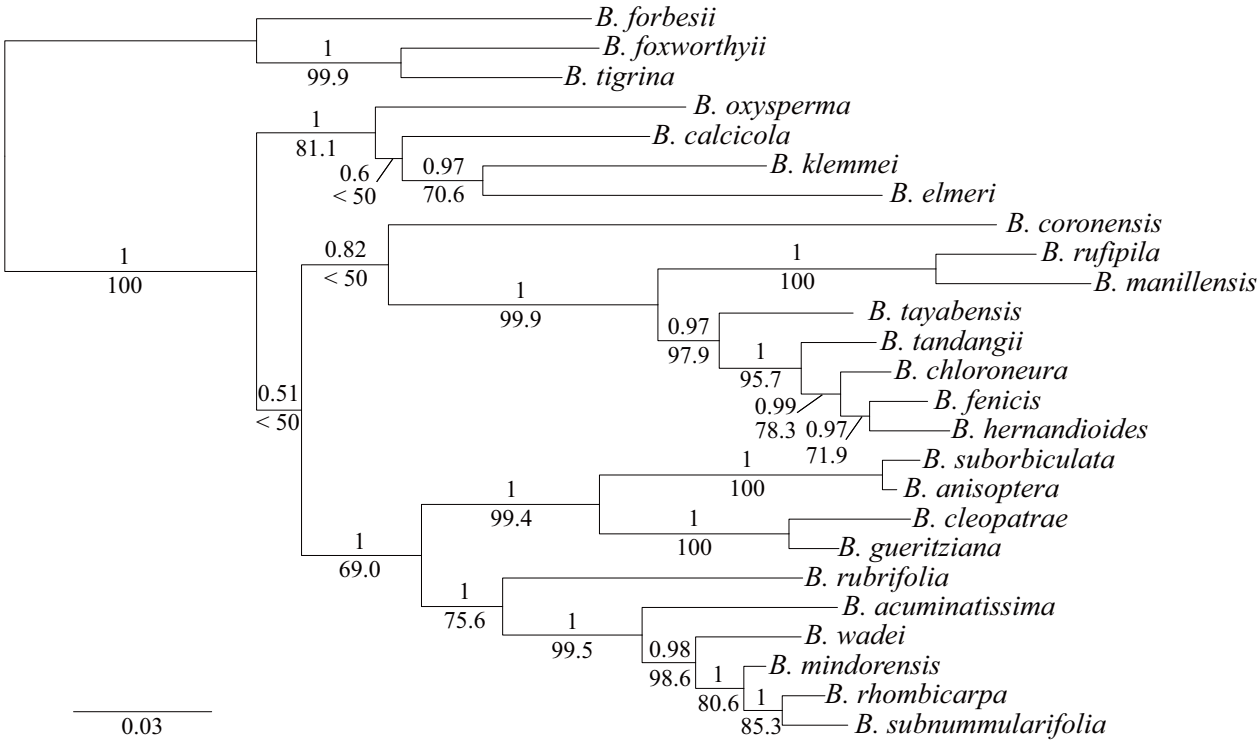

FIGURE 5. Bayesian majority-rule consensus tree with mean branch length based on ITS for *Begonia* sect. *Baryandra*. The numerals on branches are Bayesian posterior probabilities (*PP*: *upper*) and bootstrap percentages (*BP*: *lower*) in the MP analysis. The topology of the MP strict consensus tree (not shown) was the same as that of the Bayesian tree, except the three nodes which collapsed in the MP tree (*BP* < 50).

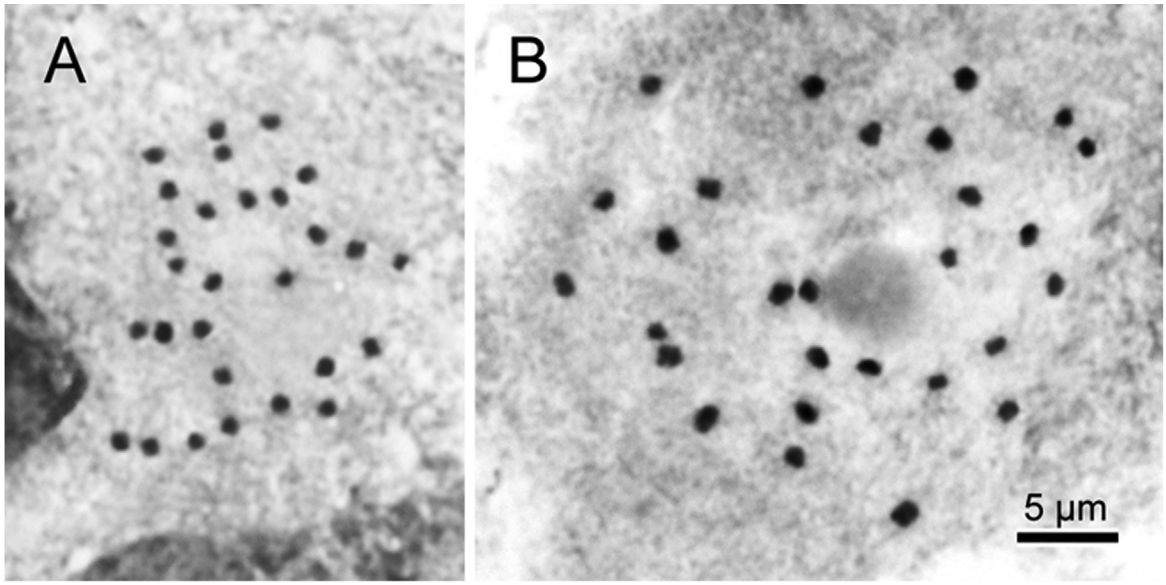

FIGURE 6. Somatic chromosomes at metaphase of *Begonia tandangii* (A, 2*n* = 28: *Ching-I Peng 23400*) and *B. fenicis* (B, 2*n* = 28: *Koh Nakamura 20100209*).

Discussion

The results of the morphological and molecular analyses support the taxonomic treatment of *Begonia tandangii* as a new species of sect. *Baryandra*. The phylogenetic analyses indicated that this new species is allied to the species found in Luzon Island (*B. chloroneura* in Isabela, *B. hernandioides* in Ilocos Norte, and

B. tayabensis in Laguna; Rubite 2010) and the northern islands (*B. fenicis* in Batan and Babuyan island groups of the Philippines, Lanyu and Lutao islands of Taiwan, and Yonaguni and Iriomote islands of Japan; Merrill 1908, Hatusima 1975, Chen 1993). *Begonia tandangii* and these four species share a noticeable morphological characteristic of five-tepalled pistillate flowers, while other species of sect. *Baryandra* have four-tepalled pistillate flowers (Hughes *et al*. 2010, Rubite *et al*. 2013).

Taxonomic Treatment

Begonia tandangii C.-I Peng & R.Rubite, *sp. nov.* (Fig. 2 & 3)

Begonia tandangii resembles *B. fenicis* in gross morphology, differing from the latter in having leaf margin sparsely fringed with minute hairs (vs. glabrous or with minute hairs only on teeth) and capsules with broadly-ovate outline, acuminate apex, and rounded base (vs. capsules with broadly-obovate outline, rounded to truncate apex, and rounded base).

Type:—PHILIPPINES. Luzon Island, Aurora Province, Baler, Barangay Zabali, elev. ca. 10 m, E121° 33' 1", N15° 45' 16", 27 October 2011, *Ching-I Peng 23400* (holotype HAST).

Monoecious perennial herbs, rhizomatous. Stems pink when young, green when mature, prostrate, to 1 cm in diameter, internodes 5–13 mm. Stipules pink to green, caducous, ovate to triangular, 1.2–2.5 × 0.8–1.2 cm, adaxially glabrous, abaxially sparsely puberulous with minute hairs, with a prominent keel, apex cuspidate. Leaves basal, alternate; petiole pink, 8.5–20 cm × 5 mm, erect, terete, succulent, glabrous to remotely puberulous; blade green, obliquely ovate to orbicular, 8.4–12.5 × 6.8–10.5 cm, adaxially glabrous, abaxially glabrous or sparsely puberulous with fine hairs, venation palmate, 8–10-veined, base oblique, cordate, basal lobes rounded, sinus narrow, apex mucronate to acuminate, margin with sparse minute hairs less than 1 mm long and irregularly denticulate, teeth small, apiculate. Inflorescences axillary, arising directly from rhizome, dichasial cymes; peduncle green, basally pink, 18–24 cm long, glabrous; bracts caducous, orbicular to oblate, 4–6 × 6–10 mm, glabrous, margin entire and often reflexed, apex obtuse to apiculate. Staminate flowers: pedicel ca. 1.5 cm, glabrous or sparsely puberulous with fine hairs; tepals 4, pinkish white, outer 2 obovate to orbicular, 10–13 × 8–12 mm, inner 2 oblanceolate to narrowly obovate, 7–10 × 3–5 mm; stamens ca. 30; filaments ca. 1.5 mm long, united at base; anthers broadly obovate, ca. 1.0 mm long, apex obtuse. Pistillate flowers: pedicel 1.5–2 cm, glabrous or sparsely puberulous with fine hairs; tepals 5, pinkish white, sometimes persistent when fruiting, oblanceolate to broadly obovate, outer pair orbicular 10–11 × 8 mm, inner three 10–11 × 5–8 mm; ovary green, glabrous, 3-loculed; placentae axile, bilamellate; styles 3, fused at base, ca. 3 mm long; stigmas 2-cleft, spiraled. Capsules nodding, brown, glabrous, 4–5 × 10–11 mm (excluding wings), broadly ovate in outline, apex acuminate, base rounded; unequally 3-winged; abaxial wing lunate, 3–5 × 10–11 mm; lateral wings lunate, 1–2 × 10–11 mm. Somatic chromosome number, $2n = 28$ (Fig. 6).

Distribution, habitat and ecology:—*Begonia tandangii* is currently known only from the type locality. The species grows on limestone rocks in semi-shaded hill of broadleaf forest at seashore and only a patch of a few square meters was observed. The species was flowering and fruiting when collected in late October. In cultivation in the greenhouse of Academia Sinica in Taipei, Taiwan, it flowered and fruited from July to December.

Etymology:—The species epithet is named after Mr. Danilo N. Tandang, Philippine National Herbarium, who guided us to the type locality.

Note:—*Begonia tandangii* resembles *B. fenicis* in gross morphology but they are distinguished basing on hairs on leaf margin and capsule shape, as described above. The ITS phylogeny clearly separated the two species. The two species are allopatric.

IUCN Red list category:—Vulnerable (VU D2). *Begonia tandangii* is known only from the type locality in Baler, Aurora Province. Although it is in the neighborhood of Aurora Memorial National Park, the type locality is not under any protection. Habitat disturbance brought about by timber harvesting and rapid development of Aurora Province may have a negative impact on the survival of the species.

Acknowledgements

We thank Mark Hughes for providing us ITS sequences used in his paper in press, Chien-I Huang for field assistance and Chiun-Jr Huang for assistance in field collection and molecular experiments, Chien-Yu Ke for the fine drawing, the curators and staff of the herbaria HAST and PNH for allowing us to use their specimens and plant databases, and our lab members for assistance in cultivation of plant materials in the experimental greenhouse. This study was supported in part by a postdoctoral fellowship from Academia Sinica, Taiwan to K.N. and research grants from National Science Council (NSC 198-2811-B-001-090-) and Academia Sinica to C.I P.

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Appendix 1. *Begonia fenicis* specimens examined for morphological comparison with *B. tandangii*.

PHILIPPINES. Babuyan Island: 17 June 1907, *E. Fénix, Bur. Sci. 3893* (PNH). Batan Island: Santo Domingo de Basco, 27 May 1907, *E.A. Mearns, Bur. Sci. 3207* (PNH); Santo Domingo de Basco, 30 May 1907, *E. Fénix, Bur. Sci. 3619* (PNH); Mt. Iraya, 17 December 2011, *Koh Nakamura 11969* (HAST); Mahatao, 18 December 2011, *Koh Nakamura 11773* (HAST); Diura, 18 December 2011, *Koh Nakamura 11720, 11725, 11754* (HAST). Sabtang Island: Sinakan, 19 December 2011, *Koh Nakamura 11968* (HAST). TAIWAN. Lanyu Island: Tienchi, 1 April 1985, *T. Y. Aleck Yang 1489* (HAST); Hongtou Village to Lungtouyen, 7 April 1987, *Ching-I Peng 10779* (HAST); subridge of Mt. Tasenshan, 7 April 1987, *Ching-I Peng 10794* (HAST); 2.0 km west of Lang Tao village, 13 March 1992, *T. G. Lammers 8529* (HAST); Bridge Chungaichiao, 23 April 1997, *T. Y. Aleck Yang 8032* (HAST); Langtao Village, 21 May 1998, *T. Y. Aleck Yang 10414* (HAST); entrance of hiking trail to Tienchih, 4 June 2001, *Ya-Yi Huang 473* (HAST); Hsiaotienchih, 5 June 2001, *Ya-Yi Huang 488* (HAST); Yujenhsi, 5 June 2001, *Ya-Yi Huang 508* (HAST); Hongtouhsi, 14 October 2001, *Wai-Chao Leong 2522* (HAST); Yunghsing Farm, 9 August 2002, *Wai-Chao Leong 3306* (HAST); entrance of Chungheng Road, 9 August 2002, *Wai-Chao Leong 3308* (HAST); near Chunchianyen, 9 August 2002, *Wai-Chao Leong 3311* (HAST); Langtao-Yunuyen, 9 August 2002, *Wai-Chao Leong 3312* (HAST); en route from Yehyuhsi, 11 August 2002, *Wai-Chao Leong 3326* (HAST); Yujenhsi, 12 August 2002, *Wai-Chao Leong 3331* (HAST); Yujenhsi, 12 August 2002, *Wai-Chao Leong 3336* (HAST); Longmen, 12 October 2010, *Koh Nakamura 20101300, 20101301, 20101303–20101306* (HAST); Shuangshiyan, 12 October 2010, *Koh Nakamura 20101279, 20101281, 20101282* (HAST); Wukongdong, 13 October 2010, *Koh Nakamura 20101470, 20101471, 20101475–20101477* (HAST); Zhongaiqiao, 13 October 2010, *Koh Nakamura 20101506, 20101507* (HAST); Zhonghenggonglu, 13 October 2010, *Koh Nakamura 20101365, 20101366* (HAST). Lutao Island: Tapaisha, 9 October 2001, *Ya-Yi Huang 803* (HAST); Kuanyintung to Haisenping, 9 October 2001, *Ya-Yi Huang 796* (HAST); 7.5 km road marker, 7 August 2002, *Wai-Chao Leong 3293* (HAST); Tapaisha, 7 August 2002, *Wai-Chao Leong 3282* (HAST); Dabaisha, 14 October 2010, *Koh Nakamura 20101504* (HAST); Niutoushan, 15 October 2010, *Koh Nakamura 20101491, 20101497* (HAST); Fudidongtian, 15 October 2010, *Koh Nakamura 20101487* (HAST). JAPAN. Iriomote Island: Shirahama, 22 March 2010, *Koh Nakamura 20100159, 20100160* (HAST); Hinai River, 23 March 2010, *Koh Nakamura 20100196, 20100207–20100209* (HAST). Yonaguni Island: Mt. Kubura, 24 March 2010, *Ching-I Peng 18366* (HAST).

Appendix 2. *Begonia* species included in the molecular phylogenetic analyses. GenBank accession numbers of the ITS sequences are shown.

Sect. *Baryandra*—*B. acuminatissima* Merr., JX656721 (as *B. camiguinensis* Elmer); *B. anisoptera* Merrill, X656720; *B. calcicola* Merrill, JX656708; *B. chloroneura* P.Wilkie & Sands, AF485134; *B. cleopatrae* C.Coyle, AF485133; *B. coronensis* Merrill, JX656715; *B. elmeri* Merrill, JX656714; *B. fenicis* Merrill, JX678218; *B. gueritziana* Gibbs, JX678217; *B. hernandioides* Merrill, JX656707; *B. klemmei* Merrill, JX656709; *B. manillensis* A. de Candolle, JX656713; *B. mindorensis* Merrill, JX656717; *B. oxysperma* A. de Candolle, JX656710; *B. rhombicarpa* A. de Candolle, JX656719; *B. rubrifolia* Merrill, JX656711; *B. rufipila* Merrill, JX656712; *B. subnummularifolia* Merrill, JX656722; *B. suborbiculata* Merrill, JX656716; *B. tandangii* C.-I Peng & R.Rubite, AB828324; *B. tayabensis* Merrill, JX656718; *B. wadei* Merrill & E.Quisumbing, JX656706.
Sect. *Reichenheimea* (out groups)—*B. forbesii* King, JX656704; *B. foxworthyii* Burkill & Ridley, JX656702; *B. tigrina* Kiew, JX656703.