Begonia difformis comb. & stat. nov. (Sect. Platycentrum, Begoniaceae), a new species segregated from B. palmata D. Don

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Abstract

Begonia palmata D. Don is one of the most widely distributed and morphologically variable species of Asian Begoniaceae. Examinations of its morphological variation indicate that two of its seven varieties, B. palmata var. difformis and B. palmata var. crassisetulosa, both distributed in the Gaoligong Mountain areas of Yunnan, China are indistinguishable and yet distinct from other varieties. Phylogenetic analyses using ITS DNA sequences further reveals that samples identifiable to these two varieties are also distantly related to samples of typical B. palmata. Based on these observations, we combine and elevate these two varieties to the status of species, Begonia difformis (Irmsch.) W.C. Leong, C.I Peng & K.F Chung, comb. & stat. nov..

Key Words: Begonia palmata var. crassisetulosa, flora of China, ITS, Yunnan

Introduction

Begonia Linnaeus (1753: 1056), comprising more than 1,700 species (Doorenbos et al. 1998, Golding & Wasshausen 2002, Hughes & Pullan 2007, Chung et al. 2014), is the 6th largest genus of flowering plants (Frodin, 2004). With relative few exceptions, most species of the genus are micro-endemic with restricted distribution (Kiew 2001, Tebbitt 2005, Chung et al. 2014). In Asia, Begonia palmata D. Don (1825: 223) is one of the most widespread Begonia species (Gu et al. 2007), occurring in forest understorey from 100 to 3200 m in central to southern China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, Jiangxi, Sichuan, Xizang, Yunnan), Taiwan, South Asia (Bangladesh, Bhutan, NE India, Nepal, and Sikkim), and Indochina (Myanmar, Laos, Thailand, and Vietnam). Begonia palmata is highly variable and seven varieties are recognized by Golding & Wasshausen (2002). The autonym Begonia palmata var. palmata is distributed in Yunnan (China), Himalayas, and northern Indochina. Begonia palmata var. bowringiana (Champ. ex Bentham 1852: 120–121) Golding & Karegeannes (1984: 494) is widespread in southern and central China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hunan, Jiangxi, Sichuan, Xizang, and Yunnan) and Taiwan. In the Flora of China (Gu et al. 2007), var. bowringiana was expanded to include B. palmata var. principalis (Irmscher 1939: 530) Golding & Karegeannes (1984: 495) that is distributed in Yunnan and Myanmar (Irmscher 1939). Begonia palmata var. difformis (Irmscher 1939: 531) Golding & Karegeannes (1984: 495) and B. palmata var. crassisetulosa (Irmscher 1939: 532) Golding & Karegeannes (1984: 495) are both distributed in Gaoligong Mountains, western Yunnan, China. Begonia palmata var. khasiana (Irmscher 1939: 529) Golding & Karegeannes (1984: 495) is found in the Himalayas. Begonia palmata var. laevifolia (Irmscher 1951: 43–44) Golding & Karegeannes (1984: 495) is only known from Hekou, southeastern Yunnan. These varieties are very similar on gross morphology, differed from one another mainly by the indumentum on stems, leaves, and flowers (Irmscher 1839, Gu et al. 2007); however, phylogenetic relationship among these varieties of B. palmata remains to be explored.

Among these varieties, B. palmata var. difformis and var. crassisetulosa are morphologically distinct in possessing red-minute setae on abaxial side of the tepals, while other varieties bearing brownish villous to tomentose hairs (Table 1). During our ongoing studies of Asian Begonia taxonomy and phylogenetics, field observation, greenhouse transplantation and specimen examination suggest that B. palmata var. crassisetulosa and var. difformis are hardly
distinguishable and should be combined. Molecular phylogenetic analysis further reveals that samples identifiable as *B. palmata* var. *difformis* (and var. *crassisetulosa*) formed a clade distantly related to samples attributable to other varieties of *B. palmata*. Based on these analyses, we combine the two varieties and elevate them to the status of species.

**TABLE 1.** Comparison of *Begonia difformis* and *B. palmata*.

<table>
<thead>
<tr>
<th>Character/Species</th>
<th><em>B. difformis</em></th>
<th><em>B. palmata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blade lobes</td>
<td>Usually divided to more than 1/3 of the leaf blade</td>
<td>Usually shallowly divided to less than 1/3 of the leaf blade</td>
</tr>
<tr>
<td>Blade indumentum</td>
<td>Adaxially hispidulous along veins</td>
<td>Adaxially hirsute, hispidulous</td>
</tr>
<tr>
<td>Outer tepals of staminate flower</td>
<td>Abaxially setulose</td>
<td>Abaxially villous or tomentose</td>
</tr>
<tr>
<td>Outer tepals of pistillate flower</td>
<td>Abaxially setulose of nearly glabrous</td>
<td>Abaxially villous or tomentose</td>
</tr>
<tr>
<td>Immature ovary</td>
<td>Setulose to nearly glabrous</td>
<td>Villous or tomentose to nearly glabrous</td>
</tr>
</tbody>
</table>

**Material and Methods**

**Morphology**

All plants studied were cultivated in the experimental greenhouse of Academia Sinica, Taipei for morphological observation and molecular analyses. Herbarium specimens (A, E, HAST, and MO) have also been examined for morphological comparison.

**Phylogenetic analyses**

To test the monophyly of *Begonia palmata*, DNA sequences of the nuclear ribosomal internal transcribed spacer (ITS) of the 26 species in Clade PLA-SPH of Chung et al. (2014) were adopted. Nine samples of *B. palmata*, including four unambiguously identifiable to *Begonia palmata* var. *difformis* or var. *crassisetulosa* collected from Gaoligong Mountains, Yunnan, China were newly sequenced for ITS sequences. The 26 samples in Clade PLA-SPH include species of China, Taiwan, Himalayas, Indochina, Peninsular Malaysia, and Java (Table 2). Three tuberous deciduous species, *B. chingii* Irmscher (1939: 519), *B. fimbristipula* Hance (1883: 202), and *B. ravenii* Peng & Chen (1988: 217), were chosen as outgroup taxa for they were placed in Grade DIP I sister to Clade PLA-SPH clade (Chung et al. 2014). The experimental procedures of DNA extraction, PCR amplification, and DNA sequencing described in Chung et al. (2014) were followed.

Multiple DNA sequence alignment was conducted by MUSCLE implemented in MEGA5.2 (Tamura et al., 2011) with subsequently manual adjustments. Phylogenetic relationships were reconstructed based on Bayesian MCMC inference. The best nucleotide substitution models were determined by MrModeltest (Nylander, 2004). The model GTR+I+G was selected. For Bayesian MCMC inference, the consensus topology with posterior clade probability of each node was obtained by MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003) based on parameters setting as follows: random starting tree, 10,000,000 generation runs with sampling occurring every 1000 generations. Bayesian clade posterior probabilities and average branch lengths were calculated based on the sampled trees after the first 25% of the sampled trees were discarded as burn-in.

**TABLE 2.** Materials, voucher information and GenBank accession numbers.

<table>
<thead>
<tr>
<th>Species</th>
<th>Section</th>
<th>Distribution</th>
<th>collector</th>
<th>GenBank Accession No.</th>
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<td><em>Begonia acetosella</em> Craib</td>
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<td>Platycenrum (PLA)</td>
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TABLE 1. (Continued)

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<th>GenBank Accession No.</th>
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<td>Peng 20288 (HAST)</td>
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<td>Java</td>
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<td>Malaysia</td>
<td>Peng 20239 (HAST)</td>
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<td>China, Guizhou</td>
<td>Peng 18779 (HAST)</td>
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<td>Taiwan</td>
<td>Peng 14835 (HAST)</td>
<td>KF636479</td>
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<td>Begonia robusta Blume</td>
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<td>Java</td>
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<tr>
<td>Begonia ruboides C. M. Hu ex C. Y. Wu &amp; T. C. Ku</td>
<td>Diploclinium (DIP)</td>
<td>China, Yunnan</td>
<td>Peng 18705 (HAST)</td>
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<td>Begonia sikkimensis A. DC.</td>
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<td>China, Tibet</td>
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<td>Begonia xanthina Hook.</td>
<td>Platycentrum (PLA)</td>
<td>Himalayas</td>
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</table>

Results and Discussion

Morphology

Begonia difformis resembles B. palmata in being hairy and palmatifid leaf, differing by the leaf shape and the indumentum on leaves and flowers. Begonia difformis has palmate leaves usually divided to more than 1/3 of total leaf length, whereas B. palmata possesses shallowly lobed leaf blades which are usually divided to less than 1/3 of total length. While adaxial surfaces of leaf blades of B. difformis are hispidulous only along veins, B. palmata is hisrute or hispidulous on the whole surfaces. Compared to the villous or tomentose flower of B. palmata, B. difformis has red-minute setae flowers. Detail comparison of B. difformis and B. palmata is provided in Table 1.

Irmscher (1939) suggested B. palmata var. crassisetulosa and var. difformis are both having sparsely-hairy leaves but tepals of the former variety has thick bristles (setae) to 2 mm long, in which the later one has no such indumentum. In our observation, the tepal indumentum can be very sparse (sometimes nearly glabrous) and could be difficult to observe on dry specimen. Density of tepal indumentum can be different between flowers within the same individual.

Phylogenetic Analyses

The aligned data matrix included 728 aligned positions. The Bayesian 50% majority rule consensus tree with mean branch length and posterior probability (PP) is depicted in Fig. 1. Samples identified to Begonia palmata (PP: 100) and B. difformis (PP: 100) were grouped into two highly supported monophyletic groups distantly related to one another. The clade composed of Begonia difformis was nested within a clade with six species from China, five species from Himalayas and two species share by the two regions (PP: 82), whereas the B. palmata clade resided in another clade with two species from Java and 3 species from Peninsular Malaysia (PP: 100). This result strongly supports that B. difformis is a distinct species.

Taxonomic treatment

Begonia difformis (Irmsch.) W.C. Leong, C.I Peng & K.F Chung, comb. & stat. nov. (Fig. 1)

Basionym:—Begonia laciniata Roxb. subsp. difformis Irmsch. (1939: 531).
Lectotype (verified by Irmscher 1956/designated here)—CHINA. Yunnan, September 1912, G. Forrest 16098 (E!), Shweli-Salwin Divide, Sep. 1924, G. Forrest 25207 (paratype, E!). See Note.

Evergreen herbs, 20–80 cm tall. Rhizomes elongate, to 2 cm in diam.; aerial stems erect, hirsute when young. Leaves bearing on rhizomes and aerial stems; stipules caducous, narrowly ovate or ovate, to 3 cm long, 2 cm wide, margin minutely dentate, apex cuspidate; petioles 10–28 cm long, hirsute or sparsely so; blade asymmetric, ovate or oblate- orbicular, 7–17 cm × 5–11 cm, adaxial surface hirsutulous along veins, abaxial surface hirsute, base oblique, slightly cordate or cordate, margin remotely and shallowly denticate, usually divided to 1/3 of the blade or more, lobes 3–7(–9), subtriangular, 2.5–5 cm long, apex acuminate to long-acuminate; venation palmate, 5–10-nerved. Inflorescences tomentose; bracts caducous, ovate, 1–1.5 × 0.5–0.8 cm, abaxial surface sparingly hirsutulous to nearly glabrous, apex acute, margin ciliate. Stamine flowers: Pedicels 1–2 cm long; tepals 4, white or pink, outer two obovate to orbicular, 1.2–2.3 × 0.8–2.0 cm, abaxial surface red-setulose or sparsely so, inner two oblanceolate to narrowly obovate, 0.7–2.3 × 0.6–1.0 cm, glabrous; stamens ca. 80–200; filaments 0.8–2.4 mm long; anthers narrowly obovate, 1.9–2.3 mm long, acute or round at apex. Pistillate flowers: Pedicels 1–2 cm long; tepals 5, unequal, oblanceolate to orbicular, the largest 1.1–2.4 × 0.8–2.1 cm, abaxially red-setulose or nearly glabrous, the smallest 0.8–2.1 × 0.4–0.9 cm wide; ovary green, red-setulose or nearly glabrous, 2-locular; placentae axile, bilamellate; styles fused at base; stigmas 2-cleft, spiralled. Capsule nodding, obovoid, 1.5–1.7 × 0.8–1.0 cm wide, unequally 3-winged, abaxial wing obliquely oblong or narrowly so, 1.2–2.9 cm long, lateral wings much shorter.

**FIGURE 2.** Bayesian 50% majority rule consensus tree base on nrITS DNA sequence. Numbers adjacent to the branches are posterior probabilities.
**Distribution & habitat:** — *Begonia difformis* occurs at the elevation at 1500–3200 m in western and southwestern Yunnan (Fig. 3), commonly found on sandstone rock crevices of the moist environments by streams or on mountain slopes under evergreen broadleaved forests or coniferous forests.

**Phenology:** — Flowering June–October; Fruiting August–November.

**Chinese name:** 刺毛紅孩兒.

**Note:** — Both *Begonia laciniata* subsp. *difformis* and subsp. *crassisetulosa* were both described without types (Irmscher 1939). In the Global Plant database of JSTOR, *Forrest 8673* (E), which bears a *determinavit* slip with Irmscher’s identification in 1956, was noted as the ‘Type’ of *B. laciniata* var. *crassisetulosa* (‘Verified by E.Irmscher, 1956/7/1’; http://plants.jstor.org/stable/10.5555/al.ap.specimen.e00299206). Irmscher’s *determinavit* slips of *B. laciniata* subsp. *difformis* were also seen on two specimens at E: *Forrest 16098* (‘det. E. Irmscher 1958’) and *Forrest 25207* (‘det. E. Irmscher 1956’). *Forrest 16098* was noted as ‘Lectotype’ of *B. laciniata* subsp. *difformis* (‘Verified by E. Irmscher, 1956/7/1’; http://plants.jstor.org/stable/10.5555/al.ap.specimen.e00299196), while *Forrest 25207* as ‘Syntype’ (‘Verified by E.Irmscher, 1956/07/01’; http://plants.jstor.org/stable/10.5555/al.ap.specimen.e00299194). Because both taxa were described by Irmscher, his verifications as noted in JSTOR are followed.

**FIGURE 3.** Distribution map of *Begonia difformis*.

**Additional selected specimen examined:** — CHINA. Yunnan: Baoshan, Gangdang, Baihualing, 2000 m, 10 August 2011, *Peng 23252* (HAST), *Peng 23253* (HAST); Baoshan, Longling, Zhenan Zhen, Lishuzhai, on the east side of Gaoligong Shan, 24°48′09″ N, 98°47′41″ E, 1930 m, 21 October 1998, *Li 10756* (HAST); Baoshan, Longling, Zhengan Zhen, Xiaoshui He, on the west side of Gaoligong Shan, 24°50′16″ N, 98°45′53″ E, 2170 m, 22 October 1998, *Li 10782* (HAST); Baoshan, Longling, Zhengan Zhen, Vicinity of Hupa village, east side of Gaoligong Shan, 24°48′48″ N, 98°49′58″ E, 1530 m, 23 August 2003, *Gaoligong Shan Biodiversity Survey 17621* (HAST); Baoshan, Longling, Lujiang, Vicinity of Nankang village, east side of Gaoligong Shan, 1908 m, 24°48′47″ N, 98°46′59″ E, 25 August 2003, *Gaoligong Shan Biodiversity Survey 17820* (HAST); Baoshan, Longling, Mangkuan, Baihualing Cun, east side of Gaoligong Shan, 25°18′00″ N, 98°46′51″ E, 2220 m, 8 September 2003, *Gaoligong Shan Biodiversity Survey 18866* (HAST); Baoshan, Longling, Mangkuan, Vicinity of Hanlong, Baihualing Cun, east side of the Gaoligong Shan, 25°17′44″ N, 98°48′09″ E, 1777 m, 2 June 2005, *Gaoligong Shan Biodiversity Survey 25364* (HAST); Baoshan,
Longling, Sanyun, Qiaojie Cun, in vicinity of Henghe village, west side of Gaoligong Shan, 24°59′38″ N, 98°43′55″ E, 1980 m, 1 September 2003, *Gaoligong Shan Biodiversity Survey 18323* (HAST); Baoshan, Tengchong, 1550 m, 6 August 2011, *Peng 23216* (HAST); Baoshan, Tengchong, Sanyun, Chuanlong Cun, on the west side of Gaoligong Shan, 25°00′13″ N, 98°43′29″ E, 1740 m, 4 November 1998, *Li 11477* (HAST); Chih-tse-lo, 2500 m, 7 September 1933, *Tsai 54180* (A), 3200 m, 11 September 1934, *Tsai 58473* (A), *Tsai 58488* (A); Dehong, Yingjiang, Xima, Qincaidagou, 24°44′44″ N, 97°44′27″ E, 1730 m, 3 November 2002, *Peng 19152* (HAST); Lincang, Fengxiang Zhen, 23°53′09″ N, 100°10′28″ E, 2100 m, 7 November 2002, *Peng 19192* (HAST); Nujiang, Fugong, Lishadi, Mi’elu Cun, east side of Gaoligong Shan, 27°15′56″ N, 98°52′14″ E, 1500 m, 12 August 2005, *Gaoligong Shan Biodiversity Survey 27354* (HAST); Nujiang, Fugong, Lishadi, Zigudu Cun, east side of Gaoligong Shan, 27°11′45″ N, 98°52′57″ E, 1610 m, 14 August 2005, *Gaoligong Shan Biodiversity Survey 27331* (HAST); Nujiang, Fugong, Maji, along the west side of the Nujiang River, east side of Gaoligong Shan, 27°17′40″ N, 98°52′18″ E, 1320 m, 19 August 2005, *Gaoligong Shan Biodiversity Survey 27882* (HAST); Nujiang, Fugong, Lumadeng, west side of the Nujiang River, 27°05′56″ N, 98°52′21″ E, 1225 m, 9 May 2004, *Gaoligong Shan Biodiversity Survey 19739* (HAST); Nujiang, Fugong, Lumadeng, Yaduo Cun, east side of Gaoligong Shan, 27°07′48″ N, 98°52′40″ E, 1090 m, 22 August 2005, *Gaoligong Shan Biodiversity Survey 28901* (HAST); Nujiang, Fugong, Yueliangshi, 1600 m, 13 August, 2011, *Peng 23278* (HAST); Nujiang, Gongshan, 27°48′05″ N, 98°34′07″ E, 1900 m, 18 September 1997, *Li 9240* (MO), *Li 9241* (MO); 1800-2000 m, 19 September 1997, *Li 9276* (MO); Nujiang, Fugong, 26°54′ N, 98°51′ E, 1600-1700 m, 30 September 1997, *Li 9784* (MO); Nujiang, Gongshan, Cikai Zhen, east side of the Gaoligong Shan, 1600 m, 20 July 2000, *Li 12974* (HAST); Nujiang, Gongshan, Dulongjiang, on the east side of the Dulong Jiang River, 27°42′40″ N, 98°20′50″ E, 1460 m, 30 October 2004, *Gaoligong Shan Biodiversity Survey 21127* (HAST); Nujiang, Gongshan, Dulongjiang, on the west side of Gaoligong Shan, 2340 m, 27°43′53″ N, 98°24′10″ E, 2 November 2004, *Gaoligong Shan Biodiversity Survey 22113* (HAST); Nujiang, Gongshan, Cikai, Vicinity of Yimaluo Cun, east side of Gaoligong Shan, 27°39′28.5″ N, 98°42′41.5″ E, 1520 m, 14 August 2006, *Gaoligong Shan Biodiversity Survey 33333* (HAST); Shang-pa Hsien, 2100 m, 25 September 1933, *Tsai 56624* (A); Shang-pa Hsien, 1600 m, 2 October 1933, *Tsai 54748* (A), 2200 m, 17 September 1933, *Tsai 54261* (A), 2800 m, 10 October 1934, *Tsai 58684* (A), 2000 m, 30 October 1934, *Tsai 59105* (A).

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