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Begonia blancii (sect. *Diploclinium*, Begoniaceae), a new species endemic to the Philippine island of Palawan

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ABSTRACT. A new species of *Begonia* in sect. *Diploclinium*, *B. blancii* M. Hughes & C.-I Peng, is described from the Bulalakaw Falls area in Palawan. A somatic chromosome number of $2n = 30$ was determined. *Begonia blancii* is allied to *B. suborbiculata* and other 2-locular species in *Begonia* sect. *Diploclinium*, but is very distinct in having triangular-spathulate leaves. It shares clustered stomata with a number of other *Begonia* species, which are likely to help it reduce water loss through transpiration during the dry season. Its IUCN status is considered to be Least Concern.

Keywords: *Begonia blancii*; *Begonia gueritziana*; *Begonia suborbiculata*; Chromosome number; Leaf anatomy; New species; Palawan; Philippines; Stomata.

INTRODUCTION

The *Begonia* of Palawan have recently been revised (Hughes and Coyle, 2009a; Hughes et al., 2010). However, given the narrow endemism prevalent in *Begonia* and the relatively low collection density of Palawan, recent exploration has brought a very distinct new species, *B. blancii* M. Hughes & C.-I Peng, to light, which is described below, bringing the total number of species known from the island to 14. It belongs to *Begonia* sect. *Diploclinium* and is allied to other Malesian species in that section with 2-locular fruit and often fleshy leaves (*B. acclivis* M. Hughes, *B. anisoptera* Merr., *B. cleopatrae* M. Hughes, *B. gueritziana* Gibbs, *B. suborbiculata* Merr. and *B. wilkiei* M. Hughes). However, it is immediately distinct from its allies in having triangular-spathulate leaves. All these species are endemic to Palawan with the exception of *B. anisoptera*, which is only found in Zamboanga, Mindanao, and *B. gueritziana*, which is endemic to Borneo. The new species is named after Patrick Blanc, who first brought this interesting plant to our attention.

MATERIALS AND METHODS

Cryo scanning electron microscopy

Fresh leaves of *Begonia blancii* and allied species, *B. gueritziana* and *B. suborbiculata*, were dissected and attached to a stub. The samples were frozen with liquid nitrogen slush then transferred to a sample preparation chamber at -160°C. After 5 min, when the temperature rose to -130°C, the samples were fractured. The samples were etched for 10 min at -85°C. After coating at -130°C, the samples were transferred to the SEM chamber and observed at -160°C with a cryo scanning electron microscope (FEI Quanta 200 SEM/Quorum Cryo System PP2000TR FEI) in Academia Sinica. Voucher specimens (*Begonia blancii*: *Peng et al. 22545*; *B. gueritziana*: *Peng et al. 21976*; *B. suborbiculata*: *Rubite 325*) are deposited at HAST.

Chromosome preparation

Root tips of *Begonia blancii*, *B. gueritziana* and *B. suborbiculata* were pretreated with 2 mM 8-hydroxyquinoline solution at 15-18°C for about 8 h and fixed overnight in ethanol-acetic acid (3:1) below 4°C. They were macerated in an enzyme mixture containing 2% Cellulase Onozuka R-10 (Yakult Honsha, Tokyo, Japan) and 1% Pectolyase (Sigma, St. Louis, MO, USA) at about 37℃ for 1 h. Chromosomes were stained with a 2% Giemsa solution (Merck, Darmstadt, Germany). Classification of the chromosome complements based on centromere position at mitotic metaphase follows Levan et al. (1964). Voucher specimens (*B. blancii*: *Peng et al. 22545*, *B. gueritziana*: *Peng et al. 21976* and *B. suborbiculata*: *Rubite 325*) are deposited in HAST.

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NEW SPECIES

Begonia blancii M. Hughes & C.-I Peng, sp. nov. – TYPE: Philippines. Palawan, en route to Bulalakaw Falls, about 25 km east of El Nido, 300-400 m, *Patrick Blanc & Rosario Rivera Rubite 09-201* (holotype: PNH; isotype: E, SING). 匙葉秋海棠 Figure 1

Species nova Begoniae suborbiculatae fructu quinquealato et biloculari habenti similis sed foliis spathulatis ad substratum adpressis differt.

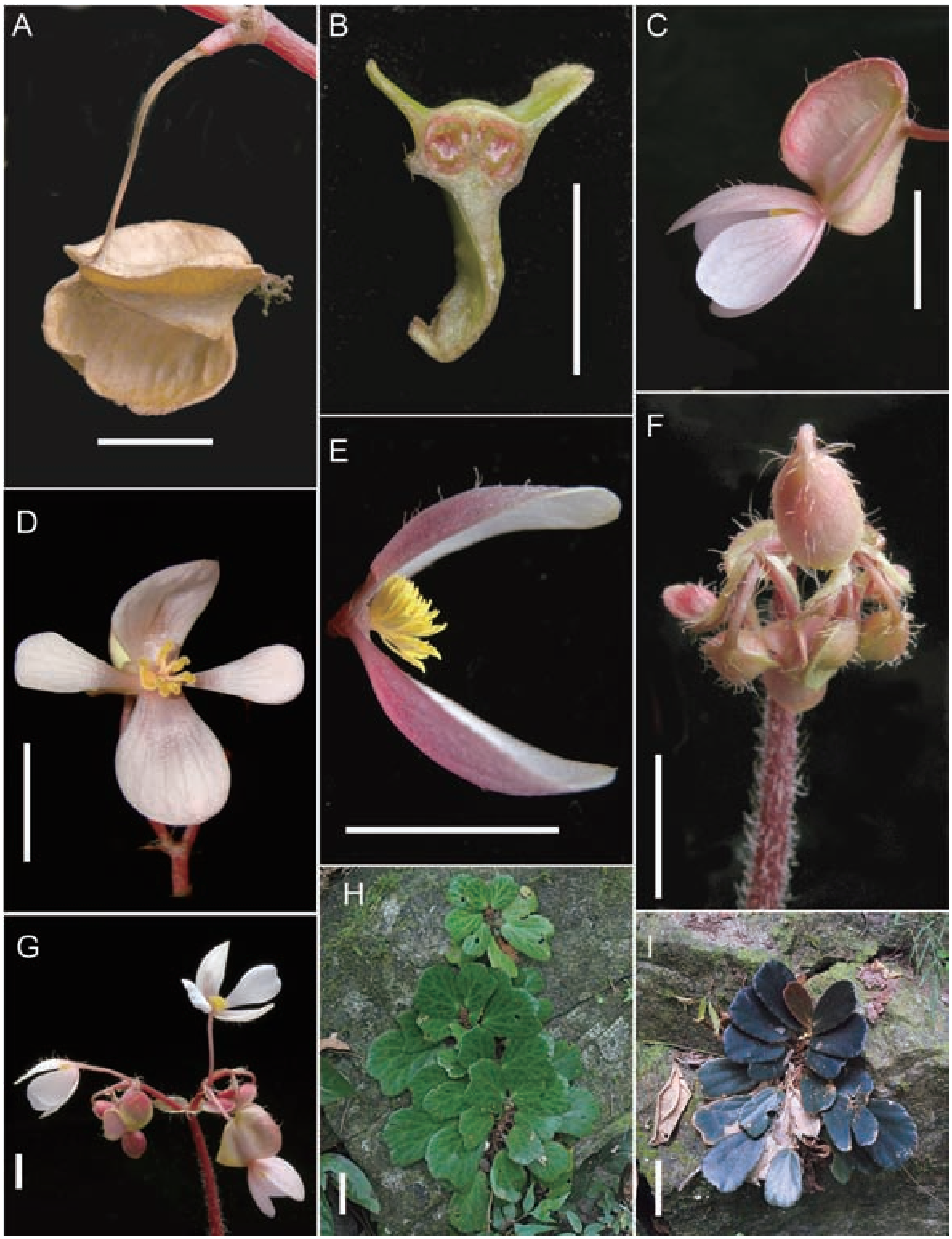

Figure 1. *Begonia blancii* M. Hughes & C.-I Peng. A, ripe fruit showing cucullate lower wing and vestigial wings below the splash cup; B, transverse section of developing fruit showing 2 locules and bifid placentae; C, opening female flower; D, female flower, showing 3 stigmas; E, side view of male flower and androecium, lateral tepals removed for clarity; F, young inflorescence; G, inflorescence. H, I, plants growing *in situ*. Scale bars for A-G are 1 cm, for H-I are 5 cm.

Lithophytic creeping herb. *Stem* succulent, repent, ca. 5-9 mm diameter, with long pale hairs up to 5 mm long, internodes up to 1 cm apart. Stipules triangular, ca. 15 × 12 mm, slightly keeled, with 2 mm long hairs along the keel and scattered on the abaxial surface, tip extended, apex fimbriate, persistent, becoming recurved and papery with age. *Leaves* appressed to the substrate when growing vertically, more erect when growing on more horizontal substrate; petiole ca. 1-2 cm on young leaves, extending to around 5-7 cm as the leaf ages reducing the overlap in the leaf mosaic; lamina distinctly fleshy, in various colour forms such as green mottled with darker green, solid dark green with slightly paler veins, or uniform blackish green, basifixed, ob-triangular spathulate, asymmetric, base minutely cordate, sinus ca. 1 mm deep, basal lobe on one side extending to ca. 5 mm, 6-11 × 4-8 cm, upper surface glabrous, underside hairy on the veins, margin entire, slightly wavy, with short hairs, apex broad, truncate or with shallow rounded lobes. *Inflorescence* up to 20 cm long, cymose, with around 10 flowers, terminal flower pairs consisting of one male and one female, male and female flowers open at the same time; primary peduncle up to 15 cm long, ca. 4 mm in diameter, with scattered long pale hairs, secondary and tertiary peduncles 10-30 mm long, with sparser hairs concentrated near to branching points; bracts triangular-lanceolate, ca. 13 × 6 mm at the first dichotomy and becoming smaller higher up, glabrous or with a small number of hairs, fimbriate. *Male flower*: pedicels ca. 15-20 mm long, glabrous or with a few hairs; tepals 4, outer 2 ovate, pink on the reverse with scattered hairs, otherwise white or very pale pink and glabrous, ca. 18 × 10 mm, inner 2 obovate to spathulate, tips rounded-truncate to slightly retuse, white, glabrous, 15 × 7 mm; androecium pale yellow, asymmetric, with around 50 stamens; filaments free, unequal, 1.75 mm long on the lower stamens reducing to 1 mm on the upper; anthers 1.75 mm long, oval-oblong, dehiscing through slits running almost the full length of the anther, slits lateral at the base tending to unifacial at the tip, connective extended, rounded. *Female flower*: pedicels ca. 15-20 mm long, glabrous or with 1 or 2 hairs; ovary 15 × 15 mm overall, with 3-5 wings, uppermost wing largest, rounded, 10 × 15 mm, markedly cucullate, two lowest wings 5 × 12, sometimes with 2 more very reduced wings running parallel along the side of the capsule; capsule ca. 10 × 5 mm, 2-locular, placentae bifid; tepals 4, outer 2 ovate, pink on the reverse with a few hairs, otherwise white or very pale pink and glabrous, ca. 18 × 10 mm, inner 2 obovate to spathulate, tips rounded-truncate to slightly retuse, pale pink, glabrous, 15 × 7 mm; styles 3, golden yellow, bifid, 4 mm long, stigmatic surface once spiralled. *Fruit* the same shape and size as the ovary or very slightly larger, recurved so the largest wing is lowermost, drying pale brown, dehiscing through the attachment of the two uppermost wings which form a splash cup.

Distribution and ecology. Known only from the type locality. Grows on the sides of small (up to ca. 1 m high) boulders in the shade of primary forest. The rocks are almost bare of soil and moss, with the plant roots growing directly on the surface. The fleshy leaves are persistent during the dry season.The petiole extends with increasing leaf age which reduces leaf overlap on the vertical

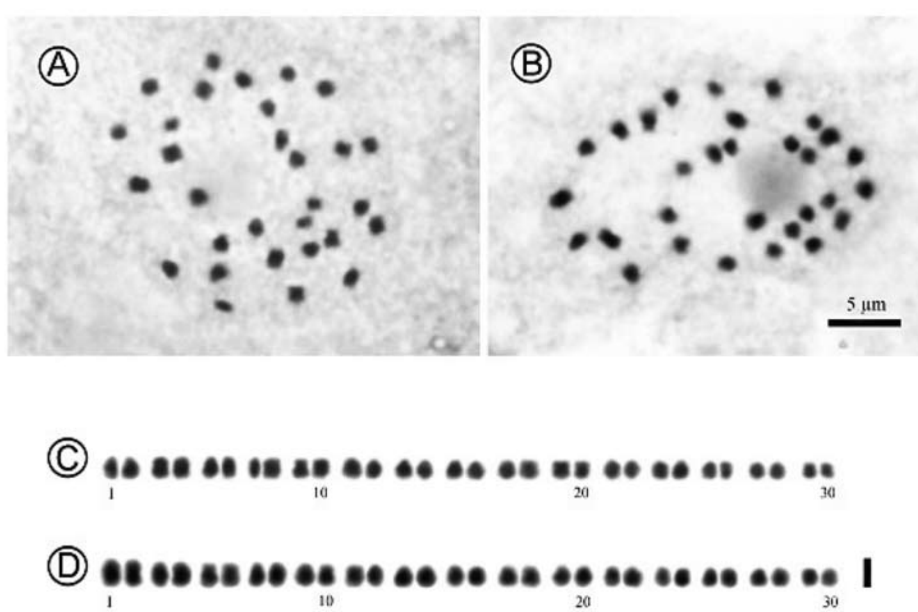

Figure 2. Somatic chromosomes at metaphase of *Begonia blancii* and *B. suborbiculata*. A and B, micrographs; A, *Begonia blancii* (2*n* = 30: *Peng et al. 22545*); B, *B. suborbiculata* (2*n* = 30: *Rubite 325*); C and D, somatic chromosomes serially arranged by chromosome length and centromere position; C, *B. blancii* (2*n* = 30: *Peng et al. 22545*); D, *B. suborbiculata* (2*n* = 30: *Rubite 325*). Scale bars for A and B are 5 μm, for C and D are 2 μm.

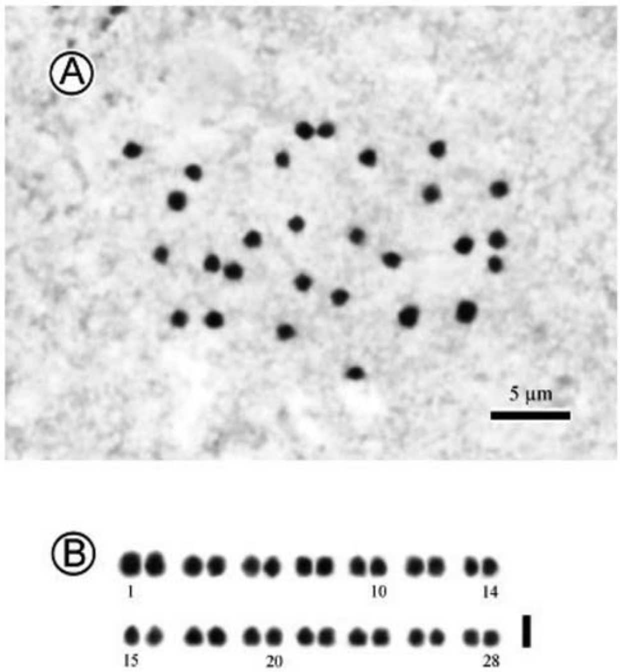

Figure 3. Somatic chromosomes at metaphase of *Begonia gueritziana* ($2n = 28$: *Peng et al. 21976*). A, micrograph; B, somatic chromosomes serially arranged by chromosome length and centromere position. Scale bars for A is 5 µm, for B is 2 µm.

substrate. This phenomenon has also been observed in the allied *Begonia suborbiculata* from Palawan (Rubite, pers. obs.), *B. kingiana* from Malaysia (Blanc, 2003) and *B. pasamanensis* from Sumatra (Hughes et al., 2009b).

IUCN category. The species is endemic to the El Nido-Taytay Managed Resource Protected Area, and although at the moment it is only known from the type collection site there are likely to be other populations in the as yet unexplored neighbouring forests. The species is 'quite common' at the type locality, which is lightly disturbed primary forest. Hence we consider this species to belong to the Least Concern IUCN category in recognition of the current successful management of the El Nido-Taytay area. Any increased degredation of the forest habitat will instantly lead to this species becoming liable to listing under the Vulnerable category.

Chromosome cytology. Somatic chromosome numbers of the three species, *Begonia blancii* and *B. suborbiculata* from Philippines, and *B. gueritziana* from Borneo, are reported here for the first time. Somatic chromosomes at mitotic metaphase of *Begonia blancii* were determined to be $2n = 30$ (Figure 2A and 2C). The 30 chromosomes gradually varied from ca. 1.0 to 1.6 µm long. The centromere positions of most chromosomes could not be determined. However, several longer chromosomes were metacentric. Satellites were not observed.

Somatic chromosomes at mitotic metaphase of *B. suborbiculata* were also determined to be $2n = 30$ (Figure 2B and 2D). The 30 chromosomes gradually varied in chromosome length (ca. 1.1-1.7 µm). The centromere positions of shorter chromosomes could not be determined, however, several chromosomes were metacentric and/or submetacentric. Satellites were not observed.

Somatic chromosomes at mitotic metaphase of *B. gueritziana* were determined to be $2n = 28$ (Figure 3A and 3B). The 28 chromosomes of the plants gradually varied from ca. 1.0 to 1.5 µm long. The centromere positions of most chromosomes were uncertain. Satellites were not observed.

Forty-four species of *Begonia* sect. *Diploclinum* are currently recognized from the Philippines (Rubite and Madulid, 2009; Hughes et al., 2010). However, cytological features in Philippine *Begonias* have rarely been reported. To our knowledge, a total of 13 species of this section have a chromosome number of $2n = 30$ (this study and our unpublished data), with the exception of *B. fenicis* ($2n = 56$) collected from Batan (Kokubugata and Madulid, 2000). The chromosome number of $2n = 30$ in *B. blancii* and *B. suborbiculata* agrees with that of other Philippine *Begonia* (unpublished data).

While *B. gueritziana*, endemic to Borneo, is allied to *B. blancii* morphologically in having a similar habit and bilocular fruit, its chromosome number ($2n = 28$) differs.

DISCUSSION

A comparison of leaf morphology and anatomical features between *B. blancii*, *B. suborbiculata* and *B. gueritziana* are shown in Figure 4 and Table 1. *Begonia blancii* shows adaptations for coping with a shaded and periodically dry habitat. The leaves are persistent and succulent, indicating a drought-tolerant strategy. By comparison, some *Begonia* species from Palawan in similar habitats die back to a small tuber or rhizome during the dry season, e.g., *B. rhombicarpa* A.DC., *B. wadei* Merr. & Quisumb. and *B. woodii* Merr., showing a drought-avoidance strategy. *B. suborbiculata* partially loses turgor in its leaves during the dry season, permitting the leaves to lie flat on the substrate which may reduce their water loss through transpiration (P. Blanc, pers. obs.).

The clustered stomata of *B. blancii* (Figure 4C) are likely to be another way in which the species copes with drought, in common with a number of other *Begonia* species from the region and beyond. In a survey of *Begonia* sect. *Dipliclinium* and the allied *Begonia* sect. *Baryandra* from the Philippines (Rubite, unpublished data), we found that out of 29 species, all except two had clustered stomata. One of the species with single stomata, *B. oxysperma* A. DC., is confined to a narrow altitudinal band, the lower limit of which coincides with the prevalent cloud base of its native Mt. Banahao and several neighbouring mountains (Tebbit, 2005) and hence grows in high and constant humidity. The species is also noted as needing very high humidity in cultivation. One other species, *B. longiscapa* Warb., had single stomatas.

A number of other *Begonia* species are known to have clustered stomata, including *B. peltatifolia* (from exposed

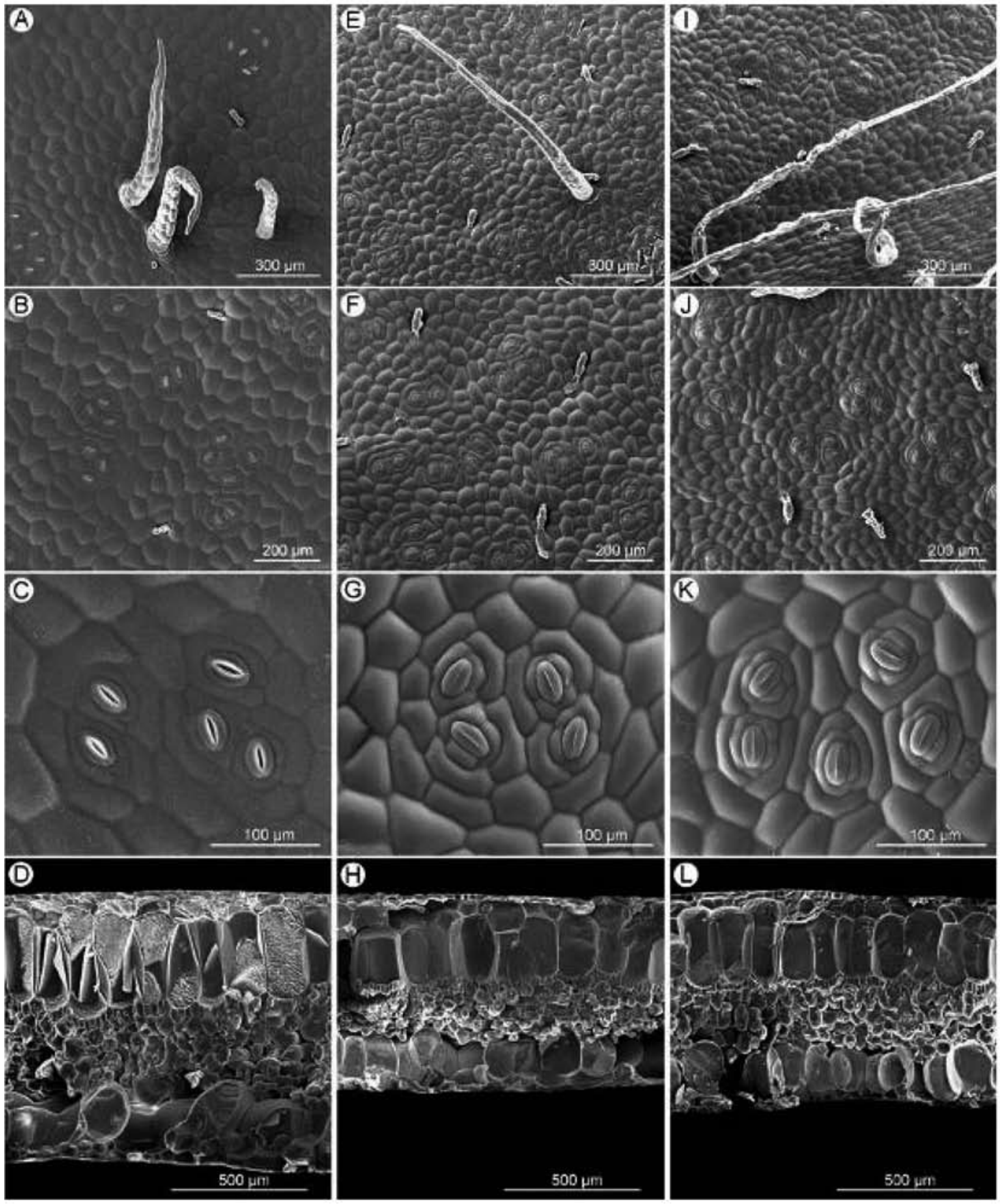

Figure 4. *Begonia* leaf SEM micrographs. A-D, *B. blancii*; E-H, *B. suborbiculata*; I-L, *B. gueritziana* (*Peng 22342*). A, E, I, abaxial surface, showing multicellular hairs and glandular hairs; B, F, J, abaxial surface, showing glandular hairs and stomatal clusters; C, G, K, close-up images of stomatal cluster; D, H, L, cross section. (A-D from *Peng 22545*; E-H from *Rubite 325*; I-L from *Peng 22342*. Vouchers are deposited at HAST)

Table 1. Leaf morphology and anatomical character comparison between *Begonia blancii*, *B. suborbiculata*, and *B. gueritziana*.

	Begonia blancii	*Begonia suborbiculata*	*Begonia gueritziana*
Abaxial trichomes	Scattered in mesophyll area, sometimes clustered, rather dense on veins; mesophyll area trichome 0.3-0.8 mm long, veins trichomes ca. 1-3 mm long (Figure 4A)	Scattered in mesophyll area and veins; 0.8-1.1 mm long (Figure 4E)	Lacking in mesophyll area , rather dense on veins, finely adpressed; shrivelled and ±twisted on mature leaves; 0.9-2.2 mm long (Figure 4I)
Stomatal complex	3-7 clustered, rarely single or in pairs (Figure 4B, C)	3-5 clustered, rarely singly or in pairs (Figure 4F, G)	3-5 clustered, rarely singly or in pairs (Figure 4J, K)
Thickness	ca. 950-1,000 µm (Figure 4D)	ca. 670-740 µm (Figure 4H)	ca. 750-800 µm (Figure 4L)

limestone mountain summits, Hainan province, China); *B. fenicis* Merr. (exposed elevated coastal coral rocks, Orchid Island, Taiwan) and *B. pseudodryadis* C.Y. Wu (limestone hills, Yunnan, China) (Peng, unpublished data). *Begonia leprosa* Hance has clustered stomata and co-occurs on limestone habitats with *B. variifolia* Y.M. Shui & W.H. Chen, with which it is known to hybridize (Peng et al., 2010). Although they co-occur, there is some evidence of niche differentiation; *B. leprosa*, with clustered stomata, is noted as occurring on the sunlit rocky entrance of caves, while *B. variifolia* is found deeper inside on shaded rock faces (Peng et al., 2010). Increased stomatal clustering with increased exposure has also been noted by Hughes and Miller (2002) in *Begonia* from the Socotra archipelago. *Begonia samhaensis* M. Hughes & A. G. Mill. grows on a very exposed rocky summit and has stomata in clusters of up to 15; its sister species, *B. socotrana* Hook. f., grows in slightly more sheltered habitats and has stomata in clusters of up to 8. Clustered stomata are correlated with multiseriate epidermis in *Begonia* (Boghda and Barkley, 1972), and hence linked to the possession of succulent leaves.

Three species of *Begonia* sect. *Petermannia* from the Philippines were examined, and all had single stomata. Species in this section tend to be humicolous and occur in microhabitats with more constant water availability, in contrast to the largely saxicolous *Begonia* sect. *Diploclinium*. Hence there is a strong correlation between the water use ecology of *Begonia* species and stomatal clustering. Variation of stomatal cluster size within species depending upon water availability has been reported for *B. heracleifolia* Cham. & Schltdl. and *B. nelumbiifolia* Cham. & Schltdl., with plants growing on rocky substrates tending to have larger clusters than those growing on soil (Hoover, 1986). Increases in stomatal cluster size in response to induced drought stress has also been reported experimentally in *Vicia faba* L. (Gan et al., 2010). Hence the stomatal clusters in *Begonia blancii* very probably form part of its drought tolerance strategy, by permitting carbon uptake whilst limiting water loss through transpiration. However the mechanism by which stomatal clusters create an advantage remains unclear.

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特產菲律賓巴拉望島之秋海棠屬（秋海棠組）一新種：匙葉秋海棠

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本文發表特產菲律賓巴拉望島的秋海棠屬（秋海棠組）一新種：匙葉秋海棠。此新種略似產於菲律賓之秋海棠組具二室子房的 *B. suborbiculata*，但新種具有平鋪於裸岩之垂直壁面的肉質三角形匙狀葉片而明顯可以區別。匙葉秋海棠為常綠性，如同若干生長於相似生境的秋海棠，其葉片具有聚生的氣孔，此殆與減少旱季時的水分蒸散有關。本文並報導本新種與近緣種之染色體數與核型：*B. blancii* ($2n = 30$)，*B. suborbiculata* ($2n = 30$)，以及 *B. gueritziana* ($2n = 28$；婆羅洲產）。根據世界自然保育聯盟（IUCN）的評估標準，匙葉秋海棠屬於「安全」等級。

關鍵詞：*Begonia blancii*；*Begonia gueritziana*；*Begonia suborbiculata*；秋海棠屬；染色體數；葉片解剖；新種；巴拉望島；菲律賓；氣孔。