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Begonia subvillosa, new running wild *Begonia* in Cibodas Botanical Gardens and Naturalized Prediction

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ABSTRACT. *Begonia* (Begoniaceae) have been widely cultivated as ornamental plants especially species of begonias introduced from abroad. Two introduced Begonia species, namely *B. hirtella* and *B. cuculata* have long been naturalized in Indonesia and one introduced Begonia species was found released from the cultivated area, namely *B. subvillosa*. Because of morphological information and distribution of B. subvillosa populations in Indonesia are still limited, this study aimed to characterize the morphology, distribution, and stomata type of *B. subvillosa* in Cibodas Botanical Garden (CBG) and compare them with two previously naturalized Begonia species. The morphological data were described based on living plants found in CBG and added from herbarium specimens and/or protologue of *B. subvillosa*. The distribution and habitat data of three species were traced using roaming method in CBG area. The stomata preparations were polished using nail polish, then the type of stomata was observed. The SLA and leaf size were measured using ImageJ. Three populations of *B. subvillosa* have been found in the CBG as the first record of the species outside its natural habitat. Morphologically, *B. subvillosa* is easily distinguished by the character of the leaf blade and petiole which are covered by brown and dense trichomes. *Begonia subvillosa* also has the clustering stomata similar to two naturalized Begonias. In addition to morphological adaptation and functional traits, good generative regeneration ability can be increases the chance of naturalization in the future.

Keywords: *Begonia subvillosa*; Cibodas Botanical Gardens; clustering stomata; naturalized species; SLA

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INTRODUCTION

Begonia is one of the six largest genera in angiosperm plants with a total of 2052 species with asymmetrical leaves characteristic. *Begonia* has long been cultivated as an ornamental plant because of its attractive shape, color, and pattern on the leaves and flowers especially the species of *Begonia* introduced from abroad or exotic species. Begonias are a common indoor plant that can grow in a variety of conditions, quite attractive, and also could be used to make food and medicine (Efendi, 2018; Hughes, Moonlight, Jara, Tebbitt, & Pullan, 2015; Siregar, 2017; Sizhuk & Andreyeva, 2018).

Begonia is an herbaceous plant with general characteristics of vines and succulents belonging to the Begoniaceae family and widely distributed in tropical and subtropical areas (Rizki, Soegianto, & Arumingtyas, 2009). *Begonia* diversity is unevenly distributed across tropical regions with the greatest diversity in America and Asia (approximately more than 600 species), while Africa (160 species) and Australia are both devoid of the species (Goodall-Copestake, Perez-Espona, Harris, & Hollingsworth, 2010; Moonlight *et al*., 2018). The number of exotic *Begonia* species in Indonesia has reached more than 100 species and cultivars (Backer & Bakhuizen Van Den Brink, 1963; Hartutiningsih & Siregar, 2013) and several of them are from Cibodas Botanical Gardens (CBG) collection.

The notes of the collection of introduced *Begonias* in CBG have been carried out since 1930 namely *B. venosa* (Dakkus, 1930) and *B. glabra* in 1963 (Nasution, 1963). Both species are exotic species from America (Doorenbos, Sosef, & De Wilde, 1998). Previously, two species of *Begonias*

from America, namely *B. cuculata* (Teijsmann & Binnendijk, 1866) and *B. hirtella* were planted in Bogor Botanical Gardens. Now both have naturalized in Java (Girmansyah, 2014; Undaharta, 2016) and are no longer recorded as collection plants in the botanical gardens.

Begonia subvillosa, one of the introduced *Begonias* from Brazil was also found wildly in CBG area. However, there are few information about the existence of *B. subvillosa*'s in Indonesia. The first recorded as a collection in botanical gardens as *B. schmidtiana* and also recorded in Flora of Java (Backer & Bakhuizen Van Den Brink, 1963). However, there has been no record of this species either as a collection plant or as a wild species (Sujarwo, Gumilang, & Hidayat, 2019). Furthermore, herbarium specimens of *B. subvillosa* have never been stored in the Herbarium Bogoriense (Doorenbos *et al*., 1998) and Herbarium Cianjur Hortus Tjibodasensis (CHTJ).

It is crucial for tracking the spread of naturalized introduced *Begonia* and the morphological record of introduced *Begonia* as a form of habitat adaptation is still unknown. Flowering duration, leaf size, seed mass, and specific leaf area (SLA) are all morphological and functional traits that have been used to distinguish naturalized exotic plant species from invasive plants (Junaedi *et al*., 2019; Gallagher *et al*., 2014). Therefore, this research was aimed to describe the morphological characteristics, including functional traits and stomata type as well as their distribution of *Begonia subvillosa* in CBG. It is hoped that this data can be used as initial information for risk assessment of naturalized species, especially *Begonia* species.

MATERIALS AND METHODS

Study area. The study was conducted in CBG area (**Fig. 1**). CBG located on the slope of Mt. Gede Pangrango National Park, West Java, at an altitude of 1300 to 1425 m above sea level. It covering an area of 85 ha, including four blocks (Sujarwo *et al*., 2019). Samples were collected during the field survey using free exploration method (Rugayah, Windadri, & Hidayat, 2004). Three collection number of *B. subvillosa* were collected from three site of CBG area namely, (1) Gardens collection in Vak. I.I and I.K., (2) Nurseries unit, and (3) Gesneriads Thematic Gardens.

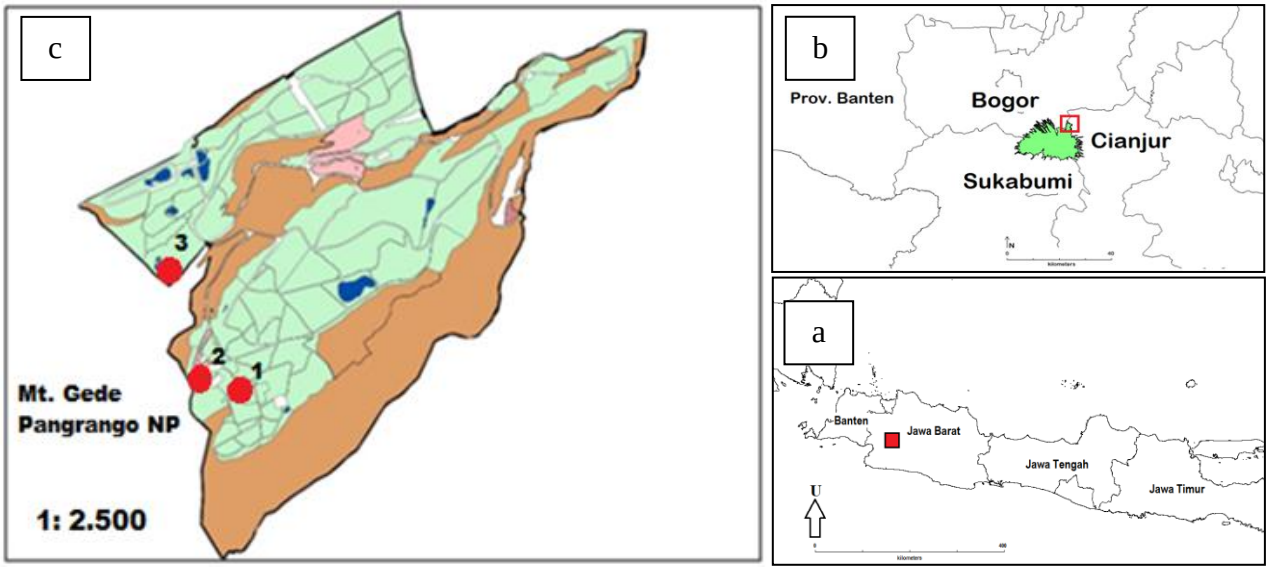

Fig. 1. Sampling location in Cibodas Botanical Gardens, Cianjur, West Java, a. Map of Java Island, b. Map of Mt. Gede Pangrango National Park (NP) (**green color**), (c) Map of Cibodas Botanical Gardens (red dot=location of *Begonia subvillosa* population, 1. Gardens collection, in Vak. I.I and I.K. (Coordinat point: 6.744005 S, 107.006920 E), 2. Nurseries unit (Coordinat point: 6.744239 S, 107.005681 E), 3. Gesneriads Thematic Gardens) (Coordinat point: 6.744005 S, 107.006920 E) (UNEP-WCMC & IUCN, 2021).

Procedures. The data was recorded including the number of individual plant and habitat type. Morphological observation was characterized based on living collection that found in CBG refers to (Doorenbos *et al*., 1998) including habit, leaves, flowers, fruit, and seed. The morphological data

were compared to specimen type or protolog accesed by online database begonia: https://padme.rbge.org.uk/begonia/ to verify the validity of the accepted names (Hughes *et al*., 2015). Specimens' observation also has been conducted in online herbarium (GBIF) and Herbarium CHTJ to record the morphological data and their distribution. Stomata preparations were made using mold methods using nail polish (Efendi, 2019). Observational data including stomata's type, stomata's distribution type, and stomata's size.

Two responses traits, namely leaf size and specific leaf area (SLA) were also analyzed (Gallagher, Randall, & Leishman, 2015; Junaedi, Zahra, & Salamah, 2019). Leaf size were measured from leaf photos using ImageJ (Schneider, Rasband, & Eliceiri, 2012). SLA measurement were using a procedure adapted from (Perez-Harguindeguy *et al*., 2016) that were obtained by comparing leaf area with leaf dry weight and measured as $mm^2\ mg^{-1}$.

Data analysis. Data were analyzed descriptively. The morphological data and response traits of *B. subvillosa* were compared with *B. hirtella* and *B. cucullata* to describe their similarity that might be reason for naturalization.

RESULTS AND DISCUSSION

Accepted names. *Begonia subvillosa* Klotzsch (Martín, Ospina, & Zanotti, 2017).
Synonyms. *B. subvillosa* var. *leptotricha* (C. DC) LB. Sm. & Wassh., *Begonia schmidtiana* Regel., *Begonia leptotricha* C.CD., *Begonia schmidtii* Haage & Schmidt.
Description. Terrestrial herbs, annual to perennial. **Stem:** erect, up to 30 cm. height, internodeus short, 1-5 cm length. Stipule persistent, entire (**Fig. 2b**) **Petiole** short, 1-2 cm length, pilose. **Leaves**: alternate, basifixed, broadly ovate, 6-14 cm x 3,5-8 cm, lower surface covering with densely brown-pilose, margin entire, venation palmate. **Inflorescence**: borne in axillar; *dichasial*; bisexual; protandrous; bracteole 3, persistent. **Male flower:** tepal 4, white, free, outer tepal smaller than inner tepal, androecium actinomorphic, filament yellow, *equal*, free, the anther is longer than the filament, connective not extended (**Fig 2d**). **Female flower:** tepal 5, free, ovary three locul, wing 3, unequal, placentae axillar, bifid each locule, stigma 3, free, one branch, persistent, in a band and spiraled (**Fig. 2c**). **Fruit:** capsule type, dry when mature, has three curved wings. **Seed** microscopic, barrel-shape, 310-350 µm. length, 195-210 µm. width. (**Fig. 2e**).

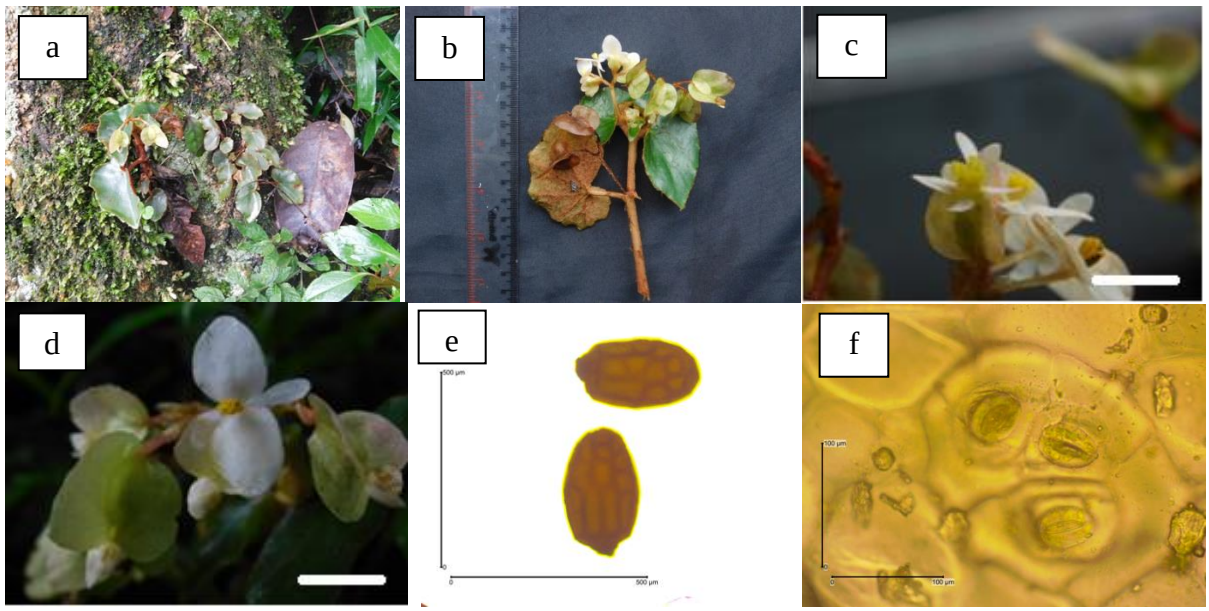

Fig. 2. Morphological characteristic of *Begonia subvillosa*. a. Population of *B. subvillosa* in their natural habitat in CBG, b. habit, c. Female flower, d. Male flower and young fruit, e. seed, f. stomata (scale bar: c – d = 1 cm; e = 500 µm; f = 100 µm).

Stomata. Anisocytic, clustering-stomata type, 3 to 7 stomata each group, hypostomatic (**Fig. 2f**)
Ecology and habitat. *Begonia subvillosa* grows in open or slightly shaded areas, attached to mossy and damp rocks, grows between the roots of *Yucca gigantea* (CBG's collection). Currently, there are no records of wild population of *B. subvillosa* around the CBG area.

Begonia subvillosa is a native species to Bolivia, Brazil, and Argentina's northwestern provinces. The entry of these *Begonias* into CBG was certainty unknown because there were no records of this species as a collection in CBG. First, it is known in gardens collection and nursery unit (Efendi, 2015). Then, it has been grown in gesneriads thematic garden (**Fig. 1**) as a new population in CBG.

Morphologically, the dense trichomes that cover the abaxial surface of the leaves distinguish *B. subvillosa* from *B. cucullata* and *B. hirtella* (**Table 1**). A curved wings shape and ovate leaf shape are more similar to *B. hirtella* than *B. cucullata* (triangular wings shape) (**Fig. 3**), although leaf thickness is thicker than *B. hirtella* and *B. cuculata*. Although they are divided into different sections based on morphology, genetically they are belonging to similar section, namely *Ephimera* (Moonlight *et al*., 2018).

Table 1. Comparison of morphological characters and the functional traits of *B. hirtella*, *B. cucullata*, and *B. subvillosa.*

No	Character	Scientific names		
		Begonia hirtella	*Begonia cucullata*	*Begonia subvillosa*
1	Plant longevity	Annual	Annual/perennial	Annual/perennial
2	Habit	Erect herbs	Erect herbs	Erect herbs
3	Upper surface of leaves	Glabrous, or sparsely trichome	Glabrous	Glabrous to sparsely trichome
4	Lower surface of leaves	Sparsely trichome	Glabrous to sparsely trichome	Densely by trichome
5	Venation	Palmate	Palmate	Palmate
6	Number of venations	9 – 12	7 – 10	5 – 7
7	Tepal of male flower	4	4	4
8	Tepal of female flower	5	5	5
9	Number of filaments	Ca. 12 – 19	Ca. 25 - 33	Ca. 8 – 12
10	Placentae borne	Axillary	Axillary	Axillary
11	Placentae	Bifid each locule	Bifid each locule	Bifid each locule
12	Wings	Triangular	Rounded	Rounded
13	Fruit type	Capsule	Capsule	Capsule
14	Stomata type	Anisocytic	Anisocytic	Anisocytic
15	Distributed Stomata type	Clustering	Clustering	Clustering
16	Number of stomata in group	3 – 11 stomata	5 - 15 stomata	2 – 7 stomata
17	Leaf area (cm^2)	20.07 – 37.26	11.30 – 25.58	9.28 – 17.37
18	SLA ($mm^2\ mg^{-1}$)	53.89 – 72.87	21.55 – 76.36	28.46 – 49.44

Based on the flowers position and their flowering period, *B. subvillosa* have a lower position of female flowers than male flowers and the ripening period of male and female flowers are relatively simultaneous allowing self-pollination. In comparison to *B. hirtella* and *B. cuculata*, *B. subvillosa* produced fewer fertile seeds (around 4–20 seeds each fruit). Because the population was low, pollination was dependent on the wind. In comparison to native *Begonias*, the three *Begonias* have a shorter reproductive time, a longer flowering period throughout the year, and more fruit production, resulting in faster distribution (Tebbitt, 2005).

In the paradermal section, the three *Begonias* have clustering stomata types with the number of stomata per group between 3-15 stomata. The clustering stomata type is reported to be more efficient in water use and tends to have a higher plasticity ability than the single type stomata especially in drought stress (Hoover, 1986). Otherwise, under suitable condition the stomata open optimally that the photosynthesis can run optimally (Papanatsiou, Amtmann, & Blatt, 2017). Trichomes, like stomata, help to keep the leaves moist and protect them from herbivores. Tolerance for a broader range of microclimate changes including temperature, humidity, and light exposure.

Fig. 3. Comparison of leaf blade morphology and fruit characters, *B. hirtella* (left), *B. cucullata* (middle), and *B. subvillosa* (right).

Will they naturalize? Although as introduced species, *B. subvillosa* grows in association with native species in the gardens collection. The periods of the plant and the frequency of planting in the past are most likely factors in the success of naturalization at this time. Because *B. hirtella* and *B. cucullata* were the first species to be imported and planted in Indonesia (Girmansyah, 2014), their chances of spreading are greater than those of *B. subvillosa*.

Begonia subvillosa also had fertile seeds. Despite the small numbers, this increases the likelihood that generative regeneration in this species will continue. The seeds are very small (310-350 µm x 195-210 µm) and produced in a capsule-fruited, which serve as an adaptation for wind dispersal (Barrera, De Guzman, & Mergilla, 2019; Rubite, 2013). Furthermore, *B. subvillosa*'s SLA values and leaf area (28.46 – 49.44 mm^2mg^{-1}) still in the range of *B. cucullata*'s values (21.55 – 76.36 mm^2mg^{-1}) and were smaller than *B. hirtella* (53.89 – 72.87 mm^2mg^{-1}). Based on the data from Junaedi *et al*. (2021), naturalized species had an average SLA value of 40.03 mm^2mg^{-1} in CBG while not naturalized species had an average of only 11.82 mm^2mg^{-1}. The SLA value has a positive relationship with the possibility of plant naturalization; the higher the SLA value, the greater the possibility of naturalization.

Begonia subvillosa have clustering stomata, unlike stomata type in most native *Begonia* of Indonesia, namely single type and single and/or small clustering (Efendi, 2019). The clustering stomata type were also found in *B. hirtella* dan *B. cucullata*. A stomatal cluster is a collection of two or more stomata that share a single stomatal chamber and are only separated by subsidiary cells. Begonia (Begoniaceae) is one of 38 genera in 19 vascular plant families where it has been found (Min, Xi, Xing, & Bai, 2002). The clustering stomata was classified as non-contagious cluster, could be increase significantly along with salt treatment/drought, was reported to be more efficient in water

use, and tends to have a higher plasticity ability than the single type stomata especially in drought stress (Hoover, 1986; Suffan & Metusala, 2021). Otherwise, under suitable condition the stomata open optimally that the photosynthesis can run optimally (Lehmann & Or, 2015; Papanatsiou *et al*., 2017). Trichomes trait, likes in stomata, help to physiological and ecological roles such as tolerance for a broader range of microclimate changes including temperature, humidity, and light exposure (Barrera *et al*., 2019; Ichie, Inoue, Takahashi, Kamiya, & Kenzo, 2016; Xing *et al*., 2017).

CONCLUSION

The distribution of *B. subvillosa* in CBG is the first record in outside of their natural habitat. The species is more likely to naturalize based on morphological parameters and functional traits, if no cutting is done. Interesting clustered stomata characters were investigated further as a trait for a species naturalization or invasiveness, particularly *Begonia* species.

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