Indonesian Journal of Forestry Research Vol. 9, No. 1, April 2022, 1-8 ISSN: 2355-7079/E-ISSN: 2406-8195

LEAF TRAITS OF THE INVASIVE SPECIES *Bartlettina sordida* (Less.) R.M. King NATURALIZED IN CIBODAS HIKING TRAIL, MT. GEDE PANGRANGO NATIONAL PARK, WEST JAVA, INDONESIA

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Received: 1 July 2021, Revised: 3 April 2022, Accepted: 13 April 2022

LEAF TRAITS OF THE INVASIVE SPECIES *Bartlettina sordida* (Less.) R.M. King NATURALIZED IN CIBODAS HIKING TRAIL, MT. GEDE PANGRANGO NATIONAL PARK, WEST JAVA, INDONESIA. Cibodas Botanical Garden (CBG) maintains not only native plant species but also introduced plant species. Some of these have been naturalized from CBG to the adjacent Mt. Gede-Pangrango National Park (GPNP). One of the reported naturalized species is *Bartlettina sordida*. Understanding species traits will give information for the future management of *B. sordida*. This research investigates the distribution of *B. sordida* in the Cibodas hiking trail and studies leaf traits variation of this species along with an altitudinal change in the Cibodas hiking trail. Samples were collected along the Cibodas hiking trail from the entrance gate to the Cibeureum waterfall. At every survey location, we recorded *B. sordida* occurrences. In the sample plots we also collected leaf samples from detected *B. sordida*. The leaves were then fixated using the hands-free method to observed stomatal type, density, and size. The leaves were also treated for specific leaf area (SLA) measurements. Stomata size is significantly correlated along the altitudinal gradient. Stomata density is negatively correlated but not significant along the altitudinal gradient. Stomata size is positively correlated with SLA. These traits are related to water-efficient adapting to GPNP climate, which is very different from its natural habitat. The ability to adapt to altitudinal gradient helps *B. sordida* to dominate the Cibodas hiking trail.

Keywords: Tropical botanical garden, environment gradient, alien species, stomatal traits

KARAKTER DAUN SPESIES INVASIF (<u>Bartlettina</u> <u>sordida</u> *(Less.) R.M. King) TERNATURALISASI DI JALUR PENDAKIAN CIBODAS, TAMAN NASIONAL GUNUNG GEDE PANGRANGO, JAWA BARAT, INDONESIA. Kebun Raya Cibodas (KRC) tidak hanya mengoleksi spesies asli Indonesia, namun juga memiliki koleksi spesies eksotik. Beberapa spesies eksotik ini telah ternaturalisasi di luar wilayah KRC dan ditemukan di wilayah Taman Nasional Gunung Gede Pangrango (TNGGP). Salah satu spesies eksotik yang ditemukan adalah* <u>Bartlettina</u> <u>sordida</u> *(Less.) R.M King. Pemahaman mengenai karakter spesies dapat memberikan informasi mengenai penanganan spesies tersebut. Penelitian ini bertujuan untuk mempelajari distribusi* <u>B</u>. <u>sordida</u> *di jalur pendakian Cibodas, dan mempelajari variasi karakter daun spesies ini terhadap gradien ketinggian di jalur pendakian Cibodas. Sampel yang dikoleksi adalah yang berada di sepanjang jalur pendakian Cibodas hingga air terjun Cibeureum. Pada setiap titik sampling, kami mengoleksi data kehadiran. Sampel daun diambil pada setiap titik ditemukannya* <u>B</u>. <u>sordida</u>. *Daun kemudian segera difiksasi menggunakan metode hands-free untuk pengamatan tipe, densitas, serta ukuran stomata. Sampel daun juga digunakan untuk pengukuran Spesific Leaf Area (SLA). Ukuran stomata secara signifikan berkorelasi positif terhadap gradien ketinggian. Densitas stomata berkorelasi negatif namun tidak signifikan terhadap gradien ketinggian. Ukuran stomata berkorelasi positif dengan SLA. Karakter daun yang ditemukan berkaitan dengan efisiensi air untuk beradaptasi dengan iklim TNGGP yang sangat berbeda dengan habitat alaminya. Kemampuan untuk beradaptasi terhadap gradien ketinggian mendukung* <u>B</u>. <u>sordida</u> *mendominasi jalur pendakian Cibodas.*

Kata kunci: Kebun raya tropis, gradien lingkungan, spesies eksotik, karakter stomata

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I. INTRODUCTION

Cibodas Botanical Garden (CBG) was first established to acclimate high economic value plants (Efendi, Hapitasari, Gresia, Rustandi, & Supriyatna, 2016). These plants were carried mostly from subtropical areas to be naturalized and planted in Indonesia during the colonial era. However, this function has recently been altered following the need to conserve plant biodiversity (Heywood, 2011), and CBG is an ex-situ plant conservation institution. It collected not only native plant species but also exotic plant species. Exotic species were collected in the past, and CBG keeps these plant collections in good condition (Mutaqien, Tresnanovia, & Zuhri, 2011).

Botanical gardens conducted integrated conservation by documenting plant information (Mounce, Smith, & Brockington, 2017). This information includes distribution, morphological characters, molecular traits, physiology to support plant species conservation (Schulman & Lehvävirta, 2011). On the other hand, botanical gardens were also responsible for distributing exotic invasive species in the past. For example, during the 19th century, the Royal Botanic Gardens Kew, London, spread quinine tree (*Cinchona* spp.) across the British islands. (Hulme, 2011). While in Indonesia, Bogor Botanical Garden introduced the water hyacinth (*Euchhornia crassipes*) for ornamental plants. It has instantly covered the water body, and the excessive were dumped into the Ciliwung river (Tjitrosoedirdjo, 2005). Invasive pathways were also facilitated by trading seeds by the colonials in the 18th and 19th centuries that drove tropical botanical gardens (Dawson, Mndolwa, Burslem, & Hulme, 2008). Although the number of naturalized species is relatively low (Galera & Sudnik-Wójcikowska, 2010), the botanical gardens should be aware of the opportunistic behavior of these introduced plant species to prevent the spreading into the adjacent areas (Reichard & White, 2001).

Cibodas Botanical Garden is located next to Mt. Gede-Pangrango National Park (GPNP). Some reports found that several CBG's living collections have been naturalized into GPNP. These naturalized species were *Bartlettina sordida*, *Brugmansia candida*, *Cestrum aurantiacum*, *Chimonobambusa quadrangularis*, and *Passiflora ligularis* (Wahyuni & Tjitrosoedirdjo, 2013; Zuhri & Mutaqien, 2013; Padmanaba, Tomlinson, Hughes, & Corlett, 2017). CBG, as a conservation institution, needs to carry out a risk assessment of introduced species to prevent the spreading and minimize the invasion risks (Hardwick et al., 2010; Andersen, Naylor, Endress, & Parks, 2015). CBG has conducted a weed risk assessment of 25 introduced threatened species with two approaches (Junaedi, Putri, & Kurniawan, 2021), namely by specific leaf area (SLA) and *Tropical Weed Risk Assessment Protocol* (TWRAP) *scoring* (Jefferson, Havens, & Ault, 2009). Invasive species risk assessment could also be conducted using other traits such as stomata density and size (Klich, 2000; Huang, Ratkowsky, Hui, Wang, Su, & Shi, 2019), seed productions, leaf N:P ratios, and biomass (Pyšek et al., 2012).

Bartlettina sordida (Less.) R.M.King & H.Rob. or blue mist plant belongs to the family of Asteraceae, and many species from this family are listed as invasive species. *B. sordida* is an evergreen shrub, with large, opposite, broadly obovate, hairy leaves, with purplish veins. Flowers are purple, blue, or pink, and each inflorescence contains 8-150 fruits to be dispersed by the wind (Csurhes, 2016). This species is originating from Mexico and well known as an ornamental plant. It was reported as invasive species in Africa (Henderson & Wilson, 2016), Australia (Randall, 2007), New Zealand (Webb, 1987), and Indonesia (Sunaryo & Tihurua, 2012; Wahyuni & Tjitrosoedirdjo, 2013).

Bartlettina sordida was firstly collected in CBG in 1899 (Mutaqien et al., 2011). There was not much information about the leaf traits of this species, and it was mostly found in the Cibodas hiking trail. We also found no data explaining the ability of this species to spread outside its native range. We obtained SLA data as a response of light captured, photosynthesis rate, plant growth and reproduction (Rindyastuti, Hapsari, & Byun, 2021) According to Junaedi and Mutaqien (2018), SLA is one of the important indicators to distinguish natural exotic plant species (which have started and/or spread to new areas and have the potential to increase plant populations in new areas. Stomata traits also showed plant adaptation ability in several environment conditions (Hong, Lin, & He, 2018). Mountain ecosystems are highly suitable for investigating potential range extention of invasive species across latitudinal gradient (Arévalo et al., 2005). Environmental gradient often triggered phenotypic plasticity (Drenovsky et al., 2012), we also found no report of *B. sordida* plasticity. Thus, this research aims to study *B. sordida* leaf traits responding to environmental gradient in the Cibodas hiking trail. Mountain ecosystems are highly suitable for investigating potential range extensions of invasive species across latitudes (Arévalo et al., 2005). This study will capture the adaptation capacity of *B. sordida* along an altitudinal gradient. This information will be useful for stakeholders to adequately manage and minimize the invasion risks of this naturalized species in GPNP in particular and tropical mountainous forests in general.

II. MATERIALS AND METHOD

A. Study Site

The research was conducted inside Mt. Gede-Pangrango National Park (GPNP) on the Cibodas hiking trail from the entrance gate to the Cibeureum waterfall. GPNP is located in Cianjur district, West Java, Indonesia. The GPNP vegetation type zonation were categorized into sub-montane zone (1.000-1.500 m asl), montane zone (1.500-2.400 m asl), and sub-alpine zone (2.400-3.019 m asl) (van Steenis, 1972)). Based on Schmidt and Ferguson classification, GPNP has a type A climate with annual rainfall 3000-4200 mm per year. Air temperature ranging from 0ºC (on the top of the mountains) to 18ºC (in Cibodas), and the relative air humidity range is 80-90% (Rozak et al., 2016). Samples were collected in December 2019.

B. Methods

Data were collected in December 2019 using the tracking method alongside the Cibodas hiking trail (Figure 1). The survey was conducted repeatedly at every 100 m distance on the hiking trail pathway. There were 18 survey points at 18 locations along the hiking

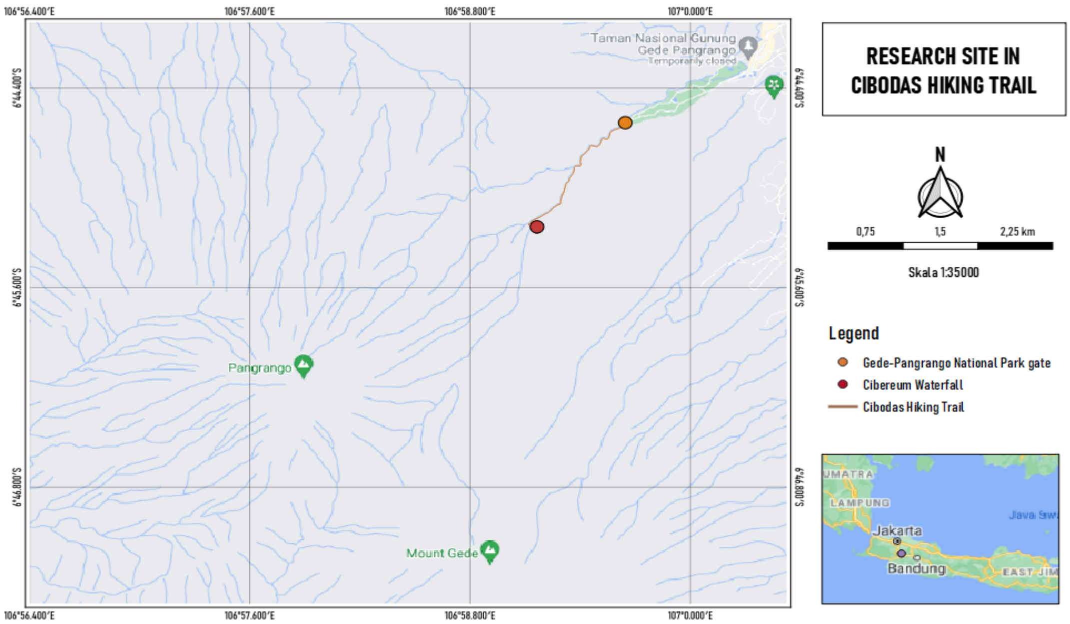

Figure 1. Research site along the Cibodas hiking trail, Mt. Gede-Pangrango National Park

trail. It was recorded *B. sordida* presence data at every survey location. In the plots we also collected leaf samples from detected *B. sordida*. The leaves were then fixated using the hands-free method to observed stomatal type, density, and size. The leaves were also treated for specific leaf area measurements. The leaf data in this study consists of stomata density, size and specific leaf area (SLA). Leaf stomata were immediately fixated from *B. sordida* leaves using the hands-free section method (Sari & Harlita, 2013). Stomata density and size were observed under 10x10 magnification using microscope Olympus CX22LED®. Stomata type and size were measured using Image Raster 3.0. Specific leaf areas (SLA) of B. sordida were measured based on the protocol of SLA data collection (Pérez-Harguindeguy et al. (2013). The leaves samples for SLA measurement were collected by choosing old leaves with dark green color. Leaves samples were stored in Ziplock to maintain humidity after the photo of the leaf was taken. Leaf area was measured from leaf photo data analyzed in ImageJ (Schneider, Rasband, & Eliceiri, 2012). To obtain dried leaves mass, leaves were dried in the oven for 72 hours at 65^{o}C temperature then weighed using digital scale. The SLA value was obtained from the ratio between the dried leaf mass and the measured leaf area.

C. Analysis

We used linear regression analysis to model the leaf traits variation (stomata size, stomata density, and SLA) along the elevational gradient (Preacher, Curran, & Bauer, 2006). We conducted stepwise regression to decide which variable should be included in the model (model 1), then conducted linier regression as follow:

$$\boldsymbol{Y = a + bx + e} \quad \text{...(1)}$$

where: $\boldsymbol{Y}$ is leaf traits (stomata density, stomata size and/or SLA), $\boldsymbol{x}$ elevation (m asl) and $\boldsymbol{e}$ is normally distributed error. All analysis conducted in R and R studio.

III. RESULT AND DISCUSSION

Based on the conducted stepwise regression result, the model analysis found that all leaf traits affected by altitude (Figure 2). The regression shows stomata density negatively correlated with altitude (Figure 3). The higher the habitat of *B. sordida*, the lower the stomata density is. Results have shown that the stomata density are not statistically significant with the elevation (Figure 3).

Bartlettina sordida was originally grown in a tropical climate with full sun and dry habitats. CBG imported it for being an ornamental plant. Recently, some reports that *B. sordida* has been naturalized in adjacent forests; one of the reported areas is Mt. Gede-Pangrango National Park (GPNP). This species was reportedly found along hiking trails, and there has been no report that it was found deeper in the forest. GPNP has a wet tropical highland climate with high rain intensity throughout the year and high

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Subset selection object
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SLA                 FALSE      FALSE
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Figure 2. Model analysis results

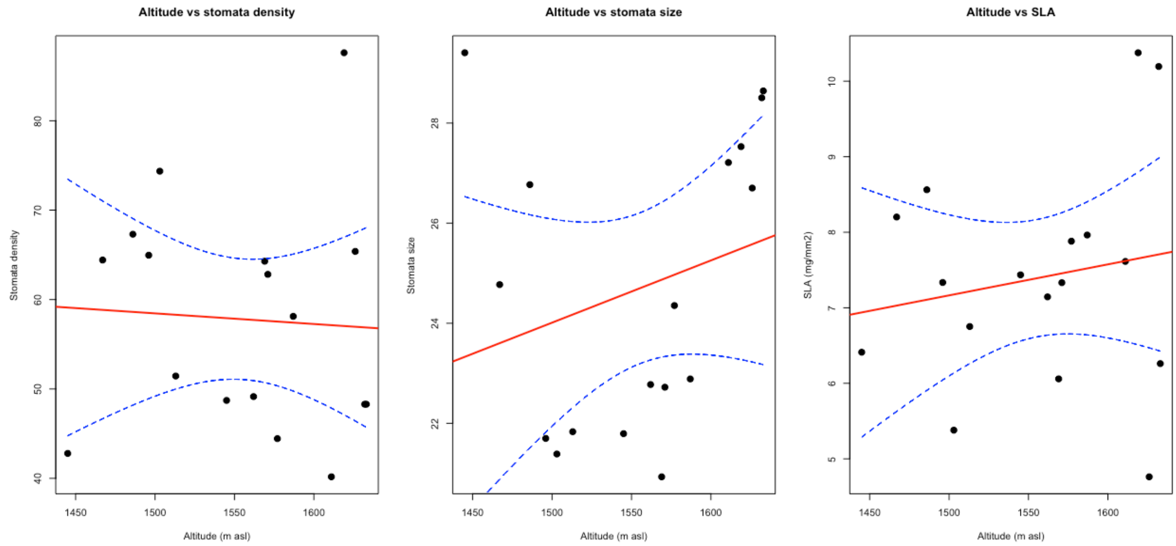

Figure 3. Predicted correlation between a) altitude and stomata size (left); b) altitude and stomata density (middle); c) specific leaf area (SLA) and stomata size (right)

UV exposure. These extreme conditions affect the leave traits of *B. sordida* inside GPNP. Data shows that *B. sordida* leaf traits are affected by the altitudinal gradient. The data shows that *B. sordida* SLA positively correlates with stomata size, which is uncommon for plant adaptation in highland areas. Usually, SLA is negatively correlated with stomata size. This is presumably a defense mechanism when sunlight is high at noon and wind velocity is also high. Small stomata means a smaller open area for leaves, reducing water loss during evapotranspiration. This plasticity is presumably because of the wet climate conditions in the Cibodas hiking trail. *B. sordida* is originally grown in full sun and dry climate; thus, it doesn't need much water for photosynthesis. *B. sordida* has shown its ability to survive in a different climate from its origin, which is why it's alarming as an invasive species (Kleunen et al., 2018). Invasive species have also shown the tendencies of high SLA to compete with native species (Mathakutha et al., 2019).

Stomata density is decreasing with higher altitude. This adaptation is common for herbs to allow efficient use of water. Highland plants are very efficient in water use due to extreme climate (low temperature, high UV intensity, high wind velocity) (Woodward et al., 2002). However, although stomata densities are correlated with altitude, statistically it is not significant. This is presumably because the main factor that drives stomata frequency is other environmental factors. Plants behave differently at higher altitudes; most of the strategies correspond to extreme climate. Highland plants tend to develop their stomata density correlated with stomata opening. Higher stomata frequency also correlated with higher stomatal conductance; species with higher conductance are most likely to distribute with a wider range. At lower elevations, shrubs and herbs tend to occur in the understory where competition for sunlight is strong, while at higher elevations, these plants commonly grow in more open habitats.

Stomata are found on the abaxial surface; the type of *B. sordida* stomata is normocytic. It means that the non-differentiated epidermis guards the stomata. This character is typical of Asteraceae and gives some advantages; it can survive through drought and the rainy season. The leaves also covered by trichomes, which have contributed to the control of transpiration and temperature, as reported in other species of Asteraceae (Borges et al., 2018). This trait helps Asteraceae grow easily in a new area and dominating it if the environment is suitable.

Not only the stomata size, but also SLA is increasing with higher altitude. High altitude caused low-temperature stress. It can break the plant carbon balance, thus lead to growth restriction, supporting the “source limitation” hypothesis (Chai et al., 2015). Highland soil is usually nutrient-deficient, especially in tropical forests; the higher the altitude, the poorer the soil nutrient. Therefore, plants develop a mechanism to keep their food storage in biomass and store carbon in leaves and the stem. These conditions also force plants to produce seeds when the environment is extreme to maintain the continuity of the species (Seipel, Alexander, Edwards, & Kueffer, 2016).

The competition at high altitudes also involving light competition, the open area along Cibodas hiking trail with partial shading provides suitable habitat for *B. sordida* to invade the area immediately. Along the Cibodas hiking trail, the side is an open area where light can reach the soil surface, supporting seedling growth (Zuhri & Mutaqien, 2013). *B. sordida* was not recorded inside the undisturbed forest due to canopy interlock. This condition creates no chance for *B. sordida* to germinate. *B. sordida* was found abundant only in a shaded area where no other invasive species were found. There were some areas where *B. sordida* was absent; in an open area, along Gayonggong bridge, was dominated by *Brugmansia* spp. until some shading at the end of the bridge. This shifting might be caused by interspecific competition of sunlight; *B. sordida* seedlings require partial shade.

Environmental stress acting as a filter against invasive species, while disturbances open another bare land area for invasive species to dominate (Dullinger et al., 2017). The highland ecosystem is a natural barrier for exotic species; however, open areas for recreational purposes create a pathway for invasive plants. Only species with particular traits such as high SLA and protected stomata will survive in extreme highland ecosystem. More studies should be performed until the last *B. sordida* is found along the hiking trails. Comparing other exotic species to native species can also compare how exotic species adapted to the highland ecosystem (El-Barougy et al., 2020). Using community-based and species-based research also has advantages in confirming environmental resistance against invasive species (Gallien & Carboni, 2017). It is suggested that botanical gardens to perform risk assessment of exotic species, before it is planted in the garden’s collections.

IV. CONCLUSION

Bartlettina sordida is one of CBG’s collections that have been naturalized in GPNP. It was brought into CBG as ornamental plants. This species native range is a dry and full sun habitat, but it dominates the Cibodas hiking trail in GPNP with a wet highland ecosystem. However, *B. sordida* can only be found along the hiking trail, not deeper in the forest. From its stomata traits, *B. sordida* has an advantage by its stomata characteristics. Like other Asteraceae, the stomata of *B. sordida* are protected by trichome to help water maintenance through extreme climate conditions. *B. sordida* shows plasticity in Cibodas hiking trail that leads to invasive character. There are two suggested ways to avoid the further possibility of plants naturalized from CBG collection spreading to the mountain forest: 1. To evaluate the negative impact of invasive plants before collections in CBG, 2. Conduct applied research to carry out appropriate invasive plant control techniques in CBG and restore natural forests that have been disturbed by these invasive plants.

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