Flowering and fruiting of *Sarcotheca macrophylla* Bl, an endemic species of Borneo and implication for conservation

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**Abstract.** *Sarcotheca macrophylla* (Oxalidaceae) is an endemic species of Borneo. Little is known about the flowering and fruiting of this endemic species. The present study aimed to investigate the flowering and fruiting of *S. macrophylla* in terms of the first-time flowering, and morphology of the flowers, fruits, and seeds. Morphology of flowers, fruits, and seeds was also recorded and documented. The results showed that the first-time flowering of *S. macrophylla* occurred at 5 months to 16 months after seed germination with the plant height ranged from 13 to 23cm. These results showed that the first-time flowering of *S. macrophylla* occurred at seedling stage, indicating early reproduction of the species. Two morphs of *S. macrophylla* (short-styled plants and long-styled plants) confirmed the heterostylous syndrome (different styly length) which is common in Oxalidaceae. Heterostylous is often associated with self-incompatibility which is commonly linked to the low seed set in this plant family. The present study has implication in the conservation of this endemic species, as the species produced low seed set associated with heterostylous syndrome of the flowers. Efforts to increase the seed set to support regeneration and survival of this endemic species are required.

1. **Introduction**

Establishment of the conservation priorities in light of the increasing of environmental degradation has placed endemic species as one of target species highly prioritized in conservation planning, as they are inherently vulnerable due to their limited distributions compared to widespread species [1,2]. In the management of conservation, understanding the biology and ecology of the target species is essential. Knowledge on the biology of the target plant species (such as the morphology, phenology, flowering and fruiting, reproductive biology, etc.) and the ecology of the species (such as distribution, habitat, microclimate, edaphic properties, etc.) is important to support the effective management of conservation of the target species [3–5].

The present study focused on the biology of *Sarcotheca macrophylla* Bl., an endemic species of Borneo that belongs to Oxalidaceae family [6,7], in terms of the flowering and fruiting of the species to support conservation of this endemic species. Other studies that have been conducted on *S. macrophylla* included phenology of *S. macrophylla* at Bogor Botanic Garden [8], morphology and viability of pollen of *S. macrophylla* [9], and general description of morphology of *S. macrophylla* [6]. However, more specific data of the (i) flowering in terms of age and plant size at the first-time flowering, as well as detailed morphology of the flowers, and (ii) detailed morphology of fruits and seeds of *S. macrophylla* are little known. Therefore, we focused the present study on (i) the first-time flowering, age and plant...
size at first reproduction (flowering), (ii) detailed morphology of flowers, fruits, and seeds of *S. macrophylla*.

The first-time flowering, age, and plant size at first-time flowering are widely recognized to associate with genetic and environmental factors [10–13]. Different plant species can have different time at the first time reproduction. Early reproduction has advantages to (i) increase investment in reproduction early in the life cycle and (ii) increase relative fitness as it can avoid a high risk of mortality before reproductive maturity or poor growth condition during late reproduction [10].

In terms of flower morphology, heterostyly (one of the characteristics of most species in Oxalidaceae family) is recognized as a syndrome of traits. This syndrome includes differences in style length and anther height that are common in Oxallidaceae and other 27 plant families [14,15]. Heterostyly has been generally recorded on some genera within Oxalidaceae such as *Oxalis, Biophytum, Averrhoa, Sarcotheca*, and *Dapania* [6]. Specific and detailed data of heterostyly have been recorded in some species within Oxalidaceae such as *Oxalis alpina, O. cytisoides*, and *Sarcotheca celebica* [14–16]. The syndrome of heterostyly has been widely recognized as a phenomenon of self-incompatibility with the polymorphisms of styly (distyly and tristyly). Distyly comprises two morphs with two types of styly (i) short styly with long stamen and (ii) long styly with short stamen. In this case, pollination occurs only between morphs (short styly in one morph correspond to short stamen from another morph, and it is reciprocal and depicting self-incompatibility in the distylious species [14,15]. Tristyly comprises of three morphs with three types of styly (i) long styly with short and medium stamen (ii) medium styly with short and long stamen (iii) short styly with medium and long stamen, and pollination occur with compatible size of styly and stamens between morphs [15]. The two forms of heterostyly (distyly and tristyly) are reportedly to be commonly associated with self-incompatibility that is often linked to the low seed production [6,19–22].

The present study aimed to investigate the first-time flowering, the morphology of flowers related to heterostylos syndrome, and fruiting of *S. macrophylla*. Data of the present study are required to support the management of conservation of this endemic species.

2. Methods

2.1. Study Species

*Sarcotheca macrophylla* Bl (Oxalidaceae) is a shrub or tree up to 15 m and 10 cm in diameter. The species is endemic to Borneo with distribution includes Indonesian Borneo and Sarawak, and occurs in primary and secondary forests [6]. This species has a long inflorescence which can consist of more than 100 clusters of flowers [9]. The flower is small, red and usually blooms in one day [8]. The flowering season of this species is all year round [9].

2.2. Age and plant size at first time flowering

To investigate age and plant size at first-time flowering of *S. macrophylla*, seed germination experiment was performed and seedling development until flowering was observed and recorded. This experiment was conducted from 2015 to 2019 and the data were already recorded for five times in different years (2015, 2016, 2017, 2018, and 2019). In 2015, we sowed 25 seeds from Katingan, Central Kalimantan, while in 2016 we sowed 10 seeds collected from the Bogor Botanic Gardens collection plants. In 2017, 2018, and 2019 we sowed seeds from young plants grown from seeds originated from Katingan, Central Kalimantan, with the number of seeds that were sown 10, 15, and 20 respectively. Parameters that were measured included the age of plant at first-time flowering after seed germination, plant height, number of branches, number of flower clusters, leaf number, and points at the flowers arise.

2.3. Morphology of flowers, fruits, and seeds

To investigate the morphology of *S. macrophylla* flowers, flowers from the two morphs (long styled and short styled plants) were collected to observe and measure the lengths of stamens and styles, the length and width of sepals and petals, the length and width of flowers under stereomicroscope. Morphology of
fruits was investigated by observing the fruits and the seeds, as well as measuring the size of the fruits and the seeds, and counting number of seeds per fruit. The number of flowers that were observed and measured for each morph was three, while the number of fruits and seeds that were observed and measured was five.

2.4. Data Analysis
To analyse the characteristics of plants with the first-time flowering, the average of plant height, number of branches, number of flower clusters, and leaf number was calculated. Detailed morphology of flowers of each morph of *S. macrophylla* was observed and the average of the length of styly and stamens, the area of petal and sepal, flower length and width of each morph was calculated. Sepal area was estimated by calculating sepal length and width in an elliptic area (modified from [23])

\[
\text{Sepal area} = \pi \times \frac{1}{2} \times \text{sepal length} \times \frac{1}{2} \times \text{sepal width}
\]

Petal area was estimated by calculating petal length and width in an elliptic area (modified from [23])

\[
\text{Petal area} = \pi \times \frac{1}{2} \times \text{petal length} \times \frac{1}{2} \times \text{petal width}
\]

The length of styly and stamens, the area of petal and sepal, flower length and width of the two morphs of *S. macrophylla* (long-styled plants and short-styled plants) were analyzed using one-way ANOVA.

3. Result
Results of the present study have shown the age and plant size at the first-time flowering, as well as the morphology of flowers, fruits, and seeds of *Sarcotheca macrophylla*. Age of *S. macrophylla* at the first time flowering was determined by recording time from seed germination, seedling development to the first-time flowering which ranged from 5 to 16 months after seed germination (table 1). There seems to be no differences in the time range of first-time flowering between seeds originated from Katingan, Central Kalimantan and Botanical Garden collection. The range of age at the first time of flowering in 2015 was 7-10 months, in 2016 (5-16 months), in 2017 (6-8 months), in 2018 (7-8 months), and in 2019 (7 months) after seed germination (table 1). More than 40% of the seeds germinated and even seeds sown in 2017 and 2018 all germinated. The percentage of the flowering plants was different. All the plants grown from seeds sown in 2015 were able to produce flowers (100%), while the percentage of the plants grown from seeds sown in 2016 and 2017 that produced flowers were 40% and 50%, respectively. Only 20% of plants grown from seeds sown in 2018 and 2019 produced flowers. Although the percentage of flowering plants in different years of experiments was different, the age of the plant at the first-time flowering was similar (table 1).
Table 1. Development of seedlings of *Sarcotecha macrophylla* from seed germination to the first time of flowering.

| Year | Number of seeds sown | Percentage of seed germination (%) | Number of seedlings grown from seed germination | Number of plants produced first-time flowers | Percentage of plants with first time flowering (%) | Age of plants produced first-time flower |
|------|----------------------|-----------------------------------|-----------------------------------------------|--------------------------------------------|-------------------------------------------------|------------------------------------------|
| 2015 | 25                   | 53.3                              | 8                                             | 8                                          | 100                                             | 7 – 10 months                            |
| 2016 | 10                   | 40                                | 4                                             | 2                                          | 50                                              | 5 – 16 months                             |
| 2017 | 10                   | 100                               | 10                                            | 4                                          | 40                                              | 6 – 8 months                              |
| 2018 | 15                   | 100                               | 15                                            | 3                                          | 20                                              | 7 – 8 months                              |
| 2019 | 20                   | 50                                | 10                                            | 2                                          | 20                                              | 7 months                                 |

Mean plant height at the first time flowering was 19.5 cm ranging from 13 to 23 cm, compared to the maximum height of the plant (15 m) [6], indicating that the first time flowering of *S. macrophylla* occurred at seedling stage (table 2). At the first time of flowering, number of branches ranged from 0-1 (table 2). The flowers of *S. macrophylla* had typical cymosa inflorescence that consisted of some clusters of inflorescences with red-coloured flowers (figure 1). The number of flower clusters ranged from 1 to 8 (mean 4.3) and the number of leaves ranged from 8 to 12. The flowers were found arising from various points (terminal, axillary, and at the main stem) (table 2). It seems that *S. macrophylla* produced flowers before producing branches (mean number of branches 0.3).

Table 2. Height, number of branches, number of flower clusters, leaf number, and points of arising of flowers of seedlings of *S. macrophylla* at the first-time flowering.

| Parameters                      | Range | Mean |
|---------------------------------|-------|------|
| Height (cm)                     | 13-23 | 19.5 |
| Number of branches              | 0-1   | 0.3  |
| Number of flower clusters       | 1-8   | 4.3  |
| Leaf number                     | 8-12  | 9.67 |
| Points of flower arising        | Terminal, axillary, and at the main stem | -    |
In more detailed morphology, flowers of *S. macrophylla* consisted of five oblong tepals and sepals (figure 2). Two morphs of *S. macrophylla* had significantly different morphology as presented in Table 3. The styly length of the two morphs was significantly different (table 3). The short-styled plant had short styly (mean styly length 1.3 ± 0.17 mm) and long stamens (mean stamen length 3.07 ± 0.12 mm), while the long-styled plants had long styly (mean styly length: 3.33 ± 0.29 mm) and short stamens (mean stamen length 1.3 ± 0.17 mm) (table 3, figure 2B). Sepal area, petal area, and flower width were not significantly different between the two morphs. The flower length was significantly different between the two morphs, with long styled-plants had longer flowers (mean flower length 6.93 ± 0.12 mm) compared to the short-styled plants (mean flower length 5.5 ± 0.00 mm) (table 3).

**Table 3.** Measurement of two morphs (long-styled and short-styled plants) of *S. macrophylla*.

| Morphs                  | Short-styled     | Long-styled     |
|-------------------------|------------------|-----------------|
| Style length (mm)       | 1.30 ± 0.17 (b)  | 3.33 ± 0.29 (a) |
| Stamen length (mm)      | 3.07 ± 0.12 (a)  | 1.30 ± 0.17 (b) |
| Sepal area mean ± SD    | 1.73 ± 0.57 (a)  | 1.56 ± 0.54 (a) |
| Petal area mean ± SD    | 5.46 ± 0.84 (a)  | 5.69 ± 0.34 (a) |
| Flower length (mm)      | 5.50 ± 0.00 (b)  | 6.93 ± 0.12 (a) |
| Flower width (mm)       | 5.40 ± 0.17 (a)  | 5.83 ± 0.29 (a) |

Note: different letters within parenthesis indicate significantly different.
Figure 2. A. Flowers of *Sarcotheca macrophylla* with five oblong sepals and tepals, B. heterostyly (distyly) of *S. macrophylla*, first with long styly and short stamens and the second with short styly and long stamens.

Observation on the fruits of *S. macrophylla* showed that fruits were arranged in clusters. The color of unripe fruits was red maroon, while the color of ripe fruits were black purple (figure 3). *S. macrophylla* fruits are ripe after approximately 40 days. Range of number of fruit clusters per individual was 2-8 fruit clusters. The number of fruits per cluster ranged from 0 to 10, with the fruit length ranged between 8 and 10 mm and the fruit width ranged from 7 to 9 mm (table 4). The seeds of *S. macrophylla* were ellipsoid and black greyish (figure 3). Number of seeds per fruit of *S. macrophylla* ranged from 0-3 seeds per fruit, with the seed length ranged from 5 to 6 mm and the seed width ranged between 3 and 4 mm (table 4).

Table 4. Measurement of fruits and seeds of *S. macrophylla*.

| Measurement                        | Range   |
|------------------------------------|---------|
| Range of number of fruits per cluster | 0-10    |
| Range of fruit length (mm)         | 8-10    |
| Range of fruit width (mm)          | 7-9     |
| Range of number of seeds per fruit | 0-3     |
| Range of seed length (mm)          | 5-6     |
| Range of seed width (mm)           | 3-4     |

Figure 3. A. Plants of *S. macrophylla* with clusters of fruits, B. fruits of *S. macrophylla*, C. seeds of *S. macrophylla*. 
4. Discussion
The present study has shown the flowering and fruiting of *S. macrophylla* in terms of first-time flowering and the morphology of flowers, fruits, and seeds. First-time flowering of *S. macrophylla* occurred at 5 to 16 months after seed germination with the plant height ranged from 13 cm to 23 cm indicating early reproduction of this endemic species. Early reproduction is advantageous because it (i) increase investment in reproduction early in the life cycle and (ii) increase relative fitness as it can avoid a high risk of mortality before reproductive maturity or poor growth condition during late reproduction [10].

Observation on the morphology of flowers of *S. macrophylla* in the present study showed that there were two morphs, namely (i) short-styled plants and (ii) long-styled plants. The short-styled plants had long stamens, while the long-styled plants had short stamens. This confirmed the heterostyly (in this case distyly, with two types of styly (short styly and long styly) in the genus *Sarcotheca*. Similar results were observed in the morphology of flowers of *Sarcotheca celebica* that had two morphs (short-styled and long-styled plants), with similar characteristics that the short-styled plants had long stamens, while the long-styled plants had short stamens [17]. In the scope of higher level within the plant family of Oxalidaceae, the present study also confirmed heterostyly (distyly; tristyly) that have been widely reported in some species within this plant family, such as *Averrhoa carambola*, *Biophytum fruticosum*, *B. radiantoides*, *B. microphyllum*, *Dapania pentandra*, and *Oxalis cytosioidea*, *O. debilis*, O. *deppei*, *O. latifolia* [6,15,24]. Other species from other plant families are also reportedly to be heterostylous, such as *Tylosemia esculentum* (Leguminosae), *Primula apennina* (Primulaceae), *Psychotria nuda* (Rubiaceae), *Polygonum equisetiforme*, *P. aviculare*, and *P. maritimum* (Polygonaceae) [22,25–27].

Observation of *S. macrophylla* flowers in the present study showed that flower size of short-styled plants and long-styled plants was significantly different, with long-styled plants had longer flower than the short-styled ones. This indicates that this heterostylos syndrome related to heteromorph and polymorphisms of the flowers. Other studies also reported the heteromorph of flowers of heterostylous plant, such as the difference of flower size between two morphs of the distylos *Primula veris* (Primulaceae), with the long-styled plants had larger flower than the short-styled ones [28].

Both morphs of *S. microphylla* were able to produce fruits, however many fruits were set with low number of seeds (0-3 seeds). This might be partly due to the characteristics of distylo syndrome of the species that is commonly associated with self-incompatibility which is often linked to the low seed set [19,22]. Self-incompatibility prevents intra-morph fertilization with which pollen from the same flower or from a flower from the same morph is inhibited in germination, thus, prevent fertilization [22,29,30]. During an incompatibility reaction, pollen tube growth may be interrupted in several regions including stylly, and regions between the stigma and the ovule [25,31]. Similar results on another species (*S. celebica*) has been reported that both long-styled and short-styled plants of *S. celebica* set fruits, however, many fruits contained low number of seeds [17].

The low seed set in this endemic species can have consequences on the low capacity for regeneration that can lead to the population decline. The present study has shown an implication in the conservation of this endemic species since the species produce extremely low number of seeds. Efforts to increase seed set in this endemic species are required. Some studies reported that hand pollination of inter-morphs of distylos plant species can increase the seed set [17]. Further study on the reproductive biology and ecology including pollination and pollinators of this species is required to increase understanding of the factors limiting the seed set in this species and to overcome the shortage of seeds in this endemic species.

5. Conclusion
The present study has shown the flowering and fruiting of *S. macrophylla*, an endemic species of Borneo, and the implication of conservation of this endemic species. The first time flowering of *S. macrophylla* occurred at 5-16 months after seed germination indicating that the first-time flowering occurred at early stage that is known to have some advantages, such as to increase investment in reproduction early in the life cycle. Observation on detailed morphology of the flowers of *S. macrophylla* has shown heterostylos syndromes (different styly length) that is commonly linked to the low seed set. Further study on the reproductive biology and ecology including pollination and pollinators of this species is required to
increase understanding of the factors limiting the seed set in this species and to overcome the shortage of seeds in this endemic species.

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