Observations on Flowers of the Genus *Bruguiera* from Setiu Mangrove Areas, Terengganu

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Abstract The purpose of this research was to study the flower morphology of mangrove trees from the genus *Bruguiera* (Rhizophoraceae) at Setiu mangrove areas, Terengganu. The objective of this study is to determine the pollination strategy of each species in this genus from their floral traits. A total of five *Bruguiera* species was recorded in this study, including the new locality record of *Bruguiera rhynchopetala*. A total of 33 flowers from 11 trees were collected for morphometric study. The results showed that *B. gymnorhiza* and *B. rhynchopetala* were the largest flowers as compared to *B. sexangula. Bruguiera rhynchopetala* however, showed overlap traits between the parents, and thus suggested the same pollination strategy. Meanwhile, *B. cylindrica* showed the smallest flower from all the measurements recorded and *B. hainesii* showed intermediate size. The small herkogamy in *B. cylindrica* indicates self-pollination, while the large herkogamy in the other four species might indicates cross-pollination by pollinating agents, possibly birds.

Keywords Flower morphometric · Herkogamy · Mangrove · Pollination

Introduction

Globally, mangrove vegetation constitute approximately 80 species from 20 families (Duke 1992). These species are mainly from two main orders, the Myrtales and Rhizophorales, which comprise about 25% of all mangrove families, and almost 50% of all species. The better known taxa of mangroves however are the members of order Rhizopholares, family Rhizophoraceae, consisting of *Rhizophora*, *Bruguiera, Ceriops* and *Kandelia* (Hogart 2007). *Bruguiera* is the largest genus in the family Rhizophoraceae. In Malaysia, genus *Bruguiera* consists of five species

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which are *Bruguiera cylindrica, B. gymnorhiza, B. hainesii, B. parviflora* and *B. sexangula*. Based on its flower size, these species is categorised into two groups which are the large-flowered group that includes *B. gymnorhiza, B. hainesii* and *B. sexangula* while the small-flowered group consists of *B. cylindrica* and *B. parviflora*.

Flower characteristics such as morphology, colour, nectar and odour, will provide the best strategy for the plant to attract specific pollinators (Nagarajan et al. 2010). For example, the large-flowered group are usually with recurved pedicels and solitary flower, which obviously suitable for the nectarivorous birds to access the flowers and also to prevent the rainfall from diluting the nectar (Tomlinson 1986). In addition, the large flowers also produce nectar in large quantity that is secreted in a deep calyx cup. For the small-flowered group, the flowers are not recurved and small in size, flat calyx cup and with a small quantity of nectar. Therefore based on the characteristics, the large-flowered group is thought to be pollinated by birds while the small-flowered group is considered as insect-pollinated.

Other than the flower size, the relationship between floral traits and its pollinators in genus *Bruguiera* is still poorly understood. *Bruguiera hainesii* for example, reported to be pollinated by birds from their regular visitation to the flowers and also large amount of nectar produced by the flower (Noske 1993). The characteristics of the flower however showed combination of characters such as relatively large flower size and in multiple-flowered inflorescences (Jamilah et al. 2013; Sheue et al. 2005). Recent study by Ono et al. (2016) showed that *B. hainesii* is a hybrid between the small-flowered *B. cylindrica* (maternal parent) and the large-flowered *B. gymnorhiza* (paternal parent). As hybridisation could occur from the sharing of pollinators, this showed that bird visitations to the small-flowered *B. cylindrica* or insect visitation to the big-flowered *B. gymnorhiza* resulted in fertilisation of the ovules. Therefore, other flower characteristics is needed to describe the pollination strategy in this genus. Thus, we examined the flower attributes in genus *Brugueira*, to infer the pollination strategy of each *Brugueira* species in Setiu.

Setiu is located in the northeast of Peninsular Malaysia in the state of Terengganu (5° 40′ N, 102° 43′ E). The mangrove areas of Setiu consist mainly of nipa palm, *Nypa* (Aracaceae) and some stands of true mangrove, *Rhizophora* (Rhizophoraceae) (Nakisah and Fauziah 2003). Previous study in Setiu mangrove areas recorded only three of the five *Bruguiera* species reported to occur in Malaysia (Mohd Lokman and Sulong 2012), namely *Bruguiera cylindrica, B. gymnorrhiza* and *B. sexangula*. The critically endangered *B. hainesii* however, was recently discovered in Setiu mangrove area by Jamilah et al. (2013), making the total to four *Bruguiera* species recorded at Setiu. The four *Bruguiera* species can be distinguished based on the colour of their calyx tube. The calyx tube is yellow-green in *B. cylindrical*, red to pink in *B. gymnorrhiza*, varied from pink-red to greenish *B. hainesii*, and yellow in *B. sexangula* (Tomlinson 1986).

Flower Morphology

During collections of flowers in May 2015, we encountered a huge tree with redish calyx flowers which we firstly identified as *B. gymnorhiza*. Further observations showed that the flowers are *B. rhynchopetala*, characterise from the presence of petal bristles (Fig. 1). *Bruguiera rhynchopetala* is a hybrid previously known to occur in China and Australia (Duke and Ge 2011). In Malaysia, the first record of this species was from Pulau Langkawi, Kedah (Wan Juliana et al. 2014), therefore the finding of this study marked the second locality record of this species in Malaysia (Mohd Razali Salam, pers. comm.). The flowers observed in this study were with light red calyx tube, although reported previously to have rosy blush to all green calyx tube by Tomlinson (1986).

The flower attributes (mean ± SD) for each *Bruguiera* species were as summarised in Table 1. Among species, *B. rhynchopetala* recorded the largest size of flower with petal length of 11.74 ± 0.29 mm, calyx tooth length of 17.63 ± 0.31 mm, calyx length of 34.28 ± 0.95 mm, style length of 20.06 ± 0.79 mm, stamen length of 11.65 ± 0.55 mm and flower disk depth of 6.55 ± 0.52 mm. *Bruguiera gymnorhiza* on the other hand, showed the largest in corolla width (5.97 ± 0.37 mm), herkogamy (9.09 ± 0.84 mm), stigma width (0.50 ± 0.04 mm), and highest count in number of calyx (13.44 ± 0.29 mm), number of petals (12.55 ± 0.60 mm) and number of stamen (21.44 ± 1.13 mm). Meanwhile, *B. cylindrica* showed the smallest size in all data recorded.

From the Principal Component Analysis (PCA), results showed that the first two components explained 85.8% of the total variance combined. The first component

Fig. 1 A flower and a close bud of *Bruguiera rhynchopetala* found at Setiu mangrove area, Terengganu. (Photograph by Mohamed Nor Zalipah)

Table 1 Mean (± SD) of flower attributes of five *Bruguiera* species recorded in Setiu, Terengganu

Characteristics/species	*B. cylindrica* (N = 9)	*B. gymnorhiza* (N = 9)	*B. hainesii* (N = 6)	*B. rhynchopetala*(N = 3)	*B. sexangula*(N = 6)
Petals length (mm)	2.44 ± 0.18^{a}	11.59 ± 2.20^{c}	5.32 ± 0.39^{b}	11.74 ± 0.50^{c}	11. 23 ± 0.35^{c}
Calyx tooth length (mm)	4.58 ± 0.21^{a}	16.15 ± 2.78^{c}	8.88 ± 0.78^{b}	17.63 ± 0.53^{c}	16.36 ± 0.57^{c}
Calyx length (mm)	9.82 ± 0.27^{a}	34.10 ± 3.37^{c}	20.39 ± 2.08^{b}	34.28 ± 1.65^{c}	32.26 ± 0.84^{c}
Corolla width (mm)	2.18 ± 0.37^{a}	5.97 ± 1.10^{d}	3.96 ± 0.64^{b}	5.55 ± 0.83^{c,d}	4.53 ± 0.16^{b,c}
Style length (mm)	3.99 ± 0.26^{a}	19.33 ± 2.87^{d}	9.70 ± 0.67^{b}	20.06 ± 1.37^{d}	16.34 ± 0.55^{c}
Stamen length (mm)	2.79 ± 0.14^{a}	10.24 ± 2.20^{c}	4.70 ± 0.57^{b}	11.65 ± 0.95^{c}	9.76 ± 0.62^{c}
Flower disk depth (mm)	3.24 ± 0.35^{a}	6.02 ± 1.12^{b}	3.64 ± 1.04^{a}	6.55 ± 0.89^{b}	4.36 ± 0.37^{a}
Stigma width (mm)	0.22 ± 0.07^{a}	0.50 ± 0.13^{b}	0.21 ± 0.08^{a}	0.38 ± 0.04^{b}	0.26 ± 0.02^{a}
Herkogamy (mm)	1.20 ± 0.31^{a}	9.09 ± 2.53^{c}	5.00 ± 0.64^{b}	8.41 ± 2.12^{c}	6.58 ± 0.98^{b,c}
Number of petals	7.56 ± 0.53^{a}	12.55 ± 1.81^{c}	10.00 ± 0.63^{b}	12.00 ± 0.00^{c}	10.33 ± 0.33^{b}
Number of calyx	7.67 ± 0.50^{a}	13.44 ± 0.88^{d}	10.00 ± 0.26^{b}	11.67 ± 0.58^{c}	10.50 ± 0.22^{b}
Number of stamen	11.11 ± 2.71^{a}	21.44 ± 3.40^{b}	14.83 ± 2.32^{a}	15.67 ± 11.02^{a,b}	11.83 ± 2.51^{a}

Different letters indicate significant differences from one another (multiple comparison following significant result from Kruskal-Wallis test. Significant results were recorded at $P < 0.001$ for all characteristic)

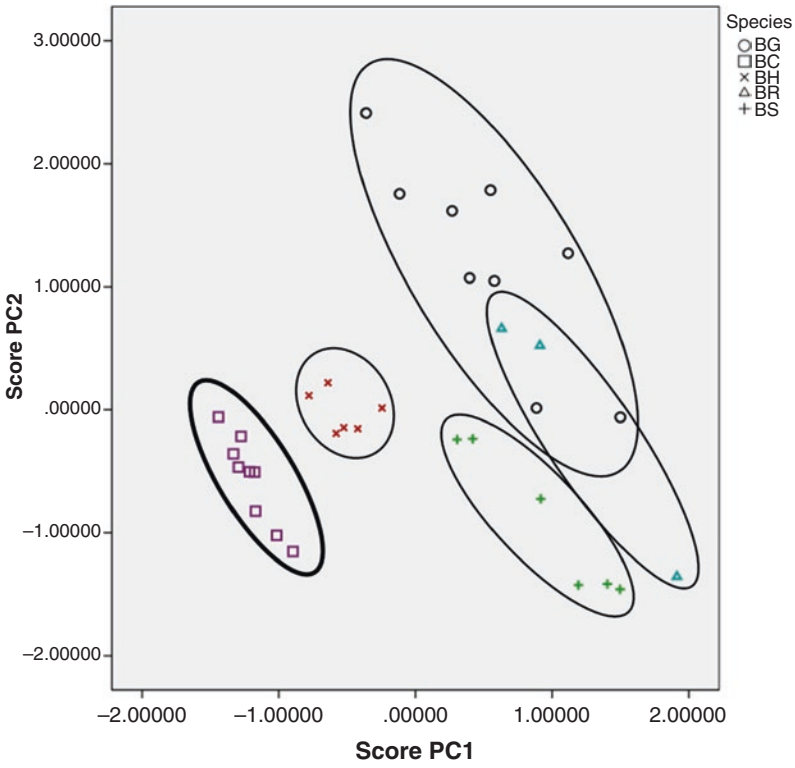

Fig. 2 Ordinations of *Bruguiera* flowers on the first two principal component axes of principal component analysis (PCA)

revealed the height of the flower which indicate by the petal length, calyx tooth length, calyx length and style length of the flowers. The second component explained the gynoecium (stigma width) and androecium (number of anthers) in the flowers. The flower morphology of *Bruguiera* species plotted in morphospace defined by the first two components of PCA showed that *B. cylindrica, B. hainesii* and *B. sexangula* were totally different with no overlap between species (Fig. 2). However *B. gymnorhiza* and *B. rhynchopetala* showed overlapping in the characteristics measured and recorded.

The smallest flower among *Bruguiera* species in Setiu, *B. cylindrica* can be found in abundance at the mangrove area (Nakisah and Fauziah 2003). The trees can be found beside the river and in swampy areas of the mangrove. With its small flowers, this species was found to be pollinated by thrips (Nagarajan et al. 2008) while Tomlinson et al. (1979) reported butterflies as their pollinators. The adaption for insect pollination in *B. cylindrica* is similar with the other small-flowered *B. parviflora*, except for the shorter floral cup and with distinct calyx cup. However, the small herkogamy (anther-stigma separation) of this species indicates efficient pollen transfer, thus self-pollination in this species is possible (Ushimaru and Nakata 2002), although reported to be insect pollinated.

According to Duke and Jackes (1987), in mangroves, the morphological attributes of the hybrids are either intermediate or shared between the maternal plants. *Bruguiera hainesii* has recently been reported as a hybrid between *B. cylindrica* and *B. sexangula* (Ono et al. 2016) and our PCA analysis showed that the flower is intermediate in size between the parents. It is interesting to note however, herkogamy for this species indicates overlapping with the large-flowered *B. sexangula,* therefore might adopt the same pollination strategy. The identity of pollinator species however, might differ from the difference in flower size. This is because, the mismatch with flower morphology might resulted in visitors failing to make contact with the reproductive organ of the flowers while feeding at the flowers (Muchhala 2003; Pandit and Choudhury 2001) thus reducing potential for fertilisation to occur. Noske (1993) however reported that the aggressive behaviour showed by sunbirds is likely the reason for selection of *Bruguiera* flowers visited by the birds, therefore restricted their potential as pollinators to certain species of *Bruguiera* in mangroves elsewhere of Peninsular Malaysia.

The other hybrid, *B. rhynchopetala* showed more overlap in morphology including herkogamy with *B. gymnorhiza* compared to *B. sexangula.* The measurements recorded showed that it is slightly bigger that *B. gymnorhiza* and with lighter red calyx tube. Larger herkogamy usually indicates cross-pollination therefore required animal pollinators for this group. The natural hybridisation between *B. gymnorhiza* and *B. sexangula* is possible from the sharing of pollinators, for this case, the birds. Wee et al. (2014) reported that two species of common sunbird in Singapore, the olive-backed sunbird (*Nectarinia jugularis*) and the copper-throated sunbird (*Nectarinia calcostetha*) as pollinators for *B. sexangula*. *Bruguiera sexangula* usually occurs in upstream reaches of freshwater dominated estuaries, while *B. gymnorhiza* is more marine and tolerates to high levels of salinity (Duke and Ge 2011). Although habitat differentiation between the two species may reflect their different level of salt tolerance, considerable overlap in geographic distribution and habitats provides opportunities for hybridisation. Other factor contributing to natural hybridisation includes the long and partially overlapping flowering periods (Duke and Jackes 1987).

Conclusion

In Setiu, based on flower morphometric analysis, the large-flowered group consists of *Bruguiera gymnorhiza. B. sexangula*, and their hybrid, *B. rhynchopetala*, while the small-flowered group was only represented by *B. cylindrica*. *Bruguiera hainesii* on the other hand, is intermediate in size between the two groups. Although previous study reported possible bird pollination for this species, their flower morphology suggests that this species might be pollinated by smaller size pollinators as compared to the other three large-flowered *Bruguiera.* Larger herkogamy in the large-flowered group including *Bruguiera hainesii,* indicates cross-pollination by birds, while small herkogamy suggested self-pollination in *B. cylindrica.* To

confirm pollination by pollinators however, more study on floral functional aspects (such as floral persistence, anthesis time, pollen production, nectar production etc.) of this genus is needed. A better understanding of the pollination biology of this important mangrove taxa is fundamental to conserving these important coastal ecosystems.

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Appendix

Eigenvectors of the first two components extracted in Principal Component Analysis (PCA) for each variable.

Variable	Component 1	Component 2
Petals length	0.948	−0.242
Calyx tooth length	0.941	−0.296
Calyx length	0.968	−0.210
Corolla width	0.868	0.139
Style length	0.979	−0.162
Stamen length	0.921	−0.201
Flower disk depth	0.851	−0.069
Stigma width	0.748	0.366
Herkogamy	0.884	−0.096
Number of petals	0.874	0.270
Number of calyx	0.930	0.198
Number of stamen	0.561	0.683

The flower of *Bruguiera hainesii*, *B. gymnorhiza* and *B. sexangula* (left to right) showed different colour of calyx (Photograph by Nur Athirah Ismail)

The difference in flower size of *Bruguiera cylindrica, B. hainesii*, *B. gymnorhiza* and *B. rhynchopetala* (left to right) (Photograph by Nur Syamimi Wahab)

Sunbird observed assessing the flowers of *Bruguiera rhynchopetala* at Setiu mangrove area (Photographs by Ahmad Fadhli Adzemi)

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