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Morphological characteristics of Indonesian *Rubus* flowers

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Abstract. *Normasiwi S, Salamah A, Surya MI. 2021. Morphological characteristics of Indonesian Rubus flowers. Biodiversitas 22: 1441-1447. Rubus* spp. are woody or herbaceous plants that can be used for fruit, ornamental and medicinal purposes. The increasing use of *Rubus* as a commercial species is highly dependent on the formation of high-quality genetic material. However, the lack of basic biological knowledge is one of the limiting factors in this development. This research aims to describe the morphological characters of Indonesian *Rubus* flowers at Cibodas Botanical Garden, Indonesian Institute of Sciences, Cianjur, West Java, Indonesia. From January to June 2020, we observed nine *Rubus* species with five replications, namely *R. alceifolius, R. chrysophyllus, R. ellipticus, R. fraxinifolius, R. linneatus, R. moluccanus, R. pyrifolius, R. rosifolius*, and *Rubus* sp (Blackberry) in Cibodas Botanical Garden, Indonesia. The results showed variations in the characteristics of the pistil, stamens, torus shape, and duration of flowering stages between species. The mean value of the stamen-pistil ratio for *R. pyrifolius* was highest among other species (8.27), and *R. fraxinifolius* was the lowest (0.16). Furthermore, a correlation analysis between stamens and pistils for nine *Rubus* species was relatively positive (r = 0.598), similarly distinctly positive between pistils and fruits with r = 0.763. Flower development duration takes ranging 10-12 days from initiation to anthesis, depending on the species.

Keywords: Anthesis, floral biology, *Rubus*, reproductive systems

INTRODUCTION

Plant reproductive systems and morphology have unique relations (Peng et al. 2014). Flowering plants have a different major in pattern sex expression depending on their form characteristics (Endress 2010; Williams 2012; Lu et al. 2015). Diversity species of flowering plants are generally indicated by the diversity of flowers and fruits, and species classification largely depends on flower and fruits idiosyncrasies (Redmond and Stout 2018; Ramírez-Barahona et al. 2020). The diversification of floral structure and function gives a convincing illustration in adaptive evolution and offers convenience for researching the functional association between floral traits and plant mating strategies.

Rubus (*Rubus* spp.), the genera that belong to Rosaceae, are woody or herbaceous plants with simple, pinnate, or palmate leaves. It is distributed in all continents except Antarctica on cultivable soils ranging from tropical to subarctic regions (Alice and Campbell 1999). In the tropics, *Rubus* are found in mountainous areas (Setyawan 1999). The prevalence of interspecific hybridization, polyploidy, and various forms of apomixis in *Rubus*, made it having a lousy reputation among taxonomists due to taxonomic classification became difficult (Kalkman 1993; Yang et al. 2015; Sochor et al. 2019; Lechowicz et al. 2020). In 1910, Focke grouped *Rubus* into twelve subgenera, namely *Chamaemorus, Cylactis, Dalibarda, Chamaebatus, Comaropsis, Orobatus, Dalibardastrum, Malachobatus, Anaplobatus, Idaeobatus, Lampobatus*, and *Rubus (Eubatus).* Furthermore, Zandee and Kalkman (1981) note four subgenera distributed in Malesia, including *Chamaebatus, Idaeobatus, Malachobatus, and Micranthobatus.* Moreover, 46 *Rubus* species were recorded in the Malesia region, 25 spread in Indonesian mountain forests. In general, these genera display similar characters, such as having an inflorescence terminal in 5-merous flowers, being mostly bisexual, rarely unisexual, and being dioecious, with numerous stamen and pistils on various type of torus. These plants most notable characteristic is their collective drupes fruit with or without the torus, rarely loose individually, with a juicy or fleshy mesocarp (Kalkman 1993).

Reproductive biology and flowering observations of some *Rubus* species (Raspberry, Blackberry) have been reported from America, North American, New Zealand, Norway, and Britain (Williams 1959; Heide and Sønsteby 2011; Nielsen et al. 2017; Hodnefjell et al. 2018; López et al. 2019). Most of these studies focused on single or commercials species. However, floral character morphology studies of wild *Rubus* are rare in South-east Asia, especially in Indonesia. Furthermore, the Indonesian *Rubus* flower's diversity has attracted little attention in the study of the functional association between floral traits and plant mating strategies. Cibodas Botanical Gardens (CBG), an ex-situ conservation institution located at the Cibodas Biosphere Reserve area, has thirteen wild species of *Rubus* as their garden collections. They namely *Rubus*

acuminatissimus, Rubus alpestris, Rubus alceifolius, Rubus chrysophyllus, Rubus ellipticus, Rubus elongatus, Rubus fraxinifolius, Rubus lineatus, Rubus moluccanus, Rubus rosifolius, Rubus sumatranus, Rubus pyrifolius, and *Rubus* sp. Besides these species, CBG also has commercials *Rubus* cultivar such as blackberry and raspberry.

Susandarini (2016) and Moreno-Medina et al. (2018) reported that *Rubus* is one of the pioneers of forest succession and is potentially used for fruit, ornamental, and medicinal purposes. The development of *Rubus* as commercial species depends on the development of high-quality genetic material. In-plant breeding program, the desired characteristics are obtained using existing variability, and the essential process for plant improvement is the understanding of pollination mechanisms (Frankel and Galun 2012; Sedgley and Griffin 2013). Furthermore, such material will require information on the species basic biology, which is currently lacking in some respect. This research aims to describe the morphological character of nine species of Indonesian *Rubus* flowers at Cibodas Botanical Garden. The information *Rubus's* floral morphology may add information on flower characters diversity for pollination knowledge and breeding systems.

MATERIALS AND METHODS

Study species and site

All observations and experiments were carried out at Cibodas Botanical Garden, Indonesian Institute of Sciences, Cianjur, West Java, Indonesia, from January to June 2020. CBG is located in the Cibodas Biosphere Reserve area with an altitude of 1200-1400 m a.s.l., an average temperature of 20°C, and air humidity up to 96 %. Nine species *Rubus* spp. were investigated, consisting of eight wild species, *R. alceifolius, R. chrysophyllus, R. ellipticus, R. fraxinifolius, R. linneatus, R. moluccanus, R. pyrifolius, R. rosifolius*, and one commercial species, namely *Rubus* sp. (Blackberry).

Flower development

Observation of the nine *Rubus* species flowering stage was carried out at each stage of development, starting with a flower bud with a 1 mm diameter until one day before the flower anthesis. Then, the number of days needed for anthesis was also calculated.

Flower morphology characters

The variations in the flower morphology traits of *Rubus* spp were observed in the flower of nine species of *Rubus*, which were randomly selected with five replications. The flower's recorded characters were the number of pistils, the number of stamens, the stamen-pistil ratio, the number of fruits, and the inflorescence half flower at anthesis. Some additional characters were the color of the pistil and stamen, flower bud diameters one day before (D-1) anthesis, flower bud growth diameters, and the torus's size and shape at one day before (D-1) anthesis; the sepal and petal colors also observed. The number of pistils, stamens, and fruits were identified by the naked eye and counting manually. The size and form of flowers were measured by Microdirect 1080P HDMI Handheld digital microscope, then calculated with a mean value of five flowers per species. The torus's shape was divided into four categories: umbrella, triangular, parallel, and bulge. The Munsell plant tissue color chart measured the flower color. Then, the data were obtained, processed by the MS. Excel and IBM SPSS Statistics 21 software to get the mean value of size parts of flowers, stamens, pistils, and fruits number correlation within species analyzed by Rank Spearman correlation (two-tailed). Some data sets were transformed to a logarithmic scale before analysis to equalize variances. Fisher's Least Significance Different (LSD) 5% was used as further statistic analysis when ANOVA shows significant differences and all the variable were analyzed by Principal Component Analysis (PCA)

RESULTS AND DISCUSSION

Flower development

The nine species of *Rubus* have different flower development (Figure 1). Time from flower initiation to anthesis (fully open flower) ranging from 10-12 days. Among the nine species of *Rubus*, the faster growth of flowering time is *R. linneatus* and *R. chrysophyllus*. Simultaneously, those that take longer to anthesis are *R. alceifolius, R. ellipticus, R. fraxinifolius*, and *Rubus* sp (Blackberry).

The appearance of half flower-cuts in Figure.2 illustrates a difference in length of pistils and stamens among the species and the torus variation shape. Anthers of those nine *Rubus* dehisced from outer to inner, which started at anthesis until D+3, and after D+3, anthers leak and dry out. Similarly, most stigmas were receptive at the anthesis stage. On the other hand, nine *Rubus* on observation produced nectar at the filaments' base as a reward.

Flower morphology characters

Comparing the number of pistils, stamen, and fruit of *Rubus* (Table 1) showed that *R. rosifolius* has the highest number of stamen and pistil among the species, with an average of 122 stamens and 404.6 number pistils. Meanwhile, *R. linneatus* had the lowest number of stamens with 42.67, and *R. pyrifolius* had the lowest number of pistil with 6.6. The number of fruits from natural-pollinated among the species showed that *R. fraxinifolius* was the highest number of fruits with 340.00 (93 %), and the lowest was *R. pyrifolius* with 1.33 (20 %) fruits. Oppositely, the mean value of the stamen-pistil ratio for *R. pyrifolius* was highest among other species (8.27), and *R. fraxinifolius* was the fewest (0.16).

Additionally, the correlation analysis by Spearman rank (Table 2) between stamens and pistils for nine species of *Rubus* was relatively positive (r = 0.598). Similarly, the correlation analysis between pistils and fruits distinctly positive with r = 0.763. On the other hand, between stamens and fruits was not any significant correlation.

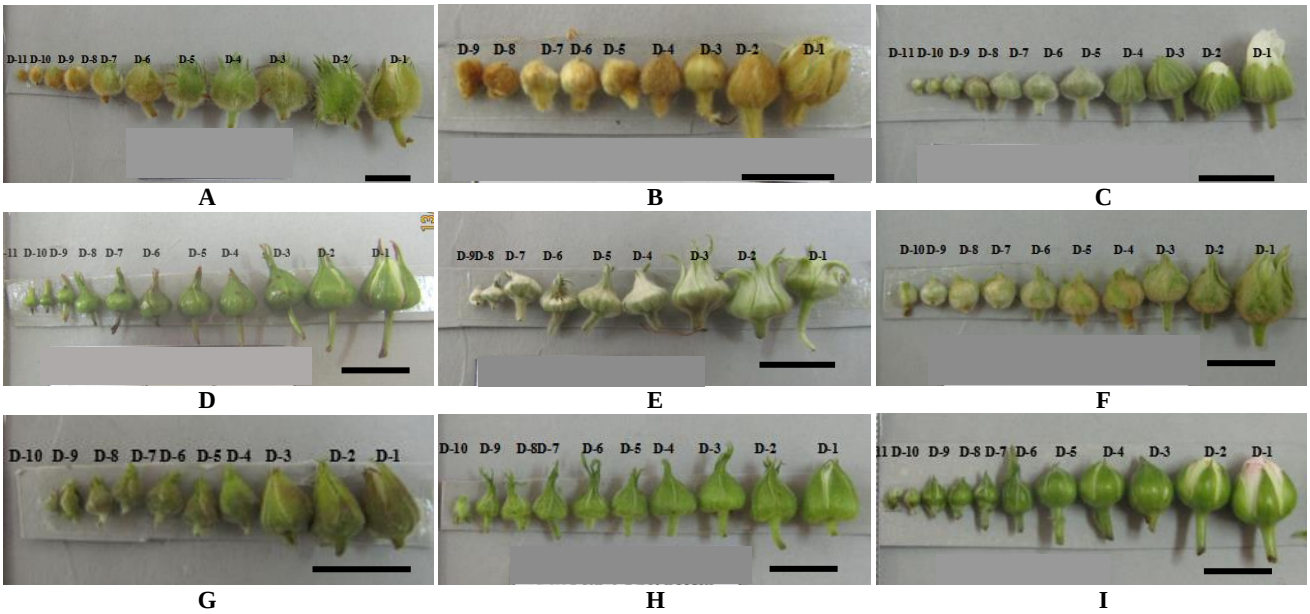

Figure 1. Flower development processes of nine *Rubus* species. A. *R. alceifolius*, B. *R. chrysophyllus*, C. *R. ellipticus*, D. *R. fraxinifolius*, E. *R. lineatus*, F. *R. moluccanus*, G. *R. rosifolius*, H. *R. pyrifolius*, I. *Rubus* sp. (blackberry). Scale bar = 1 cm

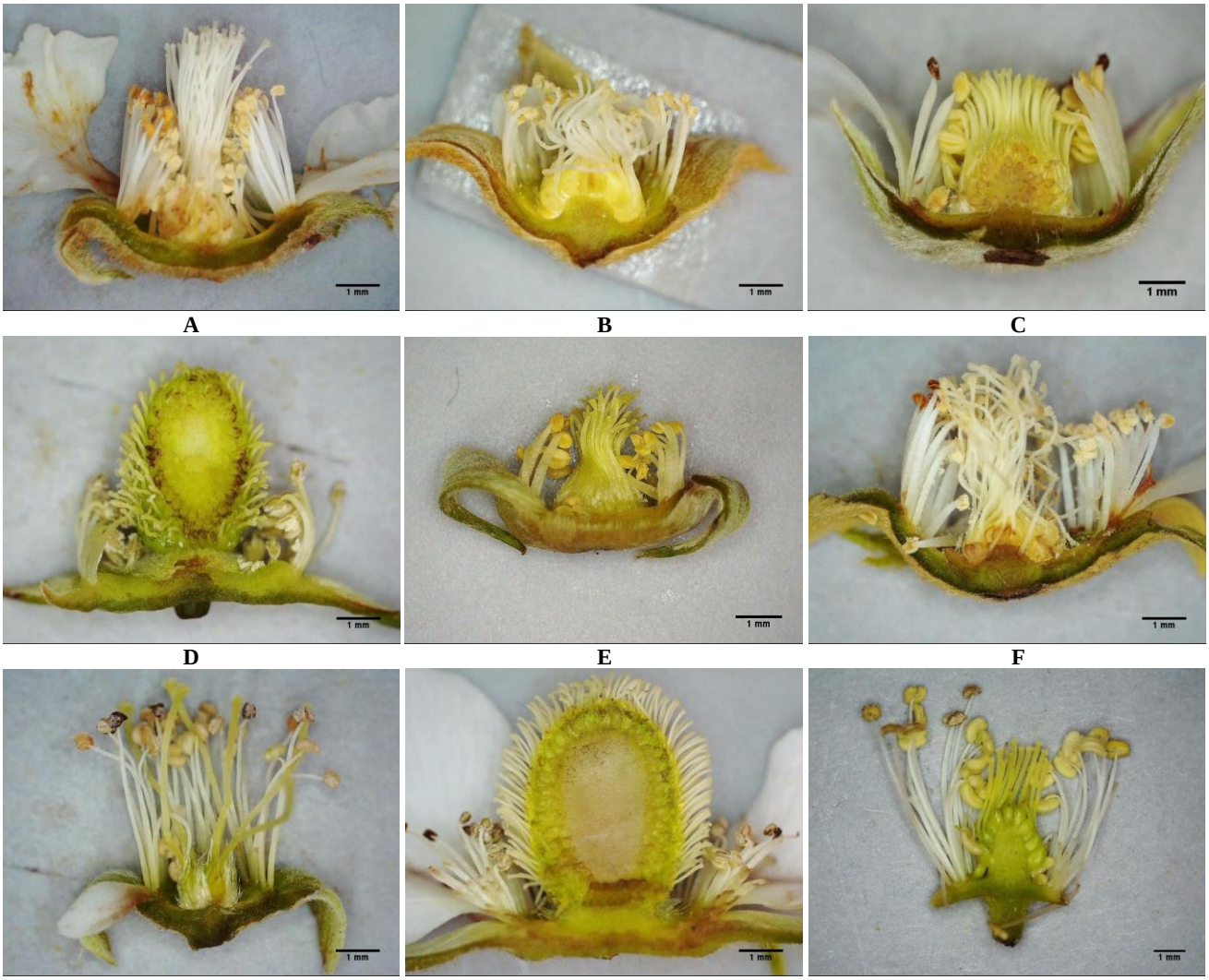

Figure 2. Half flower-cuts of nine species *Rubus*. A. *R. alceifolius*, B. *R. chrysophyllus*, C. *R. ellipticus*, D. *R. fraxinifolius*, E. *R. lineatus*, F. *R. moluccanus*, G. *R. pyrifolius*, H. *R. rosifolius*, I. *Rubus* sp. (blackberry). Scale bar = 1 mm. One day old flower (D1)

Table 1. The number of stamen, pistil, and fruit of nine species *Rubus*

Species of *Rubus*	Number of stamens	Number of pistils	Stamen-pistil ratio	Number of fruits
R. alceifolius	95.20b	73.20d	1.30bc	17.00c
R. chrysophyllus	56.25d	39.33f	1.99b	35.67bc
R. ellipticus	80.00bc	138.50c	0.58bc	58.33b
R. fraxinifolius	61.60cd	365.33b	0.16c	340.00a
R. lineatus	42.67d	67.33de	0.65bc	36.67bc
R. moluccanus	95.20b	73.20d	1.30bc	35.67bc
R. pyrifolius	53.60d	6.60g	8.27a	1.33d
R. rosifolius	122.00a	404.60a	0.30c	292.00a
Rubus sp. (Blackberry)	64.00cd	49.00e	1.33bc	26.00bc

Note: The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%

Table 2. Spearman rank correlation coefficients number of stamen, pistils, and fruits for nine species of *Rubus*

Correlation coefficient	Number of stamens	Number of pistils	Number of fruits
Number of stamens	1.00	0.598**	0.218
Number of pistils	0.598**	1.00	0.763**
Number of fruits	0.218	0.763**	1.00

** Correlation is significant at the 0.01 level

Table 3. Characteristics (length and color) stamen and pistil of nine species *Rubus*. The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%.

Species of *Rubus*	Stamen		Pistil	
	Length (mm)	Color	Length (mm)	Color
R. alceifolius	3.95b	Light yellow-green	7.32a	Grayish yellow-green
R. chrysophyllus	2.23de	Pale yellow	4.25de	Light yellow-green
R. ellipticus	2.73cd	Light yellow-green	1.90f	Light yellow-green
R. fraxinifolius	1.91ef	Pale yellow	1.82f	Grayish yellow-green
R. lineatus	1.35f	Strong yellow	4.70cd	Pale yellow
R. moluccanus	3.01c	Pale yellow-green	5.64b	Light yellow-green
R. pyrifolius	4.84a	Pale yellow	5.12bc	Strong yellow-green
R. rosifolius	2.52cde	Light yellow-green	1.81f	Strong yellow-green
Rubus sp. (Blackberry)	5.59a	Pale yellow	3.95e	Grayish yellow-green

Table 4. Characteristic flower of nine species *Rubus* (mean±s.e). The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%

Species of *Rubus*	Flower bud diameters D-1 (mm)	Flower bud growth diameters (mm/day)	Torus D-1		Flower color	
			Diameters (mm)	Forms	Petal color	Sepal color
R. alceifolius	5.83a±1.031	0.41a±0.035	1.84a±0.248	Umbrella	Grayish yellow-green	Strong yellow-green
R. chrysophyllus	3.74a±0.182	0.42a±0.063	1.35a±0.066	Triangular	Grayish yellow-green	Strong yellow-green
R.ellipticus	3.71a±0.283	0.34a±0.062	0.93a±0.071	Triangular	Pale yellow-green	Moderate yellow-green
R. fraxinifolius	5.04a±0.678	0.43a±0.361	1.52a±0.176	Bulge	Grayish yellow-green	Moderate yellow-green
R. lineatus	5.05a±0.551	0.37a±0.037	1.60a±0.140	Parallel	Grayish yellow-green	Moderate yellow-green
R. moluccanus	4.32a±0.342	0.34a±0.053	2.40a±1.185	Umbrella	Grayish yellow-green	Moderate yellow-green
R. pyrifolius	3.51a±0.105	0.25a±0.057	0.99a±0.119	Umbrella	Grayish yellow-green	Strong yellow-green
R. rosifolius	5.11a±0.439	0.30a±0.007	1.85a±0.184	Bulge	Grayish yellow-green	Strong yellow-green
Rubus sp (blackberry)	5.81a±0.132	0.48a±0.019	1.22a±0.557	Bulge	Grayish yellow-green	Strong yellow-green

Note: *D-1= one day before anthesis. ns = not significant

Table 5. Eigenvalue and percentage of variance values and factor loadings generated by PCA

	PC1	PC2	PC3
Eigenvalue	2.980	1.658	1.449
Variability (%)	37.254	20.728	18.109
Cumulative (%)	37.254	57.982	76.092
Number of pistils	0.817	0.488	-0.164
Number of stamens	0.297	0.784	-0.31
Number of fruits	0.89	0.192	0.036
Length of pistil	-0.884	0.163	0.053
Length of stamen	-0.606	0.041	-0.074
Flower bud diameter	-0.01	0.503	0.755
Flower bud growth diameter	0.044	-0.222	0.89
Torus diameter	-0.116	0.789	0.187

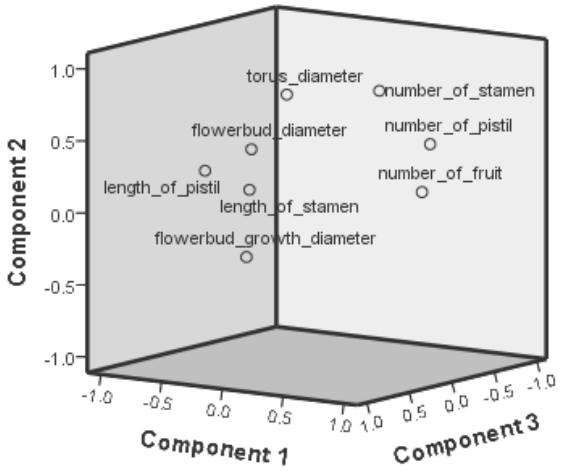

Figure 3. The component plot in rotate space by PCA

Furthermore, Table 3 gives information about the stamen and pistil's characteristics (length and color). The longest stamen and pistil, namely Blackberry (5.59 mm) and *R. alceifolius* (7.32 mm), respectively. The shortest stamen and pistil, namely *R. lineatus* and *R. rosifolius* with 1.35 mm and 1.81 mm in length, respectively.

The highest mean of flower bud diameters (5.83 mm) was found in *R. alceifolius*, followed by Blackberry with 5.81 mm in widths (Table 4). The *R. pyrifolius* has the smallest flower bud, with 3.51 mm in diameters. Variations of the floral form of the nine species of *Rubus* are also shown in the torus character. *Rubus alceifolius, R. moluccanus,* and *R. pyrifolius* have an umbrella-shaped torus; *R. chrysophyllus* and *R. ellipticus* are triangular, and *R. fraxinifolius, R. rosifolius,* and *Rubus* sp (Blackberry) have similar torus form, namely bulge. On the other hand, the torus of *R. linneatus* is parallel. Additionally, petal and sepal colors among the nine species look similar in that most petals are greyish yellow-green, while sepals' colors are moderate to intense yellow-green.

The flower morphology characters of *Rubus* were investigated by PCA (Principal Component Analysis). Table 5 and Figure 3 explain that the PCA examinated divided into PC1, PC 2, and PC 3, which a total variance of 76.091%. The PC1 alone was 37.254%, and PC 2 was 20.728%. When Eigenvalue (the length of a principal component that measures the principal component's variance) was examined in Table 5, they were 2.980 in PC1, 1.658 in PC2, and 1.449 in PC3. The PC1 was found to be associated with the number of pistils, the number of fruits, length of the pistil, and length of a stamen; PC2 was found to be the number of stamens, flower bud diameter, and torus diameter; and PC3 was found to be flower bud diameter and flower bud growth diameter.

Discussion

The morphological characteristics of all *Rubus* species reported in this study are similar and relatively consistent with those described by Kalkman (1993) and Graham and Woodhead (2011). Kalkman (1993) described the *Rubus* species as hermaphrodites, wherein elaborated thyrsoid with whole ovate sepal formed by five imbricated pieces. Moreover, Graham and Woodhead (2011) reported that *Rubus* flowers are pentamer flowers with small, white to pink petals, many stamens, and an apocarpous gynoecium of many carpels, a cone-like receptacle. Recent PCA phenetic analysis represents that similar flower forms of *Rubus* did not indicate they in the same clades (Real and Madulid 2019); instead, the homoplasy of flower forms may be correlated with pollination agents (Van der Niet et al. 2014; Souto-Vilarós et al. 2018). On the other hand, the separation of the structure of reproductive organs from their function is practically impossible because of its associated functionally and indivisible (Dafni 1992).

The development of racemose flower in *Rubus* not concurrent but alternating; one flower and the others in the raceme have a range of 1- 10 days. The onset and duration of stages in the flower development affected by endogenously sources used in the flowering process and strongly influenced by the climatic condition under which the plants were growing (Donders et al. 2014; Hatfield and Prueger 2015; Hodnefjell et al. 2018).

Nine species of *Rubus* of CBG collection used in this study divided into two subgenera are *Ideobatus* and *Malachobatus*. The species included subgenera *Ideobatus*, namely *R. ellipticus*, *R. fraxinifolius*, *R. rosifolius*, and Blackberry. On the other hand, *R. alceifolius*, *R. chrysophyllus*, *R. lineatus*, *R. moluccanus*, and *R. pyrifolius* include subgenera *Malachobatus*. The characteristics of the subgenera *Ideobatus* and *Malachobatus* were showed in the length of stamen and pistils. The length of the stamen of *R. alceifolius*, *R. chrysophyllus*, *R. lineatus*, and *R. moluccanus* (subgenera of *Malachobatus*) were longer than their pistils and have elevated torus shape. In otherwise, *R. ellipticus*, *R. fraxinifolius*, *R. rosifolius*, and *Rubus* sp (Blackberry) (subgenera Ideobatus) have the stamens shorter than the pistils and a more varied torus shape from elevated to flat (Zandee and Kalkman 1981).

The different lengths of pistil and stamen might indicate the divergence of pollination behavior for each species. Jiang et al. (2010) and Kuester et al. (2017) reported that a field investigation validated the stamen-pistil ratio showing

their outcrossing levels of the breeding system. The higher stamen-pistil ratio indicated they a nearly obligate outcrossing species; on the opposite, the getting lower of the stamen-pistil ratio indicates that they are facultative autogamous species. The positive correlation between stamen-pistil and pistil-fruit described that pistils have a significant role in pollination. The average percentage of natural pollinated-fruitfulness of each species was different. Pawar et al. (2017) reported that the self-pollination in *R. ellipticus* resulted in the highest percentage of fruit set (96.66%), followed by natural pollination (76.66%). Hiregoudar et al. (2019a) observed that the initial fruit set percentage of natural pollination was 68.75% in *R. paniculatus*.

Hiregoudar et al. (2019a,b) reported that the appearance of stigma turned to a thoroughly creamy color on the day of anthesis indicates the highest amount of sugary secretion and fresh display. The pollination conducted at this stage led to the highest fruit set. The styles became completely straight and showed peak receptivity. Further analysis of stigma receptivity and morphological change may lead to visible associations between stigma receptivity and flower development stages that could be employed in controlled pollination methods in *Rubus*.

A fundamental comprehension of flower structure, sexuality, and phenology is essential for understanding the floral life cycle needed for any pollination study (Dafni 1992; Feijo et al. 2012). Flower morphology shows the pollination mechanisms of *Rubus* and determines the most pollinator visits often. Likewise, investigation of the fertility behavior of internal verticils (androecium and gynoecium) allows the designing of improvement for breeding programs of the species (López et al. 2019). Our study shows that the variety in the floral morphology of *Rubus* would straightly impact the diversity of flower characters for pollination knowledge and breeding systems. On the other hand, information on *Rubus* spp pollination systems is critical to enhancement breeding programs with controlled pollination.

In conclusion, the nine species of Indonesian *Rubus* have a specific character of floral morphology. The flower characteristics variation shows on the pistil, stamens, torus shape, and duration of flowering stages between species. Flower development duration takes ranging 10-12 days from initiation to anthesis. Furthermore, a positive correlation was presented between the number of stamens and pistil and between the number of pistils and fruits. The variety in *Rubus*' floral morphology would directly impact flower characteristics' diversity for pollination knowledge and breeding systems.

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