



editors@smujo.id



Tulis

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Berbintang

Ditunda

Penting

Terkirim

Draf

86

Meet

Rapat baru

Gabung ke rapat

Hangout



Agus



Tidak ada kontak HangoutsCari

## [biodiv] Submission Acknowledgement

Kotak Masuk x

**Ahmad Dwi Setyawan** <smujo.id@gmail.com>

kepada saya

Inggris

Indonesia

[Terjemahkan pesan](#)

AGUS SUSATYA:

Thank you for submitting the manuscript, "The growth, mortality of flower buds and the life history of the Giant and Sumatra, Indonesia" to Biodiversitas Journal of Biological Diversity. With the online journal management system through the editorial process by logging in to the journal web site:

Submission URL: <https://smujo.id/biodiv/authorDashboard/submission/4563>

Username: agussusatya

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

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[Biodiversitas Journal of Biological Diversity](#)

Balas

Teruskan



Agus Susatya &lt;agussusatya@gmail.com&gt;

**[biodiv] Editor Decision**

2 pesan

**Smujo Editors** <smujo.id@gmail.com>  
Balas Ke: Smujo Editors <editors@smujo.id>  
Kepada: AGUS SUSATYA <agussusatya@gmail.com>

23 Desember 2019 11.30

AGUS SUSATYA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The growth, mortality of flower buds and the life history of the Giant and Rare Flower of *Rafflesia arnoldii* R.Br. In Bengkulu, Sumatra, Indonesia".


Our decision is: Revisions Required

Smujo Editors  
[editors@smujo.id](mailto:editors@smujo.id)

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[Biodiversitas Journal of Biological Diversity](#)

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7861K

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**Agus Susatya** <agussusatya@gmail.com>  
Kepada: Smujo Editors <editors@smujo.id>

6 Januari 2020 20.03

Bengkulu, January 5<sup>th</sup>, 2020

Dear To editor.

Attached filed is the second revision version.

Following notes are my respond to the comments.

1. Title and abstract has been changed and rewritten according to the comments.
2. Comment a5: been replaced by herb article
3. A6: been added by species name
4. Methods: revised as suggested
5. A8 : added reference
6. A9 : conformed with abstack
7. A10: Added scale
8. A11: Corected
9. A12 and 13: added references
10. A14: There was no available reference, but diameter tentatively categorized
11. A16: High coefficient variation has been checked, and was correct.
12. A17: added at Method
13. A18. Table 1 contained 5 stages. One stage, anthesis, was not included into the table because it occurred once. It was explained atline 138.
14. A19. Revised and the growth model was the result of the analysis.
15. A20. This general morphology of species of *Rafflesia*, and can be found at all the species of rallesia

16. A21: added figure 2.
17. A22: corrected
18. A23. The figure 3. Has detailedly been explained at the last paragraph of materials and methods.
19. A24: added species nama and reference
20. A26: added explanations
21. A27, A28, A29: been revised, rewritten, and corrected according to the comments.

Thank for your comments, and hopefully can soon be published

Best regard

Agus Susatya

[Kutipan teks disembunyikan]



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Agus Susatya &lt;agussusatya@gmail.com&gt;

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**[biodiv] Editor Decision**

2 pesan

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**Smujo Editors** <smujo.id@gmail.com>  
Balas Ke: Smujo Editors <editors@smujo.id>  
Kepada: AGUS SUSATYA <agussusatya@gmail.com>

8 November 2019 12.46

AGUS SUSATYA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The growth, mortality of flower buds and the life history of the Giant and Rare Flower of *Rafflesia arnoldii* R.Br. In Bengkulu, Sumatra, Indonesia".

Our decision is: Revisions Required

Smujo Editors  
[editors@smujo.id](mailto:editors@smujo.id)

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Reviewer A:

Authors should improve sentence construction/syntax and grammar. With these errors, there is a tendency that the ideas of the authors are misunderstood.

Some wrong spellings: devided, copule, innoculate, growth, consequive and many more

et al. - always with a "."

All scientific names/genus names should be italicized

**For Introduction:**

Focus more on life histories of parasitic plants or specific plants.

Number of species from Barcelona (2009) - is outdated. Please find more updated sources.

**Results and Discussion:**

Can be improved by dividing the section into 3 parts: Growth pattern, survivorship, life history (the authors tend to mix up these three).

Growth pattern - better represented by a line graph to show temporal differentiation in growth

Survivorship - better represented by a line graph; compare with other species of plants for further discussion or parasitic plants

Would have been perfect if microclimate data was also obtained and correlations be made with the growth patterns.

Include in discussion possible causes of bud mortality – are there possible predators? Can the relationship with the host vine also be accounted for, i.e. the more buds into a host, the lesser is the survival?

**References**

Follow format

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IN GENERAL, THIS RESEARCH IS A GOOD ONE AS IT PROVIDES VALUABLE INFORMATION NEEDED FOR THE CONSERVATION OF THE SPECIES.

Recommendation: Revisions Required

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Reviewer E:

This manuscript has valuable information, especially for *Rafflesia* study.

Bengkulu is the largest distribution center of *Rafflesia* in Indonesia, and the data presented is the result of long observation. Monitoring is very needed to know condition habitat of *Rafflesia*.

To improve the manuscript, the author can read again paragraph by paragraph and follow the instructions from the journal biodiversity.

Recommendation: Revisions Required

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Reviewer M:

The methodology and experimental design of this research are largely sound and I recommend that as such the science is acceptable and publishable.

As an English-language publication the syntax, grammar, and writing need to be significantly improved before this should be published. I am attaching a Word document where I started making some corrections to the writing, however the need for improvement is so significant, and in some cases it will be necessary to consult with the authors as to exactly what they mean, that these corrections should be done by the authors in close consultation with an English-language editor.

In addition to the problematic writing, there are extensive variations in the format, such as the use of "et al" instead of "et al.", the inconsistent use of commas (,) and semicolons (;), and a wide variety of other problems with the formatting that do not require a deep understanding of English-language writing. These should be corrected, because they detract from the research and make the article look messy, sloppy, and of low-quality.

Recommendation: Revisions Required

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Reviewer N:

Evaluation of the manuscript titled "The growth, mortality of flower bud, and the life history of the Giant and Rare flower of *Rafflesia arnoldii* R.Br. in Bengkulu, Sumatra, Indonesia"

The manuscript shows interesting topic on life history and population structure of *Rafflesia arnoldii* in Bengkulu. A relatively scarce study on the particular species which is endemic to Sumatra and protected under the government regulation. I study *Rafflesia* also and quite familiar with its life history. This manuscript provides decent observations and analysis on bud development of *R. arnoldii*. New classification on its life stage particularly during bud development is interesting. However, criteria used to determine each life stage is kind of arbitrary resulting odd (decimal) range of bud size. Exact range value is more common to be used. In addition, former study (i.e. Nikolov & Davis 2017) coined different term to name some life stage for example *protocorm* and *cormus* while this manuscript seems to coin another new term, probably explaining the different terminology with the former ones is necessary.

In the manuscript, a number of flowers (buds) within a host is called a population, while in another study by Susatya et al. 2017 the same situation was identified as a subpopulation. This also needs some explanation. Visualizing with photographs or illustrations will help the reader understand and differentiate each life stage. Another important thing is the results from direct observation on buds (diameter, age) and estimation from growth modelling is a bit unclear. Furthermore, an exponential model or J-shape graph plotting all measurements is important to be added as Figure.

I did not conduct grammar review nor language check, yet I corrected some typos. Some level of language correction is still required.

In general, this manuscript is publishable with some clarifications and revisions.

Recommendation: Revisions Required

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Reviewer P:

The said manuscript was reviewed, and a brief report about it has been prepared and uploaded for the author/editor.

Recommendation: Revisions Required

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Reviewer Q:

I have found your manuscript as an interesting issue regarding life history of *Rafflesia arnoldii* and I recommend your paper for publication with major revisions.

Please refer to a few remarks placed below:

There are too many editorial errors to list them all. That's why I'm just giving examples:

- Multiple double or triple spaces (e.g. in abstract, line 12, 17, 19 and 20).
- Different way of quoting literature in the text. Some do not comply with the Editorial Board's recommendations E.g. There are two ways to cite it in the line: 31 (Lind et al, 2013, Liu et al. 2017), the action recommends the following: Liu et al. 2017 or Line 27: Lime, 2017 > Lime 2017
- Please validate the record: Line 92: 3<sup>0</sup> 41' 42" S and 102<sup>0</sup> 32' 01" 1E. Longitude and latitude are recorded differently.
- Please validate the record. Line 107: 80%-100 % > 80%-100% , similarly throughout the manuscript
- Please validate the record: Line 179 *Keithii* > *R. keithii*.
- t.c.

The entire manuscript requires in-depth editorial correction!

- Figure 1: The figure is unclear. Needs improvement
- Figure 2: The charts should be marked, e.g. with the letters: a, b, c
- REFERENCES > the bibliography is not prepared in accordance with the editors' recommendations

Other comments are included in the manuscript.


Recommendation: Revisions Required

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Biodiversitas Journal of Biological Diversity

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**Agus Susatya** <agussusatya@gmail.com>  
Kepada: Smujo Editors <editors@smujo.id>

23 November 2019 16.13

Dear Editor,


Attached file, a revised version of the article. Hopefully, I will be published soon.

best regards

agus susatya  
[Kutipan teks disembunyikan]

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**2 lampiran**

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8729K

 **Revisions and corrections AGUS SUSATYA.docx**  
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## Responses to Reviewer's Report

Agus Susatya

Revisions and corrections have been carried out according to Reviews suggestions. Following is suggestions and my responses to them.

a. The title of the manuscript contains a two relative terms. To better serve the academic community, the title ought to be focused. Hence, it is suggested that the terms "Giant" and "Rare" in the title are to be omitted. The title of the manuscript contains a two relative terms. To better serve the academic community, the title ought to be focused. Hence, it is suggested that the terms "Giant" and "Rare" in the title are to be omitted. ***It has been revised. (line 2-3)***

b) While the abstract generally reflected the content of the manuscript, it is 20% longer than the stipulated guideline. ***It has been corrected by deleting some tenses.***

c) Under MATERIALS AND METHODS, a list of visible stages was provided. However, this list had not been introduced in the previous section nor is there an accompanying citation associated to it. ***It was revised and improved by providing pictures of all stages (line 69-79), and a reference to define a stage (Hidayati et al. 2000 and Sofiyanti et al. 2007).Line 63.***

d) The RESULTS AND DISCUSSION does not state matters regarding the Anthesis Stage be it in

Table 1 or the text. ***Anthesis or flowering bud, was not put in the table, because it occurred once. Information of anthesis has been added. Line 131-132.***

e.) One (1) table and three (3) figures were made available in this manuscript. The table presented sufficient information but it was not displayed to benefit the readers, and this is due to the format used. As for the figures, while the information was adequate, Figure 2 did not display sufficient guide to convenient the readers; the three mini-figures ought to be labeled as a), b), and c). ***Correction of the table has been done to put a, b, and c. Figure 2 became Figure 3.***

f). The author(s) claimed 11 buds died. Some explanations were provided in a previous paragraph. However, the explanation did not account the death of all 11 buds. ***Cause of the death of buds was not detailedly observed. But we explained possible causes. Line 295-296. Bud's mortality related stage has been explained. Line 309-319.***

g) This manuscript lacks a concluding section. ***Conclusion has been added***

h) 22 references were used in this manuscript and the references were appropriate. However, for the benefit of BIODIVERSITAS' subscribers, it would be good for the author to refer to the article Sofiyanti N, Mat-Salleh K, Purwanto D, Syahputra E. 2007. The note on morphology of *Rafflesia hasseltii* Surigar from Bukit Tiga Puluh National Park, Riau. Biodiversitas 9: 257-261. Yes. ***We added this article. Thank !***

**Concerning species ephithat *R. arnoldii*.** There has been some discussion about it regarding the proper use of species name *R. arnoldii* or *R. arnoldi*. I decided use *R. arnoldii* , because I



followed Meijer (1997), the authority of Rafflesia, who revised Rafflesiaceae. And this name was widely used in Rafflesia word.

The number of species of Rafflesia. ***We replaced Barcelona (2009) by Hidayati and Walck (2016).***

I have also gone through check, and recheck with grammarly software, and corrected bibliography in accordance to editor's recommendation.

Thank you for the reviewers. And hopefully, it will be published soon.

Best regard

Agus susatya





Comment [A1]: According to contents of the manuscript, it is better to change into... Life history, flower population structure, and flower bud mortality

# Growth, flower bud mortality, and life history of the *Rafflesia arnoldii* R.Br. (Rafflesiaceae) in Bengkulu, Sumatra, Indonesia

**Abstract.** The life history of *R. arnoldii* is the reflection of the complex interaction between flower bud development and the external environments in order to reach its optimal survivorship. The objectives of the study were to determine the growth, mortality of buds at various development stages, and to reconstruct the life history of *R. arnoldii*. The study was carried out at Taba Penanjung, Bengkulu Province. Two populations of *R. arnoldii* were selected for the research, where all buds were mapped, measured their diameters, and recorded their every two weeks for six months. All buds were categorized into five stages. The exponential model of growth development was applied to reconstruct the life history. Buds from the perigone stage respectively grew 3.5 and 12 times faster than those from the bract and cupule stages. The bud's mortality was higher at earlier development than the latter stages. The population structure of *R. arnoldii* changed dynamically over time. This was mainly influenced by the high mortality of small buds and the low flower bud recruitment. The complete history of *R. arnoldii* required 3.5 to 5 years, where a female flower needed a longer time than a male flower.

Comment [A2]: Please rewrite

Comment [A3]: Please add research method and how many individuals were observed?

Comment [A4]: Please rewrite

**Key words:** copule, exponential, perigone, population, *Rafflesia arnoldii*

**Running title:** Growth, mortality, and life history of *Rafflesia arnoldii*

## INTRODUCTION

Life history or life cycle of plants is generally referred to as the dynamics of entire growth stages of plants to adapt to surrounding environments (Lime 2017). Life history involves complex responses of plants to environments acting as natural selection. Such plant-environment interaction leads to plants to develop strategies to the trade-off between their functional traits such as plant's statures, leaf area, wood density, seed productions and their demographic attributes including growth rates and their survivorships in to cope the environmental pressures (Lind et al. 2013; Liu et al. 2017). The ultimate goal of the strategies is to reach the plant's maximum fitness (Lind et al. 2013). The fitness itself reflects the growth and mortality rates at different development stages (Crawley 1986), mode reproduction, fecundity, new recruitment, and the energy allocated to reproduction (Smith 1986). The growth rate appears to have a close relationship with mortality. Schemske et al. (1994) recorded that either low or high growth caused the low mortality of trees. Furthermore, the development stage, which was expressed by the plant's size, apparently also influences mortality. Advanced development stages or larger plants tended to have a lower mortality (Wunder et al. 2008). Furthermore, the inability plant to cope with the environmental pressures at various stages can lead to the failure of the plants to survive (Wunder et al. 2008).

Comment [A5]: It is better to use refere on *Rafflesia* or other herb species

*Rafflesia arnoldii* R.Br. (Rafflesiaceae) is well known to have the biggest single flower among the plant kingdom. Like other members of Rafflesiaceae, it is a holoparasitic plant, which its life entirely depends on its hosts on certain species of *Tetrastigma* (Vitaceae) (Meijer 1997; Zuhud et al. 1998; Susatya 2011). It has unique biological characteristics such as trunkless, leafless, and true root system. The only visible organ is a flower (Mat-Salleh 1991; Meijer 1997; Nais 2001). Such characteristics could lead to an interesting pattern of its life history. Unfortunately, the life history of species of *Rafflesia* was rarely carried out, because of the length of its life history, relatively small population, high mortality, the uncertainty of a single bud to reach maturity, and the remoteness of its location (Nais 2001; Hidayati and Walck 2016). Among 36 recognized species of *Rafflesia* (Hidayati and Walck 2016), a detailed life history was only provided by Hidayati et al. (2000) for *R. Patma* Blume, and Nais (2001) for *R. keithii* Meijer, *R. pricei* Meijer, and *R. tengku-adlinii* Mat-Salleh & Latiff. It required 256 to 512 days for *R. patma*, (Hidayati et al. 2000), 270 to 390 days for *R. tengku-adlinii*, 300 to 450 days for *R. pricei*, and 360-480 days for *R. keithii* (Nais 2001) to complete its respective life history. Both Hidayati et al. (2000) and Nais (2001) further divided the life history of *Rafflesia* species into 7 sequential development stages, and estimated time required to reach each stage. The stages included pollination, fruit and seed formation, seed dispersal, inoculation its seeds to the host, the emergence of flower bud, mature bud, and anthesis (Hidayati et al. 2000; Nais 2001). Basically, the life history of *Rafflesia* can be divided into two components; invisible and visible stages. The former includes the growth of *Rafflesia*'s seed inside its host plant, which can take 2-3 years (Hidayati et al. 2000), the

Comment [A6]: Please mention species name

48 latter consists of several flower bud developments. The visible one is also the only structure that is exposed to external  
49 environments. Due to the combination of the exposure of the various external environments and various flower bud sizes,  
50 it is expected that the different bud sizes will show different growth rates (Hidayati et al. 2000), and mortality rate (Susatya  
51 et al. 2017).

52 Please mention research problem. The main objectives of this research were to reconstruct the life history according to  
53 flower development stages, and to determine the growth and mortality rate of the flower buds, and to know the change of  
54 the population structure of *R. arnoldii* over time according to flower development stages.

## 55 MATERIALS AND METHODS

56 The research site was located in Taba Penanjung Conservation Area (TPCA) within Bukit Daun Protection Forest,  
57 Central Bengkulu, Bengkulu Province, with coordinate between 3°41'42.00" S and 102°32'0.10"E. TPCA was established  
58 to protect 4 populations of *R. Arnoldii*, however, two of them were perished due to their host plants was cut down. Data  
59 from the nearest climate station of Kepahiang Regency showed that the site received an average of the annual rainfall of  
60 2.717 mm. The average monthly rainfall reached 226 mm.

61 To carry out the study, two populations from two different host plants consisting of 17 flower buds, were observed  
62 their buds every two weeks for six months. Each bud was recorded its coordinate, mapped and labeled, vertically  
63 photographed and measured its diameter at every observation. For the life history study, the observation was limited to  
64 *Rafflesia*'s visible structure, flower buds. Furthermore, the flower bud development stage of *R. arnoldii* was grouped  
65 according to the combination of the size of buds (Hidayati et al. 2000; Sofiyanti et al. 2007) and the morphology of *R.*  
66 *arnoldii*. Based on these two criteria, the life history was then more detailedly categorized into eight stages consisting of  
67 six visible flower development stages, mature fruit, and one an invisible stage. The invisible stage included inoculation and  
68 seed germination processes within its host. The visible stage contained copule, copule-bract transition (CBT), bract, bract-  
69 perigone transition (BPT), perigone, and anthesis stages (Figure 1).

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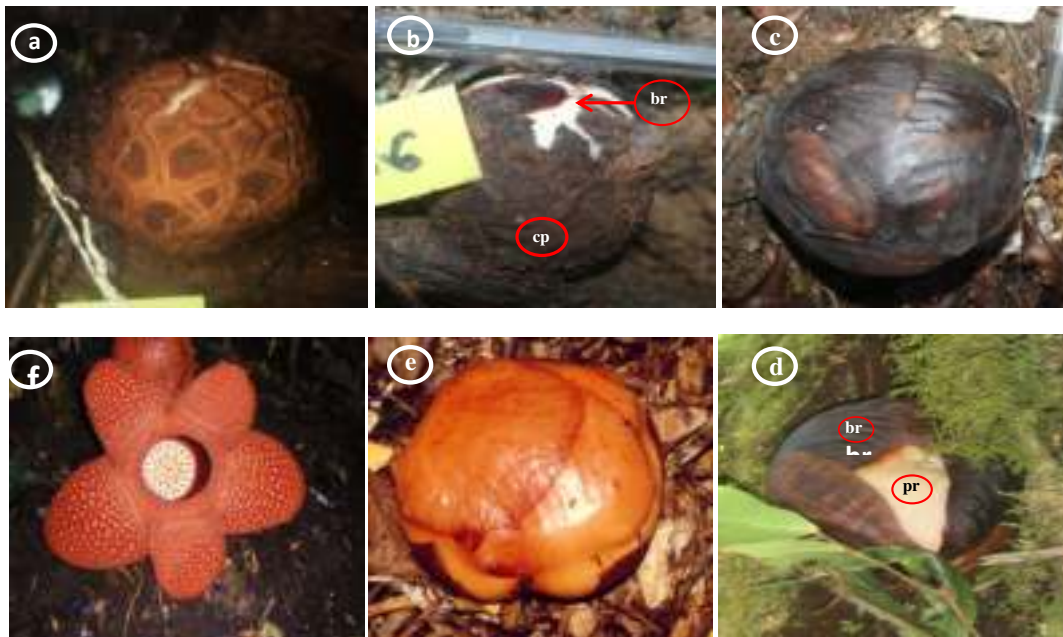
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82 **Figure 1:** The flower development stage of *R. arnoldii*. (a) Copule stage-(a), (b) Copule-bract transition stage, CBT,-(b). In  
83 CBT, parts of copule (cp) are still largely seen, and gradually replaced by a bract (br). (c) Bract stage-(c), a stage where a  
84 visible bud is fully covered by a bract, a similar structure to sepal. (d) Bract-perigone transition stage, BPT,-(d), a stage  
85 where bracts are still largely recognized (br) and gradually replaced by a perigone (pr). Perigone stage (pr). A visible bud  
86 from this stage is all covered by a perigone, a similar structure to petal. Anthesis stage (f).

87

Comment [A7]: Please separate into 3 parts:  
Plant Materials  
Procedures  
Data analysis

Comment [A8]: Please mention referen

Comment [A9]: Please conform with abstract

Comment [A10]: Figure 1e?  
Please add scale on each figure

88 To categorize a bud into a certain stage, each bud was vertically photographed. Copule, bract, and perigone stages were  
 89 defined by the 80%-100% of the images of vertically photographed bud respectively covered by copule, bract, and  
 90 perigone structures. A bud was categorized into CBT, if it grew between copule and bract stages, and the coverage of the  
 91 images of the photographed bud by bract reached 40% to 80%. Meanwhile, a bud was grouped into BPT, if it grew  
 92 between bract and perigone stages with the coverage of the images of the photographed bud by perigone reaching to 40%  
 93 to 80%. Any bud had less 40% of the coverage by either bract or perigone, was also respectively categorized into either  
 94 copule or bract stages. To know the growth of each stage, we averaged the differences of two consecutive measurements  
 95 of the diameter of all buds at each stage.

Comment [A11]: It is better do not use word 'we'

96 Because the bud growth followed the exponential model or J-shape (Hidayati et al. 2000; Meijer 1958; Nais 2001), the  
 97 exponential equation was used to construct a mathematical model for bud's growth. The following equation was applied to  
 98 construct a model showing a relationship between age or time (day) and bud diameter (cm).  $D_t = c e^{kt}$ , where  $D_t$ ,  $c$ ,  $e$ ,  $k$ ,  
 99 and  $t$  respectively explained bud's diameter at  $t$ , constant value, the base value for natural log (ln, 2.719), constant value  
 100 expressing bud growth rate, and time required to reach a certain diameter. To calculate the constants,  $c$ , and  $k$ , the  
 101 exponential equation was transformed into the linear model through converting diameter and time values by natural  
 102 logarithm (ln), and then run it into regression analysis.

Comment [A12]: Reference?

103 The model was developed into two steps, and used to estimate the time (age) of a bud according to its diameter from all  
 104 visible stages. The first step was to select buds representing the *Rafflesia*'s life history from the smallest diameter (copule  
 105 stage) to a diameter just before blooming (perigone stage), then followed their cohorts, and measured their diameters every  
 106 two weeks for six months. Buds were grouped according to their size categories, respectively representing the smallest,  
 107 small, medium, large, and largest categories. The range of recorded diameter data for six months was respectively from  
 108 0.580 cm to 1.145 cm (smallest), 1.580 cm to 2.300 cm (small), 2.395 cm to 5.552 cm (medium), 5.68 cm to 10.986 cm  
 109 (large), and 9.050 cm to 22.4 cm (largest). The largest bud was 22.4 cm, which was a diameter of *R. arnoldii* just before  
 110 flowering. The second step was to develop a series of regression models to estimate the age of a certain bud at each  
 111 category according to its corresponding diameter. The results of the estimation, containing all data of diameter and its  
 112 corresponding estimated age from all size categories, were then used to develop the growth model. The growth model  
 113 further was employed to estimate time (age) of each development stage according to its diameter range. In addition to the  
 114 age of each visible stage, to reconstruct the complete life history of *R. arnoldii*, it required to know the time to reach fruit  
 115 maturity and the time for a seed to inoculate, germinate, and grow within the host plant. For these purposes, information  
 116 from Meijer (1997) and Hidayati et al. (2000) was used to determine those times.

Comment [A13]: Reference?

Comment [A14]: Any reference used to classify diameter size?

## 117 RESULTS AND DISCUSSION

Comment [A15]: Results and Discussion should be written as a series of connecting sentences. It should be divided into subtitles and please conform with manuscript title

118 Not much studied on life history of *Rafflesia* species are available, eventhough it is essential for conservation purposes  
 119 (Hidayati and Walck 2016). The first study on life history was conducted by Meijer (1958), who collected composite data  
 120 from different bud sizes of *R. arnoldii* and then followed their fates for a certain time, not the whole life. A similar  
 121 approach then was used to study the life history of *R. patma* (Hidayati et al. 2000). It was Nais (2001) who observed the  
 122 cohort of buds from their emergences to anthesis for *R. keithii*, *R. pricei*, and *R. tengku-adlinii*. Furthermore, Both Hidayati  
 123 et al. (2000) and Nais (2001) divided life history into 7 sequential stages. However, Meijer (1958), Hidayati et al. (2000),  
 124 and Nais (2001) did not study more detail on the growth and mortality of buds at each stage. Since the only visible  
 125 structure of *Rafflesia* is flower buds, which is also subjected to external environments, and varies in their sizes, then it is  
 126 expected that buds of each stage will show different growth and mortality.

127 [Please explain Table 1 here](#)

128 Table 1 : The mean of growth and its coefficient variation of the bud diameter according to its stage.

Flower development stage (the range of diameter of buds)	Growth mean (cm day <sup>-1</sup> )	Standart deviation (cm day <sup>-1</sup> )	Coefficient variation (%)
Copule (0.58-3.03 cm)	0.0143	0.0146	104.059
Copule-Bract transition (3.04-6.97 cm )	0.0435	0.0271	64.664
Bract (7.98-12.03 cm)	0.0572	0.0622	111.320
Bract-perigone (12.04-17.56 cm)	0.1357	0.0735	54.686
Perigone (15.57-21.89 cm)	0.1841	0.1276	69.731

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Comment [A16]: Please check, since standard deviation high enough

Comment [A17]: How to calculate coefficient variation? Please mention in Materials and Methods

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The visible buds of *R. arnoldii* was categorized into 6 development stages consisting of copule, copule-bract transition (CBT), bract, bract-perigone transition (BPT), perigone, and anthesis stages. Anthesis occurred once during the observation at bud's diameter of 22.40 cm. The results of the analysis showed that the diameter range of copule, CBT, bract, BPT, and perigone stages respectively was 0.58-3.03 cm, 3.04-6.97 cm, 6.98-12.03 cm, 12.04-17.56 cm, and 17.57-21.80 cm (Table 1). The mean growth rate of bud diameters varied across the stages, where the copule stage showed the lowest rate (0.0143 cm day<sup>-1</sup>), meanwhile the perigone stages exhibited the highest value (0.1841 cm day<sup>-1</sup>). This showed that buds at smaller stages exhibited slower diameter growth rates than that of larger stages. This was also observed at *R. patma* (Hidayati et al. 2000). Buds at both copule and bract stages also had the highest coefficient variations, while those at the other three stages showed much lower coefficient variations. This indicated that buds at earlier stages of the development showed higher growth variations than that of the later stages. The comparison among the mean diameter growth rates of buds across stages displayed that the growth of flower buds followed the exponential model (Table 1). The exponential growth was also mentioned by Hidayati et al. (2000), Meijer (1958), and Nais (2001). The mathematical growth model showing the relationship between the diameter of bud (y) and time (x) was expressed by  $Y = 0.785 e^{0.0052x}$ .

We used the information on the diameter range of each stage (Table 1) and the growth model to estimate the age of the diameter of buds, and later to reconstruct the life history. Growth development of bud's *Rafflesia* was not in a discrete pattern, where one stage was replaced completely by the next stage. It consisted of a series of the overlapping development stages, where before one stage was complete, the following stage had already developed. This was a basic reason why we introduced the transition stages. The first visible structure was copule, which was basically the bark of the host plant covering the true *Rafflesia* structure (Mat-Salleh 1991; Meijer 1997; Nais 2001). The first visible *R. arnoldii* at Taba Penanjung had the diameter range from 0.58-3.03 cm. The start of the development of the inner structures of *Rafflesia* was still unknown, but the result of examining a dead bud of *R. arnoldii* (5 cm in diameter) showed that inner structures such as column, disc, processes, perigone lobes, and ramenta had already developed. It was predicted that all these inner structures began to develop in the late copule stage. The remnant cupule was still seen through bract, perigone stages, and mature fruits at the base of the bud. As the bud grew, the upper cupule started to crack to allow the first true structure of *Rafflesia* or bract to be visible. Bract was originally ivory white, but turned black as it grew older. Bract consisted of three series of 5 imbricate and whorl scales (Meijer 1997). From copule to fully developed bract, it required 231 days to 351 days. Bract was gradually replaced by light orange perigone lobes, when it started to drop. The fully developed perigone stage consisted of buds with diameter of 17.57 cm to 21.89 cm, and was reached in 109 days to 145 days from bract stage. It further took 339 to 497 days for a small copule of flower bud of *R. arnoldii* to reach anthesis. This appeared to be similar with *R. keithii*, which reached its anthesis in 360-480 days (Nais 1997). Both *R. arnoldii* and *R. keithii* were considered to have a similar size of their flowering buds (Meijer 1997). However, it was quite longer than the smaller sizes of flowering *Rafflesia* such as *R. patma*, *R. tengku-adlinii*, and *R. pricei*. To reach anthesis, those three species respectively took 221 days (Hidayati et al. 2000), 270 days to 390 days, and 300-450 days (Nais 2001). Fully Orange perigone lobes of bud marked anthesis to occur within 4-5 days. Anthesis took place when a bud reached 22.4 cm. Field observation showed that if the upper layer of the perigone lobe was slightly raised, then the anthesis would take place within 1-2 days, and lasted between 5 to 7 days. All flower structures decomposed after a month. Column was the only female structure that did not decomposed and further developed into mature fruit within 6-8 months (Meijer 1997; Hidayati et al. 2000). Seeds appeared to have high viability. Latifah et al. (2017) recorded the seed viability of *R. arnoldii* and *R. patma* respectively reached 78.75% and 93.24%. It was still unknown how seed inoculated to the host plant. However, it was estimated that seed inoculation and germination within the host plant required between 2 and 3 years (Meijer 1997; Hidayati et al. 2000). Overall, the complete life history of female *R. arnoldii* was estimated between 3 years and 5 months and 5 years and one month, while male *R. arnoldii* needed shorter time and only required between 2 years and 11 months to 4 years and 5 months. These values were within the range of the life history predicted by Meijer (1958), who estimated 4.5 to 5 years for *R. arnoldii* to complete its life history (Figure 2).

Comment [A18]: Please conform with table 1 (Table 1 is written 5 stages)

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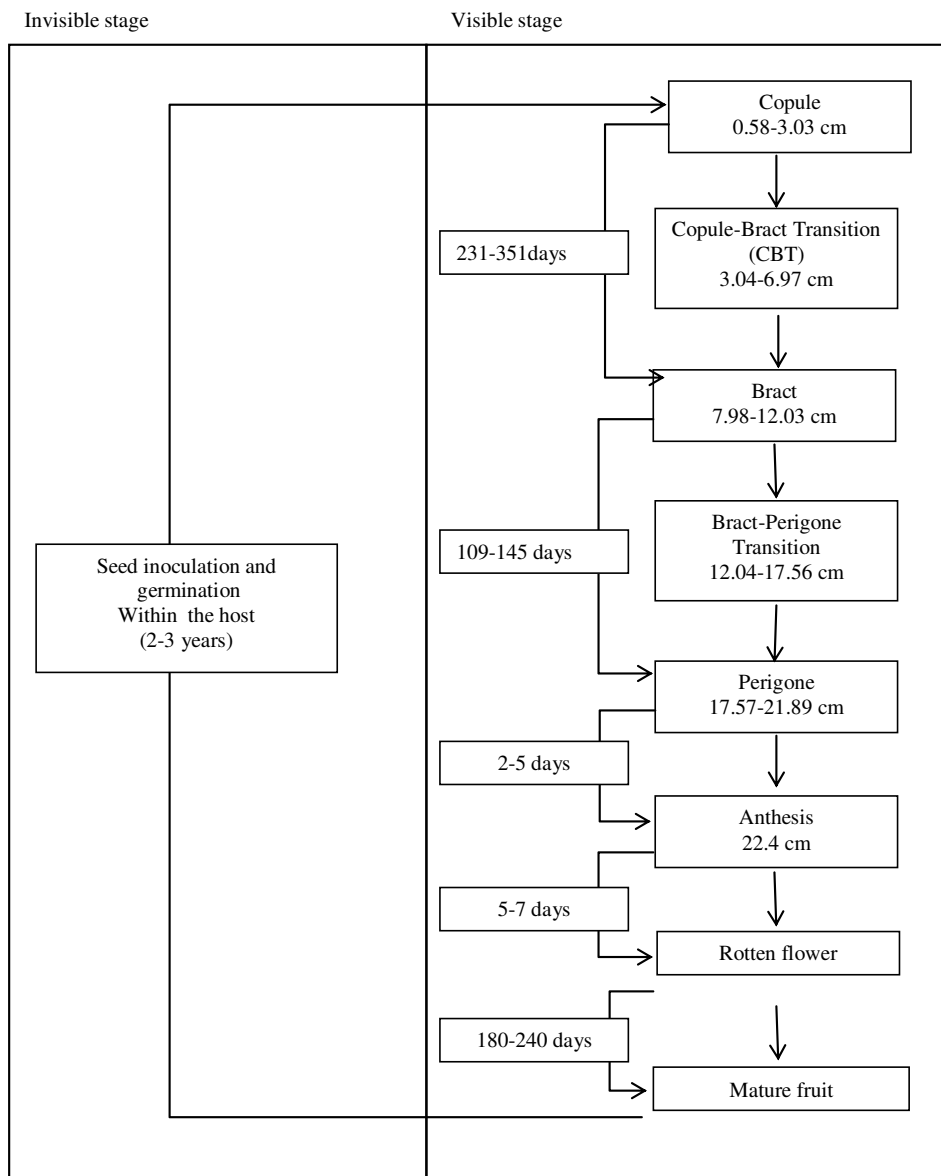


Figure 2: The Reconstruction of the complete life history of *R. arnoldii*. Time required to reach a given stage was estimated by the exponential model. Time to reach mature fruit and for a seed inoculate, germinate, and grow in the host was based on Meijer (1997) and Hidayati et al. (2000)

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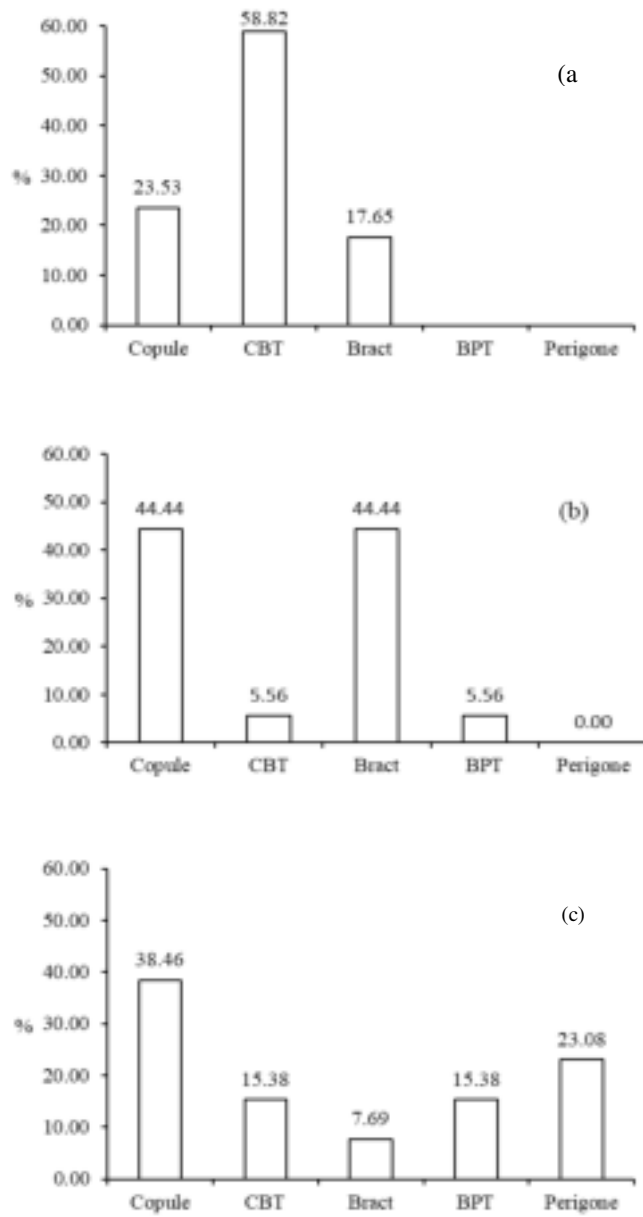


Figure 3: The population structure of *R. arnoldii* according to its flower bud growth development stage. (a) The population structure at the initial (a), (b) after 3 months (b), and (c) after 6 months (c) observations. Copule bract transition stage (CBT). Bract perigone transition stage (BPT).

273 The population of *Rafflesia* seems exceptionally small compared to the other higher plants. Susatya (2011) and Susatya et  
 274 al. (2017) founds that the average population size of *R. arnoldii* was only 12.5 flower buds. The very small population size  
 275 was also found at the other species of *Rafflesia* (Lau 2003; Barcelona et al. 2009; Munirah 2017; Susatya et al. 2017). The  
 276 population size of *R. arnoldii* at the research was slightly higher than its average population size. The initial observation  
 277 showed the total population reached up 17 flower buds and belonged to only copula, CBT, and bract stages. Larger stages  
 278 such as BPT and perigone contained no buds. The initial population structure was dominated by buds belonging to CBT.  
 279 Buds at CBT reached up to 58.82% of the total buds. Meanwhile, buds at copule and CBT, were fewer (Figure 3a). Within  
 280 3 months, the population structure was significantly changed due to mortality, new recruitment, and growth a bud from a  
 281 stage to the next growth development stages. The population structure of this period was shifted toward to both copule and  
 282 bract stages (Figure 3b). During these 3 months, 4 buds belonging to copule and bract transition died, but 5 new bud  
 283 recruitments emerged, which automatically belonged to the copule stage. It was interesting that after 3 months the pattern  
 284 of population structure appeared to be opposite to the previous one. The dominant CBT structure at the initial observation  
 285 became the least dominant, while less dominant copule and bract stages at the initial observation developed into the  
 286 dominant ones. Of the 18 flower buds at the second observation, both copule and bract stages contributed to 44.44% of  
 287 the total buds, while BCT only had 5.5% (Figure 3b). During this period, it was also noted the emergence of the bract-  
 288 perigone transition stage (BPT), which consisted of 5.56% of the total buds. This was due to the growth of a bud from  
 289 bract stages into BPT. After 6 months of observation, the population structure showed a different pattern and appeared to  
 290 be a better structure than those of the two previous observations. Within this period, the population structure consisted of  
 291 all growth stages. Unlike the two previous observations, this last observation showed that no stage was distinctively more  
 292 dominant than the others. In this last observation, perigone stage interestingly became the emerging category to shape  
 293 population structures. Three factors such as the loss of buds (7 buds), low new recruitment (2 buds), an incident of  
 294 flowering *Rafflesia* had attributed the change of the population structure of *Rafflesia arnoldii* (Figure 3c). The cause of bud  
 295 mortality was not further studied in detail. —Hidayati et al. (2000) recorded that bud's mortality was caused by the  
 296 predations by the squirrel. Field observations showed that injured parts of flower buds caused by various factors were  
 297 immediately followed by a rotting process that led to the bud's mortality.

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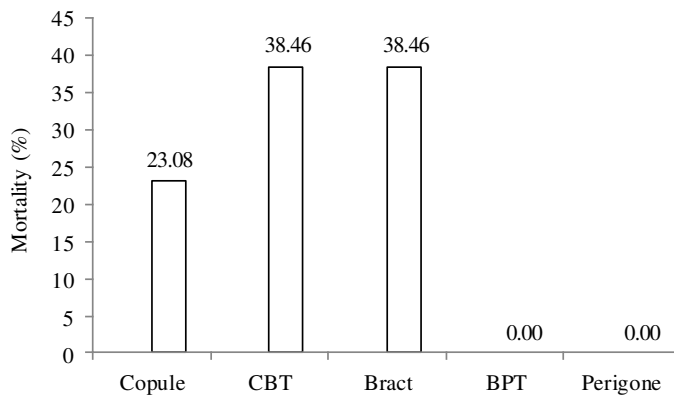


Figure 4 : The bud mortality of *R. arnoldii* according to its growth development stage.

310 Total buds died during the six months reached up to 11 buds or 47.82% of all recorded buds. However, in the same  
 311 period, the recruitment was only recorded 7 buds. This was the first time the bud recruitment was recorded for the species  
 312 of *Rafflesia*. Comparison between the high mortality and low recruitment of buds of *R. arnoldii* indicated an alarming  
 313 signal concerning the future population of *R. arnoldii*. The mortality of *R. arnoldii* was far less than that of *R. patma* and  
 314 *R. bengkuluensis*. *Rafflesia patma* and *R. Bengkuluensis* respectively suffered the loss of 75% of its buds (Hidayati et al.  
 315 2000), and 67% (Susatya et al. 2017). Sofiyanti et al. (2007) summarized that the mortality of *Rafflesia*'s bud generally  
 316 varied from 60% to 90%. Detailed analysis of the bud's mortality showed an interesting pattern, where all losses occurred  
 317 at buds belonging to copule, CBT, and bract stages. Among these three stages, buds at both CBT and bract stages showed  
 318 the highest mortality rates (38.46%) (Figure 4). Meanwhile, the larger buds at both BPT and perigone stages were not  
 319 recorded any losses. This pattern indicated that the buds at larger stages survived better than smaller ones (Susatya et al.  
 320 2017).

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## CONCLUSION

The life history of *R. arnoldii* was reconstructed based on the growth model of its flower buds. It was estimated that female and male flowers of *R. arnoldii* respectively took from 3 years and 5 months to 5 years and one month, and from 2 years and 11 months to 4 years and 5 months to complete their life histories. The exponential growth of buds of *R. arnoldii* was confirmed, and developed into a mathematical model. The growth rate of buds at copule stage was far slower than those of bract and perigone stages. *R. arnoldii* experienced high mortality rate at copule, copule-bract transition, and bract stages. Buds at bract-perigone transition and perigone stages had very high survivorship and likely would undergo anthesis. Population structure was changed in the short period of time and caused by combination between low recruitment and high mortality of buds.

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## ACKNOWLEDGEMENTS

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# The growth of flower bud, life history, and population structure of *Rafflesia arnoldii* R.Br. (Rafflesiaceae) in Bengkulu, Sumatra, Indonesia

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**Abstract.** The life history of *R. arnoldii* is the reflection of the complex interaction between flower bud development and the external environments in order to reach its optimal survivorship. The objectives of the study were to determine the growth of flower buds at various development stages, to reconstruct the life history, and to know the population structure of *R. arnoldii*. The study was carried out at Taba Penanjung, Bengkulu Province. Two populations consisting of 17 individual buds of *R. arnoldii* were selected for the research. All buds were categorized into six visible stages, mapped, measured their diameters, and recorded their fates every two weeks for six months. The exponential model of growth development was applied to reconstruct the life history. The results showed that buds from the perigone stage respectively grew 3.5 and 12 times faster than those from the bract and cupule stages. The exponential growth of flower bud was confirmed, and explained by  $Y = 0.785 e^{0.0052 X}$ , where Y and X was respectively diameter and age of flower bud. The complete life history of *R. arnoldii* required 3.5 to 5 years, where a female flower needed a longer time than a male flower. The population structure of *R. arnoldii* was not constant, but changed dynamically over time. The dynamics of population structure was mainly caused by the high mortality of small buds and the low flower bud recruitment.

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**Key words:** copule, exponential, perigone, population, *Rafflesia arnoldii*

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**Running title:** Growth, population, and life history of *Rafflesia arnoldii*

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## INTRODUCTION

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Life history or life cycle of plants is generally referred to the dynamics of entire growth stages of plants to adapt to surrounding environments (Lime 2017). Life history involves complex responses of plants to environments acting as natural selection. Such plant-environment interaction leads to plants to develop the trade-off strategies between their functional traits such as plant's stature, leaf area, wood density, seed productions and their demographic attributes including growth rates and survivorships to cope the environmental pressures (Lind et al.2013; Liu et al.2017). The ultimate goal of the strategies is to reach the plant's maximum fitness (Lind et al.2013). The fitness itself reflects the growth and mortality rates at different development stages (Crawley 1986), reproduction, fecundity, new recruitment, and the energy allocated to reproduction (Smith 1986). Furthermore, the growth rate appeared to affect the survival of seedlings of *Calathea ovandensis*, a neotropical herb (Horvits and Schemske 2002). The development stage, which was expressed by the plant's size, apparently also influenced mortality. Advanced development stages or larger plants tended to have a lower mortality (Wunder et al. 2008). Moreover, the inability plant to cope with the environmental pressures at various stages could lead to the failure of the plants to survive (Wunder et al. 2008).

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*Rafflesia arnoldii* R.Br. (Rafflesiaceae) is well known as the biggest single flower among the plant kingdom. It is a holoparasitic plant, which its life entirely depends on its hosts consisting of *Tetrastigma leucostaphyllum* (Dennst.) Alston ex Mabb., *T. curtisii* (Ridl) Suess, *T. pedunculare* (Wall. ex. Lamson) Planch (Vitaceae) (Meijer 1997; Zuhud et al. 1998; Susatya 2011). It has unique biological characteristics such as trunkless, leafless, and no true root system. The only visible organ is a flower (Mat-Salleh 1991; Meijer 1997; Nais 2001). Such characteristics could lead to an interesting pattern of its life history. Unfortunately, the life history of species of *Rafflesia* was rarely studied, because of the length of its life history, relatively small population, high mortality, the uncertainty of a single bud to reach maturity, and the remoteness of its location (Nais 2001; Hidayati and Walck 2016). Among 36 recognized species of *Rafflesia* (Hidayati and Walck 2016), a detailed life history was only provided by Hidayati et al. (2000) for *R. patma* Blume, and Nais (2001) for *R. keithii* Meijer, *R. pricei* Meijer, and *R. tengku-adlinii* Mat-Salleh & Latiff. It required 256 to 512 days for *R. patma*, (Hidayati et al. 2000), 270 to 390 days for *R. tengku-adlinii*, 300 to 450 days for *R. pricei*, and 360-480 days for *R. keithii* (Nais 2001) to complete its respective life history. Both Hidayati et al.(2000) and Nais (2001) further divided the life history of *Rafflesia* species into 7 sequential development stages, and estimated time required to reach each stage. The stages included pollination, fruit and seed formation, seed dispersal, inoculation its seeds to the host, the emergence of flower

47 bud, mature bud, and anthesis (Hidayati et al. 2000; Nais 2001). Basically, the life history of *Rafflesia* can be divided into  
48 invisible and visible stages. The former includes the growth of *Rafflesia*'s seed inside its host plant, which can take 2-3  
49 years (Hidayati et al. 2000), the latter consists of several flower bud developments. The visible one is also the only  
50 structure that is exposed to external environments. Due to the combination of the exposure of the various external  
51 environments and various flower bud sizes, it is expected that the different bud sizes will show different growth rates  
52 (Hidayati et al. 2000), and mortality rate (Susatya et al. 2017).

53 The main objectives of this research were to reconstruct the life history according to flower development stages, and to  
54 determine the growth and mortality rate of the flower buds, and to know the change of the population structure of *R.*  
55 *arnoldii* over time according to its flower development stages.

## 56 MATERIALS AND METHODS

### 57 Study area

58 The research site was located in Taba Penanjung Conservation Area (TPCA) within Bukit Daun Protection Forest,  
59 Central Bengkulu, Bengkulu Province, with coordinates between 3°41'42.00''S and 102°32'0.100''E. TPCA was  
60 established to protect 4 populations of *R. arnoldii*, however, two of them were perished due to their host plants was cut  
61 down. Data from the nearest climate station of Kepahiang Regency showed that the site received an average of the annual  
62 rainfall of 2.717 mm. Meanwhile, the average monthly rainfall reached 226 mm (BPS Kab. Kepahiang 2018).

### 63 Plant materials and Procedures.

64 To carry out the study, two populations from two different host plants consisting of 17 flower buds were observed their  
65 buds every two weeks for six months. Each bud was recorded its coordinate, mapped and labeled, vertically photographed  
66 and measured its diameter at every observation. For the life history study, the observation was limited to *Rafflesia*'s visible  
67 structure, flower buds. Furthermore, the flower bud development stage of *R. arnoldii* was grouped according to the  
68 combination of the size of buds (Hidayati et al. 2000; Sofiyanti et al. 2007) and the morphology of *R. arnoldii*. Based on  
69 these two criteria, the life history was then more detailedly categorized into eight stages consisting of six visible flower  
70 development stages, mature fruit, and one an invisible stage. The invisible stage included inoculation and seed germination  
71 processes within its host. The visible stage contained copule, copule-bract transition (CBT), bract, bract-perigone transition  
72 (BPT), perigone, and anthesis stages (Figure 1).



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98 Figure 1: The flower development stage of *R. arnoldii*. (a) Copule stage. (b) Copule-bract transition stage, CBT. In CBT,  
99 parts of copule (cp) are still largely seen, and gradually replaced by bracts (br). (c) Bract stage, a stage where a visible bud  
100 is fully covered by bract, a similar structure to sepal. (d) Bract-perigone transition stage, BPT, a stage where bracts are still  
101 largely recognized (br) and gradually replaced by perigones (pr). (e) Perigone stage. A visible bud at this stage is all  
102 covered by perigone, a similar structure to petal. (f) Anthesis stage.

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**Data analysis.**

To categorize a bud into a certain stage, each bud was vertically photographed. Copule, bract, and perigone stages were defined by the 80-100% of the images of vertically photographed bud respectively covered by copule, bract, and perigone structures. A bud was categorized into CBT, if it grew between copule and bract stages, and the coverage of the images of the photographed bud by bract reached 40% to 80%. Meanwhile, a bud was grouped into BPT, if it grew between bract and perigone stages with the coverage of the images of the photographed bud by perigone reaching to 40% to 80%. Any bud had less 40% of the coverage by either bract or perigone, was also respectively categorized into either copule or bract stages. To know the growth of each stage, the differences of two consecutive measurements of the diameter of all buds at each stage were averaged. All data of the growth were then calculated their means, standard deviations, and coefficient variations. The coefficient variation of each stage was the percentage of its standard deviation to the mean value.

Because the bud growth followed the exponential model or J-shape (Hidayati et al. 2000; Meijer 1958; Nais 2001), the exponential equation was used to construct a mathematical model for bud's growth. The following equation was applied to construct a model showing a relationship between age or time (day) and bud diameter (cm).  $Y_t = c e^{kX}$ , where  $Y_t$ ,  $c$ ,  $e$ ,  $k$ , and  $X$  respectively explained bud's diameter at  $t$ , constant value, the base value for natural log (ln, 2.719), constant value expressing bud's growth rate, and time required to reach a certain diameter (Susatya 2011). To calculate the constants,  $c$ , and  $k$ , the exponential equation was transformed into the linear model through converting diameter and time values by natural logarithm (ln), and then run it into regression analysis.

The model was developed into two steps, and used to estimate the time (age) of a bud according to its diameter from all visible stages (Susatya 2011). The first step was to select buds representing the *Rafflesia*'s life history from the smallest diameter (copule stage) to a diameter just before blooming (perigone stage), then followed their cohorts, and measured their diameters every two weeks for six months. Buds were tentatively grouped according to their size categories, respectively representing the smallest, small, medium, large, and largest categories. The range of recorded diameter data for six months was respectively from 0.580 cm to 1.145 cm (smallest), 1.580 cm to 2.300 cm (small), 2.395 cm to 5.552 cm (medium), 5.68 cm to 10.986 cm (large), and 9.050 cm to 22.4 cm (largest). The largest bud was 22.4 cm, which was a diameter of *R. arnoldii* just before flowering. The second step was to develop a series of regression models to estimate the age of a certain bud at each category according to its corresponding diameter. The results of the estimation, containing all data of diameter and its corresponding estimated age from all size categories, were then used to develop the growth model. The growth model further was employed to estimate time (age) of each development stage according to its diameter range. In addition to the age of each visible stage, to reconstruct the complete life history of *R. arnoldii*, it required to know the time to reach fruit maturity and the time for a seed to inoculate, germinate, and grow within the host plant. For these purposes, information from Meijer (1997) and Hidayati et al. (2000) was used to determine those times.

**RESULTS AND DISCUSSION**

**The growth of flower bud and the life history of *Rafflesia arnoldii***

Not many studies on the life history of *Rafflesia* species are available, eventhough it is essential for conservation purposes (Hidayati and Walck 2016). The first study on life history was conducted by Meijer (1958), who collected composite data from different bud sizes of *R. arnoldii* and then followed their fates for a certain time, not the whole life. A similar approach was then used to study the life history of *R. patma* (Hidayati et al. 2000). It was Nais (2001) who observed the cohort of buds from their emergences to anthesis for *R. keithii*, *R. pricei*, and *R. tengku-adlinii*. Furthermore, Both Hidayati et al. (2000) and Nais (2001) divided life history into 7 sequential stages. However, Meijer (1958), Hidayati et al. (2000), and Nais (2001) did not study more detail on the growth and mortality of buds at each stage. Since the only visible structure of *Rafflesia* is flower buds, which is also subjected to external environments and varies in their sizes, then it is expected that buds of each stage will show different growth and mortality.

Table 1 : The mean of growth and its coefficient variation of the bud diameter according to its stage.

Flower development stage (the range of diameter of buds)	Growth mean (cm day <sup>-1</sup> )	Standard deviation (cm day <sup>-1</sup> )	Coefficient variation (%)
Copule (0.58-3.03 cm)	0.0143	0.0146	104.059
Copule-Bract transition (3.04-6.97 cm )	0.0435	0.0271	64.664
Bract (7.98-12.03 cm)	0.0572	0.0622	111.320
Bract-perigone (12.04-17.56 cm)	0.1357	0.0735	54.686
Perigone (15.57-21.89 cm)	0.1841	0.1276	69.731

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Table 1 showed the mean growth rate ( $\text{cm day}^{-1}$ ), standard deviation, and coefficient variations of buds at different development stages. The visible bud of *R. arnoldii* was categorized into 6 development stages consisting of copule, copule-bract transition (CBT), bract, bract-perigone transition (BPT), perigone, and anthesis stages. Anthesis occurred once during the observation at bud's diameter of 22.40 cm. The results of the analysis showed that the diameter range of copule, CBT, bract, BPT, and perigone stages respectively was 0.58-3.03 cm, 3.04-6.97 cm, 6.98-12.03 cm, 12.04-17.56 cm, and 17.57-21.80 cm (Table 1). The mean growth rate of bud diameters varied across the stages, where the copule stage showed the lowest rate ( $0.0143 \text{ cm day}^{-1}$ ), meanwhile the perigone stages exhibited the highest value ( $0.1841 \text{ cm day}^{-1}$ ). This showed that buds at smaller stages exhibited slower diameter growth rates than that of larger stages. The slower growth rate at a smaller diameter of flower bud was also observed at *R. patma* (Hidayati et al. 2000). Buds at both copule and bract stages also had the highest coefficient variations, while those at the other three stages showed much lower coefficient variations. This indicated that buds at earlier stages of the development showed higher growth variations than that of the later stages. The comparison among the mean diameter growth rates of buds across stages displayed that the growth of flower buds followed the exponential model (Table 1). The exponential growth was also mentioned by Hidayati et al. (2000), Meijer (1958), and Nais (2001). The exponential growth was also confirmed by the result of the analysis of the growth model. The result of the analysis of the growth model of flower bud was  $Y = 0.785 e^{0.0052 X}$  and its coefficient of determination ( $R^2$ ) was 0.92. This result also indicated that the growth model showed a strong relationship between the diameter of bud (Y) and time required to reach the diameter (X). It meant that the model could be used to estimate the age of a flower bud from its diameter.

The information on the diameter range of each stage (Table 1) and the growth model was used to estimate the age of the diameter of buds, and later to reconstruct the life history. The growth development of *Rafflesia*'s bud was not in a discrete pattern, where one stage was replaced completely by the next stage. It consisted of a series of the overlapping development stages, where before one stage was complete, the following stage had already developed (Meijer 1997). This was a basic reason why transition stages were introduced at this research. The first visible structure was copule, which was basically the bark of the host plant covering the true *Rafflesia* structure (Mat-Salleh 1991; Meijer 1997; Nais 2001). The first visible *R. arnoldii* at Taba Penanjung had the diameter range from 0.58-3.03 cm. The start of the development of the inner structures of *Rafflesia* was still unknown, but the result of examining a dead bud of *R. arnoldii* (6 cm in diameter) showed that inner structures such as column, disc, processes, perigone lobes, and bracts had already developed (Figure 2). It was predicted that all these inner structures began to develop in the cupule stage.



Table 2: The inner structures of *R. arnoldii* at a small flower bud (6 cm diameter). (1) Column. (2) Disc. (3) Processes. (4) Perigone lobes. (5) Bracts.

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The remnant cupule was still be seen through bract, perigone stages, and mature fruits at the base of the bud. As the bud grew, the upper cupule started to crack to allow the first true structure of *Rafflesia* or bract to be visible. Bract was originally ivory white, but turned black as it grew older. Bract consisted of three series of 5 imbricate and whorl scales (Meijer 1997). From copule to fully developed bract, it required 231 days to 351 days (Figure 3). Bract was gradually replaced by light orange perigone lobes, when it started to drop. The fully developed perigone stage consisted of buds with diameter of 17.57 cm to 21.89 cm, and was reached in 109 days to 145 days from bract stage. It further took 339 days to 497 days for a small copule of flower bud of *R. arnoldii* to reach anthesis (Figure 3). This appeared to be similar with *R. keithii*, which reached its anthesis in 360-480 days (Nais 1997). Both *R. arnoldii* and *R. keithii* were considered to have a similar size of their flowering buds (Meijer 1997). However, it was a quite longer than the smaller sizes of flowering *Rafflesia* such as *R. patma*, *R. tengku-adlinii*, and *R. pricei*. To reach anthesis, those three species respectively took 221 days (Hidayati et al. 2000), 270 days to 390 days, and 300-450 days (Nais 2001). Fully Orange perigone lobes of bud marked anthesis to occur within 4-5 days. Anthesis took place when a bud reached 22.4 cm. Field observation showed that if the upper layer of the perigone lobe was slightly raised, then the anthesis would take place within 1-2 days, and lasted between 5 to 7 days. All flower structures decomposed within a month after flowering. Column was the only female structure that did not decomposed and further developed into mature fruit within 6-8 months (Meijer 1997; Hidayati et al. 2000). Seeds appeared to have high viability, where the seed viability of *R. arnoldii* and *R. patma* respectively reached up to 78.75% and 93.24% (Latifah et al. (2017). It was still unknown how seed inoculated to the host plant. However, it was estimated that seed inoculation and germination within the host plant required between 2 and 3 years (Meijer 1997; Hidayati et al. 2000). Overall, the complete life history of female *R. arnoldii* was estimated between 3 years and 5 months and 5 years and one month, while male *R. arnoldii* needed a shorter time and only required between 2 years and 11 months to 4 years and 5 months. These estimated values were within the range of the life history predicted by Meijer (1958), who estimated 4.5 to 5 years for *R. arnoldii* to complete its life history (Figure 3).

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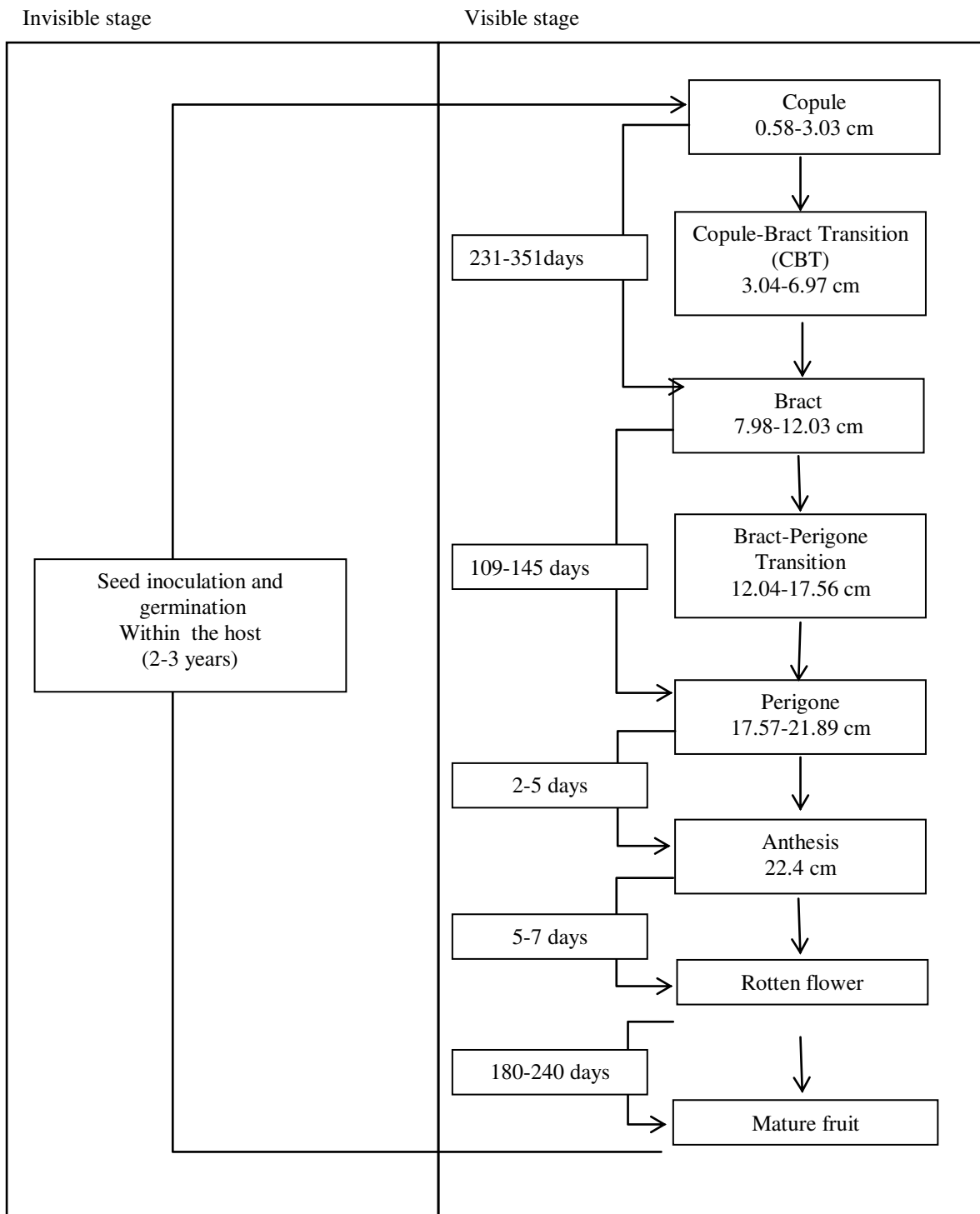


Figure 3: The result of the reconstruction of the complete life history of *R. arnoldii*.

**The population structure of *R. arnoldii***

The population of species of *R. arnoldii* seems to be small compared to the other higher plants. Susatya (2011) and Susatya et al. (2017) found that the average population size of *R. arnoldii* was only 12.5 flower buds. The population size of *R. arnoldii* at the research site (17 flower buds) was slightly higher than its average population size. The small population size was also reported at the other species of *Rafflesia* such as *R. kerrii* Meijer (Lau 2003), *R. manillana* Teschem., *R. schadenbergiana* Gopp., *R. speciosa* Barcelona et Fernando (Barcelona et al. 2009), *R. cantleyi* Meijer (Munirah 2017), and *R. bengkulensis* Susatya, Arianto et Mat-Salleh (Susatya et al. 2017).

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284 The initial observation showed that the total population reached up 17 flower buds and belonged to only copula, CBT,  
 285 and bract stages. Larger stages such as BPT and perigone contained no buds. The initial population structure was  
 286 dominated by buds belonging to CBT. Buds at CBT reached up to 58.82% of the total buds. Meanwhile, buds at copule  
 287 and CBT were fewer (Figure 4a). Within 3 months, the population structure was significantly changed due to mortality,  
 288 new recruitment, and growth a bud from a stage to the next growth development stages. The population structure of this  
 289 period was shifted toward to both copule and bract stages (Figure 4b). During these 3 months observation, 4 buds  
 290 belonging to CBT died, but 5 new bud recruitments emerged, which automatically belonged to the copule stage. It was  
 291 interesting that after 3 months the pattern of population structure appeared to be opposite to the previous one. The  
 292 dominant CBT structure at the initial observation became the least dominant, while less dominant copule and bract stages  
 293 at the initial observation developed into the dominant ones. Of the 18 flower buds at the second observation, both copule  
 294 and bract stages contributed to 44.44% of the total buds, while BCT only had 5.5% (Figure 4b). During this period, it was  
 295 also noted the emergence of the bract-perigone transition stage (BPT), which consisted of 5.56% of the total buds. This  
 296 was due to the growth of a bud from bract stages into BPT. After 6 months observation, the population structure showed a  
 297 different pattern and appeared to be a better structure than those of the two previous observations. Within this period, the  
 298 population structure consisted of all growth stages. Unlike the two previous observations, this last observation showed  
 299 that no stage was distinctively more dominant than the others. In this last observation, perigone stage interestingly became  
 300 the emerging category to shape the population structure. Three factors such as the loss of buds (7 buds), low new  
 301 recruitment (2 buds), and an incident of flowering *Rafflesia* had attributed the change of the last population structure of *R.*  
 302 *arnoldii* (Figure 4c).  
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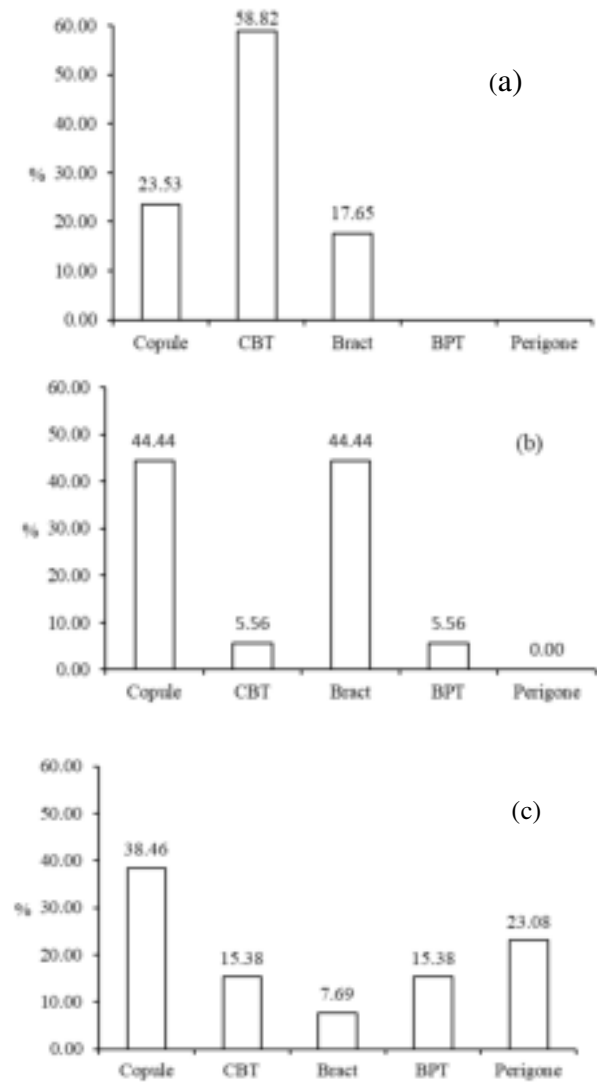


Figure 4: The population structure of *R. arnoldii* according to its flower bud growth development stage. (a) The population structure at the initial, (b) after 3 months, and (c) after 6 months observations. CBT and BPT respectively referred to copule bract transition and bract perigone transition stages.

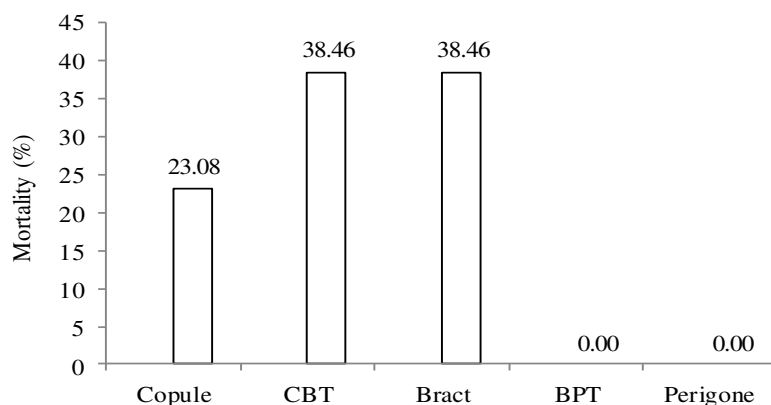


Figure 5: The bud mortality of *R. arnoldii* according to its growth development stage.

Total buds died during the six months observation reached up to 11 buds or 47.82% of all recorded buds. However, in the same period, the recruitment was only recorded 7 buds. This was the first time the bud recruitment was recorded for the species of *Rafflesia*. Comparison between the high mortality and low recruitment of buds of *R. arnoldii* indicated an alarming signal concerning the future population of *R. arnoldii*. The mortality of *R. arnoldii* was far less than that of *R. patma* and *R. bengkuluensis*. *Rafflesia patma* and *R. bengkuluensis* respectively suffered the loss of 75% of its buds (Hidayati et al. 2000), and 67% (Susatya et al. 2017). Sofiyanti et al. (2007) summarized that the mortality of *Rafflesia*'s bud generally varied from 60% to 90%. The cause of bud's mortality was not further observed in this research. However Hidayati et al. (2000) provided information the cause of the mortality. They reported that bud's mortality was caused by the predations from the squirrel, and the injured parts of flower buds were immediately followed by a rotting process that led to the bud's mortality. Detailed analysis of the bud's mortality showed an interesting pattern, where all losses occurred at buds belonging to copule, CBT, and bract stages. Among these three stages, buds at both CBT and bract stages showed the highest mortality rates (38.46%) (Figure 5). Meanwhile, the larger buds at both BPT and perigone stages were not recorded any losses. This pattern indicated that smaller sizes of buds showed high mortality, meanwhile larger size survived better (Susatya et al. 2017).

## CONCLUSION

The exponential growth of buds of *R. arnoldii* was confirmed in this research. The growth rate of buds at copule stage was far slower than those of bract and perigone stages. The life history of *R. arnoldii* was reconstructed based on the growth model of its flower buds. It was estimated that the female flowers of *R. arnoldii* respectively took from 3 years and 5 months to 5 years and one month to complete its life history. The male flower took a shorter time, and needed 2 years and 11 months to 4 years and 5 months to reach its complete life history. The population structure of *R. arnoldii* was dynamically changed in the short period of time and mainly caused by the combination between the low recruitment and high mortality of buds. *Rafflesia arnoldii* experienced high mortality rates at copule, copule-bract transition, and bract stages. Buds at bract-perigone transition and perigone stages had very high survivorships and likely would undergo anthesis.

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**[biodiv] Editor Decision**

2 pesan

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Our decision is to: Accept Submission

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