Differences in microhabitat and morphology of two sympatric congeneric species *Palaquium maliliensis* and *Palaquium obovatum* in the education forest of Hasanuddin University

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Abstract. Two species of *Palaquium* (*P. maliliensis* and *P. obovatum*) were found coexist in same habitat in the educational forest of Hasanuddin University (Unhas). Two or more species are living in a habitat could be formed from similar parent through genetic isolation process without geographic isolation and was classified as sympatric congeneric species. So far, sympatric congeneric species is still controversial among researchers. Based on this case, the purpose of this study was to determine the microhabitat and morphological differences between the two species. The methods of the study were conducted by purposively selecting 21 samples of *P. maliliensis* and 20 samples of *P. obovatum* at the tree level. In each samples found, the microhabitat characteristics (topographic position and slope) and morphological characteristics (leaf, buttress root, and morphological in general) were observed. Morphological measurement data were analyzed with correlation test for intra-species variable and real difference test for inter-species variable. This study concluded that the slope was one of the major microhabitat factors that has driven the process of sympatric speciation between *P. maliliensis* and *P. obovatum*. In morphological characteristics, differences between *P. maliliensis* and *P. obovatum* appeared in the form of leaves.

1. Introduction

Evolution is a theory in biology which explained that various species of flora and fauna derived from pre-existing species and the differences which occur due to inherited modification. It can be said that unlimited variation of species is a result of evolution. Every species has intimated relationship based on the similar ancestor [1].

One important result of the evolution process is speciation, it is a formulation process of new species where the process will be focused on divergent species, namely one ancestor inherits to be more then one species [2]. During their evolution, a massive morphological differences is happened. This speciation is supported by two significant process, they are geographical isolation and reproduction isolation [3].

Furthermore, Gavrilets, S. (2006) stated that there are four kinds of natural speciation which consist of allopatric, peripatric, parapatric, and sympatric speciation [4]. Among the four models of speciation, sympatric is a rare/unique speciation and still a matter of controversy among researchers. According to Papadopulos et. al. (2011) in sympatric speciation new species can be formed in the similar geographical area with parental species where genetic isolation evolved in various ways, without geographic isolation [5]. With the uniqueness, it is interesting to do research about sympatric speciation [6].
Research about sympatric speciation has been done by Yamada et al. (2005). They characterize the growth strategies of two sympatric congeneric species *Pterospermum diversifolium* BI and *P. javanicum* Jungh (*Sterculiaceae*) and the coexistence of both in the forest communities in East Kalimantan [7].

Indonesia is a nation with the very highest rate of biodiversity in the world. Supported by such condition, sympatric speciation process very easily happen in this nation. For instance, in the Experimental Forest of Hasanuddin university (Unhas) was discovered two species of *Palaquium* which have similar morphological characteristics and live in the same habitat. Theoretically, the existence of these two similar genus species in a habitat can be expected is sympatric congeneric species [1,8]. Sympatric congeneric species were identified, namely *Palaquium maliliensis* and *Palaquium obovatum* (*Sapotaceae*). Referring to the findings, therefor of this research would discuss about the growth strategy by focusing attention on microhabitat and morphological variation both of sympatric congeneric species *Palaquium maliliensis* and *P. obovatum* (*Sapotaceae*) and the relationship between them in affecting the formation of sympatric speciation, and also the coexistence of both in the forest communities.

2. Materials and methods

2.1. Study site and species

This research was performed for five months, from March to July 2014 in the experimental forest of Hasanuddin University (Maros, South Sulawesi, Indonesia; latitude 04°57′56″- 04°58′17″N, longitude 119°46′25″-119°46′40″E). The objects of this research were *Palaquium maliliensis* and *Palaquium obovatum* at the tree level (a diameter of more than 20 cm).

![Figure 1. Map of educational forest of Hasanuddin University as a research site.](image)
2.2. Method
Observation of the microhabitat was conducted by making two line transects that directly cut the contour (166 degrees East) starting from the edge of the river to the top of the hill with a bandwidth was 25 m. Line transect then divided into small plots with size was 25 m x 10 m. In each plot was measured slope and counting the number of *P. maliliensis* and *P. obovatum* species.

![Figure 2](image_url)

**Figure 2.** Measurement of leaf morphology (A), buttress morphology (B), stem diameter, total height, and bole height (C), and broad canopy (D)

Observation of the morphological parameters were applied only to the individual trees that had a diameter more than 20 cm. For sufficient number of samples, some trees of *P. maliliensis* and *P. obovatum* that were outside of line transects also measured the morphologic parameters. Thus, the overall number of samples for *P. maliliensis* were 21 samples and for *P. obovatum* were 20 samples. Parameters of morphology measurement were divided into three models, namely leaf morphology,
buttress morphology, and morphology of trees in general. For measurement of leaf morphology, the measured parameter which consisted of leaf length, leaf base width, leaf mid width, and width at the apex leaf. To buttress morphology measurements, parameters measured were height, width, and thick of buttress. As for the morphological measurements of trees in general were measured included total height, bole height, broad canopy, and diameter of tree trunk. Parameters of Morphology measurement can be seen in Figure 2.

2.3. Data analysis
Data analysis was performed by using SPSS (Statistics Product and Service Solutions) 17.0 with 0.05 confidence level. The tests consist of;
1. Linear regression test, was used to determine the average leaf form of both species where long leaves used as the independent variable (X) and width of the leaves as the dependent variable (Y). The formula is \( Y = a + b \times X \).
2. Pearson correlation test, was used to determine changes in intra-species that occur on leaf form and leaf size in line with age (diameter) of trees.
3. Significant Difference Test, was used to compare the morphology of the tree by comparing the ratio (leaf morphology, size buttress, total height, bole height, and broad canopy) to the diameter of the stem between the two species.

3. Result
3.1. Microhabitat
Distribution data of the two species sympatric *P. maliliensis* and *P. obovatum* which were the object of this study indicate that, although growing in the same habitat, but each species was found growing on specific different microhabitat. *P. maliliensis* were found growing in steep slopes area (slope ranges between 24 - 43 percent) in both of the lower slope area (along the river) and the upper slopes area (along the hilltop). Instead, *P. obovatum* were found on sloping area (slope ranges between 2-7 percent) between the lower and upper slopes area. In the transition area between these species were often found individual who were difficult to distinguish between *P. maliliensis* and *P. obovatum*. The species were named *P. maliliensis / obovatum* in Figure 3.

3.2. Leaf morphology
Average form of *P. maliliensis* and *P. obovatum* leaves could be determined by linear regression analysis of the leaf length (independent variable) against the leaf base width, leaf mid width, and leaf apex width (Figure 2). The regression analysis of 1050 samples of *P. maliliensis* leaves indicated that, the regression equation between length to base width, length to middle width, and length to apex leaves was successively \( Y = 2.192 + 0.12X \), \( Y = 2.451 + 0.190X \), and \( Y = 2.227 + 0.126X \). Thus, if the specified length of leaf was 10 cm then based on the regression equation derived from the value of base width, middle width, and the width of apex leaf was consecutive 3.462 cm, 4.351 cm, and 3.487 cm. Based on the calculation of the width, form of *P. maliliensis* could be designed (as shown in Figure 4A left). With reference to Harrington and Durrell (1957), leaf morphology was categorized as an *elliptical* shape [3].

For *P. obovatum*, regression model of 1000 leaves samples between leaf length against width of leaf base showed the regression equation \( Y = 2.272 + 0.135X \), between leaf length against width of leaf middle showed the regression equation \( Y = 3.470 + 0.203X \), and between leaf length against width of apex leaf showed the regression equation \( Y = 3.280 + 0.181X \). Thus, if the specified length of the leaf was 10 cm, value of base width obtained was 3.622 cm, width of middle was 5.50, and width of apex was 5.09 cm. Based on the results of these calculations could be designed leaf form of *P. obovatum* (as shown in Figure 4B right). With reference to (Harrington and Durrell 1957), leaf morphology was categorized as an *obovate* shape.
To determine the extent of change in the shape and size of leaves in line with the age of the trees both of two *Palaquium* species, Pearson correlation test was performed between stem diameter and shape of leaf, also stem diameter and leaf size. Ratio of apex leaf width with base leaf width was used as a representation of the leaf shape. According to Figure 4, if the value of the ratio was equal to 1 or close to the value 1, the leaf shape was *elliptical* but if the value of the ratio approached 1.5, the shape of the leaf was *obovate*. Representation of the leaf size was the average value of leaf width (width of base, middle and apex) multiplied by the length of leaf.

Figure 3. The distribution area of *P. maliliensis* and *P. obovatum*, first line transects (A) and second line transect (B). Each symbol is a representation of one species: ● = *P. maliliensis*, △ = *P. obovatum*, dan □ = *P. maliliensis*/obovatum

Result of Pearson correlation test for *P. maliliensis* showed that, there was no real and direction relationship between stem diameter and ratio of the apex width with base width (r = 0.029, p = 0.343). This showed that the shape of *P. maliliensis* leaf unchanged (still *elliptical*) in line with the aging of trees. In contrast to the results of correlation for *P. obovatum*, it founded a real and unidirectional relationship between stem diameter and ratio of apex width with base width (r = 0.096, p = 0.002). It showed that the greater of the *P. obovatum* tree, leaf shape tended to become increasingly *obovate*. 
Figure 4. Average leaves shape of *P. maliliensis* (A) and *P. obovatum* (B) which are based on the regression equation of the length against width of base, middle and apex of the leaf

Statistically, the average leaves shape between the two species of *Palaquium* could be inequality tested by comparing ratio of apex leaf width with base leaf width (leaf shape) between the two species by using a real difference test (1050 leaves samples for *P. maliliensis* and 1000 leaves samples for *P. obovatum*). Significant difference test results showed that the value of correlation coefficient was 1072.471 and the probability value was 0.000 (the ratio of apex leaf width with base leaf width of *P. maliliensis* between *P. obovatum* was highly significant). This means that in morphology, leaf shape of these species was significantly different.

To determine changed in the shape and size of leaves both species *Palaquium* based on their distribution area, the measurement of leaves samples from a number of individuals of each species along the transect was done. To avoid the influence of age on the shape and size of leaf, then the observed samples tree was limited with a range of diameter between 25 cm - 35 cm. In keeping with the results of studied that showing the distribution pattern of both species, these observations also showed that the morphological changes. Shape of leaf tended to be *elliptical* when the tree grown at an increasingly steep slope areas and become *obovate* on sloping areas. The size of the leaf tended to become larger and leaf were likely to become increasingly *acuminate* when *Palaquium* tree grown on the lower slopes area close to the river.

3.3. Buttress morphology
Both *P. maliliensis* and *P. obovatum* are a tree species that have quite large of buttress form (Martawijaya et al. 2005). To determine the differences in the morphology of buttress between the two species, real differences test was performed between ratio of the height buttress, width buttress, and thick buttress against stem diameter of the two species. Real difference test result of ratio of height buttress against stem diameter, indicated that there was no real difference between the two species ($f = 3.381, p = 0.074$). Based on the results of these tests that in similar stem diameter, statistically buttress of *P. maliliensis* and *P. obovatum* had an equal height, although the average value of the height buttress of *P. maliliensis* was higher than *P. obovatum*.

Result of real difference test of ratio of width buttress against stem diameter was showed that there was no real difference between the two species ($f = 1.705, p = 0.199$). Instead, real difference test results of ratio of thick buttress against stem diameter between the two species indicated that there was real difference ($f = 4.363, p = 0.043$). The test results showed that for the same stem diameter, the
average width buttress of *P. maliliensis* almost equal to the average width buttress of *P. obovatum*. However, the buttress of *P. maliliensis* significantly was thicker than the buttress of *P. obovatum*.

### 3.4. Morphology of trees in general

The morphology of tree in general was a combination of stem diameter, total of tree height, bole height, and wide canopy. To determine whether there were differences in the morphology between the two species, real difference test could be done by comparing the ratio of the total height, bole height, and wide canopy against stem diameter between the two species. The test results showed that there was no real difference to the ratio of the total height of the tree against stem diameter (*f* = 0.335, *p* = 0.0566) and the ratio of the width canopy against stem diameter (*f* = 0.860, *p* = 0.0359). Instead, for the ratio of the bole height against stem diameter was found significant differences (*f* = 5.270, *p* = 0.027). Based on this result, for the same diameter, *P. maliliensis* had a similar height tree and wide canopy between *P. obovatum*, but bole height of *P. maliliensis* was lower than bole height of *P. obovatum*.

![Figure 5. Changes in the shape and size of leaf without differentiating the species in line with changes in the distribution area](image)

In addition to some of ratio of the tree size variables against the stem diameter as mentioned above, also compared the ratio of the total height against the bole height (Top Crown Ratio) between the two species. The test results showed that there was no real difference between the ratio of the total height against the bole height between the two species (*F* = 0.947, *p* = 0.0337). It also showed that there was no difference Top Crown Ratio between the two species.

### 4. Discussion

Based on the statistical analysis result of the morphology of two species *Palaquium*, it can be concluded that differences in leaf morphology both of species was one of the main bases for dividing these into different taxon levels. Leaf form of *P. maliliensis* was *elliptical*, whereas leaf form of *P. obovatum* was *obovate*. As can be seen from the contents of the questions in Thonner's Analytical Key to the Families of Flowering Plants, morphology of plants is distinguished by group of taxon level of the family based on the similarity or dissimilarity characteristics of the interest organs [9]. Instead, for clustering plants at the level of species was generally used form of leaf [10]. Taxon classification system based on morphology is known as artificial classification system. Other taxonomic groups of scientists adhere classification system that distinguishes on the basis of genetic kinship is known as phylogeny classification system. Gene is a determinant of the nature of an organism, while the morphology of trees such as leaf shape is phenotype which is a reflection of the nature of genes. Therefore, the result of artificial classification is generally similar to the classification results in phylogeny. On the basis of the theory, can be assumed that the real difference in the form of a leaf between the two species showed that there has been a fairly significant genetic changed between the two species.
Besides of leaf morphology and size of the buttress, visually differences between the two species also can be seen from the appearance of the stem. Stem of *P. maliliensis* seems had more knurr, it was not overly impressed straight, and also having bole height tended to be lower. On the other hand, stems from *P. obovatum* appeared straight and smooth without having a lot of knurrs. The difference in appearance between the stem characteristics of both species indicated that the growth of *P. maliliensis* was slower but more robust than the growth of *P. obovatum*. According to the local community (Syarifuddin, pers. Com. July 14, 2014), the wood of *P. maliliensis* was stronger and darker in color (red) compared with the wood of *P. obovatum*

Although no previous phylogenetic studies that indicated the origin of the two species, the similarities and also differences in morphology, demonstrated by the results of a statistical test between *P. maliliensis* and *P. obovatum*, has strengthen the notion that these species were sympatric congeneric species. As reported by Savolainen et al. (2006), a palm plant in phylogeny which are known from similar parent about 6.9 million years ago was placed as a distinct species-level taxon, because it shows difference in flowering time as a result of differences in soil types preference in the same habitat. The results of genetic research on these species showed differences in the genome, though not much but it was enough to claim the palm into two distinct taxa [8].

Data on the distribution of topographic of both species showed that although growing in the same habitat, two species were occupying of different niche/microhabitat. *P. maliliensis* was found growing on a steep slope area both on the lower slopes (near the river) and the upper slope (near the top hill). Instead, *P. obovatum* was found on sloping area between the lower slopes of the upper slope. In the transition area between the microhabitat, sometimes found individuals which were not easy or confusing to distinguish between *P. maliliensis* and *P. obovatum*. Difficult to distinguish between these species, mainly occurs on leaves morphology that was midway between the elliptical shape and *obovate* shape. Most of the leaves tended to be elliptical and some were likely to *obovate* in addition to the morphology of leaves, difficult to determine *P. maliliensis* or *P. obovatum* also occur on the stem characteristics, which were found many knurrs as in *P. maliliensis* but buttress form was more similar to *P. obovatum*. The data was reinforcing the notion that aspects of slope level as one of the most dominant factors influencing the process of sympatric speciation in produce both of these *Palaquium* species and the speciation process is currently underway.

Cox and Moore (2005) explains that, if the environment in which to grow was changing / distinct there will be a natural selection that produce a morphological adaptation of species to new environments [1]. According to the Chiatante et. al. (2002), the amount of slope directly alters the soil depth and water content. Steep slopes usually have shallow soil and lacking of water supply, while the sloping area that has a moderate to deep soil depth where water supply is usually plenty [11]. Associated with these explanations, can be presumed that *P. maliliensis* who was occupy the steeper areas grow more slowly produce stronger stems compared with *P. obovatum* which grows in shallower areas with deeper soil layers and more water supply.

5. Conclusion

The results of this study concluded that the degree of slope was one of the major microhabitat factors that has driven the process of sympatric speciation between *P. maliliensis* and *P. obovatum*. *P. maliliensis* only found on the steep slopes (ranging between 24 - 43 percent), while *P. obovatum* found on sloping areas (ranging from 2 - 7 percent).

In morphological characteristics, differences between *P. maliliensis* and *P. obovatum* appeared in the form of leaves, size of buttress, and form of stem. Statistical analysis showed that leaf of *P. maliliensis* had elliptical shape whereas leaf of *P. obovatum* had *obovate* shape. Size buttress of *P. maliliensis* significantly thicker than buttress of *P. obovatum*, and *P. maliliensis* actually had bole height lower than *P. obovatum*. Visually, the stem form of *P. maliliensis* was more solid, not straight and had a lot of knurrs, than the stem form of *P. obovatum* was straight and smooth with little knurr.

The difficulty in determining *Palaquium* species that found in the transition area between the steep topography and slope topography, has strengthen the notion that sympatric speciation is still in process.
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