Characterising the diversity of Zingiberaceae leaf shape.

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Abstract

Zingiberaceae is the largest and popular family in the Zingiberales of monocots with a worldwide distribution. It includes around 50 genera and 1300 species. Many species in Zingiberaceae are used in many fields of human's life because of their high economic value. Zingiberaceae has high diversity of the morphology characters for both flower and leaf because of the number of species in this family. These morphological features provide a reliable basis for the identification of species in Zingiberaceae. At present, there are few reports on the study of their morphology, especially the leaf shape. In this project found that the leaf shape in Zingiberaceae has significant difference between species from both statistic and phylogeny results by using herbarium samples of *Zingiber*, *Aframomum*, *Boesenbergia*, *Curcuma*, *Globba* and *Hedychium*.

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1. Introduction

1.1 Leaf, an important organ for plants with significant fundamental functions.

The leaf is the main photosynthetic organ in plants and is an important structure for transpiration. Photosynthesis is the chemical process by which plants produce organics and oxygen by using carbon dioxide, water and light. As photosynthesis uses carbon dioxide and produces oxygen, it also has close relationship with and very important influence for both human beings and the ecosystem (Sujatha, 2015). Different leaf shapes influence photosynthesis by impacting on the shape and proportion of palisade cells and spongy mesophyll cells which carry out distinct functions (Shabala, et al., 2002). Therefore, different shape and structure of leaf would influent the photosynthetic efficiency of plants (Adams & Terashima, 2018). The shape of the leaf also influences the number of stomata which impacts on transpiration rates and gas exchange. Generally, the larger leaf blade is, the more stomata and the stronger transpiration exist. Given these effects of leaf shape on photosynthesis and transpiration, leaf shape also plays an important role in how plants adapt to different environments. For example, in the tropical rainforest area, there are more broad leaf plants, it helps them enhance their transpiration efficiency and temperature regulation. In the cold or dry areas, there are more plants with small leaves or needle-point leaves, which helps them enhance their photosynthesis efficiency and reduce water evaporation. Hence, the leaf is one of the most important organs for plants. It is important to research the leaf and the leaf shape of plants.

1.2 The classification of the monocots.

True leaves are characteristic of vascular plants (Evert, et al., 2007), most vascular plants are part of the flowering plants which is also called the angiosperms. In this group of plants, the monocots is one of the most important independent groups distinguished from the eudicots, with distinct morphological characters. The APG IV system which classifies plants using molecular-based characters, defines the monocots as including 8 orders, 62 families and around 67,000 species (Robinson, 2016 & Anon, 2016).

1.2.1 The fundamental differences between monocots and eudicots.

There are many morphological differences between monocots and eudicots (Fay, 2013 & Robinson, 2016) (Figure 1). Firstly, in their seeds, monocots only have a single cotyledon but eudicots usually have two. Secondly, the arrangement of vascular bundles in their stem is different, in monocots vascular bundles are scattered and in eudicots they are arranged in distinct ring. Thirdly, the number of petals for monocots is in multiples of three, and it is usually in multiples of four or five in eudicots. There are also distinct morphological differences in the leaf. For monocots, their leaves consist of the proximal sheath and distal blade with the ligule as their anatomical marker (Kaplan, 2001). Monocots do not have petiole as eudicots. Monocots' leaves are also usually linear in shape with parallel veins, whereas eudicots' leaves have net pattern leaf veins.

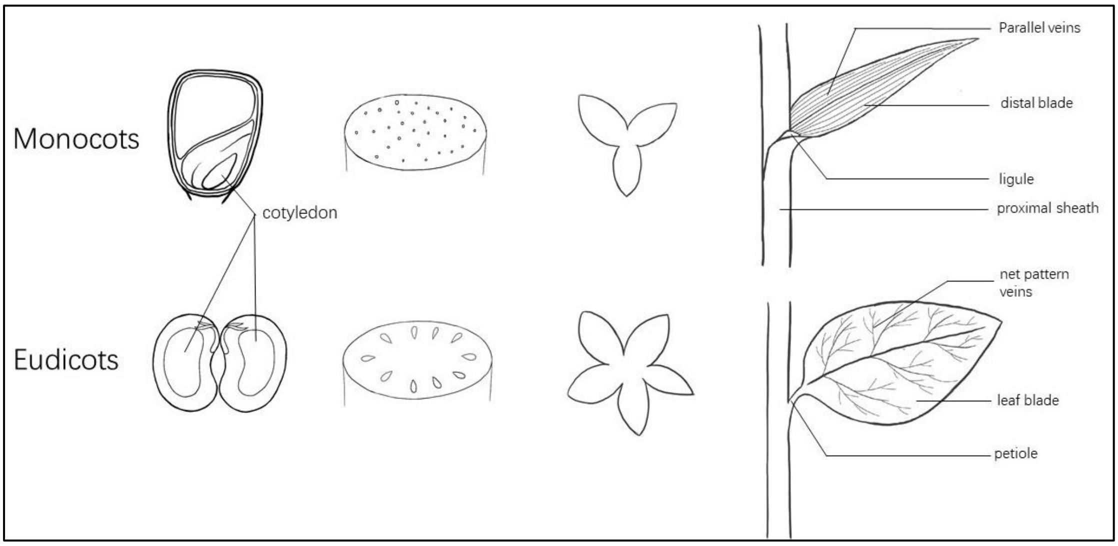

Figure 1 Basic morphologic differences between monocots and eudicots. (The monocots usually have only one cotyledon, the scattered vascular bundles in their stem, the number of petals is usually three and the different leaf structure with eudicots.)

1.2.2 Monocots, a valuable clade in different fields.

As an important clade of angiosperms, the monocots have a great deal of values for human society, including in agriculture, manufacturing and horticulture. For instance, there are many important crops from the grass (Poaceae) family. In this family, the wheat (*Triticum aestivum*), rice (*Oryza sativa*) and corn (*Zea mays*) are seen as the three major cereal grain crops of the world, and they are a staple food source. Grasses are also important fodder source for livestock. Monocots also provide more luxury crops such as fruit. For example, banana is from *Musaceae*, pineapple is from Bromeliaceae, coconut is from Arecaceae, and sugarcane is from Poaceae. Many other monocot plants are significant raw materials for manufacturing products, such as bamboos for papermaking and construction. Moreover, because of the high diversity in morphology, monocots have a great ornamental value in horticulture. For example, the *Liliaceae* and the Orchidaceae have high floral diversity with special petal shapes and varied colours, the Arecaceae is an economically important monocot family as they are widely used to decorate street and gardens, because of their attractive huge palmate leaves.

1.3 The Zingiberaceae.

The Zingiberaceae family is a highly diverse group of monocots with significant economic value due to its widespread use in horticulture, its use as a spice and its use as a component of traditional medicines (Tamokou, Mbaveng, Kuete, 2017). More recently research has also focussed on the extraction of compounds from members of the *Zingiberaceae* for their medicinal properties (Tamokou, Mbaveng, Kuete, 2017), as well as compounds involved in scent (Raj, et al.,2013; Kanjilal, et al., 2010; Jena, et al., 2016; Chane-Ming, & Chalchat, 2003).

1.3.1 Zingiberaceae, a family with wide distribution.

Zingiberaceae is the largest family in the Zingiberales of monocots and made up with around 50 genera including more than 1300 species (Christenhusz & Byng, 2016). In the Zingiberaceae, there are four main tribes (see the appendix VII the phylogeny poster of Zingiberaceae), these are the Globbeae, the Zingibereae, the Riedelieae and the Alpinieae. Genus Zingiber is the type genus of the Zingiberaceae and ginger is from this genus. The distribution of the Zingiberaceae is worldwide but concentrated in the tropical and sub-tropical areas of the world. The Zingiberaceae species are native to Asia especially the Southeast Asia, and have a wide distribution all over the world, their wild species also can be found in Africa, America and Australia (Fig.2). Most Zingiberaceae are in tropical and sub-tropical areas, but some species can also be found in the warm-temperate zones (Delin Wu & Kai Larsen, 2008). The Zingiberaceae is one

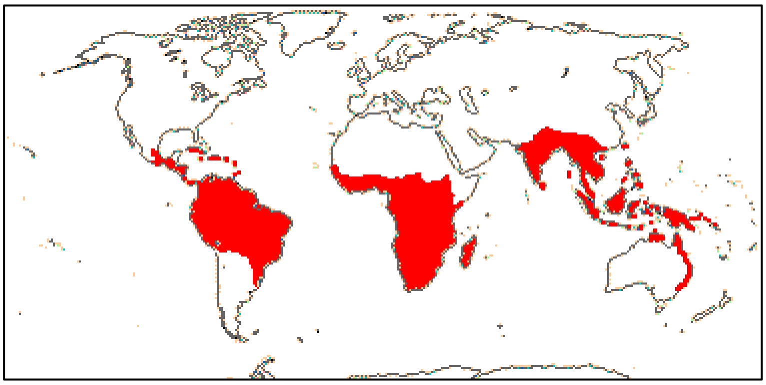

Figure 2 Distribution of Zingiberaceae. The Zingiberaceae is a worldwide living family and mainly distributes in the tropical areas. The distribution map comes from the Angiosperm Phylogeny Website.

of the familiar families known by publics, because of their wide distribution.

1.3.2 Zingiberaceae economic value.

Most Zingiberaceae species have highly aromatic odours, especially in the foliage or roots. Therefore, there are numerous species usually used as spices in cuisine and these spices have a long history of trade in the world. For example, ginger from the genus Zingiber and turmeric from the genus *Curcuma* (Zhou, et al., 2018). In some countries, Zingiberaceae species are used as traditional medicines, and some research shows that chemical compounds extracted from Zingiberaceae have medical activity (Tamokou, Mbaveng, Kuete, 2017). Many species in this family have special odour, and it usually comes from the essential oil in their leaves. In addition, many Zingiberaceae leaf research (Raj, et al.,2013; Kanjilal, et al., 2010; Jena, et al., 2016; Chane-Ming, & Chalchat, 2003) are focusing on the essential oil. Therefore, Zingiberaceae is a great family with high diversity and economic value.

1.3.3 The sister group of Zingiberaceae.

The Costaceae is the closest sister family to the Zingiberaceae. Both two families have worldwide distribution (Fig.3), but the Zingiberaceae is distribute wider than the Costaceae, especially in temperate area in the Asia and the North America. In the southeast Asia and the Pacific islands, the distribution of the Zingiberaceae are more intensive. In addition, there are more Zingiberaceae species distribute at the east coast of Australia.

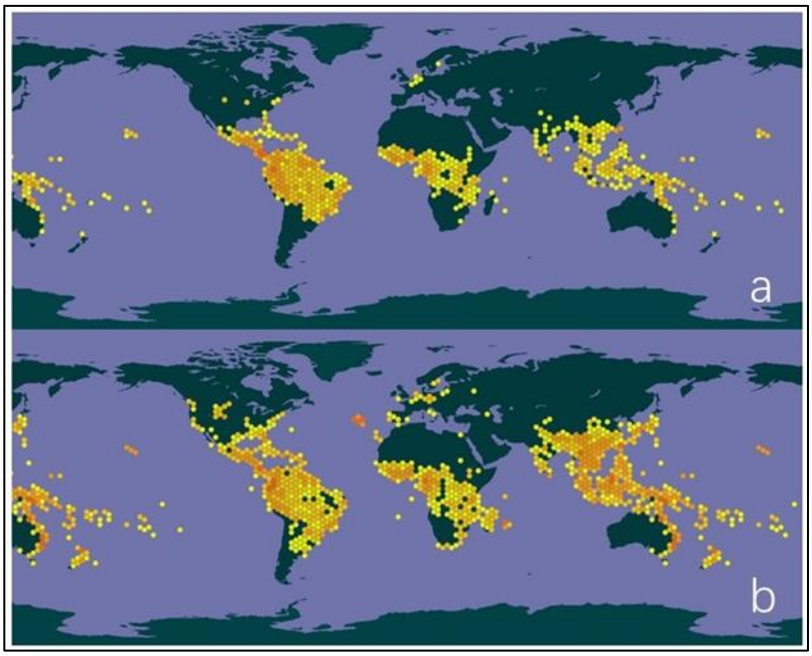

Figure 3 Distribution of Costaceae and Zingiberaceae. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). The distribution of Zingiberaceae is wider than the Costaceae. a. The distribution of Costaceae. b. The distribution of Zingiberaceae.

1.3.4 Ligule morphologic difference in the two families.

A main difference between these two families is the morphology of their ligule. It also is a key character to distinguish these two families. The Costaceae has complete ligule surrounding the stem. The ligule of Zingiberaceae is lobed (Fig.4). Therefore, the morphological character from leaf also is important for classification. The family

Zingiberaceae is a good sample with great morphological characters to be explored.

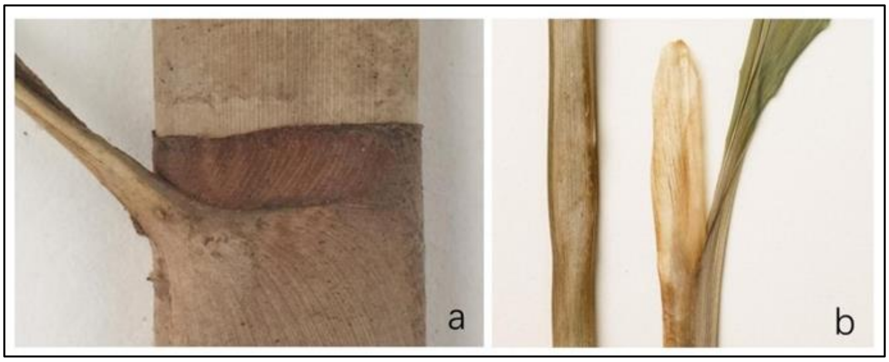

Figure 4 Ligule of Costaceae and Zingiberaceae. The ligule of Costaceae is complete and in Zingiberaceae it is lobed. The specimen images come from RBGE herbarium catalogue. a. Costaceae; *Costus dubius* (Afzel.) Schum.; (E00680767) b. Zingiberaceae; *Hedychium cylindricum* Ridl. (E00421687).

1.3.5 Leaf morphology between Zingiberaceae and Costaceae.

Zingiberaceae is a large monocots family with more than 1300 species and a worldwide distribution. However, there is a blank for the leaf morphology of the Zingiberaceae. The morphology research for Zingiberaceae are focusing on the seeds and pollens, few of them focusing on the leaves. Zingiberaceae has high diversity on the morphology on the seeds (Benedict, et al., 2015.; Anon, 2015) and pollens (Chen & Xia, 2011; Sakai, et al., 2013; Saensouk, et al., 2009). Chen and Xia (2010) find that there are good differences on the *Curcuma* leaf epidermal morphology. Therefore, Zingiberaceae is a good family to do more morphological research on their leaves, and it could have good diversity of the leaf shape.

1.4 The morphology of the Zingiberaceae.

1.4.1 Zingiberaceae, an attractive family characterised by their flowers.

As a monocot family in horticulture field, the Zingiberaceae (ginger) family has high morphological diversity with great ornamental value (Fig. 3). The Zingiberaceae are also important evolution models, some research shows that they should have 6 stamens, but 5 of them have disappeared or become petaloid (Kirchoff, B.K. et al., 2009). The

Zingiberaceae are mainly classified by their flower structures' characters.

Figure 5 Flowers in Zingiberaceae. The Zingiberaceae flowers have various variation with many shapes and colours. These pictures are from the http://www.botany.hawaii.edu/faculty/carr/zingiber.htm, taken by G. D. Carr.

1.4.2 Leaf morphology of Zingiberaceae.

The leaf of Zingiberaceae has a high diversity and is characteristic. The monocots leaf consists of the leaf blade and the sheath with the ligule as their anatomical marker. However, not all the monocots have the ligule. In the Zingiberales, Zingiberaceae and Costaceae are the two families with obvious ligule. While, for the morphology of the ligule, in the Zingiberaceae their ligules are lobed, but in the Costaceae they are complete (Poulsen, & Lock, 1997). This is also an important character to distinguish these two sister families.

1.5 Aims.

Therefore, this project is focusing on the leaf shape of the Zingiberaceae. This project aims to answer the question: whether there are significant differences in leaf shape between Zingiberaceae species and how does their leaf shapes vary in the evolution context. There are abundant herbarium online resources. Using online herbaria resources for observing the leaf shapes of Zingiberaceae and collecting the leaf shape data for quantitative analysis of their leaf shape to observe leaf shape and place this in a phylogenetic context.

2. Methods

2.1 Genera selection.

To select Zingiberaceae genera for analysis, we looked at the published Zingiberaceae phylogeny (Theodor, 2020). There are 4 main tribes in the Zingiberaceae, the Globbeae, Zingibereae, Riedelieae and Alpinieae. The Globbeae and the Riedelieae are smaller tribe who have fewer species comparing with the other tribes. We used two main criteria to select the genera for analysis. The first criteria was the species number in the genus (we chose a cut-off of over 50 species), the second was the specimen condition in digital herbaria. There are 8 genera that contain more than 50 species, these are *Globba* from the Globbeae, *Curcuma*, *Boesenbergia*, Zingiber and *Hedychium* from the Zingibereae, Redychium from the Riedelieae, and the *Aframomum* and Renealmia from the Alpinieae. We chose to focus on the larger tribes, with wide global distributions and those with commercially important species. We therefore chose to exclude Riedelieae from the analysis.

Ultimately, we selected 6 genera for analysis, *Globba* from the Globbeae; *Curcuma*, *Boesenbergia*, *Zingiber* and *Hedychium* from the Zingibereae; and *Aframomum* from the Alpinieae. The simplified phylogeny relationship of these 6 genera is showed in the Fig 6.

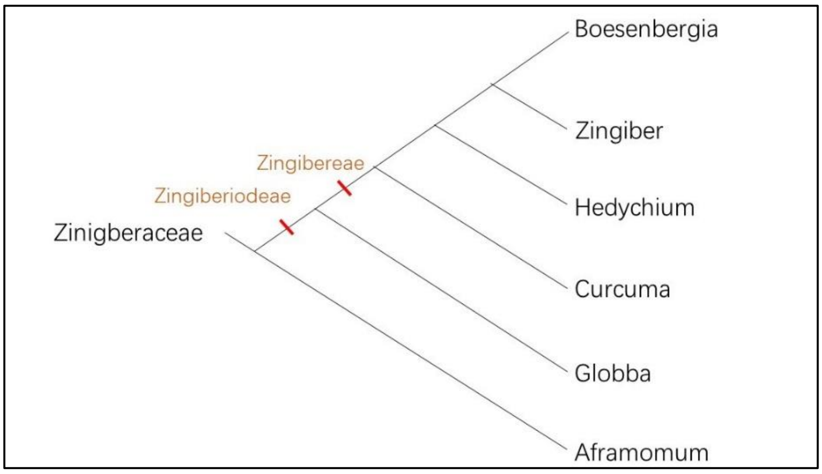

Figure 6 The simplified phylogeny tree of *Aframomum, Globba, Zingiber, Boesenbergia, Hedychium* and *Curcuma*.

2.2 Digital herbarium selection.

In order to analyse leaf shape in a broad ranges of species remotely, we chose to analyse images from digital herbaria: the Index Herbariorum, Harvard Herbarium specimen database, MNHN Herbarium Catalogue (the Muséum national d'Histoire naturelle digital herbarium), the Kew Herbarium Catalogue and the RBGE herbarium catalogue (the Royal Botanic Garden of Edinburgh digital herbarium). In these digital herbarium databases, the Kew garden herbarium, Harvard herbarium and the RBGE herbarium have more specimen records for the *Zingiber*aceae. However, most specimen records in the Harvard herbarium database are descriptions without images, we therefore did not use the Harvard herbarium for this study. Both the Kew garden herbarium and the RBGE herbarium have a large number of specimen images. The Kew garden has more type specimens which is a very important resource to identify species. However, in this digital herbarium, the preservation of the specimens is poor which affects our ability to quantify our observations. For example, the Figure 7 demonstrates the same species' specimens, *Aframomum leptolepis* (K.Schum.) K.Schum., in the Kew Herbarium catalogue and the RBGE herbarium catalogue. Figure 7.a. is from Kew Herbarium. Even though this image is of a type specimen, the leaf is folded at the edge making shape analysis difficult. Figure 7.b is the *A. leptolepis* specimen from the RBGE herbarium catalogue, whose leaf is well-pressed and we can see more morphological details of the leaf. Some labels of digitised specimens are also limited in useful information. In the RBGE herbarium, most specimens have been collected in past 50 years, and have better preservation with more information on their labels. These conditions are helpful to observe more morphological information from the specimens. Therefore, in this project, most specimen images used came from the RBGE herbarium catalogue.

Figure 7 The comparation of Specimens in Kew Herbarium catalogue and RBGE Herbarium catalogue. These two specimen are the same species, *Aframomum leptolepis* (K.Schum.) K.Schum.. a. *Aframomum leptolepis* (K.Schum.) K.Schum. from the Kew Herbarium. (K000743719), the leaf edge is folded which is not ideal enough to observe the leaf shape. b. *Aframomum leptolepis* (K.Schum.) K.Schum. from the RBGE Herbarium. (E00930403). The leaf is well-pressed on the flat and can see the detail of leaf shape, it is better specimen sample.

2.3 Species, specimens and leaves selection.

To select species for analysis we began browsing all of the species specimens in the genus phylogeny in the RBGE herbarium catalogue, identifying those with digital images – specimens without confirmed names were excluded at this stage. We chose to carry out analysis of three biological replicates, therefore only species with three or more independent samples, that were of a good quality image had at least one complete leaf without any folded edge, out of shape, crumpled blade and damaged structure are reserved (the Fig.8 shows the specimens with bad quality). The .tiff images of the selected species were then downloaded for shape analysis (see appendix I: Specimens information for a list of all species analysed in this study). However, for the *Curcuma*, many species in this genus has large leaves and the leaf specimens of them are usually folded. Therefore, in *Curcuma*, the species with large leaves which can be measured all the data are reserved but these species could only have 2 measured leaves in total.

Figure 8 Bad quality specimens. a. damaged leaf structure and shape（E00183020）. b. folded leaf edge and overlapped leaves (E00643275). c. crumpled leaf blade and folded leaf (E00499907). The specimen images come from the RBGE herbarium catalogue.

2.4 Measurement of the leaves.

To measure different features of the leaves we used the image analysis software package FIJI (Schindelin et al., 2012) using the following protocol.

We used FIJI software to open the .tiff images of the specimens and using the line tool, chose 2 cm on the ruler on the specimen and clicked 'Analyse'- 'Set Scale' in the tool bar to set the accurate scale which is showed on Fig.9.

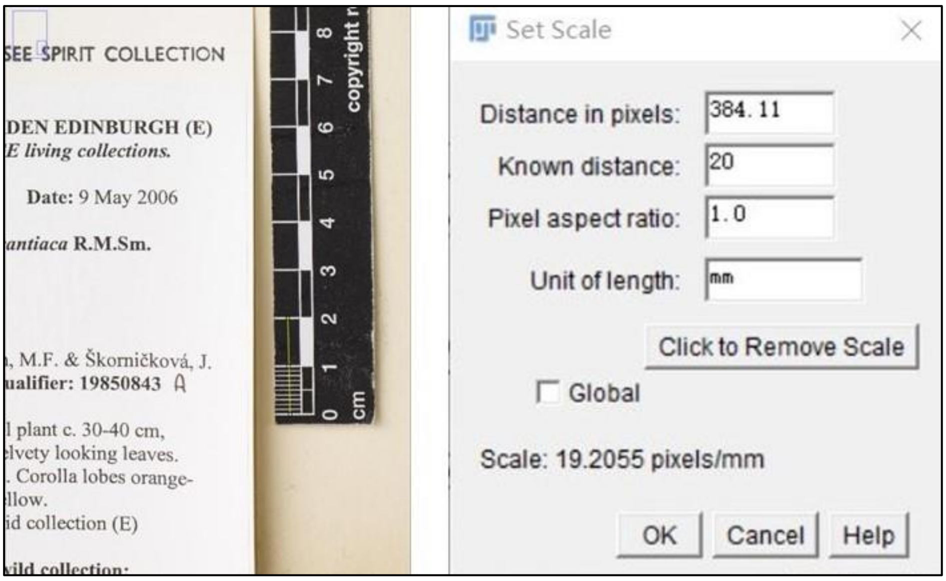

Figure 9 Set scale in the FIJI. The left photo shows using line tool to choose 2 cm on the origin scale on the specimen. The right photo is setting scale in tool bar.

Then, we used the line tool to choose the distance which would like to be measured and clicked ‘Analyse’- ‘Measure’ in the tool bar. The data measured were the length of ligule, length of whole leaf distal blade, the width which cross the mid-point of the leaf blade without pseudo-petiole, the widths which cross the upper and lower quartile and the pseudo-petiole length (Fig.10).

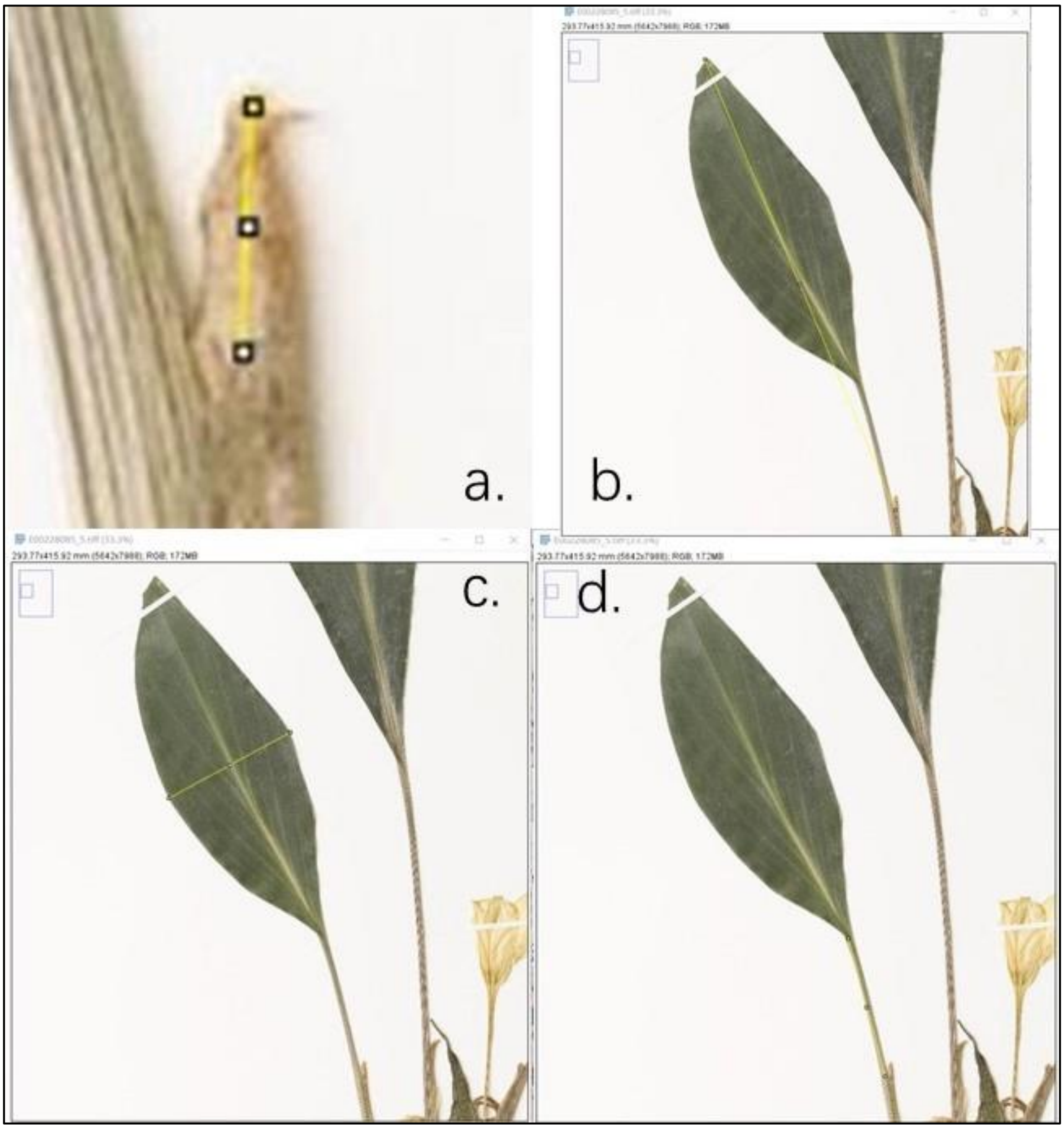

Figure 10 Measure data of the specimens’ leaf. a. measuring the ligule length b. measuring the whole leaf length (from the tip to the base of ligule) c. measuring the leaf width (across the mid-point of the lamina) d. measuring the petiole length. The specimen showed in this figure is the *B. aurantiaca* (E00228085)

After that, we used the angle tool to measure the angle between the main vein and lateral veins (Fig.11). This tool was used to select the angle between the main veins and the lateral veins passing through the midpoint of the blade length and then clicked 'Analyse'- 'Measure' to get result. All the measured results were shown in the 'Results' window.

Finally, we counted the number of veins of all the measured leaves if it was possible (can see the veins clearly).

Figure 11 Measurement of the angle between the main vein and the lateral veins.

2.5 Statistics analysis.

2.5.1 ratio calculation.

To statistic and calculate the collected data of leaves, we used the data statistic software the Microsoft Excel (Quirk & Rhiney, 2020).

All the measurement results were put into Excel for calculation. There are the parameters calculated in this study.

1) Ligule - leaf ratio: using the ligule length data divided the whole leaf blade length data. Because the leaf size has great difference between Zingiberaceae species, some big leaves have big ligules, this parameter would show the relative size of their ligule in the family and demonstrate the species which have real big or small ligules.

2) Pseudo-petiole - leaf ratio: using the pseudo-petiole length data divided the whole leaf blade length data. Because not all the genera and species in the Zingiberaceae have obvious pseudo-petiole structure, this parameter would show which species have conspicuous pseudo-petiole.

3) Length-width ratio: using the leaf width data (across the mid-point of the lamina) divided the whole leaf blade length data and calculating the approximate result of this ratio which helps to make the results seem more straightforward. The ratio of the leaf width and length will show a general impression of the leaf shape, the leaves look linear or roundish.

The excel was also used to make box plot graph for each genus. We selected the data which should be graphed and insert the box plot graph in the excel.

2.5.2 One-way ANOVA.

Excel was used to run one-way ANOVA to explore if the ligule-leaf ratio, pseudo-petiole ratio and the width-length ratio have significant difference between species within the same genus.

2.5.3 Post-hoc analysis: Tukey-test q value calculation.

The Tukey-test can quantize the difference between groups, but in the excel, there are no automatic program to do this test. However, this would not affect the calculation of Tukey-test in excel (Zaiontz, 2020). To calculate the q value of the Tukey-test, the difference is the absolute value of the average number of the compared two groups. The *n* is the count number of each group, SE=$\sqrt{\frac{1}{2}MSw(\frac{1}{n_1}+\frac{1}{n_2})}$ (*MSw* is the MS value of the within groups which can be looked up in the ANOVA table), q=$\frac{Difference}{SE}$. Then check the Q table to confirm the Q value and compare the q with the Q.

2.6 Establishment of the phylogeny tree.

To build the phylogeny tree of the selected species and find out the characters evolution in a phylogenetic context, we used the R package and the nuclear ribosomal internal transcribed spacer (ITS) data from the Genbank database.

Download the ITS DNA data and select the sequence accession number for each species in the Genbank database (https://www.ncbi.nlm.nih.gov/genbank/) (it is showed in appendix V). The accession data include an outgroup species to help build the phylogeny tree of the picked species. The species picked in this project is the *Tamijia flaggellaris* which is the only species in the tribe Tamijioideae in Zingiberaceae. A file was built with only accession number of the species and saved as “Ginger_final.csv” in the excel.

However, in the Genbank, it is lacking the data for the *A. chrysanthum*, the *C. larsenii* and the *Z. nudicarpum*. In the built phylogeny tree which is not including these three species. There are the steps we built the phylogeny tree.

1. Use R to read the genbank data and renamed it to “renamed_ITS.fasta”. (The used commands are recorded in the appendix VI part 1.)
2. Align the DNA sequence by MUSCLE (Madeira et al 2019), copy the aligned DNA

data and save them as “Ginger_aligned.fas” in the word pad.

3. The aligned DNA data was checked in the software Bioedit.
4. The RAxML BlackBox online was used and uploaded the aligned DNA data, chick the Bootstrap and the Boostopping cut off value was 0.03 and submit. The phylogeny tree with an outgroup should be build up.
5. The “result-raxml.support” file was renamed to “LZ-tree.tre”.
6. Back to R, found the storage location of the file “LZ_tree.tre” and did the command recorded in the appendix IV Part 2.
7. The excel was used to type-in the morphology characters data and save the file as “Ginger_character_matrix.csv”
8. Back to R to do the character analysis and build trees. The commands used in this step are recorded in the appendix VI part 3.
9. Run the Blomberg’s K (Blomberg et al. 2003) tests in R by using the commands recorded in the appendix VI part 4. This phylogenetic signal is helping to estimate the genetic relationship between species.

3. Results

There are 87 digital herbarium specimens for 29 species from 6 genera used to assess leaf morphology. In order to quantify shape, in FIJI, it is measured that the ligule length, the length of whole leaf (from the base of ligule to the leaf tip), the leaf width (across the mid-point, upper quartile and lower quartile separately), pseudo-petiole length and the angle between the main vein and lateral veins. For *Boesenbergia*, the veins number is also counted. These data would describe and help us quantify the leaf shape differences between species and provides the basis for phylogenetic analysis.

3.1 *Zingiber*: A worldwide genus with conspicuous lobed ligule and relatively low diversity leaf shape.

Zingiber is the type genus of the Zingiberaceae and belongs to the Zingibereae tribe. The species *Zingiber officinale* Roscoe. is the type species of the genus which is commonly known as ginger. In this genus, there are 100 to 150 species (Theerakulpisut et al., 2012), with a worldwide distribution (Fig.12) concentrated in the South and Southeast Asia and north of the South America. In the North America, west of Africa, north of Australia and Europe, they also have sporadic distribution. In the RBGE digital herbarium, there are 556 specimens with digital images and most of them come from Southeast Asian countries.

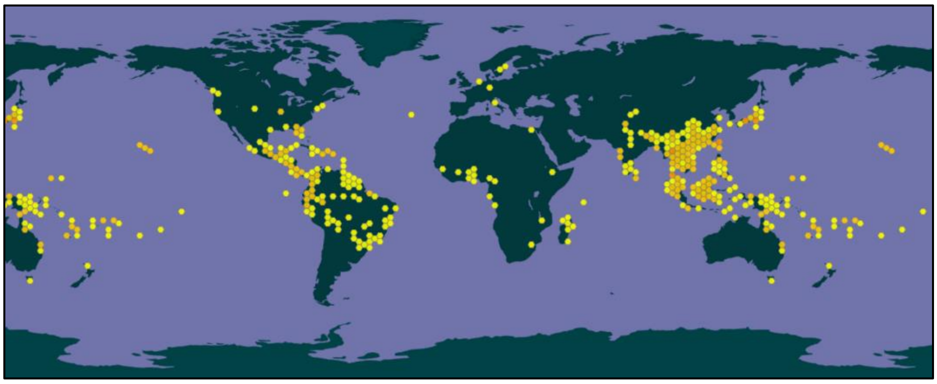

Figure 12 The worldwide distribution of *Zingiber*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). *Zingiber* is mainly distribute in the South and Southeast Asia and north of the South America.

3.1.1 Ligule of *Zingiber.*

Generally, the diversity of ligule shape in this genus is high, some species have conspicuous ligule structure and the variation of the size and shape are high. Ligule shapes can be rotundate (Fig.13.b), obtuse (Fig.13.d.), wedge-shape (Fig.13.e.) or acuminate (Fig.13.a.). *Zingiber* ligules are clearly lobed which is an important typical character for the Zingiberaceae to distinguish from its sister group. *Zingiber* ligules can also be deeply lobed in the middle or from two separate lobes (Fig.13.a.,d.) which is not a common character to the Zingiberaceae. The ligule length of the *Zingiber* has large difference, it can vary from 2 mm (*Z. officinale*) to 27 mm (*Z. bradleyanum*) in different species (appendix II. Table.23). The *Z. bradleyanum* and the *Z. zerumbet* have more varied ligule-leaf length ratio (Fig.15).

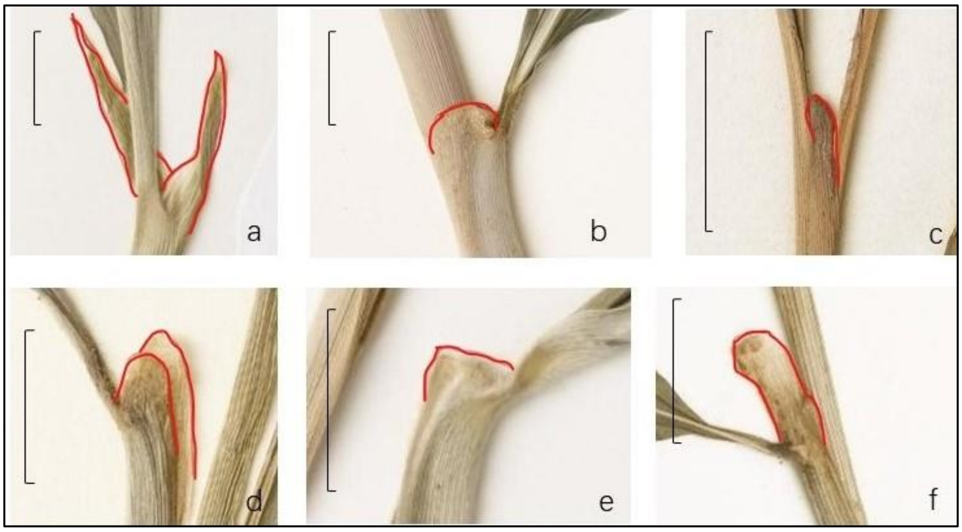

Figure 13 Different ligule shape in *Zingiber. Zingiber* has various ligule shape. The red line indicates the ligule shape of each specimens. The specimen images come from RBGE herbarium catalogue. The scale bar represents 1 cm for each specimen. a. *Zingiber bradleyanum* Craib. (E00294288) b. *Zingiber nudicarpum* D.Fang (E00421674) c. *Zingiber officinale* Roscoe.(E00412174) c. *Zingiber* sp. (E00318742) d. *Zinigbe*r sp. (E00424513) e. *Zinigber* sp. (E00435753) f. *Zingiber* sp. (E00318743).

3.1.2 Leaf of *Zingiber*

In *Zingiber*, the type genus, we measured leaf traits in species: *Zingiber bradleyanum* Craib. (Fig.14.a), *Zingiber nudicarpum* D.Fang.(Fig.14.b), *Zingiber officinale* Roscoe. (Fig.14.c) and *Zingiber zerumbet* (L.) Sm. (Fig.14.d). The leaf size of this genus varies a lot with several kinds of shapes visually (Fig. 14). For example, the typical leaf shape in this genus are oblong (such as *Z. bradleyanum*, *Z. nudicarpum* and *Z. zerumbet*) and linear (*Z. officinale*) (appendix I Table 11). The *Z. officinale* Roscoe is the only one which leaf length over 10 times than the width (appendix II Table 24). However, the *Z. bradleyanum* has the most varied width-length ratio (Fig.15) For the width of the leaf blade, the width across the mid-point is usually the widest, and the width cross the upper and lower quartile are similar in this genus (appendix II Table 23.).

The pseudo-petiole in the *Zingiber* is a small proportion of the total leaf length (Fig.15), the *Z. bradleyanum* and the *Z. zerumbet* have larger ligule ratio and they have bigger variation in species.

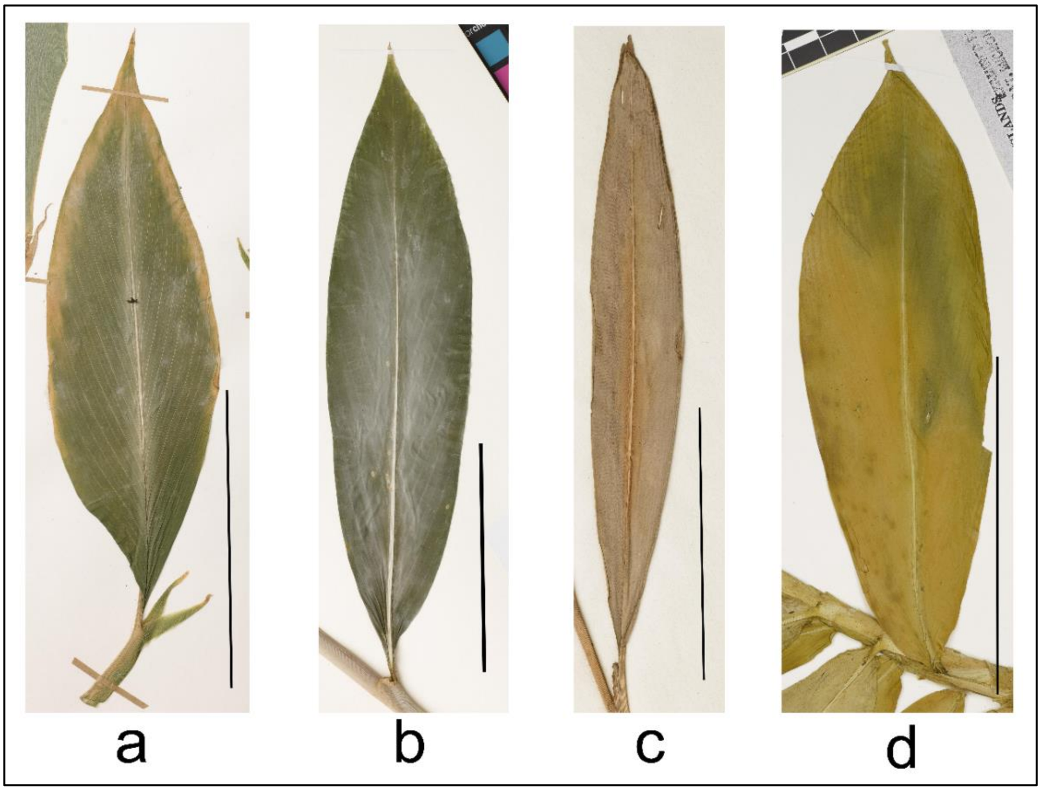

Figure 14 Leaf shape of Zingiber. The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. The leaf size of this genus has widely difference. a. *Zingiber bradleyanum* Craib. (E00077500) b. *Zingiber nudicarpum* D.Fang (E00421674) c. *Zingiber officinale* Roscoe.(E00412174) d. *Zingiber zerumbet* (L.) Sm.(E00770319).

According to the ANOVA results, the P-value for ligule-leaf ratio, pseudo-petiole-leaf ratio and the width-length ratio are less than 0.05 (appendix III table 55-60), which means the ligule length, pseudo-petiole length and the ratio of width-length have significant difference between species in this genus. In addition, the Tukey-test shows that the *Z. officinale* has more difference with other species on the pseudo-petiole-leaf ratio and the width-length ratio. The *Z. nudicarpum* has more difference with others on ligule-leaf length ratio (appendix III Table. 65. 68. 71).

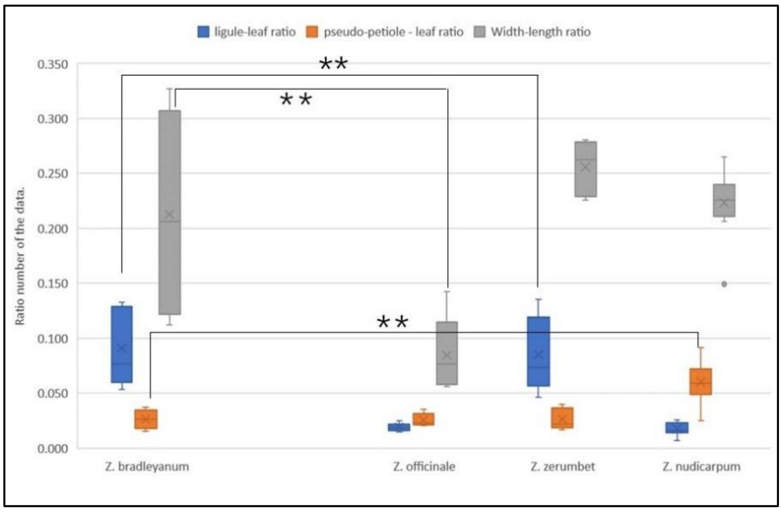

Figure 15 *Zingiber* ratio data box plot graph. The brackets and "**" mean the ANOVA and Tukey-test results show a significant difference between the bracketed species and the P-value from ANOVA test is less than 0.01. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

3.2 *Aframomum*: A concentrated African genus with long aristate apex.

Aframomum is an Alpinieae tribe genus of the Zingiberaceae with around 60 species. Comparing with other genera mentioned, it is a small genus, In RBGE digital herbarium, there are 357 specimens with images. Different from the other genera, this genus has concentrated distribution and major distributes in the west and middle areas of Africa and some islands at east of Africa (Fig.16). They can also be found in south Asia, the north of the South America and pacific islands. It is the largest Africa Zingiberaceae genus and one of the largest Africa rainforest herb genera (Harris, et al., 2000). However, the distribution for every single species is limited. For example, the *A. glaucophyllum*, *Aframomum daniellii* and *A. giganteum*. are found around the gulf of West Africa.

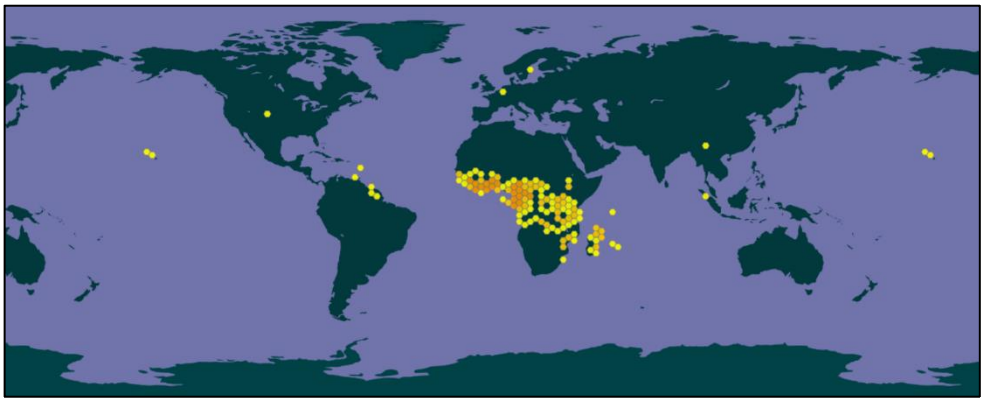

Figure 16 The distribution of *Aframomum*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). This genus mainly distributes in the tropical Africa, especially in the west coast.

3.2.1 Ligule of *Aframomum*

The ligule shape of *Aframomum* in different species have similar shape and does not have conspicuous variation and differences of the shape in the observed species (Fig.17). However, t For the ligule length of the five observed and measured species, they usually come from around 5 to 10 mm, only in the *A. longiligulatum* which has longer ligule (reach to over 17 mm maximum) comparing with other species (appendix II table 13). The Tukey-test results also shows that *A. longiligulatum* has the most difference with other species in this genus (appendix III Table 27). Even though this genus has conspicuous and regular ligule structure, the ligule does not occupy a lot of the whole

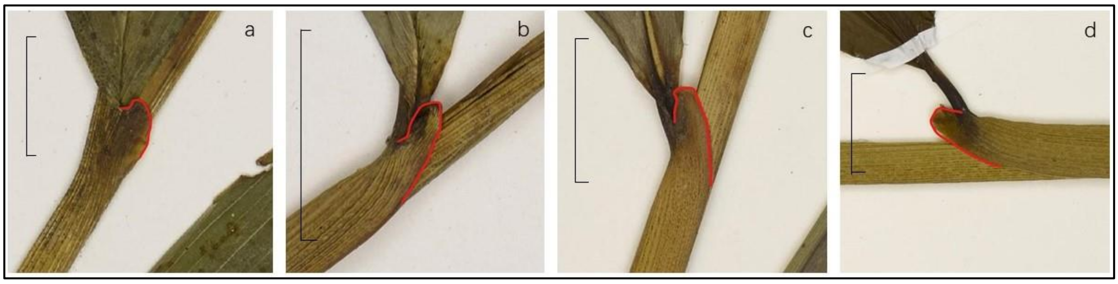

Figure 17 Ligule of *Aframomum*. The red line indicates the ligule shape of each specimens. The specimen images come from RBGE herbarium catalogue. The scale bar represents 1 cm for each specimen. a. *Aframomum angustifolium* (Sonn.) K.Schum. (E00957875). b. *Aframomum chrysanthum* Lock. (E00607558). c. *Aframomum glaucophyllum* (K.Schum.) K.Schum. (E00983168). d. *Aframomum leptolepis* (K.Schum.) K.Schum. (E00930401).

leaf blade. For the ligule-leaf ratio, this data usually would not over than 3% (appendix II table 14), but for several leaves (in *A. longiligulatum*) it can reach around 8%. Except the *A. longiligulatum*, the ligule length in the other species of *Aframomum* has several variations in average (appendix III Table 25).

3.2.2 Leaf of *Aframomum*

We analysed 5 species in this genus: *Aframomum angustifolium* (Sonn.) K.Schum. (Fig.18.a), *Aframomum chrysanthum* Lock. (Fig.18.b), *Aframomum daniellii* (Hook.f.) K.Schum. (Fig.18.c), *Aframomum leptolepis* (K.Schum.) K.Schum. (Fig.18.d) and *Aframomum longiligulatum* Koechlin. (Fig.18.e). The observed leaf shape in the analysed *Aframomum* species is similar. For example, except the *A. longiligulatum*, the other species in this genus have similar width-length ratios (appendix III Table 31). Most species observed in this genus are looked oblong, but from the data of their width measurements, the middle width is usually longer than the lower quartile and the upper quartile width, so their leaf shape are more like elliptic.

Generally, the observed species have either no, or very small pseudo-petioles. The occupation of the pseudo-petiole length for the whole leaf blade is only around 1% or 2%, no more than 3% (appendix II table 14.). Even though the ANOVA result shows that they have significant difference on the pseudo-petiole-leaf length ratio, the Tukey-test results shows they do not have too much difference with each other (appendix III Table 33). Moreover, it is special that the leaves in this genus usually have a long and thin tip (Fig.18), which can be a leaf shape character for the genus. Form the box plot graph (Fig. 19), the width-length ratio for the *A. longiligulatum* and the *A. daniellii* have larger variation. In addition, the *A. longiligulatum* has larger width-length ratio number, which means this species has wider leaf relatively. From the ANOVA results of this genus (appendix III Table 26-33), the P-value of the three ratios are less than 0.05 which shows these ratios have significant difference between species in this genus. The Tukey-test result of the width-length ratio shows that the *A. longiligulatum* has more difference with other species in this genus. Therefore, there are clear shape differences between species in the *Aframomum*.

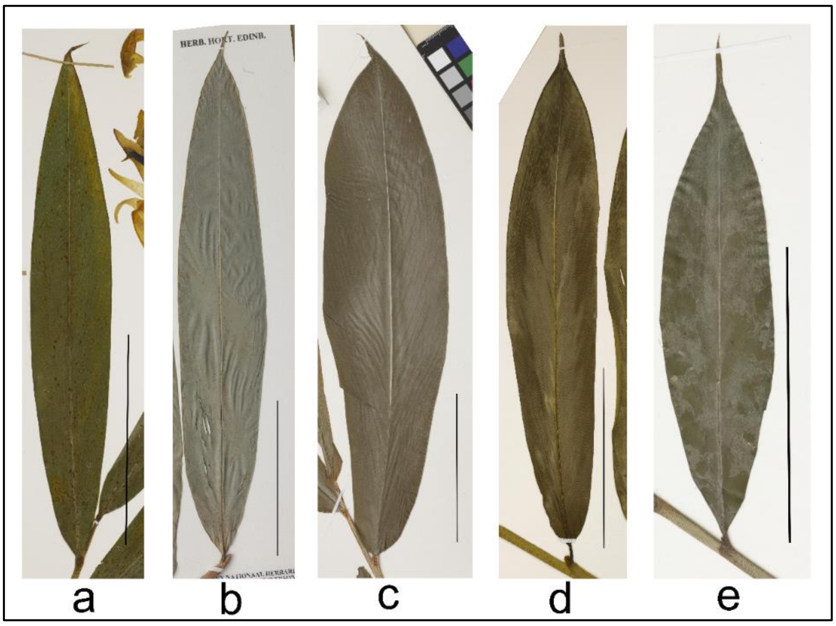

Figure 19 Leaf shape of *Aframomum.* The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. a. *Aframomum angustifolium* (Sonn.) K.Schum.(E00957875) b. *Aframomum chrysanthum* Lock. (E00643480) c. A*framomum daniellii* (Hook.f.) K.Schum. (E00486322) d. *Aframomum leptolepis* (K.Schum.) K.Schum. (E00930401) e. *Aframomum longiligulatum* Koechlin. (E00509461).

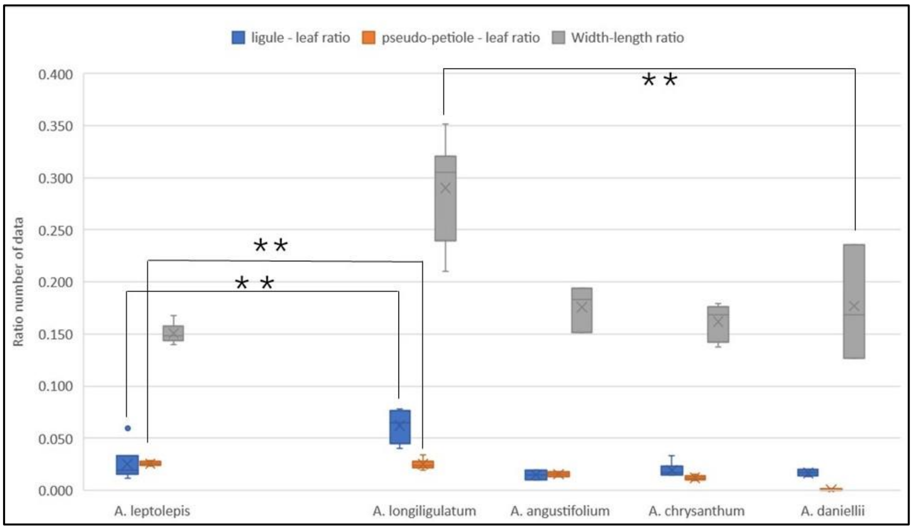

Figure 18 *Aframomum* ratio data box plot graph. The brackets and "**" mean the ANOVA and Tukey-test results show a significant difference between the bracketed species and the P-value from ANOVA test is less than 0.01. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

3.3 *Boesenbergia*: A limited distribution genus in the Southeast Asia with high leaf shape diversity.

Boesenbergia is another genus belonging to the Zingibereae tribe, with around 50 species native to South and Southeast Asia living in the tropical area. This genus has a concentrated distribution in the Southeast Asia countries, such as Thailand, Malaysia, Lao's and Indonesia (Fig.20). In the RBGE digital herbarium, there are 236 specimens with images, and most of them come from Thailand and Malaysia. Single species in this genus can have very limited distributions, such as the *B. cordata* which are only native to one province in Malaysia (see appendix I Table 4.).

Figure 20 The distribution of *Boesenbergia*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). The *Boesenbergia* mainly concentrates in limited areas in the tropical Southeast Asia.

3.3.1 Ligule of *Boesenbergia*

The ligule in *Boesenbergia* is varied in shape, the ligule can have a wedge-shape (Fig. 21. b.) or an acuminate shape ligule (Fig. 21. a.). The ligules generally short usually around 1 or 2 mm except for a few specimens (appendix II Table 15). However, within the *B. orbiculata.*, the ligule-leaf ratio varies, and their ligule length can occupy up to 5% of the leaf length (appendix II Table 16.). Even though the visual observation has many differences, the ANOVA result shows that they do not have significant difference on the ligule-leaf length ratio, which means they do not have many differences on the ligule (appendix III Table 35). The box plot graph shows that the ligule of different species occupies a similar proportion in the whole leaf (Fig. 23).

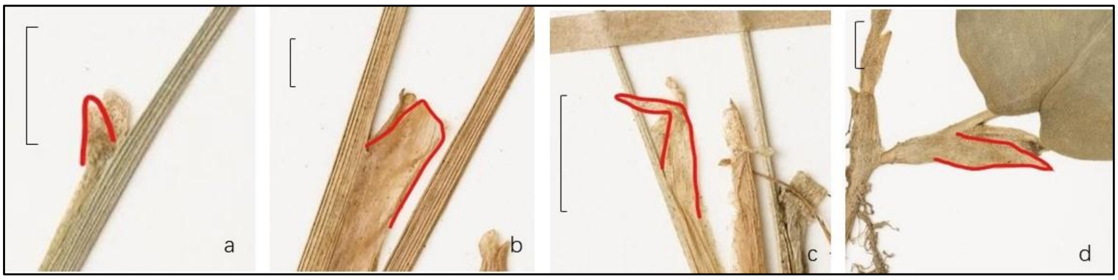

Figure 21 Ligule of *Boesenbergia*. The red line indicates the ligule shape of each specimens. The specimen images come from RBGE herbarium catalogue. The scale bar represents 0.5 cm for each specimen. a. *Boesenbergia aurantiaca* R.M.Sm.(E00228085). b. *Boesenbergia basispicata* K.Larsen ex Sirirugsa. (E00211999). c. *Boesenbergia flavorubra* R.M.Sm. (00389721). d. *Boesenbergia orbiculata* R.M.Sm. (00389730).

3.3.2 Leaf of *Boesenbergia*

Species in the *Boesenbergia* genus have a varied leaf shapes (Fig.22). To quantify the difference in leaf shape, we measured five species: *Boesenbergia aurantiaca* R.M.Sm. (Fig.22.a), *Boesenbergia basispicata* K.Larsen ex Sirirugsa. (Fig.22.b), *Boesenbergia cordata* R.M.Sm. (Fig.22.c), *Boesenbergia flavorubra* R.M.Sm. (Fig.22.d) and *Boesenbergia orbiculata* R.M.Sm.(Fig.22.e). In the observed species in this research, the shape of lamina can have oblong, cordiform and the rotund leaf blade shape (Fig.11). The cordiform and rotund leaf shapes are only found in this genus in this research. Another atypical character in this genus is a long pseudo-petiole, which was observed in all five species analysed here. Generally, the pseudo-petiole length is around one third of whole leaf length (appendix II Table 16). The box plot graph shows that the *B. cordata* has larger average pseudo-petiole to leaf length ratio (Fig.23). From the ANOVA results (appendix III Table 34-41), in this genus, the P-value of the width-length ratio is less than 0.05, so ratio of their leaf width and length have significant difference between species. The Tukey-test result shows that the *B. orbiculata* has larger difference with other observed species, which also can be represented on the box plot graph (Fig.23). The number of width-length ratio for *B. orbiculata* are higher than other species. Statistical analysis of pseudo-petiole to leaf-length ratio shows that the pseudo-petiole-leaf ratio have significant difference between species. Tukey-test results demonstrates that the *B. cordata* and the *B. orbiculata* are significantly different from the other analysed species based on pseudo-petiole to leaf-length ratio (appendix III Table 38).

Based on this analysis of traits, the pseudo-petiole to-leaf ratio and the width to length ratio, could be used to differentiate between species in the *Boesenbergia* genus, for example the *B. orbiculata* is significant different from other species based on these two measured traits.

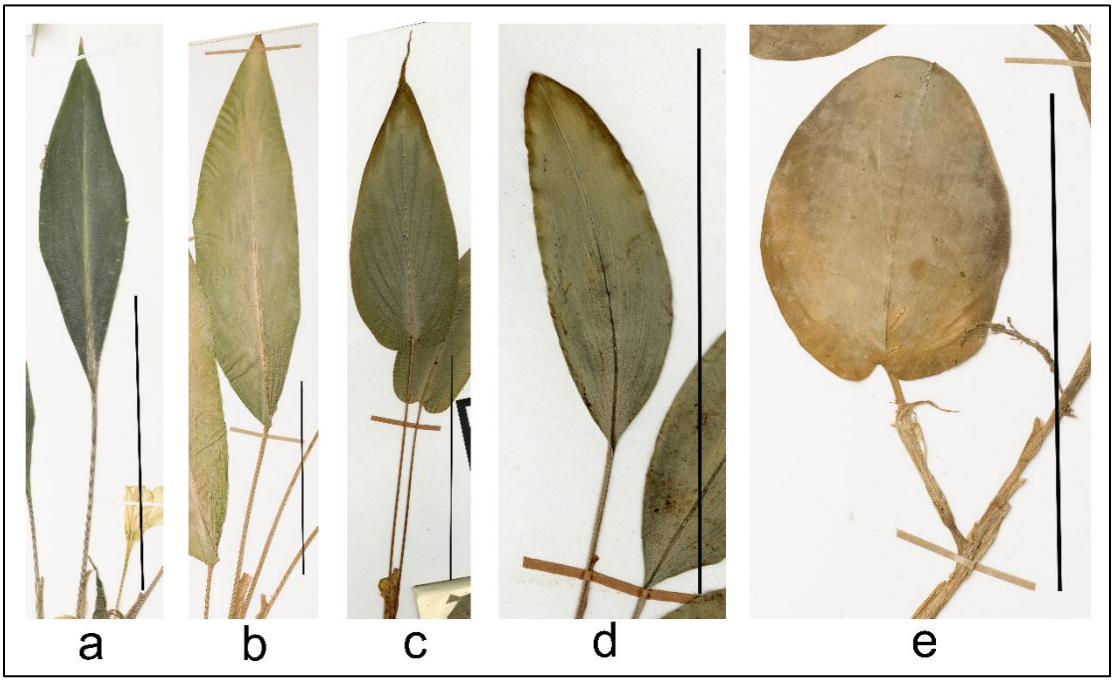

Figure 22 Different leaf shape in *Boesenbergia*. The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. Their leaves have clear wide sheath, obvious ligule, long pseudo-petiole and widely different leaf blade shape. a. *Boesenbergia aurantiaca* R.M.Sm. (E00228085) b. *Boesenbergia basispicata K.Larsen ex Sirirugsa* (E00211999) c. *Boesenbergia cordata R.M.Sm.* (E00149736) d. *Boesenbergia flavorubra R.M.Sm.* (E00149738) e. *Boesenbergia orbiculata R.M.Sm.* (E00389727).

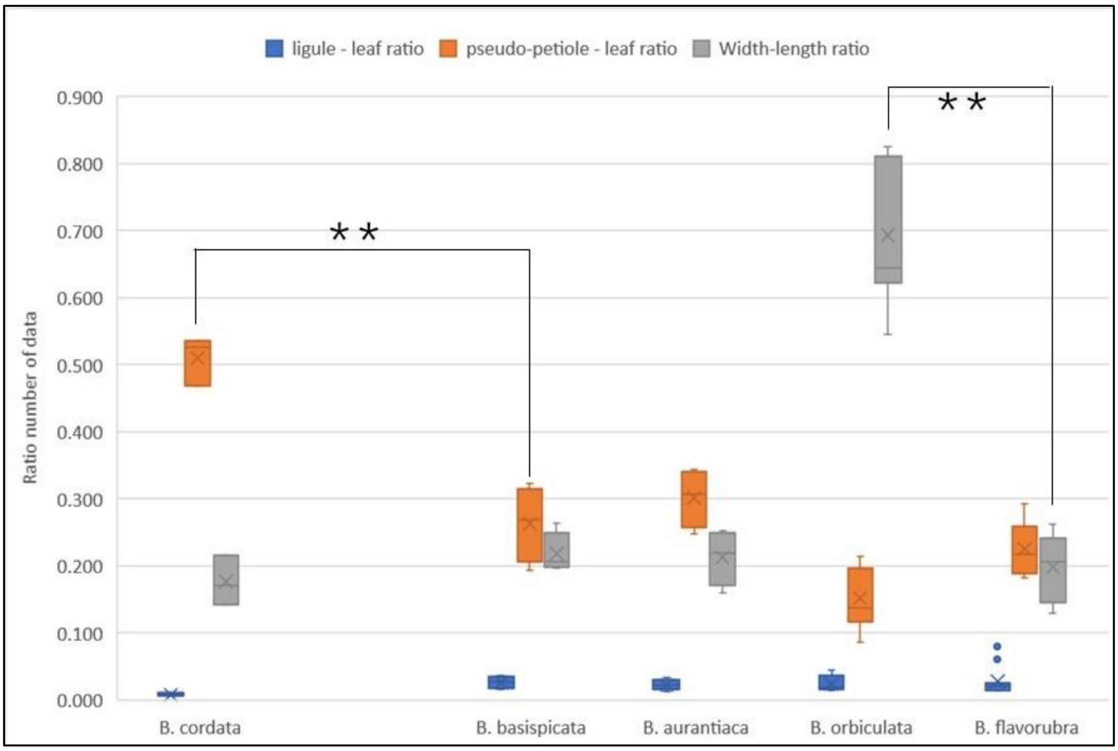

Figure 23 *Boesenbergia* ratio data box plot graph. The brackets and "**" means the ANOVA and Tukey-test results show a significant difference between the bracketed species and the P-value from ANOVA test is less than 0.01. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

3.4 *Hedychium*: A Zingibereae genus with various leaf shape and conspicuous ligule.

Hedychium belongs to the tribe Zingibereae which is the largest tribe of the Zingiberaceae, with about 50-80 species. In the digital herbarium of RBGE, there are 648 specimens with images. Their origin countries are mainly from Thailand, Viet Nam, China and Pacific Islands. According to the GBIF database, they have a worldwide distribution, they have a wide distribution in South America except the South and Southeast Asia (Fig.24). They also have a sporadic distribution in the southeast of

Africa, the east of Australia, the south of North America and Europe.

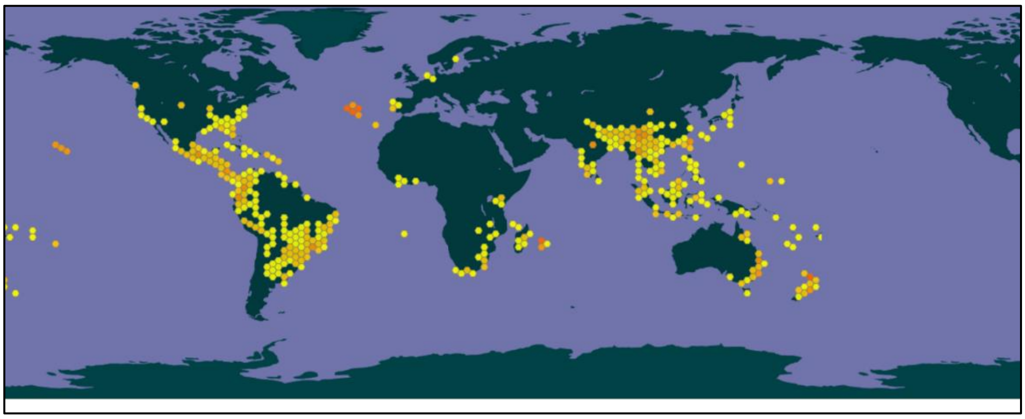

Figure 24 The distribution of *Hedychium*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). The *Hedychium* mainly distributes in the south Asia and the South America, especially in the tropical area.

3.4.1 Ligule of *Hedychium*

Hedychium is a genus with conspicuous ligule structure. Their ligules are varied in both size and shape and they are usually longer than the pseudo-petiole (Fig.25) which is not a common phenomenon in the family. Ligule shape can be long and thin, or piliferous and some ligules have different pigmentation (such as it is creamy in Fig.25.a, green in Fig.25.b and brown in Fig.25.c.). The length of ligule is stable and smaller with smaller ligule to leaf ratio, such as the *H. densiflorum*, the *H. coccineum*, the *H. ellipticum* and the *H. greenii* (Fig.26). Usually in the ginger family, their ligule is shorter or has the similar length with the pseudo-petiole, but in *Hedychium* their ligule is longer than the pseudo-petiole which are showed in the Fig.25. Therefore, the conspicuous ligule is one of good characters to distinguish this genus from others. The ligule-leaf ratio is fluctuant in this genus, especially the *H. coronarium* (Fig.26), and their ligule can occupy 1% to nearly 20% of leaf in total (appendix II Table 22). The usual ratio of ligule-leaf length of this genus is larger than the other genus, which means that the *Hedychium* have larger ligule than the other genus relatively (see appendix II). However, P-value of the ligule-leaf ratio in the ANOVA result is over 0.05, which means that there is not significant difference between species on the ligule-leaf ratio in this genus, due to the large variation observed (appendix III Table 57).

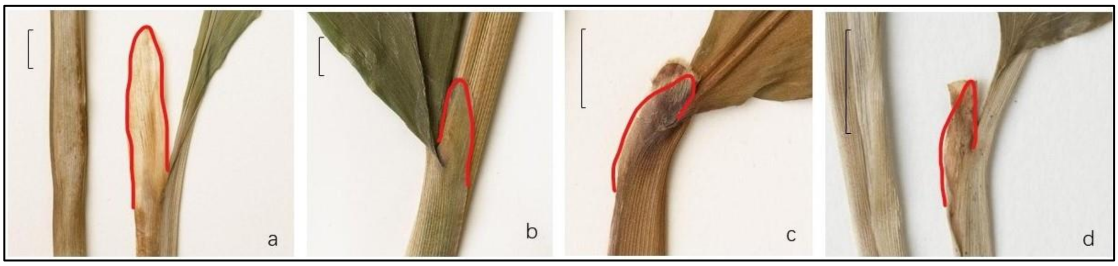

Figure 25 Different ligule in *Hedychium*. The red line indicates the ligule shape of each specimens. The specimen images come from RBGE herbarium catalogue. The scale bar represents 1 cm for each specimen. a. *Hedychium cylindricum* Ridl. (E00421687). b. *Hedychium densiflorum* Wall. (E00212283). c. *Hedychium greenii* W.W.Sm. (E00211530). d. *Hedychium ellipticum* Buch.-Ham. ex Sm. (E00646993).

3.4.2 Leaf of *Hedychium*

Hedychium is a big leaf genus in the ginger family relatively. The five measured species in this genus are the *Hedychium coccineum* Buch.-Ham. ex Sm. (Fig.27.a), *Hedychium coronarium* J.König. (Fig.27.b), *Hedychium densiflorum* Wall. (Fig.27.c), *Hedychium ellipticum* Buch.-Ham. ex Sm. (Fig.27.d) and the *Hedychium greenii* W.W.Sm. (Fig.27.e). The leaf shape in *Hedychium* is usually oblong or oblanceolate but linear

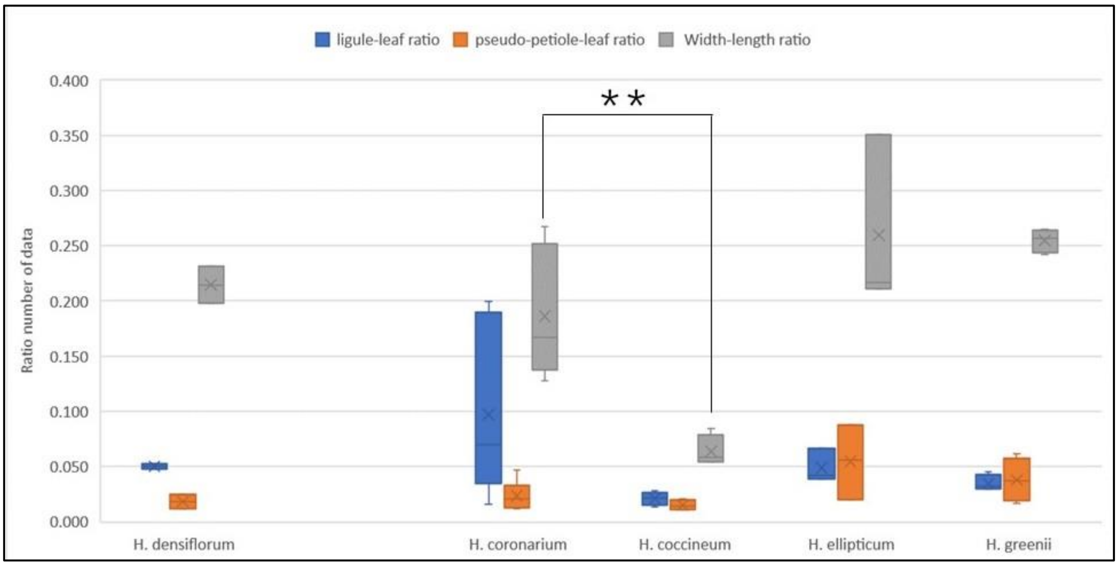

Figure 26 *Hedychium* ratio data box plot graph. The brackets and "**" mean the ANOVA and Tukey-test results show a significant difference between the bracketed species and the P-value from ANOVA test is less than 0.01. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

leaf can also be found in this genus (appendix I Table 9). The length of the leaf is around 250 to 300 mm and it can reach to over 450 mm in several species, such as the *H. coronarium*. For the species with oblanceolate shape leaves, the shapes can easily be characterised by comparing the width across the upper and lower quartile of the leaf blade. The width across the upper quartile usually is larger than the lower quartile. This oblanceolate shape represented typically in the *H. ellipticum* (appendix II Table 21). *H. coronarium* and *H. ellipticum* have more variation of the leaf width-length ratio (Fig.26). According to the ANOVA results of this genus (appendix III Table 56-62), the ratio of leaf width-length has significant difference between species because of the P-value is less than 0.05. In addition, the Tukey-test results of the *Hedychium* leaf width-length ratio demonstrates that the *H. coccineum* is the species who has more difference on this ratio in this genus (appendix III Table 62). The pseudo-petiole in *Hedychium* species is generally short, on average it is 14 mm. Statistical analysis shows that the pseudo-

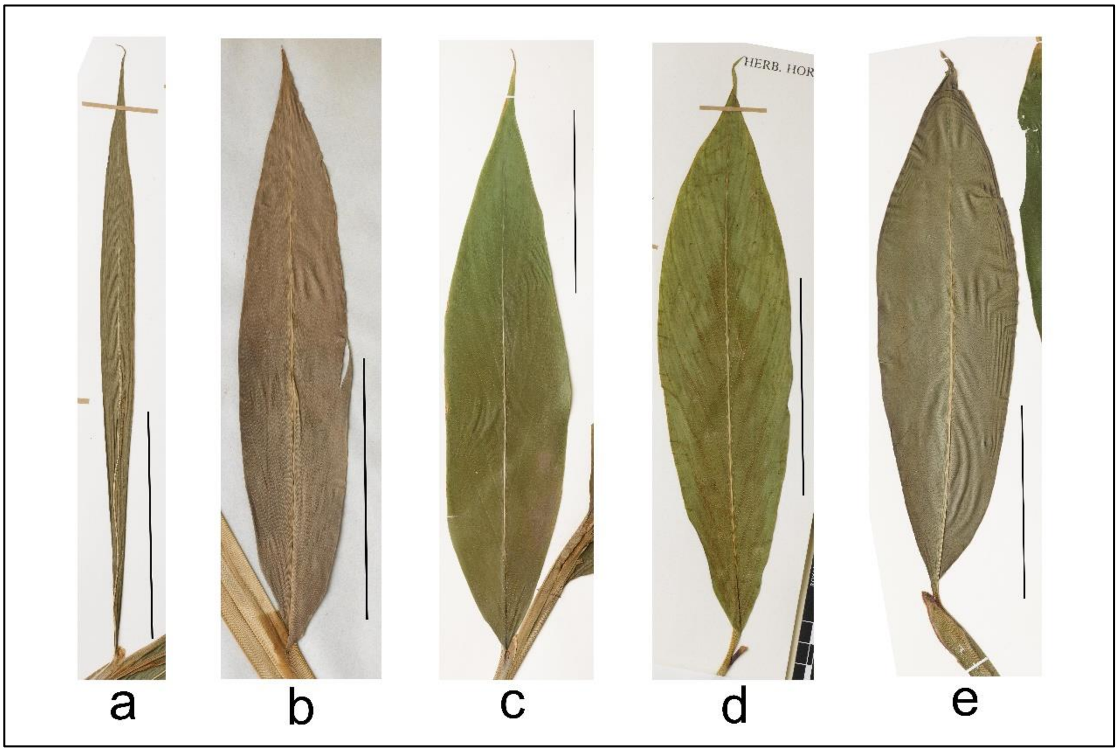

Figure 27 The leaf shape of *Hedychium*. The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. a. *Hedychium coccineum* Buch.-Ham. ex Sm. (E00531053) b. *Hedychium coronarium* J.König. (E00504164) c. *Hedychium densiflorum* Wall. (E00212282) d. *Hedychium ellipticum* Buch.-Ham. ex Sm. (E00499883) e. *Hedychium greenii* W.W.Sm. (E00247016).

petiole length does not significantly vary. The angle between the main vein and the lateral veins is average 15 degrees (appendix II Table 22), only the linear leaf species, the *H. coccineum*, is under 10 degrees.

3.5 *Curcuma*: A high economic value species with typical long pseudo-petiole.

Curcuma belongs to the Zingibereae tribe of Zingiberaceae and it has around 100 species and it is a genus with high economic value. A widely known species with high economic value is the turmeric (*Curcuma longa* L.). According to the GBIF, it also is a worldwide distribution genus but concentrate in the South and Southeast Asia, especially in the tropical area. They can be found in the north of Australia, the north of South America, west of Africa and sporadically in the North America as well (Fig.28). In RBGE digital herbarium, there are around 600 specimens with images. The 5 measured species in this genus are *Curcuma aeruginosa* Roxb. (Fig.31.a), *Curcuma harmandii* Gagnep. (Fig.31.b), *Curcuma larsenii* Maknoi & Jenjitt. (Fig.31.c), *Curcuma parviflora* Wall. (Fig.31.d) and *Curcuma vamana* M.Sabu & Mangaly (Fig.31.e).

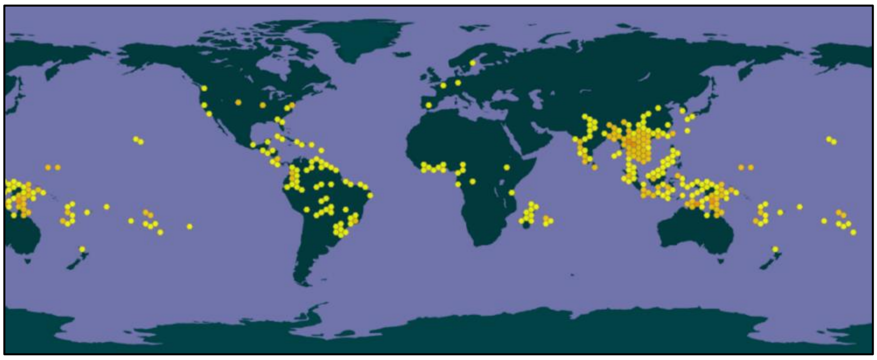

Figure 28 The distribution of *Curcuma*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). The *Curcuma* mainly distribute in the tropical South Asia.

3.5.1 Ligule of *Curcuma*

Curcuma ligule length and the ratio of ligule-leaf length shows that this is genus has a small ligule. Their ligules are only around 1 to 2 mm long. In the observed *Curcuma* species, only *Curcuma parviflora* Wall. have longer ligules which can reach to 4.4 mm maximum, but compared with the other genera, their ligules are short, which can be represented on their ligule-leaf length ratio. For example, the ligule-length ratio of *Curcuma* is no more than 0.1% (appendix II Table 18), but for the other genera which are mention above are between 1% to 4% (appendix II).

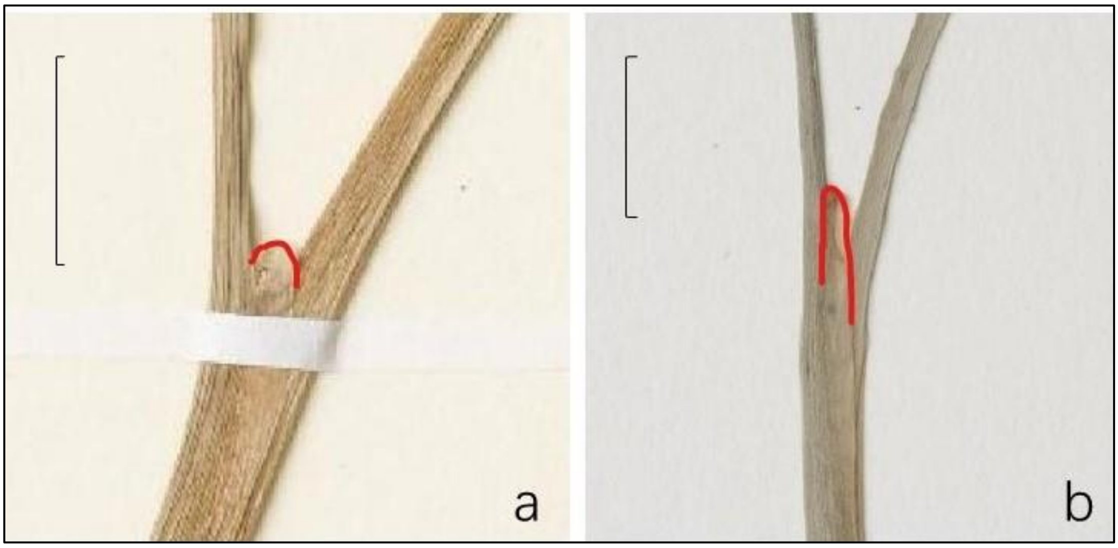

Figure 29 Ligule of *Curcuma*. The red line indicates the ligule shape of each specimens. The specimen images come from RBGE herbarium catalogue. The scale bar represents 1 cm for each specimen. a. *Curcuma* sp. (E00428024) b. *Curcuma albiflora* Thwaites. (E00643438).

3.5.2 Leaves of *Curcuma*

The leaves in *Curcuma* usually oblong (Fig.31. a. d. e.) but they can also have linear and oblanceolate leaves in some species. There is a large variation in leaf length in this genus, it can from 167 mm (*C. parviflora*) to 664 mm (*C. aeruginosa*)(appendix II Table 17 & Fig. 30).We observed several species with leaves over 900 mm in length (for example, Fig.31.a), this large size meant that herbarium preservation was difficult and affected the measurement of the leaf, resulting in folding of the leaves. The *C. aeruginosa* which is measured, their leaves can become over 660 mm. There are some species with smaller size leaves, such as *C. larsenii*. Whose leaves is around 230 mm long (appendix II Table 17). Overall, comparing with other genera, *Curcuma* is a genus with large leaf size (the leaf length is over 300 mm in average) (appendix II Table 17).

In addition, to variation in leaf length, width-length ratio has varied widely in *Curcuma* (Fig.30). The leaf width-length ratio of this genus varies from 4% to 31% (appendix II Table 18), this data shows their leaf shape could vary from linear to elliptic. From the width of the leaves, there also are big difference between species. In some species, such as *C. larsenii,* the widths across the mid-point, the upper quartile and the lower quartile are similar (around 10 mm) (appendix II Table 17), but for *C. harmandii,* its width across the mid-point can be around 1.3 times to the width across the upper quartile and the lower quartile (appendix II Table 17). The angle between the main vein and the lateral vein usually is between 15 to 20 degrees in average, but for *C. larsenii*, a linear leaf shape species, their angle is usually under 5 degrees.

The pseudo-petiole length to leaf length ratio also shows huge variation in *Curcuma* species. *Curcuma* generally has a long pseudo-petiole and the pseudo-petiole length can occupy nearly 20% to 50% (appendix II Table 18) of the whole leaf length. This pseudo-petiole to leaf length ratio is similar to the *Boesenbergia* (around 10 to 50%) (appendix II Table 16).

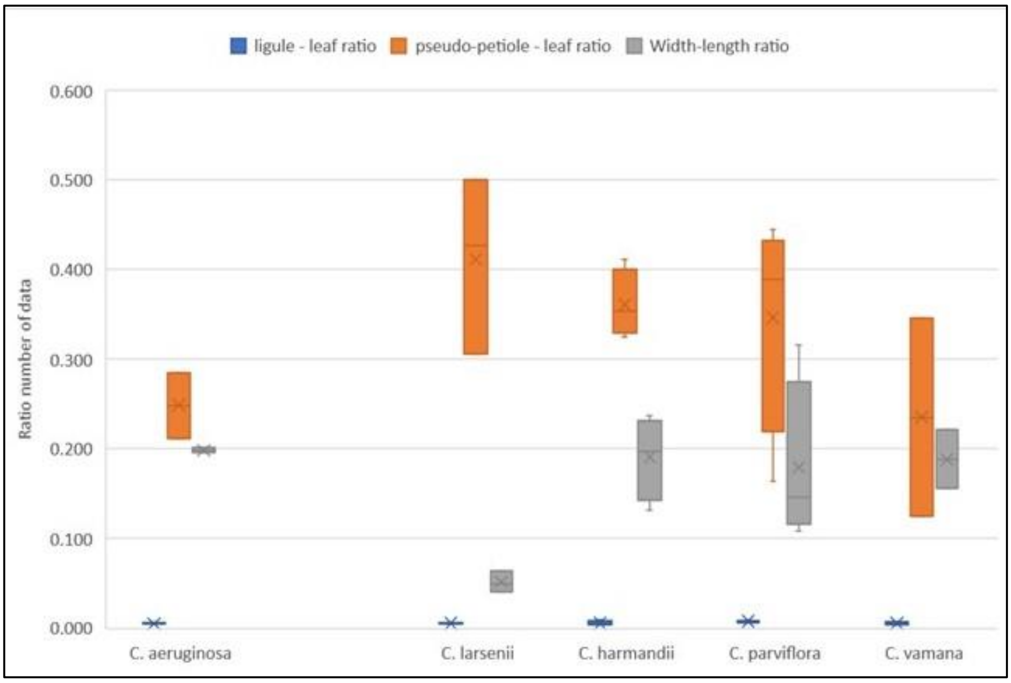

Figure 30 *Curcuma* ratio data box plot graph. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

Statistical analysis of the measured ratios: the ligule-leaf length ratio, pseudo-petiole length to leaf length ratio and the leaf width-length ratio, shows that there is no significant difference between species using these traits (appendix III Table 42-47). This may be because of large variation within the species themselves.

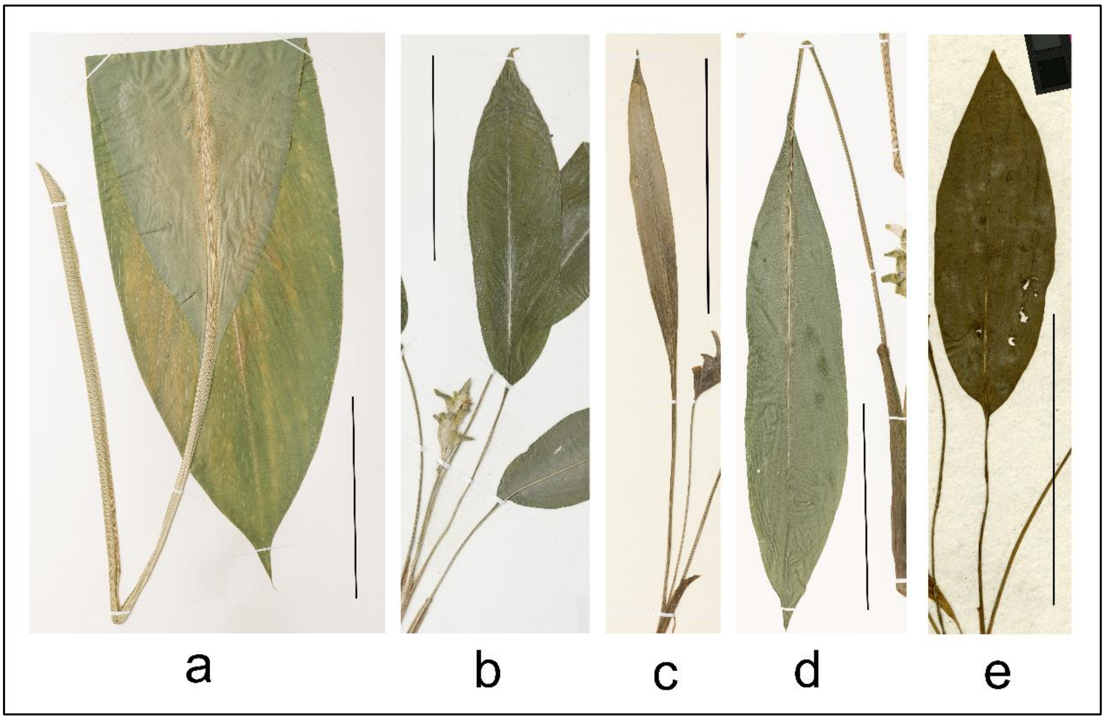

Figure 31 Leaf shape in *Curcuma*. The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. a. *Curcuma aeruginosa* Roxb. (E00211386) b. *Curcuma harmandii* Gagnep. (E00894175) c. *Curcuma larsenii* Maknoi & Jenjitt. (E00375668) d. *Curcuma parviflora* Wall. (E00211372) e. *Curcuma vamana* M.Sabu & Mangaly.(E00097613).

3.6 *Globba*: a high diversity genus in Globbeae tribe with short ligule.

Globba is one of the three genera belong to the Globbeae tribe in Zingiberaceae, it also is one of the largest genera with over 100 species in this family. In the digital herbarium of the Royal Botanic Garden of Edinburgh (RBGE herbarium), there are 738 specimens with digital images. Their mainly origin countries are Thailand, Viet Nam, Indonesia and Lao's. According to the data from the Global Biodiversity Information Facility (GBIF), the main distribution of this genus is the tropical Southern Asia, the South-eastern Asia, but they are also found sporadically in Europe, Australia, North and South America (Fig.32). In this research, the observed specimens are come from Thailand, Viet Nam and Indonesia, concentrating in the tropical South-east Asia. The measured species in this genus are *Globba albiflora* Ridl. (Fig.35.a), *Globba atrosanguinea* Teijsm. & Binn. (Fig.35.b)., *Globba brachyanthera* K.Schum. (Fig.35.c), *Globba marantina* L. (Fig.35.d). and *Globba pendula* Roxb. (Fig.35.e).

Figure 32 The distribution of *Globba*. The map comes from GBIF database. The number of species in the location is demonstrated by the colour scale from yellow (low species number) to dark orange (higher number of species). They mainly distribute in the tropical Southern Asia and the South-eastern Asia.

3.6.1 Ligule of *Globba*

The ligule of the *Globba* is small (Fig.33), they are usually no longer than 2 mm of the species observed (appendix II Table 19). The ligule shape varies and can be acuminate (Fig.33. a.), obtuse (Fig.33. b.) or wedge-shape (Fig.33. c. d.). In some species (Fig.33. A. *Globba* sp., E00421684) the thin edges of the ligule can have different pigmentation to the thick middle and some species have a hairy margin (Fig33. B. *Globba* sp., E00933672). In addition, there is no distinct difference on the ligule-leaf length ratio in this genus, the length of their ligule is consistent within species (Fig.34). Statistical analysis ligule to leaf length ratio shows that there no significant difference between species in their ligule-leaf ratio (appendix III Table 49).

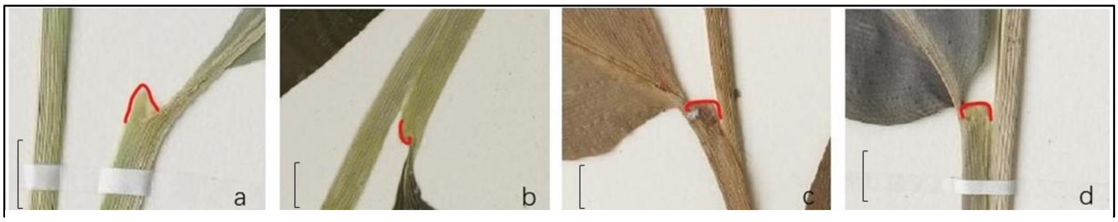

Figure 33 Different ligule shape in *Globba*. The red line indicates the ligule shape of each specimens. The scale bar represents 0.5 cm for each specimen. The specimen images come from RBGE herbarium catalogue. a. *Globba* sp. (E00421684). b. *Globba* sp. (E00933672). c. *Globba* sp. (E00220151). d. *Globba* sp. (E00226832).

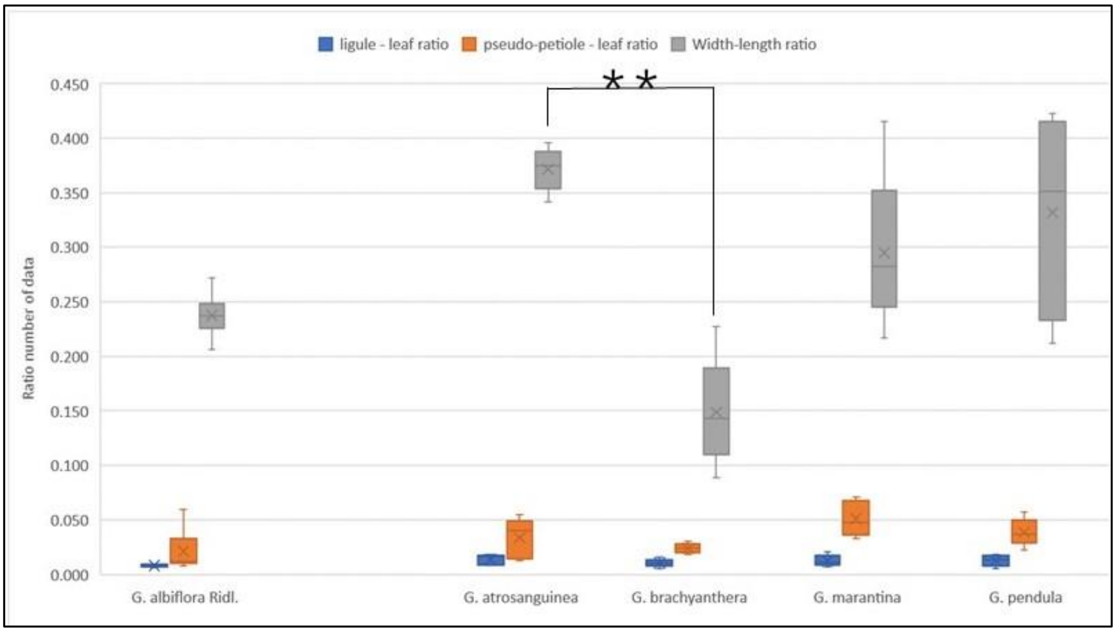

Figure 34 *Globba* ratio data box plot graph. The brackets and "**" mean the ANOVA and Tukey-test results show a significant difference between the bracketed species and the P-value from ANOVA test is less than 0.01. The middle quartile marks the mid-point of the data and is shown by the line. The upper and lower quartile represent the seventy-five and twenty-five percent of data fall below the upper and lower quartile. The × in the box means the average number of the data.

3.6.2 Leaves of *Globba*

Generally, *Globba* leaf length is small usually no more than 220 mm, although there are few species -that have larger leaves, such as *G. albiflora* (appendix II Table 19). The length of their leaves is usually around 100 to 200 mm in average, and the width across the mid-point of the leaf blade is usually less than 50 (appendix II Table 19). For all the measured leaves in this genus, their width across the upper quartile is less than the width across the lower quartile, which is because they usually have a long leaf apex (Fig.35. a. c. e.). The length of the pseudo-petiole in this genus is not very long as well, with small fluctuation (Fig.23). The ratio of the pseudo-petiole in this genus shows that they usually have a short pseudo-petiole and the length of it only occupies 1% or 2% of the whole leaf blade length (appendix II Table 20). The angle between their main vein and the lateral vein is between 10 to 20 degrees without a dramatic fluctuation. According to the ANOVA results of this genus, except the P-value of the leaf width-length ratio is less than 0.05, both P-values of the ligule-leaf ratio and the pseudo-petiole-leaf ratio are larger than 0.05, so there is significant difference between species for the leaf width-length ratio. There is less relationship between the species difference and ligule length ratio and the pseudo-petiole length. In addition, the Tukey-test results show that the *G. brachyanthera* has more difference with other species (appendix III Table 55).

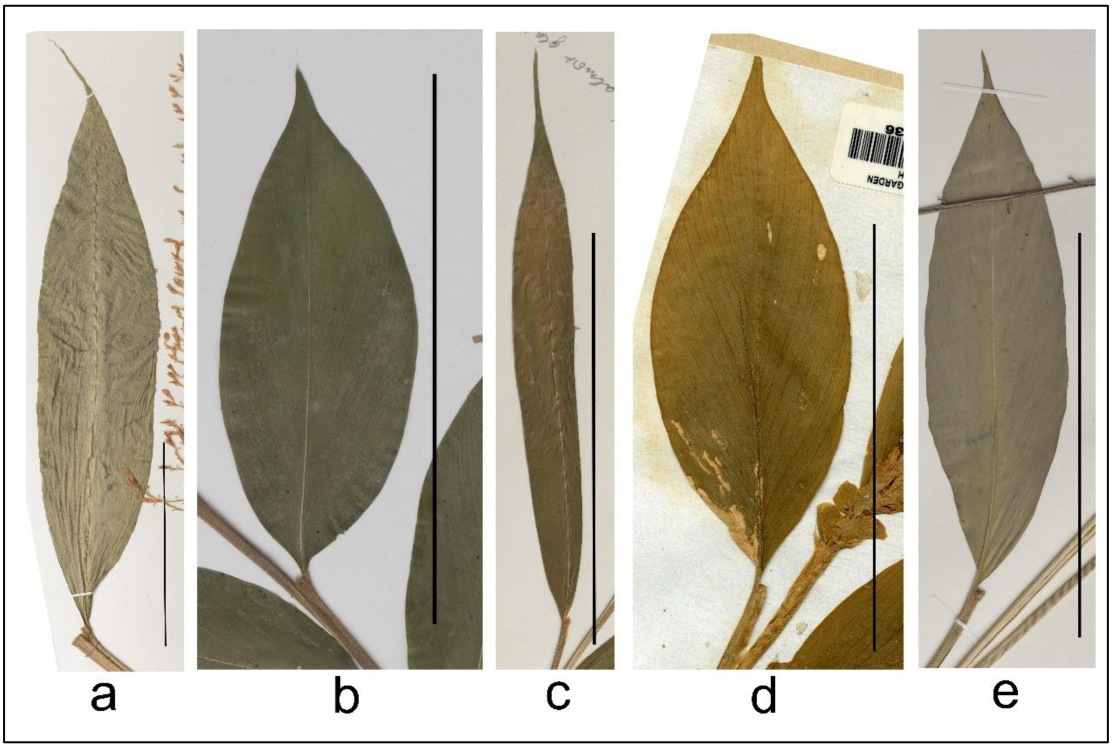

Figure 35 Leaf shape in Globba. The specimen images come from RBGE herbarium catalogue. The scale bar represents 10 cm for each specimen. a. *Globba albiflora* Ridl. (E00287262) b. *Globba atrosanguinea* Teijsm. & Binn. (E00176650) c. *Globba brachyanthera* K.Schum. (E00149831) d. *Globba marantina* L. (E00149936) e. *Globba pendula* Roxb. (E00208613).

3.7 The phylogeny of Zingiberaceae.

In order to explore whether the ratio characters could be used as trait inform phylogeny we built up the phylogeny tree of Zingiberaceae by using the ITS DNA sequence of our observed Zingiberaceae species. This is to observe leaf shape and place this in a phylogenetic context. For results, there are three phylogeny trees shows the same phylogenetic relationship of species with different morphology characters, the ligule-leaf length ratio, pseudo-petiole-leaf length ratio and the leaf width-length ratio. From the phylogeny it has different genetic relationship showed on the phylogeny of the whole family Zingiberaceae, and the *Aframomum* has the farthest genetic relationship with other genera (Fig 36., Fig 37., Fig 38.). The *C. aeruginosa* has the largest difference with other *Curcuma* species which did not be perceived in the morphology observation. In *Zingiber*, the Z. zerumbet and the Z. bradleyanum have larger difference on the ligule-leaf length ratio with other *Zingiber* species, which is similar as the statistic results. Both the phylogeny and the statistic results (ANOVA and Tukey-test) show that there are some species has significant difference with other same genus species. It also appears in the pseudo-petiole-leaf length ratio and the width-length ratio of *Boesenbergia*. On the pseudo-petiole-leaf length ratio, the B. cordata is has the most significant difference with other species, and on the width-length ratio, the B. orbiculata is the species who has the farthest genetic relationship with other species in the same genus. Therefore, the results between the statistic and the phylogeny are similar

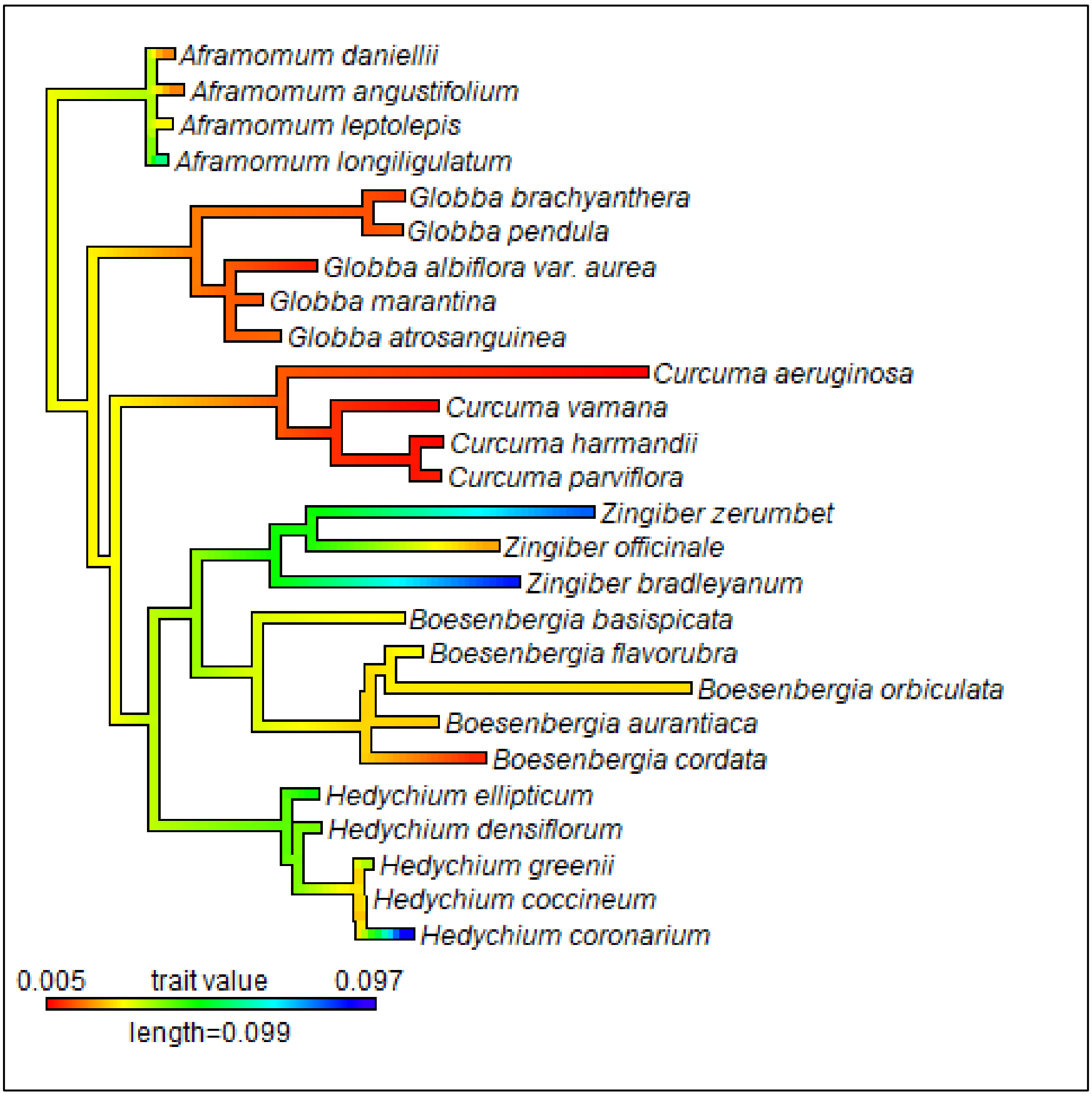

Figure 36 Phylogeny tree of Zingiberaceae with ligule-leaf length ratio.

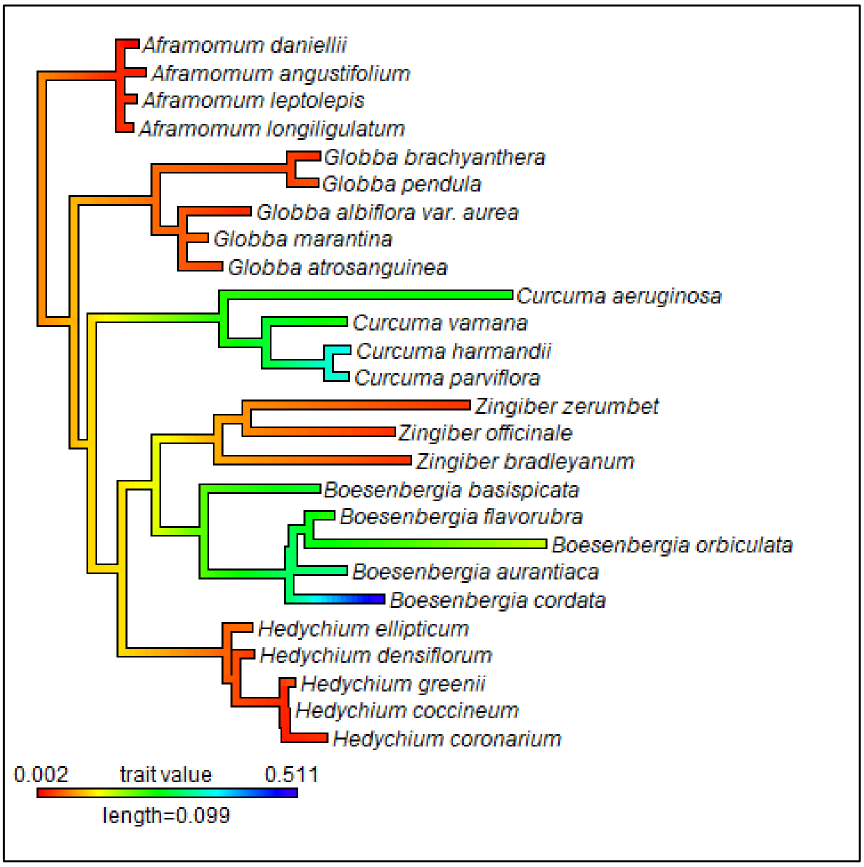

Figure 37 Phylogeny of Zingiberaceae with pseudo-petiole-leaf length ratio.

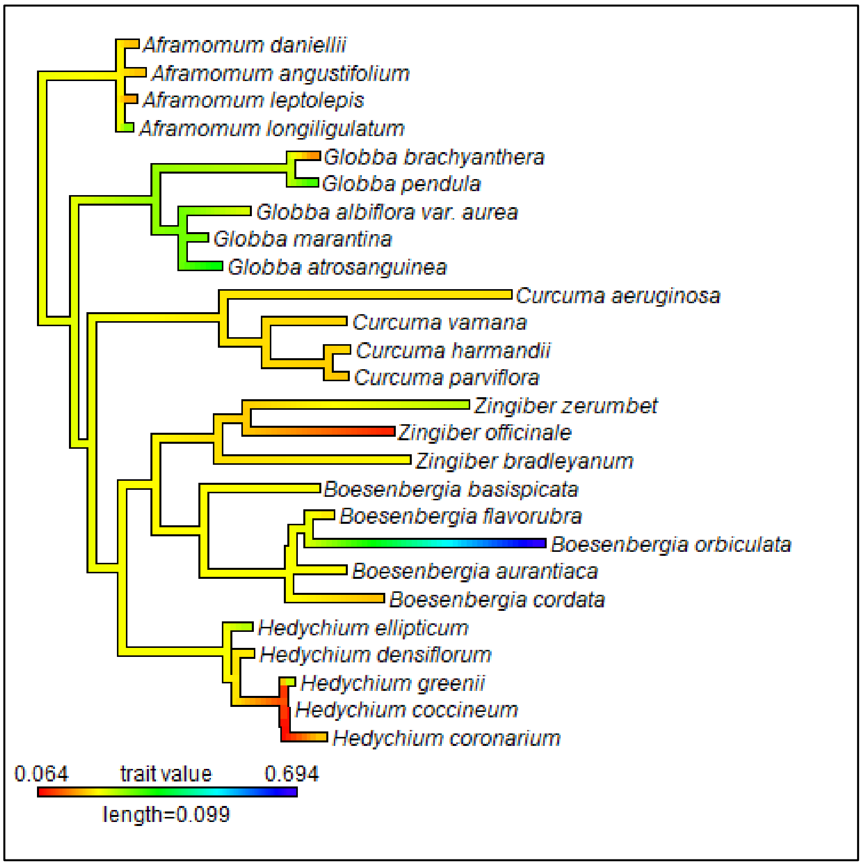

Figure 38 Phylogeny of Zingiberaceae with leaf width-length ratio.

Blomberg's K (Blomberg et al. 2003) which tests if species close together on the tree are similar in terms of a character state. It was tested the ligule-leaf ratio, pseudo-petiole to leaf ratio and the leaf width-length ratio (Fig.39). The K statistic ligule-leaf length ratio and width-length showed that the results show that there are significant differences between the species in these traits (K values < 1). This data shows that leaf traits can be used to inform phylogeny construction in the Zingiberaceae, in particular the ligule-leaf length ratio and the leaf width-length ratio.

```
> phylosig(final_tr, Ligule_leaf, method="K", test=T)

Phylogenetic signal K : 0.224997
P-value (based on 1000 randomizations) : 0.223

> phylosig(final_tr, Pseudopetiole_leaf, method="K", test=T)

Phylogenetic signal K : 1.60984
P-value (based on 1000 randomizations) : 0.001

> phylosig(final_tr, Width_length, method="K", test=T)

Phylogenetic signal K : 0.184413
P-value (based on 1000 randomizations) : 0.459
```

Figure 39 the Blomberg's K test results for each character ratio.

4. Discussion and Conclusion.

This research aimed to answer the question: are there significant differences in leaf shape between Zingiberaceae species and how does their leaf shape vary in an evolutionary context. To address this we analysed leaf shape in 29 species from 6 genera, using visual observation of digital herbarium images. We semi-quantified leaf shape by measuring different leaf regions and carried out statistical analyse to define key traits that could distinguish between species. We then constructed phylogenies based on these traits and compared them to published phylogenies base on gene sequences and floral morphology. The visual observation, distribution comparison, and statistics analysis carried out during this project demonstrates that Zingiberaceae leaf shape has significant difference between species and the phylogenetic analysis shows there are obvious relationship of leaf morphology between genera and species.

4.1 Distribution of the genera.

The genera distribution in Zingiberaceae has large difference between tribes. Most genera come from the Zingiberoideae are distributed in the Eurasia and Australia, a few genera are living in the America and the Africa. The *Aframomum* genus, part of the Alpinioideae clade is the only genus located solely in Africa analysed in this project and it does not have coincident distribution with other genera. Our analysis showed that the *Aframomum* genus was possible to distinguish clearly from the other genera, based on differences in the measured leaf traits (Fig. 19 & appendix II Table 13. 14). This distinctions of *Aframomum* genus, may results from its different distribution and relative isolation.

4.2 The significance of measured data.

For the statistics analysis, the data used to compare the difference between species are the ligule-leaf length ratio, the pseudo-petiole-leaf ratio and the leaf width-length ratio. The ligule-leaf length ratio shows the proportion of leaf in the whole leaf. In the

Zingiberaceae, the leaf shape varies a lot between different genera and species with different ligule shapes which is mentioned in the results part. This data can judge the relative size of the ligule for each species and show which species has the real big or small ligule and make the results more objective. For example, the average ligule-leaf ratio of genera shows that *Hedychium* has the biggest relative ligule and the *Curcuma* have the smallest (appendix IV Table 72). The ANOVA and the Tukey-test results further demonstrate there are significant difference between genera on their ligule proportion, and the ligule of *Hedychium* and the *Zingiber* have more difference (appendix IV Table 73 &74), which is consistent with the phylogeny result (Fig.36).

In addition, the pseudo-petiole-leaf ratio shows the proportion of the pseudo-petiole in the Zingiberaceae, and the statistic results (appendix IV Table 75-77) combine with the phylogeny result (Fig.37) demonstrate which genera have more typical pseudo-petiole (*Curcuma* and *Boesenbergia*). Both results demonstrate these two genera have more significant difference with the other genera. Combining with the visual observation, these two genera have longer pseudo-petiole in the family.

Furthermore, the leaf width-length ratio could give people a rough impression of the lamina shape, the leaf looks more linear or roundish. In the statistics results (appendix IV Table 78-80) of this data, the ANOVA result shows there is significant difference between genera, but in the Tukey-test, it has an opposite result. However, the Tukey-test result is more consistent with the result of phylogeny (Fig.38). Except for the *B. orbiculata*, the trait values between species are similar.

Moreover, the angle between the main vein and the lateral vein. Usually this data is less than 10 degree in the linear leaves (appendix II). Therefore, it can be a valuable parameter in leaf shape analysis. However, the results of this data in this research do not have enough reliability because of the inconsonant leaf age. It could have difference between different age leaves.

4.3 Drawbacks of the herbarium samples.

First of all, the preservation condition of specimens has important effect the sample selection. One of the important influences is that it is hard to control the leaf age of the measured leaf samples. The same leaf age of the samples is an important condition for error reduction of the measurement, because it is common that the young and the old leaves differ greatly in shape and important for the leaf shape research. The ideal situation is choosing all leaf samples in the same leaf age which can be controlled by counting the number of the leaves in the same branch. In addition, on some herbarium samples, it is hard to confirm the age of the leaves and only can conclude the leaf position roughly, such as the upper leaf and basal leaf.

Secondly, there are too much damaged samples in the herbarium and make it is hard to collect enough samples in some species. For example, in the *Curcuma*, the *C. vamana* only have two measured leaves meet the sample selecting conditions. It is not enough in the general sample selection. Therefore, the measured data is not enough to describe the shape of the leaves. In addition, this makes the results unconvincing of these species to a certain degree. Moreover, it may have a big discrepancy between the result and the reality and cannot demonstrate the accurate leaf shape in the family because of the lacked data.

Thirdly, the herbarium samples have lost much information of leaf shape. For example, we only can measure the ligule length but cannot observe detail ligule shape of the leaves. The figure 40 shows the ligule of A. daniellii, we can see the ligule shape is

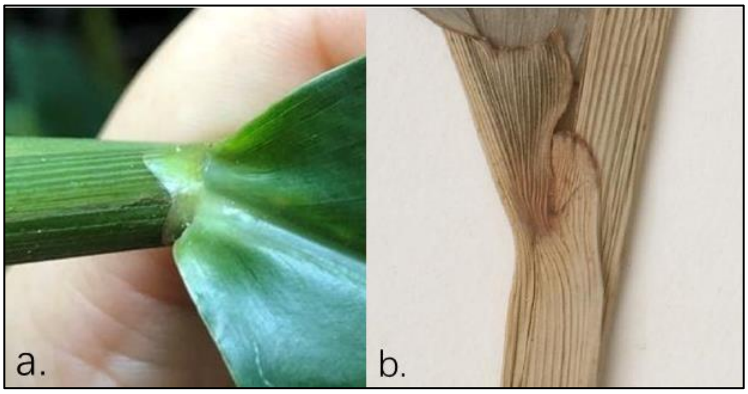

Figure 40 The ligule of *A. daniellii* in living collection and herbarium collection. The living collection is from the RBGE, the herbarium image is from the RBGE herbarium catalogue. a. the living collection of *A. daniellii*. (Coll. BERGA 61) b. The herbarium collection of *A. daniellii* (E00486322).

slight bilobed on the living collection (Fig.40. a), but this shape detail was lost on the herbarium sample (Fig.40. b).

To solve this problem, the best way is using the living collection samples. The living collection samples will be easier to control the leave age and the sample quality. The reason why we did not collect enough samples in this study is the uneven quality of herbarium specimens. Therefore, this research would have better results if the living collection samples are used.

4.4 Problems in leaf measurement.

The sample number is too less and makes it is a little hard to find a regular rule from the measurement data. The results data is not enough to describe the shape of the leaves. For example, for the width of leaf, the width across the mid-point, the upper and lower quartile are measured. These three numbers show difference of the leaf shape in some species. However, the data is still too less to describe an accurate shape of leave and only can give a vague impression of them.

Moreover, the number of veins is not accurate. It is hard to see the veins clearly on the herbarium sample digital images, because of the uneven quality of specimens. For instance, the veins number cannot be counted in the damaged leaves. In addition, on the herbarium specimens, there are dirt and stains covered on some samples and affect the number count of the veins. Therefore, the veins number counted in this research does not have any indicative value. However, it is easy to count on the living collection samples. Therefore, it would have better results if the living collections are used.

4.5 Morphometric analysis

Except for the sample selection problems, the quantitative analysis also affects the results of the study. The measured data is not enough to describe an accurate leaf shape and only can give a rough impression of leaves. For instance, the leaf width and length data can describe the leaf shape and the size roughly, but the real leaf shape and size cannot be illustrated. Just for the leaf size, it cannot be calculated directly by multiplying the width with length which is obvious not accurate enough. There is a same dilemma on the shape description. Therefore, a new method should be found out to solve this problem. The morphometric analysis can help.

The geometric morphometric analysis helps to allow the identification and quantification of the shape feature in plant leaves (Klein & Svoboda, 2017; Mitteroecker & Gunz, 2009, Andres et al., 2016, Chitwood et al., 2016). These research show that morphometric analysis is an available method to analyse the leaf shape of the Zingiberaceae. This method can help to get more accurate data about the shape. For example, binary images are common to be used in this method and can show the leaf shape more direct. The Fig. 41 to Fig.46 are the binary images of the leaf for observed species made by Photoshop. These images give more clear shape impression and eliminate the interference caused by sample quality problems. Therefore, to improve the accuracy of the results in this research, morphometric analysis is an ideal method.

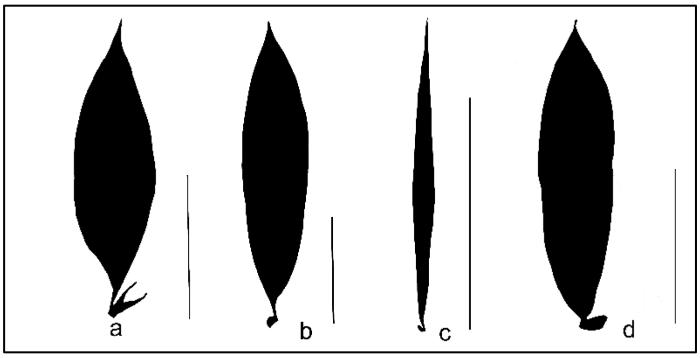

Figure 46 The binary images for *Zingiber* leaves. The scale bar represents 10 cm for each leaf. a. *Z. bradleyanum* (E00077500) b. *Z. nudicarpum* (E00421674) c. *Z. officinale* (E00177158) d. *Z. zerumbet* (E00770319).

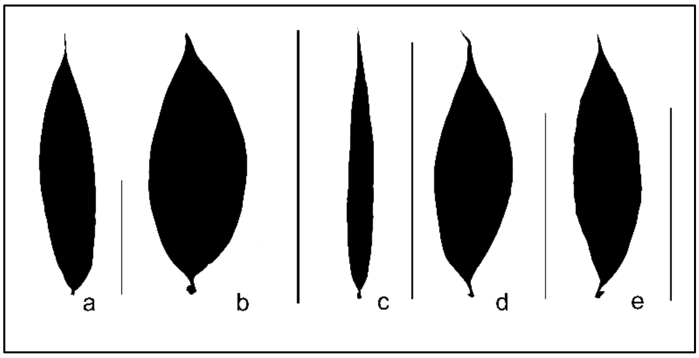

Figure 45 The binary images for *Hedychium* leaves. The scale bar represents 10 cm for each leaf. a. *H. coccineum* (E00531053) b. *H. coronarium* (E00211112) c. *H. densiflorum* (E00212282) d. *H. ellipticum* (E00499883) e. *H. greenii* (E00247016).

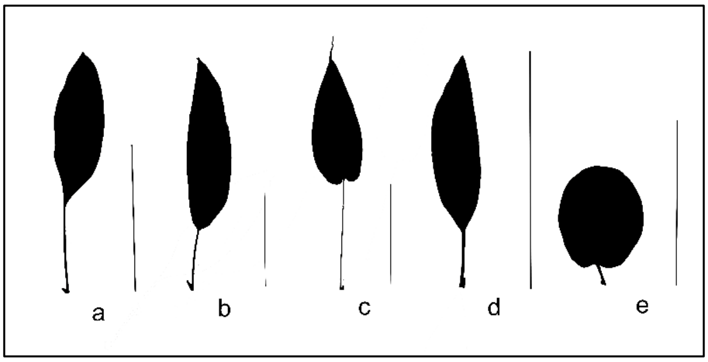

Figure 44 The binary images for *Boesenbergia* leaves. The scale bar represents 10 cm for each leaf. a. *B. aurantiaca* (E00228085) b. *B. basispicata* (E00211999) c. *B. cordata* (E00149736) d. *B. flavorubra* (E00149738) e. *B. orbiculata* (E00149745).

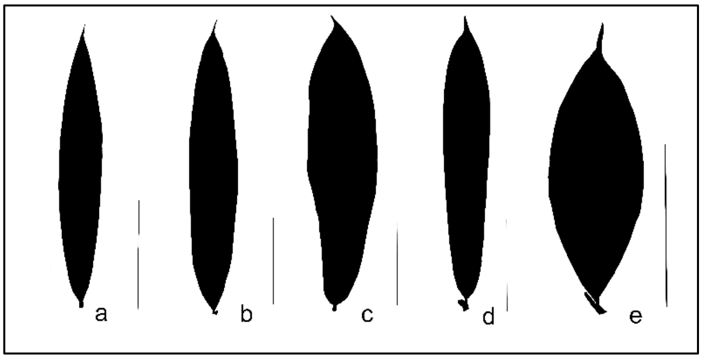

Figure 43 The binary images for *Aframomum* leaves. The scale bar represents 10 cm for each leaf. a. *A. angustifolium* (E00957875) b. *A. chrysanthum* (E00643480) c. *A. daniellii* (E00486322) d. *A. leptolepis* (E00930389) e. *A. longiligulatum* (E00509455).

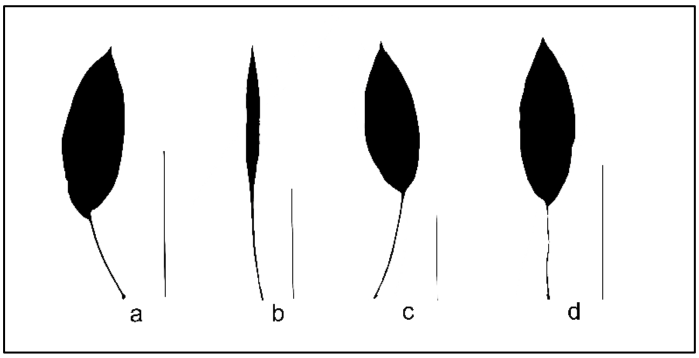

Figure 42 The binary images for *Curcuma* leaves. The scale bar represents 10 cm for each leaf. a. *C. harmandii* (E00894175) b. *C. larsenii* (E00097673) c. *C. parviflora* (E00211374) d. *C. vamana* (E00097613)

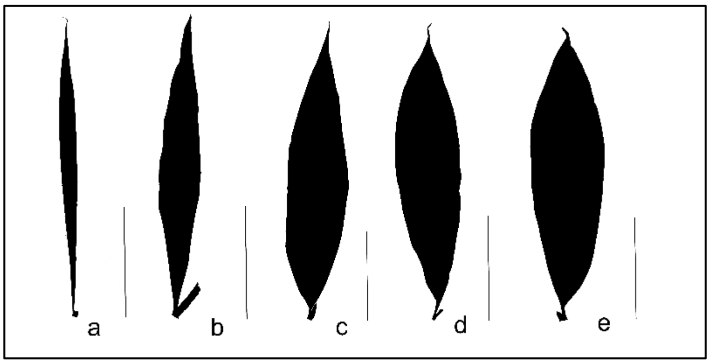

Figure 41 The binary images for *Globba* leaves. The scale bar represents 10 cm for each leaf. a. *G. albiflora* (E00183030) b. *G. atrosanguinea* (E00176650) c. *G. brachyanthera* (E00119502) d. *G. marantia* (E00421681) e. *G. pendula* (E00208613).

4.6 Conclusion: The leaf shape diversity between genera and species.

The Zingiberaceae is a high leaf shape diversity family. Generally, there are several kinds of leaf shapes in each genus. The main leaf shape of Zingiberaceae is oblong and linear. In the genus *Boesenbergia*, there are more special leaf shape, such as the roundish leaf for *B. orbiculata*. There also are a lot of leaf shape characters in the family, such as the ligule, pseudo-petiole and the long leaf tip in the *Aframomum*. According to the statistic and phylogeny results, on ligule and the pseudo-petiole, they both shows significant difference between genus and species. In addition, from the results of the statistics analysis and the phylogeny, there are obvious relationship of leaf morphology between genera and species. Therefore, the leaf shape is a reliable support to help distinguish species of Zingiberaceae.

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Appendix I

Specimens information.

Species	Barcode	leaf shape	edge	pattern of parallel vein
Aframomum leptolepis (K.Schum.) K.Schum.	E00930389	oblanceolate	entire	transversed
	E00930401	oblanceolate	entire	transversed
	E00930402	oblanceolate	entire	transversed
	E00930403	oblanceolate	entire	transversed
Aframomum longiligulatum Koechlin	E00509455	oblong	entire	transversed
	E00509457	oblong	entire	transversed
	E00509458	oblong	entire	transversed
	E00509461	oblong	entire	transversed
Aframomum angustifolium (Sonn.) K.Schum.	E00482328	oblong	entire	transversed
	E00957858	oblong	entire	transversed
	E00957875	oblong	entire	transversed
Aframomum chrysanthum Lock	E00607558	oblong	entire	transversed
	E00643480	oblong	entire	transversed
	E00679426	oblong	entire	transversed
Aframomum daniellii (Hook.f.) K.Schum.	E00982949	oblong	entire	transversed
	E00228504	oblong	entire	transversed
	E00486322	oblong	entire	transversed
	E00982934	oblong	entire	transversed

Table 1. Aframomum leaf shape recording.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Aframomum leptolepis (K.Schum.) K.Schum.	E00930389	Cameroon:Sud-Ouest	5782	27 January 1998	http://data.rbge.org.uk/herb/E00930389
	E00930401	Cameroon:Sud-Ouest	6733	02 July 1999	http://data.rbge.org.uk/herb/E00930401
	E00930402	not specified	5794	02 June 1998	http://data.rbge.org.uk/herb/E00930402
	E00930403	not specified	5794	02 June 1998	http://data.rbge.org.uk/herb/E00930403
Aframomum longiligulatum Koechlin	E00509455	Congo:Likouala	5226	19 April 1995	http://data.rbge.org.uk/herb/E00509455
	E00509457	Central African Republic:Sangha Economique:	1346	06 October 1988	http://data.rbge.org.uk/herb/E00509457
	E00509458	Central African Republic:Sangha Economique	3594	23 October 1993	http://data.rbge.org.uk/herb/E00509458
	E00509461	Cameroon:East:Lobeke Reserve	6536	24 November 1998	http://data.rbge.org.uk/herb/E00509461
Aframomum angustifolium (Sonn.) K.Schum.	E00482328	Central African Republic:Sangha-Mbaere	1191	24 September 1988	http://data.rbge.org.uk/herb/E00482328
	E00957858	Malawi	213	05 November 1983	http://data.rbge.org.uk/herb/E00957858
	E00957875	Malawi	6680	22 October 1985	http://data.rbge.org.uk/herb/E00957875
Aframomum chrysanthum Lock	E00607558	Côte d'Ivoire	834	01 September 1975	http://data.rbge.org.uk/herb/E00607558
	E00643480	Liberia:Grand Gedeh	9177	21 January 2010	http://data.rbge.org.uk/herb/E00643480
	E00679426	Liberia	11839	09 March 2013	http://data.rbge.org.uk/herb/E00679426
Aframomum daniellii (Hook.f.) K.Schum.	E00982949	Central African Republic	1117	19 March 1988	http://data.rbge.org.uk/herb/E00982949
	E00228504	Central African Republic:Sangha-Mbaere	1981	16 August 2006	http://data.rbge.org.uk/herb/E00228504
	E00486322	Central African Republic:Sangha-Mbaere	5666	07 December 1997	http://data.rbge.org.uk/herb/E00486322
	E00982934	Cameroon	2795	07 March 1991	http://data.rbge.org.uk/herb/E00982934
	E00930389	Cameroon:Sud-Ouest	5782	27 January 1998	http://data.rbge.org.uk/herb/E00930389

Table 2. Aframomum specimens information.

Species	Barcode	leaf shape	edge	pattern of parallel vein	Type of
Boesenbergia cordata R.M.Sm.	E00149736	cordiform	entire	transversed	
	E00389710	cordiform	entire	transversed	
	E00389713	cordiform	entire	transversed	
Boesenbergia basispicata K.Larsen ex Sirirugsa	E00211999	oblong	entire	transversed	
	E00428231	oblong	entire	transversed	
Boesenbergia aurantiaca R.M.Sm.	E00228085	oblong	entire	transversed	
	E00389714	oblong	entire	transversed	
Boesenbergia orbiculata R.M.Sm.	E00149745	rotund	entire	transversed	isotype
	E00389727	rotund	entire	transversed	
	E00389730	rotund	entire	transversed	
	E00149738	oblong	entire	transversed	isotype
	E00389718	oblong	entire	transversed	
	E00389721	oblong	entire	transversed	

Table 3. Boesenbergia leaf shape information.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Boesenbergia cordata R.M.Sm.	E00149736	Malaysia:Sarawak	B.8258	13 June 1975	http://data.rbge.org.uk/herb/E00149736
	E00389710	Malaysia:Sarawak:Betong [2nd Division]	S.30786	27 September 1971	http://data.rbge.org.uk/herb/E00389710
	E00389713	Malaysia:Sarawak:Fourth Division	2273	24 June 1962	http://data.rbge.org.uk/herb/E00389713
Boesenbergia basispicata K.Larsen ex Sirirugsa	E00211999	Thailand		September 1987	http://data.rbge.org.uk/herb/E00211999
	E00428231	Thailand:Nakhon Si Thammarat:Nopphitam	5521	24 September 2010	http://data.rbge.org.uk/herb/E00428231
Boesenbergia aurantiaca R.M.Sm.	E00228085	Malaysia:Sabah:Segama Lahad Datu Dist.	1471	9 May 2006	http://data.rbge.org.uk/herb/E00228085
	E00389714	Malaysia:Sabah:Lahad Datu	AN 112115	13 October 1985	http://data.rbge.org.uk/herb/E00389714
Boesenbergia orbiculata R.M.Sm.	E00149745	Malaysia:Sarawak	B.8275	14 June 1975	http://data.rbge.org.uk/herb/E00149745
	E00389727	Malaysia:Sarawak	959	12 April 1978	http://data.rbge.org.uk/herb/E00389727
	E00389730	Malaysia:Sarawak	1146A	April 1978	http://data.rbge.org.uk/herb/E00389730
	E00149738	Malaysia:Sarawak	B.8245	12 June 1975	http://data.rbge.org.uk/herb/E00149738
	E00389718	Malaysia:Sarawak:4th Dision	S.49061	19 October 1984	http://data.rbge.org.uk/herb/E00389718
	E00389721	Malaysia:Sarawak	RK 390	23 April 1978	http://data.rbge.org.uk/herb/E00389721

Table 4. Boesenbergia specimens information.

Species	Barcode	leaf shape	edge	pattern of parallel vein	Type of?
Curcuma aeruginosa Roxb.	E00211325	oblong	entire	transversed	
	E00211386	oblong	entire	transversed	
Curcuma larsenii Maknoi & Jenjitt.	E00097673	linear	entire	transversed	
	E00097674	linear	entire	transversed	
	E00375668	linear	entire	transversed	
Curcuma harmandii Gagnep.	E00894175	oblanceolate	entire	transversed	
	E00097675	oblanceolate	entire	transversed	
	E00097676	oblanceolate	entire	transversed	
Curcuma parviflora Wall.	E00097696	oblong	entire	transversed	
	E00211372	oblong	entire	transversed	
	E00894173	oblong	entire	transversed	
Curcuma vamana M.Sabu & Mangaly	E00097613	oblong	entire	transversed	isotype
	E00211320	oblong	entire	transversed	

Table 5. Curcuma leaf shape imformation.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Curcuma aeruginosa Roxb.	E00211325	Indonesia			http://data.rbge.org.uk/herb/E00211325
	E00211386	Thailand:Trat	87 22/3		http://data.rbge.org.uk/herb/E00211386
Curcuma larsenii Maknoi & Jenjitt.	E00097673	Thailand:Sakon Nakhon	62	01 August 1999	http://data.rbge.org.uk/herb/E00097673
	E00097674	Thailand:Ubon Ratchathani	67	05 August 1999	http://data.rbge.org.uk/herb/E00097674
	E00375668	Viet Nam:Dong Nai	48	19 June 2008	http://data.rbge.org.uk/herb/E00375668
Curcuma harmandii Gagnep.	E00894175	Thailand:Chaiyaphum	2642	09 August 2013	http://data.rbge.org.uk/herb/E00894175
	E00097675	Thailand:Chachoengsao	46	19 July 1999	http://data.rbge.org.uk/herb/E00097675
	E00097676	Thailand:Chachoengsao	48	21 July 1999	http://data.rbge.org.uk/herb/E00097676
Curcuma parviflora Wall.	E00097696	Thailand:Kamphaeng Phet	56	08 October 1904	http://data.rbge.org.uk/herb/E00097696
	E00211372	Thailand		24 August 2005	http://data.rbge.org.uk/herb/E00211372
	E00894173	Thailand:Mae Hong Son	2586		http://data.rbge.org.uk/herb/E00894173
Curcuma vamana M.Sabu & Mangaly	E00097613	India:Kerala	CU 37343	20 July 1984	http://data.rbge.org.uk/herb/E00097613
	E00211320	Thailand			http://data.rbge.org.uk/herb/E00211320

Table 6. Curcuma specimens information.

Species	Barcode	leaf shape	edge	pattern of parallel vein
Globba albiflora Ridl.	E00183029	oblong	entire	transversed
	E00183030	oblong	entire	transversed
	E00955827	oblong	entire	transversed
Globba atrosanguinea Teijsm. & Binn.	E00176650	oblanceolate	entire	transversed
	E00183017	oblanceolate	entire	transversed
	E00507882	oblanceolate	entire	transversed
Globba brachyanthera K.Schum.	E00119502	linear	entire	transversed
	E00128136	linear	entire	transversed
	E00149831	linear	entire	transversed
Globba marantina L.	E00095579	oblong	entire	transversed
	E00421681	oblong	entire	transversed
	E00421690	oblong	entire	transversed
	E00507886	oblong	entire	transversed
Globba pendula Roxb.	E00149876	oblong	entire	transversed
	E00189268	oblong	entire	transversed
	E00421683	oblong	entire	transversed
	E00673945	oblong	entire	transversed

Table 7. Globba leaf shape information.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Globba albiflora Ridl.	E00183029	not specified	L-92.0125	1995	http://data.rbge.org.uk/herb/E00183029
	E00183030	not specified	L-92.0125	1995	http://data.rbge.org.uk/herb/E00183030
	E00955827	Thailand:Phetchabun:Nam Nao	5852	01 August 2015	http://data.rbge.org.uk/herb/E00955827
Globba atrosanguinea Teijsm. & Binn.	E00176650	Brunei Darussalam:Temburong	331	1991	http://data.rbge.org.uk/herb/E00176650
	E00183017	not specified	L-90.0008	1995	http://data.rbge.org.uk/herb/E00183017
	E00507882	not specified		13 December 1997	http://data.rbge.org.uk/herb/E00507882
Globba brachyanthera K.Schum.	E00119502	Indonesia:East Kalimantan [Kalimantan Timur]	PK2583	15 March 1999	http://data.rbge.org.uk/herb/E00119502
	E00128136	Malaysia:Sarawak:Miri [4th Division]	MW121	31 October 1990	http://data.rbge.org.uk/herb/E00128136
	E00149831	Malaysia:Sarawak:Kuching [1st Division]	B2503	14 July 1962	http://data.rbge.org.uk/herb/E00149831
Globba marantina L.	E00095579	India:Karnataka	HFP803	14 September 1970	http://data.rbge.org.uk/herb/E00095579
	E00421681	Viet Nam:Kien Giang Prov.	LY258	13 July 2010	http://data.rbge.org.uk/herb/E00421681
	E00421690	Viet Nam:Kien Giang Prov.	LY258	June 2008	http://data.rbge.org.uk/herb/E00421690
	E00507886	Indonesia	7357		http://data.rbge.org.uk/herb/E00507886
Globba pendula Roxb.	E00149876	Malaysia:Sabah	89S88	08 July 1989	http://data.rbge.org.uk/herb/E00149876
	E00189268	Thailand:Songkhla	00-200	28 June 2000	http://data.rbge.org.uk/herb/E00189268
	E00421683	Viet Nam:Dong Nai Prov.	2435	date: 13 July 2010	http://data.rbge.org.uk/herb/E00421683
	E00673945	Malaysia	FRI54915	19 March 2007	http://data.rbge.org.uk/herb/E00673945

Table 8. Globba specimen information.

Species	Barcode	leaf shape	edge	pattern of parallel vein	Type of
Hedychium densiflorum Wall.	E00149991	oblong	entire	transversed	
	E00212282	oblong	entire	transversed	
	E00212283	oblong	entire	transversed	
Hedychium coronarium J.König	E00211110	oblong	entire	transversed	
	E00211112	oblong	entire	transversed	
	E00504164	oblong	entire	transversed	
Hedychium coccineum Buch.-Ham. ex Sm.	E00211095	linear	entire	transversed	isotype
	E00531053	linear	entire	transversed	
Hedychium ellipticum Buch.-Ham. ex Sm.	E00148648	oblanceolate	entire	transversed	
	E00499883	oblanceolate	entire	transversed	
Hedychium greenii W.W.Sm.	E00211530	oblanceolate	entire	transversed	
	E00247016	oblanceolate	entire	transversed	

Table 9. Hedychium leaf shape information.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Hedychium densiflorum Wall.	E00149991	China	8844	August 1912	http://data.rbge.org.uk/herb/E00149991
	E00212282	China:Yunnan:Nujiang Lisu Aut. Pref.	5	15 August 2005	http://data.rbge.org.uk/herb/E00212282
	E00212283	China:Yunnan:Nujiang Lisu Aut. Pref.	5	15 August 2005	http://data.rbge.org.uk/herb/E00212282
Hedychium coronarium J.König	E00211110	India:Sikkim:East District	1010	01 August 1992	http://data.rbge.org.uk/herb/E00211110
	E00211112	Myanmar:Mandalay	4277	05 October 1908	http://data.rbge.org.uk/herb/E00211112
	E00504164	India	6539A		http://data.rbge.org.uk/herb/E00504164
Hedychium coccineum Buch.-Ham. ex Sm.	E00211095	India:Assam	5	22 June 1808	http://data.rbge.org.uk/herb/E00211095
	E00531053	not specified	3683	14 August 1987	http://data.rbge.org.uk/herb/E00531053
Hedychium ellipticum Buch.-Ham. ex Sm.	E00148648	Viet Nam:Lam Dong:Huyen Lac Duong Distr.	49	30 August 2001	http://data.rbge.org.uk/herb/E00148648
	E00499883	India:West Bengal	1288	11 August 1992	http://data.rbge.org.uk/herb/E00499883
Hedychium greenii W.W.Sm.	E00211530	not specified	C4373	September 1964	http://data.rbge.org.uk/herb/E00211530
	E00247016	not specified	11	25 October 2005	http://data.rbge.org.uk/herb/E00247016

Table 10. Hedychium specimens information.

Species	Barcode	leaf shape	pattern of parallel vein	Type of?
Zingiber bradleyanum Craib	E00077500	oblong	transversed	
	E00294288	oblong	transversed	
	E00141461	oblong	transversed	
Zingiber officinale Roscoe	E00177158	linear	transversed	
	E00389842	linear	transversed	
	E00412174	linear	transversed	
Zingiber zerumbet (L.) Sm.	E00683210	oblong	transversed	
	E00770317	oblong	transversed	
	E00770319	oblong	transversed	
	K000255220	oblong	transversed	Unknown type material
Zingiber nudicarpum D.Fang	E00421674	oblong	transversed	
	E00421675	oblong	transversed	
	E00421773	oblong	transversed	
	E00421788	oblong	transversed	

Table 11. Zingiber leaf shape information.

Species	Barcode	Country of origin	Collection number	Collecting date	Citation
Zingiber bradleyanum Craib	E00077500	Thailand:Chiang Mai	849	10 October 1997	http://data.rbge.org.uk/herb/E00077500
	E00294288	Thailand:Phetchaburi:Amphoe Kaeng Krachan	1125	28 June 2000	http://data.rbge.org.uk/herb/E00294288
	E00141461	Thailand:Chiang Mai	849	10 October 1997	http://data.rbge.org.uk/herb/E00141461
Zingiber officinale Roscoe	E00177158	Malaysia:Sarawak:Betong [2nd Division]	S.44798	06 May 1985	http://data.rbge.org.uk/herb/E00177158
	E00389842	Sri Lanka:Western		1838	http://data.rbge.org.uk/herb/E00389842
	E00412174	not specified			http://data.rbge.org.uk/herb/E00412174
Zingiber zerumbet (L.) Sm.	E00683210	Papua New Guinea:Milne Bay	13383	17 January 2009	http://data.rbge.org.uk/herb/E00683210
	E00770317	Micronesia (Federated States of)	3514	09 August 1946	http://data.rbge.org.uk/herb/E00770317
	E00770319	Micronesia (Federated States of)	52	May 1946	http://data.rbge.org.uk/herb/E00770319
	K000255220	Jawa	118	1902	http://specimens.kew.org/herbarium/K000255220
Zingiber nudicarpum D.Fang	E00421674	Viet Nam:Lam Dong Prov.:Da Hoai District	2441	24 February 2011	http://data.rbge.org.uk/herb/E00421674
	E00421675	Viet Nam:Lam Dong Prov.:Da Hoai District	2441	24 February 2011	http://data.rbge.org.uk/herb/E00421675
	E00421773	Lao People's Democratic Republic:Attapu	2442	16 March 2011	http://data.rbge.org.uk/herb/E00421773
	E00421788	Viet Nam:Lam Dong	2454	22 June 2008	http://data.rbge.org.uk/herb/E00421788

Table 12. Zingiber specimens information.

Appendix II.

Specimens original measurement data.

Species	Barcode	Leaf position	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width(mid point)	leaf width (lower quartile)
Aframomum leptolepis (K.Schum.) K.Schum.	E00930389	unknown	6.416	312.397	44.869	45.444	34.653
	E00930401	upper	3.313	292.851	43.272	49.193	37.453
	E00930402	unknown	12.788	215.047	30.470	33.132	30.071
	E00930403	unknown	4.765	274.515	35.720	38.319	28.619
		unknown	6.594	271.734	39.167	39.820	31.250
		unknown	5.821	329.376	54.283	49.675	40.922
Aframomum longiligulatum Koechlin	E00509455	upper	9.952	174.133	36.027	55.774	41.159
		upper	6.635	163.663	52.347	57.582	39.920
	E00509457	unknown	17.31	221.784	47.839	67.691	51.327
	E00509458	unknown	16.654	227.316	55.347	72.000	45.680
		unknown	broken ligule	228.756	50.290	65.637	37.873
	E00509461	upper	broken ligule	171.229	21.518	36.008	27.127
		upper	broken ligule	221.017	35.288	52.884	42.119
Aframomum angustifolium (Sonn.) K.Schum.	E00482328	unknown	3.503	344.646	60.654	66.745	58.836
	E00957858	upper	broken ligule	241.728	40.345	44.174	32.074
	E00957875	upper	4.839	249.197	26.651	37.668	31.932
Aframomum chrysanthum Lock	E00607558	upper	5.046	311.241	37.455	52.331	48.727
		upper	4.498	314.226	41.025	52.893	44.115
		upper	6.512	329.377	34.565	47.326	43.016
	E00643480	unknown	5.086	342.135	50.326	61.241	60.329

		unknown	5.931	334.175	41.454	58.545	47.951
	E00679426	unknown	10.923	327.176	54.114	44.942	34.421
Aframomum danielii (Hook.f.) K.Schum.	E00982949	unknown	9.27	615.494	70.234	77.921	64.811
	E00228504	upper	6.024	299.995	39.614	50.557	unavailable
	E00486322	upper	4.52	346.742	71.472	81.592	52.200
	E00982934	unknown	9.22	491.614	83.919	unavailable (overlapped)	72.902

Table 13. Aframomum leaves measurement data_1.

Species	Barcode	pseudo petiole length	angle of main vein &lateral vein	ligule - leaf ratio	pseudo-petiole - leaf ratio	Width-length ratio	Width-length ratio (approximate)
Aframomum leptolepis (K.Schum.) K.Schum.	E00930389	8.028	8.546	0.021	0.026	0.145	1:7
	E00930401	8.309	10.929	0.011	0.028	0.168	1:6
	E00930402	5.907	13.191	0.059	0.027	0.154	1:6
	E00930403	6.753	11.654	0.017	0.025	0.140	1:7
		6.514	11.867	0.024	0.024	0.147	1:7
		7.702	13.168	0.018	0.023	0.151	1:7
Aframomum longiligulatum Koechlin	E00509455	4.394	19.073				1:3
		3.635	22.101	0.057	0.025	0.320	1:3
	E00509457	6.248	22.230	0.041	0.022	0.352	1:3
	E00509458	4.31	18.660	0.078	0.028	0.305	1:3
		5.471	16.943	0.073	0.019	0.317	1:3
	E00509461	5.865	17.177		0.024	0.287	1:5
		4.847	15.976		0.034	0.210	1:4
Aframomum angustifolium (Sonn.) K.Schum.	E00482328	6.093	14.287		0.022	0.239	1:5
	E00957858	3.696	12.632				1:5
	E00957875	3.223	12.836	0.010	0.018	0.194	1:7
Aframomum chrysanthum Lock	E00607558	3.195	13.246		0.015	0.183	1:6
		3.919	12.750	0.019	0.013	0.151	1:6
		4.539	10.940				1:7
	E00643480	3.15	11.745	0.016	0.010	0.168	1:6
		3.416	12.552	0.014	0.012	0.168	1:6
	E00679426	5.129	10.680	0.020	0.014	0.144	1:6

Aframomum daniellii (Hook.f.) K.Schum.	E00982949	1.056	11.960	0.015	0.009	0.179	1:8
	E00228504	0	16.337	0.018	0.010	0.175	1:6
	E00486322	0	19.116	0.033	0.016	0.137	1:4
	E00982934	0	13.709				

Table 14. Aframomum leaves measurement data_2.

Species	Barcode	Leaf number	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width(mid point)	leaf width (lower quartile)
Boesenbergia cordata R.M.Sm.	E00149736	basal	1.763	165.636	13.453	35.749	46.681
	E00389710	basal	1.909	229.384	10.001	32.589	42.906
	E00389713	basal	1.682	280.377	20.920	48.188	58.072
Boesenbergia basispicata K.Larsen ex Sirirugsa	E00211999	basal	7.550	207.197	37.661	54.699	44.429
		basal	6.055	271.508	36.433	53.575	46.354
		basal	7.261	232.784	31.570	47.395	42.850
	E00428231	basal	4.100	247.003	36.991	51.618	45.930
Boesenbergia aurantiaca R.M.Sm.	E00228085	basal	2.618	114.726	18.489	29.060	23.018
		upper	2.476	182.149	18.009	29.261	18.274
		basal	3.466	161.548	24.908	32.595	25.764
	E00389714	basal	5.070	154.232	31.556	36.502	31.501
Boesenbergia orbiculata R.M.Sm.	E00149745	basal	1.306	69.071	37.989	43.961	34.995
		basal	1.467	67.023	45.244	51.627	41.709
		basal	1.069	68.186	37.127	43.961	32.704
	E00389727	basal	1.023	69.681	31.027	38.023	26.206
		basal	3.420	74.984	38.180	46.678	27.402
		basal	1.168	69.404	46.458	57.268	49.593
		basal	2.483	68.335	45.152	55.431	43.708
Boesenbergia flavorubra R.M.Sm.	E00149738	basal	2.073	90.595	17.300	21.733	20.133
		upper	1.468	93.503	14.071	19.804	17.617
		upper	4.892	80.462	13.513	19.023	17.089
	E00389718	upper	1.453	103.743	18.914	27.205	18.079

	unknown	1.515	105.890	13.847	18.311	13.505
	upper	2.393	92.898	12.749	16.263	13.204
	upper	0.710	48.412	7.666	11.831	10.834
	basal	2.044	107.116	16.326	21.366	17.817
	basal	1.774	93.088	19.517	22.537	16.173
E00389721	basal	7.753	96.391	7.748	13.156	11.773
	basal	2.427	101.440	9.267	13.560	12.812
	basal	2.550	101.310	8.879	13.201	12.899

Table 15. Boesenbergia leaves measurement data_1

Species	Barcode	pseudo petiole length	veins number	angle of main vein &lateral vein	ligule - leaf ratio	pseudo-petiole - leaf ratio	Width-length ratio	Width-length ratio (approximate)
Boesenbergia cordata R.M.Sm.	E00149736	77.752	30	14.569	0.011	0.469	0.216	1:5
	E00389710	122.856	22	10.336	0.008	0.536	0.142	1:7
	E00389713	147.676	32	14.194	0.006	0.527	0.172	1:6
Boesenbergia basispicata K.Larsen ex Sirirugsa	E00211999	40.025		10.783	0.036	0.193	0.264	1:4
		79.054		10.109	0.022	0.291	0.197	1:5
		57.178		7.720	0.031	0.246	0.204	1:5
	E00428231	79.675		12.592	0.017	0.323	0.209	1:5
Boesenbergia aurantiaca R.M.Sm.	E00228085	28.419	14	8.729	0.023	0.248	0.253	1:4
		60.078		7.645	0.014	0.330	0.161	1:6
		55.522		10.058	0.021	0.344	0.202	1:5
	E00389714	43.953		9.294	0.033	0.285	0.237	1:4
Boesenbergia orbiculata R.M.Sm.	E00149745	8.639	20	17.157	0.019	0.125	0.636	1:1.5
		9.245	18	13.825	0.022	0.138	0.770	1:1.3
		5.913	22	16.165	0.016	0.087	0.645	1:1.5
	E00389727	14.962	19	21.764	0.015	0.215	0.546	1:1.5
		14.747	21	22.501	0.046	0.197	0.623	1:1.6
		8.052	20	20.928	0.017	0.116	0.825	1:1.2
		12.666	18	32.498	0.036	0.185	0.811	1:1.2
Boesenbergia flavorubra R.M.Sm.	E00149738	22.436	24	14.931	0.023	0.248	0.240	1:4
		19.637	12	7.692	0.016	0.210	0.212	1:5
		17.452	12	10.945	0.061	0.217	0.236	1:4
	E00389718	20.232	18	12.781	0.014	0.195	0.262	1:4

	27.892	12	10.507	0.014	0.263	0.173	1:6
	16.901	16	9.435	0.026	0.182	0.175	1:6
	10.512	17	11.734	0.015	0.217	0.244	1:4
	24.407	16	7.453	0.019	0.228	0.199	1:5
	17.443	16	12.315	0.019	0.187	0.242	1:4
E00389721	27.198	6	9.444	0.080	0.282	0.136	1:7
	29.715	7	11.560	0.024	0.293	0.134	1:7
	18.818	9	6.715	0.025	0.186	0.130	1:8

Table 16. Boesenbergia leaves measurement data_2.

Species	Barcode	Leaf position	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width (mid-point)	leaf width (lower quartile)
Curcuma aeruginosa Roxb.	E00211325	unknow	3.075	664.398	100.005	133.756	108.915
	E00211386	unknow	3.386	575.787	93.754	112.685	93.112
Curcuma larsenii Maknoi & Jenjitt.	E00097673	basal	1.462	278.852	9.865	11.378	11.692
	E00097674	basal	1.281	201.536	7.036	10.063	6.150
	E00375668	basal	1.064	212.844	10.097	13.616	11.359
Curcuma harmandii Gagnep.	E00894175	upper	1.455	266.459	32.629	47.737	39.720
		basal	1.635	176.060	27.616	41.746	35.768
	E00097675	basal	1.282	317.425	27.677	41.648	33.240
	E00097676	basal	1.344	314.196	66.220	67.931	45.020
Curcuma parviflora Wall.	E00097696	basal	2.294	290.355	32.158	44.194	35.801
	E00211372	upper	3.046	426.991	43.894	46.402	33.810
		unknow	4.462	484.656	56.226	67.826	52.008
	E00894173	basal	1.030	167.438	34.661	52.910	39.056
Curcuma vamana M.Sabu & Mangaly	E00097613	basal	1.457	193.030	29.262	42.707	35.227
	E00211320	unknow	1.937	496.243	57.615	77.293	57.615

Table 17. Curcuma leaves measurement data_1

Species	Barcode	pseudo petiole length	veins number	angle of main vein &lateral vein	ligule - leaf ratio	pseudo-petiole - leaf ratio	Width-length ratio	Width-length ratio (approximate)
Curcuma aeruginosa Roxb.	E00211325	140.624		17.445	0.005	0.212	0.201	1:5
	E00211386	164.341		18.705	0.006	0.285	0.196	1:5
Curcuma larsenii Maknoi & Jenjitt.	E00097673	139.576		4.865	0.005	0.501	0.041	1:24
	E00097674	61.773		4.109	0.006	0.307	0.050	1:20
	E00375668	91.016		4.281	0.005	0.428	0.064	1:16
Curcuma harmandii Gagnep.	E00894175	109.578	44	16.711	0.005	0.411	0.179	1:6
		60.269	59	15.177	0.009	0.342	0.237	1:5
	E00097675	116.191	37	16.125	0.004	0.366	0.131	1:8
	E00097676	102.001	41	12.136	0.004	0.325	0.216	1:5
Curcuma parviflora Wall.	E00097696	111.466	34	12.034	0.008	0.384	0.152	1:7
	E00211372	190.150	56	9.851	0.007	0.445	0.109	1:9
		190.936		15.954	0.009	0.394	0.140	1:7
	E00894173	27.474	42	18.319	0.006	0.164	0.316	1:3
Curcuma vamana M.Sabu & Mangaly	E00097613	66.795	56	13.277	0.008	0.346	0.221	1:5
	E00211320	61.779		17.979	0.004	0.124	0.156	1:6

Table 18. Curcuma leaf measurement data_2

Species	Barcode	Leaf position	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width(mid point)	leaf width (lower quartile)
Globba albiflora Ridl.	E00183029	unknow	1.479	219.111	28.076	52.112	49.758
		unknow	1.330	217.404	27.976	51.239	45.452
		unknow	1.611	195.091	29.954	47.039	38.912
	E00183030	upper	1.629	212.082	39.075	49.236	32.707
		upper	2.215	227.616	27.695	46.902	40.889
	E00955827	upper	1.170	126.390	23.770	34.326	32.697
Globba atrosanguinea Teijsm. & Binn.	E00176650	basal	1.669	95.360	21.226	36.199	30.661
		basal	0.806	95.197	20.752	35.658	32.225
	E00183017	upper	0.870	97.041	21.513	35.547	26.318
	E00507882	basal	1.540	91.160	16.580	31.130	24.675
		basal	1.326	73.692	14.773	29.207	22.23
Globba brachyanthera K.Schum.	E00119502	upper	1.346	108.270	7.409	12.884	11.922
		middle	0.547	103.255	5.368	9.194	9.649
		basal	1.013	98.248	8.688	16.107	15.947
	E00128136	upper	0.657	85.363	7.774	13.657	10.762
		basal	1.101	118.232	16.916	26.850	23.816
		basal	1.774	125.388	12.714	24.746	21.427
	E00149831	upper	1.786	115.223	9.569	14.546	10.584
		upper	1.256	139.836	8.574	14.892	12.216
Globba marantina L.	E00095579	middle	1.667	177.689	26.625	50.168	43.938
	E00421681	upper	1.536	141.295	17.171	40.758	32.849
		upper	1.023	144.890	16.894	39.578	32.043
	E00421690	upper	3.808	186.213	37.552	77.332	69.58

	E00507886	basal	1.072	77.733	8.340	16.836	16.626
Globba pendula Roxb.	E00149876	upper	1.543	90.009	19.707	31.988	26.648
		upper	1.737	93.507	22.059	39.511	36.21
	E00189268	basal	0.773	146.829	17.197	31.177	24.99
	E00421683	upper	1.400	154.846	21.426	37.223	36.457
	E00673945	upper	1.414	139.259	29.688	48.267	36.61
		basal	2.155	130.707	31.770	53.996	40.537

Table 19. Globba leaves measurement data_1.

Species	Barcode	pseudo petiole length	veins number	angle of main vein &lateral vein	ligule - leaf ratio	pseudo-petiole - leaf ratio	Width-length ratio	Width-length ratio (approximate)
Globba albiflora Ridl.	E00183029	2.487	54	17.399	0.007	0.011	0.238	1:4
		1.621	71	14.892	0.006	0.007	0.236	1:4
		2.282	98	14.613	0.008	0.012	0.241	1:4
	E00183030	5.032	57	12.365	0.008	0.024	0.232	1:4
		2.581	55	14.791	0.010	0.011	0.206	1:5
	E00955827	7.571	68	12.952	0.009	0.060	0.272	1:4
Globba atrosanguinea Teijsm. & Binn.	E00176650	3.823	57	18.509	0.018	0.040	0.380	1:3
		5.203	76	23.128	0.008	0.055	0.375	1:3
	E00183017	4.268	63	20.123	0.009	0.044	0.366	1:3
	E00507882	1.456	36	18.159	0.017	0.016	0.341	1:3
		0.929	33	21.731	0.018	0.013	0.396	1:3
Globba brachyanthera K.Schum.	E00119502	3.251	62	10.839	0.012	0.030	0.119	1:8
		2.038		13.918	0.005	0.020	0.089	1:11
		2.073	76	12.503	0.010	0.021	0.164	1:6
	E00128136	2.374	86	13.513	0.008	0.028	0.160	1:6
		3.136		14.943	0.009	0.027	0.227	1:4
		3.381		17.106	0.014	0.027	0.197	1:5
	E00149831	2.279	84	11.577	0.016	0.020	0.126	1:8
		2.516	102	11.638	0.009	0.018	0.106	1:9
Globba marantina L.	E00095579	5.838	54	16.703	0.009	0.033	0.282	1:3.5
	E00421681	6.742	48	17.750	0.011	0.048	0.288	1:3.5
		10.241	62	15.088	0.007	0.071	0.273	1:3.7

	E00421690	12.102	42	17.586	0.020	0.065	0.415	1:2.4
	E00507886	3.051	58	18.052	0.014	0.039	0.217	1:5
Globba pendula Roxb.	E00149876	3.241	102	18.392	0.017	0.036	0.355	3:1
		3.526	87	22.734	0.019	0.038	0.423	2.4:1
	E00189268	3.226	96	15.000	0.005	0.022	0.212	5:1
	E00421683	4.821	84	18.155	0.009	0.031	0.240	4:1
	E00673945	7.996	93	23.776	0.010	0.057	0.347	3:1
		6.242	98	22.232	0.016	0.048	0.413	2.4:1

Table 20. Globba leaves measurement data_2.

Species	Barcode	Leaf position	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width(mid point)	leaf width (lower quartile)
Hedychium densiflorum Wall.	E00149991	upper	2.259	135.096	13.294	24.096	20.568
	E00212282	upper	15.769	331.134	32.763	65.517	60.179
	E00212283	unknown	14.086	266.168	33.649	61.541	52.909
Hedychium coronarium J.König	E00211110	upper	12.921	320.094	37.409	45.146	32.031
		upper	4.204	260.864	33.256	33.256	23.465
	E00211112	upper	18.102	277.902	25.157	40.876	30.583
		upper	28.538	143.207	20.317	38.282	34.376
	E00504164	unknown	35.143	468.150	58.325	87.297	73.849
		unknown	63.073	337.550	54.900	83.373	74.715
Hedychium coccineum Buch.-Ham. ex Sm.	E00211095	unknown	10.252	359.492	24.512	30.273	24.667
	E00531053	unknown	5.911	266.636	13.404	14.482	10.215
		upper	5.524	268.516	12.995	14.584	7.740
		upper	3.135	239.547	11.136	14.998	10.249
Hedychium ellipticum Buch.-Ham. ex Sm.	E00148648	unknown	16.406	246.345	72.216	86.310	46.450
	E00499883	unknown	10.808	257.977	54.465	54.465	31.519
		upper	10.870	281.171	60.888	60.888	42.405
	Average		12.695	261.831	62.523	67.221	40.125
	Standard deviation		3.214	17.730	8.988	16.841	7.722
Hedychium greenii W.W.Sm.	E00211530	upper	10.570	231.749	38.844	60.806	40.828
		upper	8.453	244.582	41.564	61.177	47.923
	E00247016	upper	8.377	282.835	53.642	68.394	51.938

	upper	7.809	265.534	49.746	70.269	49.318

Table 21. Hedychium leaves measurement data_1

Species	Barcode	pseudo petiole length	angle of main vein &lateral vein	ligule-leaf ratio	pseudo-petiole-leaf ratio	Width-length ratio	width-length ratio (approximate)
Hedychium densiflorum Wall.	E00149991	7.546	15.681	0.017	0.056	0.178	1:9
	E00212282	8.262	12.741	0.048	0.025	0.198	1:5
	E00212283	3.206	13.980	0.053	0.012	0.231	1:4
Hedychium coronarium J.König	E00211110	4.392	10.320	0.040	0.014	0.141	1:7
		7.465	13.516	0.016	0.029	0.127	1:8
	E00211112	3.352	9.393	0.065	0.012	0.147	1:7
		6.721	14.371	0.199	0.047	0.267	1:4
	E00504164	6.029	11.710	0.075	0.013	0.186	1:5
		9.288	14.311	0.187	0.028	0.247	1:4
Hedychium coccineum Buch.-Ham. ex Sm.	E00211095	6.399	7.628	0.029	0.018	0.084	1:12
	E00531053	3.047	6.181	0.022	0.011	0.054	1:18
		5.633	6.276	0.021	0.021	0.054	1:18
		2.554	6.213	0.013	0.011	0.063	1:16
Hedychium ellipticum Buch.-Ham. ex Sm.	E00148648	4.988	17.550	0.067	0.020	0.350	1:3
	E00499883	22.631	15.821	0.042	0.088	0.211	1:5
		15.644	16.915	0.039	0.056	0.217	1:5
Hedychium greenii W.W.Sm.	E00211530	6.701	14.867	0.046	0.029	0.262	1:4
		3.996	16.576	0.035	0.016	0.250	1:4

E00247016	12.858	16.144	0.030	0.045	0.242	1:4
	16.403	14.747	0.029	0.062	0.265	1:4

Table 22. Hedychium leaves measurement data_2

Species	Barcode	leaf position	ligule length/mm	whole leaf length/mm	leaf width (upper quartile)	leaf width (mid point)	Leaf width (lower quartile)
Zingiber bradleyanum Craib	E00077500	basal	15.159	120.378	28.422	39.325	36.142
	E00077500	unknown	25.954	195.757	45.274	56.301	41.006
	E00294288	upper	28.83	432.509	40.549	48.654	38.022
	E00294288	unknown	22.505	422.775	52.144	55.371	48.940
	E00141461	upper	26.443	345.31	65.255	71.194	58.070
Zingiber officinale Roscoe	E00177158	upper	3.37	133.653	4.669	11.763	8.199
	E00177158	upper	1.808	122.026	4.939	9.329	6.538
	E00389842	upper	3.552	186.863	6.403	10.532	10.742
	E00389842	basal	3.262	180.705	7.757	10.871	9.591
	E00412174	upper	4.443	232.031	29.394	33.000	26.848
Zingiber zerumbet (L.) Sm.	E00683210	upper	7.305	156.67	30.848	35.340	27.350
	E00770317	upper	9.164	137.632	25.066	38.158	25.066
	E00770319	basal	14.485	197.158	40.613	45.934	40.487
	E00770319	basal	19.431	188.348	42.491	52.811	36.375
	K000255220	basal	15.755	116.048	25.283	30.469	21.799
Zingiber nudicarpum D.Fang	E00421674	unknown	6.567	267.252	50.981	62.738	47.433
	E00421674	unknown	6.08	277.288	48.770	62.159	47.750
	E00421675	upper	1.361	88.677	11.006	19.566	16.520
	E00421675	upper	2.424	103.855	13.291	21.457	17.218
	E00421675	upper	2.762	106.938	20.628	25.684	19.539
	E00421675	upper	2.245	124.069	17.320	28.002	22.348
	E00421675	upper	1.706	128.553	21.996	30.405	25.935
	E00421675	upper	1.96	136.475	19.328	33.488	23.423
	E00421773	unknown	2.347	330.479	46.075	49.337	44.256

	E00421788	upper	1.266	81.042	10.855	21.486	18.664
	E00421788	upper	2.849	176.093	24.327	37.172	25.811

Table 23. Zingiber leaves measurement data_1.

Species	Barcode	pseudo petiole length	angle of main vein &lateral vein	ligule-leaf ratio	pseudo-petiole - leaf ratio	Width-length ratio	Width-length ratio (approximate)
Zingiber bradleyanum Craib	E00077500	3.206	14.285	0.126	0.027	0.327	1:3
	E00077500	7.272	18.945	0.133	0.037	0.288	1:4
	E00294288	8.993	10.837	0.067	0.021	0.112	1:9
	E00294288	13.782	12.063	0.053	0.033	0.131	1:7
	E00141461	5.432	11.846	0.077	0.016	0.206	1:5
Zingiber officinale Roscoe	E00177158	2.983	3.633	0.025	0.022	0.088	1:11
	E00177158	2.851	3.655	0.015	0.023	0.076	1:13
	E00389842	3.827	4.267	0.019	0.020	0.056	1:18
	E00389842	5.019	4.879	0.018	0.028	0.060	1:17
	E00412174	8.168	5.336	0.019	0.035	0.142	1:7
Zingiber zerumbet (L.) Sm.	E00683210	5.156	11.043	0.047	0.033	0.226	1:4
	E00770317	2.814	13.446	0.067	0.020	0.277	1:4
	E00770319	3.262	15.44	0.073	0.017	0.233	1:4
	E00770319	4.138	11.235	0.103	0.022	0.280	1:4
	K000255220	4.667	11.491	0.136	0.040	0.263	1:4
Zingiber nudicarpum D.Fang	E00421674	14.898	14.06	0.025	0.056	0.235	1:4
	E00421674	13.659	12.297	0.022	0.049	0.224	1:4
	E00421675	5.243	8.549	0.015	0.059	0.221	1:5
	E00421675	9.142	8.81	0.023	0.088	0.207	1:5
	E00421675	7.495	13.476	0.026	0.070	0.240	1:4
	E00421675	11.35	6.649	0.018	0.091	0.226	1:4
	E00421675	9.298	13.391	0.013	0.072	0.237	1:4

E00421675	6.762	6.121	0.014	0.050	0.245	1:4
E00421773	8.364	13.288	0.007	0.025	0.149	1:7
E00421788	5.293	12.823	0.016	0.065	0.265	1:4
E00421788	6.6	9.087	0.016	0.037	0.211	1:5

Table 24. Zingiber leaves measurement data_2.

Appendix III.

One-way ANOVA and Tukey-test results of genera.

One-way ANOVA Aframomum

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
A. leptolepis	6	0.150614035	0.025102339	0.000301544
A. longiligulatum	4	0.249004904	0.062251226	0.000289399
A. angustifolium	2	0.029582424	0.014791212	0.000042821
A. chrysanthum	6	0.116297076	0.019382846	0.000051010
A. daniellii	4	0.066931588	0.016732897	0.000010586

Table 25. Aframomum ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.006044652	4	0.001511163	9.49523742	0.000319	2.964708
Within groups	0.002705543	17	0.00015915			
Total	0.008750195	21				

Table 26. Aframomum ligule-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	10 levels	df=17	Q=5.108		
		Difference	n (Group 1)	n (Group 2)	SE	q
A. leptolepis	A. longiligulatum	0.037148887	6	4	0.005758	6.451536
A. leptolepis	A. angustifolium	0.010311127	6	2	0.007284	1.415675
A. leptolepis	A. chrysanthum	0.005719493	6	6	0.00515	1.110529
A. leptolepis	A. daniellii	0.008369442	6	4	0.005758	1.453496
A. longiligulatum	A. angustifolium	0.047460014	4	2	0.007725	6.143402
A. longiligulatum	A. chrysanthum	0.04286838	4	4	0.006308	6.796162
A. longiligulatum	A. daniellii	0.045518329	4	4	0.006308	7.216273
A. angustifolium	A. chrysanthum	0.004591634	2	6	0.007284	0.630412
A. angustifolium	A. daniellii	0.001941685	2	4	0.007725	0.251339
A. chrysanthum	A. daniellii	0.002649949	6	4	0.005758	0.460209

Table 27. Tukey-test results of Aframomum ligule-leaf length ratio.

Pseudo-petiole-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
A. leptolepis	6	0.153494606	0.025582434	0.000003950
A. longiligulatum	7	0.174674931	0.024953562	0.000025093
A. angustifolium	3	0.045902465	0.015300822	0.000005630
A. chrysanthum	6	0.071623492	0.011937249	0.000006172
A. daniellii	1	0.001715695	0.001715695	

Table 28. Aframomum pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.001142466	4	0.000285616	24.20149958	0.000000492	2.927744
Within groups	0.000212429	18	0.000011802			
Total	0.001354895	22				

Table 29. Aframomum pseudo-petiole-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	10 levels	df=18	Q=5.071		
		Difference	n (Group 1)	n (Group 2)	SE	q
A. leptolepis	A. longiligulatum	0.000628873	6	7	0.004273757	0.147147
A. leptolepis	A. angustifolium	0.010281613	6	3	0.005431851	1.892838
A. leptolepis	A. chrysanthum	0.013645186	6	6	0.004435087	3.076644
A. leptolepis	A. daniellii	0.023866739	6	1	0.008297289	2.87645
A. longiligulatum	A. angustifolium	0.00965274	7	3	0.005300943	1.820948
A. longiligulatum	A. chrysanthum	0.013016313	7	6	0.004273757	3.045637
A. longiligulatum	A. daniellii	0.023237867	7	1	0.008212186	2.829681
A. angustifolium	A. chrysanthum	0.003363573	3	6	0.005431851	0.619231
A. angustifolium	A. daniellii	0.013585127	3	1	0.008870175	1.531551
A. chrysanthum	A. daniellii	0.010221554	6	1	0.008297289	1.231915

Table 30. Tukey-test results of Aframomum pseudo-petiole-leaf length ratio.

Width-length ratio

SUMMARY

Group	Count	SUM	Average	Variance
A. leptolepis	6	0.904460862	0.150743477	0.000095616
A. longiligulatum	7	2.030576577	0.290082368	0.002437212
A. angustifolium	3	0.527562587	0.175854196	0.000487255
A. chrysanthum	6	0.971700468	0.161950078	0.000296615
A. daniellii	3	0.530435668	0.176811889	0.003006026

Table 31. Aframomum width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.083360225	4	0.020840056	17.68279894	0.000002382	2.866081
Within groups	0.023570993	20	0.00117855			
Total	0.106931217	24				

Table 32. Aframomum width-length ratio one-way ANOVA results.

Tukey-test Alpha=0.05 10 levels df=20 Q=5.008

		Difference	n (Group 1)	n (Group 2)	SE	q
A. leptolepis	A. longiligulatum	0.139338891	6	7	0.01350536	10.3173
A. leptolepis	A. angustifolium	0.025110719	6	3	0.01716501	1.4629
A. leptolepis	A. chrysanthum	0.011206601	6	6	0.01401517	0.79961
A. leptolepis	A. daniellii	0.026068412	6	3	0.01716501	1.5187
A. longiligulatum	A. angustifolium	0.114228172	7	3	0.01675133	6.81905
A. longiligulatum	A. chrysanthum	0.12813229	7	6	0.01350536	9.48752
A. longiligulatum	A. daniellii	0.113270479	7	3	0.01675133	6.76188

A. angustifolium	A. chrysanthum	0.013904118	3	6	0.01716501	0.81003
A. angustifolium	A. daniellii	0.000957693	3	3	0.01982044	0.04832
A. chrysanthum	A. daniellii	0.014861811	6	3	0.01716501	0.86582

Table 33. Tukey-test results of Aframomum leaf width-length ratio.

One-way ANOVA Boesenbergia

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
B. cordata	3	0.024965176	0.008321725	0.000005393
B. basispicata	4	0.106531115	0.026632779	0.000078794
B. aurantiaca	4	0.090740334	0.022685083	0.000062659
B. orbiculata	7	0.169929414	0.024275631	0.000142755
B. flavorubra	12	0.335787176	0.027982265	0.000432064

Table 34. Boesenbergia ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.000960792	4	0.000240198	0.99347556	0.42938	2.75871
Within groups	0.006044383	25	0.000241775			
Total	0.007005175	29				

Table 35. Boesenbergia ligule-leaf ratio one-way ANOVA results.

Pseudo-petiole-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
B. cordata	3	1.53171095	0.510570317	0.001290068
B. basispicata	4	1.0525338	0.26313345	0.003173013

B. aurantiaca	4	1.206207912	0.301551978	0.001916119
B. orbiculata	7	1.062488544	0.151784078	0.002253413
B. flavorubra	12	2.708135868	0.225677989	0.00144862

Table 36. Boesenbergia pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.289509993	4	0.072377498	38.25220518	0.000000000	2.75871
Within groups	0.047302827	25	0.001892113			
Total	0.336812821	29				

Table 37. Boesenbergia pseudo-petiole-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	10 levels	df=25	Q=4.897		
		Difference	n (Group 1)	n (Group 2)	SE	q
B. cordata	B. basispicata	0.247436867	3	4	0.023491835	10.53288818
B. cordata	B. aurantiaca	0.209018339	3	4	0.023491835	8.897488963
B. cordata	B. orbiculata	0.358786239	3	7	0.021225058	16.90389896
B. cordata	B. flavorubra	0.284892328	3	12	0.019854224	14.34920492
B. basispicata	B. aurantiaca	0.038418528	4	4	0.021749213	1.766433045
B. basispicata	B. orbiculata	0.111349372	4	7	0.019278616	5.775796854
B. basispicata	B. flavorubra	0.037455461	4	12	0.017758158	2.109197454
B. aurantiaca	B. orbiculata	0.1497679	4	7	0.019278616	7.768602091
B. aurantiaca	B. flavorubra	0.075873989	4	12	0.017758158	4.272627266
B. orbiculata	B. flavorubra	0.073893911	7	12	0.014628362	5.051413967

Table 38. Tukey-test results of Boesenbergia pseudo-petiole-leaf ratio.

Width-length ratio

SUMMARY

Group	Count	Sum	Average	Variance
B. cordata	3	0.529769086	0.176589695	0.001376736
B. basispicata	4	0.873896953	0.218474238	0.000943635
B. aurantiaca	4	0.852378465	0.213094616	0.001683883
B. orbiculata	7	4.855954534	0.693707791	0.01159936
B. flavorubra	12	2.384752629	0.198729386	0.002299943

Table 39. Boesenbergia width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	1.302370503	4	0.325592626	77.13157414	0.000000000	2.75871
Within groups	0.105531564	25	0.004221263			
Total	1.407902067	29				

Table 40. Boesenbergia width-length ratio one-way ANOVA results.

Tukey-test Alpha=0.05 10 levels df=25 Q=4.897

		Difference	n (Group 1)	n (Group 2)	SE	q
B. cordata	B. basispicata	0.041884543	3	4	0.035088484	1.193683461
B. cordata	B. aurantiaca	0.036504921	3	4	0.035088484	1.040367579
B. cordata	B. orbiculata	0.517118095	3	7	0.031702723	16.31147275
B. cordata	B. flavorubra	0.022139691	3	12	0.029655182	0.746570732

B. basispicata	B. aurantiaca	0.005379622	4	4	0.032485624	0.165600079
B. basispicata	B. orbiculata	0.218474238	4	7	0.028795428	7.587115561
B. basispicata	B. flavorubra	0.019744852	4	12	0.026524401	0.744403338
B. aurantiaca	B. orbiculata	0.480613174	4	7	0.028795428	16.6906072
B. aurantiaca	B. flavorubra	0.014365231	4	12	0.026524401	0.541585491
B. orbiculata	B. flavorubra	0.494978405	7	12	0.021849594	22.65389517

Table 41. Tukey-test results of Boesenbergia width-length ratio.

One-way ANOVA Curcuma

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
C. aeruginosa	2	0.010508897	0.005254448	0.000000784
C. larsenii	3	0.016598075	0.005532692	0.000000523
C. harmandii	4	0.023063443	0.005765861	0.000005896
C. parviflora	4	0.030392374	0.007598093	0.000001662
C. vamana	2	0.011451379	0.00572569	0.000006642

Table 42. Curcuma ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groupsc	0.000012082	4	0.000003020	0.969740731	0.465543	3.47805
Within groups	0.000031147	10	0.000003115			
Total	0.000043229	14				

Table 43. Curcuma ligule-leaf ratio one-way ANOVA results.

Pseudo-petiole ratio

SUMMARY

Group	Count	Sum	Average	Variance
C. aeruginosa	2	0.497076048	0.248538024	0.002720528

C. larsenii	3	1.234667265	0.411555755	0.009605117
C. harmandii	4	1.44424223	0.361060558	0.001406703
C. parviflora	4	1.387267641	0.34681691	0.015564173
C. vamana	2	0.470527739	0.235263869	0.024540174

Table 44. Curcuma pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.055698317	4	0.013924579	1.429869502	0.293725	3.47805
Within groups	0.097383566	10	0.009738357			
Total	0.153081883	14				

Table 45. Curcuma pseudo-petiole-leaf ratio one-way ANOVA results.

Width-length ratio

SUMMARY

Group	Count	Sum	Average	Variance
C. aeruginosa	2	0.39702514	0.19851257	0.000015753
C. larsenii	3	0.154706268	0.051568756	0.000136208
C. harmandii	4	0.763677218	0.190919305	0.002159116
C. parviflora	4	0.716823108	0.179205777	0.008652406
C. vamana	2	0.377001756	0.188500878	0.002144408

Table 46. Curcuma width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.045156817	4	0.011289204	3.23777745	0.060008	3.47805
Within groups	0.034867141	10	0.003486714			
Total	0.080023959	14				

Table 47. Curcuma width-length ratio one-way ANOVA results.

One-way ANOVA Globba

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
G. albiflora	6	0.047794686	0.007965781	0.000001969
G. atrosanguinea	5	0.06982122	0.013964244	0.000023137
G. brachyanthera	8	0.083679245	0.010459906	0.000011530
G. marantina	5	0.061553458	0.012310692	0.000026663
G. pendula	6	0.076665748	0.012777625	0.000028746

Table 48. Globba ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.000126038	4	0.000031510	1.817246244	0.157042	2.75871
Within groups	0.000433479	25	0.000017339			
Total	0.000559517	29				

Table 49. Globba ligule-leaf ratio one-way ANOVA results.

Pseudo-petiole-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
G. albiflora	6	0.125471517	0.02091192	0.000395434
G. atrosanguinea	5	0.16730512	0.033461024	0.000336148

G. brachyanthera	8	0.189934606	0.023741826	0.000020851
G. marantina	5	0.255491967	0.051098393	0.000265279
G. pendula	6	0.231995072	0.038665845	0.000155540

Table 50. Globba pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.003380803	4	0.000845201	3.981891339	0.012368	2.75871
Within geoups	0.005306528	25	0.000212261			
Total	0.008687331	29				

Table 51. Globba pseudo-petiole-leaf ratio one-way ANOVA results.

Tukey-test Alpha=0.05 10 levels df=25 Q=4.897

		Difference	n (Group 1)	n (Group 2)	SE	q
G. albiflora	G. atrosanguinea	0.012549104	6	5	0.019726763	0.636146166
G. albiflora	G. brachyanthera	0.002829906	6	8	0.017593956	0.160845356
G. albiflora	G. marantina	0.030186474	6	5	0.019726763	1.53022948
G. albiflora	G. pendula	0.017753926	6	6	0.01880873	0.943919422
G. atrosanguinea	G. brachyanthera	0.009719198	5	8	0.018572133	0.523321598
G. atrosanguinea	G. marantina	0.017637369	5	5	0.020603932	0.856019601
G. atrosanguinea	G. pendula	0.005204821	5	6	0.019726763	0.26384569
G. brachyanthera	G. marantina	0.027356568	8	5	0.018572133	1.472990079
G. brachyanthera	G. pendula	0.01492402	8	6	0.017593956	0.848246953

G. marantina	G. pendula	0.012432548	5	6	0.019726763	0.630237623

Table 52. Tukey-test results of Globba pseudo-petiole-leaf ratio.

Width-length ratio

SUMMARY

Group	Count	Sum	Average	Variance
G. albiflora	6	1.424433549	0.237405592	0.000439116
G. atrosanguinea	5	1.858309631	0.371661926	0.000404907
G. brachyanthera	8	1.189159735	0.148644967	0.002228537
G. marantina	5	1.475830672	0.295166134	0.005324431
G. pendula	6	1.990361213	0.331726869	0.007649812

Table 53. Globba width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.201020595	4	0.050255149	15.91123142	0.000001341	2.75871
Within groups	0.078961752	25	0.00315847			
Total	0.279982347	29				

Table 54. Globba width-length ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	10 levels	df=25	Q=4.897		
		Difference	n (Group 1)	n (Group 2)	SE	q
G. albiflora	G. atrosanguinea	0.134256335	6	5	0.024063517	5.57924831

G. albiflora	G. brachyanthera	0.088760625	6	8	0.021461831	4.135743261
G. albiflora	G. marantina	0.057760543	6	5	0.024063517	2.400336731
G. albiflora	G. pendula	0.094321277	6	6	0.022943663	4.110994731
G. atrosanguinea	G. brachyanthera	0.223016959	5	8	0.022655052	9.844027752
G. atrosanguinea	G. marantina	0.076495792	5	5	0.025133523	3.043576118
G. atrosanguinea	G. pendula	0.039935057	5	6	0.024063517	1.659568629
G. brachyanthera	G. marantina	0.146521168	8	5	0.022655052	6.467483212
G. brachyanthera	G. pendula	0.183081902	8	6	0.021461831	8.53058149
G. marantina	G. pendula	0.036560734	5	6	0.024063517	1.519342949

Table 55. Tukey-test results of Globba width-length ratio.

One-way ANOVA Hedychium

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
H. densiflorum	3	0.11726411	0.039088037	0.000382222
H. coronarium	6	0.582821084	0.097136847	0.005956100
H. coccineum	4	0.084346352	0.021086588	0.000040217
H. ellipticum	3	0.147152613	0.049050871	0.000233534
H. greenii	4	0.139197334	0.034799334	0.000057609

Table 56. Hedychium ligule-leaf ratio summary

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.017708891	4	0.004427223	2.12130029	0.12855	3.055568
Within groups	0.031305489	15	0.002087033			
Total	0.04901438	19				

Table 57. Hedychium ligule-leaf ratio one-way ANOVA results.

Pseudo-petiole-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
H. densiflorum	3	0.092852224	0.030950741	0.000506864
H. coronarium	6	0.141725557	0.023620926	0.000186112

H. coccineum	4	0.060867739	0.015216935	0.000024991
H. ellipticum	3	0.16361164	0.054537213	0.001139191
H. greenii	4	0.152487751	0.038121938	0.000390858

Table 58. Hedychium pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.003184447	4	0.000796112	2.183034718	0.120448	3.055568
Within groups	0.005470218	15	0.000364681			
Total	0.008654665	19				

Table 59. Hedychium pseudo-petiole-leaf ratio one-way ANOVA results.

Width-length ratio.

SUMMARY

Group	Count	Sum	Average	Variance
H. densiflorum	3	0.607429625	0.202476542	0.000714265
H. coronarium	6	1.116397834	0.186066306	0.003458611
H. coccineum	4	0.255447423	0.063861856	0.000199325
H. ellipticum	3	0.778037263	0.259345754	0.006220374
H. greenii	4	1.018956163	0.254739041	0.000114837

Table 60. Hedychium width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit

Between groups	0.095366077	4	0.023841519	11.1392245	0.000213287	3.055568276
Within groups	0.032104819	15	0.002140321			
SUM	0.127470896	19				

Table 61. Hedychium width-length ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	10 levels	df=15	Q=5.198		
		Difference	n (Group 1)	n (Group 2)	SE	q
H. densiflorum	H. coronarium	0.016410236	3	6	0.023131802	0.709423173
H. densiflorum	H. coccineum	0.138614686	3	4	0.024985201	5.54787146
H. densiflorum	H. ellipticum	0.056869212	3	3	0.026710304	2.129111398
H. densiflorum	H. greenii	0.052262499	3	4	0.024985201	2.091738144
H. coronarium	H. coccineum	0.12220445	6	4	0.021116349	5.787195887
H. coronarium	H. ellipticum	0.073279449	6	3	0.023131802	3.16790925
H. coronarium	H. greenii	0.068672735	6	4	0.021116349	3.252112099
H. coccineum	H. ellipticum	0.195483898	4	3	0.024985201	7.823987285
H. coccineum	H. greenii	0.190877185	4	4	0.023131802	8.25172148
H. ellipticum	H. greenii	0.004606713	3	4	0.024985201	0.184377681

Table 62. Tukey-test results of Hedychium width-length ratio.

One-way ANOVA Zingiber

Ligule-leaf ratio

SUMMARY

Group	Count	Sum	Average	Variance
Z. bradleyanum	5	0.454977833	0.090995567	0.001294031
Z. officinale	5	0.096239465	0.019247893	0.000014184
Z. zerumbet	5	0.42560722	0.085121444	0.001212855
Z. nudicarpum	11	0.195644553	0.017785868	0.000031830

Table 63. Zingiber ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.030088931	3	0.010029644	21.21130316	0.000001088	3.049125
Within groups	0.010402575	22	0.000472844			
Total	0.040491506	25				

Table 64. Zingiber ligule-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	6 levels	df=22	Q=4.405		
		Difference	n (Group 1)	n (Group 2)	SE	q
Z. bradleyanum	Z. officinale	0.071747674	5	5	0.009724649	7.377919039
Z. bradleyanum	Z. zerumbet	0.005874123	5	5	0.009724649	0.604044702
Z. bradleyanum	Z. nudicarpum	0.073209698	5	11	0.008293209	8.827668718

Z. officinale	Z. zerumbet	0.065873551	5	5	0.009724649	6.773874337
Z. officinale	Z. nudicarpum	0.001462025	5	11	0.008293209	0.176291785
Z. zerumbet	Z. nudicarpum	0.067335575	5	11	0.008293209	8.11936352

Table 65. Tukey-test results of Zingiber ligule-leaf ratio.

Pseudo-petiole ratio

SUMMARY

Group	Count	Sum	Average	Variance
Z. bradleyanum	5	0.132903194	0.026580639	0.000074778
Z. officinale	5	0.129139851	0.02582797	0.000034662
Z. zerumbet	5	0.132086959	0.026417392	0.000096365
Z. nudicarpum	11	0.663700759	0.060336433	0.000401139

Table 66. Zingiber pseudo-petiole-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.007364112	3	0.002454704	11.17019041	0.000117	3.049125
Within groups	0.004834608	22	0.000219755			
Total	0.012198719	25				

Table 67. Zingiber pseudo-petiole-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	6 levels	df=22	Q=4.405		
		Difference	n (Group 1)	n (Group 2)	SE	q
Z. bradleyanum	Z. officinale	0.000752669	5	5	0.006629555	0.113532298

Z. bradleyanum	Z. zerumbet	0.000163247	5	5	0.006629555	0.024624139
Z. bradleyanum	Z. nudicarpum	0.033755794	5	11	0.005653704	5.970563128
Z. officinale	Z. zerumbet	0.000589422	5	5	0.006629555	0.088908159
Z. officinale	Z. nudicarpum	0.034508462	5	11	0.005653704	6.103691548
Z. zerumbet	Z. nudicarpum	0.033919041	5	11	0.005653704	5.99943749

Table 68. Tukey-test results of Zingiber pseudo-petiole-leaf ratio.

Width-length ratio

SUMMARY

Group	Count	Sum	Average	Variance
Z. bradleyanum	5	1.06392286	0.212784572	0.008842
Z. officinale	5	0.423205758	0.084641152	0.001198
Z. zerumbet	5	1.278742598	0.25574852	0.000636
Z. nudicarpum	11	2.4594419	0.223585627	0.000875

Table 69. Zingiber width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.088989489	3	0.029663163	12.68415	0.000050279	3.049125
Within groups	0.051449229	22	0.002338601			
Total	0.140438718	25				

Table 70. Zingiber width-length ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	6 levels	df=22	Q=4.405		
		Difference	n (Group 1)	n (Group 2)	SE	q
Z. bradleyanum	Z. officinale	0.12814342	5	5	0.02162684	5.925203201
Z. bradleyanum	Z. zerumbet	0.042963947	5	5	0.02162684	1.98660312
Z. bradleyanum	Z. nudicarpum	0.010801055	5	11	0.018443431	0.585631554
Z. officinale	Z. zerumbet	0.171107368	5	5	0.02162684	7.911806321
Z. officinale	Z. nudicarpum	0.138944476	5	11	0.018443431	7.533548173
Z. zerumbet	Z. nudicarpum	0.032162892	5	11	0.018443431	1.743867092

Table 71. Tukey-test results of Zingiber leaf width-length ratio.

Appendix IV.

One-way ANOVA and Tukey-test results Between genera.

Ligule-leaf length ratio.

SUMMARY

Group	Count	Sum	Average	Variance
Zingiber	26	1.172469071	0.045094964	0.00161966
Aframomum	22	0.612430027	0.027837729	0.000416676
Boesenbergia	30	0.727953215	0.024265107	0.000241558
Curcuma	15	0.092014168	0.006134278	0.000003088
Globba	30	0.339514357	0.011317145	0.000019294
Hedychium	20	1.070781494	0.053539075	0.002579704

Table 72. All genera ligule-leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.036582437	5	0.007316487	9.468362812	0.000000090	2.280308674
Within groups	0.105864001	137	0.00077273			
Total	0.142446437	142				

Table 73. All genera ligule-leaf ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	15 levels	df=137	Q=4.898		
		Difference	n (Group 1)	n (Group 2)	SE	q
Zingiber	Aframomum	0.01725724	26	22	0.00569405	3.03074739
Zingiber	Boesenbergia	0.02082986	26	30	0.00526679	3.9549472
Zingiber	Curcuma	0.03896069	26	15	0.00637321	6.11319268
Zingiber	Globba	0.03377782	26	30	0.00526679	6.41336569
Zingiber	Hedychium	0.00844411	26	20	0.00584623	1.44436771
Aframomum	Boesenbergia	0.00357262	22	30	0.00551733	0.64752777
Aframomum	Curcuma	0.02170345	22	15	0.00658177	3.29750911
Aframomum	Globba	0.01652058	22	30	0.00551733	2.99431016
Aframomum	Hedychium	0.02570135	22	20	0.00607291	4.23212685
Boesenbergia	Curcuma	0.01813083	30	15	0.00621583	2.91688144
Boesenbergia	Globba	0.01294796	30	30	0.0050752	2.55122143
Boesenbergia	Hedychium	0.02927397	30	20	0.00567425	5.15909258
Curcuma	Globba	0.00518287	15	30	0.00621583	0.83381786
Curcuma	Hedychium	0.0474048	15	20	0.00671386	7.06073651
Globba	Hedychium	0.04222193	30	20	0.00567425	7.4409744

Table 74. Tukey-test results of all genera ligule-leaf ratio.

Pseudo-petiole-leaf length ratio.

SUMMARY

Group	Count	Sum	Average	Variance
Zingiber	26	1.057830762	0.040685799	0.000487949
Aframomum	26	0.447411189	0.017208123	9.4365E-05
Boesenbergia	30	7.561077074	0.252035902	0.011614235
Curcuma	15	5.033780923	0.335585395	0.01093442
Globba	30	0.970198282	0.032339943	0.000299563
Hedychium	20	0.611544911	0.030577246	0.000455509

Table 75. All genera pseudo-petiole -leaf ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	2.022812994	5	0.404562599	109.3214311	0.000000000	2.27840268
With groups	0.521794546	141	0.003700671			
Total	2.54460754	146				

Table 76. All genera pseudo-petiole-leaf length ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	15 levels	df=141	Q=4.898		
		Difference	n (Group 1)	n (Group 2)	SE	q
Zingiber	Aframomum	0.02347768	26	26	0.01193036	1.96789326
Zingiber	Boesenbergia	0.2113501	26	30	0.01152582	18.3370941
Zingiber	Curcuma	0.2948996	26	15	0.01394713	21.1441053
Zingiber	Globba	0.00834586	26	30	0.01152582	0.72410063

Zingiber	Hedychium	0.01010855	26	20	0.01279389	0.79010809
Aframomum	Boesenbergia	0.23482778	26	30	0.01152582	20.3740571
Aframomum	Curcuma	0.31837727	26	15	0.01394713	22.8274391
Aframomum	Globba	0.01513182	26	30	0.01152582	1.31286242
Aframomum	Hedychium	0.01336912	26	20	0.01279389	1.04496184
Boesenbergia	Curcuma	0.08354949	30	15	0.0136027	6.14212393
Boesenbergia	Globba	0.21969596	30	30	0.01110656	19.7807366
Boesenbergia	Hedychium	0.22145866	30	20	0.01241751	17.8343812
Curcuma	Globba	0.30324545	15	30	0.0136027	22.2930277
Curcuma	Hedychium	0.30500815	15	20	0.0146926	20.7593049
Globba	Hedychium	0.0017627	30	20	0.01241751	0.14195252

Table 77. Tukey-test results of all genera pseudo-petiole-leaf ratio.

Width-length ratio.

SUMMARY

Group	Count	Sum	Average	Variance
Zingiber	26	5.225313116	0.200973581	0.005617549
Aframomum	25	4.964736162	0.198589446	0.004455467
Boesenbergia	30	9.496751666	0.316558389	0.048548347
Curcuma	15	2.409233489	0.160615566	0.005715997
Globba	30	7.938094799	0.26460316	0.009654564
Hedychium	20	3.776268307	0.188813415	0.006708995

Table 78. All genera leaf width-length ratio summary.

ANOVA

Source of Variance	SS	df	MS	F	P-value	F crit
Between groups	0.413039538	5	0.082607908	5.397321839	0.000143265	2.278868816
Within groups	2.142749203	140	0.015305351			
Total	2.555788741	145				

Table 79. All genera leaf width-length ratio one-way ANOVA results.

Tukey-test	Alpha=0.05	15 levels	df=140	Q=4.898		
		Difference	n (Group 1)	n (Group 2)	SE	q
Zingiber	Aframomum	0.00238413	26	25	0.03465374	0.06879879
Zingiber	Boesenbergia	0.11558481	26	30	0.03314884	3.48684301
Zingiber	Curcuma	0.04035802	26	15	0.04011264	1.0061171
Zingiber	Globba	0.06362958	26	30	0.03314884	1.91951137

Zingiber	Hedychium	0.01216017	26	20	0.03679585	0.33047652
Aframomum	Boesenbergia	0.11796894	25	30	0.03350213	3.52123756
Aframomum	Curcuma	0.03797388	25	15	0.04040508	0.93982928
Aframomum	Globba	0.00977603	25	30	0.03350213	0.29180331
Aframomum	Hedychium	0.00977603	25	20	0.03711444	0.26340237
Boesenbergia	Curcuma	0.15594282	30	15	0.03912205	3.9860592
Boesenbergia	Globba	0.05195523	30	30	0.03194302	1.62649692
Boesenbergia	Hedychium	0.12774497	30	20	0.03571339	3.5769494
Curcuma	Globba	0.10398759	15	30	0.03912205	2.65803003
Curcuma	Hedychium	0.02819785	15	20	0.04225665	0.66729972
Globba	Hedychium	0.07578974	30	20	0.03571339	2.12216633

Table 80. Tukey-test results of all genera leaf width-length ratio.

Appendix V.

The sequence accession number for each species.

Species	Sequence accession number
T. flaggelaris	AF478797
A. leptolepis	FJ848585
A. longiligulatum	FJ848580
A. angustifolium	FJ848587
A. chrysanthum	
A. daniellii	AF478705
B. cordata	AJ388277
B. basispicata	AY424743
B. aurantiaca	AF202409
B. orbiculata	AJ388278
B. flavorubra	AY296726
C. aeruginosa	DQ395332
C. larsenii	
C. harmandii	AY424754
C. parviflora	AY424755
C. vamana	JQ409867
G. albiflora Ridl.	AY339693
G. atrosanguinea	AF478753
G. brachyanthera	AB097235
G. marantina	KX065412
G. pendula	AY339678
H. densiflorum	AF202402
H. coronarium	MF076969
H. coccineum	KX065421
H. ellipticum	KX065423
H. greenii	AF478759
Z. bradleyanum	DQ064579
Z. officinale	KR816714
Z. zerumbet	KC582876
Z. nudicarpum	

Table 81. The sequence accession number for each species.

Appendix VI.

The commands used in R.

Part 1.

```
#call the correct library
install.packages("ape")
library(ape)

#read in genbank data
its <- read.table("GINGERS_final.csv", quote="\"", stringsAsFactors=FALSE)
as.list(its)$V1 -> itsL
its_gen<-read.GenBank(itsL,species.names=T)
names_its <- data.frame(species = attr(its_gen,"species"), accs = names(its_gen))
names(its_gen) <- attr(its_gen,"species")
write.dna(its_gen,"renamed_ITS.fasta", format="fasta")
```

Part 2.

```
library(ape)
#trfn: this is the base tree file name
trfn = "LZ_tree.tre"

#this reads in the tree
tr <- read.tree(trfn)

#draw the tree to check it is in
plot(tr, cex=0.5)

#this is a list of tips on the tree
tr$tip.label

#this roots the tree on tip 27 (which is Tamijia)
tree_rerooted = root(tr, 17)
plot(tree_rerooted, cex=0.5)

#remove Tamijia from the tree
final_tr <- drop.tip(tree_rerooted, "Tamijia_flagellaris", trim.internal=T)
plot(final_tr, cex=0.5)

write.tree(final_tr, file="final_rooted_tree.tre")
```

Part 3.

```
install.packages("phytools")
install.packages("caper")
library(phytools)
library(caper)
```

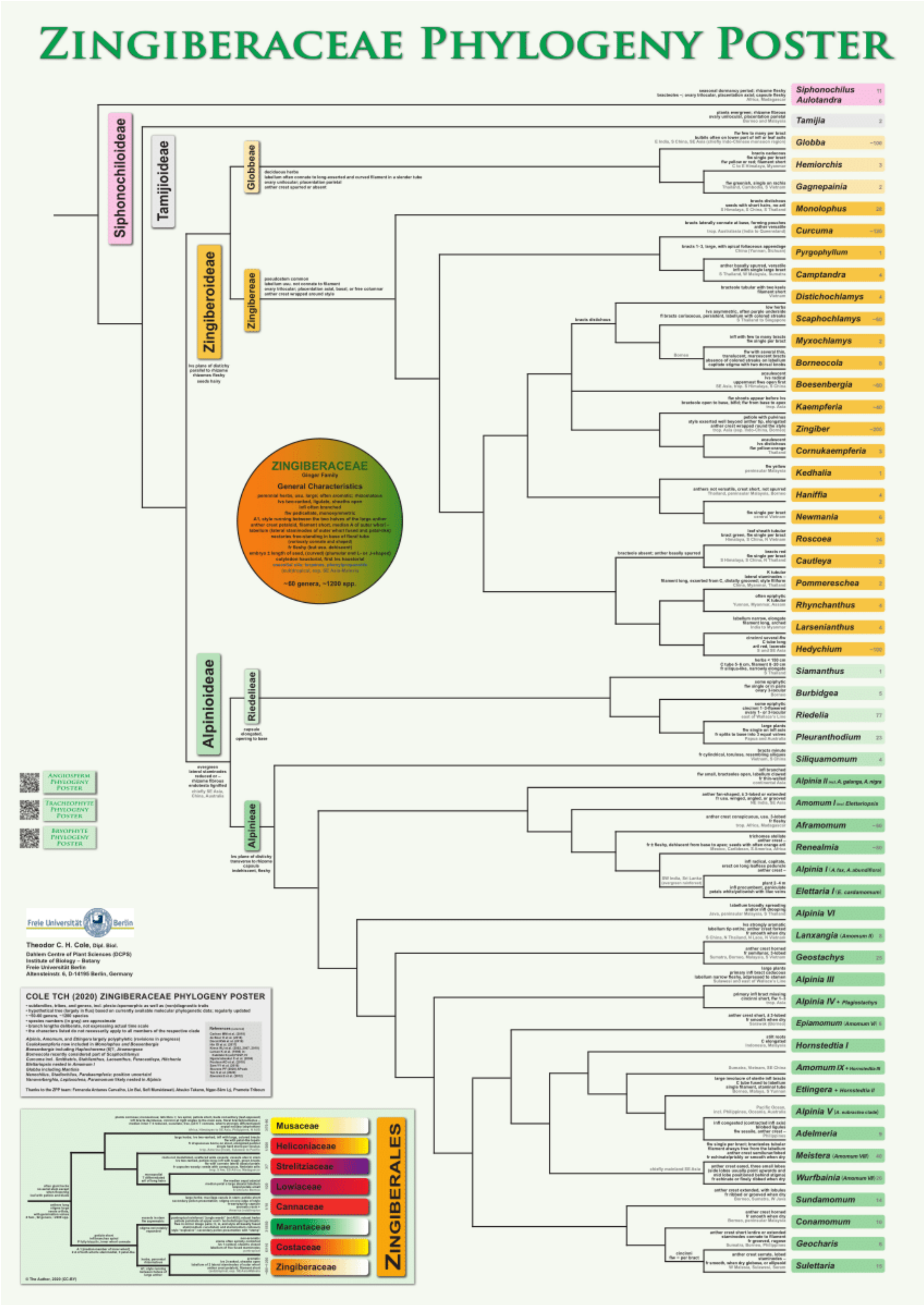
```
#this reads in your data matrix
gingerdata <- read.csv("Ginger_character_matrix.csv")
#this reads in your data one character at a time
Ligule_leaf<-as.matrix(read.csv("Ginger_character_matrix.csv", row.names=1))[,1]
obj<-contMap(final_tr, Ligule_leaf)
#save the plot of the characters to a .pdf and .png file
pdf("Ligule_leaf.pdf")
plot(obj)
dev.off()

png("Ligule_leaf.png")
plot(obj)
dev.off()

gingerdata <- read.csv("Ginger_character_matrix.csv")
Pseudopetiole_leaf<-as.matrix(read.csv("Ginger_character_matrix.csv",
row.names=1))[,2]
obj<-contMap(final_tr, Pseudopetiole_leaf)

pdf("Pseudopetiole_leaf.pdf")
plot(obj)
dev.off()

png("Pseudopetiole_leaf.png")
plot(obj)
dev.off()

gingerdata <- read.csv("Ginger_character_matrix.csv")
Width_length<-as.matrix(read.csv("Ginger_character_matrix.csv", row.names=1))[,3]
obj<-contMap(final_tr,Width_length)

pdf("Width_length.pdf")
plot(obj)
dev.off()

png("Width_length.png")
plot(obj)
dev.off()
```

Part 4.

```
phylosig(final_tr, Ligule_leaf, method="K", test=T)
phylosig(final_tr, Pseudopetiole_leaf, method="K", test=T)
phylosig(final_tr, Width_length, method="K", test=T)
```

Appendix VII.

Zingiberaceae Phylogeny poster.



Resource from:

https://www.researchgate.net/publication/314205060_Zingiberaceae_Phylogeny_Poster_ZPP_2020

Cole, Theodor, 2020. Zingiberaceae Phylogeny Poster (ZPP), 2020