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Diversity and Epidermal Characteristic of Lauraceae Leaf in Two Forest Locations, Bogor Regency, West Java

Nurul Amalia Fadhila*[1], Sulistijorini[2], Nina Ratna Djuita[3]

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[1,2,3]Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, West Java, Indonesia, 16680

e-mail:
[1*]amalia.mhs.ipb[19]amalia@apps.ipb.ac.id
[2]sulistijorini@gmail.com
[3]nrdjuita@gmail.com

*Corresponding author

Abstract. *Lauraceae is a group of tropical plants with a wide distribution. The abundance and species richness of the Lauraceae family includes the under to the upper canopy of the forest. The Lauraceae family can be utilized in various sectors, especially economically. Limiting characters as a significance in the Lauraceae classification was needed to provide additional information regarding taxon grouping. This research aimed to study epidermal characteristics, leaf venation variations, and the diversity of Lauraceae species under different forest environmental conditions. The research procedure involved: collecting ecological field data and morphological identification using a purposive sampling method based on the presence of species. Soil sampling and environmental microclimate were measured on each plot. The anatomical process was carried out by recording characters. Correlation between environmental factors and plant targets used canonical correspondence analysis. The kinship between members of Lauraceae was expressed in a dendrogram. The result of this study showed that Lauraceae could live at various elevations. The analysis data indicated differences in environmental factors that affect the existence of Lauraceae species. Leaf venation studies support clustering in the family using characters such as primary, secondary, tertiary veins, and areola. Observation of leaf venation can be used as a taxonomic significance in the family Lauraceae. Epidermal cell walls in leaf samples are straight, curved shallow, deep, and sharp. Other characters that can be found are anomocytic, laterocytic, and brachyparasitic types of stomata, with stellate and glandless trichomes The anatomical dendrogram separated Lauraceae into clusters on a similarity coefficient scale.*

Keywords: *Lauracaeae, paradermal character, species diversity, species kinship*

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INTRODUCTION

Lauraceae is a plant group that has a wide distribution in the Malesia region, to date 50 genera with 2500-3000 species have been found (Steenis, 1951). According to Hara et al. (2002), the abundance and richness of Lauraceae species can be found in the upper canopy to the lower canopy layer. Lauraceae stature is dominated by trees, shrubs, and some herbs. Members of this family can thrive at various elevations, both highlands and lowlands. The elevation is one of the most important variables to predict the presence or absence of a species from the family Lauraceae (Ngeryuang et al., 2003). Telaga Warna Nature Reserve (TWNR) is represented one of the highland forest areas that store a wealth of Lauraceae species (BBKSDA, 2016). Pierlot (1966) suggests that upland forests have the potential for higher sunlight availability. These conditions will be advantageous for some families.

Economically, Lauraceae are widely used for various purposes, such as building materials, food ingredients, cooking spices, and medicines. According to Hwang et al. (2017), the use of Lauracae as a building material has been applied since ancient times in China as coffins for the genus Phoebe. Meanwhile, the Japanese have used *Cinnamomum cahmpora* wood as the primary material for making statues. Therefore, Lauraceae has played an important role in historical and economic development. One type of member of the Lauraceae family that is commonly used in medicine is *L. cubeba* which contains essential oils rich in monoterpenes and hydrocarbon bioactivity, such as antioxidants and antimicrobial activity (Sivamaruthi et al., 2022).

Meanwhile, the Arboretum of the Environmental and Forestry Education and Training Center Rumpin District Bogor is located in a lowland forest area with a gentle slope and directly adjacent to a river bank (BDLHK, 2020). These different ecological conditions will provide an overview of species distribution in each habitat, especially the Lauraceae family. According to Whitmore (1984), the spatial distribution of plants can be influenced by environmental factors such as varying light intensity, temperature, and humidity. In addition, the soil's physical and chemical characteristics also a certain plant growth properties. Erfanzade & Alemzade (2011) explained that soil factors directly affect the survival and growth of plant species. The variation in environmental factors is thought to affect the morphology and distribution of the family Lauraceae.

The stem, leaf, fruit, and flower morphology can be used for taxon characterization. Taxon validation in Lauraceae is rarely known because identification requires key characters such as flowers, while the flowering phenology of plants is different. Villiers et al. (2010) explained that the structure and arrangement of leaves obtained from exploration could be related to species boundaries. The adaxial part of the Lauraceae leaves is green with light green hair's underside (abaxial). The marginal line varies from 1-60 cm. The leaves are oval to lanceolate. In some species of specific genera, the marginal venation pattern is between the primary venation (Kubitzki, 1993). Leaf venation traits and leaf size can be used as the main characteristics of the taxon (Beerling & Franks, 2010).

However, another limiting character is needed in the Lauraceae classification to provide additional information regarding plant grouping. Taxonomically, epidermal characters are efficient as descriptors. The anatomical character of the leaves will provide a strong basis for grouping plants by performing semi-permanent preparations using paradermal section, this can provide additional support for morphological characters (Rugayah, 2002; Hafiz et al., 2016).

Research related to the relationship between leaf characteristics and taxonomic studies, and plant ecology is very important. The study aimed to examine the epidermal characteristics, leaf venation variations, and the diversity of Lauraceae species under different forest environmental conditions. Data from this study are expected to be used by conservation actors and taxonomists in conducting evaluations to maintain the sustainability of Lauraceae in the future.

MATERIALS AND METHODS

Sampling of Specimens

The study was carried out using an exploratory method from April to July 2022, where plant samples were collected randomly from five different sites on Java Island. The respective altitudes and coordinates of the sites can be seen in Table 1.

The research procedures included: field data collection and morphological identification by adapting to Harris & Harris (2006) and Ash et al. (1999). Sampling was done at the Telaga Warna Nature Reserve (TWNR) and the Arboretum of the Environmental and Forestry Education and Training Center in Rumpin District, Bogor Regency. TWNR has an area of 368.25 Ha with a topography of mountainous and hilly regions, with a 20º-80º elevation slope of 1.400 -1.800 (masl), while the Arboretum in Rumpin district has sloping land contours with a slope of 0º-24º which is directly adjacent to cliffs and river cliffs with an altitude of 80-100 (masl). Sampling was carried out by purposive sampling at both research sites, with observation plots measuring 2 m × 2 m seedling phase, 5 m × 5 m sapling phase, 10 m × 10 m pole phase, and 20 m × 20 m tree phase based on the presence of Lauraceae. The Telaga Warna Nature Reserve plots used a range of elevations (1400, 1500, 1600, and 1700 masl), with five repetitions at each altitude. Data retrieval at the Arboretum of the Rumpin District Education and Training Center has been carried out three times without differences (99-100) masl in altitude stations.

Leaf Incision Preparation Section

The research procedure aimed at observing paradermal characteristics was adapted from Lestari (2005). The leaves were fixed in 70% alcohol for at least 24 hours. Then the fixative solution was removed and replaced with distilled water. Furthermore, the leaves were soaked in 50% HNO3 solution for 30-60 minutes to soften the mesophyll tissue. The leaves were washed using distilled water, then cut into small pieces with a size of 2 x 2 cm. To remove chlorophyll from mesophyll, the epidermal sections were immersed in sodium hypochlorite solution for 1-5 minutes and then washed with distilled water. The incision was then soaked with 0.25% safranin dye for the abaxial section and 0.1% methylene blue for the adaxial section for 1 minute. The preparation in the form of an epidermal layer was placed on an object glass, then dropped with 10% glycerin and covered with a cover glass. The ImageJ application assists measurement of the length and width of the epidermis. Quantitative and qualitative data were converted into numerical scoring. The kinship relationship between members of Lauraceae is expressed in a dendrogram using the NTSys program v.2.20 with *Unweighted Pair Group Method with Arithmetic Mean* (UPGMA).

Leaf Venation Study

The method used to observe the leaf venation of Lauraceae using the stain technology method was adapted from Fuchs (1963). The sample was put into a solution of concentrated ethanol and HCl in a ratio of 3:1 within 15 minutes until the leaves turned grayish-yellow. Dehydration with graded alcohol with percentages of 50%, 70%, and 95% for 3-5 days. Next, the sample was dried slowly and then placed in a fuchsin solution for 12 hours at a temperature of 60°C. The Fuchsin solution was prepared by mixing 400 mL of distilled water with 0.25 g of fuchsin in boiling water without being filtered. Then 2.5 g of solid NaOH was added after the solution had cooled. The leaf samples that have turned transparent were then observed for the leaf veins, such as tertiary bone type, areola type, areola veins, and areola development.

RESULTS AND DISCUSSION

Lauraceae Morphological Character

At the study site, 11 species of members of the Lauraceae family originated from two different locations (TWNR Bogor Regency and the Arboretum of the Environmental and Forestry Education and Training Center Rumpin District Bogor). There are one species in common at both locations, namely *Cinnamomun iners*. The species found in the Nature Reserve were *Cinnamomum iners, Cryptocarya densiflora, Cryptocarya ferrea, Litsea subumbelliflora, Lindera aggregata, Litsea cubeba, Litsea mappacea, Machilus rimosa* and *Neolitsea javanica*. While the species in the Arboretum *Persea americana* var. *Miki, Cinnamomum sintoc* and *Cinnamomum iners* were intentionally planted. Differences in morphological characteristics can be seen in the leaves, fruit, and stems. Spiral leaf buds can also be a diagnostic feature of members of the Lauraceae of the genus *Neolitsea*. The *Cinnamon* group in the genus *Cinnamomum* has fruit with hypanthium, and this character is not found in other Lauraceae fruit. This study has resulted in detailed observations of leaf venation on leaf samples of the *Lauraceae* group in the form of marginal venation on leaf areolations (Figure 1). According to Fang et al. (2002), leaf vein pattern is an essential feature for classification because character orientation is relatively stable at the species level. The research data showed that *C. iners* found in both locations did not have different types of leaf venation.

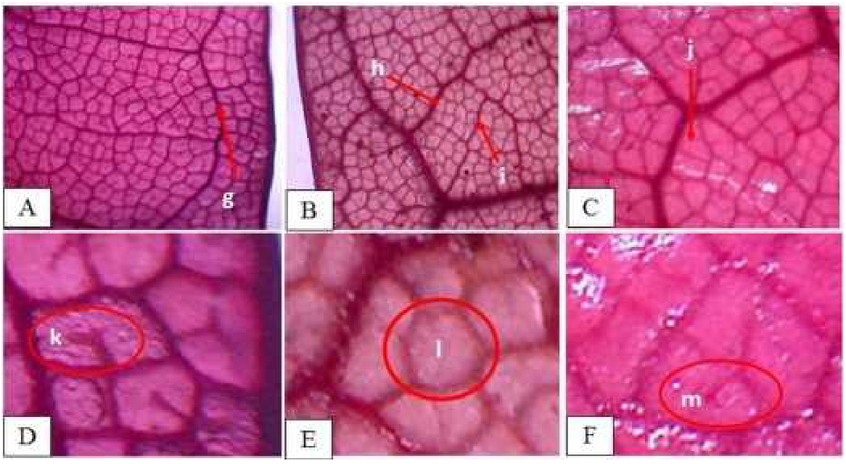

Figure 1.Leaf venation patterns of three species of the family *Lauraceae*. A. *M. rimosa*, B. *C. ferrea*, C. *C. densiflora*. D. areolation *M. rimosa*. E. areolation *C. ferrea*. F. areolation *C. densiflora*; (g) marginal venation, (h) secondary venation, (i) tertiary venation, (j) areolation, (k) areola 2- branch, (l) areola no branch, (m) areola 1-branch

Analysis of Distribution and Components Affecting Diversity

The quantitative data obtained were then analyzed to find the important value index (IVI) referred to by Mueller & Ellenberg (1974), the evenness index (E), the species richness index (R), and the Shannon-Wiener species diversity index (H'). The correlation between environmental factors (micro) and target plants was analyzed Canonical correspondence analysis (CCA) using the CANOCO v.4.5 application. Based on field observations, Lauraceae stature is a tree with a vertical growth direction and sympodial branches with oval to round crowns. Some species, such as *Machilus rimosa* can coexist with pioneer plants in shaded environments, such as epiphytic ferns.

The results of field observations showed that members of the Lauraceae family were spread over all altitude ranges. The dominance of the tree phase in TWNR is presented in Table 1. At an altitude of 1600-17000 masl, N. javanica trees are common and dominate the community with IVI of 82.51% and 40.41%, respectively. *N. javanica* is an endemic species found in Java and Sumatra. At elevations below 1400 and 1500 masl, no tree phase of *N. Javanica* was found. It is presumably due to lower soil nutrient content such as N/P at both elevations. However, this species is a type that can quickly adapt to any altitude**.** *N. javanica* was also found on slope conditions of 30º-40º.

Cryptocarya ferrea species can be found at almost all altitudes ranging from 1400-1700 masl (Table 1). Based on De Kok (2015), the distribution of Cryptocarya is generally in peninsular Malaysia and other areas in the Sunda Highlands. Meanwhile, another study by Backer & Van Den Brink (1965) revealed that the natural habitat distribution of *C. ferrea* on the island of Java is at an altitude of 90-1500 masl. The results showed that the presence of *C. ferrea* dominated at an altitude of 1400 and 1500 masl with an IVI value of 37.45% and 19.68%, respectively. The tree phase is mainly found in the lower mountain forest. The distribution pattern can be related to habitat suitability and growth characteristics of *C. ferrea*. The high value of IVI for a species is caused by its high density and frequency throughout the forest area. The difference in values indicates that these species play an essential role in the composition of the stand during a specific period and maintain the ecological balance of the forest. The composition of trees from the Lauraceae, Fagaceae, and Myrtaceae families that dominate forest vegetation is one of the characteristics of montane rainforest (Culmsee et al., 2011).

Table 1. Density, frequency, and important value index of Lauraceae trees in the Telaga Warna nature reserve, Bogor regency

Altitude (masl)	**Species**	**n**	**RD (%)**	**RF (%)**	**IVI (%)**
1400	*Cryptocarya densiflora* Blume	2	4.17	7.14	21.44
	Cryptocarya ferrea Blume	2	8.33	7.14	37.45
	Litsea mappacea Boerl.	1	8.33	7.14	28.35
1500	*Cryptocarya ferrea* Blume	1	4.17	7.14	19.68
	Litsea mappacea Boerl.	1	4.17	7.14	18.09
1600	*Machilus rimosa* Blume	11	4.17	7.14	21.26
	Neolitsea javanica (Blume) Backer	1	45.83	21.43	82.51
1700	*Cryptocarya ferrea* Blume	3	4.17	7.14	14.53
	Lindera aggregata (Sims) Kosterm.	1	4.17	7.14	16.27
	Neolitsea javanica (Blume) Backer	1	12.50	21.43	40.41

Remarks: n=number of individuals; RD= relative density; RF= relative frequency; IVI= important value index

Similar studies were also conducted at the Arboretum of the Environmental and Forestry Education and Training Center Rumpin District Bogor (Table 2). The calculations that have been carried out for all growth phases show that IVI *C. iners* is at a maximum value of 300% in the pole phase. This case occurred because *C. iners* dominated the entire research plot, and no other species were found. According to Soh (2011), *C. iners* can be naturalized and is often found on roadsides, open areas to secondary forests. *C. iners* can thrive in the arboretum forest area with a soil pH of 6 and an altitude of 99 masl. Other species deliberately planted in the arboretum area are *P. americana* and *C. sintoc*. The data obtained show that the regeneration phase, such as seedlings and saplings, can still be found even though the area has minimal shade. The composition of artificial forest vegetation is different from natural forest vegetation. Forest stands are intentionally made with certain species.

Table 2. Density, frequency, and important value index of Lauraceae in Arboretum of the Environmental and Forestry Education and Training Center Rumpin District Bogor

Growth phase	Species	N	RD (%)	RF (%)	IVI (%)
Seedling	*Cinnamomum iners* Blume	2	28.57	33.33	61.90
	Persea americana Mill.	5	71.43	66.67	138.10
Sapling	*Cinnamomum sintoc* Blume	1	10	33.33	43.33
	Persea americana Mill.	9	90	66.67	156.67
Pole	*Cinnamomum iners* Blume	5	100	100	300
Tree	*Cinnamomum iners* Blume	15	93.75	49.25	175.28
	Persea americana Mill.	1	6.25	49.25	123.17

Remarks: n=number of individuals; RD= relative density; RF= relative frequency; IVI= important value index

The results of the CCA analysis showed that Lauraceae member species in TWNR were affected by microclimatic conditions (Figure 2A). The relationship between species and their abiotic environment has a different effect on each species, especially those with the highest important value index (Aini et al., 2022). Variables affecting growth and abundance were analyzed based on the presence of altitude, wind speed, humidity, and elements such as N, P, and the N/P ratio have opposite vector directions, which have negative values so that they do not affect the distribution of species such as *C. ferrea, L. mappacea* and *C. densiflora*. Meanwhile, the distribution of *N. javanica* is influenced by N/P (Figure 2A). The comparison concentration of N and P can be caused by the composition of plant species, decomposition of organic matter, and availability of groundwater so that nutrients such as N and P can be used as the main limiting elements in an area (Reich & Oleksyn, 2004; Jing et al., 2015). At an altitude of 1600 masl, *N. javanica* and *M. rimosa* can grow well with a humidity of 67.7%. The influence of magnesium (Mg), calcium (Ca), and potassium (K) as soil macronutrients plays a role in the distribution of species at an altitude of 1400-1500 masl, such as *C. densiflora* and *L. mappacea*.

The distribution of *P. americana* var. Miki in Arboretum is influenced by environmental variables such as temperature and soil pH, as evidenced by the vector line in the direction of the circle symbol indicating the species (Figure 2B). The CCA results that affected the growth and distribution of *C. iners* were humidity and altitude (Figure 2B). The analysis results can be seen by paying attention to the vector lines still in one quadrant with the ordinance graph. *C. iners* can grow at 56.1% humidity. Differences in tree distribution patterns were shown by each species, *P. americana* and *C. iners*. The average soil temperature and pH were also environmental factors that influenced the growth of P. americana, with values of pH 6.5, respectively. Figure 2B shows that *P. americana* and *C. iners* are not affected by the intensity of sunlight in their survival. This difference is presumably because the intensity of sunlight received in each plot differs. Wolstenholme & Whiley (1999) concluded that the two primary keys to the productivity of *P. americana* are better overall canopy light interception and good root health in environments where stress is reduced to a minimum.

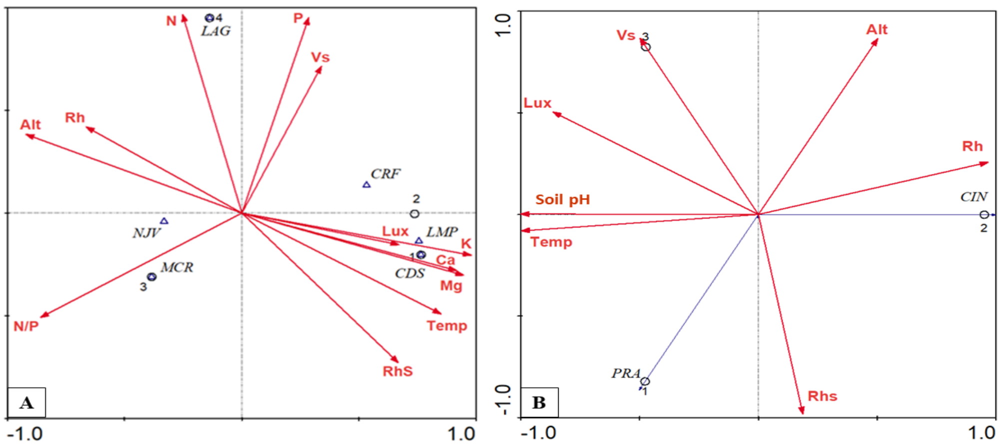

Figure 2. A. Ordination graph (CCA) of Lauraceae for each phase of the target species tree along with environmental factors in TWNR. B. Ordination graph (CCA) of Lauraceae target species in tree phase in Arboretum and environmental factors. Lux= light intensity (Cd), RhS= Soil moisture (Rh), Mg = Magnesium, Temp = Temperature (ºC), K = Potassium, Ca = Calsium, P = Phosphor, Vs = Wind velocity (m/s), N = Nitrogen, Rh = Humidity (Rh), Alt = Altitude (masl), N/P= Nitrogen and Phosphorus ratio, NJ = *N. javanica*, CF = *C. ferrea*, LA = *L. aggregata*, MR = *M. rimosa*, CD = *C. densiflora* , LM = *L. mappacea*, CI = *C. iners*, PA = *P. americana*

The distribution of Lauraceae species in Bogor Regency, especially in TWNR, represents a natural habitat, while in the Rumpin District, Arboretum represents an artificial habitat. This condition can be related to the annual macro climate. The relationship between variables that influence each other is depicted in Figure 3. Field sampling was carried out in September-October 2020. The average wind speed for the two years 2019-2020 fluctuated, in September the wind speed was 0.4 m/s² with a decrease in speed in October of 0.2 m/s². Meanwhile, the average air temperature around the study site was 22℃. Purwantara (2015) described several factors affecting temperature changes, including the location's altitude. The higher the place, the lower the ambient air temperature

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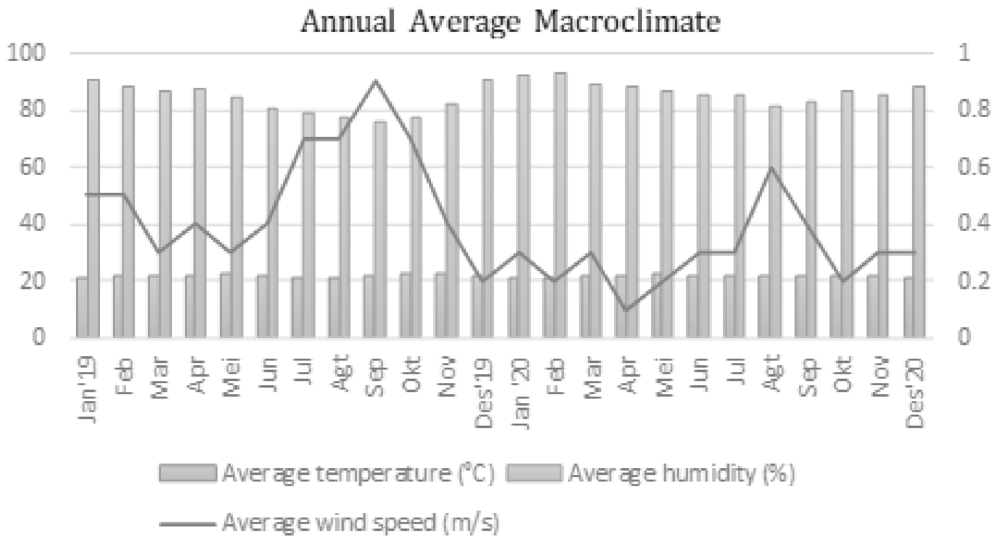

Figure 3. Annual Average Macroclimate in Bogor Distric 2019-2020. Source : Meteorological, Climatological, and Geophysical Agency Citeko.

Research carried out at both locations gave results in the form of variations in the shape and indentation of the epidermal cell walls on the lower surface of the 8 leaf samples (Figure 4). The adaxial epidermis is the upper leaf's outermost part, which protects the underlying leaf tissue from environmental stresses (Tobing et al., 2022). The shape of epidermal cells is divided into two types, 4-5 sides and irregular. *C. iners* arboretum and *C. densiflora* had similar epidermal cell shapes, 4-5 sides, while the epidermal cell shapes of *L. aggregata* and *C. iners* TWNR were irregular (Figure 4). Based on the results of anatomical observations of Lauraceae leaves, 4 types of indentation of the epidermal cell wall, namely straight, shallow grooves, deep grooves, and sharp curves. This observation is almost similar to the classification of cell wall types proposed by Sunarti (2008) that the basic pattern of cell walls is of four types: straight, shallow grooves, straight grooves, and deep grooves. The results showed that the straight and shallow, grooved cell wall types were most commonly found in several species, including *L. cubeba, P. americana* and *L. mappacea*, which had straight cell walls. Meanwhile, the cell wall type with shallow grooves can be observed in the leaf slices of *C. densiflora, C. ferrea* and *C. iners* arboretum. The cell wall types with deep grooves and sharp grooves were not found in the sample, but they can be observed in Figure 4. Cell walls with deep grooves can be seen in the *L. aggregata* incision sample. Sharply curved cell wall types can be found in the *C. iners* TWNR incision.

In the observation, *C. iners* arboretum and *C. iners* TWNR are still one species but have different cell types (Figure 4A; Figure 4H). This phenomenon is thought to be due to the difference in height at the two locations so that the light intensity received can also affect the anatomical structure of the leaves. According to Pereira et al. (2009), the difference in the shape of the periclinal cell wall is caused by the influence of light distribution on the leaves due to differences in geographical areas with contrasting light environments. The amount of light received can cause variations in growth physiologically, morphologically, and anatomically.

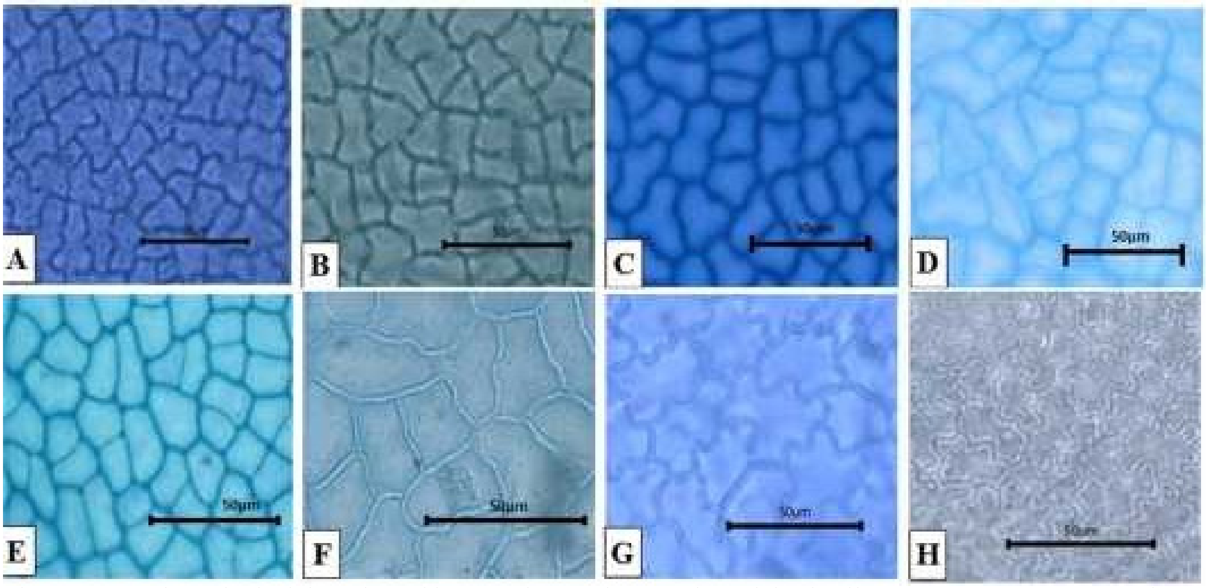

Figure 4. Paradermal adaxial section of leaves of 8 species member of the family Lauraceae. A. *C. iners* arboretum, B. *C. densiflora*, C. *C. ferrea*, D. *L. cubeba*, E. *P. americana*, F. *L. mappacea*, G. *L. aggregata*, H. *C. iners* TWNR

Anatomical relationships (Figure 5.) of 12 leaf samples from two forest sites were analyzed using 10 characters of leaf anatomical characteristics. The result showed that 12 leaf samples are arranged to form 4 clusters with A and B that are separated based on the type of primary venation. *C. densiflora, C. ferrea*, and *C. iners* arboretum were clustered I with a scale of 54%. The similarity can be seen in the shape of the epidermal cells, the type of epidermal cell wall, the length of epidermis, the width of the epidermis, the shape of the trichomes, the length of the stomata and the width of the stomata (Table 3). *C. ferrea* and *C. iners* arboretum were more closely related, with a similarity scale of 92%. Meanwhile, *C. densiflora* has different characteristics of trichome length, stomata index, and stomatal density, so this species is separated into clusters

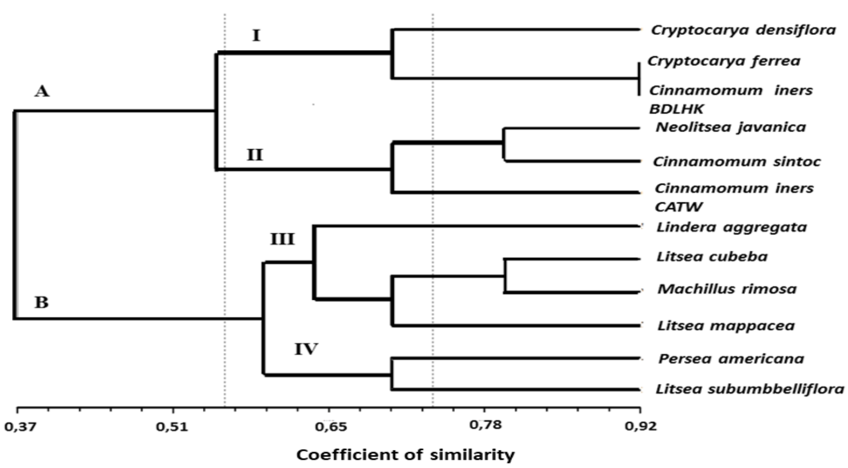

Figure 5. Dendrogram of 12 samples of Lauraceae leaves using anatomical data using the UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*) method at 2 forest locations.

Table 3. The characteristics and character traits of the anatomical observations of Lauraceae used in making the dendrogram

Characteristic	Character traits	Score
Epidermal cell shape	4-5 sides	0
	Irregular	1
Epidermal cell wall	Straight	0
	Shallow grooves	1
	Deep grooves	2
	Sharp curves	3
Epidermal length (μm)	21.4 – 30	0
	30.1 – 38.6	1
Epidermal width (μm)	9.3 – 16.1	0
	16.2 – 22.9	1
Trichome shape	There is 1 type of trichome (no gland)	0
	There are 2 types of trichomes (non- glandular and star)	1
Stomata type	Laterocytic	0
	Brachyparasitic	1
	Anomocytic	2
	Staurocytic	3
	Paracytic	4
	Amfipericytic	5
Stomata length (μm)	14.9 – 19.2	0
	19.3 – 23.4	1
Stomata width (μm)	10.5 – 18.3	0
	18.4 – 26.1	1
Stomata index	6.5 – 15.2	0
	15.3 – 23.9	1
Stomata density (mm-^{2})	147.6 – 542.9	0
	543 – 938.1	1

Cluster II is closely related because it has a similarity coefficient of 71%. *N. javanica* and *C. sintoc* were grouped because they had almost all of the characters in joint except for the type of epidermal cell wall and stomatal density. The type of stomata of the two species is also the same: anomocytic. It can be used as the basis for grouping species by involving the character of their stomata. The dendrogram in Figure 5. also grouped *L. aggregata* and *M. rimosa* in cluster III at 80%. This grouping is based on the stomata type and the trichomes shape. *P. americana* and *L. subumbelliflora* in cluster IV have a similarity level of 71%. These two types have six similarities out of ten leaf anatomical characters. The grouping of leaf samples of members of the Lauraceae family using the UPGMA dendrogram method can be seen well when analyzing the anatomical characters from qualitative and quantitative aspects. Leaf epidermal anatomy was an essential tool for identifying dicot species, and the environment also significantly influences stomata development (Khan et al., 2014). Evidence from commonly used approaches, such as the shape and density of stomata, trichomes, and epidermal cells can be used as taxonomic characteristics because they are relatively constant (Olaniran & Olamide, 2014).

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CONCLUSION

The species belonging to the Lauraceae family originated from the Telaga Warna Nature Reserve (TWNR)-Bogor and the Arboretum of the Forestry Environment and Training Center of Rumpin District, Bogor have varied leaf paradermal characters. Observation of leaf venation can be used as a taxonomic significance in the family Lauraceae. Leaf veins are relatively stable at the species level. The Lauraceae group is spread over the entire altitude range in the TWNR and is dominated by *N. javanica*. Meanwhile, *C. iners* dominated the Lauraceae species found in the arboretum. This species was also found in both locations, and its distribution was influenced by humidity and altitude. Further research is needed to complete the anatomical data in the form of leaf transverse section at both forest locations.

AUTHOR CONTRIBUTION

N.A.F. conceptualized the research and conducted the fieldwork under direction from S. and N.R.D.

N.A.F. also analyzed the sample, data processing and wrote the manuscript with revision and editing assistance from S. and N.R.D.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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