Phenetic kinship of eight ferns from Filicinae class based on morphological and anatomical characteristic

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Abstract. Filicinae is one of the Pteridophyta class with the most species. Thus, the different classification may occur for the same type. Consequently, it is necessary to simplify the classification; one of them is through phenetic kinship analysis. This study aims to identify phenetic kinship of eight ferns from filicinae class based on morphological and anatomical characteristics. The study applied descriptive and survey methods with a qualitative approach. Data was collected in August 2018 and analyzed using an Analysis Cluster method which is presented in the form of a phenogram. The finding suggests that 7 clusters are formed from 8 species of ferns from filicinae class. The closest kinship is shown in the species of *Nephrolepis cordifolia* (L.) Schott and *Nephrolepis biserrata* (Sw.) Schott which indicates that both species with the smallest taxonomic distance value have similar morphological and anatomical characters; while the furthest kinship is the species of *Nephrolepis cordifolia* (L.) Schott, *Nephrolepis biserrata* (Sw.) Schott., *Davalia denticulata* (Burm. F) ex. Kuhn., *Pteris vitata* L., *Pyrrosia piloseloides* (L.) M.G. Price., and *Drynaria bonni* Crist., *Adiantum caudatum* L., *Cryptinus enervis* (Cav.) Copel.

1. Introduction
Ferns have unique characteristics in sporophyte and gametophyte generation which is mutually independent. One of the ferns’ unique characteristics lies in the morphological character of the sporophyte, which is used as a determinant of ferns in terms of shape, size, location, and spores types [1]. Ferns play important roles in maintaining the forest ecosystem [2]; therefore, it is required to improve conservation and inventory of filicinae kinship to prevent biodiversity extinction. Kinship is a pattern of relationship or total similarity between groups based on certain characteristics from each group. A kinship approach used to determine a close relationship between two groups of plants is phenetic kinship. The phenetic approach offers scientific and explicit procedures in the classification. Moreover, the approach emphasizes more in taxonomic characteristic knowledge, types of characters that will be used, numbers of characters, the difference in one character to another, and the importance of examining as many characters as possible that have positive phonetic results [3].

The phenetic approach is still debating among taxonomists proven by the difference in the classification of different organisms for the same type. The difference of opinion occurs because the approach used is subjective. To reduce the subjectivity in the grouping process, it is necessary to conduct analysis regarding the selection of as many characters as possible, make description and measurement of states of characters as precisely as possible, and compare the state of individual characters using rigidly defined numerical procedure [4]. Rideng [5] argued that qualitative phenetic
kinship is determined by comparing the similarities and differences in the characteristics of each taxon by using several characteristic similarities in morphology, anatomy, palynology, cytology, chemistry, developmental biology, ecology, and physiology [4,8]. The greater similarities between living things, the closer the kinship will be, or vice versa. Since there are a lot of ferns in Indonesia; thus, it is necessary to simplify the object of study so that the classification of ferns (Pteridophyta) will be easier. In addition, many studies on kinship have been carried out [6,7,8]; however, only a few studies on fern kinship have been conducted [9].

2. Method and Research Design
The study applied the descriptive method. Descriptive method is a problem-solving procedure investigated by describing a state of subject or object in a research based on the facts that arise. The study applied a qualitative approach that uses analysis, refers to data and uses existing theories as supporting materials. The study was conducted in Banda Aceh and Aceh Besar from March to August in 2018. The Parameters used in the study were morphological characteristics of habitat, stems, roots, leaves, sorus, and anatomical characteristic of stems, roots, leaves, and stomata. The steps of data collection were:

- Preparation step, finding out references regarding species included to filicinae class, whether written (article, journal, or others) or pictures; designing observation table (table of Operational Taxonomy Unit) which is a modification of Nurhayati [9]; coordinating with laboratory staff regarding the availability of tools, materials, and schedules needed for research, preparing tools and materials for use in the field; conducting preliminary survey regarding the location of data collection; and finding out fern species from filicinae class that have intact parts and sorus.

- Implementation Step: sampling was conducted through finding out fern plants from filicinae class from various resources such as private collections used as ornamental plants or those found living freely on the roadside or housing areas around Banda Aceh and Aceh Besar. Then the samples were photographed and given comparisons as documentation. Furthermore, data collection was carried out through matching 100 characteristics in the Operational Taxonomy Unit: 1) The description of Operational Taxonomy Unit feature can be described as number 0 if the feature does not have number 1 as well as if the observed characteristics are in that type; 2) The identification of sample was conducted in Biology Education Laboratory, Teacher Training and Education Faculty, Syiah Kuala University; 3) Analysis of sample data that has been identified in accordance with Operational Taxonomy Unit was then analyzed using Taxonomic Distance formula to identify the kinship which will be presented in the form of phenogram.

The data obtained were analyzed descriptively by describing and interpreting the kinship of eight types of ferns from the filicinae class based on morphological and anatomical features. The next step is identifying kinship through analyzing data using Taxonomic Distance formula. Finally, the results of the analysis were presented in the form of a phenogram.

3. Results and Discussion

3.1. The Classification of Taxonomic Distance
Kinship among plant species can be conducted in various ways including Similarity Index and Dissimilarity Index, Cluster Analysis and Taxonomic Distance. The calculation of Taxonomic Distance can be used to identify the distance relationship between plant species. Taxonomic Distance is used because it is simple and easy to display using only the difference among plant organs and between the two plant species observed [3]. The result of observation on eight species of ferns from filicinae class [Drynaria bonni H. Crist H. Crist, Nephrolepis cordifolia (L.) Schott (L.) Schott, Adiantum caudatum L., Pyrrosia piloselloides (L.) M.G. Price, Nephrolepis biserrata (Sw.) Schott, Cryptinus enervis (Cav.) Copel, Pteris vitata L., and Davalia denticulata (Burm. F.) Mett. ex Kuhn]
showed varied results for each plant organ. The morphological observation of the species include habitat (the type of habitat), stem (color and fiddlehead; color, shape, and stipe characteristics; color and shape of the rachis surface), leaf (type, location, structure, shape, venation, and costa), root (rhizome and rhizoid) and sorus (location, shape, and color). On the other hand, the anatomical observation consists of stem (the shape of stem, the shape of stele and the size of the space between cells) leaf (size of space between cells), stomata (the number of neighboring cells, the number of closed-cell, and the pattern of division of neighboring cells against the closed cells) and roots (types of tissue, shape and size of cortex, the shape of the stele, amount of root hair cells, and the size of the space between cells).

The results show that eight ferns species belong to filicinae class, based on the morphological characteristics (habitat, stem, leave, root, sorus), and anatomical characteristics (stem, leave, stomata, root), indicate several similar characteristics. This opinion is in accordance with that expressed by Oktarika [10]. Moreover, the difference in the distance coefficient value of the groups is based on the morphological variation value. In addition, the influence of diversity in a type is caused by two factors, namely the environment and traits inherited by genetics. This is in line with Lovelles’ [11] opinion that the classification is based on the characters’ correlation so that two plants that have similar characteristics are considered closer kinship than two plants that have few characters in common.

3.2. The classification of Taxonomy Distance Using Cluster Analysis

Cluster Analysis is an analysis that aims to summarize data through grouping objects that have certain characters. A Cluster Analysis Method used in this study is the hierarchy method which begins through grouping data that has the smallest taxonomic distance value, namely the species of Nephrolepis cordifolia (L.) Schott. and Nephrolepis biserrata (Sw.) Schott. Both species have the closest kinship because they have the smallest taxonomic distance value (1.73). The species of Drynaria bonni. Crist. and Adiantum caudatum L., have the second closest kinship with a taxonomic distance value of 2,24, and the third closest kinship is found in the species of Crypsinus enervis (Cav.) Copel. and Davalia denticulata (Burm. F) ex. Kuhn., with taxonomic distance value of 2,25. Then the process is continued to identify other objects that have close kinship. Consequently, the combination that has the farthest kinship is found in the species of Nephrolepis cordifolia (L.) Schott, Nephrolepis biserrata (Sw.) Schott., Crypsinus enervis (Cav.) Copel., Davalia denticulata (Burm. F) ex. Kuhn., and Drynaria bonni. Crist., Adiantum caudatum L., and Pyrrosia piloseloides (L.) M.G. Price., Pteris vitata L., with taxonomic distance value of 4,36. This indicates that both species have many similar morphological and anatomical characteristics. Since the kinship sequence was found, thus the grouping forms a tree with a clear hierarchy among objects. In this grouping, the two objects that have the closest distance are first grouped. At each stage, the number of clusters will decrease by one. The result of the grouping can be presented in the phenogram form (picture 1). According to Picture 1, there are seven phenomes/clusters combination of species with each level of kinship: 1) The combination of species B and E as phenome “a”; 2) The combination of species A and C as phenome “b”; 3) The combination between F and H as phenome “c”; 4) The combination of species F, H, and A, C as phenome “d”; 5) The combination of species B, E, and F, H, A, C as phenome “e”; 6) The combination of species D and G as phenome “f”; 7) The combination of species B, E, F, H, A, C and D, G as phenome “g”

Phenome “a” is the combination of species with the closest kinship with the lowest taxonomic distance value (1.73). The pair included in the combination are the species of Nephrolepis cordifolia (L.) Schott. and Nephrolepis biserrata (Sw.) Schott. Both species have many similar characteristics such as terrestrial habitat. The fiddleheads are smooth and scaly, the rachis surface is smooth and has a blackish brown color, the stem anatomy is cylindrical, large space between stem cells and central vascular cylinder (stele) is cylindrical. The leaves are monomorphic, pinnately compound, alternate leaves, thick and fleshy leaves, visible costa, rough leaf venation, and contain a large interocular space. The neighboring cells consist of two narrow cells. The rhizoid has formed the real roots,
collenchyma tissue is found at the roots, central vascular cylinder (stele) of the root is cylindrical, and some root hair cells were found. The sorus is located under the leaf in a paired pattern and brown.

Phenome that has a close kinship is another phenome “b” with a taxonomic distance value of 2.24. The combination of species is Drynaria bonni H. Crist, and Adiantum caudatum L. Both species have terrestrial habitat. The fiddlehead is green, the surface of the rachis is smooth, the stem anatomy is cylindrical, the intercellular space is large, and the central vascular cylinder is cylindrical. The leaves are monomorphic, the leaves are located along the rachis, the leaves are flat, the leaf venation is rough, and the leaves contain large intercellular space. The stomata guard cells are kidney shaped and the neighboring cells become larger. The collenchyma tissue was found, cortex space is bigger than pith space, and some root hair cells were found. The sorus has blackish brown color.

Phenome “c” is the combination of species Cypinus enervis (Cav.) Copel., and Davalia denticulata (Burm. F.) Mett. Ex Kuhn., This phenome also includes ferns that has close kinship with taxonomic distance value of 2.25. Both species have terrestrial habitat. The fiddlehead is smooth and scaly and has blackish brown color, the rachis surface is green, and the stipe is brown. The leaves are monomorphic, the leaves are flat, the costa is visible, the leaf venation is rough and branched. The stomata guard cells are kidney shaped, the neighboring cells do not divide two equal parts, and the neighboring cells become larger. The rhizome root spreads, the sclerenchyma tissue was found, and the central vascular cylinder of the root is cylindrical. The sorus is located under the leaves.

Phenome “d” consists of four species that are Cypinus enervis (Cav.) Copel., Davalia denticulata (Burm. F.) Mett. Ex Kuhn., and Drynaria bonni H. Crist, Adiantum caudatum L., with Taxonomy Distance of 3.11. The species have terrestrial habitat. The leaves are monomorphic, the leaves are flat, the costa is visible, and the leaf venation is rough. The stomata guard cells are kidney-shaped with larger neighboring cells.

Phenome “e” has a distant kinship with a taxonomic distance value of 3.40 which consists of six species: Nephrolepis cordifolia (L.) Schott., Nephrolepis biserrata (Sw.p) Schott., and Cypinus enervis (Cav.) Copel., Davalia denticulata (Burm. F.) Mett. Ex Kuhn., Drynaria bonni H. Crist, Adiantum caudatum L. The species characteristics are terrestrial habitat, monomorphic leaves, fleshy leaves, visible costa, and rough leaf venation. Then their stomata guard cells are kidney-shaped.

Phenome “f” is a phenome with another distant kinship with a taxonomic distance value of 3.46 which consists of Pyrrosia piloseloides (L.), and Pteris vitata L species. Their characteristics are smooth scaly fiddlehead, longleaf structures, and thick leaves. The stomata guard cells are cylindrical with larger neighboring cells, the sclerenchyma tissues are found at the root, and the cortex space is bigger than pith space. The sorus is paired and located along the edge of the leaves and has thickened lines.

![Figure 1](image_url)  
**Figure 1.** Kinship of Filicinae Ferns Based on Taxonomic Distance According to Morphological and Anatomical Characteristics.

Description:
A = Drynaria bonni H. Crist, B= Nephrolepis cordifolia (L.) Schott, C= Adiantum caudatum L. D=Pyrrosia piloseloides (L.), M.G. Presl., E= Nephrolepis biserrata (Sw.) Schott., F= Cypinus enervis (Cav.) Copel., G=Pteris vitata L., H=Davalia denticulata (Burm. F.) Mett. Ex Kuhn
 Phenome “g” is a phenome that has a very distant kinship with a Taxonomy Distance value of 4.21 which consists species of Nephrolepis cordifolia (L.) Schott., Nephrolepis biserrata (Sw.) Schott., Cryptinus enervis (Cav.) Copel., Davalia denticulata (Burm. F.) Mett. Ex Kuhn., Drynaria bonni H. Crist, Adiantum caudatum L. and Pyrossia piloseloides (L.), M.G. Presl., Pteris vitata L. According to the shape, the position of closed-cell wall thickening, and the direction of stomata opening, these eight species have identical stomata (Mnium) with different stomata types. The data is presented in Figure 1.

The level of genetic similarity of a population can be described by the genetic distance from individual members of the population. The smaller the distance between individuals in a population, the more uniform the population will be. An Individual who is joined in one cluster means that the individual has a close kinship. This reveals the individual has many similarities or in other words has a small distance [12]. The farther the relationship between accessions, the wider the diversity. A similar opinion is also purposed by Hartati [13] that the similarity value is inversely proportional to distance, the greater the similarity the smaller the distance. This indicates that the more similarities of characteristics they have, the smaller the number of taxonomic distance value from plant combinations. Moreover, similar idea is also argued by Sukartini [14] which concluded that the grouping based on the percentage of similarity of qualitative and quantitative characters observed produce a picture of the position of each accession in a dendrogram, genetic distance values as well as showing the closeness of relationships or the similarity of characters between accessions.

4. Conclusions
It is concluded that there is seven phenomes (cluster) combinations of fern species with each level of kinship. The phenome that has the closest kinship has the smallest taxon distance (1.73). The combination included in this group are Nephrolepis cordifolia (L.) Schott. and Nephrolepis biserrata (Sw.) Schott. Both species have many similar morphological and anatomical characteristics. The phenome that has the most distant relationship is the species of Nephrolepis cordifolia (L.) Schott., Nephrolepis biserrata (Sw.) Schott. Cryptinus enervis (Cav.) Copel., Davalia denticulata (Burm. F.) Mett. Ex Kuhn., Drynaria bonni H. Crist, Adiantum caudatum L. and Pyrossia piloseloides (L.), M.G. Presl., Pteris vitata L., with taxonomic distance value of 4.36.

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