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Phenetic Relationship of Lichens Grown on Tea Plants (*Camellia sinensis* (L.) Kuntze) in Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District

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ABSTRACT

Lichen is a mutualism symbiotic organism between fungi (mycobiont) and photosynthetic symbiont in the form of algae (photobiont). Lichen can be found from the lowlands to the highlands, growing epiphytes in soil, rocks, weathered wood, and on tree bark, such as on the surface of tea plants (*Camellia sinensis* (L.) Kuntze) in PT Sarana Mandiri Mukti, Tea plantation Kepahiang District, Bengkulu Province. This study aims to determine the phenetic relationship of lichen on tea plants (*Camellia sinensis* (L.) Kuntze) which was conducted in May-October 2020 in Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District. Sampling using a *purposive sampling method*. The samples were identified in the Laboratory of Plant Biosystematics Basic Sciences, Faculty of Mathematics and Natural Sciences, Bengkulu University. Lichens were analyzed based on 51 characters and converted into matrix data using the MS Excel program and the *Numerical Taxonomy System* (NTSYS) version 2.02. There are 20 species of lichens consisting of 12 species of thallus crustose type, and 8 species of thallus foliose type; belonged to Ascomycota division, 2 classes (Lecanoromycetes and Eurotiomycetes), 5 orders, and 7 families. The lichen relationship phenogram shows a similarity coefficient ranging from 0.38 to 0.94 which is divided into 2 main groups A and B. The closest similarity coefficient value, 0.94, was found in *Graphis intricata* with *Graphis dichotoma, Glyphis cicatricosa* with *Sarcographa labyrinthica*, species *Parmotrema tinctorum* with *Parmotrema crinitum*.

Keywords: Phenetic Relationship, The Tea Plant, Lichens, Kepahyang

1. INTRODUCTION

Kinship in biosystematics is defined as a pattern of relationships or similarities between plant groups based on the characteristics, or characteristics of each plant group to determine the extent of kinship between the two plant groups. Methods that using whole parts of an organism to determine relationships and form classifications based on inherited characters are called phenetics [6]. Phenetic kinship is determined qualitatively by comparing the similarities and differences in the characteristics of each taxon using many similarities in character (morphology, anatomy, embryology, palynology, cytology, chemistry, reproductive biology, ecology, and physiology). The term phenetic was first proposed by [1] which aims to show kinship using the same characteristics.

Lichens are symbiotic organisms between fungi (mycobionts) and photosynthetic symbionts in the form of algae (photobionts) so that morphologically and physiologically they form a unity. The fungus in lichen functions to provide water and minerals to maintain moisture and strengthen the body. Meanwhile, algae function as partners that produce carbohydrates with the help of sunlight for photosynthesis. The form of symbiosis between fungi and algae is symbiotic mutualism [5]. Based on the morphology of the thallus, lichens are grouped into 4 main groups; *crustose, foliose, fruticose,* and *squamulose. Crustose* lichens have thallus like crusts and are firmly attached to the substrate surface, *foliose* lichens have leaf-like talus or grooved sheets, *fruticose* lichens have bush-like thallus, and *squamulose* lichens have talus like overlapping scales [2].

Based on observations in Tangsi Baru Village, Kabawetan District, it was found that there were many variations in the shape, size, and color of lichen growing on the surface of the tea plant stems. It is suspected that until now there has been no information regarding the phenetic relationship of lichens, especially in Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District. Based on this, a study was conducted to determine the phenetic relationship of lichen on tea plants (*Camellia sinensis* (L.) Kuntze) in Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District.

2. MATERIALS AND METHODS

The study used a *purposive sampling method*, only lichens that grown on Tea plants barks. It was conducted in May – October 2020 in Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District. Lichen samples were identified at the Plant Biosystematics Laboratory of *Basic Science*, Faculty of Mathematics and Natural Sciences, Bengkulu University. Tangsi Baru Village Map, Kabawetan Subdistrict was showed in Figure 1.

The grouping of the collected lichens was analyzed based on morphological and anatomical characters. Of the 51 morphological and anatomical character statuses used, they were analyzed qualitatively (type, shape, and color) and quantitatively (length and thickness). The characters are converted into matrix data using the MS program. Excel. Matrix data were analyzed using NT Edit and



Figure 1. Map of Tangsi Baru Village, Kabawetan Subistrict (Source: Kepahiang District Profile and Google Earth)



processed using the *Numerical Taxonomy and Multivariate Analysis System* (NTSYSpc) version 2.02 program. Data analysis was made based on the *Simple Matching* (SM) similarity coefficient with the *Unweighted pair group method with arithmetic average* (UPGMA) methodology [4].

3. RESULT AND DISCUSSION

The results showed that there were 20 species of lichen, belonging to the division of Ascomycota, 2 classes (Lecanoromycetes and Eurotiomycetes), 5 orders, and 7 families. Classification and types of the thallus of Lichens grown on Tea plants (*Camellia sinensis* (L.) Kuntze) are presented in Table 1.

Phenograms of lichen relationship grown on the surface of the stem (bark) of Tea plants (*Camellia sinensis* (L.) Kuntze) were obtained from analysis based on 51 phenetic characters including thallus, prothallus, *rhizine, cilia, photobiont, soredia, isidia, perithecia,* and *apothecia. Apothecia* are divided into *apothecia* in the form of plates and *lirellae*. The *apothecia* plate consisted of a flat, concave plate. Meanwhile, *lirellae* consist of striped and round *lirellae. Lirellae* lines consisted of branched and unbranched *lirellae*, and some have labia and no labia.

The results of study, the lichen relationship analysis showed a phenogram with similarity efficiency values ranging from 0.38 to 0.94. Phenogram of 20 lichen species relationship can be seen in Figure 2.

Table 1. Classification, Types of the thallus of lichens found in Tea Plants (*Camellia sinensis* (L.) Kuntze) in

 Tangsi Baru Village, Kabawetan Subdistrict, Kepahiang District

No	Class/Ordo/Familia	Genus	Species	Type of thallus
1	Ostropales/Graphidaceae	Graphis	Graphis scripta (L.) Ach	Crustose
2			Graphis vulgata (Ach.) Wallr.	Crustose
3			<i>Graphis allugallenensis</i> Weerakoon, Wijeyaratne & Lucking	Crustose
4			Graphis intricata Fee	Crustose
5			Graphis dichotoma (Mull.Arg.) Lücking, Fieldiana	Crustose
6			Graphis chrysocarpa (Raddi) Spreng.	Crustose
7		Glyphis	Glyphis cicatricosa (Ach.) Zahlbr.	Crustose
8		Diorygma	<i>Diorygma junghuhnii</i> (Mont. & Bosch) Kalb, Staiger & Elix	Crustose
9		Sarcographa	Sarcographa labyrinthica (Ach.) Mull. Arg	Crustose
10	Lecanorales/Stereocaulaceae	Lepraria	Lepraria incana (L.) Ach.	Crustose
11	Lecanorales/ Parmeliaceae	Parmelia	Parmelia sulcata Taylor	Foliose
12			Parmelia trabelucata (Aht.) Essl.	Foliose
13		Parmotrema	Parmotrema perlatum (Huds.) M.Choisy	Foliose
14			Parmotrema robustum (Degel.) Hale	Foliose
15			Parmotrema tinctorum (Despr. Ex Nyl) Hale	Foliose
16			Parmotrema crinitum (Ach.) Hale	Foliose
17	Pertusariales/	Pertusaria	Pertusaria multipuncta (Turner) Nyl.	Crustose
	Pertusariaceae			
18	Teloschistales/	Heterodermia	Heterodermia obscurata (Nyl.) Trevis	Foliose
	Physciaceae			
19	Teloschistales/	Dirinaria	Dirinaria applanata (Fee) D.D. Awasthi	Foliose
	Caliciaceae			
20	Pyrenulales/	Pyrenula	Pyrenula macrospora (Degel.) Coppins & P.James	Crustose
	Pyrenulaceae			





Figure 2. Phenogram of lichens grown on Tea Plants bark based on phenetic characters

The higher the phenogram similarity coefficient value produced, the closer the lichen relationship coefficient. On the other hand, the lower the resulting phenogram similarity coefficient value, the further the lichen relationship is. All of lichens species belong to one group with a similarity of 0.38. At the similarity value of 0.38, there are 20 species and divided into two main groups, those are group A and group B. The main group A and B are separated on the characters of the thallus type, variations in the thallus, abaxial thallus, species color, thallus margins, prothallus, lobes, *rhizin, cilia*, and photobiont.

The main group A consisted of 12 species of lichens; Diorygma junghuhnii, Graphis scripta, Graphis vulgata, Graphis allugallenensis, Graphis intricata, Graphis dichotoma, Graphis chrysocarpa, Glyphis cicatricosa, Sarcographa labyrinthica, Pyrenula macrospora, Lepraria incana and Pertusaria multipuncta. It has similar characters in the type of thallus (crustose), pressence of prothallus, pressence of apothecia, lirellae, soredia, isidia, perithecia At the similarity coefficient value of 0.61. The main group A was divided into two groups (A1 and A2). Groups A1 and A2 are separated by the character of the presence or absence of *apothecia*, *perithecia*, *isidia*, and *soredia*.

Group A1 consisted of 9 species of lichens of the genera *Diorygma*, *Graphis*, *Glyphis*, and *Sarcographa*. The similar characters including the type of thallus (crustose), variations in the thallus leprose, margins of the thallus, presence of prothallus, *rhizin*, *cilia*, *soredia*, *isidia*, and *perithecia*, apothecia in the form of striped and round *lirellae*. It has photobiont of the genus *Trentepohlia*. At a similarity coefficient of 0.80 group A1 is divided into A1a and A1b. The characters that makes the A1a and A1b groups separate is the *apothecia* type.

Group A1a is the lichen from the species Diorygma jungjuhnii which has the character of the crustose thallus type, variations of the leprose thallus, flat margin of the thallus, white prothallus, does not have rhizines, cilia, soredia, isidia, and perithecia. It has *Trentepohlia* photobiont and has *apothecia* in the form of line *lirellae* which sinks to the surface of the thallus. Lirellae are scattered all over the surface of the thallus, short branched, closely spaced, and have a thickness of 0.1 mm lirellae.

Group A1b consisted of 8 species of lichens of the genera Graphis, Glyphis, and Sarcographa. It has similar characters in the type of thallus (crustose), variations in the talus leprose, the flat margin of the thallus, black prothallus, without rhizine, cilia, soredia, isidia, and perithecia. Has apothecia in the form of striped and round lirellae. At a coefficient of 0.86, the genus Graphis is divided into two groups, namely groups of the species Graphis scripta, Graphis allugallenensis, Graphis intricata with Graphis dichotoma which have similar apothecia characters in the form of black lirellae lines, 0.05 mm thickness lirellae, white lirellae margins, and labia in black, and photobiont of the genus Trentephlia. Meanwhile, the Graphis group of the species Graphis vulgata and Graphis crysocarpa have similar black prothallus color characters, apothecia in the form of line lirellae, scattered on the surface of the thallus, the distance between the apothecia is tight, short lirellae has a prominent type (protrudes on the thallus surface), the thickness of the lirellae is 0.05 mm., lirellae 0.3 mm long, has a blacklabia, and photobiont of the genus Trentephlia.

At a coefficient of 0.94, the species of *Graphis intricata* and *Graphis dichotoma* have similar characters in the type of thallus *crustose*, variations in the *leprose* thallus, whitish-gray talus color, black prothallus, black *apothecia*, appearing on the surface of the talus. *Apothecia* is a long, branched line, 0.05 mm thick, black labia, and photobiont of the genus *Trentepohlia*. The species *Glyphis cicatricosa* and Sarcographa labyrinthica have similar characters, namely the type of talus *crustose*, variations in the thallus leprose, black prothallus, having apothecia in the form of round lirellae submerged in the surface of the talus, the distance between the apothecia is rare, the thickness of the *lirellae* is 0.05 mm, the rim of the lirellae is white, black labia, and photobiont of the genus Trentepohlia. The species characters of Graphis intricata, Graphis dichotoma, Sarcographa labyrinthica, and Glyphis cicatricosa can be seen in Figure 3. Group A2 consisted of 3 species of lichens, namely Pyrenula macrospora, Lepraria incana, and Pertusaria multipuncta which have similar characters, including the type of thallus crustose, the margins of the thallus are flat, presence or absent of prothallus, has apothecia in the form of a cup, perithecia, isidia, and soredia. At a coefficient of 0.74 group A2 is divided into groups A2a and A2b. Group A2a and A2b are separated by the characters of the presence or absence of *soredia*, *perithecia*, and isidia.

The A2a group; *Pyrenula macrospora* species, have characters including the type of thallus *crustose*, variations in the *perithecia leprose*, flat thallus margins, black prothallus, presence of black scattered *perithecia* over the thallus surface, and photobiont genus *Trentepohlia*.

Group A2b consisted of 2 lichen species; Lepraria incana and Pertusaria multipuncta. It has similar characters including the type of crustose thallus, flat margin of the thallus, green adaxial thallus, no rhizines, cilia, and apothecia, has soredia, isidia. Photobiont of the genus Trebouxia.



Figure 3 Character species *Graphis intricata* (A); *Graphis dichotoma* (B), *Sarcographa labyrinthica* (C), and *Glyphis perithecia*.

The main group B consisted of 8 species of lichens belonging to the *foliose* thallus lichens, photobiont genus *Trebouxia*. Most of this group has color on the surface of the adaxial and abaxial thallus, presence of prothallus, *perithecia*, rhizin, *cilia*; some species are not found cilia. At a similarity coefficient of 0.66, the main group B is divided into two groups (B1 and B2). Groups B1 and B2 were separated by abaxial color characters of the thallus, *apothecia, soredia, isidia, rhizine,* and *cilia*.

Group B1 consisted of 7 species of lichens which have similar characters including the type of foliose thallus, presence of rhizin, and *Trebouxia* photobiont. At the coefficient of 0.85 group B1 is divided into groups B1a and B1b. Groups B1a and B1b were separated by the characteristics of the presence of soredia and isidia.

Group B1a consisted of 5 species of lichens; Dirinaria *applanata, Parmelia sulcata, Parmotrema perlatum, Parmotrema robustum,* and *Heterodermia obscurata.* Characterized by the type of thallus *foliose*, photobiont genus *Trebouxia* does not have *apothecia, isidia,* and *perithecia,* has *rhizine, soredia,* and *cilia, Dirinaria applanata* species do not have *cilia.* At a coefficient of 0.90, the *Parmelia sulcata* species are separated from the group because it has squarrose rhizine, unbranched *rhizin,* black cilia, unbranched cilia, sparse between cilia are rare, do not have apothecia, have a green powder type soredia scattered on the surface of the talus, and photobiont of the genus *Trebouxia.*

At a similarity coefficient of 0.94, Parmotema perlatum and Parmotrema robustum species are closely related, have similar characters, namely black simple rhizin, unbranched rhizin, unbranched cilia, black cilia, the dense distance between cilia, do not have *apothecia*, has no *isidia*, has green powder type soredia, and the photobiont genus Trebouxia. The characters that distinguish Parmotema perlatum and Parmotrema robustum species are the adaxial thallus color, the sparse and dense distance between *rhizines*, the location of Parmotrema perlatum soredia was in the middle of the thallus surface, while at Parmotrema robustum soredia is located at the lobe margins. The characters of the Parmotrema perlatum and Parmotrema robustum species can be seen in Figure 4.

Group B1b at a coefficient of 0.83 consists of 2 species of lichen, namely *Parmotrema tinctorum* and *Parmotrema crinitum*. The *Parmotrema tinctorum* and *Parmotrema crinitum* species are separated from their groups because they have similar characters including the *foliose* thallus type, greenish-gray adaxial thallus color, absent of prothallus, has simple black *rhizin* type, tight distance between *rhizines*, unbranched *rhizin*, absent of *apothecia and soredia*, have scattered *isidia* over thallus surface, photobiont genus is *Trebouxia*. *Parmotrema tinctorum* has *cilia* and green- fingerlike *isidia*, whereas *Parmotrema crinitum* has black cilia and gray – coralloid *isidia*. The characters of *Parmotrema tinctorum* and *Parmotrema crinitum* species can be seen in Figure 5.



Figure 4. *Rhizin* distance in *Parmotrema perlatum* (A); *rhizin* distance in *Parmotrema robustum* (B); location of *soredia Parmotrema perlatum* (C); Location of *Soredia* on *Parmotrema robustum* (D)





Figure 5 Color of *Parmotrema tinctorum* thallus A); color of *Parmotrema crinitum* the thallus (B); fingerlike *isidia* of Parmotrema *tinctorum* (C); corraloid *isidia* of *Parmotrema crinitum* (D); *rhizin Parmotrema tinctorum* (E); *cilia* and *rhizines* of *Parmotrema crinitum* (F)

Group B2 at a coefficient of 0.66 consists of *Parmelia trabeculata*. The *Parmelia trabeculata* species is separated from its group due to its foliose thallus type, simple *rhizin*, black *cilia*, the distance between *cilia* is tight, has *apothecia* in the form of brown plates, the distance between the *apothecia* is tight, the *apothecia* are scattered on the surface of the thallus, the margins of the *apothecia* wavy green color, has no *soredia*, has no *isidia*, and photobiont of the genus *Trebouxia*.

The lichens that have the closest coefficient value are 0.94, first in the species *Graphis intricata* and *Graphis dichotoma*, the second in the species *Glyphis cicatricosa* and *Sarcographa labyrinthica*, the third in the species *Parmotrema tinctorum* and *Parmotrema crinitum* have a parallel phenogram line. Parallel lines indicate that the two species are closely related and show many similarities in phenetic characters. This is by the opinion of [3], the more similarities one has between living things, the closer the relationship is and vice versa.

4. CONCLUSION

Analysis of the lichen relationship based on phenetic characters resulted in a phenogram of kinship with a similarity coefficient of 0.38 - 0.94. At the similarity coefficient value of 0.38, the phenogram was divided into two large groups (A and B). Group A consisted of 12 species of the thallus crustose type, while group B consisted of 8 species of the thallus foliose type. The closest similarity coefficient value is 0.94, found in species between *Graphis intricata* and *Graphis dichotoma*, species *Glyphis cicatricosa* with *Sarcographa labyrinthica*, species *Parmotrema tinctorum*, and *Parmotrema crinitum*.

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