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Diversity and phenetic relationship of lichen genus *Usnea* in Cisanti Forest area, Bandung District, West Java, Indonesia

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Abstract. *Kusmoro J, Raihan R, Hermawan W, Hasan R. 2025. Diversity and phenetic relationship of lichen genus* Usnea *in Cisanti Forest area, Bandung District, West Java, Indonesia. Biodiversitas 26: 583-600.* The Cisanti pine forest in Bandung District, West Java, Indonesia, is the area of an educational eco-agrotourism area and is a natural habitat for *Usnea*. However, there is no report yet about the richness of *Usnea* in this location that is important to provide information such as its biodiversity and chemical content beneficial resources. This study aimed to explore the diversity and analyze phenetic relationships of *Usnea* in the Cisanti forest area, based on morphological, anatomical, and chemical characters. The sampling method was carried out by exploring the forest area purposively on pine trees that *Usnea* grew. *Usnea*'s phenetic relationship was analyzed using the NTSYS version 2.01e software. The results showed 23 species of *Usnea* were found, namely *U. angulata, U. baileyi, U. barbata, U. ceratina, U. chaetophora, U. cornuta, U. dasaea, U. diffracta, U. flammea, U. flavocardia, U. hirta, U. lapponica, U. maculata, U. mutabilis, U. pangiana, U. robusta, U. rubicunda, U. shimadae, U. sinensis, U. steineri, U. subcornuta, U. subflorida,* and *U. wasmuthii*. Based on the 41 characters analyzed, in general *Usnea* divided into 3 clusters and cluster separation corresponds with subgenera, namely *Usnea, Eumitria*, and *Dolichousnea.* The closest relationship is between *U. sinensis* and *U. maculata*, and between *U. barbata* and *U. lapponica* by a similarity index of 0.814. The most distant relationship is between *U. baileyi* and *U. barbata* and between *U. angulata* and *U. chaetophora,* as well as *U. subcornuta* and *U. pangiana* by a similarity index of 0.616.

Keywords: Cisanti forest area, phenetics relationship, species diversity, *Usnea,* West Java

INTRODUCTION

Usnea is a lichen that grows epiphytically on the branches of tall wooden trees, formed from the association between algae and fungi. It is commonly used as herbal medicine in Indonesia and is formed as a single or mixed formula (Jannah et al. 2022). *Usnea* thallus is radially symmetrical with an axis that contains usnic acid. The axis of *Usnea* is a special characteristic that can be observed by stretching and pulling the thallus in opposite directions so that it looks like a thread in the middle of the thallus (Wang et al. 2022; Engin et al. 2023). The color of *Usnea*'s thallus varies from light green, dark green, yellowish green, and red to brownish red (Temu et al. 2022). Lychenic acid and morphological traits, such as thallus base color and thallus-substrate contact are crucial characters in *Usnea* classification (Truong et al. 2011). Anatomical characteristics, such as the cortex, medulla, and axis, are also an important part of *Usnea* identification (Shukla et al. 2014a). The cortex types of *Usnea* thallus are distinguished into loose, dense, and thick types. The medulla has two types, dense and loose. There are two types of axis, i.e. hollow and solid (Tina-Picaza and Picaza 2023).

Usnea produces a variety of bioactive compounds, including usnic acid, depsides, depsidones, and phenolics (Salgado et al. 2018; Suharno et al. 2020; Jannah et al. 2022). Usnic acid has been subject to extensive research and analysis, and possesses extensive array of pharmacological properties encompassing anti-inflammatory, antibacterial, antiviral, anticancer, antioxidant, and photoprotective effects. These diverse pharmacological activities contribute to its significance as a crucial constituent in various applications including dietary supplements, cosmetics, and medicinal products (Luzina and Salakhutdinov 2018; Wang et al. 2022). In addition, *Usnea* produces other significant metabolites, such as salazinic acid, stictic acid, norstictic acid, and barbatic acid, which are known for their biological and physiological significance (Shukla et al. 2014b; Reddy et al. 2019). Barbatic acid has demonstrated potent cytotoxic activity against lung cancer cells, indicating its potential as an anticancer agent (Reddy et al. 2019). Additionally, *U. longissima* contains several compounds, such as useanol, lecanorin, and evernic acid, which exhibit antimicrobial, antiproliferative, and anticancer properties, further highlighting the genus's potential in developing natural therapeutic agents (Dandapat and Paul 2019).

Studies on *Usnea* in Indonesia are poorly reported and mostly focus on its chemical content and medicinal potential. More than 50 species of *Usnea* have been identified throughout Indonesia, and specifically in Java, there are at least 20 species, in West Java is estimated around 10-15 species of *Usnea* have been documented (Noer et al. 2015; Jannah et al. 2021, 2022; Agustini et al. 2023). Currently, more than 360 *Usnea* species are recognized in the world (Prateeksha et al. 2016) which are divided into three subgenera, namely *Usnea, Eumitria,* and *Dolichousnea* (Lücking et al. 2020).

The research of *Usnea* that focuses on diversity and relationships is important to be conducted. It can contribute to tracing the lychenic acid content of *Usnea* which will facilitate its use in the field of pharmacology to be developed as an herbal medicine. The Cisanti is a forest area located in Bandung District, which has a lake that is the source of the prominent Citarum River in West Java, Indonesia, which is the source of three large national reservoirs objects, namely Cirata, Saguling, and Jatiluhur (Aziz and Sekarwulan 2021). However, a report regarding *Usnea*'s diversity in Cisanti has not been reported. For this reason, this study is conducted to explore *Usnea* diversity based on morphology, anatomy, and chemical characteristics as well as analyzing their phenetic relationships.

MATERIALS AND METHODS

Study site

The study was conducted in the Cisanti forest area that dominated by pine trees (*Pinus merkusii* Jungh. & de Vriese) and is located in Kertasari District of Bandung District, West Java, Indonesia, is about 50 km from Bandung City (Figure 1). This location is upstream of the Citarum River and irrigates three vital national hydroelectric power plant reservoirs, Saguling, Cirata, and Jatiluhur. Cisanti Lake has an area of approximately 5 ha with a depth of around 1 to 3 m, at an altitude of 1500-1700 m above sea level (Iskandar et al. 2017; Aziz and Sekarwulan 2021). Bandung District Regional Regulation Number 4 of 2019 assigned Cisanti is included in the Citarum River upstream ecotourism development plan area, the target of which is implementing the concept of developing natural adventure tourism areas and educational agrotourism. The temperature in the Cisanti forest area was 22-24°C, air humidity was 76-89.5%, and light intensity was 450-920 lux.

Procedures

Sample collection

The research method used in observing the *Usnea* lichen was descriptive explorative. Data collection techniques are carried out by exploring the research area purposively. The sampling method was purposively by choosing *Usnea* that was grown in pine trees (*P. merkusii*) along the left side of the public road towards Cisanti Lake, around 5-20 m inward from the roadside at the pine forest of Cisanti (7°12'53.6"S, 107°39'27.7"E) at 13.00-16.00 local time. *Usnea* collection is carried out from the surface of main trunk of the pine tree, by cutting off part of the tree bark where lichens grow. The sample was taken from the base to the tree trunk at a height of 1.5 m above ground, then placed in a sample envelope and transferred to the laboratory to be observed and identified based on morphology, anatomy, and chemical content characteristics. *Usnea* identification was conducted at the Plant Taxonomy and Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang, Indonesia.

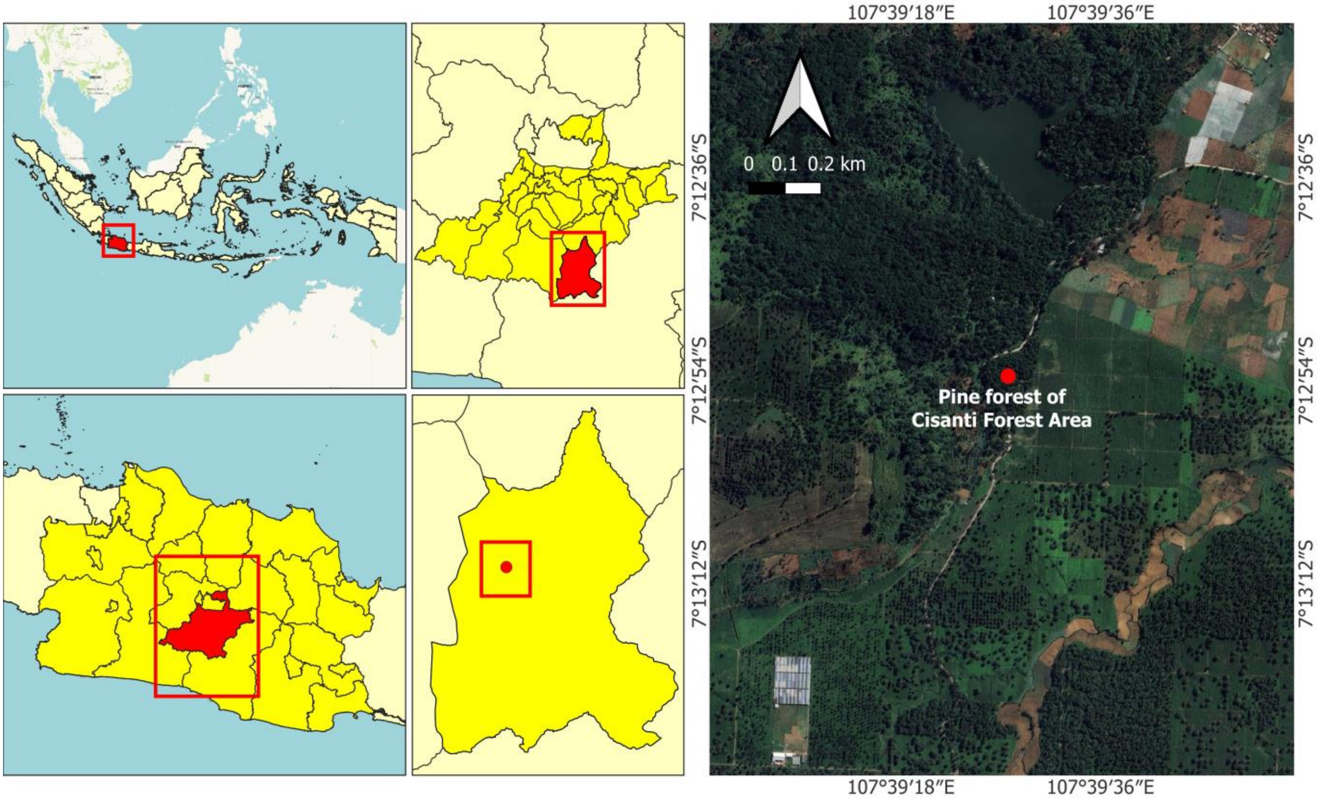

Figure 1. Research locations in the pine forest of Cisanti forest area, Bandung District, West Java Province, Indonesia

Table 1. Morphological character of *Usnea* observed

Characters	Traits and code	Reference
Thallus color	light green (0), dark green (1), yellowish green (2), brownish red (3)	Cao et al. (2018), Hájek et al. (2021)
Thallus shape	rounded (0), faceted (1)	Oštádal and Hazdrová (2016), Androsova and Virolainen (2022)
Branching type	dichotomous isomatic (0), dichotomous anisomatic (1)	Wirth and Anderegg (1995), Gola (2014)
Growth form	erect (0), sub pendent (1), pendent (2)	Ohmura (2001)
Thallus base color	white (0), black (1)	Saco and Ganzon-Fortes (2022)
Lateral branch	cylinder (0), shrinking (1), widening (2)	Molins et al. (2021)
Fibril	short (0), long (1),	He et al. (2012), Hájek et al. (2021)
Cleft/segment	none (0), there is a cleft (1), there are segments (2)	Cao et al. (2018), Ohmura (2020)
Papillae	absent (0), presents (1)	Ohmura (2001)
Reproductive structure	isidia (0), soredia (1)	Jayalal et al. (2013), Nadel and Clerc (2022)

Table 2. Anatomical characters of *Usnea* observed

Characters	Traits
Cortex thickness	thin (0), thick (1)
Medulla thickness	thin (0), thick (1)
Axis diameter	small (0), big (10)
Medulla structure	loose (0), dense (1), compact (2)
Axis shape	hollow (0), round (1)
Pigment of thallus inner part	white (0), yellow (1), orange (2), pink (3), red (4)

Morphological observation

Morphological identification of *Usnea* consisting of 10 characters, includes thallus color, thallus shape, branching type, growth form, color of thallus base, lateral branch, fibril, cleft/segment, papilla, and reproductive structure (Table 1). Samples of *Usnea* were identified morphologically at the laboratory using a stereomicroscope. The thallus was incised with a razor blade and then measured using a ruler and observed under a stereo microscope. The color grouping of the *Usnea* thallus was determined based on the Munsell color scale. The colors of *Usnea* thallus were divided into 4 color levels, light green (HUE 2.5 GY 5/2 to 8/6), dark green (HUE 7.5 GY 5/2 to 8/ 4), yellowish green (HUE Y 7/4) and brownish red (HUE R 7/4). Thereafter, the identification refers to Brodo and Sharnoff (2001) and Ohmura (2012) in the laboratory.

Anatomical observation

Anatomical characteristics were performed under a light microscope based on the longitudinal and transverse sections of fresh samples. Six anatomical parameters observed include the shape of the cortex, medulla, axis, ratio, and color pigmentation of the inner part of *Usnea* thallus (Table 2) (Cao et al. 2018; Kusmoro et al. 2018; Androsova and Virolainen 2022). Identification of the pigment color of the inside of the thallus was done by matching it with the Munsell color chart as was also done in identifying the morphological color of the thallus.

Chemical contents

Spot test. The spot test to determine the sample response toward reagents K, C, and KC (Table 3) was carried out according to Dandapat and Paul (2019). The thallus was cut off about 2 cm long and placed on a drip plate which was then treated with calcium hypochlorite (C or $CaHCl_3$) and potassium hydroxide (K or KOH) reagents and waited for several minutes until the thallus color changed. Positive results with the KOH reagent produced a yellow color in the cortex (atranorin acid) and reddish yellow or orange in the medulla (salazinic acid). Positive results on the $CaHCl_3$ reagent produced a pink color (gyrophoric acid) and a red color (lecanoric acid).

Microcrystal test. Lychenic acids such as usnic acid, haemathamnolic acid, and placodiolic acid, are secondary metabolites in *Usnea* that accumulate to form microcrystals. Each compound forms different crystals, such as stars, rods, or prisms, which can be used to identify and quantify metabolites present in lichens. Chemical analysis was carried out to determine the lychenic acid content in each type of *Usnea* to determine the species' name. This test includes a microcrystal test and a spot set test. The microcrystal test to identify the contained 25 lychenic acid type of *Usnea* was carried out according to Ohmura (2020) (Table 3). The lichen was cut off into two pieces 1 cm long, placed on a glass object, dripped with acetone until it was submerged in 1-2 drops, waited until a white circle formed around the thallus, and then fixed over a burning Bunsen flame. After the white circle dried, the thallus was separated. The dry white circle was then dripped with test reagents, let stand until dry, then covered with a cover glass, and finally observed under a light microscope.

Data analysis

The phenetic analysis based on morphological, anatomical, and biochemical characters (Tables 1, 2, and 3) was carried out using the NTSYS for PC version 2.10e software (Rohlf 2015). A dendrogram construction using NTSYS includes several stages, i.e. entering and storing binary data in ntedit.exe as a basic reference for NTSYS operations, creating similarity or dissimilarity data intervals to create a matrix, and creating cluster analysis (groups) with Sequential Agglomerative Hierarchical Nested (SAHN). The Simple Matching Coefficient (SMC) formula was used to determine the similarity value or similarity index among traits and the Unweighted Pair Group Method with Arithmetic mean (UPGMA) method to construct a dendrogram (Moulton et al. 2018).

Table 3. The chemical character of *Usnea* observed

Characters	Traits
Atranoric acid	absent (0), present (1)
Caperatic acid	absent (0), present (1)
Consistent acid	absent (0), present (1)
Diffractaic acid	absent (0), present (1)
Eumitrine acid	absent (0), present (1)
Fumarprotocetraric acid	absent (0), present (1)
Galapaginic acid	absent (0), present (1)
Gangaleoidin acid	absent (0), present (1)
Haemathamnolic acid	absent (0), present (1)
Isousnic acid	absent (0), present (1)
KC reagent reaction	absent (0), present (1)
Lobaric acid	absent (0), present (1)
Norsictic acid	absent (0), present (1)
Perlatolic acid	absent (0), present (1)
Physodalic acid	absent (0), present (1)
Placodiolic acid	absent (0), present (1)
Protocetraric acid	absent (0), present (1)
Psoromic acid	absent (0), present (1)
Reagent C reaction	absent (0), present (1)
Reagent K reaction	absent (0), present (1)
Retigeric acid	absent (0), present (1)
Salazine acid	absent (0), present (1)
Squamatic acid	absent (0), present (1)
Stictic acid	absent (0), present (1)
Usnic acid	absent (0), present (1)

RESULTS AND DISCUSSION

Species diversity of *Usnea*

Fourty samples of *Usnea* from 40 pine trees were collected in this study. The identification based on morphological, anatomical, and chemical characters analysis showed that *Usnea* in the Cisanti forest area consisted of 23 species (Figure 2), namely *U. angulata* Ach. 1814, *U. baileyi* (Stirt.) Zahlbr. 1909, *U. barbata* (L.) Weber ex F.H.Wigg 1780, *U. ceratina* Ach. 1810, *U. chaetophora* Stirt. 1883, *U. cornuta* Körb. 1859, *U. dasaea* Stirt. 1881, *U. dasaea* Stirt. 1881, *U. diffracta* Vain. 1921, *U. flammea* Stirt. 1881, *U. flavocardia* Räsänen 1936, *U. hirta* (L.) F.H.Wigg. 1780, *U. lapponica* Vain. 1925, *U. maculata* Stirt. 1882, *U. mutabilis* Stirt. 1881, *U. pangiana* Stirt. 1883, *U. robusta* Stirt. 1882, *U. rubicunda* Stirt. 1881, *U. shimadae* Asahina 1970, *U. sinensis* Motyka 1936, *U. steineri* Zahlbr. 1930, *U. subcornuta* Stirt. 1883, *U. subflorida* Motyka 1937, and *U. wasmuthii* Räsänen 1932.

The identification of 23 *Usnea* species in the Cisanti forest area, Bandung District, West Java exemplifies a significant degree of lichen biodiversity. In comparison to other locations in West Java, the *Usnea* diversity documented in Cisanti underscores the favorable environmental parameters and ecological niches conducive to lichen proliferation, especially within coniferous forests. For instance, the variety of *Usnea* species exceeds the *Usnea* species diversity previously documented in Lembang and Langkaplancar, Pangandaran, West Java where 13 *Usnea* species were recorded, categorized into three subgenera, namely *Usnea*, *Eumitria*, and *Dolichousnea*. The notable species consist of *U. rubrotincta, U. fragilescens, U. intermedia, U. esperantiana, U. hesperina, U. mutabilis, U. barbata, U. flammea, U. longissima, U. hirta, U. dasypoga, U. pectinata*, and *U. baileyi* (Jannah et al. 2022). Additionally, a study conducted in the Priangan region of West Java identified 11 species belonging to three subgenera, namely *Dolichousnea*, Eumitra, and *Usnea*. Noteworthy species include *Usnea filipendula, U. hirta, U. longissima, U. trichodea, U. baileyi, U. ceratina, U. cornuta, U. flexilis, U. flexuosa, U. florida,* and *U. glabra* (Noer et al. 2015).

Morphological characteristics

Thallus color and thallus shape

The results showed that the color of thallus is light green (HUE 2.5 GY 5/2 to 8/6), dark green (HUE 7.5 GY 5/2 to 8/4), yellowish green (HUE Y 7/4) or brownish red (HUE R 7/4), with the shape of thallus is rounded or faceted (Table 4; Figures 3 and 4). Among the 23 *Usnea* species, the thallus color type is dominated by green (15 species) compared to brownish red (8 species). However, the green color thallus of these *Usnea* is separated into three color types, namely yellowish green, dark green, and light green, with a ratio of 6:5:4 (Table 4). The color difference of thallus is caused by the algae layer and the color of the pigment contained in the thallus. The red color is caused by the red pigment found in the medulla of the thallus and the green color comes from the pigment of the algae layers that make up the thallus (Hájek et al. 2021). The color difference in the thallus of *Usnea* lichens is primarily attributed to the presence of various lichen compounds, particularly usnic acid, which is a dibenzofuran derivative widely distributed among mycobiont genera (Salgado et al. 2018). Usnic acid is known for its strong absorption of UV-B and the shortest PAR wavelengths, contributing to the bright greenish color observed in these lichens. This compound forms a dense sleeve around the trebouxioid photobiont in the outermost parts of the medulla, protecting against harmful radiation. Additionally, *Usnea* species contain a range of UV-B absorbing depsidones and depsides, although these are typically confined to the medulla and contribute less to the surface coloration (Nybakken and Gauslaa 2007).

The *Usnea* in the Cisanti consisted of two shape types of thalli, namely rounded and faceted (Figure 4), while the flattered type was notably absent. The most common shape of the thallus is the faceted that is found in 14 species (Table 4). This observation aligns with the broader morphological diversity of *Usnea* species, which can exhibit significant variability in thallus structure and chemistry. For instance, *U. oreophila*, is characterized by a thick and fistulose central axis and branches with numerous cracks and downturned edges, indicating a complex morphology that does not fit neatly into the flat category (da Cruz Lima Gerlach et al. 2019). The absence of flat thall could be genetic or be influenced by environmental factors, as lichen morphology is known to vary with habitat conditions. Studies on other lichen species, such as those in Antarctica, have shown that thallus morphology, including diameter and cortex thickness, can be significantly affected by environmental conditions like temperature and hydration (Oštádal and Hazdrová 2016; Hájek et al. 2021).

Additionally, the growth stage and geographic area can influence microalgal diversity within the thalli, which may indirectly affect thallus morphology (Molins et al. 2021).

Thallus branching type and growth form

Usnea in the Cisanti area has three forms of thallus growth and two types of branching (Table 4). The branching type of thallus of *Usnea* mostly has the anisotomic dichotomous branching type that was found in 21 species. Meanwhile, only 2 species of *Usnea* (*U. chaetophora* and *U. diffracta*) have the isotomic dichotomous branching type. The anisotomic dichotomous branching type is an irregular thallus branching, generally, the main thallus will only form one lateral branch. The isotomic dichotomous branching type is a thallus that forms two regular branches, the main thallus forms two lateral branches, and the lateral branch forms two fibrils.

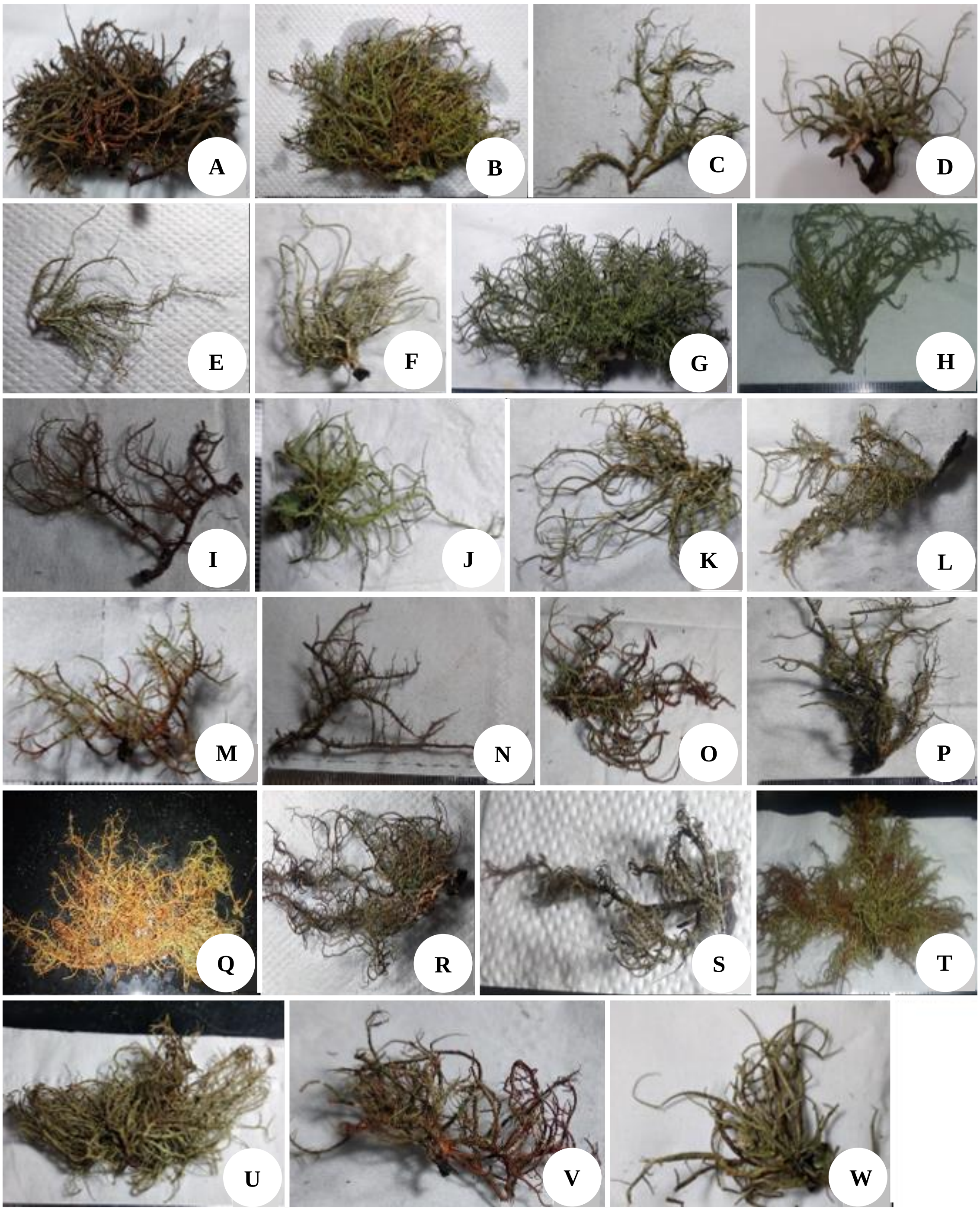

Figure 2. Diversity of *Usnea* in the Cisanti, Bandung District, West Java, Indonesia: A. *U. angulata*; B. *U. baileyi*; C. *U. barbata*; D. *U. ceratina*; E. *U. chaetophora*; F. *U. cornuta*; G. *U. dasaea*; H. *U. diffracta*; I. *U. flammea*; J. *U. flavocardia*; K. *U. hirta*; L. *U. lapponica*; M. *U. maculata*; N. *U. mutabilis*; O. *U. pangiana*; P. *U. robusta*; Q. *U. rubicunda*; R. *U. shimadae*; S. *U. sinensis*; T. *U. steineri*; U. *U. subcornuta*; V. *U. subflorida*; and W. *U. wasmuthii*

Table 4. Morphological characteristics of *Usnea* in the Cisanti forest area, Bandung District, West Java, Indonesia

Species	Subgenus	Thallus color	Thallus shape	Branching type	Growth form	Thallus base color	Lateral thallus branch shape	Fibrils	Cleft/ segment	Papillae	Reproductive structure
U. angulata Ach. 1814	*Eumitria*	Brownish red	Faceted	AD	Pendent	Black	Cylinder	Long	None	+	Soredia
U. baileyi (Stirt.) Zahlbr. 1909	*Eumitria*	Dark green	Faceted	AD	Sub pendent	Black	Shrinkage	Short	None	+	Soredia
U. barbata (L.) Weber ex F.H.Wigg 1780	*Eumitria*	Yellowish green	Faceted	AD	Pendent	Black	Shrinkage	Short	Cleft	+	Isidia
U. ceratina Ach. 1810	*Dolichousnea*	Yellowish green	Rounded	AD	Erect	White	Cylinder	Short	Cleft	+	Soredia
U. chaetophora Stirt. 1883	*Usnea*	Light green	Rounded	ID	Pendent	White	Widened	Short	Segment	+	Isidia
U. cornuta Körb. 1859	*Eumitria*	Yellowish green	Rounded	AD	Erect	White	Cylinder	Short	Cleft	+	Soredia
U. dasaea Stirt. 1881	*Usnea*	Light green	Rounded	AD	Sub pendent	White	Shrinkage	Short	Segment	+	Isidia
U. diffracta Vain. 1921	*Dolichousnea*	Light green	Faceted	ID	Erect	White	Cylinder	Long	Segment	+	Isidia
U. flammea Stirt. 1881	*Eumitria*	Brownish red	Faceted	AD	Erect	Black	Shrinkage	Long	Cleft	-	Soredia
U. flavocardia Räsänen 1936	*Eumitria*	Light green	Rounded	AD	Sub pendent	White	Shrinkage	Short	Cleft	+	Soredia
U. hirta (L.) F.H.Wigg. 1780	*Usnea*	Yellowish green	Rounded	AD	Pendent	White	Widened	Long	Cleft	-	Soredia
U. lapponica Vain. 1925	*Eumitria*	Yellowish green	Faceted	AD	Sub pendent	White	Shrinkage	Short	Segment	+	Isidia
U. maculata Stirt. 1882	*Eumitria*	Brownish red	Faceted	AD	Erect	Black	Cylinder	Short	None	+	Soredia
U. mutabilis Stirt. 1881	*Eumitria*	Brownish red	Faceted	AD	Sub pendent	Black	Shrinkage	Short	None	+	Isidia
U. pangiana Stirt. 1883	*Dolichousnea*	Brownish red	Faceted	AD	Sub pendent	White	Shrinkage	Short	Segment	+	Soredia
U. robusta Stirt. 1882	*Eumitria*	Dark green	Rounded	AD	Sub pendent	Black	Shrinkage	Short	Cleft	+	Soredia
U. rubicunda Stirt. 1881	*Usnea*	Brownish red	Faceted	AD	Sub pendent	White	Cylinder	Short	None	+	Isidia
U. shimadae Asahina 1970	*Usnea*	Dark green	Rounded	AD	Sub pendent	White	Widened	Long	Cleft	+	Isidia
U. sinensis Motyka 1936	*Eumitria*	Dark green	Faceted	AD	Erect	Black	Cylinder	Short	None	+	Soredia
U. steineri Zahlbr. 1930	*Eumitria*	Brownish red	Faceted	AD	Sub pendent	Black	Shrinkage	Short	Segment	+	Isidia
U. subcornuta Stirt. 1883	*Usnea*	Brownish red	Rounded	AD	Sub pendent	Black	Shrinkage	Long	Segment	-	Isidia
U. subflorida Motyka 1937	*Eumitria*	Yellowish green	Faceted	AD	Sub pendent	White	Widened	Short	Segment	+	Isidia
U. wasmuthii Räsänen 1932	*Eumitria*	Dark green	Faceted	AD	Pendent	Black	Cylinder	Short	None	-	Isidia

Note: AD: Anisotomic Dichotomous; ID: Isotomic Dichotomous; +: Present; -: Absent

The isotomic dichotomous branching of *Usnea* lichens contributes to their shrub-like morphology. Anisotomic dichotomous branching features unequal thallus bifurcation, with one branch predominating (Truong and Clerc 2016). This branching configuration increases surface area, enhancing photosynthesis and nutrient uptake (Sanders and de Los Ríos 2012). *Usnea* species exhibit significant branching variation across different geographical areas. For instance, species in East Fennoscandia and the Antarctic Neuropogon group display unique morphological and chemical characteristics (Seymour et al. 2007). The branching diversity underscores the ecological adaptability of *Usnea* species, facilitating their survival in diverse climates and ecosystems, ranging from tropical forests to Antarctic environments (Kadosumi et al. 2005; Seymour et al. 2007). Such branching structures optimize light and air exposure, vital for lichen survival across varied climatic conditions (Kaasalainen et al. 2020).

The growth form of the *Usnea* thallus was dominated by the sub pendent form (12 species). A sub pendent growth form is characterized by the growth of the main thallus upright while the lateral branches and fibrils pendulate downwards (Figure 5.A). Pendent growth forms were found in 5 species of *Usnea*, characterized by the main thallus and fibrils growing pendulously downwards (Figure 5.B). The erect *Usnea* growth form is found in 6 species of *Usnea* in the Cisanti area. The upright growth form is characterized by the main thallus to the lateral branches and the fibrils growing upright and the direction of growth is to the side, not pendulous downwards (Figure 5.C).

Usnea displays diverse growth forms that are essential for species identification and classification, indicating adaptations to varying environmental conditions. These growth forms serve as morphological traits that affect ecological functions and geographical distribution. Pendent form of *Usnea* species are identified by their hanging, beard-like morphology, suited for humid habitats where this structure optimizes air and moisture access. For instance, *U. beckeri* exemplifies this growth form, attaining lengths up to 25 cm typical of species in such conditions (Nadel and Clerc 2022). Sub-pendent *Usnea* species possess a partially hanging growth form, advantageous in environments benefiting from both air exposure and structural integrity. This sub pendent form serves as a transitional morphology between fully pendent and erect types (Clerc and Otte 2018).

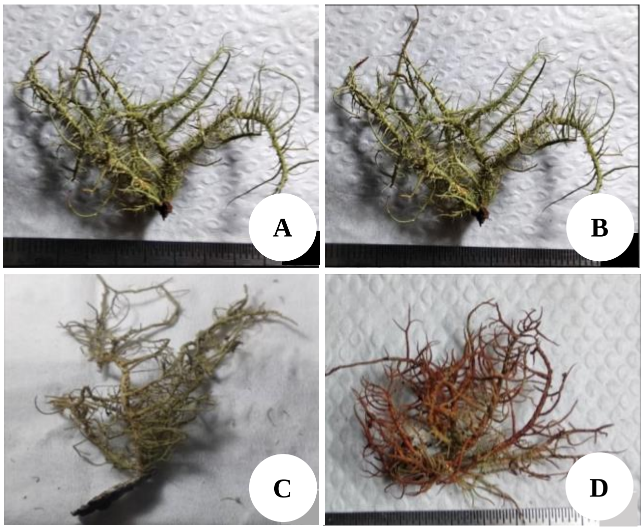

Figure 3. Thallus color representation of *Usnea* found in the Cisanti, Bandung District, West Java, Indonesia: A. Light green in *U. flavocardia*; B. Dark green in *U. sinensis*; C. Yellowish green in *U. lapponica*; and D. Brownish red in *U. rubicunda*

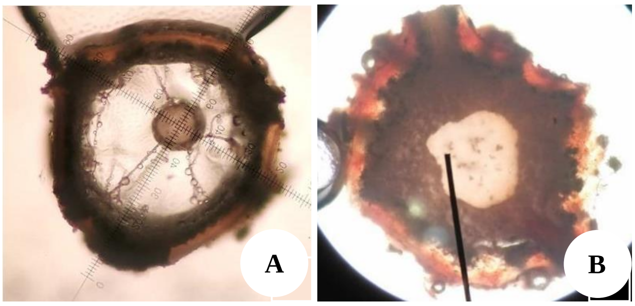

Figure 4. Thallus shape representation of *Usnea* in Cisanti forest area, Bandung District, West Java, Indonesia: A. Round thallus shape in *U. cornuta*; and B. Faceted thallus in *U. maculata*

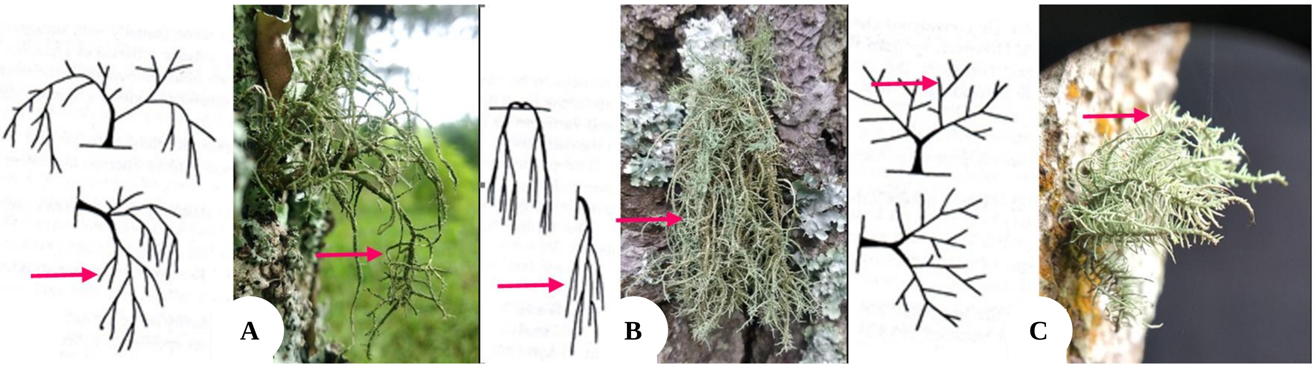

Figure 5. Thallus growth form of *Usnea*: A. Sub pendent form in *U. shimadae*; B. Pendent form in *U. angulata*; C. Erect form in *U. diffracta*

Erect *Usnea* species are characterized by their upright growth, commonly found in more exposed or arid environments where verticality minimizes water loss and enhances wind resistance. *Usnea longiciliata* exemplifies a shrubby, erect form, typically under 6 cm tall, contrasting with the elongated pendent species (Nadel and Clerc 2022). The growth forms of *Usnea* are not merely taxonomic traits but also shape their ecological roles. For example, pendent forms thrive in tropical, humid locales, whereas erect forms are adapted to endure harsher, fluctuating climates (Truong and Clerc 2016). The variety in growth forms underscores the intricate symbiotic relationships within *Usnea*, as different morphologies may sustain distinct microbial communities and secondary metabolites, pivotal for their survival and ecological interactions (Cao et al. 2018).

Thallus base color and lateral thallus branch shape

The thallus base color is either black or white, with 10 species having a black base and 13 species showing a white base (Table 4). Lateral branches exhibit three primary shapes: cylindrical, shrinkage, and widened. Nine species possess cylindrical branches, ten species show a shrinkage shape, and four species have widened branches. The variations in thallus base color and branch morphology serve as significant taxonomic indicators for the identification and classification of *Usnea* species, elucidating the diversity and structural composition of lichen communities in this forested region.

The base of the thallus is the area where the thallus meets the substrate. Lateral branches are thallus that emerge from the main thallus, generally smaller than the main thallus. The base of the lateral branch is the opening between the lateral branch of the thallus and the main thallus. The color of the base and the shape of the lateral branches as the characters for identifying *Usnea* (Molins et al. 2021). The color of the base of the thallus, which is the area where the thallus meets the substrate, is divided into black and white (Figure 6). Lateral branches are divided into three shapes, i.e. cylindrical, contracted, and widened (Figure 7).

Usnea in the Cisanti area reveals intriguing variations in thallus base color and lateral branch morphology. The color of the thallus base in 11 species of *Usnea* is black, while in the other 12 species is white. This variation in thallus base color could be linked to different environmental adaptations or genetic variations. For instance, the color differences might be associated with varying levels of pigmentation, which could influence the species' ability to absorb light and perform photosynthesis efficiently, as seen in other macroalgal studies where thallus morphology impacts photosynthetic capacity (Saco and Ganzon-Fortes 2022). Additionally, the shape of the lateral branches in *Usnea* species from Cisanti is categorized into three distinct forms, i.e. cylindrical, widened, and constricted. Among these, 8 species exhibit cylindrical lateral branches, 11 species have constricted lateral branches, and 4 species display widened lateral branches. The predominance of constricted lateral branches suggests a possible adaptive advantage in the Cisanti environment, potentially related to water retention or mechanical stability, like how the structural features of *Peltigera praetextata* thalli vary across ontogenetic stages to optimize physiological functions like water holding capacity (Androsova and Virolainen 2022).

The variability in branch morphology could also be a response to different ecological pressures, such as light availability or substrate type, which influence growth patterns and structural adaptations in lichens and other thalloid organisms (Hájek et al. 2021). Furthermore, the presence of different branch shapes within the same genus highlights the morphological plasticity of *Usnea*, which may be a crucial factor for survival in diverse habitats. This plasticity is comparable to the variability observed in the thallus structure of *Toniniopsis subincompta*, where different morphotypes are recognized as separate species based on distinct thallus characteristics (Gerasimova et al. 2021).

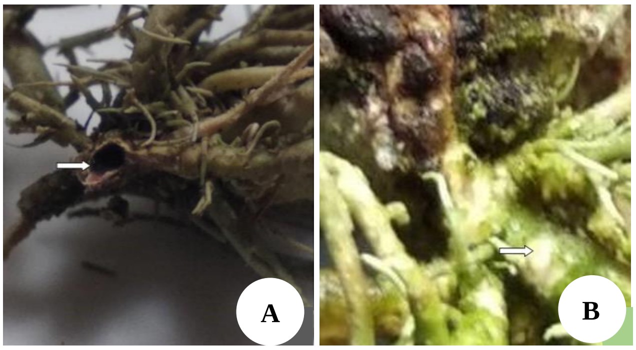

Figure 6. Colors of the base of thallus: A. Black in *U. wasmuthii*; and B. White in *U. flavocardia*

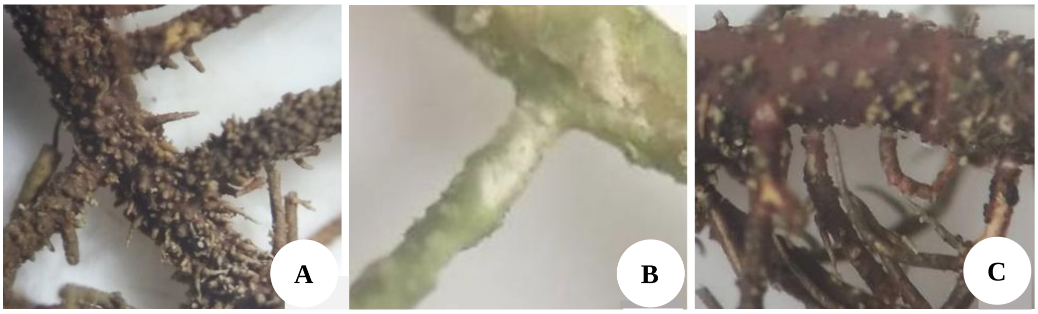

Figure 7. The shape of the lateral thallus branches of *Usnea* in the Cisanti forest area, Bandung District, West Java, Indonesia: A. Cylindrical shape in *U. flammea*; B. Contracted form in *U. chaetophora*; C. Widened shape of *U. hirta*

The dominance of constricted lateral branches in *Usnea* species could be an evolutionary trait that enhances their resilience in the specific microclimatic conditions of the Cisanti area, much like how the thallus morphology of *Pyropia yezoensis* is optimized for efficient spore production and attachment in aquaculture settings (Chen et al. 2020). This study underscores the importance of morphological diversity in lichen adaptation and survival, reflecting broader patterns observed in other thalloid organisms where structural variations are closely tied to ecological and physiological functions

Fibrils

Fibrils are parts of the thallus that emerge from lateral branches, generally smaller and shorter than lateral branches. Fibrils as presented as red arrows in Figure 5 can be used as a taxonomic character to determine the species of *Usnea*. *Usnea* with short fibrils (17 species) dominated *Usnea* with long fibril (6 species) (Table 4).

Comparatively, research in other regions also highlights the significance of fibrils in *Usnea*. For instance, a study in the Republic of São Tomé and Príncipe identified 15 species, including *U. articulata*, *U. baileyi*, *U. beckeri*, *U. bicolorata, U. erinacea, U. exasperata, U. firmula, U. krogiana*, *U. longiciliata, U. nodulosa, U. pectinata*, *U. orediosula*, *U. submollis*, and two undetermined species. Notably, *U. beckeri* and *U. longiciliata* were described as new species, characterized by their dense, brittle thallus and long cilia-like fibrils, which are crucial for their identification (Nadel and Clerc 2022). Similarly, in China, *U. sulphuridiscoidea*, was identified based on its unique morphological characteristics, including numerous papillae and fibrils, further emphasizing the importance of fibrils in *Usnea* taxonomy (Han et al. 2020). The fibrils are composed of a central axis surrounded by a cottony medulla and a dense cortex, which contains usnic acid, a compound that provides UV protection and contributes to the lichen's pale greenish or yellowish-green color (Nybakken and Gauslaa 2007; Ohmura 2014). The central cord or axis of the fibrils is particularly noteworthy for its thickened cell walls, which continue to grow and accumulate layers of electron-dense and electron-transparent materials, as observed in *U. longissima*. This growth pattern is essential for the structural transformation of the thallus, allowing for the development of long axes from corticated branches (Sanders and de Los Ríos 2012).

Cleft, segment, and papillae of thallus

The availability of cleft, segment, and papillae on the thallus of *Usnea* found in the Cisanti forest area, is shown in Table 4. A cleft of thallus is an open area in the thallus caused by the splitting of the cortex to the medulla (Figure 8.A). The segment of thallus is a partition that appears in the thallus, generally shaped like a ring (Figure 8.B). Thallus of *Usnea* which have clefts or segments or having neither is comparable. Papillae are small protrusions on the surface of the thallus (Figure 8.C). The number of papillae varies and can be used as a taxonomic character is the presence or absence of papillae in a species of *Usnea* (Ohmura 2001). Most *Usnea* at Cisanti forest has papillae. Only 3 of 23 species have no papillae, namely *U. flammea, U. hirta*, and *U. subcornuta.*

The presence of clefts, segments, and papillae on the *Usnea* thallus is one of the characteristics used in identifying the *Usnea* species. The effectiveness of those morphological characters for determining the species of *Usnea* was enhanced when combined with molecular data and careful methodological approaches to character coding. This integrated approach allows for a more accurate and comprehensive understanding of *Usnea* species diversity and phylogenetic relationships (Truong and Clerc 2016; Jannah et al. 2021).

Reproductive structure

The result reveals a diverse lichen community in the Cisanti forest area, with a notable distinction in vegetative reproduction structure, isidia, and soredia (Figure 9). Specifically, 12 species of *Usnea* in the Cisanti forest area possess isidia vegetative reproduction organs, and the rest 11 species exhibit soredia vegetative reproduction organs (Table 4). Interestingly, no *Usnea* species in the Cisanti forest area have been identified with generative reproduction organs of apothecia.

Isidia represents diminutive outgrowths on the lichen thallus, comprising both fungal and algal cellular constituents. These structures play a crucial role in vegetative reproduction and facilitate the dispersal of the lichen by detaching and forming new colonies in distant locales. Soredia are defined as fine, powdery propagules encapsulating a limited number of algal cells ensconced within fungal hyphae. Soredia likewise contribute to vegetative reproduction and exhibit a greater prevalence than isidia within *Usnea* species. Apothecia constitute the sexual reproductive formations of *Usnea*. These structures are typically positioned laterally or terminally, characterized by a thalloid, fibrillose margin, and a pale disk. The spores generated are hyaline and nonseptate (McCune 2005).

This finding aligns with the broader research on *Usnea* species in other regions, such as the biodiversity hotspot of São Tomé and Príncipe in tropical West Africa, where 15 species were recorded, including new species like *U. beckeri* and *U. longiciliata*, characterized by unique morphological features and reproductive structures (Nadel and Clerc 2022). The absence of apothecia in the Cisanti area contrasts with the findings in São Tomé and Príncipe, where different reproductive structures were noted. However, the absence of apothecia in *Usnea* was also reported by Jayalal et al. (2013) who studied morphological and anatomical-based identification of *Usnea* collection at the herbarium of the Korean Lichen Research Institute. The reproductive apothecia serve as a crucial determinant for the identification of species at the taxonomic level; however, the extended duration of growth throughout the life cycle presents a significant challenge to identification (Clerc and Naciri 2021) due to it rarely observed (McCune 2005).

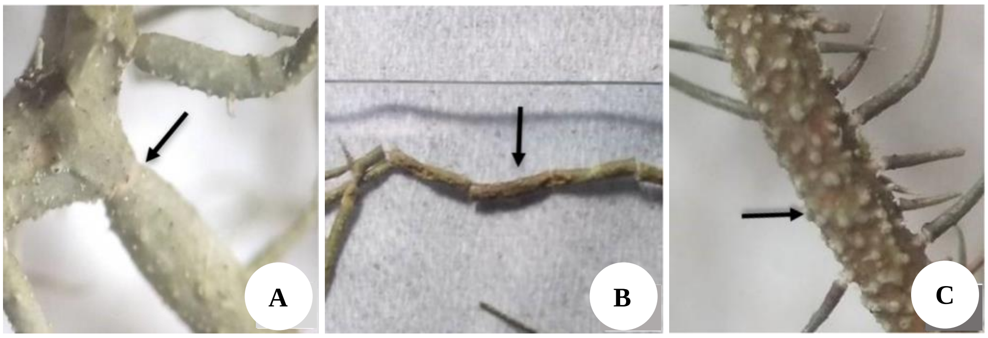

Figure 8. *Usnea* thallus in Cisanti forest area, Bandung District, West Java, Indonesia: A. Cleft in *U. subcornuta*; B. Segment in *U. hirta*; and C. Papillae in *U. subcornuta*

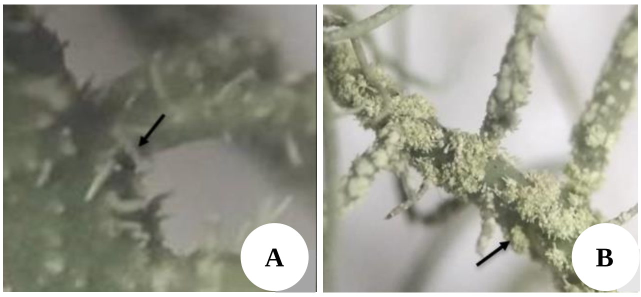

Figure 9. *Usnea* vegetative reproduction organ: A. Isidia in *U. dasaea*; and B. Soredia in *U. flavocardia*

Anatomical characters

Thallus thickness ratio

Thrower (1988) used the ratio of Cortex thickness (C), Medulla thickness (M), and Axis diameter (A) as taxonomic characters to determine the species of *Usnea* based on anatomical characters (Table 5). The thickness of the cortex layer is determined based on the middle (median) value of the thickness of the layer. *Usnea* cortical thickness in Cisanti ranges 4-20 µm so the middle value is 12 µm. Therefore, the thin cortex with a thickness of ≤12 µm consists of 18 species, while, the thick cortex with a thickness of ≥12 µm consists of 5 species (Table 5). The thickness of the *Usnea* medulla in the Cisanti area ranges from 7-28 µm with an average value of 14 µm, so it is divided into groups with thick medulla (>14 µm) and groups with thin (≤14 µm). *Usnea* with thin medulla consists of 12 species. Meanwhile, *Usnea* with thick medulla includes 11 species (Table 5). Meanwhile, the diameter of the thallus axis of *Usnea* in the Cisanti area ranges from 7-47 µm with a median value of 21 µm. Based on the median value of the axis diameter, 11 species of *Usnea* are grouped into small axis (≤21 µm) and 12 species with large axis diameters (≥21 µm).

Based on the cortex and medulla characteristics, *Usnea* in the Cisanti area is dominated by thin categories with 18 species of *Usnea* having a thin cortex, and 12 species of thin medulla. Based on the axis diameter, *Usnea* with a large axis diameter (≥21 µm) is dominant with 12 species. This categorization is crucial for understanding the ecological and physiological adaptations of these species. For instance, the dominance of *Usnea* with thin cortex and medulla, comprising 18 species with thin cortex and 12 species with thin medullary layers, suggests a possible adaptation to the local environmental conditions, such as humidity and air quality, which are essential for the growth and development of *Usnea* species (Aristarkhova et al. 2022).

The *Usnea* thallus, a lichen characterized by its fruticose and tufted morphology, exhibits a complex internal structure comprising a cortex, medulla, and central axis. The cortex, which contains usnic acid and various other lichen substances, forms the outermost layer and is dense and protective (Miller 2011). The medulla, a cottony layer beneath the cortex, surrounds the central axis, which is a dense cord of fungal cells that continually deposit wall layers of electron-transparent and electron-dense materials, contributing to the structural integrity of the thallus (Sanders and de Los Ríos 2012). This study provides valuable insights into *Usnea* diversity based on thallus characteristics in the Cisanti area.

Medulla structure and axis shape

The medulla of the *Usnea* thallus comprises layers of algae and a network of hyphae. The *Usnea* medulla is distinguished into three types, i.e. loose, dense, and compact (Figure 10). *Usnea* in the Cisanti area has all three types of medullary structures. *Usnea* loosely includes 8 species, namely *U. barbata*, *U. ceratina*, *U. chaetophora*, *U. flavocardia*, *U. hirta*, *U. pangiana*, *U. subcornuta*, and *U. wasmuthii*. *Usnea* with dense medulla includes 11 species, namely *U. cornuta*, *U. dasaea*, *U. diffracta*, *U. lapponica*, *U. maculata*, *U. robusta*, *U. rubicunda*, *U. Shimadae*, *U. sinensis*, *U. Steineri*, and *U. subflorida*. Compact *Usnea* includes 4 species, *U. baileyi*, *U. angulata*, *U. mutabilis*, and *U. flammea*. A dense medulla or tightness occurs because the hyphae are very tightly woven together, while a loose medulla, as in *Usnea pangiana*, is caused by the hyphae not being tightly woven together.

The shape of the *Usnea* axis that can be divided into round and hollow is also used as a character for identification. *Usnea* in the Cisanti area with a round axis includes 19 species, namely *U. barbata*, *U. caetophora*, *U. ceratina*, *U. cornuta*, *U. dasaea*, *U. diffracta*, *U. flammea*, *U. flavocardia*, *U. hirta*, *U. lapponica*, *U. maculata*, *U. pangiana*, *U. robusta*, *U. rubicunda*, *U. shimadae*, *U. subcornuta*, *U. subflorida*, and *U. wasmuthii*. *Usnea* with a hollow axis includes 4 species, including *U. angulata*, *U. baileyi*, *U. mutabilis*, and *U. sinensis*.

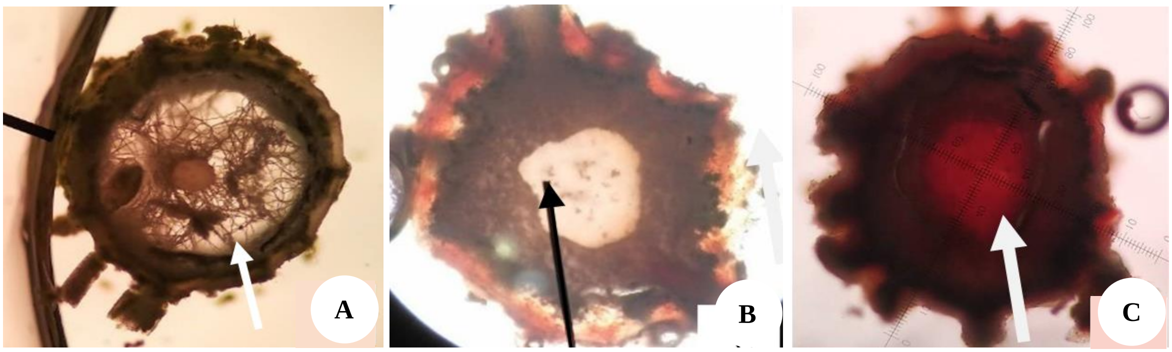

Figure 10. Structure of the medulla (arrow) of *Usnea* in the Cisanti Forest Area, Bandung District, West Java, Indonesia: A. Loose medulla in *U. pangiana*; B. Dense medulla in *U. rubicunda*; and C. Compact medulla in *U. angulata*

Table 5. Anatomical characters of the *Usnea* in the Cisanti pine forest area, Bandung District, West Java, Indonesia

Species	**Cortex (µm)**	**Medulla (µm)**	**Axis (µm)**	**Thallus (µm)**	**Ratio (C:M:A)**
U. angulata Ach. 1814	7	25	11	95	7:25:11
U. baileyi (Stirt.) Zahlbr. 1909	6	10	29	90	6:10:29
U. barbata (L.) Weber ex F.H.Wigg 1780	7	10	29	101	7:10:29
U. ceratina Ach. 1810	9	22	18	94	9:22:18
U. chaetophora Stirt. 1883	4	20	8	68	1:5:2
U. cornuta Körb. 1859	20	15	42	105	20:15:42
U. dasaea Stirt. 1881	14	14	21	78	2:2:3
U. diffracta Vain. 1921	15	18	24	106	5:6:8
U. flammea Stirt. 1881	6	9	12	43	2:3:4
U. flavocardia Räsänen 1936	4	10	15	44	4:10:15
U. hirta (L.) F.H.Wigg. 1780	5	25	22	78	5:25:22
U. lapponica Vain. 1925	8	10	19	60	8:10:19
U. maculata Stirt. 1882	4	11	16	47	4:11:16
U. mutabilis Stirt. 1881	7	8	21	60	7:8:21
U. pangiana Stirt. 1883	5	15	7	93	5:15:7
U. robusta Stirt. 1882	5	7	10	48	5:7:10
U. rubicunda Stirt. 1881	9	20	47	105	9:20:47
U. shimadae Asahina 1970	7	14	14	60	1:2:2
U. sinensis Motyka 1936	9	8	37	111	9:8:37
U. steineri Zahlbr. 1930	17	9	18	60	17:9:18
U. subcornuta Stirt. 1883	9	22	33	97	9:22:33
U. subflorida Motyka 1937	10	8	31	82	10:8:31
U. wasmuthii Räsänen 1932	7	28	38	74	7:28:38

The medulla structure and axis shape of the *Usnea* thallus exhibit significant complexity and variability across different species. The genus *Usnea*, characterized by a fruticose morphology, typically features a central axis surrounded by a medulla and a cortex, with the presence of usnic acid in the cortex being a common trait. The central axis, often referred to as the central cord, is dense and cartilaginous, providing structural support to the thallus. This axis is surrounded by a cottony medulla, which is less dense and serves as a cushion-like layer (Ohmura 2014). In *U. longissima*, the central cord exhibits unique features such as cubic and needle-like secretions, densely distributed holes, and vessel-like structures, which are analog to plant vessels, indicating a complex internal organization not commonly observed in other *Usnea* species (He et al. 2012). The medulla is composed of loosely interwoven hyphae, which can vary in thickness and density among different species. For instance, in *U. aurantiaco-atra* and *U. sphacelata*, the Medulla Thickness (MT) and Central Cord Area (CCA) differ significantly, with *U. aurantiaco-atra* having a larger CCA compared to *U. sphacelata* (Hájek et al. 2021). The medulla also houses the symbiotic algae, *Trebouxia*, which is distributed throughout the algal layer (Hájek et al. 2021). The anatomy of the thallus, including the medulla and central axis, can be influenced by environmental factors such as altitude and climate, as seen in the distinct morphological adaptations of *Usnea* species in different geographic regions (Rajeshwari et al. 2020). Additionally, the central axis in some *Usnea* can produce filamentous outgrowths consisting solely of mycobiont tissue, which resemble the natural thallus structure and contain usnic acid, further highlighting the role of the mycobiont in lichen morphogenesis (Mark et al. 2016). The sustained diffuse growth of the central cord in species like *U. longissima* leads to the accumulation of thickened cell walls, which undergo structural transformations over time, contributing to the overall morphology of the thallus (Sanders and de Los Ríos 2012).

The pigment of thallus

The pigment is a color found in the inner part of the thallus and can be seen by cutting the thallus transversely. The color pigment in *Usnea* is a special character to distinguish between species (Noer et al. 2015). The pigment colors of the thallus of the *Usnea* in this study (Figure 11) are white or non-pigmented pigments of 6 species including *U. ceratina, U. chaetophora, U. diffracta, U. flavocardia, U. lapponica*, and *U. subcornuta*. *Usnea* with red pigments of 6 species, *U. angulata, U. baileyi, U. flammea, U. mutabilis, U. robusta*, and *U. subflorida*. *Usnea* with orange pigments includes 5 species, *U. hirta, U. maculate, U. rubicunda, U. shimadae,* and *U. steineri*. *Usnea* with yellow pigment includes 5 species, *U. barbata, U. cornuta, U. dasaea, U. sinensis*, and *U. wasmuthii*. *Usnea* with pink pigment is only found in 1 species, *U. pangiana.*

The pigment of the *Usnea* thallus, primarily usnic acid, is a significant feature of this lichen genus, contributing to its characteristic coloration and various physiological activities. Usnic acid, a yellow pigment, is consistently present in the cortex of *Usnea* species and is a key diagnostic feature of the genus. This pigment not only imparts a pale greenish or yellowish-green hue to the thallus but also plays a role in the lichen's defense mechanisms against herbivory and microbial attack (Ohmura 2014). The pigment stability in *Usnea* species can be influenced by various environmental factors such as pH, temperature, illumination, and presence of metal ions and food additives (Hájek et al. 2021).

Chemical character *Usnea*

Spot test (color test)

The spot test showed that all 23 *Usnea* species reacted positively to reagent K (producing a yellow or reddish yellow color in the cortex and/or medulla) and reacted negatively to reagent C. Eight species of *Usnea* reacted positively to K and KC reagents, namely *U. dasaea, U. diffracta, U. mutabilis, U. pangiana, U. rubicunda, U. shimadae, U. steineri,* and *U. subcornuta* (Table 6).

Microcrystal test

Lychenic acid found in *Usnea* in the Cisanti area is dominated by usnic acid with 16 species (Table 6). According to Noer et al. (2015), usnic acid is a lychenic acid commonly contained in *Usnea*. Meanwhile, acids that are rarely found are eumitrin acid, thamnolic acid, and squamatic acid.

The chemical character of *Usnea* species plays a crucial role in their taxonomy, aiding in the differentiation and classification of various species within this hyper-diverse genus. *Usnea* is known for their complex phytochemistry, which includes a wide array of secondary metabolites, such as depsides, depsidones, depsones, lactones, quinones, phenolics, polysaccharides, fatty acids, and dibenzofurans (Salgado et al. 2018). These compounds are not only significant for their ecological roles but also for their utility in chemotaxonomic studies. For instance, the presence of specific compounds like usnic acid, polyphenols, and depsides has been linked to antibacterial and antifungal activities, which are pivotal in distinguishing *Usnea* species (Prateeksha et al. 2016). Salgado et al. (2018) employed advanced techniques, such as UHPLC-ESI-OT-MS-MS to identify and characterize these metabolites, revealing a total of 73 metabolites in species like *U. barbata, U. antarctica, U. rubicunda, and U. subfloridana*, with some metabolites being reported for the first time.

Table 6. Thallus pigment and lychenic acids content of *Usnea* in the Cisanti forest area, Bandung District, West Java, Indonesia

Species	**Pigment of thallus**	**Microcrystal as stated in Table 3**
Usnea angulata Ach. 1814	Red	Nor, Cap
Usnea baileyi (Stirt.) Zahlbr. 1909	Red	Hae, Phy, Bar, Fum
Usnea barbata (L.) Weber ex F.H.Wigg 1780	Yellow	Usc, Sal, Pro
Usnea ceratina Ach. 1810	White	Hae, Pla, Atr, Gan, Bar, Dif
Usnea chaetophora Stirt. 1883	White	Usc, Phy, Atr, Iso, Per,
Usnea cornuta Körb. 1859	Yellow	Usc, Hae, Iso, Sal, Pro
Usnea dasaea Stirt. 1881	Yellow	Usc, Sal, Nor, Pso
Usnea diffracta Vain. 1921	White	Atr, Bar, Iso, Dif
Usnea flammea Stirt. 1881	Red	Usc, Sti, Lob
Usnea flavocardia Räsänen 1936	White	Usc, Atr, Gal, Nor, Pso
Usnea hirta (L.) F.H.Wigg. 1780	Orange	Usc, Hae, Nor
Usnea lapponica Vain. 1925	White	Usc, Atr, Bar, Sal
Usnea maculata Stirt. 1882	Orange	Usc, Phy, Pro
Usnea mutabilis Stirt. 1881	Red	Usc, Nor, Eum
Usnea pangiana Stirt. 1883	Pink	Hae, Atr, Gal, Bar, Iso, Ret, Con
Usnea robusta Stirt. 1882	Red	Usc, Atr, Gal, Bar, Iso, Sal
Usnea rubicunda Stirt. 1881	Orange	Usc, Hae, Pla, Atr, Gal, Gan, Sal, Nor
Usnea shimadae Asahina 1970	Orange	Usc, Atr, Sal, Nor
Usnea sinensis Motyka 1936	Yellow	Usc, Iso, Sal
Usnea steineri Zahlbr. 1930	Orange	Usc, Dif, Cap
Usnea subcornuta Stirt. 1883	White	Usc, Hae, Atr, Gan, Iso, Nor
Usnea subflorida Motyka 1937	Red	Hae, Phy, Atr,Gal, Squ, Tha
Usnea wasmuthii Räsänen 1932	Yellow	Hae, Bar,

Note: Atr: Atranorin acid; Bar: Barbatic acid; Cap: Caperatic acid; Con: Constictic acid; Dif: Diffractaic acid; Eum: Eumitrin acid; Fum: Fumarprotocetraric acid; Gal: Galapagin acid; Gan: Gangaleoidin acid; Hae: Haemathamnolic acid; Iso: Isousnic acid; Lob: Lobaric acid; Nor: Norstic acid; Per: Perlatolic acid; Phy: Physodalic acid; Pla: Placodiolic acid; Pros: Protocetraric acid; Pso: Psoromic acid; Ret: Retigeric acid; Sal: Salazinic acid; Squ: Squamatic acid; Sti: Stictic acid; Usc: Usnic acid

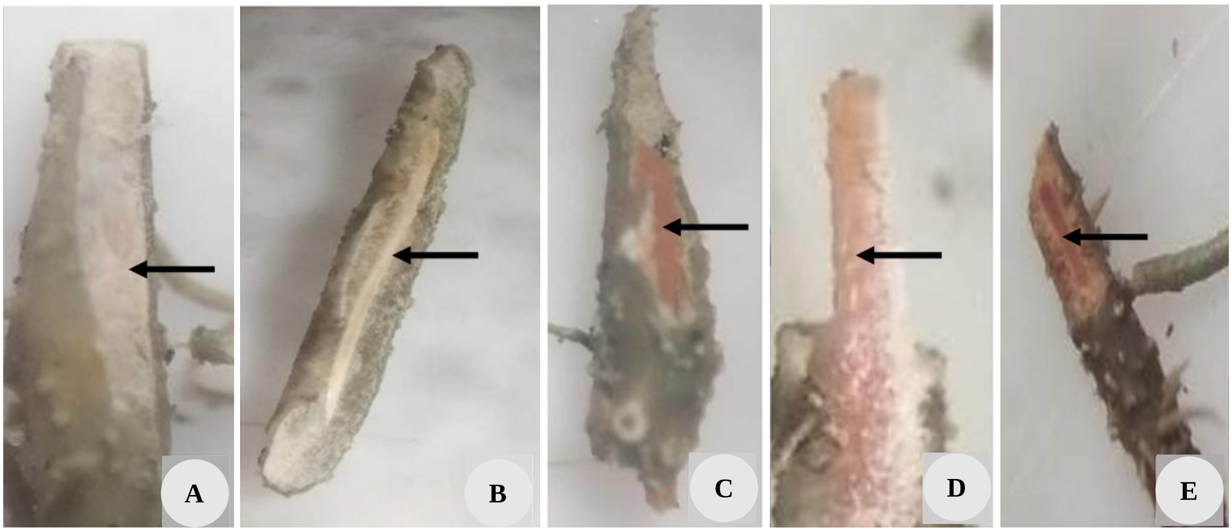

Figure 11. The colors of thallus pigment of *Usnea* in the Cisanti forest area, Bandung District, West Java, Indonesia: A. White in *U. lapponica*; B. Yellow in *U. barbata*; C. Orange in *U. shimadae*; D. Pink in *U. pangiana*; and E. Red in *U. mutabilis*

Molecular data, including ITS rDNA, nuLSU, RPB1, and Mcm7 markers, have further supported the chemical differentiation of species, as presented in *Usnea clerciana, U. aranea, U. subaranea, U. rubriglabrata,* and *U. subglabrata,* which exhibit unique chemical profiles (Truong and Clerc 2016). Additionally, the study of *U. pectinata* aggregate has shown considerable genetic and chemical variation, with six distinct chemotypes observed, indicating the potential for these chemotypes to represent separate species within the complex (Temu et al. 2022). The discovery of new species such as *U. oreophila*, characterized by a complex chemistry with squamatic acid as the main medullary compound, further underscores the importance of chemical traits in taxonomy (da Cruz Lima Gerlach et al. 2019). Similarly, *U. viktoriana* is distinguished by the presence of alectorialic acid, highlighting the role of specific secondary compounds in species identification (da Cruz Lima Gerlach et al. 2019). In Brazil, the identification of *Usnea* species has been enhanced by detailed chemical analyses, with species like *U. grandispora and U. subparvula* being characterized by their unique chemical compositions, including protocetraric and salazinic acids (da Cruz Lima Gerlach et al. 2017). The integration of chemical data with morphological and molecular analyses provides a comprehensive approach to the taxonomy of *Usnea*, enabling the accurate classification and discovery of new species within this diverse genus.

Phenetic relationships

The similarity coefficient value represents similarity index among species of *Usnea* is presented in Table 7 and dendrogram of phenetic relationships is presented in Figure 12. *Usnea* phenetic relations found in Cisanti are divided into three main branches and separated into subgenera, namely *Usnea, Eumitria,* and *Dolichousnea.* The subgenus *Usnea* (cluster I) is characterized by a solid axis, such as *U. rubicunda* and *U. chaetophora,* the subgenus *Eumitria* (cluster II) is characterized by a hollow axis, for example, *U. baileyi* and *U. wasmuthii.* The subgenus *Dolichousnea* (cluster III) is characterized by transverse clefts or segments in its thallus, for example *U. pangiana*, *U. ceratina,* and *U. diffracta.*

The relationship among 23 species of *Usnea* can be seen from their similarity coefficient values. The genus *Usnea* exhibits significant phenetic relationships based on morphology, anatomy, and chemical characteristics. The phenetic relationship between Cluster I (subgenus *Usnea*) and Cluster II (subgenus *Eumitria*) is characterized by a similarity index of 0.634, indicating a relatively close relationship between these two subgenera. This is supported by molecular studies, such as ITS rDNA sequences (Lücking et al. 2020; Jannah et al. 2021). However, the separation of these two subgenera from Cluster III (subgenus *Dolichousnea*) is marked by a slightly lower similarity index of 0.627, suggesting a more distinct divergence. This divergence is further corroborated by the comprehensive analysis of ITS barcoding markers, which shows that while *Dolichousnea* and subgenus *Neuropogon* are well-represented, *Eumitria* and *Usnea* are less simple and exhibit different evolutionary patterns (Lücking et al. 2020).

Morphologically, *Usnea* species are characterized by their shrubby thallus, which can vary significantly among species, leading to challenges in species identification based solely on morphology (Jannah et al. 2021; Aristarkhova et al. 2022). For instance, *U. nipparensis* and *U. sinensis*, despite their different thallus forms, share similar detailed morphological and chemical features, indicating a close relationship within the genus (Ohmura 2020). Additionally, the presence of secondary metabolites, such as lichen acids, flavonoids, and saponins in *Usnea* species further aids in their identification and classification, with certain species like *U. esperantiana* showing a high diversity of lichen acids (Jannah et al. 2022).

Table 7. Matrix of similarity coefficient among species of *Usnea* in Cisanti forest area, Bandung District, West Java, Indonesia

Species	*U. angulata*	*U. baileyi*	*U. barbata*	*U. ceratina*	*U. chaetophora*	*U. cornuta*	*U. dasaea*	*U. diffracta*	*U. flammea*	*U. flavocardia*	*U. hirta*	*U. lapponica*	*U. maculata*	*U. mutabilis*	*U. pangiana*	*U. robusta*	*U. rubicunda*	*U. shimadae*	*U. sinensis*	*U. steineri*	*U. subcornuta*	*U. subflorida*	*U. wasmuthii*
U. angulata	1.000																						
U. baileyi	0.672	1.000																					
U. barbata	0.681	**0.616**	1.000																				
U. ceratina	0.622	0.756	0.620	1.000																			
U. chaetophora	**0.616**	0.624	0.733	0.628	1.000																		
U. cornuta	0.678	0.728	0.734	0.639	0.613	1.000																	
U. dasaea	0.637	0.629	0.642	0.674	0.738	0.736	1.000																
U. diffracta	0.621	0.789	0.626	0.654	0.713	0.654	0.716	1.000															
U. flammea	0.721	0.704	0.663	0.619	0.653	0.747	0.776	0.660	1.000														
U. flavocardia	0.680	0.683	0.673	0.768	0.705	0.792	0.739	0.714	0.743	1.000													
U. hirta	0.637	0.653	0.714	0.619	0.619	0.650	0.745	0.718	0.800	0.744	1.000												
U. lapponica	0.680	0.755	**0.814**	0.795	0.715	0.715	0.700	0.715	0.630	0.773	0.645	1.000											
U. maculata	0.670	0.691	0.798	0.694	0.786	0.718	0.791	0.731	0.774	0.795	0.669	0.718	1.000										
U. mutabilis	0.767	0.714	0.620	0.795	0.664	0.714	0.729	0.730	0.632	0.771	0.786	0.746	0.622	1.000									
U. pangiana	**0.616**	0.624	0.642	0.721	0.733	0.645	0.685	0.686	0.723	0.622	0.741	0.662	0.687	0.707	1.000								
U. robusta	0.679	0.724	0.632	0.748	0.626	0.791	0.721	0.779	0.625	0.672	0.636	0.680	0.662	0.779	0.677	1.000							
U. rubicunda	0.637	0.722	0.731	0.608	0.743	0.689	0.634	0.674	0.770	0.746	0.633	0.642	0.739	0.798	0.718	0.791	1.000						
U. shimadae	0.636	0.723	0.651	0.657	0.658	0.769	0.660	0.687	0.761	0.634	0.724	0.637	0.676	0.643	0.766	0.737	0.704	1.000					
U. sinensis	0.743	0.789	0.693	0.624	0.637	0.740	0.732	0.778	0.714	0.704	0.715	0.789	**0.814**	0.733	0.726	0.643	0.625	0.656	1.000				
U. steineri	0.743	0.736	0.649	0.659	0.717	0.659	0.658	0.761	0.681	0.631	0.648	0.748	0.625	0.653	0.775	0.789	0.641	0.717	0.626	1.000			
U. subcornuta	**0.616**	0.672	0.632	0.624	0.604	0.763	0.724	0.741	0.624	0.640	0.787	0.698	0.623	0.624	0.655	0.746	0.685	0.773	0.743	0.637	1.000		
U. subflorida	0.672	0.687	0.622	0.664	0.766	0.679	0.686	0.620	0.739	0.624	0.790	0.645	0.736	0.752	0.760	0.651	0.675	0.624	0.679	0.629	0.671	1.000	
U. wasmuthii	0.671	0.740	0.731	0.683	0.631	0.776	0.627	0.784	0.691	0.759	0.707	0.651	0.691	0.624	0.637	0.643	0.693	0.703	0.713	0.698	0.788	0.753	1.000

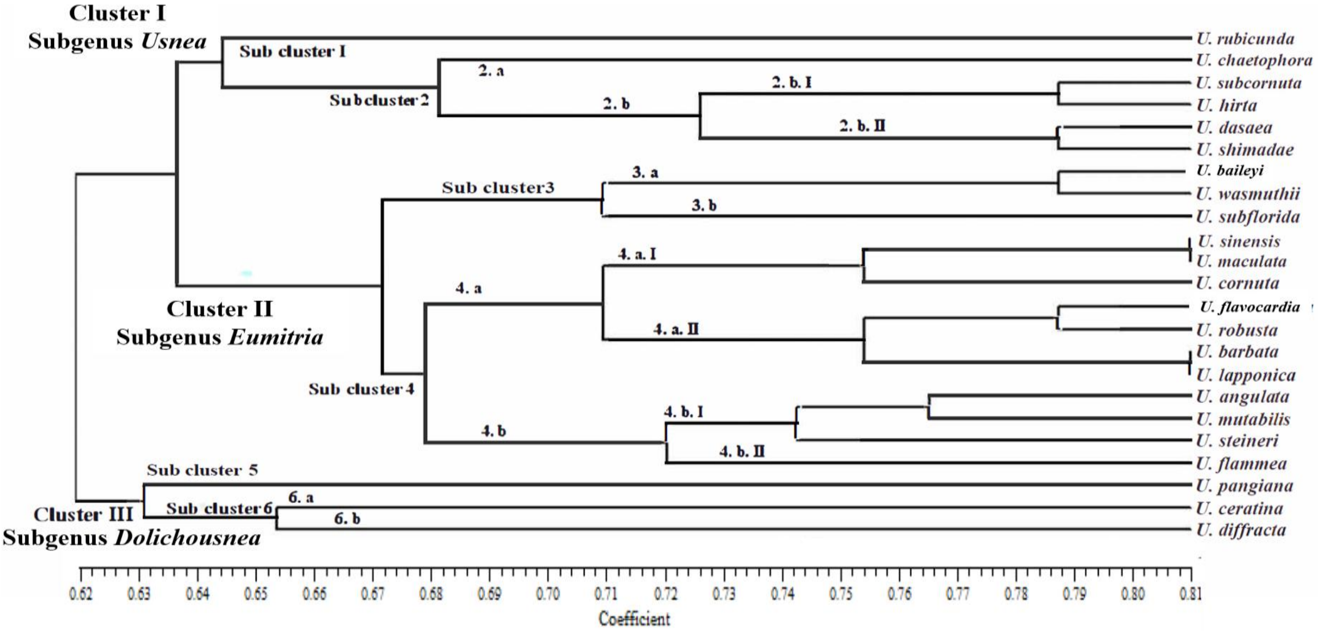

Figure 12. Dendrogram of phenetic relationships among 23 *Usnea* species found in the Cisanti pine forest area, Bandung District, West Java, Indonesia

The ecological distribution of *Usnea* species also plays a role in their phenetic relationships, as seen in the Moscow region where species like *U. hirta* and *U. dasopoga* are widespread, while others like *U. lapponica* and *U. glabrescens* are rare, reflecting their adaptation to specific microclimatic conditions and minimal pollution levels (Aristarkhova et al. 2022). The use of multiple genetic markers, such as IGS and RPB1, alongside ITS, enhances the resolution of phylogenetic trees and helps in distinguishing closely related species and resolving in *U. cornuta* complex (Lücking et al. 2020). Furthermore, the discovery of new species like *U. sulphuridiscoidea*, based on unique morphological characteristics and ITS sequence data, highlights the ongoing need for detailed morphological and molecular studies to fully understand the diversity and relationships within the genus (Han et al. 2020). The phenetic relationships within the genus *Usnea* are complex and multifaceted, requiring a combination of morphological, anatomical, chemical, and molecular data to accurately delineate species and understand their evolutionary relationships (Lücking et al. 2020; Ohmura 2020; Han et al. 2020; Jannah et al. 2021, 2022; Aristarkhova et al. 2022).

Cluster I is divided into two distinct subclusters. Subcluster 1 is exclusively comprised of *U. rubicunda*, which exhibits a similarity index of 0.641 with subcluster 2, differing in 17 distinct characters and highlighting its unique evolutionary path. Sub-subcluster 2.A is represented only by *U. chaetophora*, which shares a similarity index of 0.68 with sub-subcluster 2.B, suggesting a moderate evolutionary relationship. Sub-subcluster 2B is divided further into two groups: sub-subcluster 2.B.I, which includes *U. subcornuta* and *U. hirta* sharing a high similarity index of 0.79 and differing in seven specific characters, including growth form, lateral branches, segments, reproduction, pigment, KC spot test, and lichenic acid; and sub-subcluster 2.B.II, comprising *U. dasaea* and *U. shimadae*, which also display a high similarity index of 0.79, indicating a close evolutionary relationship. This hierarchical structure reflects the varying degrees of similarity and divergence among species within Cluster I.

Those characters are critical in distinguishing species within the genus *Rubus*, as highlighted by the extensive morphological and molecular analyses conducted in recent studies (Ohmura 2012; Ferrer-Gallego and Van de Beek 2022; Huang et al. 2023). The high similarity index within sub subcluster 2.B.I indicates a close evolutionary relationship, likely reflecting recent divergence or ongoing gene flow between these species. Conversely, sub subcluster 2.B.II, comprising *U. dasaea* and *U. shimadae*, also shares a similarity index of 0.79, suggesting a similarly close relationship. The detailed phylogenetic analysis and character evolution studies within *Rubus* have shown that such high similarity indices often correlate with monophyletic groupings, where species share a common ancestor and exhibit minimal divergence in key morphological and genetic traits (Aukhadieva et al. 2021; Huang et al. 2023). The use of molecular data, such as chloroplast genomic sequences, alongside traditional morphological characters, has been instrumental in resolving these complex taxonomic relationships and ensuring accurate classification within the genus (Huang et al. 2023). The findings from these studies emphasize the importance of comprehensive taxonomic revisions and the need for stability in nomenclature to avoid confusion and maintain consistency in species identification and classification. The detailed examination of Cluster I and its sub subclusters highlights the intricate evolutionary dynamics within the *Usnea* genus and underscores the value of combining morphological and molecular data to elucidate phylogenetic relationships accurately.

Cluster II consists of two subclusters, namely subclusters 3 and 4, highlighting intricate phenetic relationships within the genus *Usnea* based on morphology, anatomy, and chemical traits. Sub-subcluster 3.a includes *U. baileyi* and *U. wasmuthii*, which share a high similarity value of 0.79, indicating close phenetic ties. In contrast, sub-subcluster 3.B, represented by *U. subflorida*, exhibits a lower similarity of 0.71 with Sub-subcluster 3.A, differing in ten distinct characters such as thallus color, growth form, and lichenic acid, reflecting significant divergence. Subcluster 4 is also divided into Sub-subclusters 4.A and 4.B, with Sub-subcluster 4.A further branching into 4.A.I and 4.A.II. Sub-subcluster 4.A.I includes *U. sinensis* and *U. maculata*, which share a high similarity value of 0.81, differing in five characters, and *U. cornuta*, which closely aligns with a similarity value of 0.755. Sub-subcluster 4.A.II comprises *U. flavocardia* and *U. robusta* (similarity 0.792) and *U. barbata* and *U. lapponica* (similarity 0.81), underscoring their phenetic coherence. Sub-subcluster 4.b is divided into 4.B.I and 4.B.II, with 4.B.I consisting of *U. angulata*, *U. mutabilis*, and *U. steineri*, and 4.B.II represented by *U. flammea*. Sub-subcluster 4.B.I shows a similarity value of 0.767 between *U. angulata* and *U. mutabilis*, differing in eight characters, while *U. steineri* aligns more distantly with a similarity of 0.744. These findings showed the complex phenetic relationships within the genus *Usnea*, driven by a combination of morphological, anatomical, and chemical characteristics, and highlight the importance of detailed phenetic analysis in understanding species relationships and taxonomy within this diverse genus (da Cruz Lima Gerlach et al. 2020; Aukhadieva et al. 2021; Clerc and Naciri 2021).

Cluster III comprises subclusters 5 (*U. pangiana*) and 6 (*U. ceratina* and *U. diffracta*) showed a moderate similarity value of 0.632 between the two subclusters indicating a genetic connection. Within Subcluster 6 share a higher similarity of 0.654, reflecting closer phenetic ties. These species differ in 11 key traits, including thallus color, branching shape, fibrils, reproduction, and lichenic acid presence, highlighting their distinct yet related characteristics. These morphological and biochemical traits are crucial for distinguishing between closely related species, as demonstrated in other studies where DNA markers and morphological data are used in phylogenetic relationships. The application of High-Throughput Sequencing (HTS) is supported to clarify taxonomic ambiguities (Hughey et al. 2022). The integration of morphological, biochemical, and genetic data provides a comprehensive framework for understanding the evolutionary relationships and distinguishing characteristics of *U. pangiana, U. ceratina*, and *U. diffracta*, thereby enhancing our knowledge of their phylogenetic placement and contributing to more accurate taxonomic classifications.

In conclusion, this research showed that *Usnea* in the Cisanti forest area has 41 combined characters based on morphological, anatomical, and chemical parameters. Based on these characteristics, *Usnea* in the Cisanti pine forest area consists of 23 species, namely *U. rubicunda, U. baileyi, U. chaetophora, U. pangiana, U. sinensis, U. subcornuta, U. ceratina, U. subflorida, U. maculata, U. flavocardia, U. wasmuthii, U. angulata, U. barbata, U. hirta, U. steineri, U. dasaea, U. shimadae, U. mutabilis, U. lapponica, U. flammea, U. cornuta, U. robusta,* and *U. diffracta.* The phenetic relationships among 23 species of *Usnea* resulted 3 clusters, Cluster I (subgenus *Usnea*) consists of 6 species, cluster II (subgenus *Eumitria*) consists of 14 species, and cluster III (subgenus *Dolichousnea*) consists of 3 species. In general, cluster separation corresponds with subgenera. The closest relationship level is between *U. sinensis* and *U. maculata* as well as *U. barbata* and *U. lapponica* which has a similarity index of 0.814. Meanwhile, the most distant relationship level is the relationship between *U. baileyi* and *U.* barbata; and between *U. angulata* and *U. chaetophora, U. subcornuta*, and *U. pangiana* with a similarity index of 0.616.

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