

SABRAO Journal of Breeding and Genetics  
 57 (6) 2637-2647, 2025  
<http://doi.org/10.54910/sabrao2025.57.6.36>  
<http://sabraojournal.org/>  
 pISSN 1029-7073; eISSN 2224-8978



## ***USNEA* DILL. EX ADANS. (PARMELIACEAE, LECANORALES) WITH NEW SPECIES RECORDS IN WEST JAVA, INDONESIA**

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### **SUMMARY**

The *Usnea* Dill. ex Adans is the second largest genus within the family Parmeliaceae, with enormous diversity in Indonesia comprising approximately 30 species. Despite this substantial diversity, relatively few taxonomic studies have progressed on *Usnea* in Indonesia. This study aimed to document the diversity of *Usnea* species in West Java and to report newly recorded taxa from the region. During the field expedition conducted from September 2023 to March 2024 in West Java, Indonesia, the identification of 18 species of *Usnea* was successful, representing three subgenera: *Usnea* Dill. ex Adans, *Eumitria* Stirt, and *Dolichousnea* (Y. Ohmura) Articus. Among these species, five species were newly documented specimens in West Java. Species identification depended on morphological, anatomical, and microchemical analyses, including spot tests and microcrystal observations. These findings significantly enhanced the taxonomic understanding of *Usnea* in the region and suggested the unexplored diversity in montane forest ecosystems. The newly recorded species also exhibited ecological specificity to high-altitude habitats, underlining their conservation importance.

**Keywords:** Beard lichen, ecosystem, genetic diversity, Kayu Angin, morphology, species, taxonomy, *Usnea* Dill. ex Adans

**Key findings:** In the identified 18 *Usnea* species, five species were the newly recorded specimens in West Java, which considerably contributed to the taxonomic and ecological status of lichens in Indonesia. The results offer valuable prospects for breeders and plant scientists in utilizing lichen-derived bioresources for plant health and environmental management.

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: April 19, 2025; Accepted: May 28, 2025.

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**Citation:** Jannah M, Susandarini R, Nugroho LH, Kasiamdari RS (2025). *Usnea* Dill. ex Adans. (Parmeliaceae, Lecanorales) with new species records in West Java, Indonesia. *SABRAO J. Breed. Genet.* 57(6): 2637-2647. <http://doi.org/10.54910/sabrao2025.57.6.36>.

## INTRODUCTION

*Usnea* Dill. ex Adans is a fruticose genus of lichens belonging to the family Parmeliaceae, characterized by its widespread global distribution and comprising over 1,200 species. The genus can be readily identified by its shrubby to pendulous thallus, with a central cartilaginous axis and the production of usnic acid in the cortex (Gerlach *et al.*, 2023). However, worldwide, the precise number of *Usnea* species remains uncertain due to hindrances in defining species boundaries, compounded by the synonymization of several species over previous decades (Truong and Clerc, 2013; Mark *et al.*, 2016; Gerlach, 2017). Taxonomic studies on *Usnea* can play a crucial role in developing comprehensive species databases to facilitate biodiversity assessment and optimize its utilization in various applications generally worldwide and particularly in Indonesia.

To date, no comprehensive literature has provided an updated overview of the diversity of *Usnea* species in Indonesia. Taxonomic research conducted in the past decade has documented 30 species (Noer *et al.*, 2013; Susilawati, 2017; Kusmoro *et al.*, 2018; Jannah *et al.*, 2020, 2021), though these studies focused only on the geographical scope, not covering all those areas where *Usnea* naturally thrives. Java Island, with its extensive montane forests occurring at elevations between 1,000 and 4,884 meters above sea level (masl) (Kartawinata, 2016), offers diverse vegetation types that serve as suitable habitats for *Usnea*. The following study also aimed to update the inventory of *Usnea* species, focusing on the five newly identified species in Java, Indonesia.

In addition to their ecological importance, *Usnea* species have attracted increasing scientific interest due to diverse pharmacological properties, which refer to secondary metabolites, such as usnic acid, depsides, and dibenzofurans. These compounds exhibited notable antimicrobial, antioxidant, and cytotoxic activities, positioning *Usnea* as a promising source of novel therapeutic agents (Sepahvand *et al.*, 2021). For instance, a comprehensive analysis of the

species *Usnea longissima*, identified with barbatic acid with pro-apoptotic activity, highlighted its potential in anticancer applications (Reddy *et al.*, 2019).

Furthermore, the species *Usnea barbata* has demonstrated significant antibacterial and antioxidant effects, reinforcing its use in traditional medicines (Popovici *et al.*, 2021). Dandapat and Paul (2019) successfully isolated secondary metabolites from the species *Usnea longissima* and evaluated their pharmacological relevance, further supporting the genus *Usnea*'s potential in pharmaceutical industries. These findings highlight the need for further research into the biochemical diversity and biological activities of *Usnea* species, particularly in diverse regions, such as Indonesia, to explore their potential application in medicine and biotechnology. In addressing these gaps, this study presents an updated taxonomic account of *Usnea* species from montane forests in West Java, Indonesia, based on detailed morphological observations.

## MATERIALS AND METHODS

Specimens of *Usnea* Dill. ex Adans attained a collection from the forests of Cikole and the Kamojang geothermal area, Indonesia, using an exploratory sampling method at altitudes ranging from 1300 to 1600 masl. The specimens preceded their placement in the Herbarium Generasi Biologi (GEN), Indonesia. The species identification took place based on 22 morphological characters, five anatomical characters, microcrystal morphology, and spot tests. Morphological observations included attributes such as thallus color, the color of the thallus base, thallus shape, branching patterns, central axis structure, types of fibrils, presence of papillae, medulla type and color, pseudocyphellae, soralia types, isidia types, and apothecia. Anatomical observations continued by examining longitudinal sections of the medulla and central axis, as described by Gerlach (2017).

Micromorphological analysis involved slicing the thallus and observing color variations in the medulla following the treatment with potassium hydroxide (KOH) and

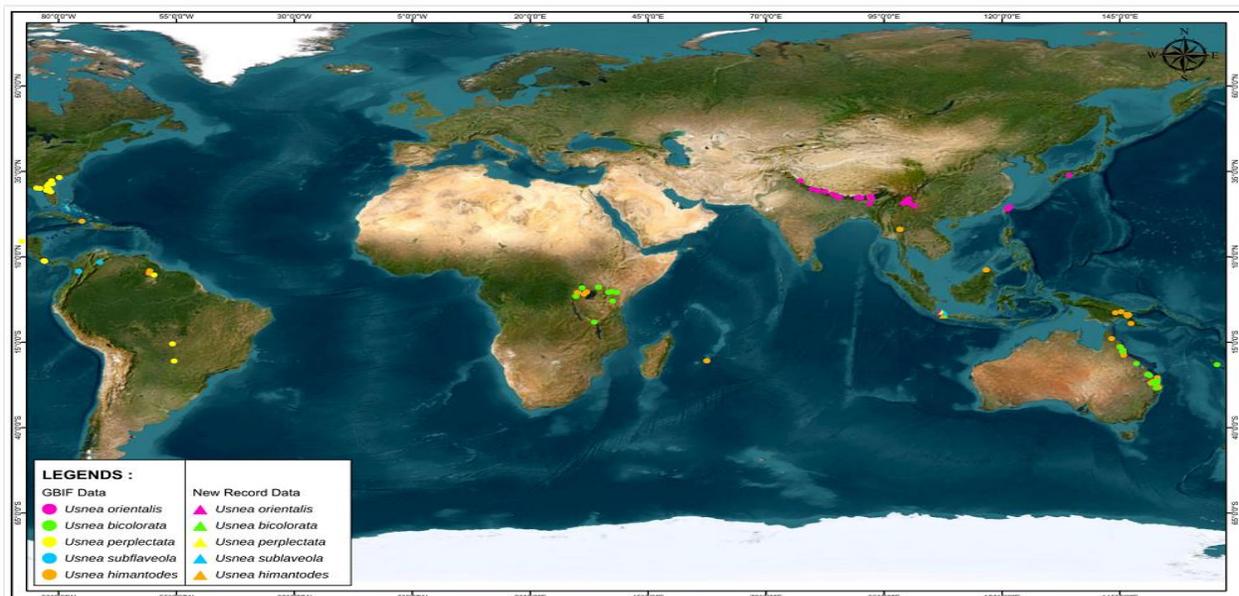
calcium hypochlorite (C). These observations ensued using a Dino-Lite microscope at a magnification of 40 × 10. Lichen acid identification, based on microcrystal morphology, transpired by extracting the thallus with acetone to induce the formation of white crystals. Crystal morphology examination microscopically continued after the addition of reagents such as G.A.An (glycerin: alcohol: aniline, 1:2:2) and G.A.OT (glycerin: alcohol: O-toluidine, 1:2:2). Further observations using G.A.W. (glycerin: alcohol: water, 1:1:1) and G.E. (glycerin: acetic acid, 1:3) reagents followed the protocol of Jannah *et al.* (2022), involving heating the crystals and examining them under a microscope. The use of crystal shapes helped identify lichen acids by comparing them with reference photographs (Huneck and Yoshimura, 1996).

Species identification had considerable support from consulting online herbarium databases, the Consortium of Lichen Herbaria (2024), and utilizing various identification keys (Randlane *et al.*, 2009; Ohmura, 2012; Truong and Clerc, 2013; Gerlach, 2017; Ohmura, 2020). Generating the *Usnea* species distribution map in West Java, Indonesia, used field data collected within the region, then processed and visualized using ArcGIS (2024)

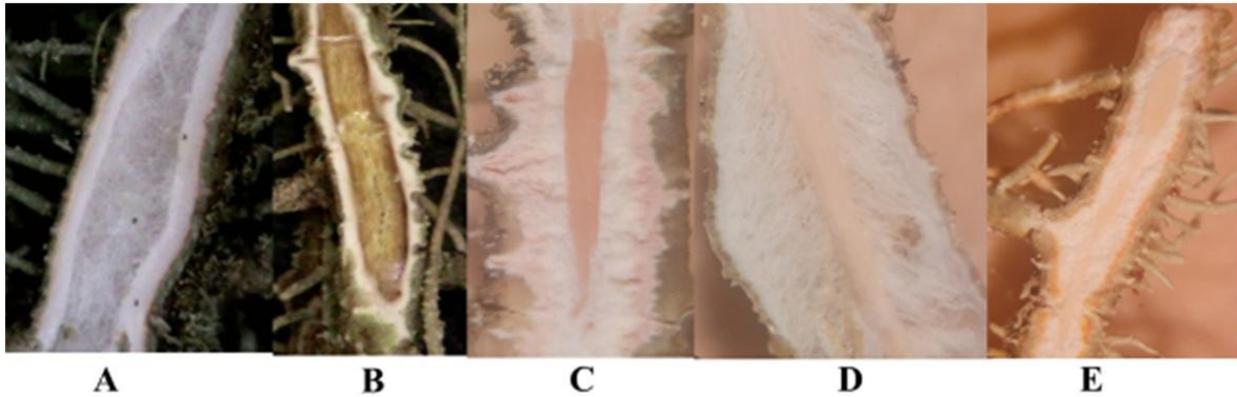
(Figure 1). Moreover, herbaria data from GBIF (2024) also served to construct the *Usnea* species distribution.

## RESULTS AND DISCUSSION

This study documented the presence of five *Usnea* species that previously had no records in West Java, Indonesia. These are *Usnea orientalis*, *U. perplectata*, *U. subflaveola*, *U. himantodes*, and *U. bicolorata*. The said group of the species represents the inaugural reports in Java Island, Indonesia (Noer *et al.*, 2013; Susilawati, 2017; Kusmoro *et al.*, 2018; Jannah *et al.*, 2021). Furthermore, a rare species, *Usnea longissima* Ach., reached its discovery in the pine forest of the Kamojang geothermal area. The identification of these *Usnea* species took place through the combination of morphological, anatomical, and biochemical characteristics, adhering to established methodologies (Randlane *et al.*, 2009; Ohmura, 2012, 2020; Gerlach, 2017; Gerlach *et al.*, 2023). The following identification key highlights the main distinguishing features of the five *Usnea* species identified in this study.



**Figure 1.** Distribution map of newly recorded *Usnea* species in West Java, Indonesia



**Figure 2.** Medulla variation in *Usnea* (fistulose axis = A, solid axis = B-F): A. Wine red pigment present in the subcortical part of medulla (*U. baileyi*); B. Central axis (*U. pectinata*); C. Strawberry pink pigment present in the subcortical part of medulla (*U. ceratina*); D. Medulla and central axis totally white (*U. bismolliuscula*); and E. Red pigment in both subcortical thin layer and parts of cortex (*U. bicolorata*).

*Usnea* species identified totaled 18 in the presented study, distributed across three subgenera, i.e., *Usnea*, *Eumitria*, and *Dolichousnea*. Species in the subgenus *Usnea* include *U. bismolliuscula* Zahlbr., *U. orientalis* Motyka, *U. rubicunda* Stirt., *U. rubrotincta* Stirt., *U. himalayana* C. Bab., *U. intermedia* (A. Massal.) Jatta, *U. barbata* (L.) Weber ex F.H. Wigg, *U. cornuta* Körb., *U. esperantiana* P. Clerc, *U. ceratina* Ach., and *U. bicolorata*. The subgenus *Eumitria* comprises the species *U. baileyi* (Stirt.) Zahlbr., *U. pectinata* Stirt., *U. perplectata*, *U. subflaveola*, and *U. himantodes*. The subgenus *Dolichousnea* includes the species *U. diffracta* Vain. and *U. longissima* Ach. The medulla can be a useful character to differentiate the species in the genus *Usnea* (Figure 2), and also a few other diagnostic characters were beneficial to further distinguish the three *Usnea* subgenera. The subgenus *Dolichousnea* showed characteristics of the presence of annular pseudocyphellae between thallus segments. However, the subgenera *Eumitria* and *Usnea* revealed distinctions of the two types of central axis, i.e., fistulose and solid, respectively (Ohmura, 2001).

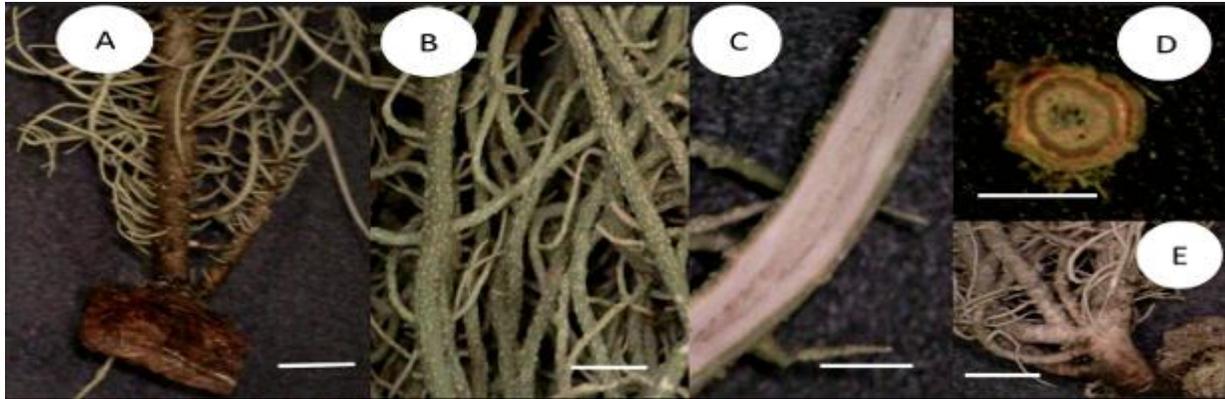
Further clarifying the taxonomic identity and ecological characteristics of these newly invented species, the paper provides the following detailed morphological descriptions,

anatomical features, biochemical profiles, and distributional data for each taxon. These diagnostic accounts hope to facilitate accurate identification, support future biodiversity surveys, and contribute to the broader understanding of *Usnea* diversity in the montane forests of Indonesia.

**Identification Key for Five *Usnea* Species from West Java**

- 1. a. Axis fistulose.....2
- b. Axis solid.....3
- 2. a. Medulla with a pink pigmentation.....  
.....*U. perplectata*
- b. Medulla white (not pigmented).....  
.....*U. subflaveola*
- 3. a. Central axis with a brown ochraceous.....*U. himantodes*
- b. Central axis white .....4
- 4. a. medulla was dense and thick with orange pigmentation near cortex.....*U. bicolorata*
- b. medulla was dense and thick without pigmentation near cortex.....*U. orientalis*

*Usnea perplectata* Motyka, Lich. Gen. *Usnea* Stud. Monogr. Pars Syst. 1:55 (1936) (Figure 3). New record: Indonesia–West Java • Bandung Regency, Subdistrict Lembang, Cikole Forest, Jl. Raya Tangkuban Parahu; 6°46'58.1"S, 107°38'36.0"E; 21.IX.2023;

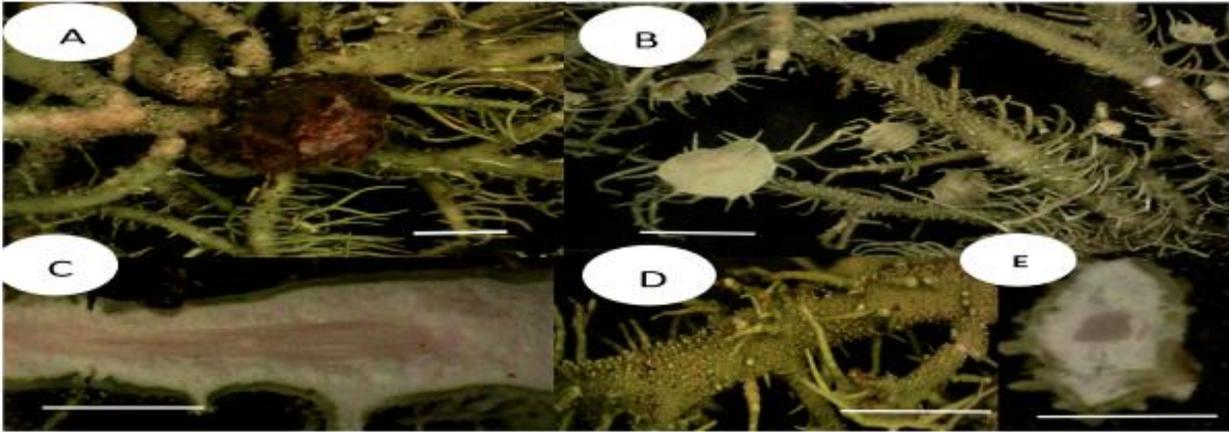


**Figure 3.** *Usnea perplectata*: A. Basal part of thallus; B. Ridged branches abundantly covered by soralia; C. Medulla compact with pink pigmentation; D. Central cross section of the thallus; and E. Trunk concolorous with thin annular crack. Scale bars: 1 mm.

Jannah 12C leg.; GEN 2344 • Putri Mount, Cikole, Lembang District; 6°46'58.933"S, 107°38'30.631"E; 21.IX.2023; Jannah 16C leg.; GEN 2345. Identification: Thallus was green and subpendent, displaying anisotomic-dichotomous branching. The thallus basal portion was brown to occasionally blackish (Figure 3A), with branch segments ranging from terete to slightly ridged. Numerous fibrils were visible on the branches, while tubercles were also evident along the primary branches, with the fiberclles sparsely distributed at the base. Soralia's position was along the ridges of the branches and contained a few isidiomorphs (Figure 3B). Papillae were infrequent, and the foveoles as well as maculae were absent. However, apothecia were nonexistent in any specimen. The cortex was thin (4.5%–8% of branch diameter), and the medulla was compact and relatively thin (6.5%–14%), often exhibiting pink pigmentation. The central axis was fistulose (Figure 3C). Biochemical spot test results were K– and C+ yellow-orange, and diffractaic acid resulted in the medulla. All the morphological and anatomical characteristics were reliant on direct measurements from field-collected specimens in Cikole, West Java (GEN 2344, 2345). Ecology: The species *Usnea perplectata* was consistently prevalent at elevations exceeding 1550 meters above sea level (masl), indicating a narrow ecological amplitude. Its altitudinal preference suggests

adaptation to high-mountain environments, which may contribute to its limited distribution. Note: In its natural habitat, *Usnea perplectata* appeared growing epiphytically on the bark of *Melaleuca* species. Although its previous treatment was as a synonym of *Usnea baileyi*, the present findings support its recognition as a distinct species. Previously, the said species garnered a recording in South America and the Galapagos Islands (Truong and Clerc, 2013). However, the presented study provides the first confirmed record of *Usnea perplectata* in Java, Indonesia. By comparing it with *Usnea baileyi*, *Usnea perplectata* can be distinctive with its subpendent thallus, terete and ridged branches, sparse fiberclles in the basal region, and pink pigmentation within the medulla, with diffractaic acid. These morphological and biochemical characteristics, along with the absence of dense basal structures, support its taxonomic separation. A longitudinal section from specimen GEN 2345 occurs to illustrate the internal anatomical features (Figure 3C).

*Usnea orientalis* Motyka, Lich. Gen. *Usnea* Stud. Monogr. Pars Syst. 2 (1):536,547 (1937) (Figure 4). New records: Indonesia–West Java • Bandung Regency, Subdistrict Lembang, Cikole Forest, Jl. Raya TangkubanParahu;6°46'54.912"S, 107°38'21.416"E; 21.IX.2023; Jannah 8C leg.; GEN 2346 • Putri Mount, Cikole, Lembang District; 6°46'55.186"S, 107°38'18.470"E;

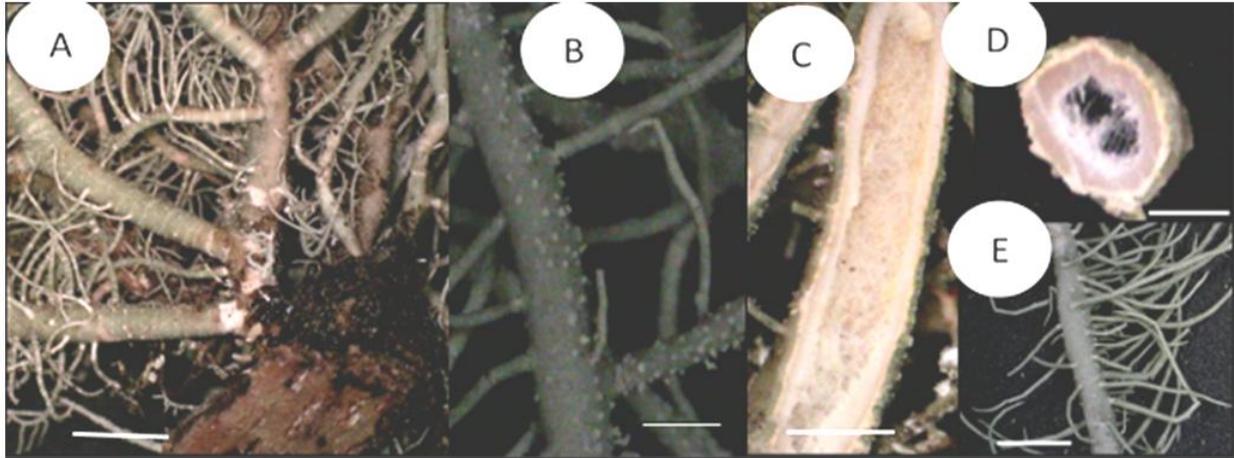


**Figure 4.** *Usnea orientalis*: A. Basal part of thallus; B. Apothecia with disc without white rim; C. Medulla dense; D. Papillae cylindrical; and E. Central cross section of the thallus. Scale bars: 1 mm.

21.IX.2023; Jannah 20C leg.; GEN 2347. Identification: The thallus was green and erect-shrubby, with anisotomic-dichotomous branching. Branch segments were terete and somewhat inflated, with a glossy surface and numerous fibrils; the basal part was dark brown to blackish (Figure 4A). Cylindrical papillae appeared on thicker branches (Figure 4D), while pseudocyphellae and maculae were absent. Apothecia were also notable, pale brown with a pruinose disc lacking a white margin (Figure 4B), while Soralia were nonexistent. The cortex was relatively thin (3.7%–8.5% of the branch diameter), the medulla was dense and thick (26.5%–37.8%), and the central axis was solid to moderately thick (12.3%–29.8%) (Figure 4C). Biochemical analysis revealed the salazinic and protocetraric acids, with spot test reactions K- and C-. All the morphological and anatomical characteristics reached documentation from field-collected specimens in West Java (GEN 2346, GEN 2347). Ecology: In Java, the species *Usnea orientalis* sat at an elevation of 1434 to 1498 masl. Within the forest, its discovery was on the bark of *Schima wallichii* (DC.) Korth. and *Pinus merkusii* Jungh. & de Vriese trees. Note: The species *Usnea orientalis* was also prevalent in Japan and Taiwan, where it grows on tree barks at elevations around 1500 masl (Ohmura, 2001). In Taiwan, it occurs between 1900 and 2900 masl. A record of the said species also

emerged in India, including the Bengal Hills of Uttarakhand and Tamil Nadu in the Western Ghats (Shukla, 2014). The specimens collected from West Java represent the first confirmed record of the species *Usnea orientalis* in Indonesia based on morphological analysis.

*Usnea subflaveola* Truong & P. Clerc, *The Lichenologist* 45(3):392 (2013) (Figure 5). New records: Indonesia–West Java • Bandung Regency, Subdistrict Lembang, Genteng; 6°46'50.590"S 107°38'00.268"E; 21.IX.2023; Jannah 3C leg.; GEN 2348 • Jayagiri, Lembang District; Jl. Gunung Putri, 6°46'54.912"S, 107°38'21.416"E; 21.IX.2023; Jannah 23C leg.; GEN 2349. Identification: The thallus was green and subpendent, exhibiting anisotomic-dichotomous branching with tapering and slightly irregular branches. Branch segments were terete to subtly ridged, and the basal part was blackish brown with fine annular fissures (Figure 5A). Papillae were notably rare, while both fibrils and fiberclcs showed an abundant development along the branches (Figure 5E). Soralia were convex and terminal, accompanied by numerous isidiomorphs (Figure 5B). Foveoles and maculae were absent, and no apothecia were evident. The cortex was thin to moderately thick (5.3%–8.4% of branch diameter), the medulla was compact, white, and thin (6.5%–13.5%), and the central axis was fistulose, comprising 48%–67.5% of the total branch diameter (Figure 5C). Biochemical analysis revealed the



**Figure 5.** *Usnea subflaveola*: A. Basal part of thallus; B. Soralia at the tip of branch thallus; C. Medulla compact; D. Central cross section of the thallus; and E. Fibrils and fiberclaves on the branches. Scale bars: 1 mm.

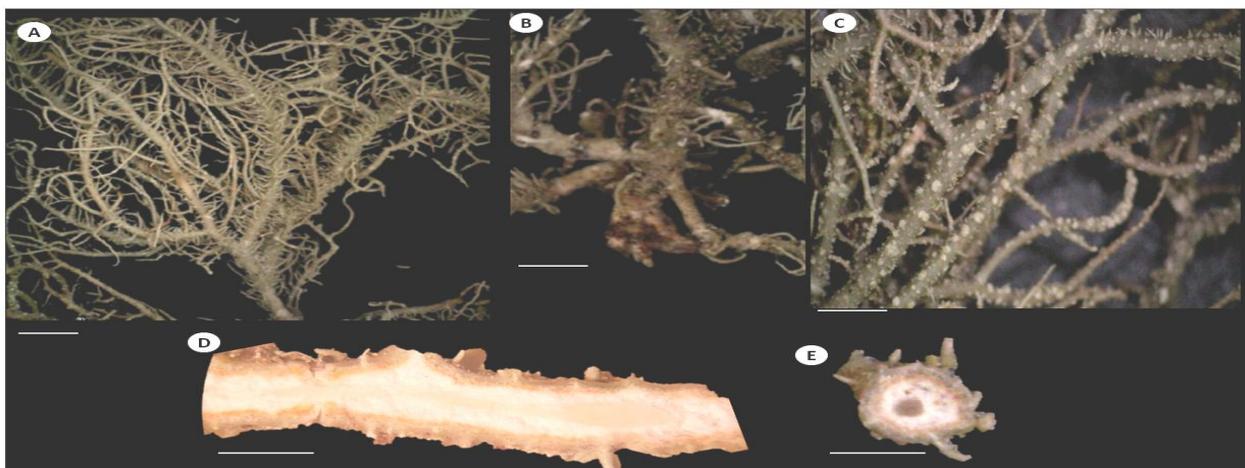
presence of norstictic and stictic acids, with spot test reactions K<sup>+</sup> yellow and C<sup>-</sup>. The description of morphological and anatomical features was according to the measurements and field observations of specimens collected from West Java (GEN 2348, GEN 2349). Ecology: In Java, the species *Usnea subflaveola* prevailed at elevations between 1487 and 1498 masl. It was primarily visible growing epiphytically on the bark of *Pinus merkusii*, particularly in relatively open forest conditions. Note: Originally described from the Neotropical Andes, *Usnea subflaveola* typically occurs at elevations between 2000 and 3100 masl and also had reports on dead wood and in humid forest microhabitats (Truong and Clerc, 2013). The species *U. subflaveola* and *U. baileyi* share some general morphological traits. Likewise, distinguishing the present specimens can be reliable by their more prominent terminal soralia, the consistent presence of isidiomorphs, a distinctly fistulose and proportionally thicker central axis, and fine annular fissures at the base of the thallus. Additionally, the co-occurrence of norstictic and stictic acids reinforces their identity. These characters were taxonomically considerable and align with the diagnostic frameworks proposed by Ohmura (2001) and Clerc (1998). Based on these distinctions, this study provides

the first confirmed record of the species *U. subflaveola* from Java, Indonesia.

*Usnea himantodes* Stirt., Scottish Naturalist 7:75 (1883) (Figure 6). New records: Indonesia–West Java • Garut Regency, Samarang Subdistrict, Sukakarya; 7°10'18.822"S, 107°48'08.573"E; 21.IX.2023; Jannah 1K leg.; GEN 2350 • Cisarua, Garut District; Jl. Gunung Putri, 7°09'53.721"S, 107°47'43.651"E; 21.IX.2023; Jannah 24K leg.; GEN 2351. Identification: The thallus was yellowish-green and pendent, with subsitomic dichotomous branching (Figure 6A). Branches ridged, with basal part pale to dark brown (Figure 6B). Fibrils were abundant on the branches and the papillae and maculae were also present, with the soralia rounded and distributed along the branches (Figure 3F). Apothecia were not evident. The cortex was thin (7.3%–18.6% of branch diameter), the medulla dense (6.5%–17%), and the central axis solid with a brown ochraceous cord and relatively thick (31%–44%) (Figure 6E). Biochemical analysis confirmed the presence of usnic acid, norstictic acid, menegazziaic acid, stictic acid, and constictic acid, with spot test reactions of K<sup>+</sup> red C<sup>-</sup>. All the morphological and anatomical characteristics depended on the observations and measurements from specimens collected in the montane forests of



**Figure 6.** *Usnea himantodes*: A. Subpendent thallus; B. Basal part of thallus; C. Ridged branches; D. Soralia; E. Medulla dense with yellow central cord; and F. Central cross section of the thallus. Scale bars: 1 mm.



**Figure 7.** *Usnea bicolorata*: A. Basal part of thallus; B. Soralia concave with granular soredia; C. Fibrils on branches; D. Medulla dense with orange pigmentation; and E. Central cross section of the thallus. Scale bars: 1 mm.

West Java (GEN 2350, GEN 2351). Ecology: In Java, the species *Usnea himantodes* occurred between 1419 and 1477 masl, growing on the bark of *Pinus merkusii*. Note: Species *Usnea himantodes* was typical to be widely distributed across tropical regions, including Australia, the Philippines, East Africa, and India (Ohmura, 2001). It has infrequent reports in Japan and Taiwan. The current findings represent the first confirmed record of the species *Usnea himantodes* in Java, Indonesia, based on

morphological and biochemical analysis of newly collected specimens.

*Usnea bicolorata* Motyka, Lich. Gen. Usnea Stud. Monogr. Pars Syst. 2(1):336 (1937) (Figure 7). New records: Indonesia–West Java • Garut Regency, Samarang Subdistrict, Sukakarya; 7°09'50.465"S, 107°47'30.565"E; 21.IX.2023; Jannah 34K leg.; GEN 2352. Identification: Thallus was grayish green and shrubby, with anisotomic dichotomous branching. Branch segments were

terete and uninflated, and the basal part was brown to black (Figure 7A). Fibrils were abundant on the branches, and papillae were sparse (Figure 7C). Soralia were concave, containing granular soredia and few isidiomorphs (Figure 7B), while pseudocyphellae and maculae were not present. The cortex was thin (7.8%–9% of branch diameter). The medulla was dense and thick (19.8%–29.5%), with orange pigmentation near the cortex (Figure 7D). The central axis was solid and thick (16.5%–27.5%), and apothecia were not evident. Biochemical analysis showed the presence of acetylportentol, barbatic acid, protocetraric acid, and physodalic acid, with spot test reactions of K<sup>+</sup> red C<sup>-</sup>. All the morphological and anatomical features were according to field observations and measurements from specimens collected in West Java (GEN 2352). Ecology: In Java, the species *Usnea bicolorata* appeared at elevations between 1450 and 1477 masl. Note: In forests, the said species was noticeable on the bark of *Schima wallichii* and *Pinus merkusii*. The species *Usnea bicolorata* also commonly occurs in Taiwan (Ohmura and Wang, 2010) and in tropical Africa (Truong *et al.*, 2011). This study presents the first confirmed record of the species *Usnea bicolorata* in Java based on morphological and biochemical analysis.

The lichen genus *Usnea* is a widely utilized plant in traditional and modern medicines in Indonesia and other surrounding regions, including Asia, Africa, Europe, and the Americas (Kusmoro *et al.*, 2018; Jannah *et al.*, 2022). Beyond its medicinal applications, *Usnea* has also been beneficial as a food source in Australia and as an ingredient in cosmetics (Shukla *et al.*, 2015). Among the Javanese people, *Usnea* has long been applicable as a traditional remedy for various conditions, including dysentery, colds, rashes, menstrual pain, childbirth facilitation, gout, heart disease, stroke, liver and kidney disorders, asthma, lung ailments, diabetes, and postpartum recovery. Additionally, *Usnea* has been useful in the pharmaceutical industry as a component of the Standardized Herbal Medicine (OHT) products (Jannah and Afifah, 2020).

Despite its wide-ranging applications, the studies on lichen diversity, particularly on *Usnea*, remain limited in Indonesian forests. Comprehensive long-term monitoring, supported by a molecular basis, is necessary to enhance one's understanding of its diversity and potential applications in Indonesia. The presented study revealed the highest diversity of *Usnea* species in the montane forests of West Java, including five newly recorded taxa that expand the distribution of the genus in Indonesia. This updated taxonomic study contributes essential data for biodiversity monitoring and ecological research in tropical highland regions. In addition to their ecological functions, *Usnea* species are known producers of bioactive secondary metabolites with antimicrobial and antioxidant properties, which can also entail further exploration for potential applications in sustainable agriculture and plant health management (Cansaran *et al.*, 2012; Popovici *et al.*, 2021). These insights provide a valuable foundation for integrating lichen-derived resources into strategies for crop protection, plant resilience, and genetic conservation.

## CONCLUSIONS

The pioneering study reports the discovery of five *Usnea* species, marking a new record for West Java and thereby expanding the distribution of the said genus in Indonesia. The findings highlight the ecological importance of montane forests as critical habitats for lichen diversity. Morphological, anatomical, and biochemical analyses proved essential for the accurate identification of species. These results contribute valuable data to the biodiversity inventories, conservation planning, and the sustainable utilization of lichen-derived resources.

## ACKNOWLEDGMENTS

The authors extend their gratitude to Ms. Vivit Nurholifah, Mrs. Pratiwi Prananingrum, and Mr. Muhammad Rifqi Hariri for their invaluable assistance during the field study. We also express heartfelt

appreciation to the editor and reviewers for their dedicated time, constructive feedback, and insightful suggestions, which have greatly enhanced the quality of this manuscript. This study received financial support from Indonesian Education Scholarship (BPI), Center for Higher Education Funding and Assessment, Ministry of Higher Education, Science and Technology of Republic Indonesia (PPAPT), and the Indonesia Endowment Fund for Education (LPDP) under Decree No. 00612/J5.2.3./BPI.06/9/2022, with their support greatly appreciated.

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