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Lejeuneaceae (Marchantiophyta) in Curug Semirang, Central Java, Indonesia

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ABSTRACT. Lejeuneaceae is one of the liverwort families with the highest species diversity. This research was conducted to determine species diversity and to describe the species of Lejeuneaceae in Curug Semirang. Lejeuneaceae samples were taken at three observation stations in the natural tourism area of Curug Semirang. The research was conducted using a purposive sampling method. The results showed that ten species of Lejeuneaceae, belonging to four genera were identified from Curug Semirang. They were *Lejeunea eifrigii*, *Lejeunea flava*, *Lopholejeunea borneensis*, *Lopholejeunea horticola*, *Lopholejeunea nigricans*, *Lopholejeunea* sp., *Lopholejeunea subfusca*, *Spruceanthus planiusculus*, *Spruceanthus semirepandus*, *Thysananthus humilis*. The habitat of Lejeuneaceae in Curug Semirang is epiphytic and terrestrial as well as lithophyte. Measurement of environmental factors shows that the area around Curug Semirang is a place that can support the growth and spread of Lejeuneaceae.

Keywords: Curug Semirang; liverworts; Lejeuneaceae diversity; Marchantiophyta; species identification

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INTRODUCTION

Curug Semirang is a natural waterfall area located on the northeastern slope of Mount Ungaran, precisely in Gintungan Hamlet, Gogik Village, West Ungaran District, Semarang Regency. Curug Semirang is located at an altitude of about 500–800 m above sea level and is managed by Perum Perhutani (Irawan *et al*., 2017). Several locations in Curug Semirang have experienced land conversion into tourism and plantation areas. This change can affect the existence of vegetation, which has a major role in maintaining the balance of the ecosystem. One type of vegetation in Curug Semirang is liverwort.

Various studies of bryophytes in the waterfall area have been carried out. Roziaty *et al*. (2019) discovered five species of epiphytic mosses belonging to five different genera in the Jumog Waterfall Area, Ngargoyoso, Tawangmangu, Karanganyar. Four orders, seven families, and seven species of Marchantiophyta (Liverworts) were discovered by Febriansah *et al.* (2019) in the Parangkikis Waterfall Area of Pagerwojo Tulungagung.

Liverworts have many benefits for the environment, including storing and maintaining the microhydrological cycle of atmospheric water in forests (Ah-Peng *et al*., 2017) also withstand heavy rain and prevent erosion (Oishi & Hiura 2017). Liverworts can be used as a bioindicator and microclimatic conditions because of their sensitivity to environmental changes, including humidity and temperature (Gehrig-Downie *et al*., 2013). Liverworts also have many uses as medicinal and commercial plants because they have intracellular organelles called oil bodies, which can be used as a potential source of drugs (He *et al*., 2013). This is in accordance with the opinion of Dey & Mukherjee (2015) that Lejeuneaceae liverworts contain terpenoids and aromatic compounds. These compounds have very strong biological activity and play an important role in plant defense against pests and diseases. Lejeunea flava, for example, has the potential to be used as an anticancer drug due to the cytotoxic properties of its oil on tumor cell proliferation.

The liverwort family with the highest diversity and widest distribution is Lejeuneaceae (Lee & Gradstein, 2013; Gradstein, 2020). There are 69 genera recorded worldwide (Wang *et al*., 2016). including 28 of which are in Java, with a total of 160 species (Siregar *et al*., 2014; Pasaribu *et al*., 2018). The Lejeuneaceae family has thallus characteristics that are shaped like stems and leaves arranged in two to three rows. The thallus contains green to yellowish chlorophyll. The branches are

of the Lejeunea type. Lateral leaves are arranged in incubus when viewed from the dorsal, where each leaf covers part of the leaf above it. Lateral leaves are divided into lobes and lobules, and have ventral leaves (except in Cololejeunea) attached to the stem. Ventral leaves have entirely rounded or divided ends (Gradstein, 2013; Heinrichs *et al.*, 2015). The stem has hyalodermis, no ocelli, small or granular oil bodies, unwinged bracts, and a smooth or toothed perianth (Bastos & Gradstein, 2020). Lejeuneaceae family has a distinguishing feature, which is that it has lobes that look like water sacs and used to catch and store water (Kraichak, 2012; Heinrichs *et al.*, 2014).

Lejeuneaceae species can grow attached to a variety of substrates. Lejeuneaceae often grow as epiphytes or attach to the bark of roots, trunks, and branches of trees. Some Lejeuneaceae members can grow terrestrially on rocky soils or epiphytically on the leaves of shrubs (Lee & Gradstein, 2013).

Research on Lejeuneaceae in Java is still limited, so there are not many publications about this family specifically. Haerida (2009) noted that there are 13 species belonging to the Lejeuneaceae tribe in Bodogol, Gunung Gede-Pangrango National Park, West Java. Meanwhile, research information specifically on Lejeunaceae in Central Java has not been found. Sulistyowati *et al*. (2014) recorded 8 species in the montane zone, Mount Ungaran, Central Java. Our previous studies also identified 17 Lejeunaceae species from the tropical to the montane zones, including plantations and forest areas on Mount Ungaran in Central Java (Khotimperwati *et al*., 2015).

Research and data collection on Lejeuneaceae liverwort species in Curug Semirang, Central Java have never been reported, so further research is needed to complete data on the diversity of Lejeuneaceae species. This study looks at how land use affects the diversity of Lejeuneaceae species in Curug Semirang, Central Java. The results can be used as a basis for further taxonomic studies of Lejeuneaceae.

MATERIALS AND METHODS

Study area. Sampling was carried out in the Curug Semirang area, Central Java, which is located at an altitude of around 500 - 800 m asl, has high humidity (76.5% - 84.3%), air of temperature (27.3°C - 28.7°C), soil moisture (70% - 80%). The vegetation is dense, rich in various types of plants with a wide canopy. Meanwhile, the identification process was carried out at the Ecology and Biosystematics Laboratory, Faculty of Science and Mathematics, Universitas Diponegoro.

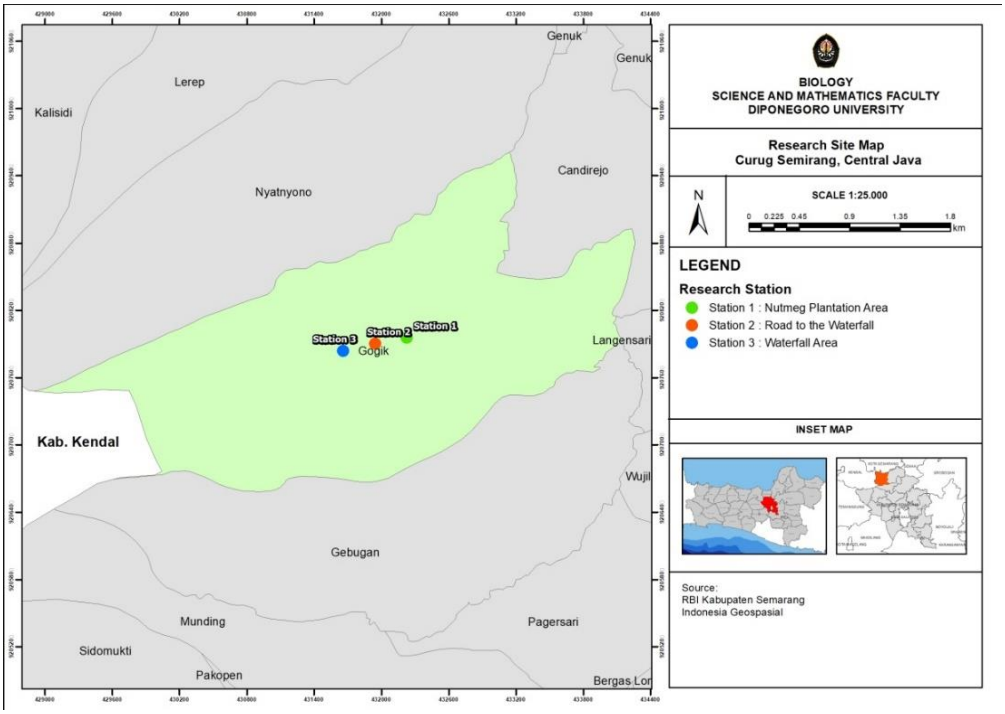

Fig. 1. Map of observation stations at Curug Semirang, Central Java: Station I Nutmeg Plantation area (green dot); Station II Road to the Waterfall (red dot); Station III Waterfall area (blue dot).

Determination of stations and measurement of environmental factors. Lejeuneaceae liverwort samples were taken along the road to the waterfall by determining three stations using a purposive method based on certain considerations such as altitude and environmental factors. Three observation stations were found, namely Station I for the nutmeg plantation area (590 masl), Station II along the road to the waterfall (671 masl), and Station III for the waterfall area (772 masl). Each observation station measured it environmental factors, composed by temperature, light intensity, air humidity, soil pH, and soil moisture.

Sampling. Sampling of epiphytic and terrestrial liverwort was carried out according to the method according to Frahm *et al.* (2003), which has been proposed. Samples of epiphytic and terrestrial Lejeuneaceae liverworts were sampled by making plots measuring 20m × 20m at each predetermined station. Each plot selected five areas of land and five host trees. For each area of soil and host trees, three samples measuring 20cm × 30cm were made. The scaled liverworts samples were separated from the substrate and put into envelopes.

Species identification. Lejeuneaceae samples were observed using a light microscope Olympus, Model CX21FSI and Optilab Viever ver. 2.2 by Miconos. The identification of samples is accomplished by comparing the characteristics of the observed species to the characteristics in the identification key. The books used as references include Gradstein (2011); Gradstein *et al.* (2001); So (1995); and Jarman & Fyhrer (1995). Web used as reference is interagency taxonomic information system (ITIS: https://www.itis.gov) and global biodiversity information facility (GBIF: https://www.gbif.org).

Data analysis. The results of the study were analyzed descriptively and qualitatively. The results of the identification of Lejeuneaceae diversity in the Curug Semirang area are presented in a species list table, and the morphological characteristics of each species are discussed in detail.

RESULTS AND DISCUSSION

Based on the identification of Lejeuneaceae liverworts in the Curug Semirang area, 10 species were found, which belong to four genera, namely *Lejeunea*, *Lopholejeunea*, *Spruceanthus*, and *Thysananthus*. The genus *Lejeunea* contains two species, namely *Lejeunea eifrigii* and *Lejeunea flava*. The Lopholejeunea genus with the highest number of species includes *Lopholejeunea borneensis*, *Lopholejeunea horticola*, *Lopholejeunea nigricans*, *Lopholejeunea sp*, and *Lopholejeunea subfusca*. The genus *Spruceanthus* contains two species, namely *Spruceanthus planiusculus* and *Spruceanthus semirepandus*. The genus *Thysananthus* only has one species, namely *Thysananthus humilis*. Identification was done by looking at the shape and arrangement of the lateral leaves, the number of lobules, the shape of the ventral leaves, and the shape of the cells.

Table 1. Lejeuneaceae liverwort species in Curug Semirang.

No	Species name	Station 1		Station 2		Station 3	
		E	T	E	T	E	T
1	*Lejeunea eifrigii*	+	+	+	-	-	-
2	*Lejeunea flava*	+	-	+	-	-	-
3	*Lopholejeunea borneensis*	-	-	+	-	+	-
4	*Lopholejeunea horticola*	+	-	-	+	-	-
5	*Lopholejeunea nigricans*	+	-	-	-	-	-
6	*Lopholejeunea* sp.	+	+	-	-	-	-
7	*Lopholejeunea subfusca*	+	-	+	+	+	-
8	*Spruceanthus planiusculus*	-	-	+	+	-	-
9	*Spruceanthus semirepandus*	+	+	+	-	-	-
10	*Thysananthus humilis*	+	-	-	-	-	-
Total	10	8		7		2	

Notes: Station I= Nutmeg plantation; Station II= Waterfall road; Station III= Waterfall.

Based on Table 1, all the Lejeuneaceae liverworts were found growing epiphytically on tree trunks, and only six species were found growing terrestrially on soil and rocks. According to Lee & Gradstein (2013), species of Lejeuneaceae are more frequently discovered growing as epiphytes or adhering to the bark of tree trunks, branches, and leaves. Additionally, according to Lai Guan-Yu *et al*. (2020), epiphytic liverworts frequently grow around the base of tree trunks, where there is enough light and humidity for liverworts to grow.

Based on Table 1. Station III had the lowest diversity of Lejeuneaceae liverworts compared to stations I and II, where only two species were found. The Station III area is the waterfall area that is most visited by tourists. This area is undergoing development and management as a tourist attraction. Various developments have been carried out and resulted in reduced vegetation and tree density, which could affect the diversity of Lejeuneaceae liverworts that grow epiphytically on trees and cause this area to be warmer. According to Király *et al*. (2013), tree vegetation density affects liverwort density because it protects liverworts from direct radiation exposure. Mukhia *et al.* (2019) also say that too much light exposure causes inconsistent humidity in the ambient air. In addition, the number of traders and tourists who litter will have an impact on the degradation of environmental quality. Therefore, management based on conservation is required to maintain the ecosystem's equilibrium at Curug Semirang.

Environmental conditions can affect the diversity and distribution of Lejeuneaceae liverworts. Based on environmental factor measurement data (Table 2), Curug Semirang can support the growth of Lejeuneaceae liverworts. This is in accordance with the opinion of García *et al.* (2016), which states that humidity, temperature, and light intensity greatly affect the diversity and distribution of liverworts. Liverworts can grow optimally at a light intensity of 1000–2000 lux. According to Glime (2020), the optimal temperature for liverwort growth is 15–25ºC but it tolerates temperatures up to 40ºC. Liverworts can live at humidity levels ranging from 23 to 100%.

Table 2. Environmental factor data

Environmental factors	Station		
	I	II	III
Humidity (%)	76.5	84.3	80
Soil Moisture (%)	70	80	80
Temperature (˚C)	28.7	27.2	28.2
Altitude (m asl)	590	671	772
Light Intensity (lux)	2180-2220	1896	1700

Description of Lejeuneaceae species at Curug Semirang

1. *Lejeunea eifrigii* Mizut.

   Synonym: *Lejeunea eifrigii* var. eifrigii

   Lejeunea eifrigii var. indica Sushil K.Singh

Thallus light green when fresh, rarely red and brownish green when a herbarium, thallus width 0.88-1.5 mm, branching Lejeunea type. The arrangement of the lateral leaves is close together (Fig. 1a); lateral leaf ovate with acute-apiculate tip, 0.43-0.75 mm long, 0.33-0.56 mm wide, curved dorsal base, ventral base flat, flat edge, blunt tip; lacks vitta; lobules small or absent attached to lateral leaves (Fig. 1b); hexagon-shaped cells, thin cell walls, 0.03 in length, 0.02 mm in width, large trigone, the oil body is not clearly visible (Fig.1d). Ventral leaf spacing, rounded shape, flat edge, 0.15-0.33 mm long, 0.15-0.30 mm wide, tip split 1/2 the lateral leaf length (Fig. 1c).

Distribution: Indonesia, Sumatra (Siregar *et al*., 2020). India, Mizoram, Kolasib, Bukpui forest (Söderström *et al.*, 2020).

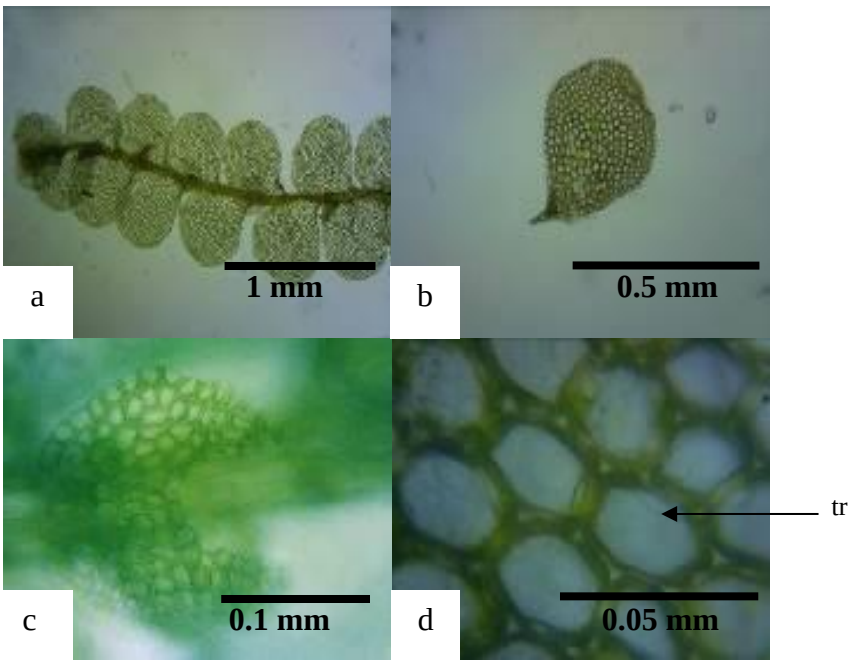

Fig. 1. *Lejeunea eifrigii*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (40×10); d. Lateral leaf cells (100×10); tr = trigone

2. *Lejeunea flava* (Sw.) Nees
   Synonym: *Eulejeunea flava* (Sw.) Steph.
   Jungermannia flava Sw.

The thallus is green, rarely red, with a width of 0.6-1.2 mm and a branching Lejeunea type. Irregular arrangement of lateral leaves (Fig. 2a); each lateral leaf is oval with a rounded tip, 0.43 mm long, 0.41 mm wide, flat edge, lacks vitta, has small ear-like lobules (auriculate) or oval located at the base of the leaf and has two teeth (Fig. 2b); Lateral leaf cells are hexagonal in shape, 0.02 mm long by 0.01 mm wide, trigon not clearly visible, large triangular shape (Fig. 2d). The ventral leaf is divided into two, spaced, rounded, flat-edged, 0.17 mm long and 0.16 mm wide, with the tip split into 2/5 of the length of the lateral leaf (Fig. 2c).

Distribution: Indonesia, Sumatra (Siregar *et al*., 2017).

Fig. 2. *Lejeunea flava*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

3. *Lopholejeunea borneensis* (Steph.) Verd.
Synonym: *Mastigolejeunea borneensis* Stephani.

The thallus is green when fresh and brownish in the specimen, rarely red, with a thallus 1-2 mm wide, branching Lejeunea type. The arrangement of the lateral leaves is tight (Fig. 3a); lateral leaf ovate, 0.62 mm long, 0.62 mm wide, flat edge, rounded tip, lacks vitta, has ovoid lobules, 1/3 length of lateral leaf (Fig. 3b); isodiametric leaf cell shape, length and width of 0.02 mm, the trigon is clearly visible in the form of a large triangle (Fig. 3d). Leaves are ventral spaced, 0.24 mm long, 0.33 mm wide, rounded and undivided, with a flat edge (Fig. 3c).

Distribution: Indonesia and Malaysia (Söderström *et al.*, 2018).

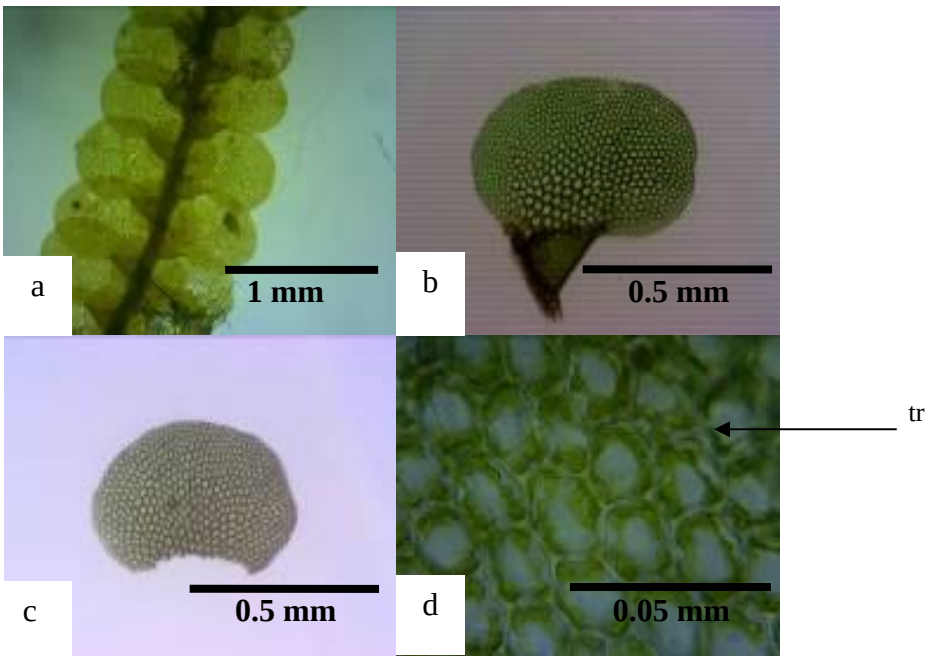

Fig. 3. *Lopholejeunea borneensis*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

4. *Lopholejeunea horticola* Schiffner

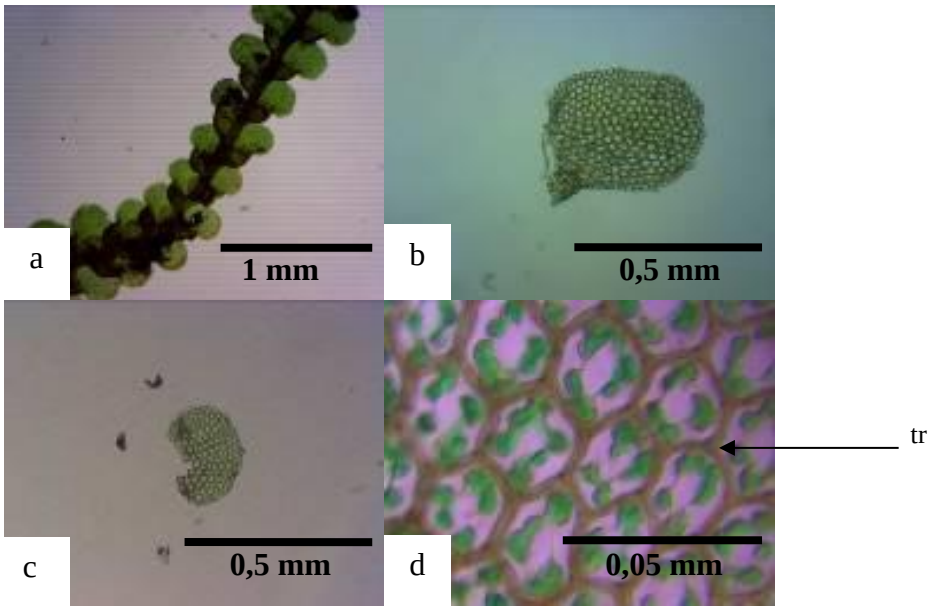

Fig. 4. *Lopholejeunea horticola*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

Thallus green to dark brown, rarely red, less than 1 mm wide, 0.52 mm thick, not dendroid, branching Lejeunea type, and irregular (Fig. 4a). The lateral leaves are ovate, 0.33 mm long, 0.41 mm wide, with a flat edge and rounded tip, and lack vitta (Fig. 4b); the leaf cell shape is isodiametric, 0.03 mm long, 0.02 mm wide, and the trigon is clearly visible with a triangular shape (Fig. 4d). Ventral leaves spaced, 0.21 mm long, 0.27 mm wide, round and undivided, flat edge (Fig. 4c).

Distribution: Indonesia (Java and Bali) (Nadhifah *et al.*, 2021).

5. *Lopholejeunea nigricans* (Lindenb.)

Synonym: *Lejeunea abortiva* Mitt.

Lejeunea atroviridis Spruce

The thallus is green to dark brown, rarely red, with a thallus width of 0.9-2 mm and a branching Lejeunea type (Fig. 5a). The arrangement of the lateral leaves is tight (Fig. 5b); the lateral leaves are ovate, 0.47 mm long, 0.49 mm wide, flat edge, rounded tip, no vitta, lobules are narrow at the tip and medium in size (Fig. 5c); isodiametric leaf cell shape, 0.02 mm long and wide, trigon not clearly visible, small triangular shape (Fig.5f); toothed perianth (Fig. 5d). leaves ventral spaced, 0.28 mm long, 0.33 mm wide, round and undivided, always intact, flat edge (Fig.5e).

Distribution: Indonesia, (Bali, Sumba) (Nadhifah *et al.*, 2021).

L. nigricans bears some resemblance to the *L. eulopha* species found in Africa. Both species have rounded lateral and ventral leaves. Both are distinguished by the shape of the lateral leaf cells. *L. nigricans* has isodiametric lateral leaf cells while *L. eulopha* is hexagonal (Fischer, 2013).

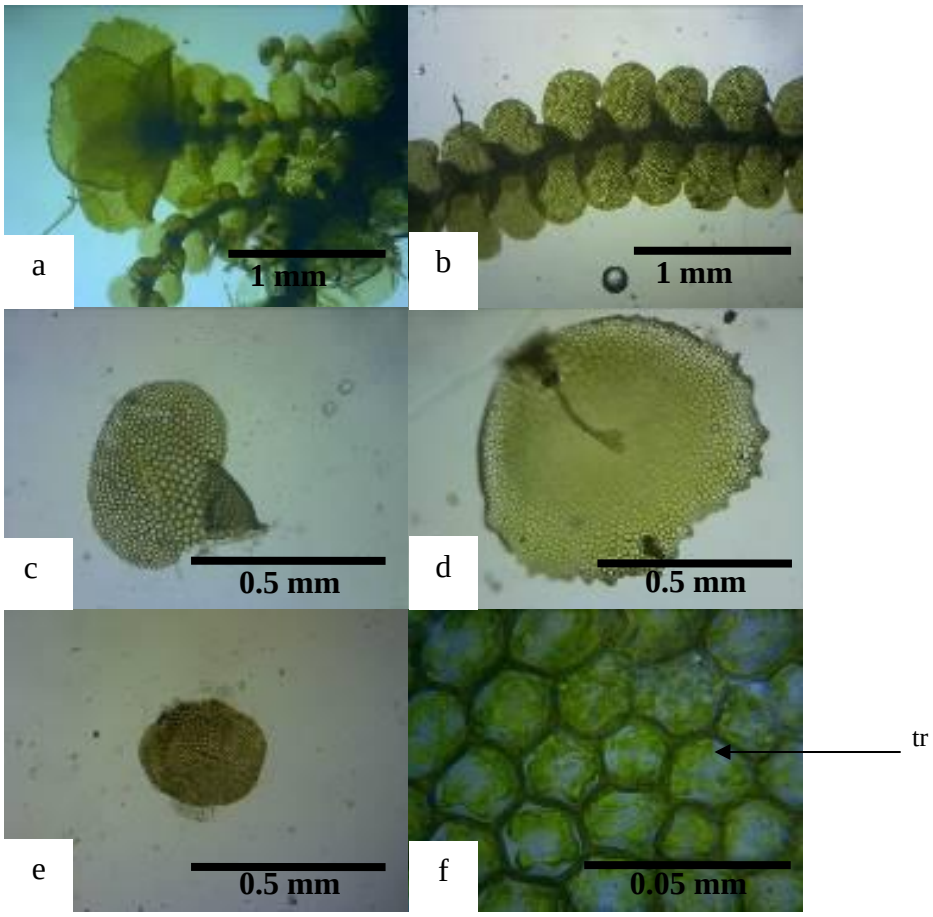

Fig. 5. *Lopholejeunea nigricans*: a-b. Habitus (4×10); c. Lateral leaves (10×10); d. Perianth (10×10); e. Ventral leaves (10x10); f. Lateral leaf cells (100×10); tr = trigone

6. *Lopholejeunea* sp.

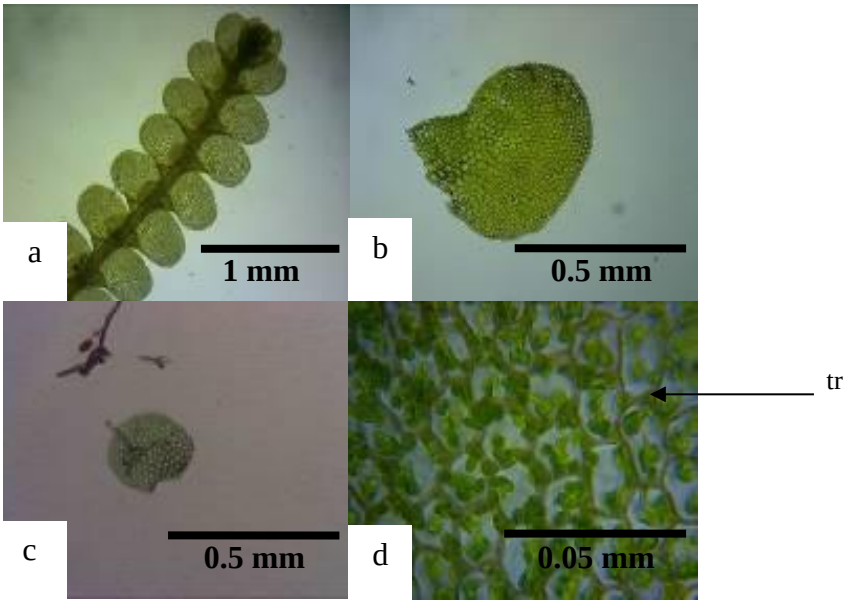

Fig. 6. *Lopholejeunea* sp.: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

The thallus is green to dark brown, rarely red. The thallus is 0.77 mm wide. The thallus is not dendroid. The branching is Lejeunea type and irregular. The lateral leaves are arranged tightly (Fig. 6a); the lateral leaf is ovate, 0.54 mm long, 0.52 mm wide, flat edge, rounded tip, lacks vitta, lobules are narrow at the tip of medium size, and have a toothed perianth (Fig. 6b). Leaf cell shape isodiametric, 0.02 mm long, 0.01 mm wide, clearly visible trigon, small triangular shape (Fig. 6d). Leaves ventral spaced, 0.2 mm long, 0.26 mm wide, round and undivided, always intact, flat edge (Fig. 6c).

Distribution: Indonesia and Malaysia (Söderström *et al.*, 2018).

7. *Lopholejeunea subfusca* (Nees) Schiffn.
   Synonym: *Archilejeunea cyclostipa* (Taylor) Steph.
   Jungermannia subfusca Nees

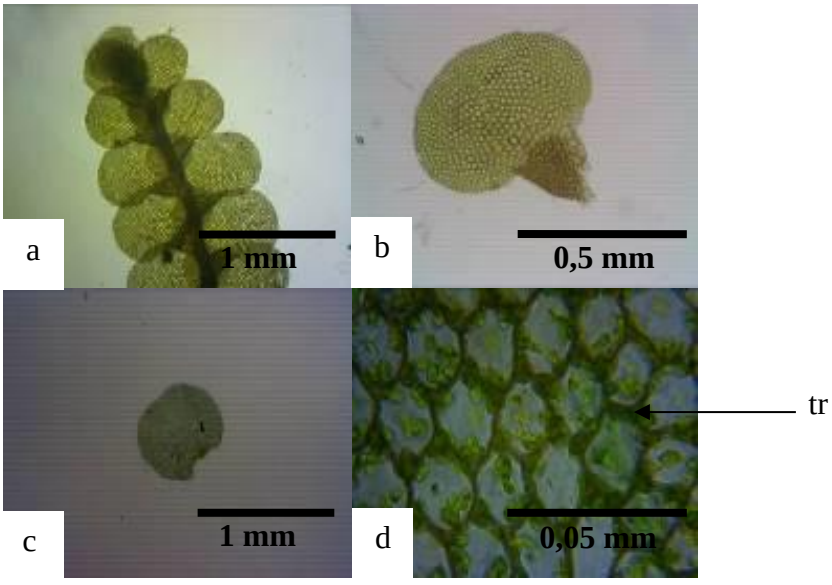

Fig. 7. *Lopholejeunea subfusca*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

Thallus green to dark brown, rarely red, 1-2 mm wide, not dendroid, irregularly branching Lejeunea type.The arrangement of the lateral leaves is tight (Fig. 7a); the lateral leaves are ovate, 0.59 mm long and wide, with a flat edge and rounded tip; they lack vitta; narrowed lobules at the tip of medium size, less than the lateral leaf length; and they have toothed perianth (Fig. 7b); isodiametric leaf cell shape, 0.02 mm long and wide, clearly visible trigon, and a large triangular shape (Fig. 7d). Ventral leaves spaced, 0.37 mm long, 0.48 mm wide, round and undivided, always intact, flat edge (Fig. 7c).

Distribution: Indonesia (Lesser Sunda, Aru Islands, Bali, Java) (Nadhifah *et al*, 2021)

8. *Spruceanthus planiusculus* (Mitt.) X.Q.Shi, R.L.Zhu & Gradst.
   Synonym: *Archilejeunea planiuscula* (Mitt.) Steph.
   Lejeunea planiuscula Mitt

Thallus is dark green and rarely red, 1.8–2 mm wide, branching Lejeunea type. Lateral leaf arrangement tight (Fig. 8a); lateral leaves are curved with a pointed tip, 1.38 mm long, 0.82 mm wide, leaf margins wavy, lack vitta, and have small lobules measuring 1/6 to 1/3 of the length of the lateral leaf (Fig. 8b); isodiametric leaf cells, 0.02 mm long and wide, triangular trigone shaped (Fig. 8d). Udivided ventral leaves with wavy edges, measuring 0.63 mm long and 0.65 mm wide (Fig. 8c).

Distribution: Indonesia (Sumatra, Java), China, India, Myanmar, Papua New Guinea, Vietnam (Siregar *et al*., 2020).

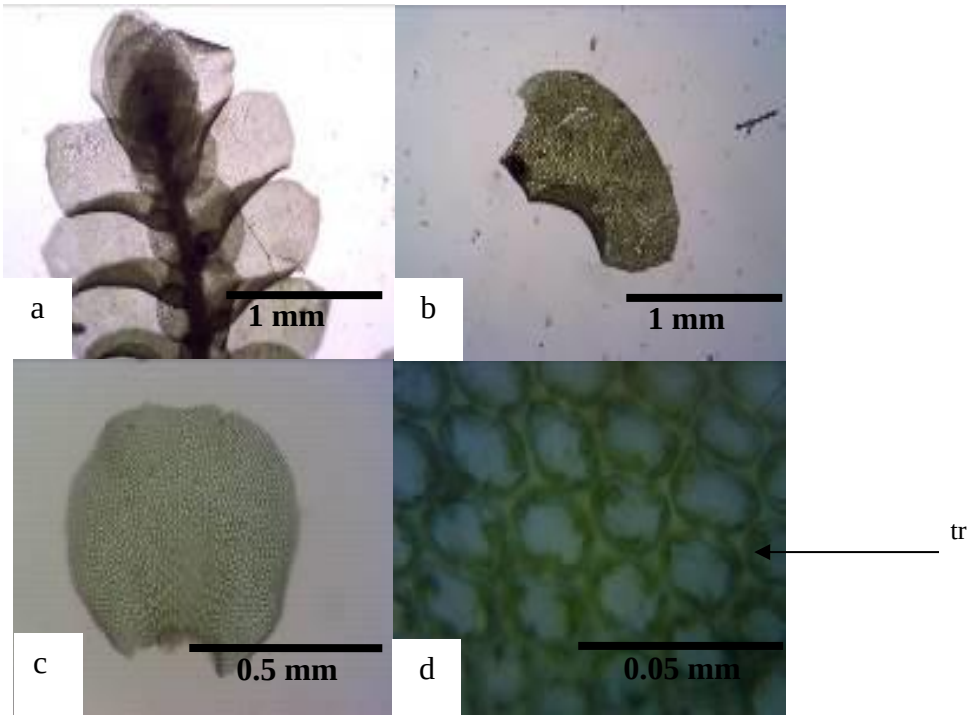

Fig. 8. *Spruceanthus planiusculus*: a. Habitus (4×10); b. Lateral leaves (4×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

9. *Spruceanthus semirepandus* (Nees) Verd.
   Synonym: *Jungermannia semirepanda* Nees
   Ptychanthus semirepandus Nees

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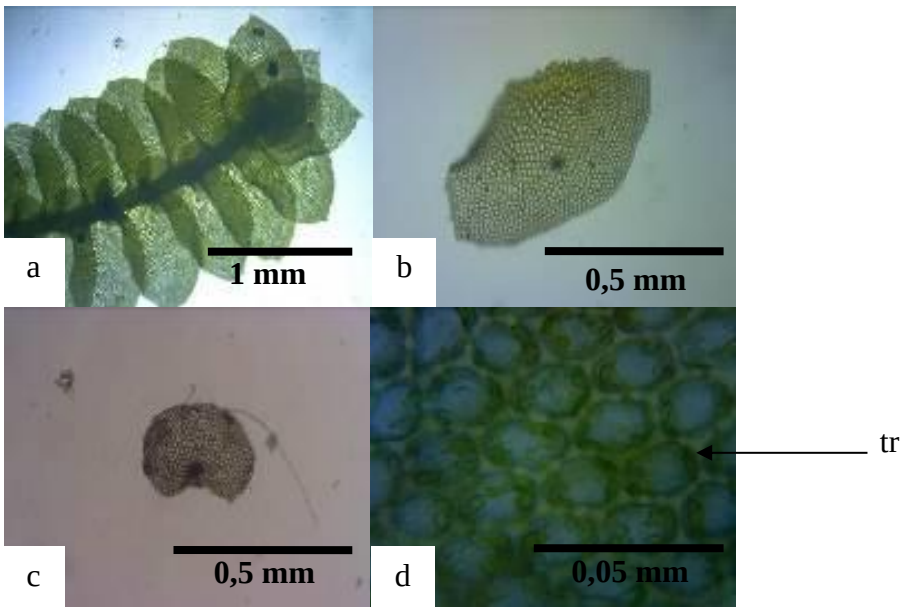

Fig. 9. *Spruceanthus semirepandus*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

The thallus is dark green or brown, rarely red; the thallus is 1.6-2 mm wide, branching Lejeunea type and irregular. Incubous lateral leaf arrangement (Fig. 9a); laterally curved leaves with a wavy tip and tapering to a pointed tip, 0.72 mm long, 0.43 mm wide, have small lobules measuring 1/6 to 1/3 of the length of the lateral leaf, lack vitta (Fig. 9b); isodiametric leaf cells with a length of 0.02 mm, a width of 0.01 mm, and have a triangular trigone (Fig. 9d). The ventral leaves are round with toothed edges, 0.27 mm long and 0.32 mm wide (Fig. 9c).

Distribution: Indonesia, Korea, China, India, Japan, New Caledonia, Papua New Guinea, Philippines (Choi, Seung *et al.*, 2012).

10. *Thysananthus humilis* (Gottsche) Sukkharak & Gradst.
    Synonym: *Mastigolejeunea humilis* (Gottsche) Schiffn.

Fig. 10. *Thysananthus humilis*: a. Habitus (4×10); b. Lateral leaves (10×10); c. Ventral leaves (10×10); d. Lateral leaf cells (100×10); tr = trigone

Thallus green to dark brown, rarely red, 1-2 mm wide, Lejeunea type, branching Incubous lateral leaf arrangement (Figure 10a); lateral leaf tip pinnate, 0.48 mm long, 0.67 mm wide, flat edge, lacks vitta, has lobules that are narrowed at the tip with 0–3 teeth, medium in size (Figure 10b); the shape of the middle leaf cell is longer; the cell length is 0.03 mm, the width is 0.02 mm, the trigon is clearly visible, the shape is a large triangle (Figure 10d). The ventral leaves are tightly packed, 0.37 mm long and 0.44 mm wide. The tip is curved and undivided. The edges are uneven (Fig. 10c).

Distribution: Indonesia (Sumatra, Java, Kalimantan, Sulawesi), China, Australia, Thailand (Wang & Zhu, 2014).

T. humilis bears some resemblance to the species *T. repletus,* found in India. Both species have 1-3 toothed lobules and curved ventral leaves. These two species are distinguished by the shape of their lobules. *T. humilis* has a rounded tip, while *T. repletus* has a tapered tip (Deo, 2020).

CONCLUSION

There are ten different Lejeuneaceae species, which are divided into four genera: *Lejeunea, Lopholejeunea, Spruceanthus,* and *Thysananthus,* in the Curug Semirang region of Central Java. *Lejeunea* comes in two species: *Lejeunea eifrigii* and *Lejeunea flava*. The *Lopholejeunea* genus with the highest number of species includes *Lopholejeunea borneensis, Lopholejeunea horticola*, *Lopholejeunea nigricans, Lopholejeunea* sp., and *Lopholejeunea subfusca*. *Spruceanthus semirepandus* and *Spruceanthus planiusculus* are the two species that make up the genus *Spruceanthus*. *Thysananthus humilis* is the only species that makes up the genus *Thysananthus*.

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