

Ultrastructure of *Lejeunea* spp. leaves surface in a lowland tropical urban forest of Universitas Indonesia Campus, Depok, Indonesia

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Abstract. Putrika A, Nabella DM, Salamah A, Nisyawati, Dwiranti A. 2020. Ultrastructure of *Lejeunea* spp. leaves surface in a lowland tropical urban forest of Universitas Indonesia Campus, Depok, Indonesia. *Biodiversitas* 21: 4184-4191. *Lejeunea* is one of liverworts genera that have a wide distribution in the world. It has many variations of the character that have not been revealed, such as variations of the cell surface. The purpose of this research was to study the ultrastructure of *Lejeunea* spp. leaves surface in a lowland tropical urban forest of Universitas Indonesia (UI) Campus, Depok, Indonesia. Six species of *Lejeunea* spp. were studied, i.e., *L. anisophylla*, *L. cocoes*, *L. exilis*, *L. papilionacea*, *L. catanduana*, and *L. curviloba*. The research methods carried out in this study consisted of the sample observation using a light microscope, sample preparation for Scanning Electron Microscope (SEM) observation (fixation, post-fixation, dehydration, drying, mounting), and observations using SEM. The results of observation using a light microscope showed the smooth cell surface of all samples studied. Meanwhile, the differences between six species of *Lejeunea* in the UI campus could be differentiated under SEM. *L. catanduana* could be distinguished from other species from its cell wall thickness and texture, ornamentation types, and the number of ornamentations per cell. The texture of the cell wall of *L. catanduana* was the roughest than the other species due to abundant ornamentation such as papillae on its the cell wall surface. Furthermore, only this species has mamillae on the cell surface. The number of papillae or mamillae was 1-4 per cell. On the other hand, *L. cocoes* has the thinnest cell wall and slightly rough texture. Only this species has the simple papillae on the cell surface. Thus, the results of this study suggested that the cell surface variations in *Lejeunea* might be potential to be used as taxonomic characters in grouping species.

Keywords: Leaf cell surface, *Lejeunea*, light microscope, scanning electron microscope, ultrastructure

INTRODUCTION

Leafy liverwort is a type of liverworts that has a widespread distribution, especially in tropical areas (Konrat et al. 2008). This group has a leaf-like structure, which is not possessed by the thallus-shaped liverworts (Gradstein 2011). One of the largest families found in leafy liverwort is Lejeuneaceae. The distribution of Lejeuneaceae is around 50-90% in the African, Australian, and Indo-Malayan regions. The largest genus in the family Lejeuneaceae is *Lejeunea* Lib., that spread mainly in tropical Asia. The genus has about 150-200 species (Lee and Gradstein 2013). *Lejeunea* is one of liverworts genera that have a wide distribution. In Indonesia, the presence of *Lejeunea* spp. was reported 5 species in Central Java (Haerida and Gradstein 2011), 2 species in Bali (Haerida 2017), 3 species Mount Patuha (Gradstein et al. 2010), and also in the Universitas Indonesia (UI) (Ariyananda 2015; Wijaya et al. 2019; Putrika et al. 2020). The previous study reported there are six species of *Lejeunea* found in the Universitas Indonesia campus, which identified as *L. anisophylla* Mont, *L. cocoes* Mitt, *L. exilis* (Reinw., Blume & Nees) Grolle, *L. papilionacea* Prantl (Wijaya et al. 2019; Putrika et al. 2020), *L. catanduana* (Steph.) H.A. Mill., H. Whittier & B. Whittier, and *L. curviloba* Setph. (Ariyananda 2015). These species were identified morphologically by using a light microscope based on the lobule, lobes, underleaves, oil bodies, etc. Although these

six species have been identified, they have character similarities in terms of morphology, which are sometimes not easily distinguished amongst them, especially between *L. cocoes* and *L. exilis*, such as leave arrangement, lobes and lobule shape, cell shape, and oil body type (Putrika et al. 2020).

Species belongs to the genus *Lejeunea* have many variations of the character that could never see under a light microscope due to the limitation in resolution and magnification. One of the characters is variations of the cell surface (Lee et al. 2011), which could make different species misinterpreted into similar species. Wax ornamentation is one character of variation in leaf cell surfaces that can help to identify *Lejeunea* at the species level. Ornaments in each species have a unique shape, such as the presence of papillae in various sizes and numbers, as reported by Lee et al. (2014), who found *L. mimula* and *L. tuberculosa* have ornaments that are relatively small in size and spread throughout the cell surface. Therefore, ultrastructure such as wax ornamentation becomes a stable character in a species that can be used to help in the identification of *Lejeunea*. Ultrastructure means the fine structure, especially within a cell, that can be seen only with the high magnification obtainable with an electron microscope. The ornamentation would be clearly identified using the high-resolution electron microscope. The scanning electron microscope is a high-resolution microscope enabling visualization up to nanometers level

with the magnification reaches 3,000,000 times (Choudhary and Priyanka 2017).

The ultrastructure of 31 species of leafy liverworts, especially *Lejeunea* in Asia was reported by Lee et al. (2014). In addition, Lee (2020) also showed the ultrastructure of 24 *Lejeunea* species in Malesian region. However, to date, the ultrastructure characters of *Lejeunea* spp. in Indonesia, particularly in Universitas Indonesia Campus, Depok, West Java, Indonesia has not been reported. Universitas Indonesia (UI), which is located in the urban area, has a vast open green space area, such as an urban forest and campus green park. This location has numerous species of Bryophyte which are adapted in the urban environment. The morphological identification of Bryophyte, including the genus of *Lejeunea* in UI has been carried out previously. Thus, this study aimed to describe the ultrastructure cell surface variance of six species of *Lejeunea*. The results of this study gave an insight into the ultrastructure variations of different *Lejeunea* species and further reinforce the possibility of using the ultrastructure characteristic for species identification, especially for those with similar characteristics.

MATERIALS AND METHODS

Study area

Six species of *Lejeunea* were taken from the Universitas Indonesia (UI) campus area of Depok, West Java, Indonesia (Figure 1). The sample collection was conducted from January until June 2019. This research evaluated 4 species of fresh specimens and 2 species of herbarium specimens. Four fresh specimens were collected from the campus main street area, according to Putrika et al. (2019) sampling sites. Meanwhile, the two herbarium samples were collected in UI Urban Forest (Ariyananda 2015). Each species consisted of 3 samples which have been observed with Scanning Electron Microscope (SEM). The sample preparation process was carried out at Integrated Laboratory Research Center (ILRC) Universitas Indonesia. The SEM was observed in the PUSPIPTEK BPPT Serpong, Tangerang Selatan, Indonesia.

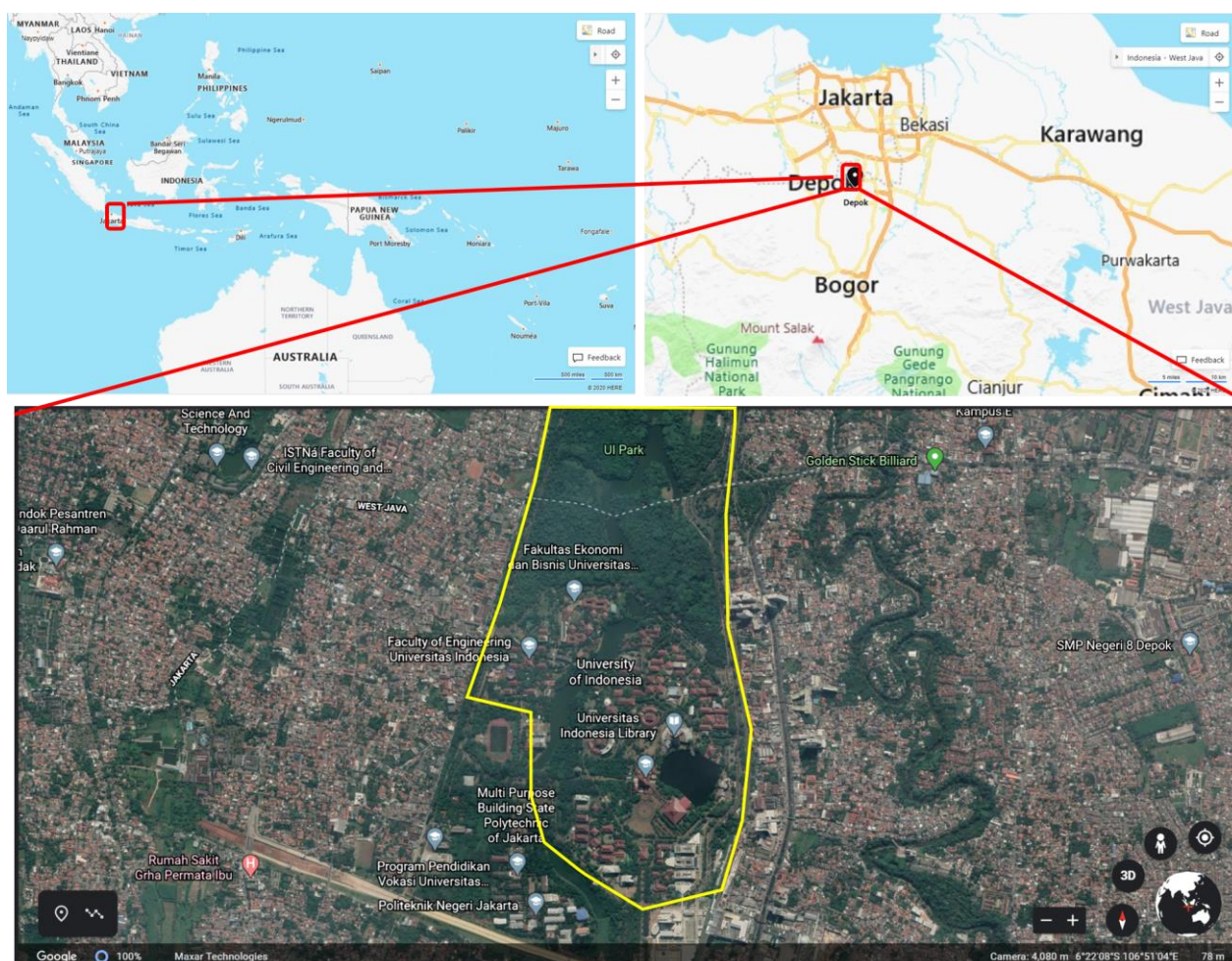


Figure 1. The area of Universitas Indonesia, Depok, West Java, Indonesia. The yellow line indicates the sampling site in this study and reported by Putrika et al. (2020). (source: google earth)

Procedure

The samples obtained from the field were observed using a stereomicroscope and an inverted microscope to ensure that the species taken belongs to the genus of *Lejeunea*. Lobe, lobule, margin leaf cell, and laminal cell shape were observed using a light microscope (Ariyananda 2015; Putrika et al. 2017). Samples that have been observed using a light microscope were then placed in a petri dish for SEM preparation. Sample preparation for observation using SEM consists of five main steps, i.e. fixation, post-fixation, dehydration, drying, and mounting. Samples were fixed using 2.5% glutaraldehyde in PBS (pH 7.0) for 30 minutes. Then the washing step was carried out using Phosphate Buffer Saline (PBS) three times, 10 minutes each. After the fixation, samples were then subjected to the post-fixation using a solution of 4% OsO₄ in PBS for 15 minutes and followed with PBS washing. Samples were then dehydrated using ethanol series (70%, 80%, 90%, 100%), and dried using hexamethyldisilazane (HMDS) (Dwiranti et al. 2019). The samples were finally mounted and observed using SEM.

Data analysis

Quantitative analysis was carried out using ImageJ software. The scale of the system was set according to the scale bar taken from the microscope. After that, by using line, the width and length of a specific area of interest were measured. The width and length appeared were derived automatically from the software using the set scale.

RESULTS AND DISCUSSION

There are four species of *Lejeunea* found in the study area, i.e. *L. cocoes*, *L. anisophylla*, *L. papilionacea*, and *L. exilis*. Another two species, *L. catanduana* and *L. curviloba*, which were reported by Ariyananda (2015), could not be found in this study. Nevertheless, Zhu et al. (2018) informed that *L. anisophylla* is accepted name, and placed *L. catanduana* as synonymous. Thus, in this study there are 2 samples of *L. anisophylla*, one specimen is a fresh material and another specimen is a dry herbarium collection from 2014. The herbarium collection of *L. anisophylla*, which has been identified as *L. curviloba*, was collected in the campus urban forest. It is found in the shaded area than the main street study site. To complete the ultrastructure data of all species formerly found in Universitas Indonesia, the two herbarium collections were observed. The fresh specimens have a green color, meanwhile, the herbarium specimen showed brown color under the light microscope. Both fresh specimens and herbarium specimen have a clear and detailed surface structure visualized by SEM. In general, those species can be distinguished by the lobes, lobule, and underleaves, using light microscopy. The detailed ultrastructure such as ornamentation of the leaf surface could only be visualized using SEM.

Leaf morphology of *Lejeunea* spp.

Lejeunea leave is divided into large dorsal lobes and small ventral lobule. Figure 2 shows the variation of leaf

arrangement and leaf lobes shape of six species of *Lejeunea*. *L. exilis* and *L. cocoes* have a similar character, such as distant of leaf arrangement, minute size and caducous leaves, as well as obtuse leaf apex (Figure 2). According to those characters, *L. exilis* sometimes interchangeably with *L. cocoes*. Lee et al. (2014) revealed that *L. exilis* and *L. cocoes* are closely related according to the phenetic analysis. Both species can be distinguished by the perianth character, leaf cell, and underleaves shape (Lee 2013).

Based on the light microscope observation, *L. anisophylla*, *L. papilionacea*, *L. curviloba*, and *L. catanduana* have a dense leaf arrangement. The leaf shape of *L. anisophylla*, *L. cocoes*, and *L. exilis* are ovate. *L. papilionacea*, *L. catanduana*, and *L. curviloba* have oblong-rectangular lobes. The lobes leaf apex of *L. cocoes* and *L. exilis* is obtuse but the other species showed round shape. Those characters in Figure 2 were visualized clearly using a light microscope and SEM. Even though *L. anisophylla* and *L. catanduana* are the same species, in this study, they showed the different lobes shape. These differences might be caused by a different point of view. The fresh *L. anisophylla* was observed from the middle site of one individual, meanwhile, *L. anisophylla*, previously known as *L. catanduana*, taken from the herbarium specimen, was observed from the apical side (Figure 2).

The lobule of *Lejeunea* was observed under light microscopy and SEM (Figure 3). There are four species that can be observed clearly under light microscopy and SEM, i.e. *L. anisophylla*, *L. cocoes*, *L. exilis*, and *L. papilionacea*. Meanwhile, lobule of *L. catanduana* and *L. curviloba* were only seen obviously under SEM. It may be because those two species are the dry herbarium specimen, so that it has brownish color in the light microscope. According to the SEM imaging, a specific character found in all *Lejeunea* observed is a single teeth lobule. Lee (2013) reported that single teeth lobule is a distinguishable character of *Lejeunea* from other genera within the Lejeuneaceae family. The leaf lobule of *L. anisophylla*, *L. cocoes*, *L. exilis*, and *L. papilionacea* have a truncate apex. The two herbarium specimens, *L. catanduana* and *L. curviloba* have a rounded lobule apex.

Margin leaf cell

According to the light microscope observation, the leaf lobes margin of *L. papilionacea* is crenulate, while the other species has the entire margin cells. The margin lobes cells of *L. papilionacea* are more swollen than other cells, thus it makes the leaf margin crenulate. Moreover, the leaf margin of *L. papilionacea* seems to have a border because of different cell shapes and sizes in the leaf lobes margin. The leaf margin of *L. anisophylla*, *L. exilis*, and *L. cocoes* is entire. They have pentagonal shapes and the sizes of the leaf margin are smaller than the other cells. Two specimens from the herbarium sample, *L. catanduana* and *L. curviloba*, have a clear shape of the margin cell visualized by SEM. The leaf margin cells of *L. catanduana* have a smaller size than in the middle cells (Figure 4). The border of each cell was distinguishable and indicated by "br" within the figure.

Laminal cell

The observation using a light microscope shows that all species of *Lejeunea* relatively similar surface without any up-and-down structure. A light microscope could facilitate the observation of the sample two-dimensionally, thus no detailed ornamentation or three-dimensional structure was visualized by using a light microscope. The median leaf cells of six species of *Lejeunea* have a pentagonal-hexagonal shape based on the light microscope observation. The cell shapes of six species of *Lejeunea* are more detailed under SEM (Figure 5). Figure 5 B' shows that *L. cocoës* has shrinkage leaf cells because of a smaller size than the other species and the thinnest cell walls (Table 1), therefore it is more easily shrunk. From the light microscope visualization, *L. cocoës* cells are not shrunk. The cell shape of the species is hexagonal that can observe clearly under a light microscope. The visualization of the median leaf cells from herbarium specimens also showed a clear structure. There was no shrinkage of the cells. The observation of herbarium specimens also shows some ornamentations on the leaf's cell surface which could not be observed under the light microscope.

The observation of six species *Lejeunea* under SEM shows that each species has a variety of size, number, and type of ornamentations on the lamina cells (Table 1). SEM imaging shows that the lamina cell surfaces are not completely smooth (Figure 6). There are two types of ornamentations such as papillae and mammillae on some

parts of the cell surfaces. Among all species, *L. catanduana* shows the roughest texture of the lamina cells. This species has two types of ornamentations, i.e. conical papillae and mamillae which are spread almost all over the cell surface. Furthermore, this species has the largest size of ornamentations compared to the others. *L. cocoës*, *L. anisophylla*, *L. papilionacea*, and *L. curviloba* only has one type of ornamentation and it is not spread on all part of the cell surface. Table 1 shows that the ornamentation type of *L. cocoës* has conical papillae in the leaf cell surface, while the other has conical papillae. Lee et al. (2013) show that most *Lejeunea* has a smooth leaf surface or slightly rough with minute papillae. *L. papilionacea*, *L. exilis*, and *Lejeunea cocoës* in Malaysia have been reported that they have a smooth leaf cell surface (Lee 2020).

Under a light microscope, *L. cocoës* and *L. exilis* are look-alike and sometimes are difficult to be distinguished. However, herewith we show that both species could be differentiated clearly under SEM due to the different dominant forms of papillae. *L. cocoës* has simple papillae, and *L. exilis* has a conical papilla (Table 1). This character could not be found in *L. cocoës* and *L. exilis* in Malaysia (Lee 2020). This difference might be induced by the different preparation methods for SEM observation. Lee (2020) prepared the sample using the critical point drying method, while in this study, the samples were dried by using Hexamethyldisilazane (HMDS) following Dwiranti et al. (2019).

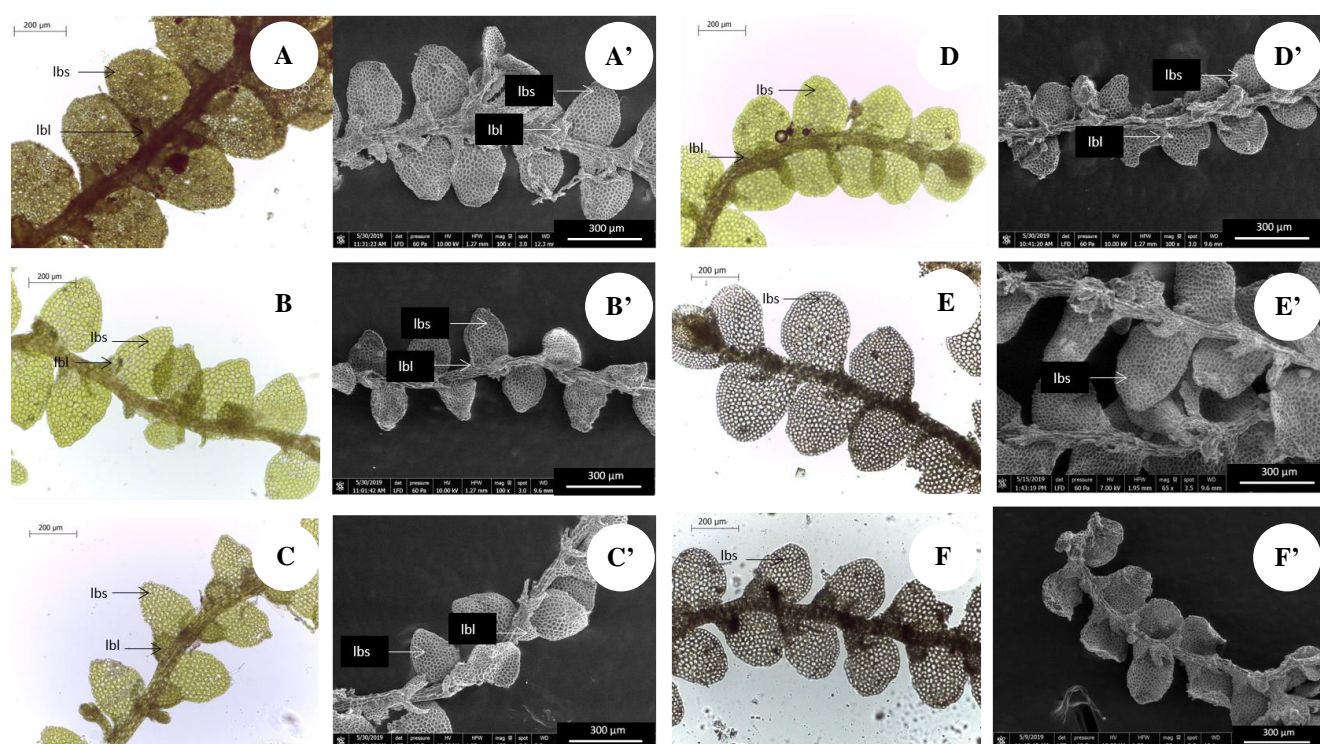


Figure 2. Visualization of *Lejeunea* spp. leaf arrangement and lobes. *L. anisophylla* (A, A'), *L. cocoës* (B, B'), *L. exilis* (C, C'), *L. papilionacea* (D, D'), *L. catanduana* (E, E') and *L. curviloba* (F, F') observed by light microscope (A-F) and SEM (A'-F'). Samples A-D were freshly prepared, while E and F were taken from the herbarium. "lbl" showed the lobule and "lbs" showed the lobes.

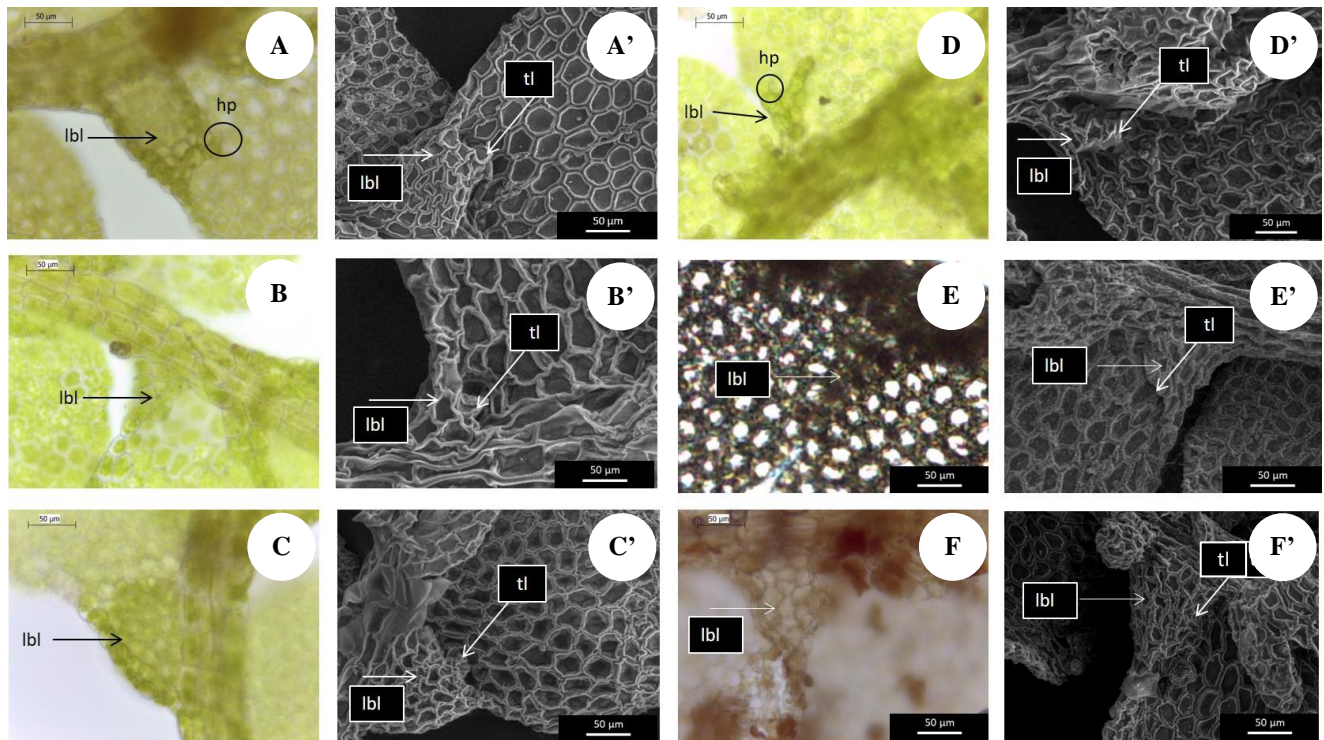


Figure 3. Visualization of *Lejeunea* spp. lobule. *L. anisophylla* (A, A'), *L. cocoes* (B, B'), *L. exilis* (C, C'), *L. papilionacea* (D, D'), *L. catanduana* (E, E') and *L. curviloba* (F, F') observed by light microscope (A-F) and SEM (A'-F'). Samples A-D were freshly prepared, while E and F were taken from the herbarium. "lbl" showed the lobule. "hp" showed the hyaline papillae. "tl" showed the teeth lobule

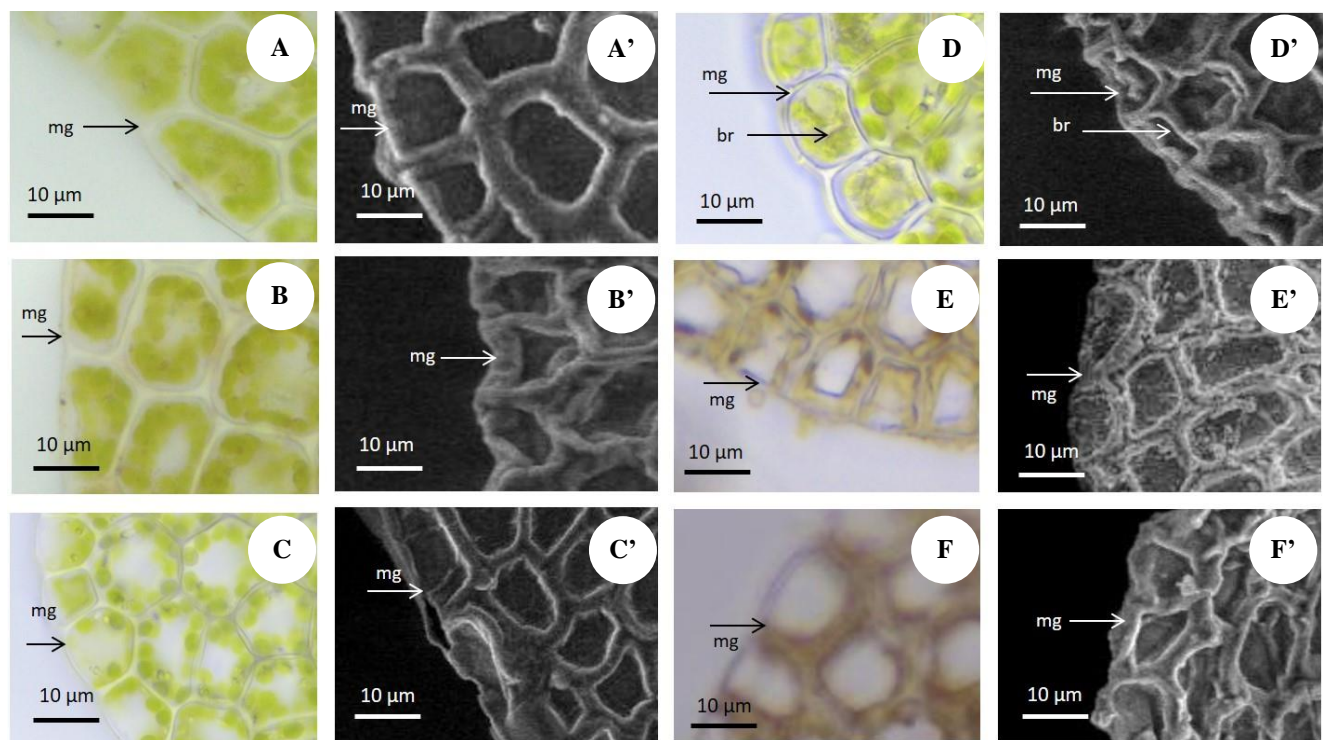


Figure 4. Visualization of *Lejeunea* spp. margin leaf cell. *L. anisophylla* (A, A'), *L. cocoes* (B, B'), *L. exilis* (C, C'), *L. papilionacea* (D, D'), *L. catanduana* (E, E') and *L. curviloba* (F, F') observed by light microscope (A-F) and SEM (A'-F'). Samples A-D were freshly prepared, while E and F were taken from the herbarium. "mg" indicates the margin. "br" indicates the border

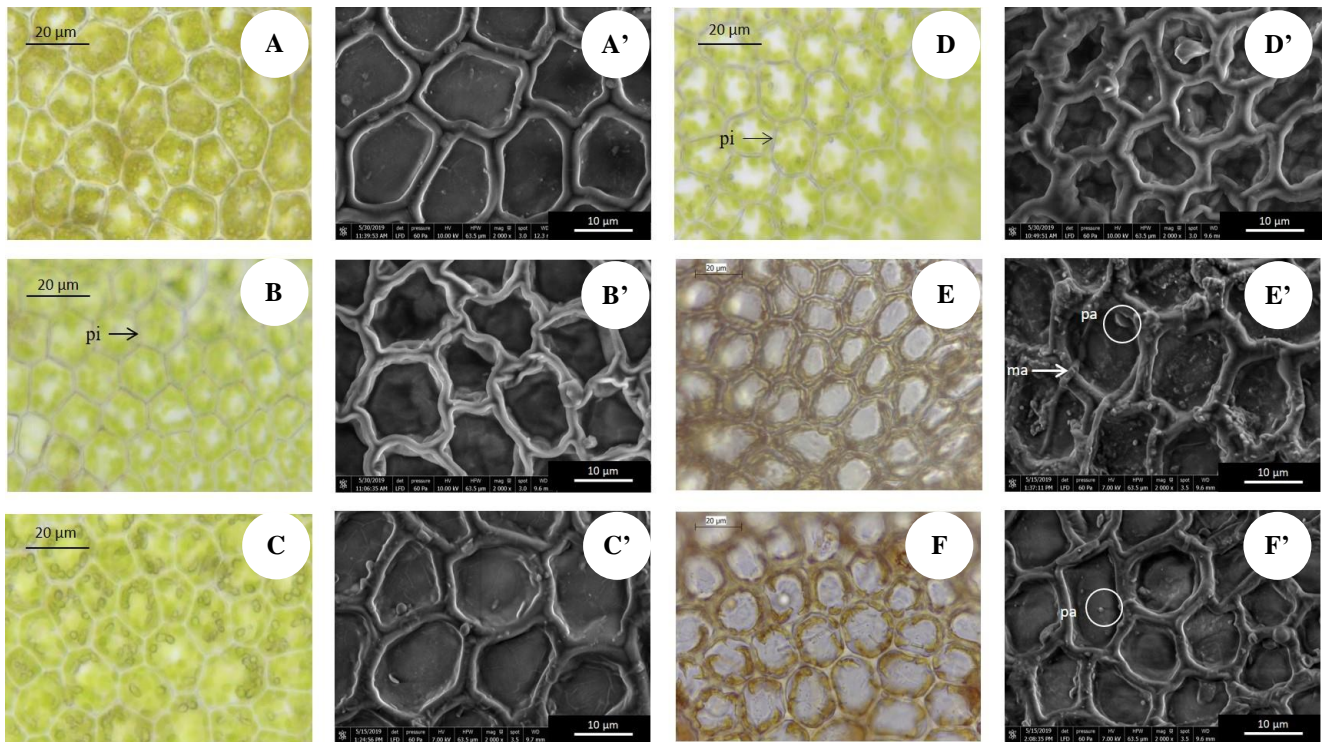


Figure 5. Visualization of the Laminal cell shape variation of *Lejeunea* spp. *L. anisophylla* (A, A'), *L. cocoas* (B, B'), *L. exilis* (C, C'), *L. papilionacea* (D, D'), *L. catanduana* (E, E') and *L. curviloba* (F, F') observed by light microscope (A-F) and SEM (A'-F'). Samples A-D were freshly prepared, while E and F were taken from the herbarium. “pa” indicates papillae, while “ma” indicates mamillae. “pi” depicted the intermediate thickening.

The papillae character was also found in true mosses carried out by Câmara and Kellogg (2010) in the family of Pottiaceae, Fissidentaceae, Rachithecaceae, and Sematophyllaceae with varying forms, namely the cone shape and baggy shape. The function of papillae in mosses is still unclear, nevertheless previous study proposed that it has a role to regulate light and temperature for adaptation to xerophyte conditions (Câmara and Kellogg 2010).

Mamillae-shaped cell surface ornamentation is only found in *L. catanduana*. The shape of the mamillae of *L. catanduana* is almost the same as the shape of the mamillae in the species *Gymnostomum calcareum* which has an elongated shape like a rope (Kou et al. 2014). Even though *Lejeunea catanduana* is a synonym of *L. anisophylla*, in this study, they have a different character in the leave cell surface. *L. catanduana* has a rough surface with abundant conical papillae and mamillae on the cell surface. It also has a thick cell wall and has ornamentalations in the cell wall surface (Figure 6E). On the other hand, *L. anisophylla* has a slightly rough surface with only conical papilla. It might be because those samples were collected from different site conditions. Further study with more sample collection from a different location would be necessary. *L. catanduana* is the most distinguishable compared to other species according to the type and number of cell surface ornamentation.

Lejeunea cocoas has the thinnest cell wall and slightly rough texture. Only this species has simple papillae on the cell surface (Table 1). According to Lee (2013), *L. cocoas* has a hyaline cell wall, thus it might be the reason for the thin cell walls. *L. cocoas* in Malaysia have been reported to have a smooth leaf cell surface (Lee 2020). The different result might be because of the different habitat conditions between Malaysia and Indonesia, especially Depok. These results indicated the use of ultrastructure would be beneficial for species identification. Further evaluation would be necessary to confirm this, especially regarding the adaptation mechanism that could induce ultrastructure differentiation. The larger number of species within the same genus from a different location with different environmental parameters would thus be necessary.

Another information that could be retrieved from the ultrastructure observation using SEM is the trigon. According to SEM observation, it is clear that all species have the trigons. *L. anisophylla*, *L. cocoas*, *L. papilionacea*, *L. catanduana* have large trigon, meanwhile, *L. exilis* and *L. curviloba* have small trigon (Figure 7). Siregar et al. (2020) reported that *L. curviloba* has an indistinct trigon as well. These results suggested that trigon development could also be used to differentiate one species from the other. This result is consistent with those reported by Lee (2013) that stated that trigon could be used to differentiate the species.

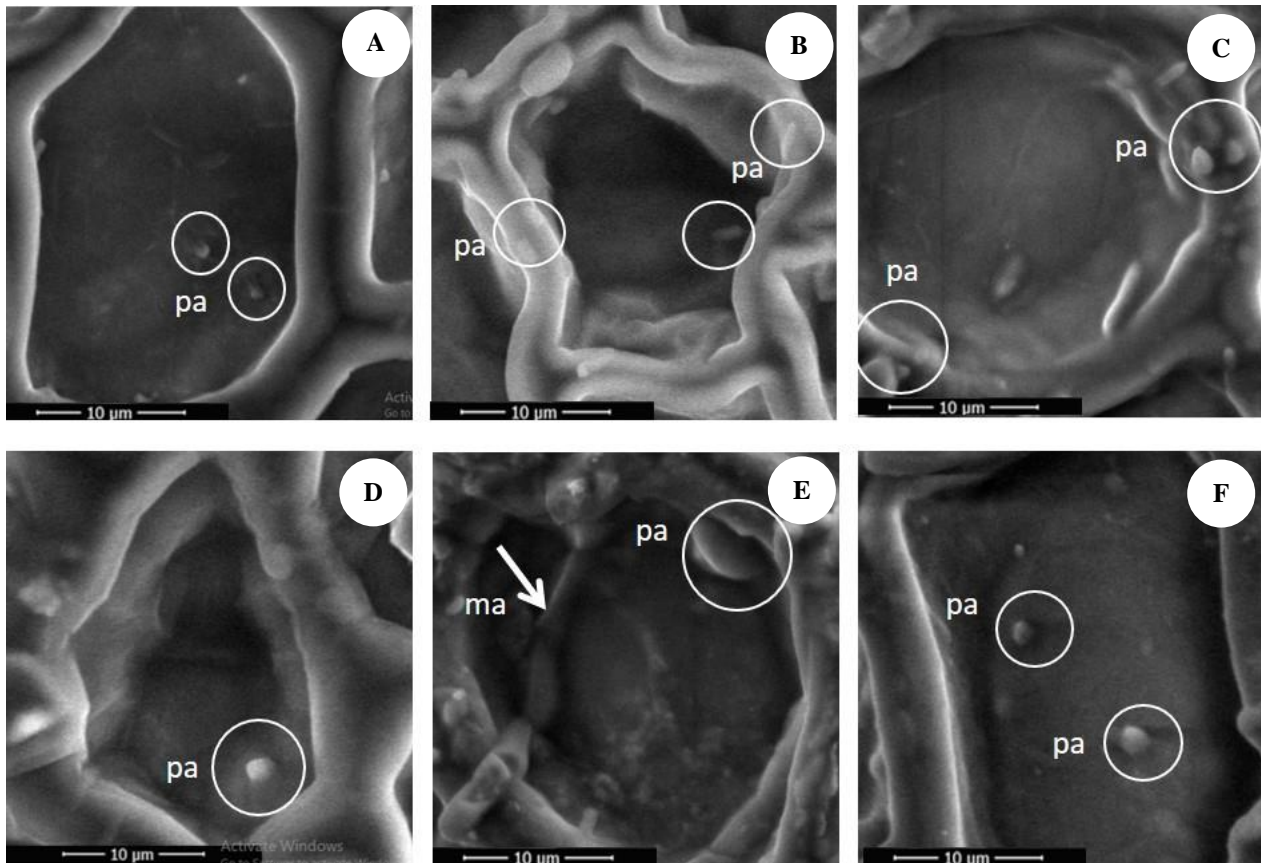


Figure 6. Visualization of the laminal cell surface ornamentation of *Lejeunea* spp. *L. anisophylla* (A), *L. cocoes* (B), *L. exilis* (C), *L. papilionacea* (D), *L. catanduana* (E), and *L. curviloba* (F) observed by SEM. ‘pa’ indicates papillae, while ‘ma’ indicates mamillae

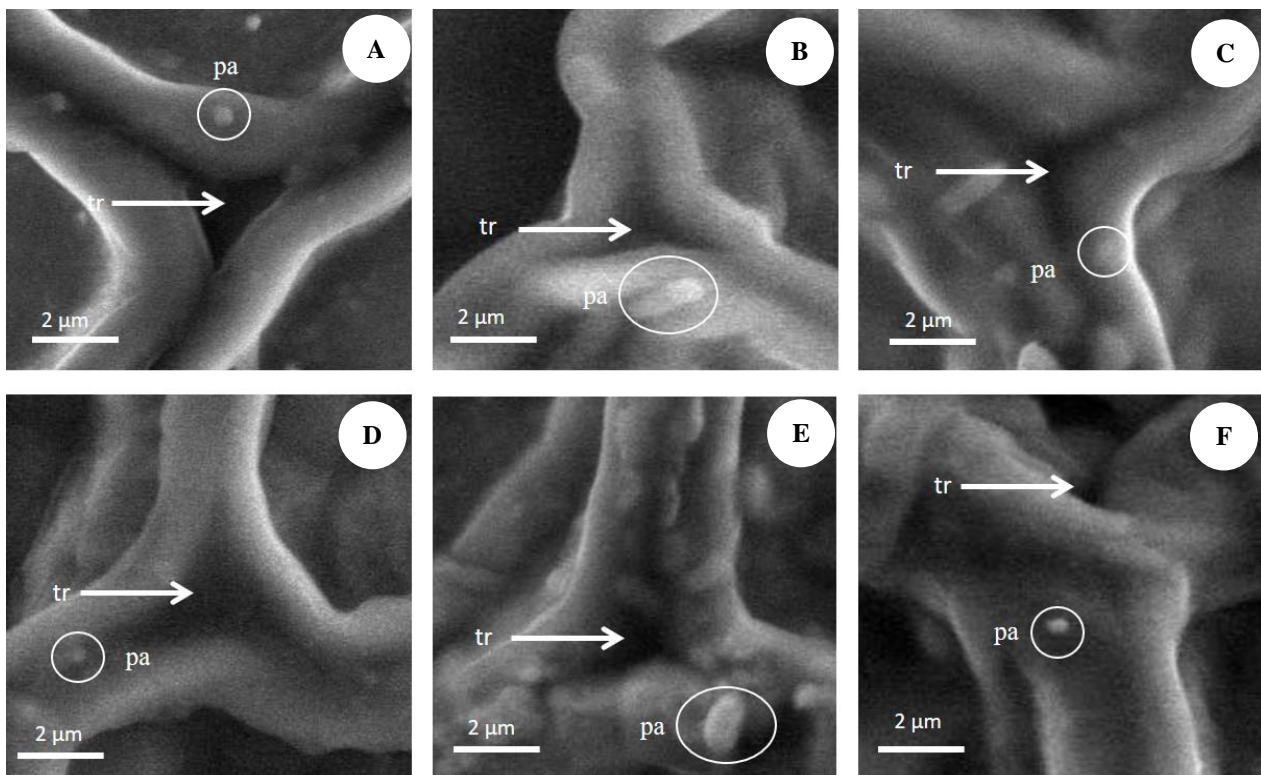


Figure 7. Visualization of the trigon of *Lejeunea* spp. *L. anisophylla* (A, A’), *L. cocoes* (B, B’), *L. exilis* (C, C’), *L. papilionacea* (D, D’), *L. catanduana* (E, E’) and *L. curviloba* (F, F’) observed by SEM. ‘pa’ indicates papillae, while ‘ma’ indicates mamillae. Trigon is depicted by the arrows (“tr”).

Table 1. Laminal cell characters of six species of *Lejeunea* in UI Campus, Depok, Indonesia under SEM

Species	Laminal cell wall surface				Laminal cell surface			
	Texture of cell wall	Type of ornamentation	Cell wall thickness (µm)	Texture of cell surface	Type of ornamentation	Ornamentation length (µm)	Number of ornamentation (per cell)	Average size of leaf cells (µm)
<i>L. anisophylla</i>	Slightly rough	Papillae	1.4-2.15	Slightly rough	Conical papillae	1.1-2.0	1-3	L: 17.14-20.02 W: 18.57-21.98
<i>L. cocoes</i>	Slightly rough	Papillae	1.08-1.19	Slightly rough	Simple papillae	1.5-2.3	1-3	L: 11.24-12.56 W: 11.20-13.05
<i>L. exilis</i>	Slightly rough	Papillae	3.15-3.81	Slightly rough	Conical papillae	1.1-3.2	1-3	L: 15.37-18.54 W: 14.55-16.78
<i>L. papilionacea</i>	Slightly rough	Papillae	1.26-1.7	Slightly rough	Conical papillae	0.7-1.8	1-2	L: 14.08-20.18 W: 10.15-11.40
<i>L. catanduanana</i>	Rough	Papillae	1.12-1.47	Rough	Conical papillae mamillae	1.5-3.6	1-4	L: 16.35-20.27 W: 10.00-12.49
<i>L. curviloba</i>	Slightly rough	Papillae	2.63-3.39	Slightly rough	Conical papillae	1.7-2.1	1-3	L: 10.27-16.54 W: 12.29-12.35

To conclude, according to the observation by the SEM, six species of *Lejeunea* in the Universitas Indonesia Campus could successfully be distinguished based on the ultrastructure characters of the laminal cell surface. Altogether, the results suggested that the ultrastructure characteristic might be potential to be used to differentiate the *Lejeunea*.

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