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Oil Bodies Variation in Ephypitic Leafy Liverworts at Universitas Indonesia, Depok

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Abstract. Oil bodies are specialized globular membrane-bound organelle containing secondary metabolites and found in liverwort cells. Oil bodies can be used as a characters identification in specific level since the early nineteenth century because it has various shape, color, size, number, and distribution between species. The aim of this study is to describe oil bodies variation of ten species of leafy liverworts in the Universitas Indonesia campus. The research started in March-June 2018 covering the area of UI's urban forest and main roads within campuses. We used quadrat method plots in the Urban Forest and broad survey along the main road of UI. We used the right and left sides of the main road of UI as observation track traces. The number of quadrate plots used in the urban forest are 12 plots of 20 × 20 meters. We placed 4 plots each in 3 zones, namely Wales Barat, Wales Timur and Vegetasi Alam, 4 plots. Variation of oil bodies were observed form the leaf cells of the liverworts under the binocular light microscope with magnification 400–1000 x. The size of leaf cells and oil bodies was measured by image J 64 software. The results obtained as many as 10 species of leafy liverworts consisting of 2 families and 6 generas. We identified variation of oil bodies from the ten species. There are four type of oil bodies. The variation showed are in oil bodies number, shape and size. Therefore, oil body variations can be used as a key species identification in leafy liverworts in Universitas Indonesia.

Keywords: Oil bodies, leafy liverworts, ephypitic liverworts

INTRODUCTION

Oil bodies are unique feature of liverworts that separating them from another non-vascular plants, mosses and hornworts. This character can be found in the gametophyte or sporophyte cells, especially from order Jungermaniales (leafy liverworts) [1]. They have a single membrane organelle which is containing the secondary metabolite such as terpenoid and aromatic compounds that can be suggested as potential resource for medicines [2]. Most species have colorless oil bodies in the cytoplasm, thus it can be easily to recognize under the light microscope because the unique color and shape that different with chloroplast [3-4]. Furthermore, this organelle has various shape, color, size, number, and distribution in the leaf cell therefore it plays an important character for taxonomy [5-6].

The analysis of liverworts oil bodies mostly has been studied in the Order Jungermaniales comprised families Lejeuneaceae and Frullaniaceae. The oil bodies variation in some liverworts from Order Jungermaniales have been studied in New Zealand [3], Fiji [4] and Eastern Himalaya, India [7]. Most of them are in the montane forest and form the subtropical region. The study of oil bodies variation from Indonesian liverworts has not been conducted yet.

Gradstein 1977 classified four main types of oil body of liverworts. They are Massula type, Bazzania-type, Jungermania-type, and Calypogaea-types [5]. Morphologically, Massula type and Bazzania type have a homogenous surface, but they recognized by the size and number per cell. Massula type is homogenous oil body, small size (2–6 µm long) and numerous in median leaf cell (more than 8 per cell). Furthermore, Bazzania type larger than Massula type (1–15 µm long) and up to 8 per cell. In addition, Jungermania-type and Calypogaea type have segmented oil

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body. Jungermania type has finely segmented, consisting of numerous minute, indistinct globules surrounded by common a membrane. Calypogaea type have coarsely segmented, consisting of distinct globules aggregate to form "grape shape" [5].

Indonesia, particularly in Java, have 568 species of liverworts that have recorded [8, 9]. It needs further study of liverworts in Java particularly in lowland and urban area. One of the urban areas in Java is Universitas Indonesia. It has an open space area encompasses urban forest and public park in the faculty region. In the 2012 there were 2 families (Frullaniaceae and Lejeuneaceae), 7 genera, and 15 species of epiphytic liverworts in UI urban forest and along main road of UI [10]. The other study of epiphytic liverworts in 2018 there are 10 species from 2 families of epiphytic liverworts in Universitas Indonesia [11]. According to the previous research of liverworts, we would like to observe the variation oil bodies of epiphytic liverworts in Universitas Indonesia. The objective of the study is to identify and describe the oil body type from 10 species of epiphytic liverworts in Universitas Indonesia.

EXPERIMENTAL

The liverworts collected in the UI 3 zones urban forest and main road inside campuses on March until June 2018. We investigated the liverworts in three zones of UI's urban forest area, that are Wales Barat, Wales Timur and Vegetasi Alam.

Sampling Method

Sampling technique in the UI's urban forest followed a sampling protocol technique from Gradstein et al. [12] with a slight modification from Putrika [10]. Sampling in the UI's urban forest was plot quadrate method. The number of plot in the urban forest were 12 plots measuring 20 x 20 m^2. Five trees which have DBH $\geq$ 20 at each quadrat were selected as a host for sampling epiphytic liverworts. Whereas the broad survey was used in the main road inside campuses. Broad survey is more flexible. It is considered as the best method which can be use for the limited region [13] like the main road of UI. Observation track were placed on the right and left sides of the main road of UI. Selection of the host tree on the main road of the UI campus is same as in the UI forest which have DBH $\geq$ 20. In each tree, 8 squares of subplot 15 x 15 cm^2 were placed at 4 different direction, north, west, south, and east at 0–200 cm high of a tree trunk. Liverworts found at each quadrat were sampled, named and sealed in the plastic bag for the laboratory identification.

Observation Technique in Laboratory

Fresh materials are needed for oil bodies observation. Therefore, the samples have to observed immediately after collection. The voucher specimens were deposited in the Herbarium of Department of Biology UI. Gametophyte characters were observed on fresh specimens from the field by microscope stereo Carton BD.B1-02-01/20/16 and light microscope Nikon SE 891575. After all of the gametophyte characters have been observed, than it matched with characters in identification book to be identified until the species level. The identification book used are from So [14], Zhu et al. [15] and Gradstein [9]. Variations of oil bodies were observed form the leaf cells of the liverworts under the binocular light microscope (Leica DM 500) with magnification 400–1000 x. The size leaf cells and oil bodies were measured by image J 64 software. After that, characters of oil bodies matched with explanation from Gradstein [5].

RESULTS AND DISCUSSION

Our results suggested that epiphytic liverworts obtained at the UI urban forest and main road inside campuses were leafy liverworts group. There were 10 species which belongs to 2 families, Lejeuneaceae and Frullaniaceae. The type of oil bodies of ten species of epiphytic liverworts is classified into 4 types, i.e massula type, jungermania type, and calypogeia type, and bazzania type (Table 1). According to the data, Lejeuneaceae have four types of oil body.

Table 1 shows that the oil body between each genera within one family is different in type, but the oil body between species in one genus is same. The oil body type of *Acrolejeunea fertilis* and *Microlejeunea ulicina* is Massula type.

Cololejeunea lanciloba is the only one species that has Bazzania oil bodies type. *Frullania campanulata* and *Lejeunea genus* have same oil bodies type which is Jungermania type. Furthermore, all species of *Cheilolejeunea* in UI campus have a Calypogeia oil body type.

Cheilolejeunea has the largest oil body size in the group of Lejeuneaceae, but the number of oil body per cell is fewer than the other family, i.e. 2-4 each cell. Three species of *Cheilolejeunea* in UI campus have difference size and different number of oil bodies. *C. intertexta* (Fig. 1a) has 2-4 oil bodies of each cells which size is 9–22 × 4–6 µm. Oil bodies of *C. serpentina* (Fig. 1b) have various sizes which are greater than the other three species, 13–22 × 4–6 µm, although the numbers of oil bodies are only 2–3 in each cell. *Cheilolejeunea* sp. (Fig. 1c) have intermediate cell size ranging from 12–24 µm, but have a few of oil bodies, about 1–3 in each cells. The results of this research are also supported by Gradstein 2011 [9] who stated that the genus *Cheilolejeunea* has oil bodies of the size (specify size) and have a shaped Calypogeia type (Table 1).

Three *Lejeunea* species in UI campus have Jungermania type oil bodies. Each species can be identified from the number and size of the oil bodies. *Lejeunea exilis* (Fig. 2b) has moderate numbers of each cells oil bodies compared to other species with 10–14 leaf cells with a length and medium width of 2–9.6 × 0.9–1.4 µm. *Lejeunea anisophylla* (Fig. 2c) has the least amount of oil bodies in each cells that is as much as 4–8 at each cells but the size is larger than *L. exilis* which is 3–12 × 1–4 µm. *Lejeunea patriciae* (Fig. 2d) has a larger cell size than other *Lejeunea* species but has the smallest size of oil bodies which is only 3.6–6.8 × 2.2–3.8 µm. The difference between cell sizes and the oil bodies sizes allows more number of oil bodies in the cells than other *Lejeunea* species, which is 13–17 each leaf cells. The oil body type of *Lejeunea* is Jungermania type. It has the same character as the Lejeuneaceae in Malayan [16].

Oil body character of Lejeuneaceae in Malaysia especially *L. patriciae* and *L. exilis* has Jungermania type. *Acrolejeunea fertilis* belongs to subfamilies Ptychanthoideae. These subfamilies member generally have homogenous oil bodies as found in the study [17]. Oil body growth is rarely influenced by the environment and can be distinguish between families, genera, and species. It has a difference between the amount, size, and shape of the oil body [3-4]. Therefore, oil body can be used as a distinguished characters between genera or families [4].

Description of Oil Bodies

Cheilolejeunea intertexta (Fig. 1a). Leaf cell hexagonal, 20–32 × 13–24 µm, thick cell walls, yellowing green, trigones small, 0.9–1.7 µm. Oil bodies opaque, 2–4 each leaf cells, disperse around cells, elongated elips, vary in size 9–22 × 4–6 µm, large granulose, Calypogeia type. Ocelli is found in center leaf cells.

Cheilolejeunea serpentina (Fig. 1b). Leaf cell rounded, 10–19 × 15–28 µm, thin cell walls, not pigmented, opaque, trigones well develop, 2.2–4.6 µm. Oil bodies greenish, 2–3 each leaf cells in the central cells, elongated elips, vary in size 13–22 × 4–6 µm, large granulose, Calypogeia type. Ocelli is found in center leaf cells. Oil bodies are fill almost in the cell lumen.

Cheilolejeunea sp. (Fig. 1c). Leaf cell hexagonal, 10–19 × 15–20 µm, thin cell walls, not pigmented, opaque, trigones well develop, 12–24 µm. Oil bodies greenish, 1–3 each leaf cells in the central cells, elongated elips, 10–15 × 3–6 µm, large granulose, Calypogeia type. Ocelli is found in center leaf cells.

Cololejeunea lanciloba (Fig. 1d). Leaf cell hexagonal, 16–30 × 19–37 µm, leaf margin bordering with a thick rectangular hyaline cells. thin cell walls, not pigmented, trigone well develop, 1.5–3.5 µm. Oil bodies greenish, 7–13 each leaf cells, elongated elips, 5.3–8.5 × 10–15 µm, homogenous vague segmented, Bazzania type.

Microlejeunea ulicina (Fig. 1e). Leaf cell hexagonal, 8–12 × 9–16 µm, thick cell walls, not pigmented opaque, trigones small, 0.21–4.24 µm. Oil bodies yellowing opaque, 3–5 each leaf cells, disperse around cell, elongated rounded, 0.7–1.4 × 0.4–0.9 µm, homogenous, Massula type.

Acrolejeunea fertilis (Fig. 1f). Leaf cell hexagonal to rounded, 28–50 × 21–28 µm, thin cell walls, not pigmented yellow brown, trigone very large, 3.1–5.8 µm. Oil bodies yellowish, 5–7 each cells, disperse around cells, elongated elips, 8–13 × 3–5 µm, homogenous, Massula type.

Frullania campanulata (Fig. 2a). Leaf cell elliptical, 14–21 × 10–15 µm, thin cell walls, not pigmented yellow brown, trigone is small, 0.69–1.40 µm. Oil bodies greenish, 2–4 per leaf cell, elongated ellipse, 8–13 × 3–5 µm, granulose, Jungermania type, located near marginal cell walls.

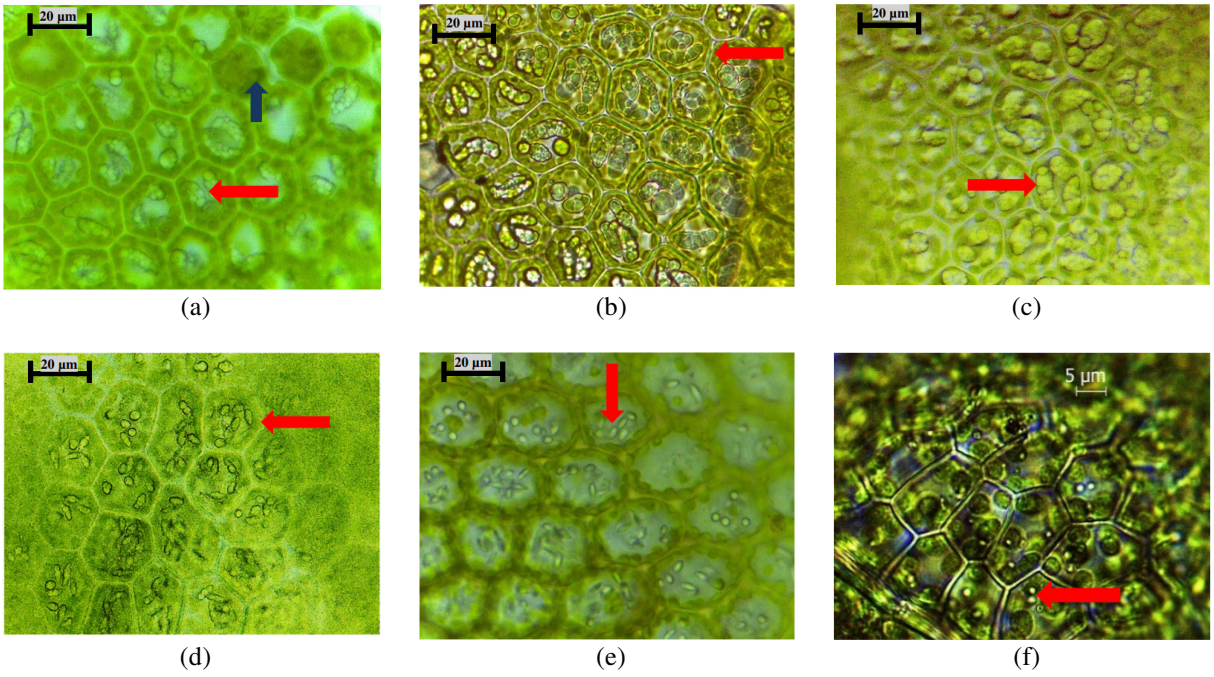
Lejeunea exilis (Fig. 2b). Leaf cell quadrate on the marginal, hexagonal on the center leaf, 19–24 × 15–20 µm, thin cell walls, not pigmented, opaque, trigones well develop, 1.2–2.4 µm. Oil bodies greenish, 10–14 each leaf cells, located near marginal cells, elongated elips, vary in size, 2–9.6 × 0.9–1.4 µm, fine granulose, Jungermania type.

Lejeunea anisophylla (Fig. 2c). Leaf cell rectangular to polyhedral, 18–30 × 15–23 µm, thin cell walls, not pigmented, opaque, trigones well develop, 1.6–3.0 µm. Oil bodies greenish, 4–8 each cells, located near marginal cells, elongated elips, 3–12 × 1–4 µm, fine granulose, Jungermania type.

Lejeunea patriciae (Fig. 2d). Leaf cell polygonal, 31–40 × 20–30 µm, thin cell walls, not pigmented, opaque, trigones very small, 1.0–1.5 µm. Oil bodies greenish, 13–17 each leaf cells disperse around the cells, rounded to elongated elips, 3.6–6.8 × 2.2–3.8 µm, fine granulose, Jungermania type.

TABLE 1. Oil bodies type of 10 species leafy liverworts in Universitas Indonesia.

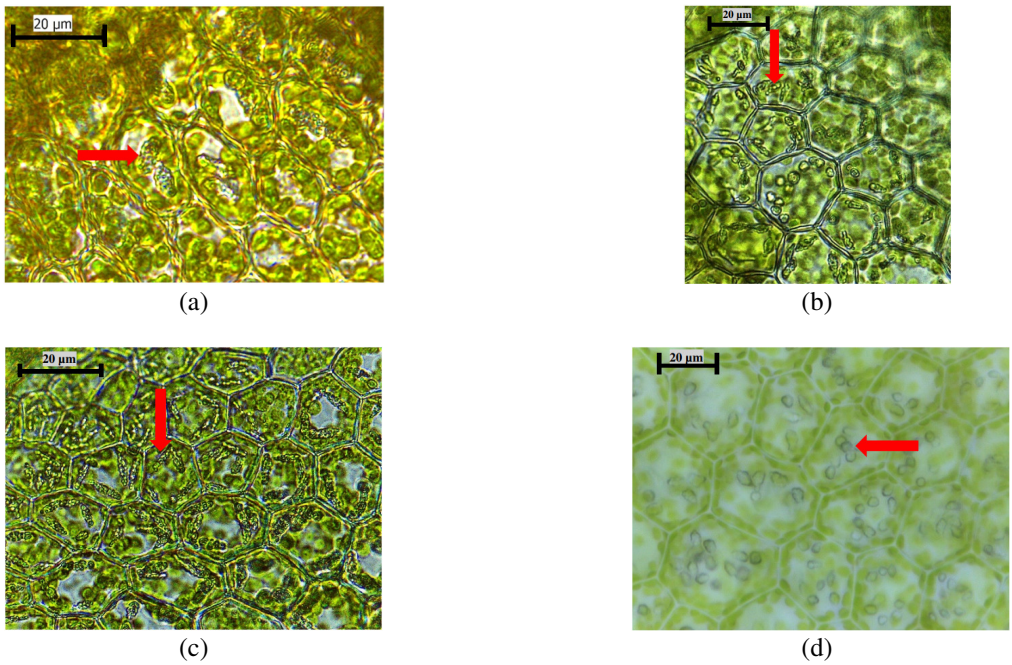
No	Families	Genera	Species	Oil Bodies Type
1	Lejeuneaceae	*Cheilolejeunea*	*Cheilolejeunea intertexta*	Calypogeia type
2	Lejeuneaceae		*Cheilolejeunea serpentina*	Calypogeia type
3	Lejeuneaceae		*Cheilolejeunea* sp.	Calypogeia type
4	Lejeuneaceae	*Cololejeunea*	*Cololejeunea lanciloba*	Bazzania- type
5	Lejeuneaceae	*Microlejeunea*	*Microlejeunea ulicina*	Massula-type
6	Lejeuneaceae	*Acrolejeunea*	*Acrolejeunea fertilis*	Massula-type
7	Frullaniaceae	*Frullania*	*Frullania campanulata*	Jungermania-type
8	Lejeuneaceae	*Lejeunea*	*Lejeunea exillis*	Jungermania-type
9	Lejeuneaceae		*Lejeunea anisophylla*	Jungermania-type
10	Lejeuneaceae		*Lejeunea patriciae*	Jungermania-type



(a) (b) (c)

(d) (e) (f)

FIGURE 1. The red arrow indicates the oil bodies while the blue arrow shows the ocelli found in: Calypogeia type; (a) *Cheilolejeuna intertexta*, (b) *Cheilolejeunea serpentina*, (c) *Cheilolejeunea sp.*, Bazzania type; (d) *Cololejeunea lanciloba*, and Massula type; (e) *Microlejeunea ulicina*, (f) *Acrolejeunea fertilis*



(a) (b) (c) (d)

FIGURE 2. The red arrow indicate Jungermania type of oil bodies found in (a) *Frullania campanulata*, (b) *Lejeunea exilis*, (c) *Lejeunea anisophylla,* and (d) *Lejeunea patriciae.*

CONCLUSION

We conclude that all oil bodies type were recorded in this research. Oil body types among the 6 genera have different features. There are 4 types of 10 oil bodies of leafy liverworts in Universitas Indonesia i.e 2 species have Massula type, 4 species have Jungermania type, 3 species have Calypogeia type, and 1 species have Bazzania type. Each species have a unique oil bodies characters. That result inform that oil bodies can be use as a great key taxonomical value for interspesific differentiating structure based on size, number, and location of oil bodies on the cells within species.

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REFERENCES

1. C. B. Stotler, R. E. Stotler and D. G. Long, in *Bryophyte Biology*, edited by B. Goffinet and A. J. Shaw (Cambridge University Press, Singapore, 2008).
2. X. He, Y. Sun and R.-L. Zhu, CRC Crit. Rev. Plant. Sci. **32**, 293-302 (2013).
3. G. H. Stewart, New. Zeal. J. Bot **16**, 185-205 (1978).
4. N. Kitagawa, Bull. Nara Univ. Ed. Natur. Sci. **35**, 59-70 (1986).
5. S. R. Gradstein, A. M. Cleef and M. H. Fulford, Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht **479**, 377-393 (1977).
6. A. Vanderpoorten and B. Goffined, *Introduction to Bryophytes* (Cambridge University Press, United States, 2009).

7. D. K. Singh, M. Dey and D. Singh, Taiwania **57**, 384-390 (2012).
8. L. Söderström, S. R. Gradstein and A. Hagborg, Phytotaxa **9**, 53-149 (2010).
9. S. R. Gradstein, in *Guide to the Liverworts and Hornworts of Java*, edited by S. S. Tjitrosoedirdjo (SEAMEO BRYOTROP, Bogor, 2011).
10. A. Putrika, N. Nisyawati and N. S. Ariyanti, Bio Site **3**, 25-38 (2017).
11. S. K. Wijaya, B.Sc. Final Project, Universitas Indonesia, Depok, 2018.
12. S. R. Gradstein, N. M. Nadkarni, T. Kromer, I. Holz and N. Noske, Selbyana **24**, 105-111 (2003).
13. J. M. Glime, *Bryophyte Ecology volume I: Physiological Ecology* (Michigan Technological University, Michigan, 2013).
14. M. L. So, *Mosses and Liverworts of HongKong Volume 1* (Hong Kong Baptist University, Hong Kong, 1995).
15. R. L. Zhu, and M. L. So, *Mosses and Liverworts of Hong Kong Volume 2* (Heavenly People Depot, Hong Kong, 1996).
16. G. E. Lee, Cryptogamie Bryol. **34**, 381-484 (2013).
17. S. Kornochalert, K. Santanachote and J. Wang, Cryptogamie Bryol. **33**, 39-63 (2012).