Visualization of *Microlejeunea ulicina* by using Transmission Electron Microscopy

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**Abstract.** Oil bodies are highly distinctive organelle uniquely found in liverworts. Besides the importance of this structure in taxonomy, many of the secondary metabolites contained within the oil bodies are also of the great value as potential sources of medicines. Thus, the investigation of liverwort ultrastructure is essential to give an insight into the oil bodies structure and function. *Microlejeunea ulicina* is one species of the leafy liverworts found in Universitas Indonesia that has not previously been reported to exist in Java island. The aim of this study is to investigate the ultrastructure of *M. ulicina* by using Transmission Electron Microscopy (TEM). *M. ulicina* were fixed with 2.5% glutaraldehyde, post-fixed with 4% Osmium tetroxide, dehydrated with Ethanol series, and then subjected to resin embedding. After samples embedding, sectioning by ultramicrotome was performed. Post-staining with Uranyl acetate and Lead Citrate was carried out following sectioning and the samples were finally observed by TEM. Our results showed the ultrastructure of *M. ulicina* for the first time. The structure of oil bodies could be distinguished with the other parts of the cells. Further analysis of the oil bodies by comparing our data with other species of leafy liverwort in Universitas Indonesia will be beneficial for the species identification as well as the exploration of their secondary metabolites.

1. **Introduction**

Leafy liverworts have a great number and variety and distributed worldwide [1]. One of the typical features in leafy liverworts is the oil body. Its distinct characteristic in every single species and its high potential metabolites has attracted the attention of many researchers [2,3]. *Microlejeunea ulicina* has been reported to exist in Indonesia, especially particularly in Sumatera and Borneo islands. Nevertheless, it was also found in Universitas Indonesia that has not been reported before. Thus further study on *M. ulicina* is of great importance to give rise into the Marchantia diversity.

The ultrastructure analysis of *M. ulicina* is essential for the taxonomy. Furthermore, the deeper exploration of the oil body which contains useful metabolites would also be achieved by the ultrastructure analysis. The metabolites contained in the leafy liverwort’s oil body have previously been studied to be potential as the antimicrobial, antioxidant, and so on. Due to its high resolution and magnification, the ultrastructure investigation can be accomplished by using Transmission Electron Microscopy (TEM). However, to date, there has been no report about the ultrastructure of *M. ulicina*...
especially its oil body appearance by using TEM. Thus, the aim of this study is to investigate the ultrastructure of *M. ulicina* by using Transmission Electron Microscopy (TEM). Several preparation methods were also optimized in this study to obtain the clear TEM image of *Microlejeunea ulicina*.

2. Materials and Methods

*Microlejeunea ulicina* were taken from Universitas Indonesia, Depok campus. The method used in this study was adopted from Pihakaski [5]. After removal of the contaminant by observing under optical microscopy, samples were fixed with 2.5% glutaraldehyde in Phosphate Buffer Saline (PBS), followed with post-fixation with 4% Osmium tetroxide and dehydrated using Ethanol series (30, 40, 50, 60, 70, 80, 90, and 100%), 10 minutes each.

After 100% EtOH treatment, samples were immersed in EtOH: Propylene oxide with the ratio of 3:1, 1:1, and 1:3, for 30 minutes each, followed with resin embedding. Two different kinds of resin and different length of time for incubation were optimized. The samples were then sectioned 80-100 nm by using ultramicrotome. Post-staining by using Uranyl acetate and lead citrate were carried out prior to observation by using TEM, 10 minutes each. Samples were finally observed by using TEM H-9500 (Hitachi) with the acceleration voltage of 120 kV and the resolution up to 0.1 nm.

3. Results and Discussions

In this study, the inner structure of *M. ulicina* was investigated by the application of TEM which provide higher resolution and magnification as compared to the optical microscopy. Since this is the first study of Microlejeunea ulicina ultrastructure, we optimized several conditions in the preparation steps, including the type of resin and the length of resin embedding incubation times (data not shown). Although it was challenging to prepare the samples as native as possible and fit with the requirement for TEM observation, we finally could observe the ultrastructure of *M. ulicina* by using TEM as depicted in Figure 1.

![Figure 1](image)

*Figure 1. Microlejeunea ulicina* observed by TEM. OB: Oil Bodies. 6000 X magnification. Bar: 1 µm.

According to the data obtained, some structures could be observed by using TEM. Each part of the cells shows a different electron intensity. Some area shows higher electron density then the rest. Oil body generally shows the electron transparent area due to its characteristics [5]. In this study, we were aiming to observe these oil bodies. The clear oil body-like structures are also shown in Figure 2.
As shown in Figure 2, some structures showed the electron transparent areas were clearly visualized by using TEM and assumed to be the oil bodies (OB). The size and shape of these oil bodies-like structures are varied. Most of them are distinctly separated from each other (single, undivided), although few of them are not (multipartite). The interior of the oil bodies-like structures is completely homogeneous and transparent. Similar results which were then concluded to be the oil bodies have also been reported by Pihakaski [5, 6]. The size of the oil bodies-like structures in this study is ranging from 1.03 µm to 4.96 µm in length and 0.47 µm to 1.72 µm in width. The oil bodies have been predicted to be developed from the vacuoles as reported from the Bazzania trilobata, they coalesce and form larger bodies which are then enclosed by a thin membrane [5]. However, Suire (1970) in He et al. [2] concluded that oil bodies originate from dilated ER cisternae as shown from the investigation of Radula complanata.

The results obtained in this study suggested that the preparation method that has been optimized in this study successfully resulted in the clear thin section showing the clear oil bodies-like structure of Microlejeunea ulicina. Different species of leafy liverworts have a distinct feature of the oil body. In addition, a different growth stage of the liverworts could also result in different feature of the oil body. Thus, further analysis including the stage of the samples and comparison of different types of Marchantiophyta is important to be assessed.

4. Conclusions

The inner structure of Microlejeunea ulicina could clearly be observed by using TEM. The oil body-like structures showing an electron transparent area were also obtained. The optimum condition for Microlejeunea ulicina TEM preparation steps including 2.5% glutaraldehyde fixation, OsO₄ post-fixation, EtOH dehydration series, EtOH: Propylene oxide, resin embedding using Spurr’s kit for 48 hours, ultramicrotome sectioning, and post-staining using Uranyl acetate and Lead citrate.

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