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TAXONOMIC SIGNIFICANCE OF MORPHOLOGICAL STRUCTURE IN AZOLLA

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ABSTRACT

Azolla is an aquatic fern which belongs to Salviniaceae family that has huge potential as biofertilizers in paddy plantation, alternative food source for livestock and phytoremediation for wastewater. This plant is widely distributed in Southeast Asia, East Asia, Australia, India, and Africa. Previous study had successfully used morphological characteristics to identify the species of Azolla and to distinguish the Asian Azolla pinnata and African Azolla pinnata. In Malaysia, Azolla species can be easily found and highly reproductive in major irrigation schemes like ponds, lake, paddy field and river. Thus, the goal of this study was to identify morphological structure of Azolla species found in two different locations in Selangor. The identification and characterization of Azolla were made by evaluating the 16 morphological features applied to all Azolla accessions, the degree of dorsal leaf lobe imbrication and the length to width ratio of ventral leaf lobes. The morphological data were compared with previous study and analysed using Multivariate Statistical Procedures (MVSP plus Version 2.2) software to calculate the similarity matrix between the species. The findings of this study identifies both Azolla as A. pinnata subsp. asiatica based on the morphological features assessed from the two Azolla accessions. This study confirms that morphological features can be used to identify Azolla and the environment of the growth region may cause variations to the Azolla species. Thus, specific future collection as to the character of the collecting site and its ecological setting is recommended.

Keywords: Azolla, A. pinnata subsp. asiatica, morphological, identification

ABSTRAK

Azolla adalah tumbuhan pakis akuatik dan tergolong dalam keluarga Salviniaceae yang mempunyai potensi besar sebagai baja biologi di sawah padi, sumber makanan alternatif untuk ternakan dan fitoremediasi untuk air kumbahan. Tumbuhan ini tersebar secara meluas di Asia Tenggara, Asia Timur, Australia, India, dan Afrika. Kajian terdahulu telah berjaya menggunakan ciri morfologi untuk mengenal pasti spesies Azolla dan membezakan antara Azolla pinnata Asia dan Azolla pinnata Afrika. Di Malaysia, spesies Azolla boleh didapati dengan mudah dan sangat aktif membiak di kawasan pengairan utama seperti kolam, tasik, sawah padi dan sungai. Justeru, matlamat kajian ini adalah untuk mengenal pasti spesies Azolla menggunakan kaedah morfologi dan mengkaji ciri morfologi Azolla yang terdapat di dua lokasi berbeza di Selangor. Pengenalpastian dan pencirian Azolla dibuat dengan menilai 16 ciri morfologi yang digunakan untuk semua aksesi Azolla, tahap imbrikasi lobus daun dorsal dan nisbah panjang kepada lebar lobus daun ventral. Data morfologi kajian lepas dibandingkan dan dianalisis menggunakan perisian Multivariate Statistical Procedures (MVSP plus Version 2.2) untuk mengira matriks persamaan antara spesies. Dapatan kajian ini didapati bahawa kedua-dua Azolla dikenalpasti sebagai A. pinnata subsp. asiatica berdasarkan ciri morfologi yang dinilai daripada dua aksesi Azolla. Kajian ink mengesahkan bahawa ciri morfologi boleh digunakan untuk mengenal pasti Azolla dan persekitaran kawasan pertumbuhan mempengaruhi variasi kepada spesies Azolla. Oleh itu, kajian berterusan untuk pengumpulan khusus mengenai ciri tapak pengumpulan dan persekitaran ekologi bagi Azolla adalah disyorkan.

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Kata kunci: Azolla, A. pinnata subsp. asiatica, ciri morfologi, identifikasi

1. INTRODUCTION

Azolla is an aquatic fern that native to the tropics, subtropics, and warm temperate regions of Asia, America, and Africa. This plant has a worldwide distribution and can be found in freshwaters habitat or moist soils. According to Sadeghi et al. (2013), *Azolla* are divided into two subgenera, *Azolla* and *Rhizosperma*. The species in *Azolla* subgenera includes *Azolla caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla* and *A. rubra*, and the species in *Rhizosperma* subgenus includes *A. nilotica* and *A. pinnata*. In Southeast Asia, *A. pinnata* is widely distributed and can be found in the ponds, river, and lake. It is easily grown and highly productive (Kannaiyan & Kumar, 2006).

This plant is small with about 2.5 cm in length and the colours differ from green, blue green or dark red. The stem has pinnate branches and is shaped in a triangular. The leaves were arranged at the side branches and coated with tiny hairs. The hairy layers make the leaves water repellent. The root part has many fine lateral roots that function as the primary mechanism to absorb water and nutrients (Farook et al., 2019).

In *Azolla* identification, the common method used to identify *Azolla* species is by using 16 morphological features applied to all *Azolla* accessions. The 16 morphological features used to characterize existing *Azolla* species includes sporophyte shape, polygonal branching pattern, deltoid branching pattern, root arrangement, rhizome indumentum, and the presence of stomata (Pereira et al., 2011). Many studies have used morphological characteristics and successfully identified *Azolla* species including *Azolla nilotica*, *A. pinnata*, *A. microphylla*, *A. filiculoides*, *A. rubra*. and *A. caroliniana*. Besides, the studies also found that the *Azolla* undergo morphological adaptations that lead to variability of some characters due to environmental effects. The wide distribution and broad geographic range of *Azolla* cause differences in some morphological features that depend on environmental factors like the leaf imbrication, leaf shape and size and sporophyte shape. Since there is limited study on identification of the *Azolla* species in Malaysia, then there is a need to identify the species of *Azolla* that is found in this country. Thus, the goal of this study was to identify the morphological structure of *Azolla* species found in two different locations in Selangor.

2. MATERIALS AND METHOD

2.1 Preparation of plant material

In this study, *Azolla* samples were obtained from two different sources from Ampang and Sekinchan, Selangor. The *Azolla* then was propagated at the Glasshouse and Nursery of International Islamic University Malaysia and the propagation of the plants was maintained weekly. (Figure 1)

Figure 1: The propagation of *Azolla* at the Glasshouse and Nursery, IIUM

2.2 Morphological structure observation

Fresh samples of *Azolla* plants were collected and hydrated for 10 minutes. The observations for plant identification and characterization were made by evaluating the 16 morphological features of the plant (Table 1), the degree of dorsal leaf lobe imbrication and the length to width ratio of ventral leaf lobes. The observations were made by using the binocular stereomicroscope and light microscope. The morpholological structure of Azolla species was determine by observing 16 morphological traits. These features are compared with a prior study conducted by Pereira et al. (2011) and Sweet and Hills (1971) to confirm the species.

The collected plants were prepared as herbarium specimen vouchers. The voucher specimens were kept in Herbarium, Department of Plant Science, International Islamic University Malaysia, Kuantan Campus, and the voucher specimen number was obtained.

Table 1: Morphological descriptors used for the *Azolla* accessions.

	Characteristics	Descriptor
1	Sporophyte shape	Polygonal (0), Deltoid (triangular) (1)
2	Polygonal branching pattern	Isotomous opposite (dichotomous) (0), Anisotomous opposite (1)
3	Deltoid branching pattern	Elongate alternate (0), Sub-pinnate alternate (1)
4	Rhizome indumentum	Glabrous (0), Pubescent (1)
5	Rhizome papillae	Unicellular (0), bi- or multicellular (1)
6	Root arrangement	Solitary (0), Fascicles (1)

7	Dorsal lobe apex shape	Sub-round (0), Round (1)
8	Apex dorsal lobe angle	Acute (0), Obtuse (1)
9	Dorsal lobe shape	Elliptical (0), Obovate (1)
10	Dorsal lobe border shape	Entire (0), Crenate (1)
11	Hyaline border symmetry	Asymmetrical (0), Symmetrical (1)
12	Number of cells of the hyaline border	2 to 6 layers (0), 3 to 4 layers (1)
13	Dorsal lobe papillae	Unicellular (0), Bicellular (1)
14	Dorsal lobe stomata	Annular without middle longitudinal ridge (0), Annular with middle longitudinal ridge (1)
15	Dorsal leaf lobe stomata type	Anomocytic (0), non-anomocytic (1)
16	Ventral lobe stomata	Absent (0), Present (1)

2.3 Data Analysis

Data were analyzed via MVSP to calculate the similarity matrix between the species and refined with Gower's General Coefficient index. A phenogram was developed by using the Unweighted Pair Group Method with arithmetic mean (UPGMA). This analysis was conducted using numerical analyses software; Multivariate Statistical Procedures (MVSP plus version 2.2) software.

3. RESULTS AND DISCUSSION

3.1 Morphological structure of *Azolla*

Based on this study, 16 morphological structure were identified and recorded. The morphological features were summarized a in Table 3.1.

Table 3.1: Morphological features of *Azolla*

No	Morphological features	*Azolla* accession 1	*Azolla* accession 2
1	Sporophyte shape	Polygonal	Polygonal
2	Polygonal branching pattern	Isotomous opposite (dichotomous)	Anisotomous opposite
3	Deltoid branching pattern	Elongate alternate	Elongate alternate
4	Rhizome indumentum	Glabrous	Glabrous
5	Rhizome papillae	Not applicable	Not applicable

6	Root arrangement	Solitary	Solitary
7	Dorsal lobe apex shape	Round	Round
8	Apex dorsal lobe angle	Obtuse	Obtuse
9	Dorsal lobe shape	Obovate	Obovate
10	Dorsal lobe border shape	Entire	Entire
11	Hyaline border symmetry	Symmetrical	Symmetrical
12	Number of cells of the hyaline border	3 to 4 layers	3 to 4 layers
13	Dorsal lobe papillae	Unicellular	Unicellular
14	Dorsal lobe stomata	Annular with middle longitudinal ridge	Annular with middle longitudinal ridge
15	Dorsal leaf lobe stomata type	Anomocytic	Anomocytic
16	Ventral lobe stomata	Absent	Absent
17	Leaf blade length	903.75 µm	926.69 µm
18	Leaf blade width	520.59 µm	546.5 µm

3.1 .1 *Azolla* accession 1

The morphological traits of the *Azolla* accessions 1 such as leaf trichomes, rhizome indumentum, hyaline borders, and stomata, are shown in Figure 2-7. In general, accession 1 has polygonal sporophyte shape and the polygonal shape showed an equal sized branching from the main axis (dichotomous). The deltoid branching pattern is elongate alternate, where the side leaves is not grown directly across from each other. Root is solitary type that grows from the stem branching points. The dorsal lobes apex is round with an obtuse apex angle, while the dorsal shape is obovate. The dorsal lobes border also have entire border shape. The dorsal lobes have annular stomata with middle longitudinal ridge (anomocytic) and has no ventral stomata. The degree of the dorsal leaf lobe imbrication of *Azolla* accessions 1 are non-imbricate and the length: width ratio is 1.7:1.

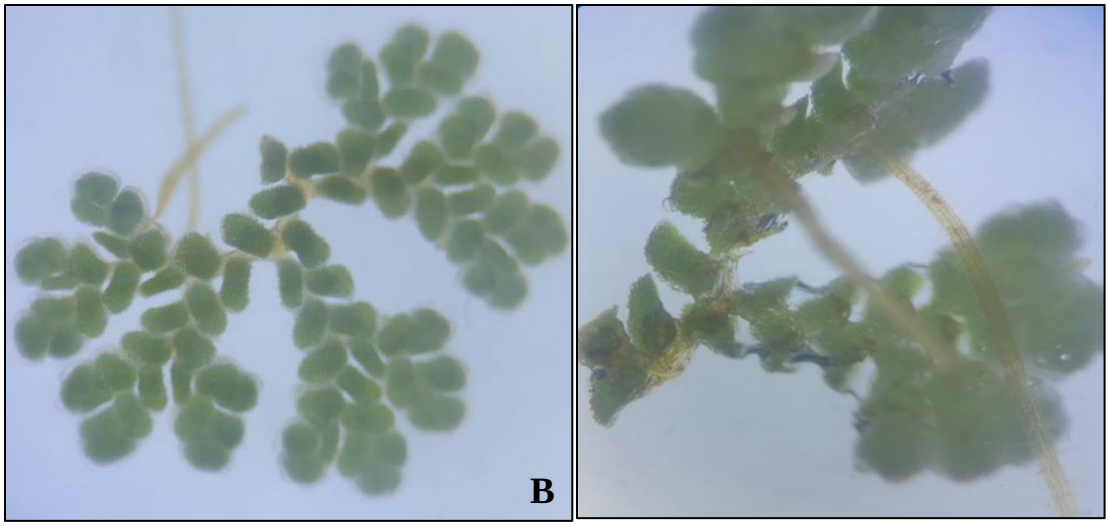

Figure 2: The stereoscopic picture of (A)Whole plant (B)Roots of *Azolla* accession 1

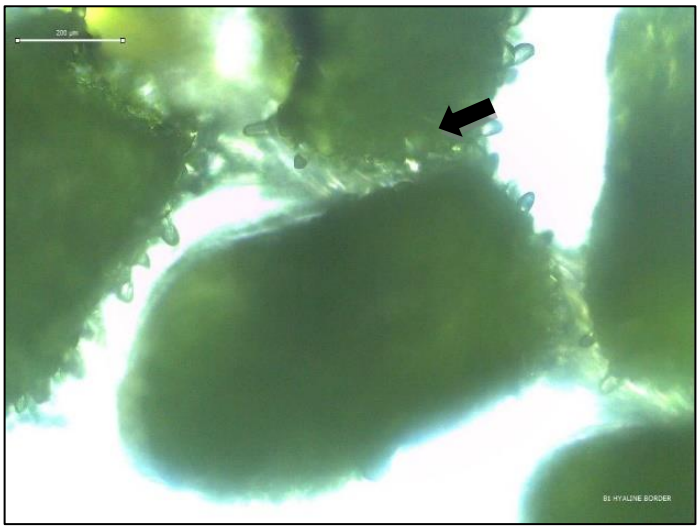

Figure 3: The unicellular dorsal lobe papillae of *Azolla* accession 1 x100

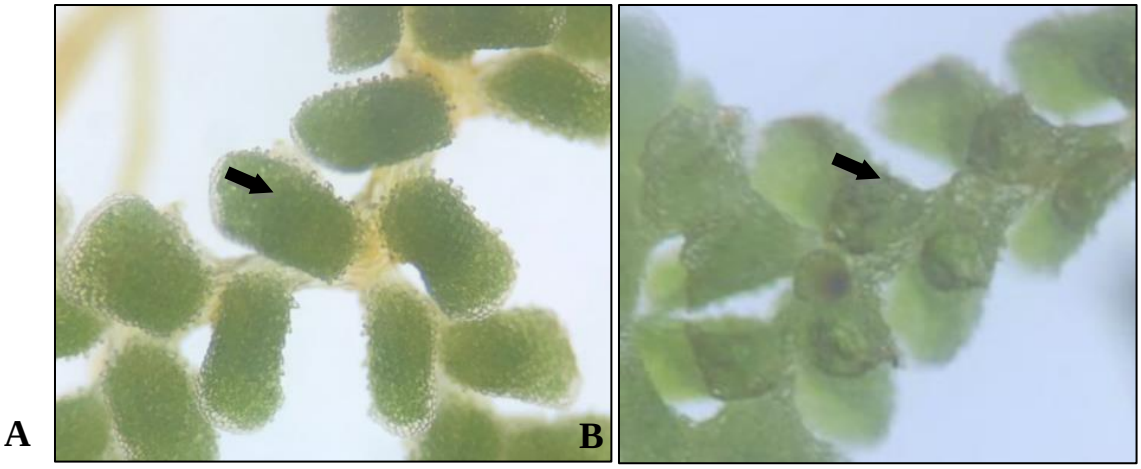

Figure 4: The stereoscopic pictures of glabrous rhizome of *Azolla* accession 1 A) Dorsal (B)Ventral

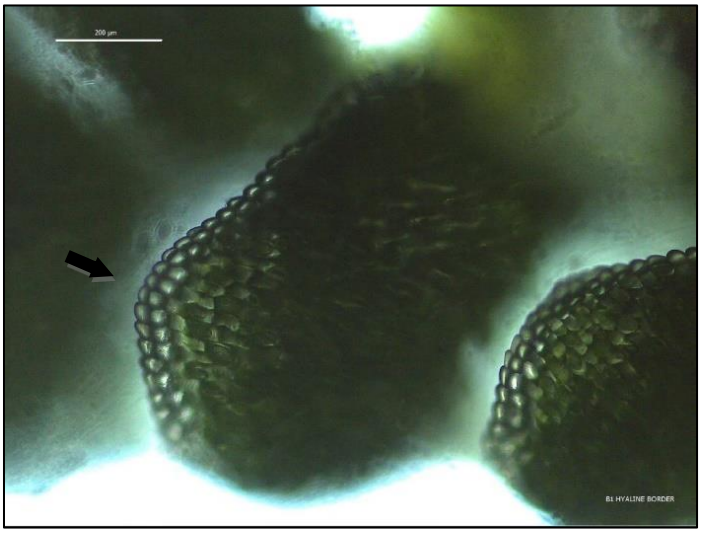

Figure 5: The symmetrical hyaline border with 3-4 layers of cells of *Azolla* accession 1 x100

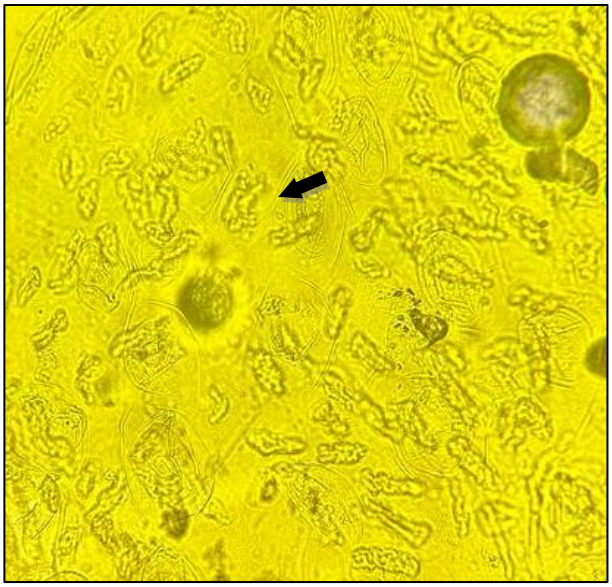

Figure 6: The annular stomata with middle longitudinal ridge of *Azolla* accession 1 x400

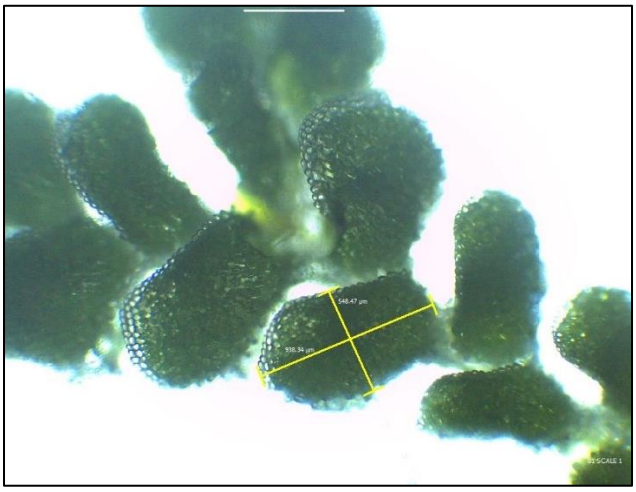

Figure 7: The length to width ratio is 1.7:1 of *Azolla* accession 1(three replicates) x100.

3.1.2 *Azolla* accession 2

For *Azolla* accession 2, the morphological traits such as leaf trichomes, rhizome indumentum, hyaline borders, and stomata, are shown in Figure 8-13. The plant has polygonal sporophyte shape, and the polygonal shape showed an unequal sized branching from the main axis (anisotomous). The deltoid branching pattern is elongate alternate, where the side leaves is not grown directly across from each other. Root is solitary type. The dorsal lobes apex is round with an obtuse apex angle, while the dorsal shape is obovate. The dorsal lobes border also have entire border shape. The dorsal lobes have annular stomata with middle longitudinal ridge (anomocytic) and has no ventral stomata. The degree of the dorsal leaf lobe imbrication of *Azolla* accessions 2 are non-imbricate and the length: width ratio is 1.7:1.

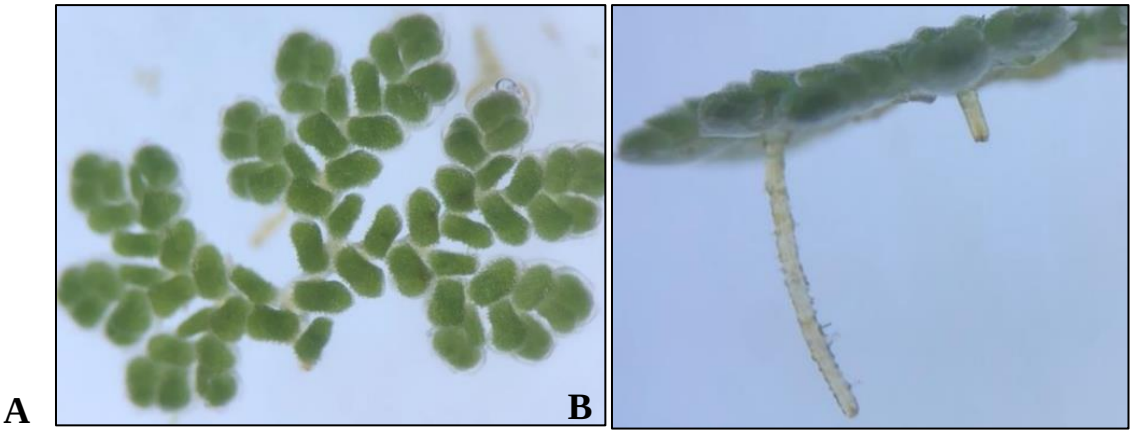

Figure 8: The stereoscopic picture of (A)Whole plant (B)Roots of *Azolla* accession 2

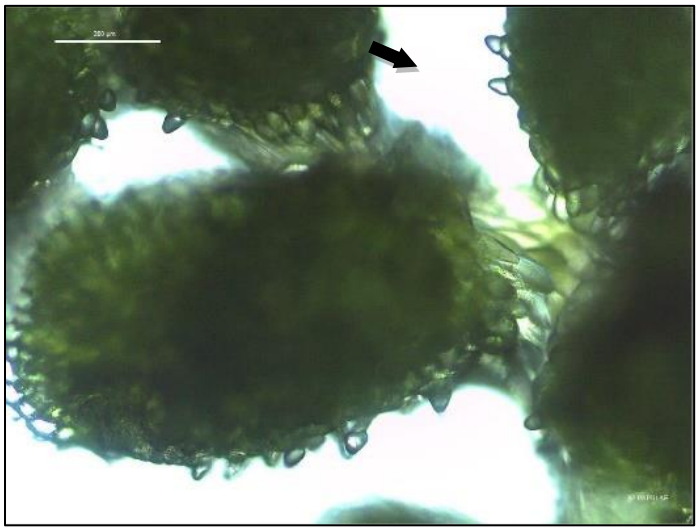

Figure 9: The unicellular dorsal lobe papillae of *Azolla* accession 2 x100

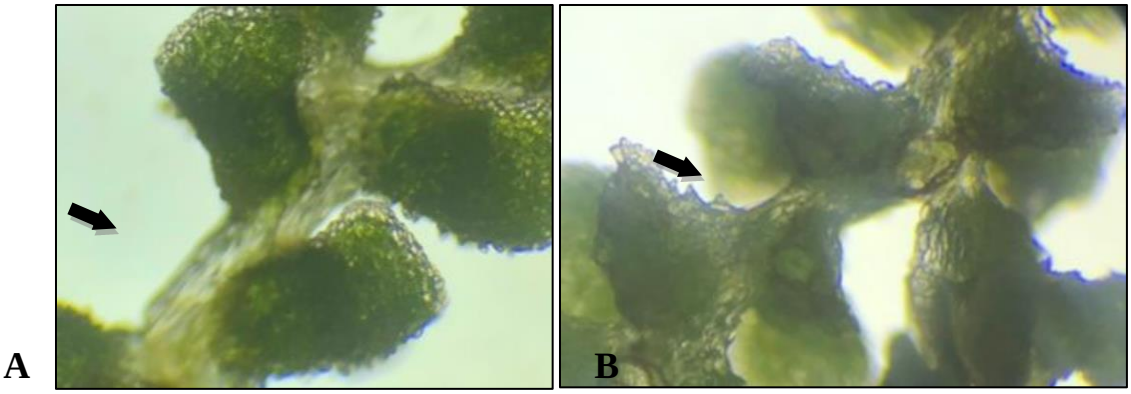

Figure 10: The stereoscopic picture glabrous rhizome for accession 2

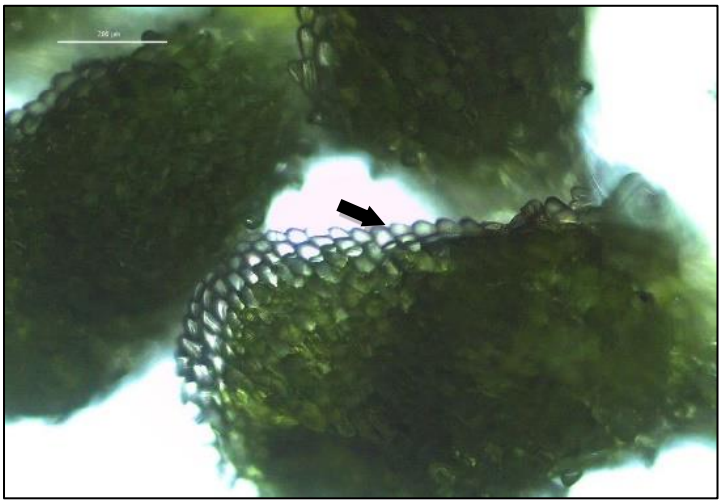

Figure 11: The symmetrical hyaline border with 3-4 layers of cells of *Azolla* accession 2 x100

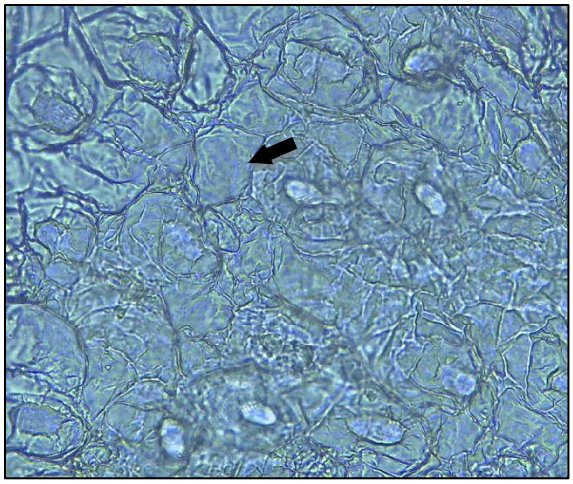

Figure 12: The annular stomata with middle longitudinal ridge of *Azolla* accession 2 x400

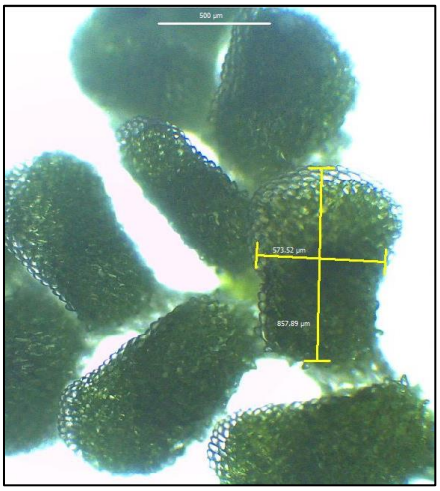

Figure 13: The length to width ratio is 1.7:1 of *Azolla* accession 2 (three replicates) x100.

3.3 Morphological structure variation of *Azolla* species

Based on the observation of the morphological features for both *Azolla* accessions referring to Saunders and Fowler (1992) identification criteria, the evaluation identifies both accessions could be classified as *Azolla pinnata* subsp. *asiatica*. Phenogram developed from by using unweighted Pair group method with arithmetic mean (UPGMA) reveal distinct variety for Azolla acession. Based on the observation in Figure 14, the data UPGMA shows that the two accessions have high similarities coefficients with 0.938. This confirms that both *Azolla* accessions belong to the same species, *Azolla pinnata* subsp. *asiatica.*

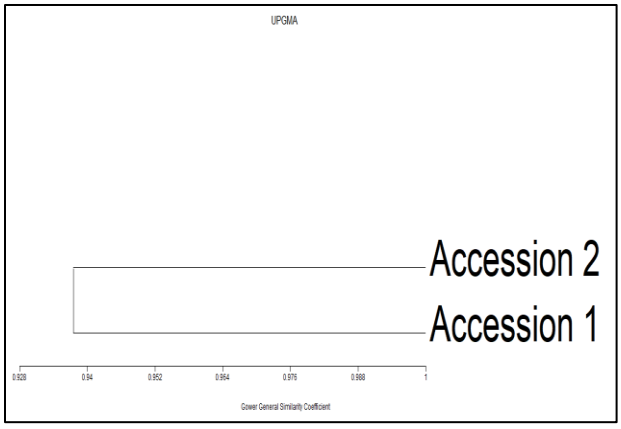

Figure 14: Phenogram (UPGMA) of *Azolla* accession 1 and *Azolla* accession 2

The morphological data obtained from this study, including polygonal sporophyte shape, elongate arrangement of branching pattern, symmetric hyaline border with 3-4 layers of cells, present of stomata on the dorsal with bicellular papillae and the absence of stomata on the ventral lobe, confirmed that both *Azolla* accession 1 and *Azolla* accession 2 belong to the species *A. pinnata*.

According to Saunders and Fowler (1992) in their study on the morphological taxonomic revision of *Azolla* Lam. section Rhizosperrna (Mey.), they observed 38 taxonomic characters morphological of *A. pinnata* and *A. nilotica*. Their findings on the morphological of *Azolla* have been referred and compares by the present research including this study. Also, the criteria observed from this study meet the criteria of *A. pinnata* based on studies conducted by Pereira *et al.*, (2011); Sweet and Hills, (1971). The criteria's that point to *A. pinnata* species includes the rounded lobes of the dorsal leaf apex, the solitary roots, and the absence of stomata on the ventral lobe (Saunders & Fowler, 1992). During observation, some of the morphological features observed in this study match with the previous study, however and there are also several variations. The variation observed includes the glabrous rhizome and the dorsal leaf shape. The morphological adaptations to the environment of the growth region may be the reason of the variations in the phenotype of the plants (Oyange *et al.*, 2020). Within the *A. pinnata* species alone, the varies in the criteria like bicellular or unicellular rhizome papillae and obovate to elliptical dorsal lobes is used to separate the *A. pinnata* into the intraspecific groups' variety (Sweet & Hills, 1971).

The identification was further verified by the degree of dorsal leaf lobe imbrication and the ventral lobe length to width ratio presented by Saunders and Fowler (1992) as to distinguish between the three intraspecific groups recognized in *A. pinnata*, which is subsp. *pinnata*, subsp. *asiatica* and subsp. *africana*. The degree of the dorsal leaf lobe imbrication for both *Azolla* accessions are non-imbricate. This observation is supported by the previous study that indicates the dorsal leaf lobe imbrication for *Azolla pinnata* subsp. *asiatica* is generally non-imbricate to slightly imbricate (Sweet & Hills, 1971; Saunders & Fowlers, 1992). Next, Saunders and Fowlers (1992) in their study also revealed that Asian specimens tend to have shorter and broader ventral lobes compare to African and Australasian specimens with length: width ratio ±1.2:1, ±1.7:1 and ±1.7:1 respectively. In this study, the ratio of length to width ratio for both *Azolla* accessions is ±1.7:1, which falls under classification of *A. pinnata*, subsp. *pinnata*, and subsp. *africana*. Then, overall evaluation on the morphological features, degree of dorsal leaf lobe imbrication and the ventral lobe length to width ratio indicates that the criteria' of both *Azolla* accessions is close to *A. pinnata* subsp. *asiatica*. The close similarity and slight difference of the morphological characters between the three intraspecific groups of *A. pinnata* indicates that the populations have the connection and

the varies in morphology may be due to environmental effects on the accessions (Pereira *et al.*, 2011; Saunders & Fowler, 1992). Based on the morphological features assessed from the two *Azolla* accession, the evaluation identifies both *Azolla* is *A. pinnata* subsp. *asiatica*.

5. CONCLUSION

This study successfully identified *A. pinnata* species based on morphological traits applied to all *Azolla* accessions. The close similarity and slight difference of the morphological characters found between the two intraspecific groups of *A. pinnata* indicates that different geographical and environmental locations affect the morphological features of *Azolla*. Therefore, it is recommended for future collections to be specific as to the character of the collecting site and its ecological setting.

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