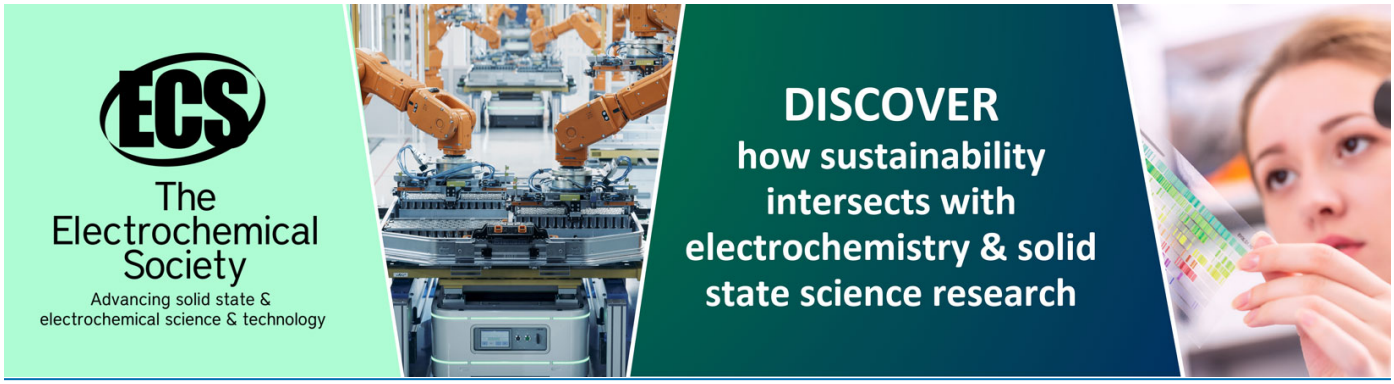
Histo-anatomy and morphology of aquatic plants *Bacopa amplexicaulis* (Pursh) Wettst., *Bacopa lanigera* (Cham. & Schltdl.) Wettst., and *Bacopa rotundifolia* (Michx.) Wettst.



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Abstract. Plants of the genus *Bacopa* are ornamental water plants used in aquascape design. This study examined the differences in morphological and anatomical characteristics in the roots, stems, and leaves of three *Bacopa* species, namely *B. amplexicaulis*, *B. lanigera*, and *B. rotundifolia*. The identification of morphological structure was based on a book entitled Morfologi Tumbuhan (Plant Morphology) by Tjitrosoepomo (2016). Anatomical identification referred to the book on Plant Anatomy by Crang et al. (2018). The data collected were analyzed descriptively and quantitatively. *Bacopa rotundifolia* had the smallest leaf size, while *B. amplexicaulis* had the largest leaf size. All of the observed species had leaf anatomical characters in the form of anomocytic stomata. *Bacopa amplexicaulis* had the largest stem diameter, measuring 4.38 cm. Trichome and aerenchyma structures were found in all stems of the test species. *Bacopa lanigera* stems had the most trichomes, 72 in one cross-section. Meanwhile, *B. amplexicaulis* had the longest trichome (1.590 µm). The aerenchyma of B. *amplexicaulis* and *B. lanigera* was flat, while that of *B. rotundifolia* was oval. *Bacopa amplexicaulis* had the longest aerenchyma at 957 µm. Only *B. lanigera* had a trichome on the root.

1. Introduction

Bacopa is a genus of aquatic plants commonly used in aquascape design. *Bacopa australis*, *B. caroliniana*, and *B. monnieri* are commonly used as ornamental plants [1]. *Bacopa monnieri* has been used for about 3000 years in Ayurvedic medicine to improve intelligence and memory [2], it contains alkaloids, bacosides, and flavonoids that can treat degenerative diseases such as Parkinson's and dementia [3]. Another species, *B. caroliniana*, contains α-Terpinolene compounds which have potential as insecticides [4]. A total of 60 species of *Bacopa* are distributed throughout the world [3,5,6]. Some botanists place the genus *Bacopa* into the Scrophulariaceae [1,3], and others place the genus *Bacopa* into the Family Plantaginaceae [6–8].

Observation of plant morphology and anatomy is essential. In biology, morphological and anatomical observations are carried out to identify taxa and to study the evolutionary history of a plant species. Observations of plant morphology and anatomy also influence research in other branches of biological sciences such as systematics, evolutionary biology, ecology, physiology, genetics, and molecular biology [9,10]. In addition, morphological and anatomical observations also contribute to other fields of science such as archeology, anthropology, paleobotany, forensics, and climatology [11].

Anatomical and morphological observations of species in the genus *Bacopa* have been carried out on several species. Anatomical observations have been made on *B. caroliniana* [12], *B. monnieri* [13,14] , *B. myriophylloides* [15], and *B. salzamanii* [16]. Meanwhile, morphological identification has also been carried out on *B. monnieri, B. salzamanii,* and *B. rotundifolia* [5]. There is no report about morphological and anatomical studies of *B. amplexicaulis, B. lanigera,* and *B. rotundifolia*. This study aimed to observe and describe the morphology and anatomy of these three species based on the leaves, stems, and roots.

2. Data and methods

The samples observed in this study were *Bacopa amplexicaulis, Bacopa lanigera,* and *Bacopa rotundifolia* (Figure 1). Samples were obtained from aquatic plant cultivators in Bogor, West Java.

Figure 1. The plant taxa studied: a) *Bacopa amplexicaulis,* b) *Bacopa lanigera,* c) *Bacopa rotundifolia*

2.1. Morphological identification

2.1.1 Leaves. Observations were made on fresh leaves. The selected leaves were mature leaves located at the fourth node from the tip of the stem apex [17]. The morphological structure of the leaves of *Bacopa* was described based on several quantitative and qualitative characters. The quantitative characters observed were the length and width of the leaves. Leaf length and width were measured using a ruler (precision 0.1 cm). The length was measured from the apex to the base of the leaf, while the width was measured at the widest part of the leaf [18]. Measurements of leaf length and width can be used as a reference to determine leaf shape by comparing the length and width of the leaf [17]. Qualitative character observations were carried out with reference to a book entitled Morfologi Tumbuhan (Plant Morphology) [19]. The qualitative characteristics observed were leaf shape (*circumscriptio*), leaf margin shape (*Margo folii*), leaf tip shape (*Apex folii*), leaf base tip shape (*basis folii*), leaf surface texture, leaf venation (*nervatio*), the arrangement of leaves on the stem (*phyllotaxis*), and leaf color.

2.1.2 Stem. Stem morphology was described based on several characteristics: stem length, internode distance, stem shape, stem color, and the presence of trichomes. The stem and internode lengths were measured with a ruler (precision 0.1 cm) and each measurement was repeated three times. Qualitative characters were compared with the same reference [19].

2.1.3 Root. Root structure was described based on the shape of the root tip.

2.2. Anatomical identification

2.2.1. Leaves. The anatomical structure of the leaves was observed by making wet preparations from fresh leaf samples for each species. The leaves taken were mature leaves located at the fourth node from the apex [17]. Leaf samples were cut in a paradermal-section using a razor blade. The sample that had been cut was then placed on a microscope slide and a drop of distilled water was added before placing a cover slip over the specimen. The prepared slides were then observed under a light microscope (Nikon

Eclipse E200) at magnifications of 40x, 100x, and 400x. The leaf anatomical character observed was the type of stomata in each species based on neighbor cells.

2.2.2. Stem. The stem sample preparations were taken 10 cm from the tip of the stem apex [20]. The stem samples were cut using a razor cross-section. The sample pieces were placed on a microscope slide and covered with a coverslip after adding enough drop of distilled water. The prepared slides were then observed under a light microscope (Nikon Eclipse E200) at magnifications of 40x, 100x, and 400x. The stem anatomical character observed was the arrangement of the layers making up the stem. Characters measured or counted were the stem diameter, number of aerenchymas, aerenchyma length, number of trichomes, and trichome. Measurements of qualitative characteristics were repeated three times.

2.2.3. Roots. The root samples were taken from the root base. Samples were cut using a razor, placed on a microscope slide and covered with a coverslip after adding enough drops of distilled water. The prepared slides were then observed under a light microscope (Nikon Eclipse E200) at magnifications of 40x, 100x, and 400x. The anatomical root characteristics observed were the arrangement of the layers making up the root and the root diameter.

3. Results and discussion

3.1. Leaf morphology

All three species had single sessile leaves growing opposite each other from a single stem node (Figure 2). The leaves facing each other at one node form a cross with the two leaves on neighboring node, a pattern called *opposita decusata* [19]. The leaves were oval (ovatus/oblongus) and had thin leaf blades. All *Bacopa* species leaves can be classified as incomplete leaf types because they only consist of leaf blades (lamina) with no leaf stems [19]. The leaves of *Bacopa rotundifolia* tended to be small compared to the other species (Table 1). This is consonant with the findings of [21] that *Bacopa rotundifolia* only has a leaf length ranging from 1.2 to 3.6 cm and a width of 0.8 to 2.6 cm. The leaves of the other two species were very similar in size.

Figure 2. Leaf morphology of *Bacopa*: a) *Bacopa amplexicaulis,* b) *Bacopa lanigera*, c) *Bacopa rotundifolia*

Table 1. Leaf morphology characteristics of *Bacopa.*

Characteristic	*Bacopa amplexicaulis*	*Bacopa lanigera*	*Bacopa rotundifolia*
Leaf length (cm)	2.83	2.43	1.17
Leaf width (cm)	2.47	2	1.03
Simple/Compound	Simple	Simple	Simple
Complete/Incomplete	Incomplete	Incomplete	Incomplete
Leaf shape *(Circumscriptio)*	Ovatus	Ovatus/ oblongatus	Ovatus
Leaf margin (*Margo folii*)	Integer	Integer	Integer
Leaf apex (*Apex folii*)	Obtusus	Rotundatus	Obtusus
Leaf base (*Basis folii*)	Emarginatus	Rotundatus	Rotundatus
Leaf venation (*Nervatio*)	Palminervis	Palminervis	Palminervis
Leaf arrangement (*Phyllotaxis*)	*Opposita decusata*	*Opposita decusata*	*Opposita decusata*
Leaf color	Green	Green	Light green
Texture	Slippery	Slippery	Slippery

All three species have finger-shaped leaf veins (*palminervis*). However, the veins in *B. amplexicaulis* were not very visible, in contrast to *B. lanigera* and *B. rotundifolia*. Leaf venation is very clearly visible in the upper (adaxial) part of *B. lanigera*. Leaf veins in *B. lanigera* have a brighter color than the leaf blade, which makes them clearly visible. In comparison, *B. rotundifolia* has a leaf venation that is very clearly visible and protrudes in the form of a finger at the bottom (abaxial) [22]. All species had a smooth adaxial leaf surface texture. A slippery leaf texture is due to a layer of wax or a thin cuticle on the leaf surface [19]. The cuticle layer protects the leaf from various external disturbances such as water-bome pathogens and contaminants in the water that could damage the cells of these aquatic plants [23].

3.2. Stem morphology

Bacopa rotundifolia had a shorter stem length than the other two species observed (Table 2). *B. rotundifolia* can grow up to 30 cm in length [8]. Trichomes are unicellular or multicellular extensions of epidermal cells and can be found in the leaves and stems of many plants, and were present in all three species studied (Table 2). Trichomes play an essential role in the adaptation of plants to the environment, in particular by increasing or maintaining the humidity of the plant relative to the surrounding air by suppressing the rate of respiration, reflecting sunlight when the light intensity is too high, and acting as a defense against herbivores [23]. However, the exact function of trichomes in aquatic plants is not known.

Table 2. Stem morphology characteristics of *Bacopa.*

Characteristics	*Bacopa amplexicaulis*	*Bacopa lanigera*	*Bacopa rotundifolia*
Stem length (cm)	22.5	21.5	16.8
Internodes (cm)	3.43	3	1.9
Stem shape	Round	Round	Round
Stem color	Light green	Light green	Yellowish-light green
Trichome	Present	Present	Present

3.3. Root morphology

The roots of *B. rotundifolia* were smaller and thinner than those of the other two species (Figure 3).

(a) (b) (c)

Figure 3. Root morphology of *Bacopa* (*scale bar* 1000µm): a) *Bacopa amplexicaulis,* b) *Bacopa lanigera*, c) *Bacopa rotundifolia*

3.4. Leaf anatomy

Based on the number of neighboring cells, the stomata in the three *Bacopa* species exhibited an anomocytic stomata type (Figure 4). Anomocytic stomata are stomata surrounded by an uncertain number of neighboring cells and difficult to distinguish from other cells [23]. The previous observations on *B. caroliniana* [12] and *B. monnieri* [13] exhibited that they also had an anomocytic type of stomata.

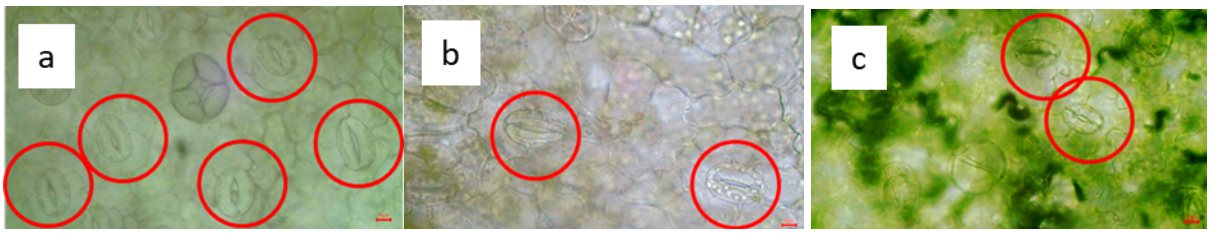

Figure 4. Leaf anatomy of *Bacopa* [scale bar 10*µ*m]: a) *Bacopa amplexicaulis*, b) *Bacopa lanigera*, c) *Bacopa rotundifolia*

3.5. Stem anatomy

Bacopa amplexicaulis had a larger diameter than other two species (Figures 5-7). The trichomes found in all *Bacopa* species are multicellular, uniseriate, and unbranched, similar to trichomes found in geraniums [23]. Although the same type of trichome was found in each species, the size of the trichomes differed between the three each species. The trichomes of *B. amplexicaulis* and *B. lanigera* were longer than *B. rotundifolia*.

Table 3. Stem anatomy characteristics of *Bacopa*.

Characteristics	*Bacopa amplexicaulis*	*Bacopa lanigera*	*Bacopa rotundifolia*
Diameter (cm)	4.38	2.01	1.5
Number of trichome	68	72	64
Type of trichome	Multicellular uniseriate	Multicellular uniseriate	Multicellular uniseriate
Length of trichome (µm)	1.590	1.317	493
Shape of aerenchyma	Flat	Flat	Oval
Number of aerenchyma	40	44	20
Length of aerenchyma (µm)	957	512	240

Aquatic plants have a distinctive form of aerenchyma structures found in stems and roots. An aerenchyma structure or tissue is formed bt parenchyma cells that differentiate to form gas-filled intracellular cavities. This structure plays a role in buoyancy as well as in oxygen and carbon dioxide

diffusion pathways between roots and leaves [23]. Each species had a different number and length of aerenchyma (Table 3). Generally, an aerenchyma structure is an elongated oval connecting the nucleus to the cortex. This structure is similar to the anatomical structure of the aquatic plant *Myriophyllum spicatum* [24].

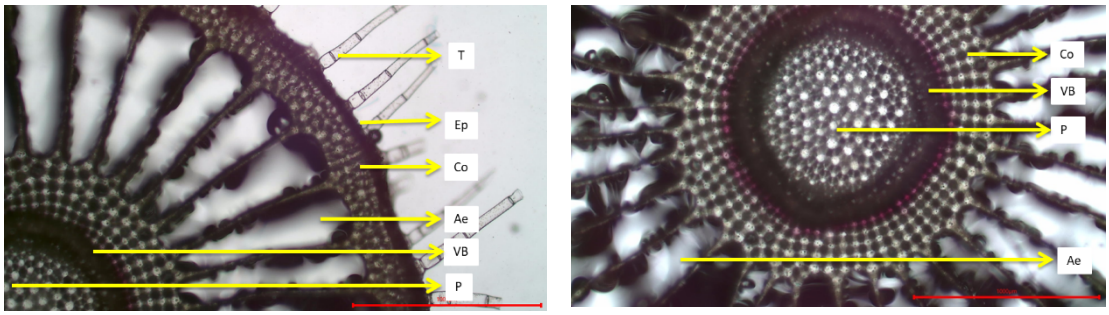

Figure 5. Stem anatomy of *Bacopa amplexicaulis* [cross section, scale bar 1000µm].
T: Trichomes, Ep: Epidermis, Co: Cortex, Ae: Aerenchyma, VB: Vascular bundle, P: Pith

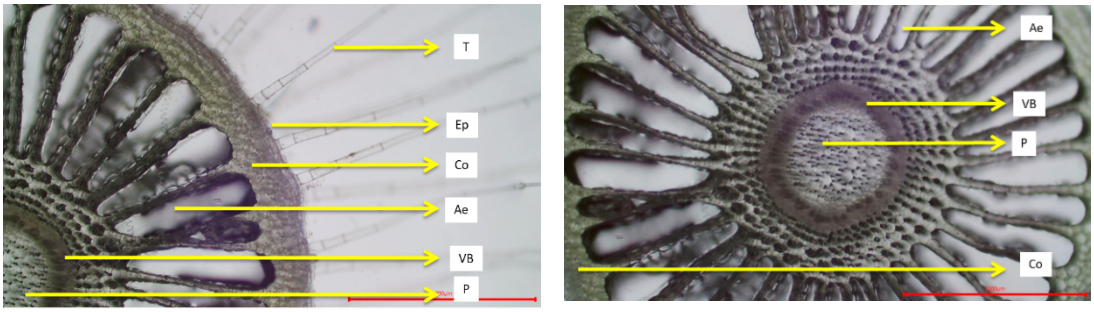

Figure 6. Stem anatomy of *Bacopa lanigera* [cross section, scale bar 1000µm].
T: Trichomes, Ep: Epidermis, Co: Cortex, Ae: Aerenchyma, VB: Vascular bundle, P: Pith

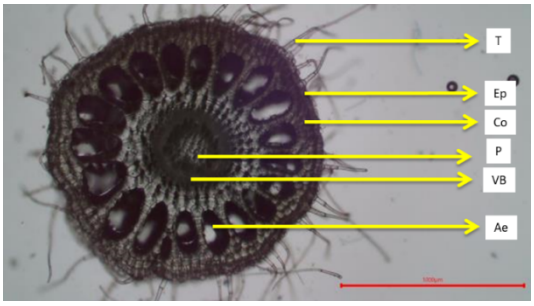

Figure 7. Stem anatomy of *Bacopa rotundifolia* [cross section, scale bar 1000µm].
T: Trichomes, Ep: Epidermis, Co: Cortex, Ae: Aerenchyma, VB: Vascular bundle, P: Pith

3.6. Root anatomy

Cross-sectional anatomical observations showed that the roots of *B. amplexicaulis* and *B. lanigera* consist of layers of epidermis, cortex, aerenchyma, as well as xylem and phloem vascular tissue (Figure 8). Trichomes were only found in the roots of *B. lanigera*, while the other two species did not have trichomes in their roots. Attempts to make cross-sectional observations on *B. rotundifolia* roots failed because the root structure was fragile and soft, making it challenging to prepare slides. Observations of the anatomy of the roots of *B. rotundifolia* were only successfully carried out on the paradermal part. In this section, only an elongated arrangement of parenchymal cells was seen. The root diameter of *B. lanigera* was 651 µm, while that of *B. amplexicaulis* was 958 µm.

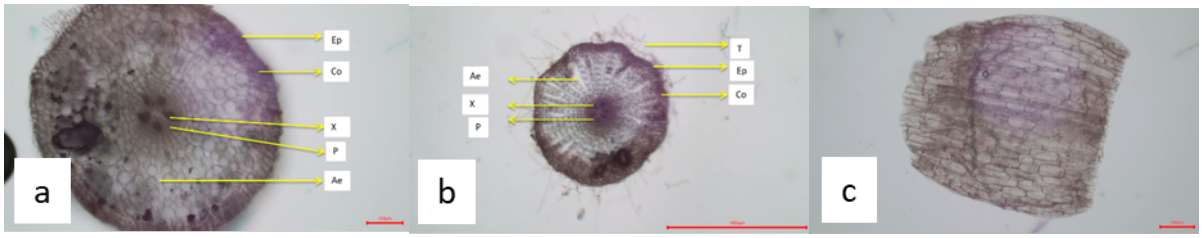

Figure 8. Root anatomy of *Bacopa.* a) *Bacopa amplexicaulis,* b) *Bacopa lanigera,* c) *Bacopa rotundifolia* [*cross section, scale bar* 100µm dan 1000µm]. T: Trichomes, Ep: Epidermis, Co: Cortex, Ae: Aerenchyma, X: Xylem, P: Phloem

4. Conclusion

The three *Bacopa* species had the same sessile leaf morphology, with a round and oval shape and anomocytic stomata. Trichomes and aerenchyma structures were found in all species observed. The trichomes found in the three species belonged to the unicellular and unbranched multicellular types. The highest number of trichomes was found in *B. lanigera* stems, while the longest trichomes were found in *B. amplexicaulis.* The highest number of aerenchyma was found in *B. lanigera*, while the largest aerenchyma size was found in *B. amplexicaulis*. The roots of *B. rotundifolia* were very soft in texture with a thin threadlike-structure, making it difficult to prepare trasverse section slides. However, the roots of the other two species were observed and showed various layers. Trichomes were only found in the roots of *B. lanigera*.

Acknowledgments

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