



Pollen Characteristics of Some Species in the Genus *Bacopa*

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ABSTRACT

Plants in the genus *Bacopa* are important herbs, which makes them suitable to be studied about pollen morphology because it can be used to identify medicinal plants. Pollens of two *Bacopa* species were examined under light and scanning electron microscopes. The two species were similar in their aperture type, shape, and exine sculpturing. The aperture type of both species is compound aperture and tricolporate. The shapes of polar view are circular and semi-angular. The shapes of equatorial view are spheroidal, oblate-spheroidal and prolate-spheroidal. On the other hand, the species differed in their number of lumina. The exine sculpturing appears to have several lumina. It was found that the number of lumina on the exine sculpturing of *B. monnieri* was larger than on *B. caroliniana*. As a result, the morphological characteristics of the pollen can be used for species identification of this genus.

INTRODUCTION

The genus *Bacopa* belongs to the family Plantaginaceae. There are approximately 100 species in the genus *Bacopa*. Most species are aquatic plants growing in warm regions. Plants in this genus are annual or perennial plants [3]. There are three species found in Thailand. Two species are used as medicinal plants, which include *B. caroliniana* (Walter) B.L. Rob. and *B. monnieri* (L.) Wettst. Both species are aquatic succulent plants. They are not only herbs but also ornamental plants in aquaria. In wetlands, they can make glamorous ground covering.

B. caroliniana is a floating aquatic or terrestrial herb. It grows on the margins of freshwater reservoirs or damp areas, margins of ponds and streams, and swamps. Its leaves are ovate to broad ovate. Its flowers are solitary with short pedicels. The colour of its flowers is either blue or purple [11]. The stem is creeping from the rhizome. The stem releases a lemon scent when it is bruised. *B. caroliniana* usually forms mats in bogs and pinelands [5]. Its leaf extract has an anti-bacterial property against *Staphylococcus aureus* and anti-fungal *Candida albicans* yeast [1].

B. monnieri is a prostrate perennial herb. The leaves are cuneate-oblong or obovate shape [13]. The flowers are solitary. The colour of the flowers is either white or pale blue. It grows on fresh water or brackish pools, wet places, sandy beaches, mud, sand in depressions among dunes, ponds, streams and ditches in wet mud on edges of

water [5,14]. The leaf extract can be used to improve the working memory of older people [12]. It is a folk medicinal plant in Cuba for treatment of rheumatism. Furthermore, it is used as a treatment for epilepsy, psychosis and mental disorders [7].

A pollen grain is a male gamete of flowering plants. The morphological properties of pollen such as size, shape, exine sculpturing pattern, and aperture type can be used to identify and classify plants [15]. Pollen morphology can be applied to other fields such as melittology, geology, climatology, forensic science, etc. [6]. For the study of pollen morphology, a light microscope is an important scientific tool for examining the shape and size of pollen grains. However, a scanning electron microscope can be used to study the fine details on exine sculpturing of pollens [9]. In this study, both light and scanning electron microscope techniques were used to observe *B. caroliniana* and *B. monnieri* pollens. The information from this study will support species identification. The light microscopic technique is an appropriate technique to study the aperture type of pollens while scanning electron microscopic technique is good to study fine details in nanometer to micrometer scales

METHODOLOGY

Plant Materials

Medicinal plants in the genus *Bacopa*, *B. caroliniana* and *B.*

monnieri were used in this study. The samples were planted in the nursery of Scientific Equipment and Research Division, KURDI. Pollens of the 2 species were examined using light and scanning electron microscopy.

Light microscopy observation

The pollens were collected from blooming flowers. The fresh pollens were boiled in 10% KOH. Then, they were stained with 1% Safanin O. The samples were mounted on a slide and covered with a cover slip. They were observed under a light microscope (Carl Zeiss; Axiostar Plus).

Sample preparation for Scanning Electron Microscope

The pollen grains of both species were primarily fixed in 2.5 % glutaraldehyde in sodium phosphate buffer pH 7.2 at 4 °C for 12 hrs, and secondarily fixed in 1% osmium tetroxide for 1 hr. The specimens were dehydrated in acetone series (20-100%) and dried in a critical point dryer (QUORUM: K850) [2]. The specimens were coated with gold particles (EIKO ENGINEER: IB-2). Finally, the specimens were observed under a field emission scanning electron microscope (HITACHI: SU8020), which was operated at 1 kV and 10 kV accelerating voltages. The description of palynological terms and pollen type were performed according to Erdman (1972) and Hoen (1999) [6,10]. The lengths of the polar axis and equatorial axis were measured from 100 grains and a range and an average value were reported. The lumina diameter and thickness of muri were measured from 100 positions and a range and an average value were reported. The numbers of lumina were counted in 10 areas.

RESULTS AND DISCUSSION

Pollen morphology of *B. caroliniana*

The pollen grains of *B. caroliniana* are monads and are classified as having a medium size. The polar axis ranged from 2028 μm and the equatorial axis ranged from 20-27 μm . The pollen shapes appear to be circular and semi-angular from the polar view, and oblate-spheroidal, spheroidal and prolate-spheroidal from the equatorial view. The compound aperture of pollen grain is a tricolporate type. The exine sculpturing has a microreticulate pattern. Lumina and muri were present on the exine (Fig 1). The lumina sizes were 134-1,070 nm and the muri sizes were 239-1,040 nm. The numbers of the lumina on the exine were 14-17 pores/ μm^2 . Table 1 lists these measurements with their averages and the corresponding standard deviations.

Pollen morphology of *B. monnieri*

The pollen grains of *B. monnieri* are monads and are classified as having a medium size. The polar axis ranged from 19-28 μm and the equatorial axis ranged from 19-29 μm . From the polar view, the pollen shapes appear to be circular and semi-angular and from the equatorial view, they appear as oblate-spheroidal, spheroidal and prolate-spheroidal. The compound aperture of pollen grain is a tricolporate type. The exine sculpturing has a microreticulate pattern. Lumina and muri are present on the exine (Fig 2). The lumina sizes were 149-1,090 nm and the muri sizes were 203-936 nm. The numbers of the lumina on the exine were 18-28 pores/ μm^2 . The measurements are also listed in Table 1.

The presented results show that similar morphological characteristics of *B. caroliniana* and *B. monnieri* pollens are size, shape, and exine sculpturing. From the polar view, there are two types of the pollen shapes; circular and semi-angular. From the equatorial view, there are three types; spheroidal, oblate-spheroidal and prolate-spheroidal. The exine sculpturing has a microreticulate pattern, which is similar to a previous report by El-Husseini and Shams (2002) who found that exine sculpturing of *B. monnieri* pollen is coarse reticulate to microreticulate pattern [8]. However, the results are different from the report by Willard et. al. (2004), who indicate that the exine sculpturing of *B. monnieri* is reticulate or homobrochate [4]. Our data of lumina diameter listed in Table 1 shows that the lumina diameters are 149 nm to 1.90 μm , which suggests that it should be heterobrochate. On the other hand, it is found that morphological differences of *B. caroliniana* and *B. monnieri* pollens include the number of lumina on the exine sculpturing. *B. caroliniana* has less lumina than *B. monnieri*. Both species have the same microreticulate pattern. The present study demonstrates that morphological characteristics of pollens can be used for identifying the two species in the genus *Bacopa*. In addition, scanning electron microscopy is one proper technique capable of distinguishing *Bacopa* species from detailed morphologies of their pollens.

CONCLUSION

This study investigated pollen morphologies of *Bacopa* for species identification. Shape, size and exine sculpturing of the two species were similar. The pollen has a circular and a semi-angular shape from the polar view, and an oblate-spheroidal, a spheroidal and a prolate-spheroidal shape from the equatorial view. The exine sculpturing is microreticulate pattern. The aperture type is tricolpate. There are three apertures on each grain. The difference in pollen characteristics of the two species is the number of lumina on the exine sculpturing. The data from this study can be further applied to species identification, proof of bee pollen products, and forensic investigation.

Table 1 The summary data of pollen morphology of *B. caroliniana* and *B. monnieri*

Pollen Characteristic	<i>B. caroliniana</i>		<i>B. monnieri</i>	
	Range	Average	Range	Average
Polar axis length (μm)	20-28	23.96 \pm 1.85	19-28	24.29 \pm 1.55
Equatorial axis length (μm)	20-27	23.64 \pm 1.56	19-29	23.30 \pm 1.81
Lumina diameter (nm)	134-1070	439.35 \pm 20.35	149-1090	455.77 \pm 18.54
Thickness of muri (nm)	239-1040	543.17 \pm 15.95	203-936	464.00 \pm 14.72
Number of lumina/ μm^2	14-17	15.00 \pm 1.11	18-28	23.40 \pm 3.20

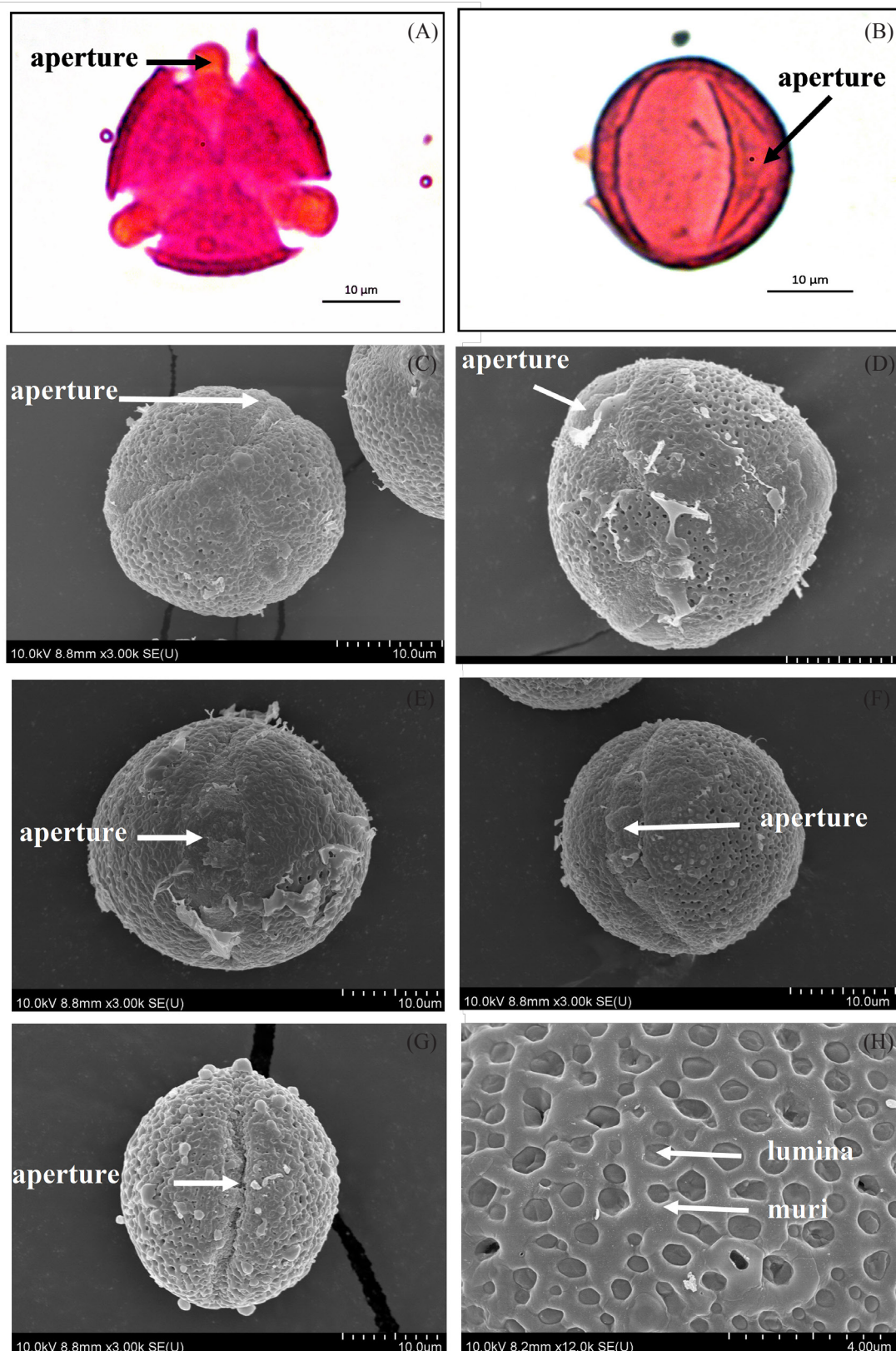


Figure 1 Pollens of *B. caroliniana*: (A) Light micrograph of a polar view of pollen grain (B) Light micrograph of an equatorial view of pollen grain (C) SEM micrograph of a polar view of pollen grain showing circular shape (D) SEM micrograph of a polar view of pollen grain showing semi-angular (E) SEM micrograph of an equatorial view of pollen grain showing oblate-spheroidal (F) SEM micrograph of an equatorial view of pollen grain showing spheroidal (G) SEM micrograph of an equatorial view of pollen grain showing prolate-spheroidal (H) Exine sculpturing showing microreticulate type.

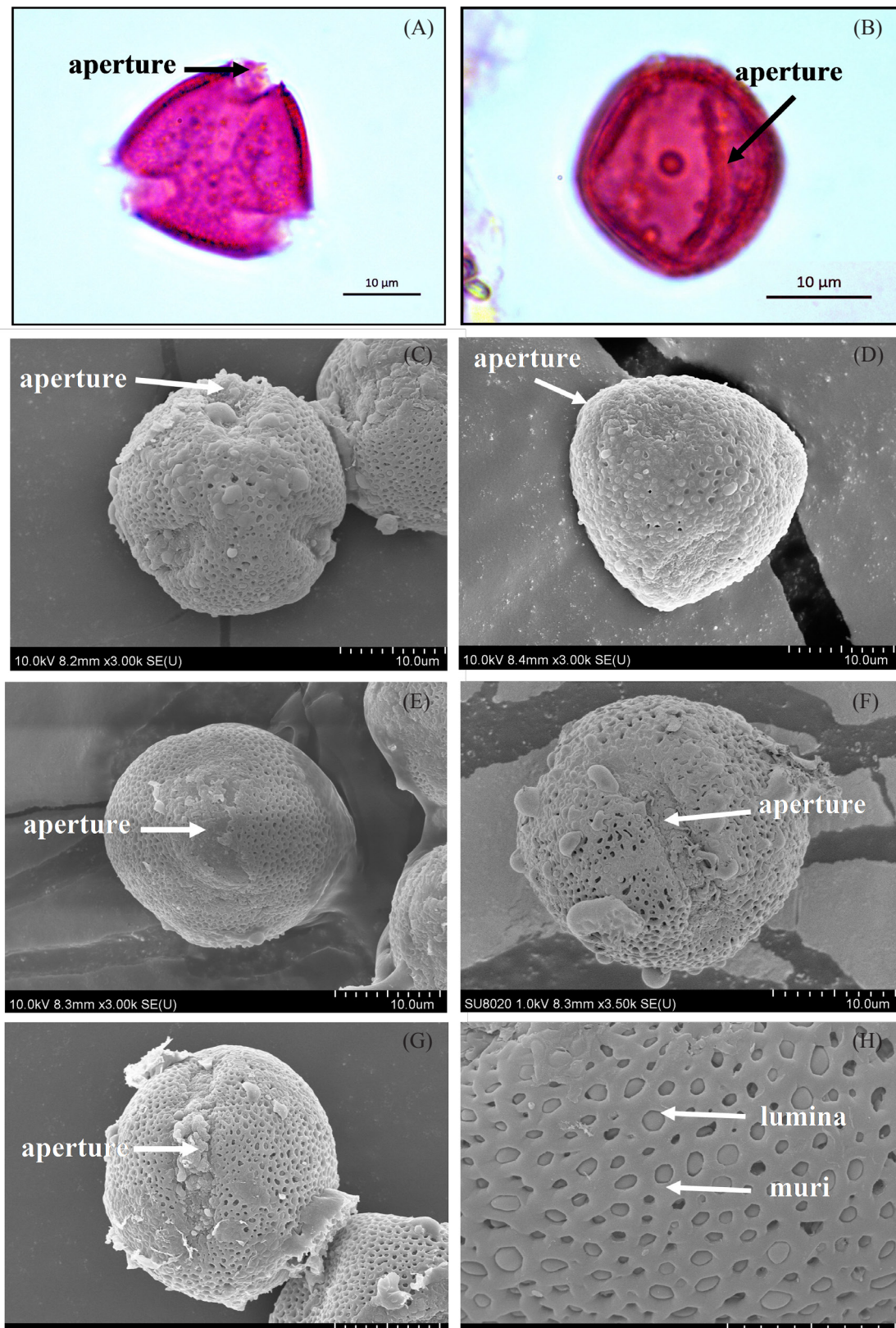


Figure 2 Pollens of *B. monnieri* : (A) Light micrograph of a polar view of pollen grain (B) Light micrograph of an equatorial view of pollen grain (C) SEM micrograph of a polar view of pollen grain showing circular shape (D) SEM micrograph of a polar view of pollen grain showing semi-angular (E) SEM micrograph of an equatorial view of pollen grain showing oblate-spheroidal (F) SEM micrograph of an equatorial view of pollen grain showing spheroidal (G) SEM micrograph of an equatorial view of pollen grain showing prolate-spheroidal (H) Exine sculpturing showing microreticulate type.

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