Agr. Nat. Resour. 54 (2020) 351–354

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Pollen Morphology of *Helicteres* (Malvaceae s.l.) from Thailand

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Article Info

Article history:
Received 29 May 2019
Revised 28 October 2019
Accepted 24 February 2020
Available online 31 August 2020

Keywords:
Helicteres,
Malvaceae,
Pollen

Abstract

The pollen morphology of seven species (eight samples of each) of Thai *Helicteres* (Malvaceae s.l.) was determined based on observations using light microscopy and scanning electron microscopy. The pollen morphology of all species has bilateral symmetry, with isopolar and triporate apertures. There are two pollen shapes: semi-trilobate and semi-triangular. The mean±SD size on the polar view was 21.97±1.47 µm to 34.63±3.24 µm and the mean±SD equatorial view pollen size range was 19.87±0.76 µm to 34.10±2.98 µm. The largest pollen was observed in *H. hirsuta* (purple flower) and the smallest pollen one in *H. lanceolata*. Pollen grains from this study can be divided into two groups based on size following Walker and Doyle (1975), being small sized and medium sized. The sculpture of the exine varies from perforate, verrucate, verrucate-perforate, verrucate-sparsely perforate, slightly verrucate-scabrate to slightly verrucate-sparsely perforate. The pollen morphology of *H. lanceolata* is similar to the pollen of *H. hirsuta* (pale pink flower). The pollen morphology of *H. hirsuta* (purple flower) is not different from the pollen of *H. hirsuta* (pale pink flower) in shape but differs in size and exine sculpturing. The pollen morphology of *H. lanceolata* was studied for the first time globally.

Introduction

In the Angiosperm Phylogeny Group (APG) IV, the families Sterculiaceae, Bombacaceae and Tiliaceae including Malvaceae s.s. were inserted into the family Malvaceae s.l. based on molecular data. Helicteroideae is a subfamily of the family Malvaceae s.l. The genus *Helicteres* belongs to this subfamily (The Angiosperm Phylogeny Group, 2016). There are about 60 species in tropical Asia and America; seven species of the genus are recognized in Thailand (Phengklai, 2001). In addition, Chantaranothai and Poompo (2019) published *H. prostrata* as a new record for Thailand. The pollen morphology of the Sterculiaceae has been investigated only by a few researchers, namely Sharma (1967), Erdtman (1972), Pire and Cristóbal (2001) and El-Husseini (2006). Sharma (1967) studied two Indian species *Helicteres* (Sterculiaceae), which had two types of pollen grains, namely the *H. isora* type, with 3-zonoporate or brevissimicolporate and granulate, papillate, negatively reticulate pollen grains with amb triangular, and the *H. hirsuta* type, characterized by 3-zono-brevissimicolporate or porate and verrucose pollen grains. Erdtman (1972) reported on the pollen morphology of *H.angustifolia* only, which is monad, subangular in shape, isopolar, with radial symmetry, 15–90 µm in size and has a perforate exine sclupture. Pire and Cristóbal (2001) investigated the pollen grains of 41 species representing 7 sections of *Helicteres*, namely the sects. *Alicteres*, *Helicteres*, *Orthocarpaea*, *Orthothecium*, *Polyandria*, *Sacarol* and *Stegogamos*. Their pollen grains were usually triporate, oblate or suboblate; amb triangular; and medium sized, and they divided into nine types on the

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https://doi.org/10.34044/j.anres.2020.54.4.02

basis of the scultpture of the exine. El-Husseini (2006) studied pollen morphology under light microscopy (LM) and scanning electron microscopy (SEM) of 11 species (6 genera) representing the Tiliaceae and Sterculiaceae in the flora of Egypt which are spheroidal to prolate or oblate-spheroidal to spheroidal, tricolporate or trizonoporate; sculpturing is reticulate-perforate or spiny-perforate; and three pollen types were recorded, namely the *Grewia*, *Hermannia* and *Melhania* types.

There are no reports on the pollen morphology of the genus *Helicteres* from Thailand. Therefore the aims of this study were to investigate the pollen morphology of the genus *Helicteres* in Thailand and to assess the implications of pollen morphology for the taxonomy of the group, including variation in *H. hirsuta*.

Materials and Methods

Eight samples of each of seven species were collected from the field in Thailand, namely *Helicteres angustifolia* L., *H. elongata* Wall., *H. hirsuta* Lour. (purple flower and pale pink flower), *H. isora* L., *H. lanata* (Teysm. and Binn.) Kurz., *H. lanceolata* DC. and *H. viscida* Blume (Table 1). Voucher specimens were deposited in the Mahasarakham University Herbarium, Thailand. Mounted samples were analyzed using an applied form of the standard methods (acetolysis) as described by Erdtman (1966). The pollen was studied using LM and SEM. For LM specimens, 2–3 drops of silicone oil were dropped onto the pollen grains. Then the pollen grains in the silicone oil were mounted on slides and sealed with paraffin. For the SEM specimens, pollen in absolute alcohol was affixed to aluminum stubs using double-sided cellophane tape. Samples were sputter-coated with a gold-palladium mixture, examined and photographed using SEM (LEO 1450 VP). Details of pollen morphology were based on 20 pollen grains under LM and SEM. The mean, range and standard error were calculated based on 20 pollen grains for each specimen under LM. The morphology of pollen grains was measured by the ratio of the length of the polar view (P) to the equatorial view (E). The ratio between the polar view and the equatorial view of pollen (P/E) indicates the shape of the pollen. Erdtman (1966) was used to classify pollen shapes and also provided the terminology used.

Results

General pollen morphology of Helicteres in Thailand

The pollen morphology of the seven species (eight samples of each) was based on observations from LM as well as features seen with SEM which were classified following Erdtman (1966) as shown in Table 2 and Figs. 1–16. Pollen grains are monad, bilateral symmetry, isopolar, triporate aperture; semi-triangular or semi-trilobate in shape; mean±SD size range polar view 21.97±1.47 to 34.63±3.24 µm, the mean±SD size range equatorial view 19.87±0.76 µm to 34.10±2.98 µm. Exine sculpturing is slightly verrucate and sparsely perforate, verrucate and sparsely perforate, slightly scabrate, perforate orverrucate.

Description of each species examined

1. *Helicteres angustifolia* L. (Table 2; Figs. 1–2): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape based on classification following Erdtman (1966), mean±SD polar view 23.20±1.38 µm, mean±SD equatorial view 20.20±1.11 µm, exine sculpturing slightly verrucate and sparsely perforate.

2. *Helicteres elongata* Wall. (Table 2; Figs. 3–4): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape, mean±SD polar view 23.30±0.97 µm, mean±SD equatorial view 20.73±1.21 µm, exine sculpturing perforate.

3. *Helicteres hirsuta* Lour. (purple flower) (Table 2; Figs. 5–6): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape, mean ± SD polar view 34.63±3.24 µm, mean±SD equatorial view 34.10±2.98 µm, exine sculpturing verrucate.

4. *Helicteres hirsuta* Lour. (pale pink flower) (Table 2; Figs. 7–8): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape, mean±SD polar view 21.97±1.47 µm, mean±SD equatorial view 21.03±1.17 µm, exine sculpturing verrucate and sparsely perforate.

5. *Helicteres isora* L. (Table 2; Figs. 9–10): Pollen grains are monad, bilateral symmetric, isopolar, triporte aperture, semi-triangular in shape, mean±SD polar view 24.67±1.32 µm, mean±SD equatorial view 23.63±1.08 µm, exine sculpturing slightly verrucate and sparsely perforate.

6. *Helicteres lanata* (Teysm. and Binn.) Kurz. (Table 2; Figs. 11–12): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-trilobate in shape, mean±SD polar view 22.57±1.05 µm, mean±SD equatorial view 21.07±0.96 µm, exine sculpturing slightly scabrate.

7. *Helicteres lanceolata* DC. (Table 2; Figs. 13–14): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape, mean±SD polar view 23.00±0.93 µm, mean±SD equatorial view 19.87±0.76 µm, exine sculpturing slightly verrucate and sparsely perforate.

8. *Helicteres viscida* Blume (Table 2; Figs. 15–16): Pollen grains are monad, bilateral symmetric, isopolar, triporate aperture, semi-triangular in shape, mean±SD polar view 32.03±227 µm, mean±SD equatorial view 31.33±2.41 µm, exine sculpturings slightly verrucate and sparsely perforate.

Discussion

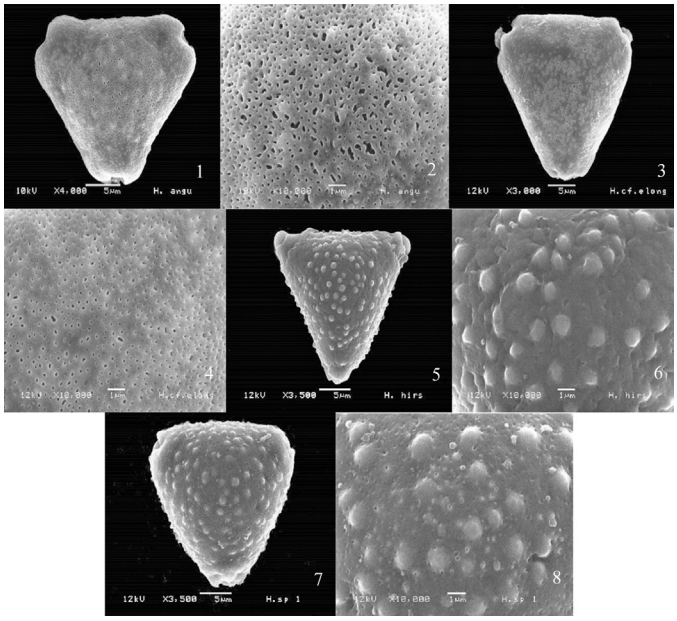
The results showed that pollens of these Thai *Helicteres* species have bilateral symmetry and a triporate aperture, are semi-triangular or semi-trilobate in shape and have negatively reticulate pollen, which agrees with the pollen morphology of *Helicteres* previously shown by Sharma (1967), Erdtman (1972), Pire and Cristóbal (2001) and El-Husseini (2006). However, the characters of brevissimicolporate and 3-zono-brevissimicolporate were not presented in the current study, whereas Sharma (1967) found that brevissimicolporate occurred in *H. isora* and 3-zono-brevissimicolporate in *H. hirsuta*.

Pollen grains from all the species and samples could be divided into two groups based on size following Walker and Doyle (1975). The first group with small-sized pollen grains (10–24 µm) were represented by *H. angustifolia*, *H. elongata*, *H. hirsuta* (pale pink flower), *H. isora*, *H. lanceolata* and *H. lanata*, while the second group with medium-sized pollen grains (25–49 µm) were represented by *H. hirsuta* (purple flower) and *H. viscida*. While Pire and Cristóbal (2001) reported medium-sized grains in all species of *Helicteres* pollen (including *H. angustifolia*, *H. elongata*, *H. isora*, *H. lanata*, *H. hirsuta* and *H. viscida*).

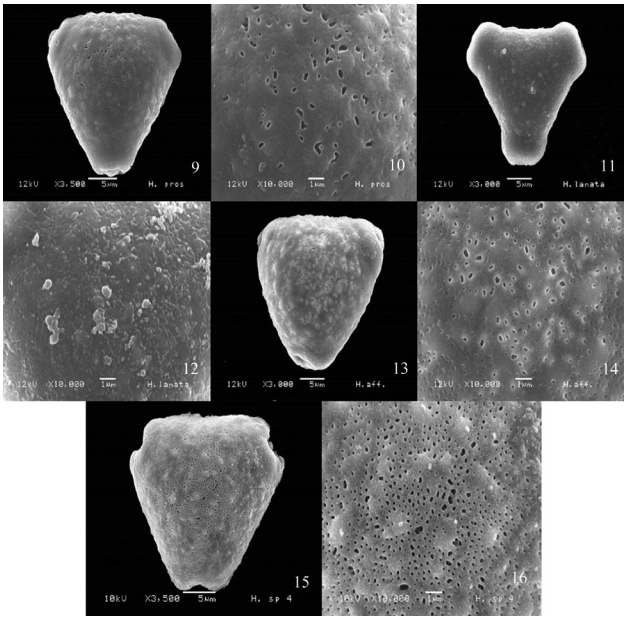
Table 2 Pollen morphology of Thai *Helicteres*

Species	Symmetry	Polar	Aperture	Shape	Average range (μm) (mean±SD)			Exine sculpturing
					P*	E*	P*/E*	
H. angustifolia Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	23.20±1.38	20.20±1.11	1.15±1.24	slightly verrucate and sparsely perforate
H. elongata Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	23.30±0.97	20.73±1.21	1.12±0.80	Perforate
H. hirsuta(purple flower) Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	34.63±3.24	34.10±2.98	1.02±1.09	verrucate
H. hirsuta(pale pink flower) Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	21.97±1.47	21.03±1.17	1.05±1.26	verrucate and sparsely perforate
H. isora (Sect.*Helicteres*)	bilateral	isopolar	triporate	semi-triangular	24.67±1.32	23.63±1.08	1.04±1.22	slightly verrucate and sparsely perforate
H. lanata Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-trilobate	22.57±1.05	21.07±0.96	1.07±1.09	slightly scabrate
H. lanceolata Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	23.00±0.93	19.87±0.76	1.16±1.22	slightly verrucate and sparsely perforate
*H. viscid*a Sect. *Orthocarpaea*	bilateral	isopolar	triporate	semi-triangular	32.03±2.27	31.33±2.41	1.02±0.94	slightly verrucate and sparsely perforate

P = Polar view; E = Equatorial view



Figs. 1–8 Scanning electron microscopy photomicrographs of pollen morphology of *Helicteres* from Thailand: (1) overall pollen morphology of *H. angustifolia* (scale bar = 5 μm); (2) pollen of *H. angustifolia* with exine sculpturing slightly verrucate and sparsely perforate (scale bar = 1 μm); (3) overall pollen morphology of *H. elongata* (scale bar = 5 μm); (4) pollen of *H. elongata* with exine sculpturing perforate (scale bar = 1 μm); (5) overall pollen morphology of *H. hirsuta* (purple flower) (scale bar = 5 μm); (6) pollen of *H. hirsuta* (purple flower) with exine sculpturing verrucate (scale bar = 1 μm); (7) overall pollen morphology of *H. hirsuta* (pale pink flower) (scale bar = 5 μm); (8) pollen of *H. hirsuta* (pale pink flower) with exine sculpturing verrucate and sparsely perforate (scale bar = 1 μm).



Figs. 9–16 Scanning electron microscopy photomicrographs of pollen morphology of *Helicteres* from Thailand: (9) overall pollen morphology of *H. isora* (scale bar = 5 μm); (10) pollen of *H. isora* with exine sculpturing slightly verrucate and sparsely perforate (scale bar = 1 μm); (11) overall pollen morphology of *H. lanata* (scale bar = 5 μm); (12) pollen of *H. lanata* with exine sculpturing slightly scabrate (scale bar = 1 μm); (13) overall pollen morphology of *H. lanceolata* (scale bar = 5 μm); (14) pollen of *H. lanceolata* with exine sculpturing slightly verrucate and sparsely perforate (scale bar = 1 μm); (15) overall pollen morphology of *H. viscida* (scale bar = 5 μm); (16) pollen of *H. viscida* with exine sculpturings slightly verrucate and sparsely perforate (scale bar = 1 μm).

Pollen grains of Thai *Helicteres* represented various scultpturing of the exine: verrucate and perforate in *H. angustifolia*; perforate in *H. elongata*; verrucate in *H. hirsuta* (purple flower); verrucate and sparsely perforate in *H. hirsuta* (pale pink flower); slightly verrucate and scabrate in *H. lanata*; slightly verrucate and sparsely perforate in *H. isora* and *H. lanceolata*; and slightly verrucate and perforate in *H. viscida*. Sharma (1967) studied two Indian species (*H. isora* and *H. hirsuta*) and distinguished two types: *H. isora* type with 3-zonoporate or brevissimicolporate and granulate, papillate, negatively reticulate pollen grains with triangular, and *H. hirsuta* type characterized by 3-zono-brevissimicolporate or porate and verrucose pollen grains. The *H. isora* type is different from the present results in the characteristics of 3-zonoporate or brevissimicolporate and granulate, papillate, while the *H. hirsuta* type is consistent with pollen grains of *H. hirsuta* (purple or pale pink flower) in the characteristics of porate and verrucose pollen grains but different in the characteristic of 3-zono-brevissimicolporate. Erdtman (1972) reported the pollen morphology of *H. angustifolia* only, which was monad, subangular in shape, isopolar, radial symmetry, 15–90 µm in size and had a perforate exine sculpture, which differs in symmetry and size from the present results perhaps because of the effects of environmental factors, while the shape is similar.

Pire and Cristóbal (2001) studied pollen grains of *H. isora*, *H. angustifolia*, *H. elongata*, *H. lanata*, *H. hirsuta* and *H. viscida,* and found that they belonged to three pollen types based on the exine sculpturing. *H. isora* was attributed to Type I (tectate-perforate, baculate) while the exine sculpturing of *H. isora* in the present study is slightly verrucate-sparsely perforate (Table 2). *H. angustifolia*, *H. elongata* and *H. lanata* were attributed to Type II (tectate-perforate, psilate to weakly verrucate), which are similar to the present study but differ being without psilate because it might have been affected by environmental factors (Table 2). *H. hirsuta* and *H. viscida* were attributed to Type VI (tectate-perforate, verrucate), which are similar in the present study (Table 2). Sharma (1967) reported the *H. hirsuta* pollen type, characterized by 3-zono-brevissimicolporate or porate and verrucose pollen grains, which is consistent with the present study in *H. hirsuta* (purple flower) except for the characteristic of zono-brevissimicolporate that is not presented in the present study but is not consistent with *H. hirsuta* (pale pink flower) which is reported by Sharma (1967). Pire and Cristóbal (2001) reported pollen of *H. hirsuta* and attributed it to Type VI (tectate-perforate, verrucate), which is consistent with pollen of both samples of *H. hirsuta* in the present study (Table 2). Moreover, the pollen morphology of *H. hirsuta* (purple flower) does not differ from the pollen of *H. hirsuta* (pale pink flower) in shape but does differ in size and exine sculpturing which might be due to environmental factors and the diversity in pollinators (insects, birds and mammals were the important pollinators) of different phyla, which reinforces the adaptability of plants to the changing environmental conditions of climate change (Girish Kumar et al., 2016).

Therefore, the pollen morphology of both *H. hirsuta* species is not different. The pollen morphology of *H. lanceolata* is similar to that of the pollen of *H. hirsuta* (pale pink flower) in shape, size and exine sculpturing. The pollen morphology of *H. lanceolata* is reported globally for the first time in the present study.

The results of this study showed that the patterns of different pollen sizes and the exine sculpturing (slightly verrucate and sparsely perforate; verrucate and sparsely perforate; slightly scabrate; perforate; verrucate) might be affected by environmental factors but it is consistent for classification.

The genus *Helicteres* belongs to the subfamily Helicteroideae, family Malvaceae s.l. and formerly this genus were in the family Sterculiaceae (The Angiosperm Phylogeny Group, 2016). The pollen morphology of *Helicteres* in the present study is not congruent with the morphological data of Phengklai (2001) who considered that the pollen morphological characters of each species in the present study could not be used for classification. The plant species in this study were taken from one specimen or only collected from the northeastern and eastern regions (some species have a much wider distribution); therefore, it may not be sufficient sampling to discuss the complete pollen characters and their variation and consequently, an artificial key of *Helicteres* in Thailand cannot be constructed usingthe current pollen morphology.

Acknowledgements

This research was financially supported by Mahasarakham University. The Walai Rukhavej Botanical Research Institute, the Faculty of Science, Mahasarakham University and herbaria (BK and BKF) provided facilities during the study. Valuable advice was provided by Dr.Chumlong Phengkhai, a specialist in the family Sterculiaceae in Thailand. Miss Weeriya Thanee provided laboratory assistance and Dr. Jolyon Dodgson assisted with reviewing and correction of an earlier version of the manuscript.

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