# Pollen and Seed Morphology of *Cleome* Species (Cleomaceae) in Vietnam (Debunga dan Morfologi Biji Spesies *Cleome* (Cleomaceae) di Vietnam)

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#### ABSTRACT

Cleomaceae is a family that includes 18 genera distributed worldwide in tropical and temperate regions. *Cleome* L. is the largest genus having 207 species, widely distributed in tropical and subtropical regions (POWO 2019). In Vietnam, the genus is represented by four species *Cleome rutidosperma* DC., *C. houtteana* Schltdl., *C. viscosa* L. and *C. gynandra* L. The present study documents the pollen and seed characteristics of all four species of *Cleome* in Vietnam, and the data obtained using light microscope and scanning electron microscope are analyzed considering their importance in taxonomic delimitation and understanding their phylogenetic relationship. The results indicated a close relationship between different pollen and aperture types. The striking differences between the pollen shapes among different species of *Cleome* could be useful in taxonomic and evolutionary studies. Similarly, seed shape and surface characters provided important clues for their delimitation. Based on the pollen and seed morphological characteristics, a taxonomic key has been provided for easy identification of the *Cleome* species in Vietnam.

Keywords: Cleome; pollen grains; scanning electron microscopy (SEM); seed coat morphology

#### ABSTRAK

Cleomaceae ialah famili yang merangkumi 18 genus yang tersebar di seluruh dunia di kawasan tropika dan sederhana. *Cleome* L. ialah genus terbesar yang mempunyai 207 spesies, tersebar secara meluas di kawasan tropika dan subtropika (POWO 2019). Di Vietnam, genus ini diwakili oleh empat spesies *Cleome rutidosperma* DC., *C. houtteana* Schltdl., *C. viscosa* L. dan *C. gynandra* L. Kajian ini mendokumenkan ciri debunga dan benih bagi keempatempat spesies *Cleome* di Vietnam dan data yang diperoleh menggunakan mikroskop cahaya dan mikroskop elektron imbasan dianalisis dengan mengambil kira kepentingannya dalam persempadanan taksonomi dan memahami hubungan filogenetiknya. Keputusan menunjukkan hubungan rapat antara jenis debunga dan apertur yang berbeza. Perbezaan ketara bentuk debunga antara spesies *Cleome* yang berbeza berguna dalam kajian taksonomi dan evolusi. Begitu juga, bentuk biji dan pencirian permukaan memberikan petunjuk penting untuk persempadanan semula mereka. Berdasarkan ciri morfologi debunga dan benih, kunci taksonomi telah disediakan untuk mengenal pasti spesies *Cleome* dengan mudah di Vietnam.

Kata kunci: Bijirin debunga; Cleome; mikroskop elektron imbasan (SEM); morfologi kulit biji

#### INTRODUCTION

Cleomaceae is a family of the order Brassicales (APG IV 2016) comprising of 18 genera and 150-180 species,

distributed worldwide in tropical and temperate regions (Melanie, Eric & Jocelyn 2014; Zhang & Tucker 2008). *Cleome* L. is the largest genus having 207 species, widely

distributed in tropical and subtropical regions (POWO 2019). In Vietnam, the genus is represented by four species Cleome rutidosperma DC., C. houtteana Schltdl., C. viscosa L., and C. gynandra L. (Ban & Dorofeev 2003). Many of the earlier studies have treated *Cleome* and other allied genera under tribe - Cleomeae of the family Capparaceae (Bentham & Hooker 1862; Candolle 1824; Eichler 1865; Takhtajan 2009) or subfamily -Cleomoideae of Capparaceae (Kubitzki & Bayer 2003; Melchior 1964; Pax 1891; Takhtajan 1997 & 1987). Lately, the monophyly of the family Cleomaceae was proved and its placement was resolved based on the molecular data (Hall 2008; Hall, Iltis & Sytsma 2004; Hall, Sytsma & Iltis 2002) and consequently it was given a distinct family status. Members of this family are distinguished from the closely related Capparaceae and Brassicaceae by their mostly herbaceous habit, palmately compound leaves, capsular fruits lacking a septum, and seeds with a testa (Melanie, Eric & Jocelyn 2014).

Several studies on the pollen morphology of Cleomaceae were carried out in the last few decades (Gamal 1993; Nyananyo 1990; Riaz et al. 2019; Richard et al. 1997; Ruiz, Xen & Nereida 1997; Solomon et al. 1973). Some studies also addressed the seed morphology of Cleomaceae and indicated the existence of characters with potential use in differentiating several genera or species (Aparadh & Karadge 2010; Arbuzova & Nikitin 2003; Puri 1971). The characters of pollens grain and the seed of Cleomaceae using scanning electron microscope (SEM) were also proved important to distinguish some species of Cleomaceae in Saudi Arabia (Wael 2016). On similar lines, our study focused on the objective to find out the important pollen and seed characters helpful in differentiating Vietnamese Cleome species using both light and scanning electron microscopy.

#### MATERIALS AND METHODS

#### MATERIALS

Specimens were collected during 2010 to 2012 in selected areas of Vietnam. Information related to collection of specimens are provided in Table 1. All specimens were identified consulting relevant literature (Chayamarit 1991; Ho 1999; Hu 2007; Jacobs 1960) and several herbarium specimens housed in HN, K, and P. Nomenclature of species followed POWO 2020 (Plant of the world online). All specimens were kept in HN (Hanoi Herbarium, Institute of Ecology and Biological Resources, Hanoi, Vietnam).

#### POLLEN STUDY

For light microscopy (LM), pollen grains were extracted from one or two flowers per sample and were acetolyzed by the standard acetolysis method (Erdtman 1960). After that, they were mounted on glycerine jelly, and observed at 1000x magnification under oil immersion TM 518. All measurements were obtained by LM and captured on a Zeiss Axio Imager A1 camera. For scanning electron microscopy (SEM), pollen grains were placed on stubs coating with gold Hitachi E-1010 sputter coater for three minutes. The surface ornamentations were examined using a Hitachi S3400-N scanning electron microscope at 20KV, installed at the International Biological Material Research Center (IBMRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB), Republic of Korea. The major morphometric parameters such as the polar axis and equatorial axis were measured on 20 pollen grains, and the exine thickness was measured in ten randomly chosen pollen grains with the arithmetic mean calculated. The terminology used for pollen description followed Hesse et al. (2009) and Punt et al. (2007).

### SEED STUDY

Mature seeds were collected from the field and were cleaned, dried, and measured for length, width, and other information (color and shape) under the light microscope (LM) Nikon, AZ100. Selected seeds were directly mounted on double-sided carbon tape and then gold ion coating in a sputter coater unit (Hitachi E-1010). Surface of seeds was observed under a Hitachi S3400-N scanning electron microscope (SEM) at 20KV. Description of the shape and surface of seeds followed the terminology used in Barthlott (1981), Jeong et al. (2017) and Murley (1951).

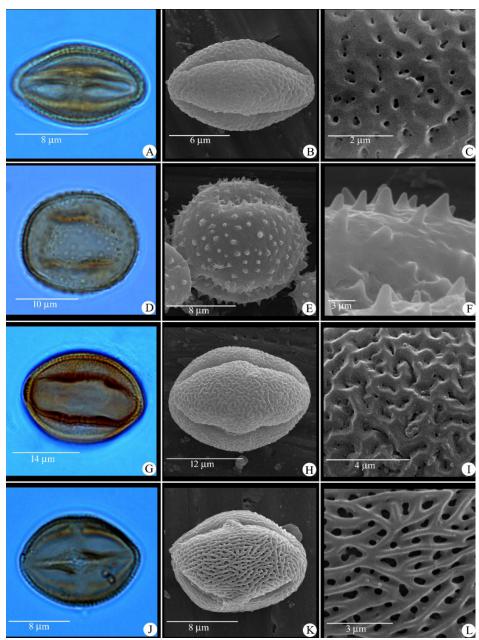
#### **RESULTS AND DISCUSSION**

#### POLLEN MORPHOLOGY

Cleome rutidosperma: The pollen grains are monad, radially symmetrical, isopolar, prolate. Aperture type tricolporate. Outline elliptic in the equatorial view (Figure 1(A) and 1(B)). Polar axis 18.62  $\mu$ m, equatorial axis 11.75  $\mu$ m. Ratio of the length of the polar axis to the equatorial axis (P/E): 1.58. Colpus length 15.10  $\mu$ m. Mesocolpium length 6.76  $\mu$ m. Exine sculpture microreticulate. Exine thickness 1.22  $\mu$ m (Table 2, Figure 1(B) and 1(C)).

## TABLE 1. List of investigated taxa and specimen details

Species name	Voucher specimen information	
Cleome rutidosperma DC.	<i>S.D. Thuong 17</i> ; Tran Chau community - Cat Ba island, Cat Hai district, Hai Phong city. N20°47'37" E106°59'26". 33 m. asl. HN!	
Cleome houtteana Schltdl.	<i>S.D. Thuong 21</i> ; 8 ward, Da Lat city, Lam Dong province. N11°57'01" E108°26'58". 1483 m. asl. HN!	
Cleome viscosa L.	<i>S.D. Thuong 18.</i> Krong Na community, Buon Don district, Dak Lak province. N11°20'11" E108°53'35". 181 m. asl. HN!	
Cleome gynandra L.	<i>S. D. Thuong 23</i> . Ca Na community, Ninh Phuoc district, Ninh Thuan province. N12°54'58" E107°44'56", 5 m. asl, HN!	



A-C. Cleome rutidosperma. D-F. Cleome houtteana. G-I. Cleome viscosa. J-L. Cleome gynandra. A, D, G, J. aperture detail (LM). B, E, H, K. general feature (SEM). C, F, I, L. surface detail (SEM)

FIGURE 1. Photomicrographs and electron micrographs of pollen grains of species of Cleomaceae in equatorial view

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Cleome houtteana: The pollen grains are monad, radially symmetrical, isopolar, prolate-spheroidal. Aperture type: tricolporate. Outline nearly circular in the equatorial view (Figure 1(D) and 1(E)). Polar axis: 19.72  $\mu$ m and equatorial axis: 17.81  $\mu$ m. The ratio of the length of the polar axis to the equatorial axis (P/E): 1.10. Colpus length: 16.46  $\mu$ m. Mesocolpium length: 12.32  $\mu$ m. Exine sculpture: echinate, spines 0.56  $\mu$ m long. Exine thickness: 1.25  $\mu$ m (Table 2, Figure 1(E) and 1(F)).

Cleome viscosa: The pollen grains are monad, radially symmetrical, isopolar, prolate. Aperture type: tricolporate. Outline elliptic in the equatorial view (Figure 1(G) and 1(H)). Polar axis: 28.90  $\mu$ m and equatorial axis: 20.12  $\mu$ m. The ratio of the length of the polar axis to the equatorial axis (P/E) is 1.43. Colpus length: 22.15  $\mu$ m. Mesocolpium length: 13.56  $\mu$ m. Exine sculpture: reticulate-rugulate. Exine thickness: 1.26  $\mu$ m (Table 2, Figure 1(H) and 1(I)). Cleome gynandra: The pollen grains are monad, radially symmetrical, isopolar, subprolate. Aperture type: tricolporate. Outline: elliptic in the equatorial view (Figure 1(J) and 1(K)). Polar axis: 17.10  $\mu$ m and equatorial axis: 13.00  $\mu$ m. The ratio of the length of the polar axis to the equatorial axis (P/E): 1.31. Colpus length: 13.34  $\mu$ m. Mesocolpium length: 9.80  $\mu$ m. Exine sculpture: striate-foveolate. Exine thickness: 1.05  $\mu$ m (Table 2, Figure 1(K) and 1(L)).

Edeoga et al. (2009) when studying pollen of three species of *C. viscosa, C. gynandra,* and *C. rutidosperma* in Nigeria documented tricolpate pollen structures of these three species. Our investigations on pollens of *C. gynandra* and *C. viscosa* showed similar characteristic, however, there is a slight difference in size of polar axis, equatorial axis, the length of colpus and the thickness of exine. This variability may be result of the geographical separation and ecological adaptations.

TABLE 2. Measurements of pollen of *Cleome* species studied (P: polar axis, E: equatorial axis, P/E: Polar/equatorial, ET: exine thickness). All units in µm except P/E

Species Characters	Cleome rutidosperma	Cleome houtteana	Cleome viscosa	Cleome gynandra
Pollen type	monad	monad	monad	monad
Aperture type	tricolporate	tricolporate	tricolporate	tricolporate
Equatorial view	elliptic	nearly circular	elliptic	elliptic
Р	18.62±1.70	19.72±1.25	28.90±2.50	17.10±2.30
Е	11.75±1.04	17.81±1.53	20.12±1.81	13.00±1.64
P/E	1.58	1.10	1.43	1.31
ET	1.22±0.13	1.25±0.24	1.26±0.33	1.05±0.28
Pollen shape	Prolate	Prolate-spheroidal	Prolate	Subprolate
Exine sculpture	microreticulate	Echinate, spine length 0.56±0.20	reticulate-rugulate	Striate-foveolate
Mesocolpium length	6.76±0.23	12.32±0.22	13.56±0.36	9.80±0.21
Colpus length	15.10±0.10	16.46±0.33	22.15±0.21	13.34±0.15

Our study also concludes that the pollen characters may prove helpful to understand the taxonomic position of studied taxa. Other characters such as the size and exine sculpture are also important to identify species.

#### SEED MORPHOLOGY

Cleome rutidosperma: Size:  $0.85 \times 0.74$  mm, asymmetrical. Shape: circular to reniform, surface rough. Color red-pink, central part white to pale yellow, surface bullate to papillate with irregular polygons, central part nearly smooth; epidermal cell shape varies from three edges to hexagonal, often with curved bristles; anticlinal cell straight to curved. Ribs wavy with irregular heights; epidermal cell papillate, bristles curved. Base of seed with white elaiosome (Table 3, Figure 2(A), 2(B) and 2(C)).

Cleome houtteana: Size:  $0.91 \times 0.76$  mm, asymmetrical. Shape: circular to reniform, with rough surface. Color: brown. Surface irregularly reticulate with irregular polygons; epidermal cell shape often rectangle, sometimes three edges to irregular four edges; anticlinal cell flat and smooth, often curved, sometimes straight (Table 3, Figure 2(D), 2(E) and 2(F)). Cleome viscosa: Size:  $0.66 \times 0.65$  mm, asymmetrical. Shape: circular to reniform, with rough surface. Color: grey-brown. Surface: verrucate with irregular polygons; epidermal cell shape varies from triangular to nearly circular, or rectangular; anticlinal cells curved. Ribs wavy; epidermal cells verrucose (Table 3, Figure 2(G), 2(H) and 2(I)).

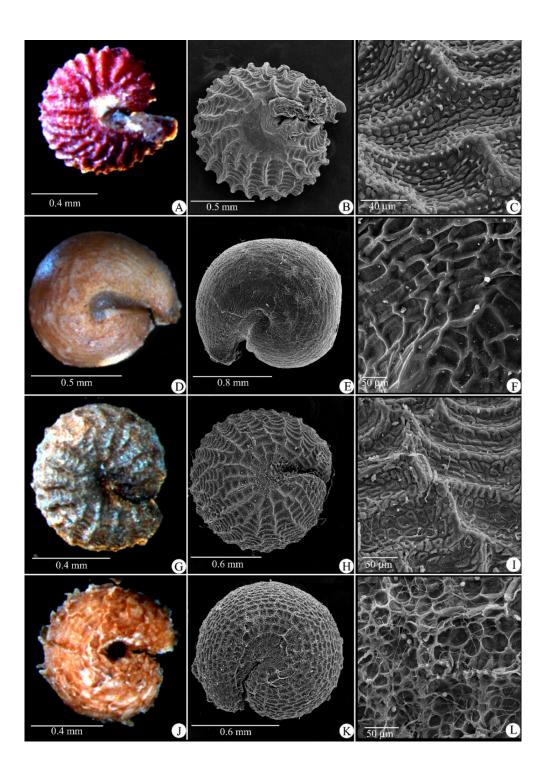
Cleome gynandra: Size:  $0.64 \times 0.67$  mm, asymmetrical. Shape: circular to reniform, with rough surface. Color bronze. Surface bireticulate with ridges running vertically or interwoven across the cells; epidermal cell shape nearly round; anticlinal cells with uneven thickness (Table 3, Figure 2(J), 2(K) and 2(L)).

As far as the seed morphology is concerned, the seed shape of the four Vietnamese *Cleome* species varies from circular to reniform. However, there is hardly any variation in size of the seeds. The color of the seeds may prove useful while identifying them to the species level. Moreover, the seed surface characters are the most important criterion for distinguishing four species.

Our research, conclusively, confirms that the pollen and seed morphological characteristics are very useful to distinguish the species of *Cleome* in Vietnam. The results also corroborates with the earlier studies and vouch for more studies on the seeds and pollen of the allied genera and family.

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Species Characters	Cleome rutidosperma	Cleome houtteana	Cleome viscosa	Cleome gynandra
Length (mm)	0.85±0.14	0.91±0.12	0.66±0.13	0.64±0.18
Width (mm)	0.74±0.13	0.76±0.17	0.65±0.11	0.67±0.15
Shape	circular to reniform	circular to reniform	circular to reniform	circular to reniform
Surface	bullate to papillate	irregular reticulate	verrucate	bireticulate
Color	red-pink	brown	grey-brown	bronze
Elaiosome	Present	Absent	Absent	Absent

#### TABLE 3. Measurements of seeds of Cleome species studied



A-C. Cleome rutidosperma. D-F. Cleome houtteana. G-I. Cleome viscosa. J-L. Cleome gynandra. A, D, G, J. aperture detail (LM). B, E, H, K. general feature (SEM). C, F, I, L. surface detail (SEM)

FIGURE 2. Photomicrographs and electron micrographs of seeds of species of Cleomaceae

## TAXONOMIC KEY TO THE *CLEOME* SPECIES IN VIETNAM BASED ON POLLEN AND SEED MORPHOLOGY

1. Pollen shape prolate2
1'. Pollen shape prolate-spheroidal or subprolate
2. Exine sculpture of pollen microreticulate. Surface of seed bullate to papillate, color red-pink,
elaiosome presentC. rutidosperma
2'. Exine sculpture of pollen reticulate-rugulate. Surface of seed verrucate, color grey-brown,
elaiosome absentC. viscosa
3. Pollen shape prolate-spheroidal; exine sculpture echinate. Surface of seed irregularly reti
culateC. houtteana
3'. Pollen shape subprolate; exine sculpture striate-foveolate. Surface of seed bireticulate
C. gynandra

#### CONCLUSIONS

In this study, the pollen and seed of all four species of Cleome L. in Vietnam, namely as C. rutidosperma DC., C. houtteana Schltdl., C. viscosa L., and C. gynandra L. were analyzed and reviewed their importance in taxonomy, and their relationships. The pollen and seed morphology of four Cleome species in Vietnam were described and provided detailed pollen and seed imaging data using the light and scanning electron microscope. The striking differences between the pollen shapes among different species of Cleome could be helpful in taxonomic and evolutionary studies. Seed shape and surface characters provided important clues for their delimitation. A taxonomy key for easy identification of the Cleome species in Vietnam has been proposed based on pollen and seed morphological characteristics.

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