

Pollen and Seed Morphology of *Rhinacanthus* Nees and *Hypoestes* Sol. ex R. Br. (Acanthaceae) of Yemen

(Morfologi Debunga dan Biji *Rhinacanthus* Nees dan *Hypoestes* Sol.
ex R. Br. (Acanthaceae) dari Yemen)

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ABSTRACT

Pollens and seeds of Hypoestes and Rhinacanthus collected from different field localities in Taiz and Soqatra Island, Yemen were investigated by using light and scanning electron microscopes. Pollen grains of Hypoestes were prolate in equatorial view, lobate trigonal to lobate circular in polar view whereas those of Rhinacanthus were subspheroidal and rounded trigonal in polar view. The aperture was tricolporate and exine ornamentation was coarsely reticulate for all species in the two genera. Scanning electron microscopy and morphological observations showed that mature dry seeds of Hypoestes and Rhinacanthus have various sizes and shapes, the surface ornamentations observed were reticulate to cristate, an addition to the tuberculum and papillae. The three Hypoestes species differ in the seed structure which are useful for identification and their high structural diversity provides an important taxonomic value for species differentiation.

Keywords: Acanthaceae; Hypoestes species; pollen morphology; Rhinacanthus scoparius; seed morphology

ABSTRAK

Debunga dan biji Hypoestes dan Rhinacanthus yang diperoleh dari lokaliti lapangan berbeza di Taiz dan Pulau Soqatra, Yemen telah dikaji menggunakan mikroskop cahaya dan mikroskop imbasan elektron. Debunga Hypoestes adalah prolata pada pandangan khatulistiwa, lobat trigon kepada lobat membulat pada pandangan kutub manakala debunga Rhinacanthus adalah subsferoid dan trigon membulat pada pandangan kutub. Apertur adalah trikolporat dan hiasan eksin adalah retikulum kasar untuk semua spesies kedua-dua genus tersebut. Mikroskop imbasan elektron dan pemerhatian morfologi telah menunjukkan biji matang kering Hypoestes dan Rhinacanthus mempunyai berbagai saiz dan bentuk, hiasan permukaan adalah retikulum hingga kristat, disamping tuberkulum dan papila. Ketiga-tiga spesies Hypoestes berbeza pada struktur biji yang berguna bagi pengelasan dan kepelbagaian struktur yang tinggi memberikan nilai taksonomi yang penting untuk membezakan spesies.

Kata kunci: Acanthaceae; morfologi debunga; morfologi kulit biji; Rhinacanthus scoparius; spesies Hypoestes

INTRODUCTION

Acanthaceae is relatively a large family of about 250 genera and 2500 species worldwide (Mabberley 1987; Scotland 1992). The family is characterized by having zygomorphic flowers and persistent 4-5-lobed calyx, gamopetalous corolla, tube cylindrical or ventricose, the limb of 5, subequally spreading lobes or strongly 2-lipped; stamens 4 and didymous or 2, epipetalous; ovary conical or oblong-cylindric, bicarpellate, syncarpous, superior, placentation axile; fruits bivalve and 2-loculed capsule rarely drupaceous (Perveen & Qaiser 2010; Wood 1997).

In Yemen, according to Alkhulaidi (2013), the family is represented by 29 genera and 94 species. Both the genera *Hypoestes* and *Rhinacanthus* are Old World origin and are found in the tropical regions. The former is more widespread and has about 40 species while the latter is more confined to the Mediterranean area and consists of about 15 species. *Rhinacanthus* is represented by one endemic species, *Rhinacanthus scoparius* (Miller

2004) and *Hypoestes* is represented by three species, one of which *Hypoestes pubescens* is endemic to the Soqatra Island. Earlier, Clarke (1900) and Wood (1997) who studied the Flora of Tropical Africa and Yemen, respectively, had described two other *Hypoestes* species - *H. triflora* and *H. forskalei*. Scotland and Vollesen (2000) divided the family into three subfamilies and both the genera belong to subfamily Acanthoideae subtribe Justiciinae as both of them have ascending cochlear aestivation and 2-4 ovules.

Generally, both light and electron microscopic studies of pollen and seed coat morphology have made significant contributions towards a better understanding of the phylogeny and systematics of the flowering plants at higher taxonomic levels (Chen & Manchester 2007; Latiff 2012; Scotland & Vollesen 2000). Previous studies on the pollen structure of Acanthaceae species in the temperate regions showed the presence of some significant variations between genera ranging from colporate, colpate to porate with several apertures and

ornamentation (Erdtman 1952; Scotland & Vollesen 2000). Furness (1995, 1990, 1989) and Furness and Grant (1996) studied the pollen morphology of different genera of Acanthaceae in Africa while Perveen and Qaiser (2010) carried out similar study on Acanthaceae in Pakistan. In addition, Carine and Scotland (1998) surveyed the pollen morphology of *Strobilanthes* from South India and Sri Lanka. However, a more comprehensive study of the pollen and seed morphology, especially those from Yemen is lacking. The three species of *Hypoestes* are slightly difficult to differentiate as they have very similar morphologically. This study was first undertaken on four species from two genera, *Rhinacanthus* and *Hypoestes* from Yemen to evaluate the taxonomic significance of pollen and seed coat morphology, as part of the comprehensive studies of all the genera in Yemen.

MATERIALS AND METHODS

Fresh materials of pollens and seeds of *Hypoestes pubescens*, *H. forskalei*, *H. triflora* and *Rhinacanthus scoparius* were collected from various localities in Yemen (Table 1). The pollen samples were washed with phosphate buffer solution (PBS) three times and dehydrated through a series of acetone and critical-point dried. Anthers were opened carefully to obtain the pollen grains, coated with gold and observed under FESEM, ZEISS Super A, 55VP with various magnifications (500-10,000 \times). Ten to twenty pollen grains of each species were examined and average measurements for the polar axis and equatorial diameter were determined for each species. Images were taken and either printed with a Sony Video Printer or transferred onto a compact disc. For light microscope, the pollens were mounted in glycerin jelly either unstained or stained by using dots of safranin and observations were made by using E 40 and 10-20 readings were taken for each taxon. Data on the parameters, apocolpium, ora diameter, polar view and equatorial view were recorded.

Seeds from the four species were obtained from dehiscent fruits in the field and they were pretreated in two ways. First, dried seeds were mounted directly on double-sided adhesive tape fixed to SEM stubs. In the

second method, the seeds were washed with phosphate buffer solution (PBS) three times, dehydrated in a graded ethanol series to critical-point and dried in CO₂. Then, the mounted specimens were coated with gold in a sputter coater and examined using a scanning electron microscope. Pollen description follows Miller (2004) and Wood (1997) while pollen terminologies follow Hesse et al. (2009). Seed coat terminologies follow Barthlott (1981) and Whiffin and Tomb (1972).

RESULTS AND DISCUSSION

MORPHOLOGY OF POLLEN GRAINS

Pollen types have been recognized based primarily on the differences in the aperture number, shape, spinules, pseudocolpi and reticulate ornamentation at both the tribal and subtribal levels (Scotland & Vollesen 2000). Earlier, Scotland (1992) referred to several taxonomic approaches being used in Acanthaceae classification, but the role of pollen morphology in the classification of the family still remains problematic until pollen morphology is better understood.

Based on this study, the pollen variation in *R. scoparius* and three *Hypoestes* species were described and illustrated in the context of discussing intergeneric and intraspecific relationships. Our aim was to discuss a range of pollen morphological variations for the two genera and species and provide a key for identification. Below are the pollen descriptions for four species studied:

Rhinacanthus scoparius (Table 2; Figure 1A-C): Spiraperturate, 3 pori with circular shape, 6 μ m diameter, spheroidal-subprolate (P/E 1.1), polar view rounded trigonal, broad ribs demarcated at poles and pseudocolpi expand till the middle part of polar view, apocolpium 8 μ m long, mesocolpium 12 μ m wide, 35 μ m long, column thickness 335 nm, reticulate ornamentation, lumina 1.7 μ m and pores 366 nm.

Hypoestes pubescens (Table 2; Figure 2D-F): Tricolporate, 3 pori with circular shape, 7 μ m diameter, prolate (P/E

TABLE 1. List of Acanthaceae specimens studied

Species	Collection
<i>R. scoparius</i>	YEMEN: Soqotra, Hadebu, Hala, A. Wahab Sabry & Anisa S., WA 340; 12-1-2011 (UKMB)
<i>H. forskalei</i>	YEMEN: Taiz, Jarah mountain, A. Wahab Sabry & Anisa S., WA 298; 10-9-2010 (UKMB); Salah, Anisa, S. AS 19, 15-11-2010 (UKMB); Alhashama, A. Wahab Sabry WA 353, 21-11-2010 (UKMB); Alhabeel, A. Wahab Sabry WA 372, 1-12-2010 (UKMB); Saber mountain, A. Wahab Sabry WA 401, 5-2-2011 (UKMB).
<i>H. pubscens</i>	YEMEN: Socotra, Hadebu, Dexam, A. Wahab Sabry & Anisa S., WA 349, 18-1-2011 (UKMB); Halah, Anisa, S., AS 68, 14-1-2011 (UKMB).
<i>H. triflora</i>	YEMEN: Taiz, Abadan, A. Wahab Sabry & Anisa S, WA 366 30-11-2010; Saber mountain, A. Wahab Sabry AS 35, 22-10-2010 (UKMB)

TABLE 2. Morphological characteristic of pollen grains of four species of Acanthaceae
P.a: Polar axis, E. l: Equatorial length, Sh: Shape, Sph: Spheroidal, Sub. Pro: Subprolate, Pro: Prolate, P. cl: Pollen class, Spir: Spiraperturate

Species	P.a (µm)	E. l (µm)	P/E	Sh	P. cl	P. v	Orn	L. S (µm)	Po. L (nm)	Psu	Ora.D (µm)	Col (nm)	Apr	E. th (nm)	M. L (µm)	Apo (µm)
<i>R. scoparius</i>	35 (34.8±0.2) 34.5	40.5 (40.2±0.6) 39.5	1.1	Sph - Sub.Pro	Spir	R. tri	Ret	1.7	366	more than 6	6	370	35 × 5	335	35	8
<i>H. forskalei</i>	23.7 (23.5±0.2) 23.3	35.7 (35.6±0.2) 35.4	1.5	Pro	Tric	L. tri	Ret	1.3	201	6	4	267	33 × 3	613	12	8
<i>H. pubscens</i>	28.7 (28.3±0.4) 27.9	44.8 (44.7±0.1) 44.6	1.5	Pro	Tric	L. cir	Ret	1.5	128	6	7	320	27 × 1.3	46	9	3
<i>H. triflora</i>	26 (25.8±0.2) 25.6	44.7 (44.3±0.4) 43.9	1.7	Pro	Tric	L. tri	Ret	1.8	638	6	3.3	468	41.4	574	11.4	9.2

Tric: Tricolporate, P. v: Polar view, R. tri: Rounded trigonal, L. cir: Lobate, circular, L. tri: Lobate, trigonal, Ret: Reticulate, Orn: Ornamentation, L. S: Lumina size, Po. L: Pores within lumina, Psu: Pseudocolpi, Ora. D: Ora diameter, Col: Columellae, Apr: Aperture, E. th: Exine thickness, M. L: Mesocolpium length, Apo: Apocolpium

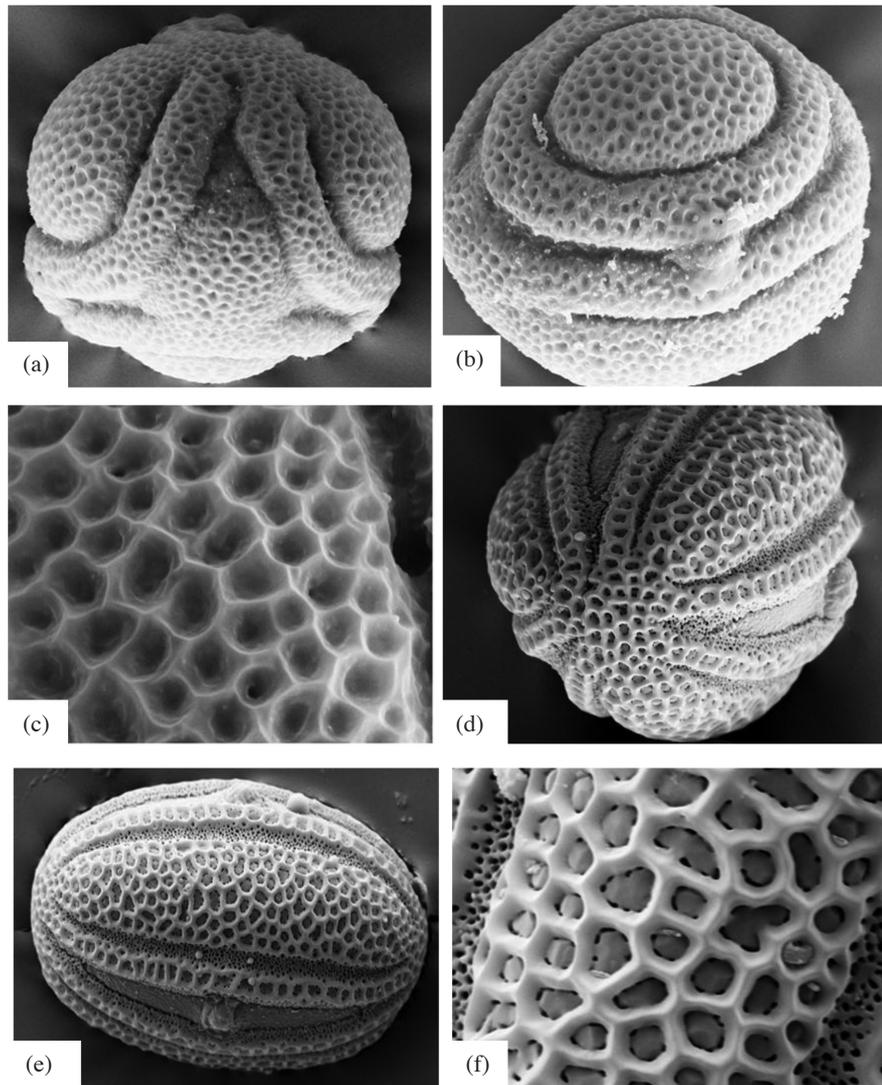


FIGURE 1. (a)-(c): Pollen grains of *Rhinacanthus scoparius*. (a) polar view, (b) equatorial view and porus, reticulation sculpture (d)-(f): Pollen grains of *Hypoestes forskalei* (d) polar view, (e) equatorial view and (f) detailed of reticulation ornamentation

1.5), polar view lobate circular, apocolpium 3 μm long, mesocolpium 9 μm long, broad ribs not demarcated at poles, reticulate ornamentation, lumina 1.5 μm and pores within lumina 128 nm.

Hypoestes forskalei (Table 2; Figure 1D-F): Tricolporate, 3 pori with circular shape, 4 μm diameter, prolate (P/E 1.5), polar view lobate, trigonal, apocolpium 8 μm long, mesocolpium 12 μm long, broad ribs not demarcated at poles, reticulate ornamentation, lumina 1.3 μm and pores 201 nm.

Hypoestes triflora (Table 2; Figure 2A-C): Tricolporate, 3 pori with circular shape, 3.3 μm diameter, prolate (P/E 1.7), polar view lobate trigonal, apocolpium 9.2 μm long, mesocolpium 11.4 μm long, broad ribs not demarcated at poles, reticulate ornamentation, lumina 1.8 μm and pores 638.3 nm.

Accordingly, two main types of pollens were observed based on the shape, type and colpium characteristic in the two genera. *R. scoparius* showed subprolate shape, spiraperture, rounded trigonal poles, reticulate ornamentation, apocolpium are more than six colpium that expand till the polar area and mesocolpium surrounded by completely colpi whereas the three *Hypoestes* species have prolate shape, reticulate sculpturing, three circular of ora and mesocolpium surrounded by incomplete colpi. Within the genus *Hypoestes*, the three species showed slightly different pollen characteristics. *H. triflora* has bigger pollen size (P/E 1.7), bigger apocolpium size (9.2 μm), higher columellae (468 nm) and longer aperture (41.4 μm) than the other two *Hypoestes* species. *H. forskalei* and *H. pubescens* have smaller pollen size (P/E 1.5), smaller apocolpium size (3-7.5 μm), shorter columellae (267-320.8 nm) and the length of aperture is (27-33 μm) (Table 2).

MORPHOLOGY OF SEEDS

Similarly, we describe and illustrate below the seed morphology of *R. scoparius* and three *Hypoestes* species in the context of discussing the intergeneric and interspecific relationships. Our aim was to discuss a range of seed morphological variation for the two genera and species studied and provide a key for identification.

Rhinacanthus scoparius (Table 3; Figure 3A-C): Seeds are tumid-tuberculate type, suborbicular, usually discoid, with rounded apex, oblique base, size is 2.0×1.6 mm, the surface covered by tiny papillae with little packs of tuberculate projections that differ in size and are somewhat spine-like or hook-like in rows, slightly pitted or grooved at dorsal surface.

Hypoestes triflora (Table 3; Figure 2, A-C): Seeds are cristate, suborbicular, discoid, rounded apex, truncate-invaginated base, size is 1.4×1.6 mm, rugose ornamentation, a whole surface covered by small reticulate crests and raised, prominent, conical and polygonal tuberculum distributed regularly on the surface, different in shape and size.

Hypoestes pubescens (Table 3; Figure 4D-F): Seeds are circumalate-papillate, subelliptic, discoid, manifestly winged, rounded apex, oblique semicordate base, size is 2.5×1.9 mm, the surface is covered with dense, small and smooth papillae arranged irregularly in reticulate shape and surrounded by either a few tuberculate or big papillae separated on marginal and central surface, margin somewhat dentate.

Hypoestes forskalei (Table 3; Figure 3H-D): Seeds are reticulated-cristate type, elliptic-ovate, discoid, ellipsoid-ovoid, prominent acuminate apex, cordate base, size is 1.6×1.1 mm, the lateral surface is smooth, papillae absent

but covered by large reticulated epidermal cells with raised anticlinal boundaries, terminal and central surfaces covered by sinuate and swollen crests.

Seed morphology does not differentiate between the two genera clearly, but it is sufficient to state that the seeds of *R. scoparius* have papillae in rows and the papillae are hooked. Within *Hypoestes*, the seed type, size and seed coat surface showed some variabilities which are valuable diagnostic taxonomic characters at species level. Despite the many SEM micrographs published in some papers and some studies describing the surface of seeds such as those of Barthlott (1981), Elisens et al. (1983), Lester (1991), Segarra and Mateu (2001) and Whiffin and Tomb (1972), some of these data are not comparable with our results because there are no standardized terminologies of the Acanthaceae pollen morphology and often no clear structural interpretation of the characters of the pollens illustrated.

This study focused on the shape and size, surface sculpturing and the types of tuberculate or papillae among the four species. Seed surface of *H. triflora* is covered with reticulate ornamentation and papillae, the randomly distributed big polygonal tuberculate spreading on the lateral and dorsal surfaces making them appear rugose. The seed surface of *H. pubescens* appears smooth but when observed at high magnification. It is covered with dense small papillae in addition to some big papillae raised on the central and lateral surfaces. On the other hand, the seed surface of *H. forskalei* showed specific characters with ovate-elliptic shape, acuminate apex and semicordate base and two types of sculpturing on the surface, i.e. reticulated on the lateral surface and cristate on the central side. To illustrate the significance of seed and pollen morphologies in both the genera and the *Hypoestes* species, a key to identification is produced below.

TABLE 3. Seed morphology of the four species of Acanthaceae

Characters	Species			
	<i>R. scoparius</i>	<i>H. triflora</i>	<i>H. pubescens</i>	<i>H. forskalei</i>
Shape	Suborbicular, discoid	Suborbicular outline, discoid	Subelliptic, discoid	Elliptic-ovate, discoid
Size (cm)	2.0 × 1.6	1.4 × 1.6	1.6 × 1.1	1.6 × 1.1
Seed apex	Rounded	Rounded	Rounded	Acuminate
Seed base	Oblique	Truncate, invaginated	Oblique-semicordate	Cordate
Seed surface	Papillate	Reticulate & papillate	Papillate	Smooth
Thickness of reticulate wall	–	1.93	–	10.13
Papillae (µm)	–	2.7	7-12	–
Tuberculum type	Hooked tumid	cristate-Polygonal	Large papillate	Reticulated-Cristate
Tuberculum size (µm)	77 × 56	89.9 × 37	58 × 47	12 × 6

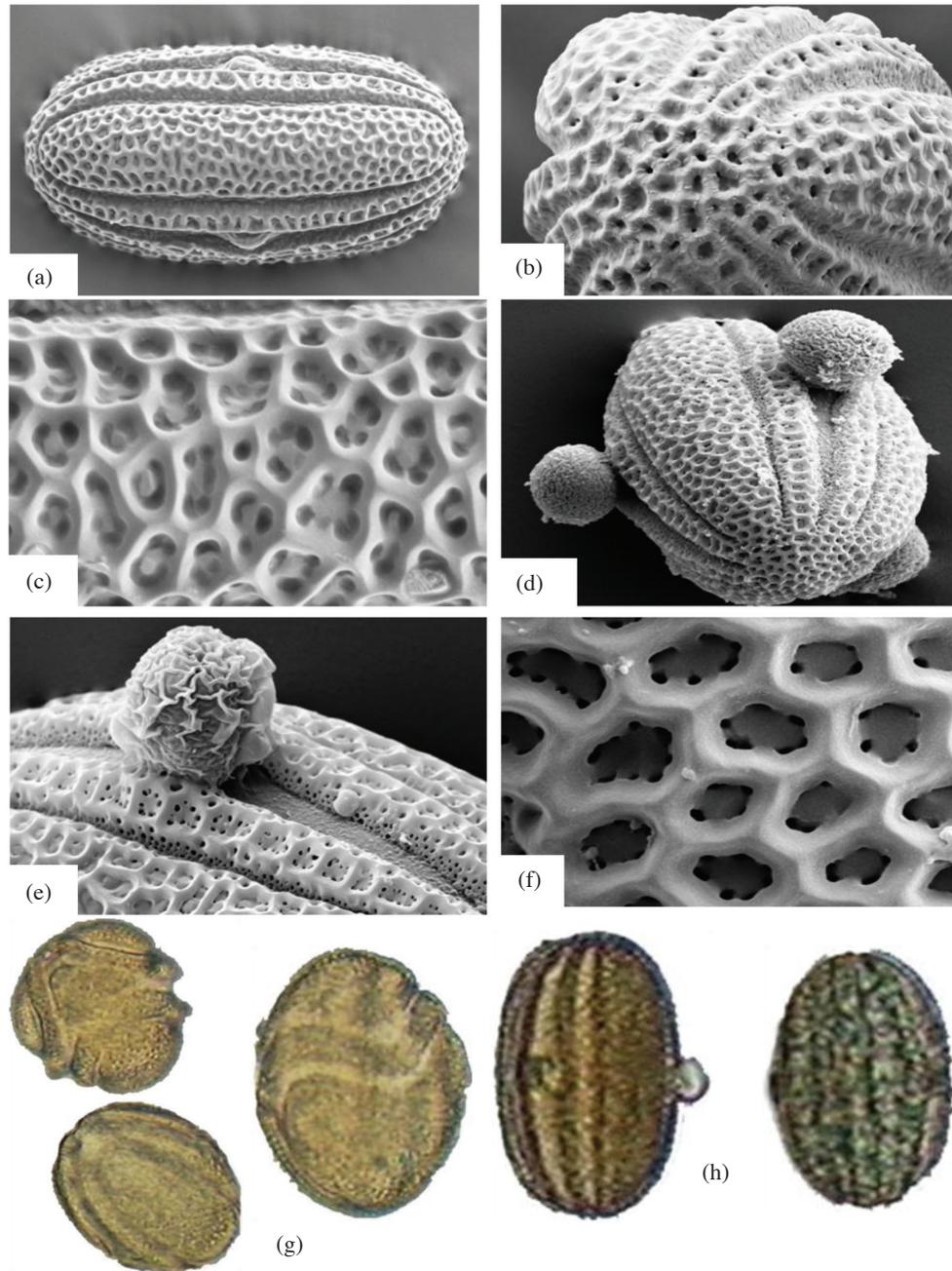


FIGURE 2. (a)-(c): Pollen grains of *Hypoestes triflora*. (a) equatorial view showing mesocolpium, (b) polar view and (c) detailed of reticulation. (d)-(f): Pollen grains of *Hypoestes pubescens*. (d) polar view and 3 pori, (e) ora in equatorial view and (f) detailed of reticulate sculpturing. (g)&(h): Light microscopy micrographs of *Rhinacanthus scoparius* (g) and *Hypoestes forskalei* (H, left) and *H. triflora* (H, right)

KEY TO SPECIES OF *RHICANANTHUS* AND *HYPOESTES*
BASED ON POLLEN AND SEED

- | | |
|---|---|
| <p>1. Pollens subprolate; rounded trigonal polar view; spiraperture; mesocolpium circular surrounded by complete colpi; seeds surface hooked tumid tuberculate; few papillae on surface
..... <i>R. scoparius</i></p> <p>1. Pollens prolate; lobate polar view; tricolporate; mesocolpium oblong surrounded by incomplete colpi; seeds surface cristate; large papillate, tuberculate; many papillae on surface 2</p> | <p>2. Size of mesocolpium 7 μm; seeds ovoid, acuminate apex; base cordate; curvature cristate at central; reticulation in lateral side
..... <i>H. forskalei</i></p> <p>2. Size of mesocolpium longer than 7 μm; seeds discoid, rounded apex; oblique base; tuberculate, with large papillae, papillae polygonal covering whole surface 3</p> <p>3. P/E of pollens is 1.7; length of pseudocolpi 41.4 μm; apocolpium 9.2 μm; seeds ca. 1.4 cm long; seed coat surface reticulate <i>H. triflora</i></p> |
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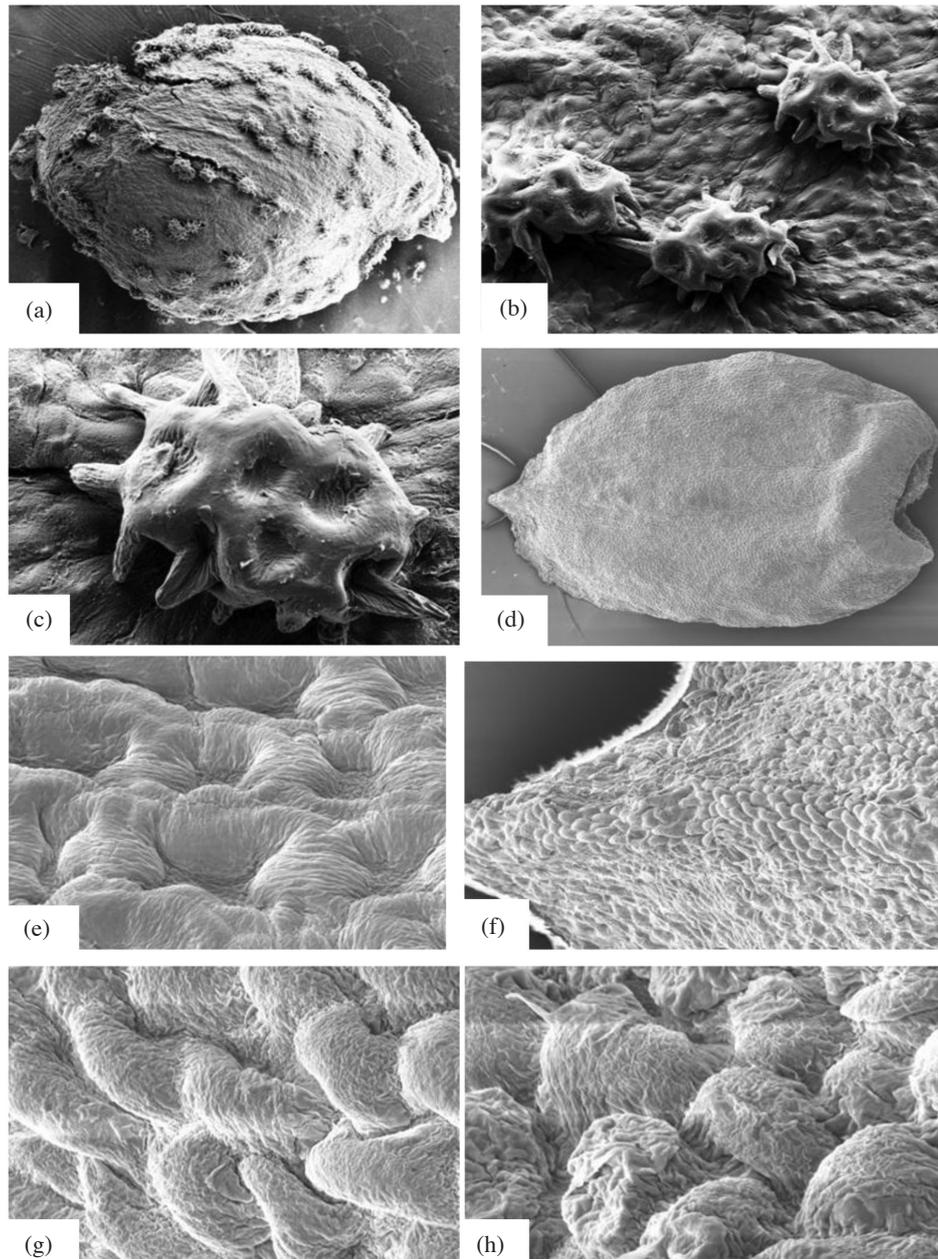


FIGURE 3. (a)-(c): Scanning electron micrographs of seeds coat of *Rhinacanthus scoparius*. (a) whole seed, (b) showing the reticulate and tuberculate structures and (c) detailed tuberculum with arms. (d)-(h): Seed of *Hypoestes forskalei*. (d) whole seed, (e) reticulate surface, (f) central sculpturing, (g) elongated cells on the surface and (h) crested structure

3. P/E of pollen is 1.5; length of pseudocolpi is 27 μm ; apocolpium 3 μm ; seeds ca. 2.5 cm long; sed coat surface papillate*H. pubescens*

CONCLUSION

Although there are some morphological descriptions of Acanthaceae species from Yemen, palynological and seed morphological studies are lacking in some genera. This study attempts to focus on the taxonomic value of pollens and seeds in two genera - *Hypoestes* and *Rhinacanthus* - as part of the whole studies of the family Acanthaceae in Yemen. Pollen morphology is uniform

among the *Hypoestes* species and similarities in shape, size, aperture and sculpturing ornamentation showed that pollen characters within a single genus is distinct and highly homoplastic. Therefore, it is difficult to be used as a taxonomic character in systematic study. However, there are detailed characteristics of pollens which can be used to differentiate the species. On the other hand, the sculpturing variation of seeds between genera and species is homogenous between the two genera or even within the same species and it would be of good taxonomic value for identification and reassessing taxonomic relationships among the *Hypoestes* species and between both the genera.

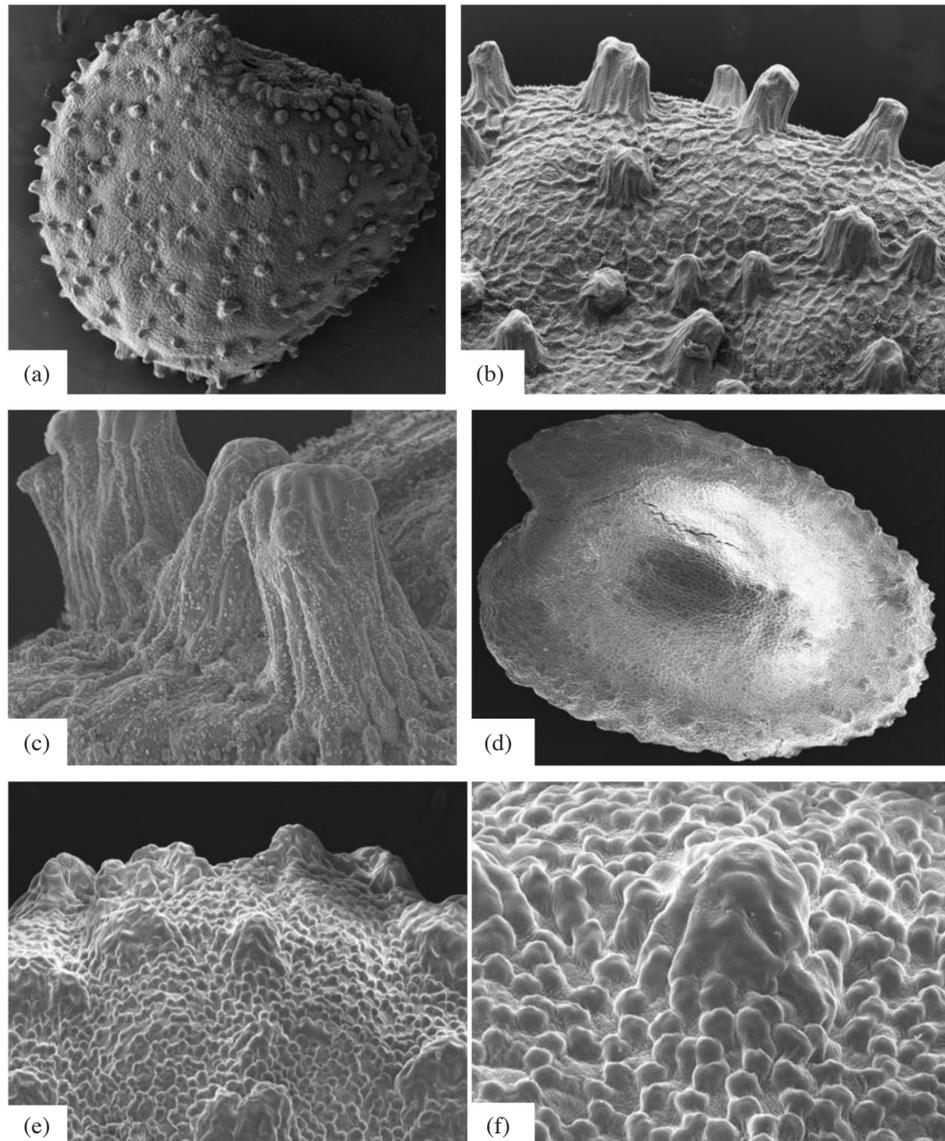


FIGURE 4. (a)-(c): Scanning electron micrographs of seed coat of *H. triflora*. (a) whole seed, (b) showing reticulate surface with polygonal tuberculum and (c) detailed of Polygonal structures. (d)-(f): Seed coat of *H. pubescens*. (d) whole seed, (e) showing small papillae on the surface and larger ones and (f) big papillae

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