Taxonomic studies of *Hippocrepis* and *Securigera* (*Fabaceae,* tribe *Loteae,* subtribe *Coronillinae*) in Egypt

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Received: February 14, 2022 ▷ Accepted: April 09, 2022

Abstract. Macro- and micromorphological features of the studied species from subtribe *Coronillinae* are examined by light and scanning electron microscopy. The study has revealed that morphological (habit, leaves, inflorescence, flowers, pods and seeds), anatomical (stem and leaf) and seed characters are of taxonomic significance for differentiation between the studied species. The results have clearly shown that *Hippocrepis areolata* Desv. is differentiated into two varieties, *glabra* Pamp. and *sinuosissima* Pomel., and these varieties are new to Egypt (var. nova).

Numerical analysis has been carried out and a phenogram illustrating the relationship between the studied taxa is drawn by calculating the average taxonomic distance. A key for identification of the the studied species is also given.

Key words: anatomy, *Coronillinae*, *Hippocrepis,* morphology, *Securigera*, seed

Citation: Aqlan, E.M., Turki, Z.A. & Shehata, F.A. 2022. Taxonomic studies of *Hippocrepis* and *Securigera* (*Fabaceae,* tribe *Loteae,* subtribe *Coronillinae*) in Egypt. -- Phytologia Balcanica, 28 (2): 209-230. -- ISSN 1310-7771 (print), 1314-0027 (online).

Introduction

The *Fabaceae* family includes about 751 genera and over 19500 species (Lewis & al. 2005; LPWG 2013). *Fabaceae* comprises three subfamilies, namely *Caesalpinoideae* DC., *Mimosoideae* DC. and *Faboideae* Rudd. (Papilionoideae); and the last subfamily splits into 10 tribes, including Loteae (Rendle 1952 and Willis 1967). According to Polhill (1981), *Papilionoideae* is subdivided into 31 tribes, including the *Loteae*.

The genus *Hippocrepis* L. belongs to the subtribe *Coronillinae*. It is composed of 21 species that form two very natural evolutionary groups: 10 woody species distributed mainly on the Iberian Peninsula and in North Africa, and 11 annual Mediterranean species, with two centers of species diversity - one on the Iberian Peninsula and North Morocco, and another in the desert areas of North Africa and Asia (Domínguez 1976).

Bornmüller (1929) made the first revision of the annual species of the genus *Hippocrepis*. Bellot (1943, 1947) published a review of the Spanish species. Tax-

DOI 10.7546/PhB.28.2.2022.6

onomy of the genus *Hippocrepis* was considered difficult (Ball 1968).

In Egypt, Täckholm (1974) and El-Hadidi & Fayed (1994/95) recorded five species of *Hippocrepis* L.*: H. areolate* Desv.*, H.constricta* Kunze*, H. cyclocarpa* Murb.*, H. multisiliquosa* L.*,* and *H. unisiliquosa* L**.** Boulos (1995, 1999, 2009) recorded five species and considered *H. unisiliquosa* subsp. *unisiliquosa* a synonym of *H. biflora* Spreng. Zareh (2005) studied some selected macromorphological characters of six *Hippocrepis* taxa from Egypt.

The genus *Securigera* DC. belongs to the subtribe *Coronillinae*, with 12 species native to the Old World (Tutin& al. 1968; Lassen 1989). Ball (1968) and Chamberlain (1970) treated *Securigera* as a monotypic genus. Meikle (1977) and Schmidt (1978, 1979) included *Securigera securidaca* in *Coronilla*. Polhill (1981) treated *Securigera* as a synonym of *Coronilla*. Lassen (1989) combined 11 species into *Securigera* from the earlier described genera *Coronilla* and *Artrolobium*, increas-

Table 1. Plant names, collection details and sources of seeds in the present study

Taxa	Source of seeds	Localities	Geographical coordinates	Date of collection
Hippocrepis areolata var.***glabra***	-	Egypt, Marsa Matrouh, 3 km westwards of Abu Lahu El-Bafri	26.430\ N 26° 50.381\ E	7/4/2016
	-	Egypt, Marsa Matrouh, Agiba beach	31° 24.858\ N 27° 00.339\ E, 15 m a.s.l.,	8/4/2016
	-	Egypt, Marsa Matrouh, Wadi Umm El-Rakham, Marsa Matrouh	31° 23.934\ N 27° 01.120\ E, 35 m a.s.l.	23/3/2017
	-	Egypt, Rosetta	Herbarial Sheet (CAI)	16/4/1988
Hippocrepis areolata var. ***sinuosissima***	-	Egypt, Marsa Matrouh, 2 km westwards of Agiba	31° 25.796\ N 26° 57.799\ E, 28 m a.s.l.	22/3/2019
	-	Egypt, Marsa Matrouh, Wadi Umm El-Rakham, Marsa Matrouh	31° 24.076\ N 27° 01.704\ E, 6 m a.s.l.	27/3/2014
	-	Egypt, 207 km eastwards of Marsa Matrouh	30° 49.693\ N 29° 21.204\ E, 20 m a.s.l.	6/4/2016.
Hippocrepis cyclocarpa	-	Egypt, Marsa Matrouh, El-Mathani El-Bahria	31° 27.999\ N 26° 45.033\ E, 15 m a.s.l.,	23/3/2018
	-	Egypt, Marsa Matrouh, Agiba beach	31° 24.848\ N 27° 00.321\ E, 17 m a.s.l.	22/3/2019
	-	Egypt, Marsa Matrouh, Wadi Umm El-Rakham	31° 23.974\ N 27° 01.137\ E, 6 m a.s.l.	7/4/2016
	-	Egypt, Marsa Matrouh, El-Kasr village	31° 21.902\ N 27° 06.957\ E, 8 m a.s.l.	27/3/2014
Securigera securidaca	Akz-Nr:SEC 1(IPK)	Iran		3,4/2014-2018

ing the number of species within the genus to 12.

In Egypt, only *Securigera securidaca* has been recorded (Täckholm 1974; El-Hadidi& Fayed 1994/95; Boulos 1995, 1999, 2009).

The present study is aimed to analyze and revising the macro- and micromorphological and the seed characters of the studied taxa in hope to find them reliable for differentiation between the taxa, and to evaluate the systematics of these characters, describing their distribution and habitat in Egypt.

Material and methods

The present study is based on available fresh material collected from natural habitats in Egypt. The vouchers are kept in the Menoufia University Herbarium and in the Cairo University Herbarium (with acronym CAI based on Theirs 2016). Seeds of *Securigera securidaca* have been obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (IPK) (Table 1). The seeds were cultivated in a greenhouse until the fruiting stage.

During field surveys, only two species of *Hippocrepis* have been recorded: *H. areolata* and *H. cyclocarpa*.

Samples for stem anatomy and mature leaves were taken from the fresh material. All plants were studied at similar developmental stages (fruiting stage) and in comparable positions for each plant. Fresh material was fixed in F.A.A. (5:5:90). After fixation, the stems and leaves were processed in ethyl alcohol series, and then embedded in paraffin wax. The stems and leaves were cut into sections of 10-15 μm; the sections were dehydrated in alcohol-xylol series. The sections were stained with Safranin and Light Green according to Sass (1961). The transverse sections were examined and photographed by a Zeiss research microscope. A planimeter was used for estimation of the width of each tissue in the section. Terminology follows Abd El-Rahman & al. (1976), Pandey (1982) and Abd El-Gawad & al. (1989).

A SEM study of the investigated seeds was carried out by mounting mature seeds on brass stubs and coating them with a thin layer of gold. JEOL JSM 530P SEM was used at the electron microscopic unit of the Faculty of Science, Alexandria University. Terminology follows Lersten (1981), Brochmann (1992), Stearn (1992), and Kirkbride & al. (2003).

For statistical analysis, the characters were binary encoded. They were encoded 0, 1 for the different character states; 60 characters included 157 character states recorded for each taxon (Appendix 1). The codes were analyzed with NTsys version 2.1 (Rohlf 2000) specialized in the numerical analysis data. A phenogram illustrating the relationship between the studied taxa was drawn by calculating the average taxonomic distance.

Results

Morphological studies *(Table 2, Fig. 1)*

Hippocrepis areolata Desv.var. ***glabra*** Pamp. in Bull. Soc. Bot. Ital.: 14 (1914). (Fig. 1a, Table 2)

Hippocrepis areolata var. ***sinuosissima*** Pomel. Nouv. Mat. Fl. Atl. 1: 195 (1874). (Fig. 1b, Table 2)

Hippocrepis cyclocarpa Murb., Acta Univ. Lund. 33 (12):30 (1897). (Fig. 1c, Table 2)

Securigera securidaca (L.) Degen & Dörfl.,Denkschr. Kaiserl.Akad.Wiss., Math.-Naturwiss. Kl. 64:718 (1897) (Fig. 1d, Table 2)

Anatomical studies (Tables 3, 4; Figs 2, 3)

Stem anatomy

Stem angular in all studied taxa. Epidermal cells isodiametric, radially elongated in *Hippocrepis areolata*, isodiametric cylindrical in *H. cyclocarpa* and radially elongated-polygonal in *Securigera securidaca*. Cortex consists of collenchyma and parenchyma in *H. areolata* var. *glabra* and *H. cyclocarpa;* collenchymas absent in *H. areolata* var. *sinuosissima* and *S. securidaca*; one-layer collenchyma in ridges, tangentially elongated, 4–5 layers parenchyma, isodiametric in *Hippocrepis areolata* var. *glabra*; 1–3 layers collenchyma in ridges, isodiametric, 4–6-gonal, 4–5 layers parenchyma, isodiametric, cylindrical in *H. cyclocarpa;* cortex consists of 6–7 parenchymatous layers, isodiametric, tangentially elongated in *S. securidaca*. Vascular cylinder of 12–13 bundles, with 3–8 sclerenchymatous layers, 3–4 layers phloem, 2-layers

Table 2. Morphological characters of the studied taxa

Character			*Hippocrepis*			*Securigera securidaca*
			areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Plant	Habit		Herb	Herb	Herb	Herb
			Annual	Annual	Annual	Annual
			Prostrate-decumbent	Prostrate-decumbent	Prostrate	Ascending
	Height (cm)		25-43	8-20	10-34	25-38
	Colour		Green - reddish green	Green - reddish green	Green - reddish green	Reddish green
Stem	Shape		Ribbed	Ribbed	Ribbed	Ribbed
	Surface		Few papillae	Few papillae	Few papillae	Few papillae
	Colour		Green - reddish green	Green - reddish green	Reddish brown	Reddish green
	Branched		At base	At base	At base	At base
	Internodes length (cm)		2.5-3.5	1.2-2	3-4	1.5-2.5
Stipule	Shape		Ovate-elliptic	Ovate-elliptic	Triangular	Ovate
	Margin		Entire, few papillae	Entire, few papillae	Entire	Entire
	Apex		Acute	Acute	Acute	Round
	Length (mm)		1.5-2	2.5-3	2-2.5	2-2.5
	Colour		Reddish green with a black spot at the margin base	Reddish green with a black spot at the margin base	Reddish brown with a black spot at the margin base	Greenish white
Leaf	Length (mm)		16-42	30-45	60-76	78-96
	Type	First	Trifoliate	Trifoliate	Trifoliate	5
		Lower / Upper	Imparipinnate	Imparipinnate	Imparipinnate	Imparipinnate
	Arrangement		Alternate	Alternate	Alternate	Alternate
	Colour		Green	Green	Green	Green - reddish
Leaf rachis	Length (mm)		6-21	13-18	32-38	32-36
	Surface		Few papillae	Few papillae	Few papillae	Few papillae

Character		*Hippocrepis*			*Securigera securidaca*
		areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Leaflet	**Number**	9-13	9-11	9-11	15-19
	Petiolule	0.5-0.8 mm	0.5 mm	0.5 mm	0.5-0.8 mm
	Insertion on stem	Opposite - alternate	Opposite - alternate	Opposite	Opposite
	Petiole surface	Few papillae	Few papillae	Few papillae	Glabrous
Limb in leaflet	**Shape**	Linear - oblong	Linear - oblong	Obovate - obcordate	Obovate-obtriangular-oblong-elliptic
	Size L × W (mm)	Terminal 9-14 × 1.5-2 Lateral 5-8 × 1-2	Terminal 11-13 × 2-2.5 Lateral 5-10 × 2-2.5	Terminal 10.5-13.5 × 6-7.5 Lateral 8-11.5 × 4-7	Terminal 9-10 × 4.5-5 Lateral 9-15 × 5-8
	Apex	Emarginate-truncate	Emarginate- truncate	Emarginate	Truncate-apiculate
	Margin	Entire	Entire	Entire	Entire
	Surface	Few papillae on midrib	Few papillae on midrib and margin	Few papillae on midrib	Glabrous
Leaf petiole	**Length (mm)**	6-10	8-18	19-28	33-52
	Surface	Few papillae	Few papillae	Few papillae	Few papillae
Peduncle	**Length (cm)**	1.5-8.4	1.1-3.2	3.4-4.3	1.8-2
	Surface	Few papillae	Few papillae	Few papillae	Few papillae
Inflorescence	**Type**	Umbellate	Umbellate	Umbellate	Umbellate
Number of flowers	**Number**	3-5	3-4	(2) 3-4	5-8
Bract	**Type**	Scarious	Scarious	Scarious	Scarious
	Shape	Triangular	Triangular	Triangular	Ovate-oblong
	Length (mm)	0.5	0.5	0.5	1
	Colour	White	White	White	White
	Apex	Acute	Acute	Acute	Acute
	Margin	Entire	Entire	Entire	Entire
	Surface	Glabrous	Glabrous	Papillate	Glabrous
Flower	**Length (mm)**	8-10	10-10.5	6-6.5	8.5-12
Pedicel	**Width (mm)**	1-1.5	1-1.5	0.5	1-1.5
	Surface	Glabrous	Glabrous	Few papillae	Glabrous

Character		*Hippocrepis*			*Securigera securidaca*
		areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Calyx	**Colour**	Green	Green with reddish blotches	Green with red smears	Green with red smears
	Shape	Campanulate	Campanulate	Campanulate	Campanulate
	Tube length (mm)	2-3	2.5	1.5-2	1-1.5
	Tube surface	Glabrous	Glabrous	Glabrous	Few papillae
	Teeth shape	Triangular	Triangular	Triangular	Triangular-lanceolate
	Teeth length (mm) **2 upper** **2 lateral** **1 lower**	 2 1.5 1.5	 1 1 1.5	 2 2 2	 1-1.5 1-1.5 1-1.5
	Teeth apex	Acute	Acute	Acute	Acuminate
	Teeth margin	Entire red	Entire	Entire, few papillae	Entire
	Teeth surface	Glabrous	Glabrous	Glabrous	Glabrous
Standard	**Shape**	Widely ovate	Widely ovate	Oblate	Elliptic
	Colour	Yellow	Yellow	Pale yellow	Yellow
	Lamina L × W (mm)	8-10 × 6-7	6-6.5 × 7-7.5	4-5.5 × 3.5-4	7-10 × 4-6
	Claw length (mm)	3-4	4	2-2.5	1.5-2
	Vein colour	Brown	Brown	Pale brown	Red
	Vein length (mm)	7.5-9.5	5.5	4.5-5	3.5-5
	Margin	**Entire**	**Entire**	**Entire**	**Entire**
	Apex	**Acute**	**Acute**	**Acute**	**Retuse**
	Surface	**Glabrous**	**Glabrous**	**Glabrous**	**Glabrous**
Wings	**Shape**	Oblong	Oblong	Oblong	Oblong-obovate
	Colour	Yellow	Yellow	Pale yellow	Yellow
	Lamina L × W (mm)	8-10 × 4-6	6.5-7 × 3-3.5	3.5-5 × 1.5-2	7.5-9.5 × 2.5-5
	Claw length (mm)	2-4	3	2	1.5-2
	Apex	Round	Round	Round	Round
	Margin	Entire	Entire	Entire	Entire
	Surface	Glabrous	Glabrous	Glabrous	Glabrous
	Auricule length (mm)	Absent	Absent	Absent	0.8-1
	Auricule apex	-	-	-	Obtuse

Character		*Hippocrepis*			*Securigera securidaca*
		areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Keel	**Colour**	Yellow	Greenish yellow	Whitish yellow	Greenish yellow
	Lamina L × W (mm)	8-9 × 3	5-5.5 × 2.5-3	3.5-5.5 × 2-3	7-9 × 2.5-3
	Claw length (mm)	2-3	3	2	1.5-2
	Apex	Acute, erect curved beak	Acute, erect curved beak	Acute, slightly curved beak	Acute, straight beak
	Surface	Glabrous	Glabrous	Glabrous	Glabrous
	Beak colour	Yellow	Yellow	Yellow	Yellow
Androecium	**Type**	Diadelphous	Diadelphous	Diadelphous	Diadelphous
Stamens	**Free length (mm)**	6-8	7	4-5	7-8
	Filaments united (free parts length mm)	5 = 4-4.5 4 = 2-3	5 = 3 4 = 2	5 = 1.5-2 4 = 1	2-3
Stamens	**Filaments united (united parts length mm)**	5-6.5	6	3.5-4	5-6
	Anthers	Uniform	Uniform	Uniform	Uniform
	Shape	Flattened, straight	Flattened, straight	Flattened, straight	Flattened, straight
	Size L × W	5-6 × 0.5	5 × 0.5	4-4.5 × 0.5	4-8 × 1-1.5
	Surface	Glabrous	Glabrous	Glabrous	Glabrous
	Colour	Green	Green	Green	Green
Style	**Length (mm)**	4-6	4.5-5	2-2.2	3-4
Stigma	**Shape**	Capitate	Capitate	Capitate	Capitate
Pod	**Shape**	Coiled circinate into 2 rings	Coiled circinate into 2 rings	Two circular turns	Lanceo-late-straight, hooked at top
	Size L × W (mm)	7.2-10.1 × 7-9 (horn 6-8 mm)	6.3-8.3 × 6-7 (horn 5-6 mm)	4.8-6.4 × 4-5 (horn 2.5-3 mm)	57-76 × 4-9
	Colour	Pale brown-brown	Pale brown-brown	Pale brown-brown	Pale brown-brown
	Surface	Reticulate-glabrous	Papillate	Reticulate-glabrous	Glabrous
	Margin	Of sinuses extended into long narrow horns	Of sinuses extended into long narrow horns	Of elevated seed chamber, not reaching the pod margin at the base	Sutures incrassate, the upper caniculate
	Beak length (mm)	4.5-5	4.8-5	2-2.2	16-23
Number of seed		7-10	7-10	9-12	4-8

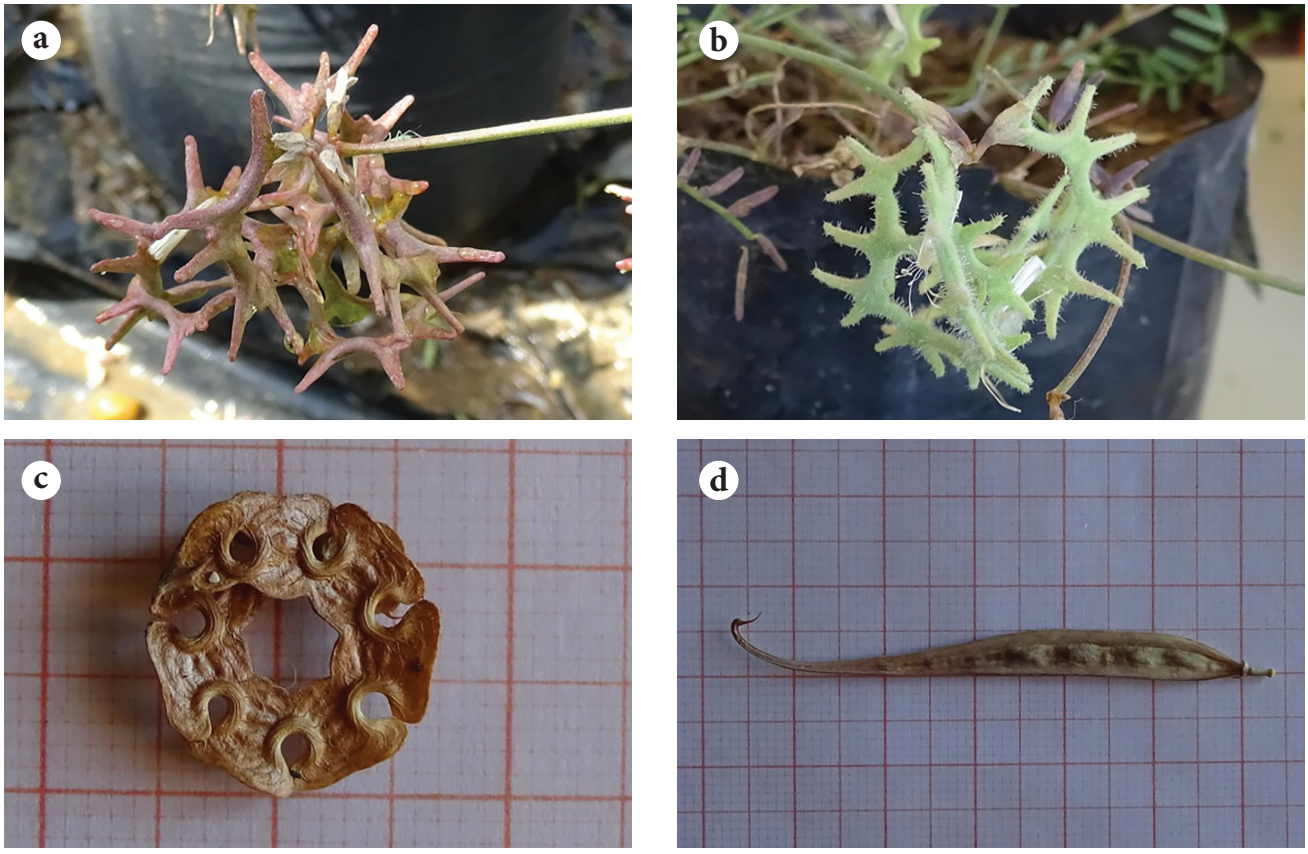

Fig. 1. Pod of *Hippocrepis areolata*: a. var. *glabra*, b. var. *sinuosissima*, c. *Hippocrepis cyclocarpa*, d. *Securigera securidaca*.

cambium, xylem with 1–6 arches, 2–7 vessels per arch in the studied species. Pith cells isodiametric, irregular, 10–14 tannin cells in *Hippocrepis areolata* var. *glabra*, 4–9 tannin cells in *H. areolata* var. *sinuosissima*; isodiametric, tangentially-radially elongated, 10–12 tannin cells in *H. cyclocarpa*, and isodiametric, radially elongated-polygonal, with 30–34 tannin cells in *S. securidaca*.

Leaf anatomy (Table 4, Fig. 3)

The leaf in midrib section is v-shaped in *Hippocrepis areolata* and *H. cyclocarpa,* and u-shaped in *Securigera securidaca*. Upper and lower epidermis uniseriate, isodiametric, tangentially-radially elongated in *H. areolata*, uniseriate, isodiametric, cylindrical in *H. cyclocarpa,* and uniseriate, isodiametric, tangentially-radially elongated in *S. securidaca*. In midrib section, 3-layers parenchyma, isodiametric, tangentially elongated in *Hippocrepis areolata*, 4-layers, isodiametric, in *H. cyclocarpa,* and 2-layers, polygonal in *Securigera securidaca*. Xylem with 2–4 arches, 1–4 vessels per arch, 2–4 layers phloem in the studied species. Phloem fibers at midrib below the main vascular bundle, one-layer in *Hippocrepis areolata* and *H. cyclocarpa*, two-layers in *Securigera securidaca*. Below the main vascular bundles 2–3 layers of parenchyma cells in *Hippocrepis areolata* and *Securigera securidaca*, four layers in *H. cyclocarpa*. Mesophyll tissue consists of palisade and spongy tissues; palisade tissue of 1–2 rows, spongy tissue of 2–5 rows in-between in *Hippocrepis areolata* and *Securiger asecuridaca*. Mesophyll tissue consists of 6–7 layers in *Hippocrepis cyclocarpa*. In *Securigera securidaca*, 10-14 tannin cells are present, but they are absent in the other species; crystals absent in all studied species.

Hippocrepis areolata var. *sinuosissima* has the same anatomical characters as *H. areolata* var. *glabra,* except for in the midrib section; parenchyma 100–112.5 µm thick, xylem arch 17.5–27.5 µm long. Below the main vascular bundles, parenchyma 55–62.5 µm thick, palisade tissue 75–87.5 µm thick, spongy tissue 112.5–150 µm thick.

Table 3. Anatomical characters of stem in the studied taxa of subtribe *Coronillinae*

Stem character			*Hippocrepis*			*Securigera securidaca*
			areolata var. *glabra*	*areolata* var. *sinuosissima*	*cyclocarpa*	
Outline			Angular	Angular	Angular	Angular
Number of ridges			6	6	6	6
Diameter (μm)			1025–1050	900-920	1100-1200	2250-2375
Cuticle thickness (μm)			2.5	2.5	2.5	2.5
Epidermal cell	**Shape**		Isodiametric, radially elongated	Isodiametric, tangentially elongated	Isodiametric, cylindrical	Radially elongated-polygonal
	Width (μm)		12.5-15	15-22.5	12.5-22.5	15-25
Cortex	**Collenchyma layers**		1 in corners	Absent	1-3 in corners	Absent
	Collenchyma	**Width (μm)**	12.5-15	Absent	15-50	Absent
		Cell shape	Tangentially elongated	Absent	Isodiametric, 4-6 gonal	Absent
	Parenchyma layers		4-5	4-5	4-5	6-7
	Parenchyma	**Width (μm)**	37.5-100	50-100	37.5-62.5	87.5-107.5
		Cell shape	Isodiametric	Isodiametric, tangentially elongated	Isodiametric, cylindrical	Isodiametric, tangentially elongated
Vascular bundles	**Number**		12-13	10-12	12	12
	Width (μm)		137.5-250	125-175	132.5-175	220-450
Pericyclic fiber	**Number of layers**		4-5	4-5	3-5	4-8
	Width (μm)		15-55	37.5-42.5	25-57.5	75-125
Phloem	**Number of layers**		3-4	3	3-4	3-4
	Width (μm)		17.5-37.5	15-20	15-32.5	30-37.5
Cambium	**Number of layers**		2	1	2	2
	Width (μm)		10-12.5	2.5	7.5-10	10-12.5
Xylem	**Number of arches**		1-4	1-4	1-5	3-6
	Number of vessels		2-5	3-5	3-7	2-6
	Width (μm)		60-142.5	55-80	105-150	95-175
Pith	**Cell shape**		Isodiametric, irregular	Isodiametric	Isodiametric, tangentially-radially elongated	Isodiametric, radially elongated-polygonal
	Diameter (μm)		490-510	450-470	520-550	1250-1300
Crystals	**In cortex**		Absent	Absent	Absent	Absent
	In pith		Absent	Absent	Absent	Absent
Tannin cells	**In cortex**		Absent	Absent	Absent	Absent
	In pith		10-14	4-9	10-12	30-34

Table 4. Anatomical characters of leaf in the studied taxa of subtribe *Coronillinae*

Character			*Hippocrepis*			*Securigera securidaca*
			areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Leaf midrib	**Shape**		V	V	V	U
	Midrib thickness (µm)		290-300	270-280	300-310	350-370
	Cuticle thickness (µm)		2.5	2.5	2.5	2.5
	Epidermal cells	**Thickness (µm)**	15-17.5 up 15-17.5 lo	22.5-25 up 15-17.5 lo	20-25 up 15-20 lo	20-25 up 12.5-15 lo
		Shape	Isodiametric, tangentially-radially elongated	Isodiametric, tangentially-radially elongated	Isodiametric, tangential-ly-radially elongated	Isodiametric, tangential-ly-radially elongated
	Xylem	**Number of arches**	2-3	3-4	2-3	3-4
		Thickness (µm)	30-32.5	17.5-27.5	37.5-40	42.5-45
		Vessels in arch	2-4	2-4	2-3	1-2
	Phloem	**Number of layers**	2	2	3	3-4
		Thickness (µm)	12.5-15	12.5-15	15-17.5	25
	Parenchyma	**Number of layers**	3 up.ep 2-3 lo. ep.	3-4 up.ep 3 lo. ep.	4 up.ep 4 lo. ep.	2 up.ep 3 lo. ep.
		Thickness (µm)	112.5-125 up.ep 37.5-50 lo. ep.	100-112.5 up.ep 55-62.5 lo. ep.	112.5-117.5 up.ep 55-60 lo. ep.	70-87.5 up.ep 57.5-62.5 lo. ep.
		Cell shape	Isodiametric, tangentially elongated	Isodiametric, tangentially elongated	Isodiametric	Polygonal
	Crystals		Absent	Absent	Absent	Absent
	Fiber number of layers		1	1	1	2
	Fiber thickness (µm)		7.5	7.5	5	12.5-17.5
	Tannin cells	**Upper vascular bundle**	Absent	Absent	Absent	Absent
		Lower vascular bundle	Absent	Absent	Absent	1-3

Legend: up. ep. - upper epidermis; lo.ep. - lower epidermis

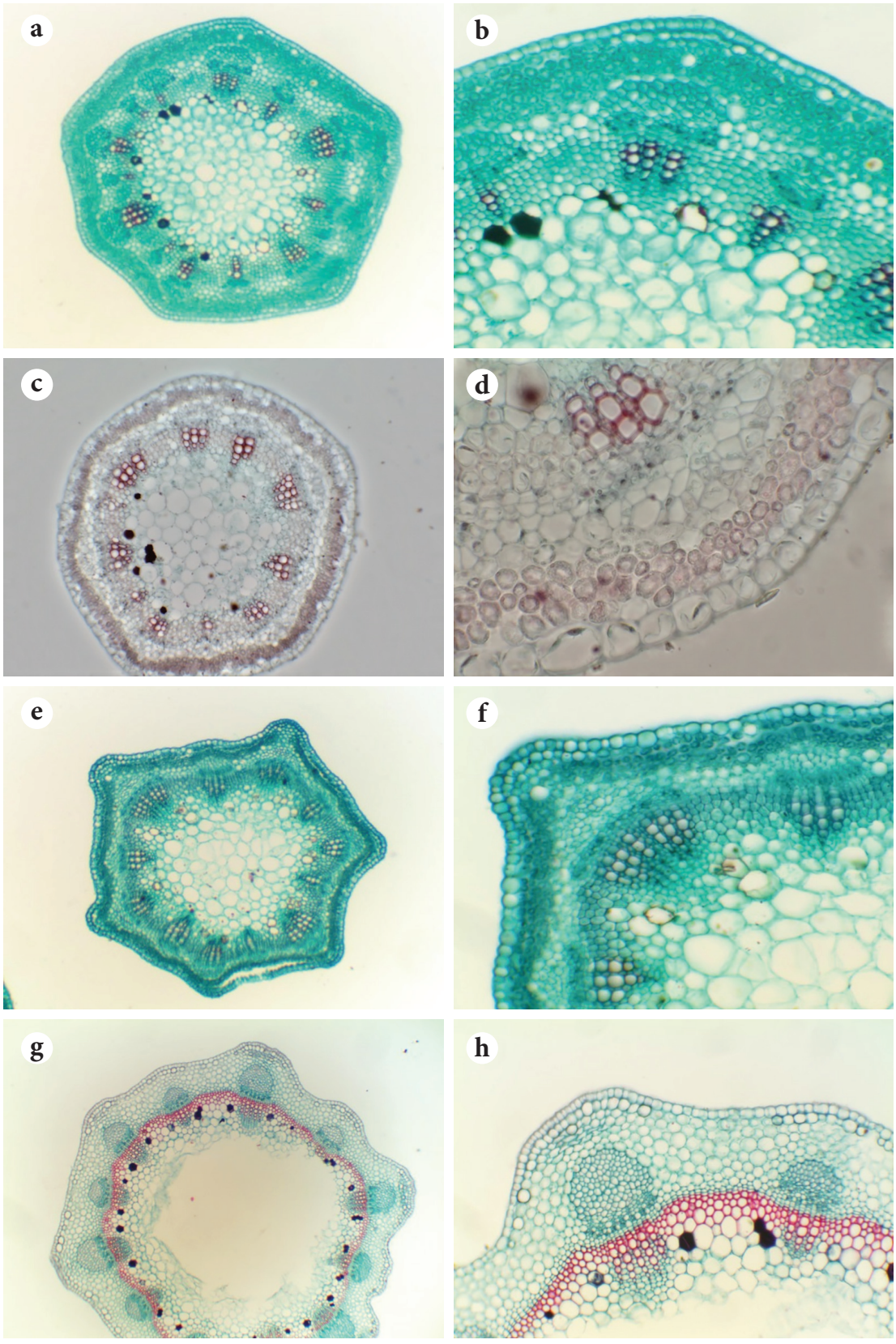

Fig. 2. Stem anatomy of the studied taxa: a,b. – *Hippocrepis areolata* var. *glabra*; c,d. - *H. areolata* var. *sinuosissima*; e,f. - *H. cyclocarpa*; g,h. – *Securigera securidaca*. (a,c,e,g ×50; b,d,f,h ×100).

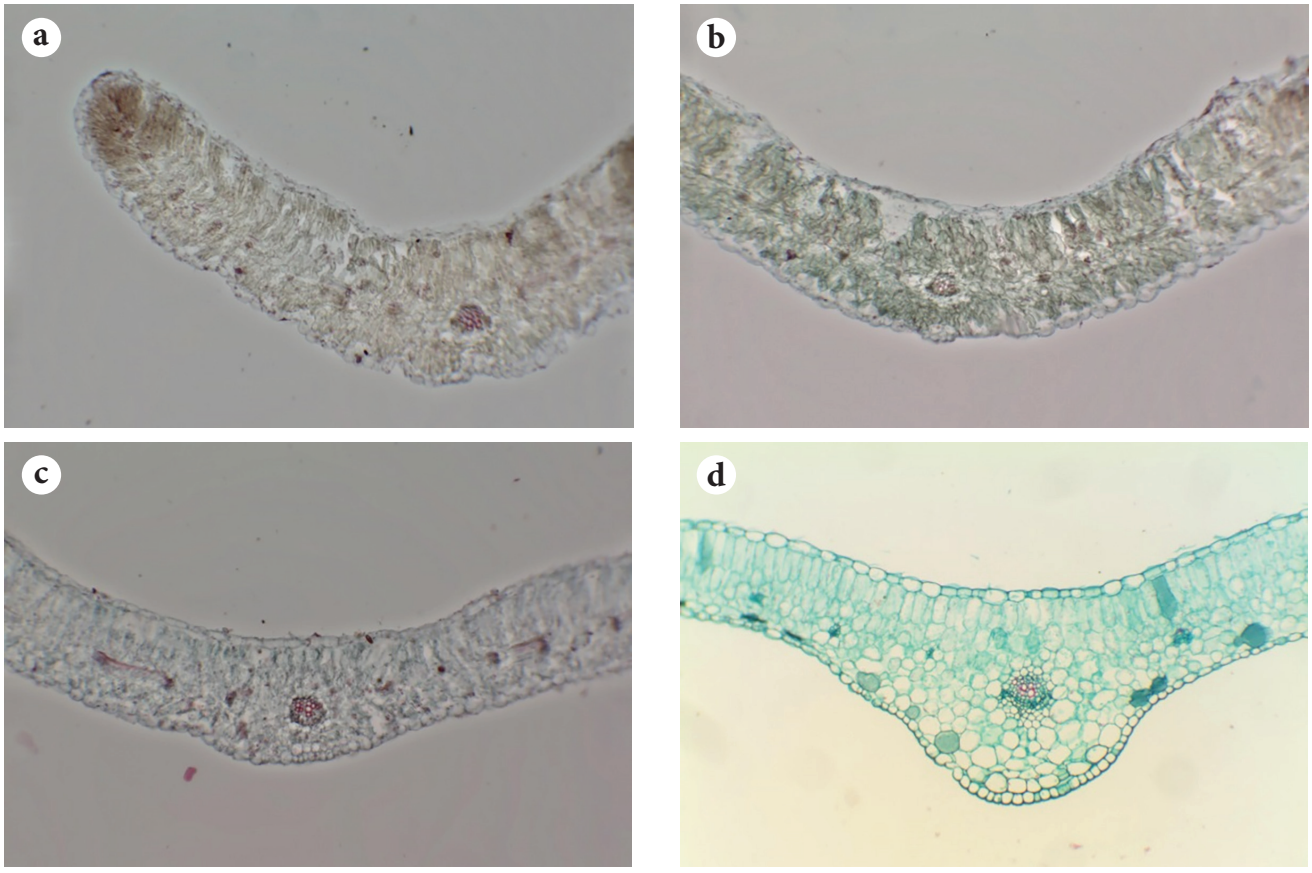

Fig. 3. Leaf anatomy of the studied taxa: a. *Hippocrepis areolata* var. *glabra*; b. *H. areolata* var. *sinuosissima*; c. *H. cyclocarpa*; d. *Securigera securidaca* (×50).

SEM of the spermoderm (Table 5, Figs 4-6)

SEM of the epidermal cells clarifies the texture and reticulation of their anticlinal (radial) walls, appearance of the outer periclinal walls and persistency of the primary cell walls.

Seed crescent-shaped, with rounded poles in *Hippocrepis areolata*, horseshoe-shaped with rounded poles in *H. cyclocarpa,* and oblong with rounded-truncate poles in *Securigera securidaca*. Dark brown in colour in *Hippocrepis areolata* var. *glabra*, brown in *H. areolata* var. *sinuosissima*, pale yellow-yellowish in *H. cyclocarpa,* and reddish-brown in *Securiger asecuridaca*. It is covered with wax in all studied species. Hilum elliptic, blackish, lateral in *Hippocrepis areolata*; elliptic, lateral in *H. cyclocarpa;* and elliptic, blackish, lateral in *S. securidaca*. Rim aril flush in the four studied taxa. Micropyle obtriangular in *Hippocrepis areolata* and *H. cyclocarpa*, and widely ovate in *S. securidaca*. Seed coat pattern with different in size micro papillae in *Hippocrepis areolata* var. *glabra*, reticulate with papillae in-between in *H. areolata* var. *sinuosissima*, micro papillate in *H. cyclocarpa,* and reticulate-foveolate in *S. securidaca*. Outline of cells isodiametric, tangentially elongated in *H. areolata* var. *glabra*, reticulate in *H. areolata* var. *sinuosissima*, isodiametric, polygonal in *H. cyclocarpa,* and polygonal in *S. securidaca*. Anticlinal wall straight in *Hippocrepis areolata* var. *glabra* and *H. cyclocarpa,* and wavy in *H. areolata* var. *sinuosissima* and *Securigera securidaca*. Relief of cell boundary channeled, thickness of cell boundary in *Hippocrepis areolata* var. *glabra* and *H. cyclocarpa* and upraised, cell boundary thick in *H. areolata* var. *sinuosissima* and *Securigera securidaca*. Curvature of outer periclinal wall concave in *Hippocrepis areolata* var. *glabra*, flat in *H. cyclocarpa*, convex in *H. areolata* var. *sinuosissima* and *Securigera securidaca*.

Table 5. Morphological aspect of the spermoderm in the studied taxa of subtribe *Coronillinae*

Character		*Hippocrepis*			*Securigera securidaca*
		areolata* var. *glabra	***areolata* var. *sinuosissima***	***cyclocarpa***	
Seed colour		Dark brown, hilum blackish	Brown, hilum blackish	Pale yellow-yellowish	Reddish brown, hilum blackish
Seed size	**L×W mm**	4.5-6 × 0.5-0.8	4.5-5.5 × 0.5-0.8	4.5-5.5 × 0.5-0.8	4-5 × 2.5-3
	L/W ratio	8.1	7.7	7.7	1.6
Seed pole		Rounded	Rounded	Rounded	Rounded- truncate
Seed shape		Crescent	Crescent	Horseshoe	Oblong
Seed coat pattern		Micro papillate different size	Reticulate with papillae in- between	Micro papillate	Reticulate-foveolate
Hilum	**Position**	Lateral	Lateral	Lateral	Lateral
	Shape	Elliptic	Elliptic	Elliptic	Elliptic
	L×W µm	69.35 × 29.03	59.67 × 37.09	59.67 × 45.16	147.72 × 131.81
Rim aril		Flush	Raised	Raised	Raised
Micropyle	**Shape**	Obtriangular	Obtriangular	Obtriangular	Widely ovate
	L×W µm	8.06 × 6.45	4.83 × 4.83	8.06 × 9.67	13.63 × 13.63
Outline of cells		Isodiametric, tangentially elongated	Reticulate	Isodiametric, polygonal	Polygonal
Anticlinal wall		Straight	Wavy	Straight	Wavy
Relief of cell boundary		Channeled	Upraised	Channeled	Upaised
Thickness of cell boundary		Moderately	Thick	Moderately	Thick
Curvature of outer periclinal wall		Concave	Convex	Flat	Convex
Wax		Present	Present	Present	Present

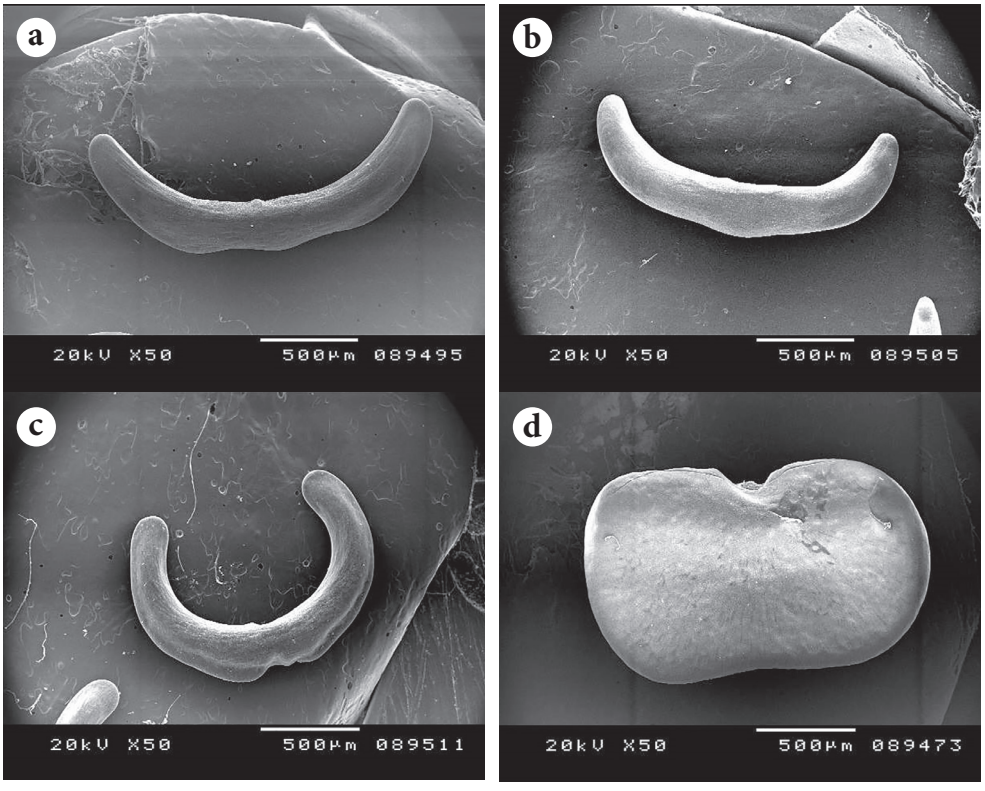

Fig. 4. SEM of seed morphology of the studied taxa: a. *Hippocrepis areolata* var. *glabra*; b. *H. areolata* var. *sinuosissima*; c. *H. cyclocarpa*; d. *Securigera securidaca*.

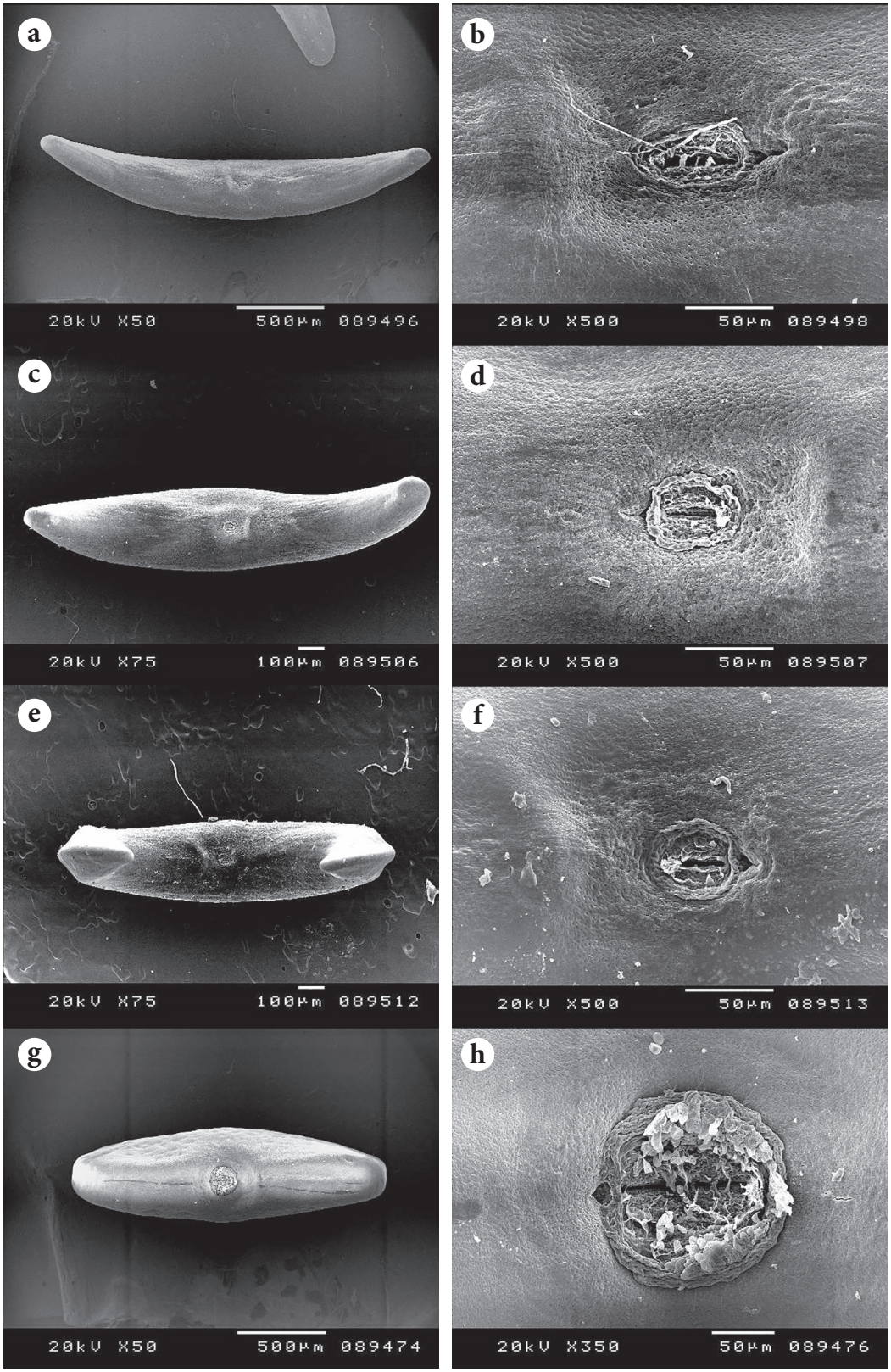

Fig. 5. SEM of hilum position and shape of the studied taxa: a,b. *Hippocrepis areolata* var. *glabra*; c,d. *H. areolata* var. *sinuosissima*; e,f. *H. cyclocarpa*; g,h. *Securigera securidaca*.

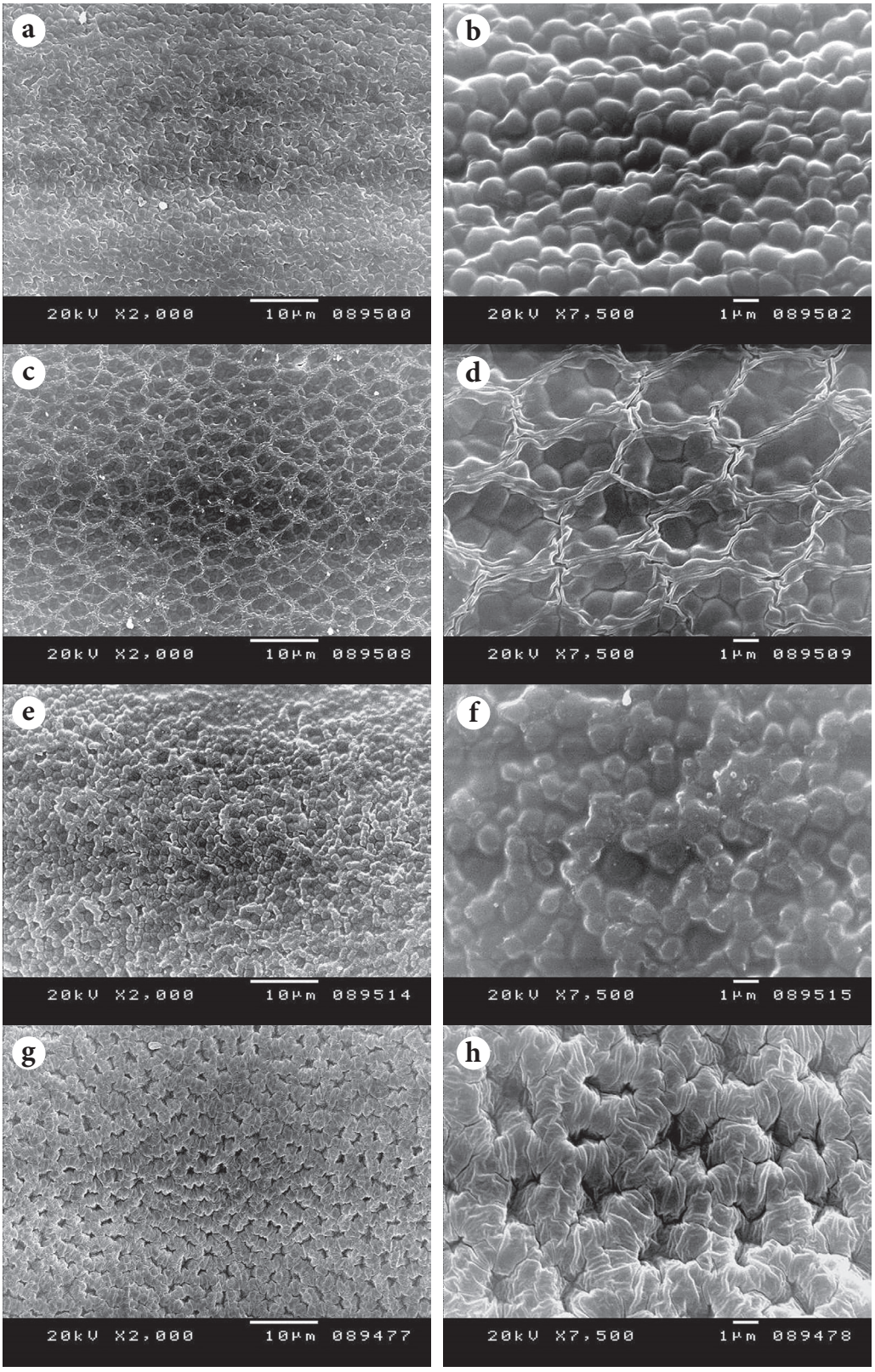

Fig. 6. SEM of spermoderm surface of the studied taxa: a,b. *Hippocrepis areolata* var. *glabra*; c,d. *H. areolata* var. *sinuosissima*; e,f. *H. cyclocarpa*; g,h. *Securigera securidaca*.

Numerical analysis

The dendrogram produced by using distance measure (Fig. 7) showed that, on the basis of all studied characters, subtribe *Coronillinae* was split off at distance level of 1.56 into two series: the first represented by *Securigera securidaca* with characteristically ascending stem, leaf with 15–19 leaflets, cortex with 6–7 parenchymatous layers, u-shaped leaf in the midrib section, tannin cells present in midrib and wings, seed oblong, reddish-brown in colour, micropyle widely ovate.

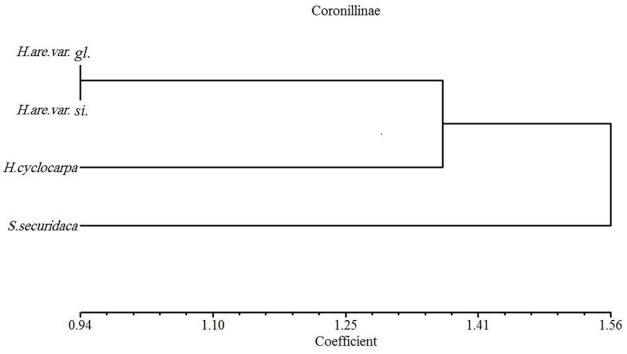

Fig. 7. A dendrogram showing characters-based clustering of the studied subtribe Coronillinae.

The second series was represented by the *Hippocrepis* sp. with characteristically prostrate stem, leaf with 8–13 leaflets, cortex with 4–5 parenchymatous layers, u-shaped leaf in midrib section, tannin cells absent in midrib and wings, seed crescent or horseshoe-shaped, yellow or brown in colour, micropyle obtriangular. The second series splits at distance level of 1.35 into two clusters. The first cluster is represented by *Hippocrepis cyclocarpa* characterized by a papillate bract, flowers 6–6.5 mm long, pods with two circular turns, margin of the elevated seed chamber not reaching the pod margin at the base, vascular bundles with 3-4 sclerenchymatous layers in stem, wings consist of mesophyll tissue, seed horseshoe-shaped. The second cluster is represented by the *Hippocrepis areolata* varieties and characterized by a glabrous bract, flowers 8–10 mm long, pods circinate, coiled with two rings, margins of sinuses extended into projecting long narrow horns, vascular bundles with 4–6 sclerenchymatous layers in stem, wings consist of palisade and spongy tissues, seed crescent-shaped. This cluster splits off at a distance level of 0.94 into two subclusters: the first is represented by *H. areolate* var. *sinuossima* and has characteristically papillate pod, upraised rim aril, and reticulate seed coat pattern, with papillae in-between. The second cluster is represented by *H. areolata* var. *glabra* and has a characteristically glabrous pod, flush rim aril, seed coat pattern micro papillate, with different in size papillae.

Discussion

Sokoloff (2003) has revealed the need for a broad-scale treatment of the genera *Coronilla* and *Hippocrepis*. The genus *Coronilla* is subdivided into the subgenera *Securigera*, *Ballia* and *Coronilla*. Subgenera *Emerus* and *Hippocrepis* are accepted within the genus *Hippocrepis*.

Täckholm (1974) and Ball (1981) considered the fruit characters major for identification. Macroscopic characters are useful for quick identification of the plant material and may serve as an essential criterion (Aguoru & Okoli 2012; Geetha & al. 2016).

The present study of the morphological characteristics (habit, leaves, inflorescence, flowers, pods, and seeds) has shown significant differences among the studied species. Shape and colour of standard are highly different in the studied taxa. Reticulate-glabrous pod surface as in *H. areolata* var. *glabra* and *H. cyclocarpa,* papillate as in *H. areolata* var. *sinuosissima* and glabrous as in *Securigera securidaca* have helped identify the species.

Furthermore, anatomical characteristics such as cortex, number of parenchyma layers, number of vascular bundles and tannin cells, have helped to identify the species. Comparison of the internal structure of leaflets has revealed differences in shape and thickness of midrib and xylem, lamina thickness, crystals and tannin cells. Turki (2007) and Kasem (2016) have reported the importance of anatomical differences in the distinction of species.

Yetisen & Özdemir (2017) have studied the morphological and anatomical features of three taxa of the *Hippocrepis* L. species and the results have shown that these characters can help differentiate the taxa. Leaflet characteristics in this study have confirmed

the taxonomic treatment of the genera *Securigera* and *Hippocrepis* in subtribe *Coronillinae*.

Gandhi & al. (2011) have studied 17 legume species belonging to three genera of *Faboideae* and the results revealed that the seed coat ornamentation/spermoderm pattern could be helpful in the identification of species. SEM spermoderm investigations indicate differences between the studied *Scorpiurus* sp. (Aqlan & *al. 2018).* Seed shape, hilum position and micropyle characteristics can be used as further tools in separating the genera *Securigera* and *Hippocrepis*. Anticlinal undulations and characteristics of cell boundaries in the seed exine are of high taxonomic significance and often help characterize the species and genus level (Barthlott &Voit 1979 and Barthlott 1981). Kaplan & al. (2007) and Fawzi & al. (2010) have reported that seed coat characters are successfully employed in the identification and classification of taxa.

In conclusion, the morphological, anatomical and seed traits investigated in this study proved useful for genus differentiation and species identification. *Hippocrepis areolata* Desv. is differentiated into two new to Egypt (new record) varieties: *glabra* Pamp. and *sinuosissima* Pomel.

Artificial key to the studied genera in subtribe *Coronillinae*

1a-Pod linear, straight, flattened, with hooked beak; u-shaped leaf in midrib section; seed oblong, micropyle widely ovate ……***Securigera securidaca***

1b-Pod loment, with horseshoe-shaped joint segments; v-shaped leaf in midrib section; seed crescent or horseshoe-shaped, micropyle obtriangular ……... ***Hippocrepis***

Artificial key to the studied *Hippocrepis* species

1a-Flowers up to 6.5 mm long, pods with two circular turns - in seed; margin of the elevated seed chamber not reaching the pod margin at the base - in leaf; wings with undifferentiated mesophyll tissue ……..……..***H. cyclocarpa***

1b-Flowers 8-10 mm long, pods circinate, coiled into two rings - in seed; margins of sinuses extended into projecting long narrow horns - in leaf; wings with palisade and spongy tissues ……..…….***H. areolata***

Artificial key to the studied *Hippocrepis areolata* varieties

1a-Pod glabrous, collenchyma present in cortex, 10-14 tannin cells in pith; seed coat pattern micro papillate, rim aril flush, anticlinal wall straight……..……**var. *glabra***

1b-Pod papillate, collenchyma absent in cortex, up to nine tannin cells in pith; seed coat pattern reticulate, with papillae in-between, rim aril upraised, anticlinal wall wavy ……**var. *sinuosissima***

Appendix 1. Data matrix of (0, 1) codes of different character states used in the statistical analysis of subtribe *Coronillinae* species (1) *Hippocrepis areolata* var. *glabra*. (2) *H. areolata* var. *sinuossima*. (3) *H. cyclocarpa*. (4) *Securigera securidaca*.

Characters		Species			
		1	2	3	4
Plant habit	Decumbent	1	1	0	0
	Prostrate	1	1	1	0
	Ascending	0	0	0	1
Plant colour	Green	1	1	1	0
	Reddish-green	1	1	1	1
Internode length	1-2.4 cm	0	1	0	1
	2.5-6 cm	1	0	1	0
Stipule	Ovate	1	1	0	1
	Elliptic	1	1	0	0
	Triangular	0	0	1	0

Characters		Species			
Stipule margin	Entire	1	1	1	1
	Papillate	1	1	0	0
Leaf type	9-13-foliate	1	1	1	0
	15-19-foliate	0	0	0	1
Leaf rachis length	6-25 mm	1	1	0	0
	26-40 mm	0	0	1	1
Leaflet shape	Linear	1	1	0	0
	Oblong	1	1	0	1
	Elliptic	0	0	0	1
	Obovate	0	0	1	1
	Obcordate	0	0	1	0
	Obtriangular	0	0	0	1
Leaflet apex	Truncate	1	1	0	1
	Emarginate	1	1	1	0
	Apiculate	0	0	0	1
Leaflet hairs	Glabrous	0	0	0	1
	Papillate	1	1	1	0
Number of bract	2-5	1	1	1	0
	5-8	0	0	0	1
Bract shape	Triangular	1	1	1	0
	Ovate	0	0	0	1
	Oblong	0	0	0	1
Bract surface	Glabrous	1	1	0	1
	Papillate	0	0	1	0
Flower length (mm)	5-7	0	0	1	0
	8-12	1	1	0	1
Pedicel surface	Glabrous	1	1	0	1
	Papillate	0	0	1	0
Tube length (mm)	1-1.5	0	0	1	1
	2-3	1	1	0	0
Tube surface	Glabrous	1	1	1	0
	Papillate	0	0	0	1
Standard shape	Oblate	0	0	1	0
	Widely ovate	1	1	0	0
	Elliptic	0	0	0	1
Standard colour	Yellow	1	1	0	1
	Pale yellow	0	0	1	0
Standard vein colour	Brown	1	1	0	0
	Pale brown	0	0	1	0
	Red	0	0	0	1
Standard apex	Acute	1	1	1	0
	Retuse	0	0	0	1
Wings shape	Oblong	1	1	1	1
	Obovate	0	0	0	1
Wings colour	Yellow	1	1	0	1
	Pale yellow	0	0	1	0
Auricule	Present	0	0	0	1
	Absent	1	1	1	0

Characters		Species			
Keel colour	Yellow	1	0	0	0
	Greenish-yellow	0	1	0	1
	Whitish-yellow	0	0	1	0
Keel beak	Straight	0	0	0	1
	Erect curved	1	1	0	0
	Slightly curved	0	0	1	0
Stamens free length (mm)	3-5	0	0	1	0
	6-8	1	1	0	1
Free filaments in stamens united	Equal	0	0	0	1
	Unequal	1	1	1	0
Stamens united length (mm)	5-8	0	0	1	1
	9-13	1	1	0	0
Style length (mm)	2-2.5	0	0	1	0
	3-5.5	1	1	0	1
Pod shape	Coiled	1	1	0	0
	Circular turns	0	0	1	0
	Lanceolate-straight	0	0	0	1
Pod surface	Reticulate	1	0	1	0
	Glabrous	1	0	1	1
	Papillate	0	1	0	0
Stem diameter (μm)	850-920	0	1	0	0
	1000-1200	1	0	1	0
	2075-2375	0	0	0	1
Epidermal cell shape in stem	Radially elongated	1	0	0	1
	Isodiametric	1	1	1	0
	Tangentially elongated	0	1	0	0
	Cylindrical	0	0	1	0
	Polygonal	0	0	0	1
Collenchyma in cortex	Absent	0	1	0	1
	Present	1	0	1	0
Collenchyma cell shape in cortex	Tangentially elongated	1	0	0	0
	Isodiametric	0	0	1	0
	4-6 gonal	0	0	1	0
Parenchyma number of layers	3-5	1	1	1	0
	6-10	0	0	0	1
Parenchyma cell shape in cortex	Isodiametric	1	1	1	1
	Tangentially elongated	0	1	0	1
	Cylindrical	0	0	1	0
Vascular bundles in stem	8-11	0	1	0	0
	12-13	1	0	1	1
Pericyclic fiber number of layers of stem	3-4	0	0	1	0
	6-9	0	0	0	1
Pith cells shape	Isodiametric	1	1	1	1
	Tangentially elongated	0	0	1	0
	Irregular	1	0	0	0
	Radially elongated	0	0	1	1
	Polygonal	0	0	0	1

Characters		Species			
Pith diameter (µm)	430-510	1	1	0	0
	520-550	0	0	1	0
	1125-1300	0	0	0	1
Leaf midrib shape	V- shape	1	1	1	0
	U- shape	0	0	0	1
Midrib thickness	270-340	1	1	1	0
	350-400	0	0	0	1
Parenchyma cells shape in midrib	Isodiametric	1	1	1	0
	Tangentially elongated	1	1	0	0
	Polygonal	0	0	0	1
Wings tissue	Mesophyll	0	0	1	0
	Palisade – spongy	1	1	0	1
Tannin cells in lower vascular	Absent	1	1	1	0
	Present	0	0	0	1
Tannin cells in wings	Absent	1	1	1	0
	Present	0	0	0	1
Seed colour	Brown	0	1	0	0
	Yellowish	0	0	1	0
	Dark brown	1	0	0	0
	Pale yellow	0	0	1	0
	Reddish-brown	0	0	0	1
Seed poles	Rounded	1	1	1	1
	Truncate	0	0	0	1
Seed L/W ratio	1.6	0	0	0	1
	7.7	0	1	1	0
	8.1	1	0	0	0
Seed shape	Oblong	0	0	0	1
	Crescent	1	1	0	0
	Horseshoe	0	0	1	0
Seed coat pattern	Reticulate	0	1	0	1
	Micro papillate	1	0	1	0
	Papillate	0	1	0	0
	Foveolate	0	0	0	1
Hilum colour	Blackish	1	1	0	1
	Same seed colour	0	0	1	0
Rim aril	Uprised	0	1	1	1
	Flush	1	0	0	0
Micropyle shape	Obtriangular	1	1	1	0
	Widely ovate	0	0	0	1
Outline of cells	Reticulate	0	1	0	0
	Isodiametric	1	0	1	0
	Tangentially elongated	1	0	0	0
	Polygonal	0	0	1	1
Anticlinal wall	Wavy	0	1	0	1
	Straight	1	0	1	0
Relief of cell boundary	Channeled	1	0	1	0
	Raised	0	1	0	1

Characters		Species			
Thickness of cell boundary	Moderately	1	0	1	0
	Thick	0	1	0	1
Curvature of outer periclinal	Concave-smooth	1	0	0	0
	Convex	0	1	0	1
	Flat	0	0	1	0

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