

## Seed morphology of nineteen *Crotalaria* L. (Fabaceae) species in Thailand

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### ABSTRACT

Seed morphology of 19 *Crotalaria* species in Thailand was studied using stereoscopic microscopy and scanning electron microscopy. Five different morphological types are described based on differences in shape, aril, fracture lines, size and seed coat surface. Each type is morphologically described, compared, illustrated, and the taxonomic implications are discussed. A key to identify the different types or some species is presented.

KEYWORDS: *Crotalaria*, Leguminosae, Papilionoideae, micromorphology, Thailand.

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### INTRODUCTION

Seed morphology often provides useful characters for generic and species identifications, and can also help understand taxonomic relationships, such as in the following families: Acanthaceae (Rueangsawang *et al.*, 2012), Asteraceae (Chehregani & Mahanfar, 2007; Inceer *et al.*, 2012), Iridaceae (Erol *et al.*, 2006), Orobanchaceae (Plaza *et al.*, 2004) and Scrophulariaceae (Kaplan *et al.*, 2007).

Various seed morphological characters in Fabaceae have been studied several times and some seed characters are very useful for faboid generic identifications (Kirkbride *et al.*, 2003). Studies based on various genera in the Fabaceae indicated that seed morphology provides valuable taxonomic characters for distinguishing some taxa in the tribe Genisteae (López *et al.*, 2000) and the genera *Colutea* L. (Mirzaei *et al.*, 2015), *Entada* Adans. (Rodrigues, 2015), *Lathyrus* L. (Günes & Ali, 2011; Günes, 2013), *Indigofera* L. (Al-Ghamdi, 2011), *Trigonella* L. (Turki *et al.*, 2014), and *Vigna* Savi (Nath & Dasgupta, 2015).

Seed morphology of nine Indian *Crotalaria* L. species was studied by Gandhi *et al.* (2011) using light and scanning electron microscopy. The seeds

varied significantly in size, colour, surface, and hilum characters. Seeds of *Crotalaria* were typically kidney-shaped. Seed colour appeared to be of less diagnostic and systematic value. All species of *Crotalaria* had a smooth surface, except *C. albida* Heyne ex Roth and *C. spectabilis* Roth. The study showed that seed coat ornamentation pattern can be helpful for species identification. Moreover, the shape of Thai *Crotalaria* seed has been described by Niyomdham (1978). However, he overlooked seed coat sculpturing. Because previous data on seed morphology of Thai *Crotalaria* species are insufficient, the current study evaluates the taxonomic significance of seed morphology of the *Crotalaria* species in Thailand for application in classification and identification of the species.

### MATERIALS AND METHODS

Mature seeds of 19 *Crotalaria* species from five sections of Le Roux *et al.* (2013) were obtained from living specimens, vouchers are stored in the KKU herbarium (Table 1). The seed were examined with microscopes and measurements were based on a sample size of 10 grains. Seeds were cleaned by ultrasonic cleaner for 2–5 minutes and dehydrated

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with an alcohol series (70%, 95%, 100%), and subsequently studied by stereoscopic microscope (SM) and scanning electron microscopy (SEM). The seed measurements were investigated under SM. For SEM, the seed was adhered onto an aluminum stub with double-sided cellophane tape and air-dried at room temperature. Finally, the samples were

sputter-coated with a gold-palladium mixture under vacuum and examined with Leo 1450 VP SEM. The terminology of seed morphology mainly follows Kirkbride *et al.* (2003) and Bojňanský & Fargašová (2007). The measurement of length and width are represented in Figure 1.

Table 1. List of specimens examined of *Crotalaria* for seed morphology

Species	Voucher Collector	Locality
1. <i>C. acicularis</i>	<i>S. Ninkaew</i> 259 (KKU)	Phetchabun
2. <i>C. alata</i>	<i>S. Ninkaew</i> 263 (KKU)	Phetchabun
3. <i>C. albida</i>	<i>S. Ninkaew</i> 305 (KKU)	Chiang Mai
4. <i>C. assamica</i>	<i>S. Ninkaew</i> 312 (KKU)	Nan
5. <i>C. bracteata</i>	<i>S. Ninkaew</i> 253 (KKU)	Nan
6. <i>C. calycina</i>	<i>S. Ninkaew</i> 325 (KKU)	Phetchabun
7. <i>C. chinensis</i>	<i>S. Ninkaew</i> 261 (KKU)	Phetchabun
8. <i>C. dubia</i>	<i>S. Ninkaew</i> 307 (KKU)	Chiang Mai
9. <i>C. filiformis</i>	<i>S. Ninkaew</i> 306 (KKU)	Chiang Mai
10. <i>C. gorrensis</i>	<i>S. Ninkaew</i> 287 (KKU)	Sakon Nakhon
11. <i>C. juncea</i>	<i>S. Ninkaew</i> 280 (KKU)	Nakhon Ratchasima
12. <i>C. lejoloba</i>	<i>S. Ninkaew</i> 318 (KKU)	Phetchabun
13. <i>C. medicaginea</i>	<i>S. Ninkaew</i> 281 (KKU)	Khon Kaen
14. <i>C. montana</i>	<i>S. Ninkaew</i> 329 (KKU)	Khon Kaen
15. <i>C. neriifolia</i>	<i>S. Ninkaew</i> 180 (KKU)	Sakon Nakhon
16. <i>C. pallida</i>	<i>S. Ninkaew</i> 273 (KKU)	Bueng Kan
17. <i>C. sessiliflora</i>	<i>S. Ninkaew</i> 291 (KKU)	Sakon Nakhon
18. <i>C. spectabilis</i>	<i>S. Ninkaew</i> 271 (KKU)	Sa Kaeo
19. <i>C. verrucosa</i>	<i>S. Ninkaew</i> 298 (KKU)	Loei

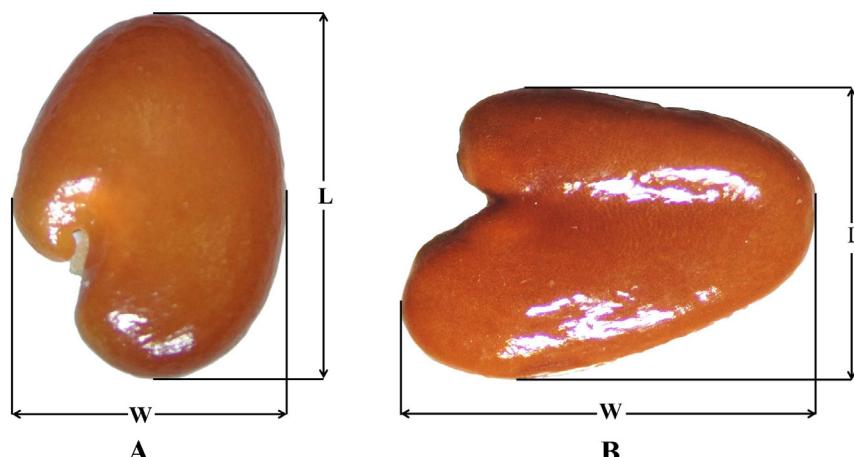


Figure 1. The measurement of length and width in each shape of *Crotalaria* seeds: A) reniform; B) harp-shaped (L = Length; W = Width).

## RESULTS

The micromorphological characters of *Crotalaria* seeds in Thailand including shape, colour, size, seed coat surface, fracture lines, hilum shape and aril features, were studied and summarized in Table 2. Among the taxa examined, two basic morphological seed shape types can be distinguished, which are reniform and harp-shaped. Both show a wide range

of variation in the characters of shape, aril, fracture lines, size and seed coat surface. Based on our SM and SEM studies we distinguish five different seed morphological types. However, the seed morphological features of each type cannot be used for species identification except in Type IV. The types are keyed out and described here, for type IV identification to species level is provided:

### KEY TO SEED TYPES

- 1. Seeds reniform
- 2. Aril present
- 2. Aril absent
  - 3. Seed coat surface with fracture lines (Fig. 6)
  - 4. Seeds 1.3–2.8 × 1–2.2 mm; seed coat surface smooth
  - 4. Seeds 3–5 × 2.8–5 mm; seed coat surface smooth and irregularly wrinkled, or colliculate
  - 3. Seed coat surface without fracture lines
  - 1. Seeds harp-shaped
- 1. Type I
- 2. Type II
- 3. Type III
- 4. Type IV
- 5. Type V

### 1. Type I

Seeds reniform, 1.8–3.5 × 1–3 mm, brown-black, with aril; seed coat surface smooth with or without fracture lines; hilum oval, ruminate. This type occurs in *C. alata* Buch.-Ham. ex D. Don (seeds slightly larger, 3–3.5 × 2.5–3 mm) and *C. lejoloba* Bartl. (seeds slightly smaller, 1.8–3 × 1–2 mm) (Figs. 2 & 3). The aril was already reported by Niymodham (1978).

### 2. Type II

Seeds reniform, 1.3–2.8 × 1–2.2 mm, brown, without aril; seed coat surface smooth with fracture lines; hilum circular or oval, ruminate. This type occurs in *C. acicularis* Buch.-Ham. ex Benth., *C. chinensis* L. and *C. montana* Heyne ex Roth (Figs. 2, 3 & 4), of which the seeds are similar in colour, size and seed coat surface. The hilum is different, circular in *C. acicularis* and *C. chinensis* and oval in *C. montana*

### 3. Type III

Seeds reniform, 3–5 × 2.8–5 mm, brown, yellow

brown or brown-black, without aril; seed coat surface smooth and irregularly wrinkled or colliculate and with fracture lines; hilum circular or oval, ruminate. This type is found in *C. assamica* Benth., *C. bracteata* Roxb. ex DC., *C. nerifolia* Wall. ex Benth. and *C. verrucosa* L. (Figs. 2 & 4). The variation in colour, seed coat surface and hilum shape cannot be used to identification of species. The hilum shape of *C. assamica* is oval, circular in the other three species. Seed coat surfaces are either smooth and irregularly wrinkled (*C. assamica* and *C. nerifolia*) or colliculate (*C. bracteata* and *C. verrucosa*).

### 4. Type IV

Seeds reniform, 3–5 × 2–4.5 mm, orange-brown, brown, brown-green, brown-black, without aril; seed coat surface smooth and irregularly wrinkled, colliculate or tuberculate, without fracture lines; hilum circular or oval, ruminate. This type is found in *C. goreensis* Guill. & Perr., *C. juncea* L., *C. pallida* Aiton and *C. spectabilis*. The species can be keyed out using colour, size, seed coat surface and hilum shapes of the seeds (Figs. 2 & 5).

### KEY TO SPECIES

- 1. Seeds smooth and irregularly wrinkled
- 2. Seeds orange-brown, 2.5–2.8 mm wide; hilum oval
- 2. Seeds brown-black, 3.5–4 mm wide; hilum circular
- 1. Seeds smooth and colliculate or tuberculate
  - 3. Seeds brown-green, 4.5–5 × 4–4.5 mm; smooth and tuberculate; hilum oval
  - 3. Seeds brown, 3–4 × 2–2.5 mm; smooth and colliculate; hilum circular
    - C. goreensis*
    - C. spectabilis*
    - C. juncea*
    - C. pallida*

Table 2. A comparison of seed characters studied for *Crotalaria* species

Seed types	Species	Shape	Colour	Size		Seed coat surface	Fracture lines	Hilum shape	Aril
				Length (mm)	Width (mm)				
I	<i>C. alata</i>	reniform	brown-black	3–3.5 (3.23±0.19)	2.5–3 (2.68±0.14)	smooth	–	oval	+
	<i>C. lejoloba</i>	reniform	brown-black	1.8–3 (2.29±0.53)	1–2 (1.60±0.46)	smooth	+	oval	+
II	<i>C. acicularis</i>	reniform	brown	1.3–1.5 (1.39±0.09)	1–1.2 (1.10±0.08)	smooth	+	circular	–
	<i>C. chinensis</i>	reniform	brown	2–2.3 (2.13±0.14)	1.5–1.7 (1.58±0.09)	smooth	+	circular	–
	<i>C. montana</i>	reniform	brown	2–2.8 (2.43±0.35)	1.5–2.2 (1.88±0.30)	smooth	+	oval	–
III	<i>C. assamica</i>	reniform	brown-black	3–5 (4±0.94)	3–5 (4.2±0.79)	smooth & irregularly wrinkled	+	oval	–
	<i>C. bracteata</i>	reniform	yellow-brown	3–3.2 (3.11±0.10)	2.8–3 (2.91±0.10)	smooth & colliculate	+	circular	–
	<i>C. neriifolia</i>	reniform	brown-black	3.5–3.8 (3.66±0.14)	2.8–3 (2.9±0.09)	smooth & irregularly wrinkled	+	circular	–
	<i>C. verrucosa</i>	reniform	brown	3.5–4.3 (3.95±0.30)	3–3.5 (3.25±0.21)	smooth & colliculate	+	circular	–
IV	<i>C. gorrensis</i>	reniform	orange-brown	4–5 (4.55±0.44)	2.5–2.8 (2.67±0.13)	smooth & irregularly wrinkled	–	oval	–
	<i>C. juncea</i>	reniform	brown-green	4.5–5 (4.74±0.24)	4–4.5 (4.26±0.24)	smooth & tuberculate	–	oval	–
	<i>C. pallida</i>	reniform	brown	3–4 (3.5±0.47)	2–2.5 (2.26±0.24)	smooth & colliculate	–	circular	–
	<i>C. spectabilis</i>	reniform	brown-black	4.5–5 (4.77±0.22)	3.5–4 (3.76±0.23)	smooth & irregularly wrinkled	–	circular	–
V	<i>C. albida</i>	harp-shaped	brown	1–1.5 (1.28±0.22)	1.3–2 (1.63±0.29)	smooth	+	circular	–
	<i>C. calycina</i>	harp-shaped	brown	1.5–2 (1.76±0.21)	2.5–3 (2.76±0.21)	smooth	+	circular	–
	<i>C. dubia</i>	harp-shaped	yellow-brown	1–1.5 (1.27±0.19)	1.6–1.8 (1.49±0.26)	smooth	+	circular	–
	<i>C. filiformis</i>	harp-shaped	brown	0.8–1 (0.94±0.08)	1–1.3 (1.22±0.12)	smooth	+	circular	–
	<i>C. medicaginea</i>	harp-shaped	brown-black	1.5–1.7 (1.61±0.09)	1.7–2 (1.90±0.12)	smooth	+	circular	–
	<i>C. sessiliflora</i>	harp-shaped	brown	1.3–1.5 (1.38±0.08)	1.5–1.7 (1.57±0.08)	smooth	+	circular	–

Notes: + = present; – = absent

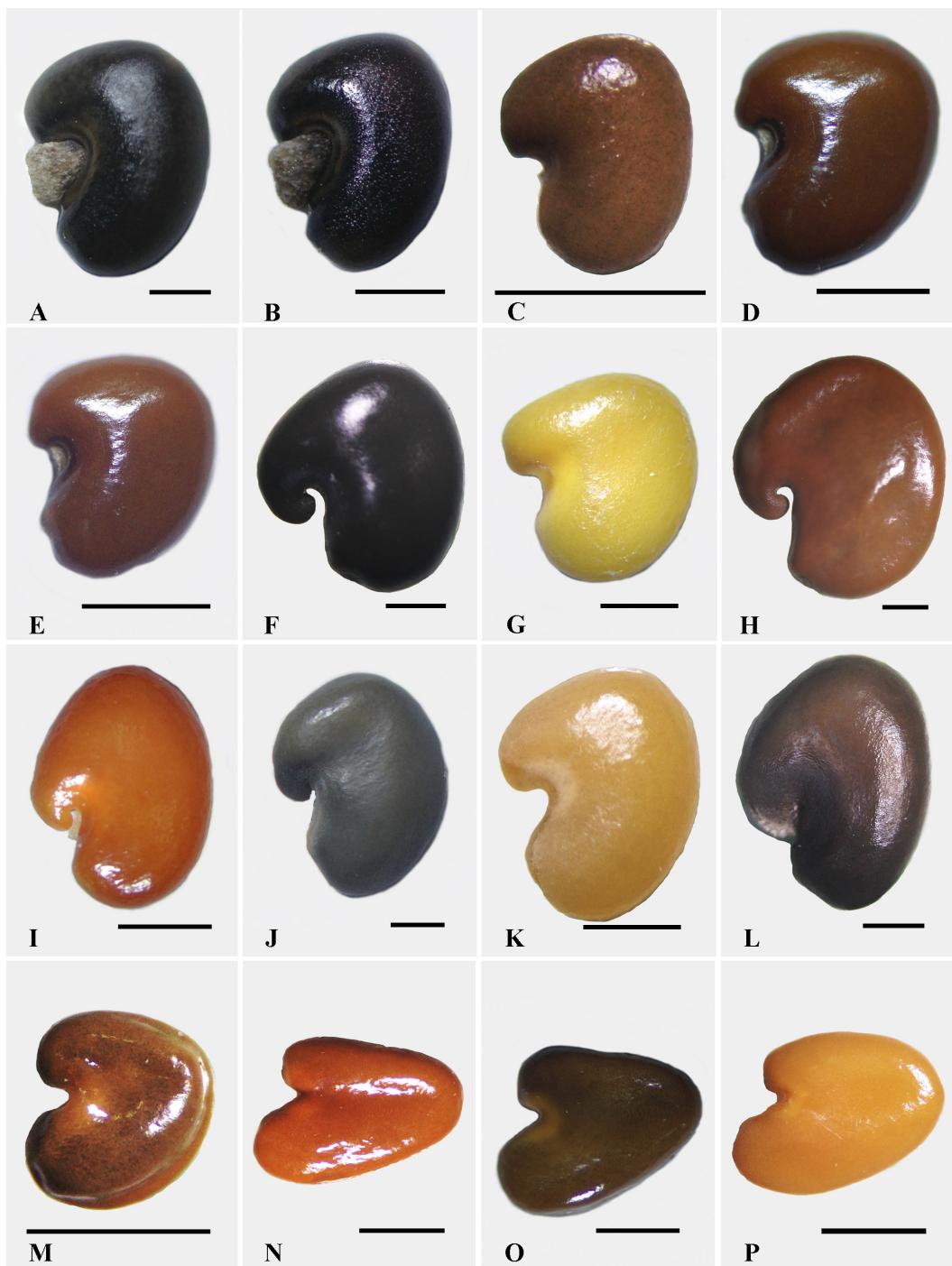


Figure 2. LM micrographs of *Crotalaria* seeds: Type I: A) *C. alata*, B) *C. lejoloba*; Type II: C) *C. acicularis*, D) *C. chinensis*, E) *C. montana*; Type III: F) *C. assamica*, G) *C. bracteata*, H) *C. verrucosa*; Type IV: I) *C. goreensis*, J) *C. juncea*, K) *C. pallida*, L) *C. spectabilis* and Type V: M) *C. albida*, N) *C. calycina*, O) *C. medicaginea*, P) *C. sessiliflora*. Scale 1 mm.

## 5. Type V

Seeds harp-shaped,  $0.8-2 \times 1-3$  mm, yellow-brown, brown, brown-black, without aril; seed coat surface smooth with fracture lines; hilum circular, ruminate. This type is found in *C. albida*, *C. calycina* Schrank, *C. dubia* Graham ex Benth., *C. filiformis* Wall. ex Benth., *C. medicaginea* Lam. and *C. sessiliflora* L. (Figs. 2 & 6). The seed of *C. filiformis* is smallest ( $0.8-1 \times 1-1.3$  mm) and of *C. calycina* largest ( $1.5-2 \times 2.5-3$  mm). The smooth seed coat surface of *C. albida* is in contrast with the undulating lines reported by Gandhi *et al.* (2011). The taxa cannot be keyed out with seed characters alone.

## DISCUSSION AND CONCLUSION

Based on external morphology, two groups of species were traditionally distinguished, reniform or harp-shaped, which agrees well with Niymandham (1978). The seed colour varies from yellow-brown, orange-brown, brown, brown-green to brown-black. Seed sizes vary between 0.8–5 mm in both length and width. The seed of *C. filiformis* is the smallest ( $0.8-1 \times 1-1.3$  mm), while that of *C. juncea* is the largest ( $4.5-5 \times 4-4.5$  mm).

Gandhi *et al.* (2011) reported that seed colour is not diagnostic, but in contrast, we found it to be an important character for identification of the species with Type IV.

Seed morphology has proven to provide useful characters for the identification and delimitation of species or species groups within *Crotalaria* as the seeds of *Crotalaria* can be divided into five types.

The seeds shape, colour and seed coat surface are quite similar in each species while seeds size is slightly variable based on standard deviation (SD; Table 2). The three species with the highest variation in seed sizes are: *C. assamica* ( $3-5 (4 \pm 0.94) \times 3-5 (4.2 \pm 0.79)$ ), *C. lejoloba* ( $1.8-3 (2.29 \pm 0.53) \times 1-2 (1.60 \pm 0.46)$ ) and *C. montana* ( $2-2.8 (2.43 \pm 0.35) \times 1.5-2.2 (1.88 \pm 0.30)$ ), respectively. However, this study is based on few specimens per species. It can be that the variability appears to greater than described here if more specimens from other areas were collected too. However, we are confident that especially the qualitative characters used in the key are stable.

The seed types do not agree with the division of *Crotalaria* into sections that are based on

morphological characters of Polhill (1982) and molecular evidence of Le Roux *et al.* (2013) and Rockinger *et al.* (2017). Seed morphological characters alone are insufficient for a full taxonomic resolution of *Crotalaria* species as the variability in morphology is too great. Nevertheless, in combination with other characters seed morphology can help to resolve taxonomic problems. Therefore, as in other Leguminosae, pollen morphology (Ridder-Numan & Van der Ham, 1997), anatomy (Ninkaew & Chantaranothai, 2015) and molecular characters (The Legume Phylogeny Working Group, 2017) should be combined to clarify the taxonomy of *Crotalaria* in Thailand.

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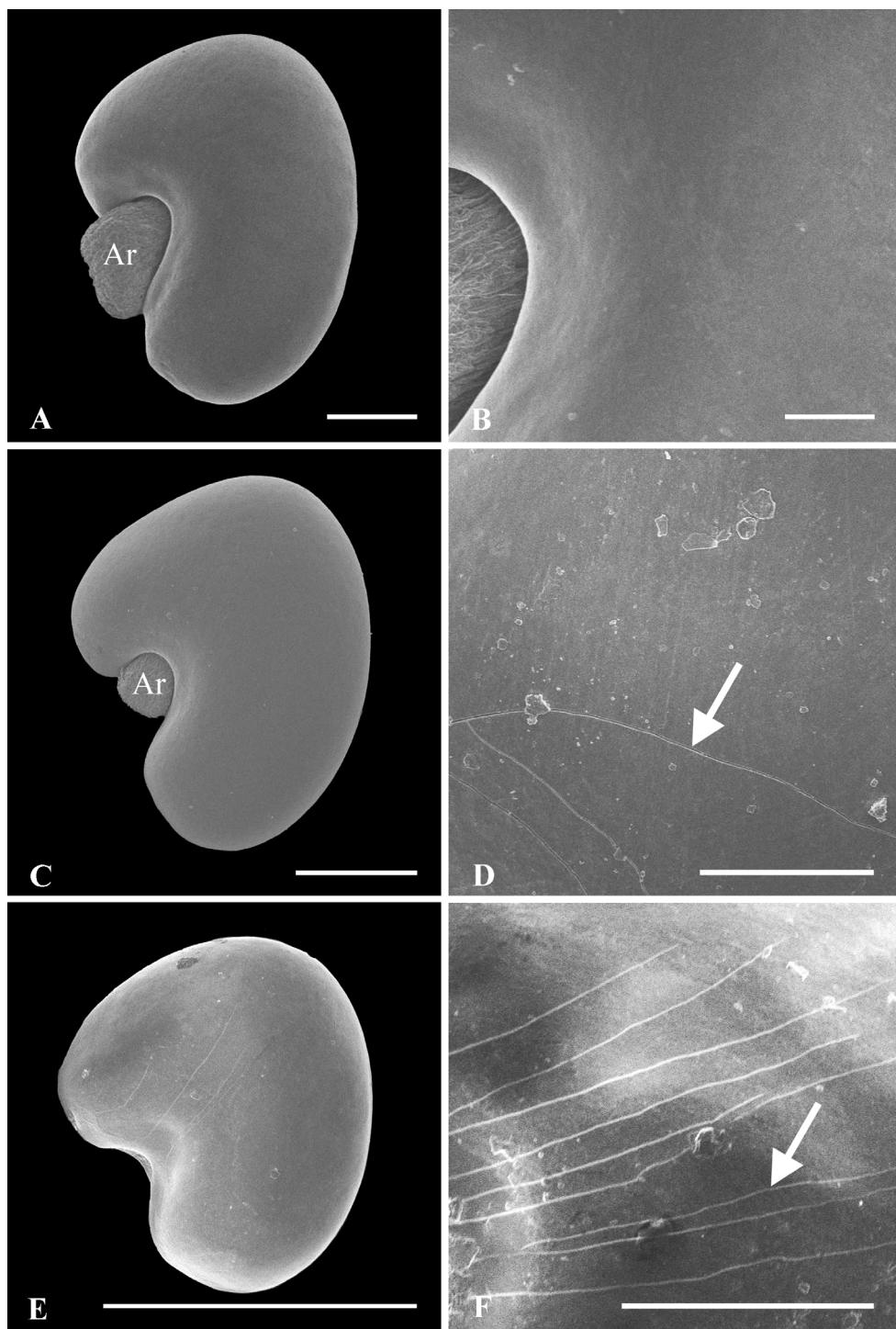


Figure 3. SEM micrographs of *Crotalaria* seeds: Type I: A & B) *C. alata*, C & D) *C. lejoloba*; Type II: E & F) *C. acicularis*. Scale bars: 1 mm (A, C, E); 200  $\mu$ m (B, D, F); Ar = Aril; white arrows = fracture lines.

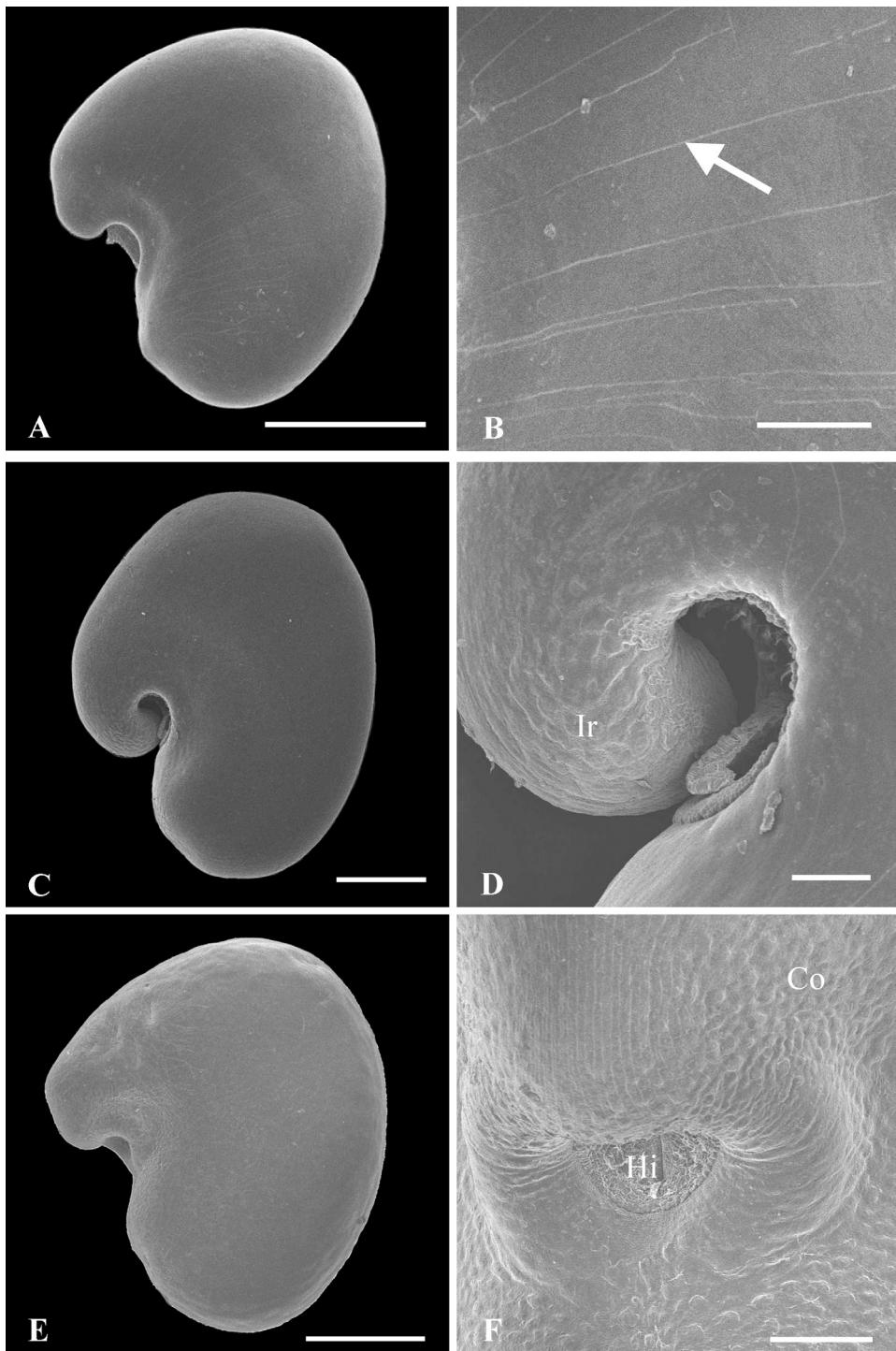


Figure 4. SEM micrographs of *Crotalaria* seeds: Type II: A & B) *C. montana*; Type III: C & D) *C. assamica*, E & F) *C. bracteata*. Scale bars: 1 mm (A, C, E); 200  $\mu$ m (B, D, F); Hi = hilum; Co = colliculate; Ir = irregularly wrinkled; white arrows = fracture lines.

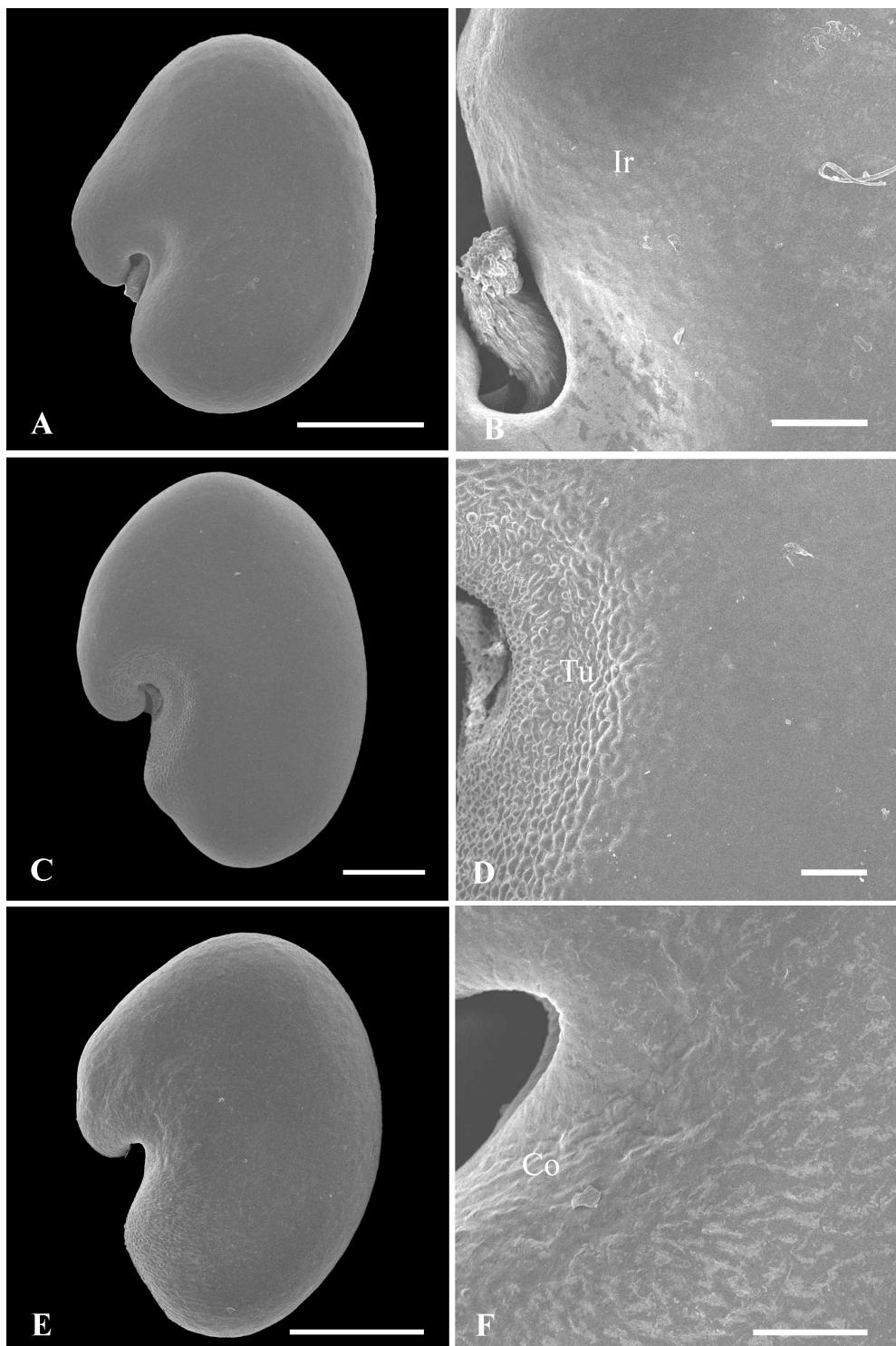


Figure 5. SEM micrographs of *Crotalaria* seeds: Type IV: A & B) *C. goreensis*, C & D) *C. juncea*, E & F) *C. pallida*. Scale bars: 1 mm (A, C, E); 200  $\mu$ m (B, D, F); Co = colliculate; Ir = irregularly wrinkled; Tu = tuberculate.

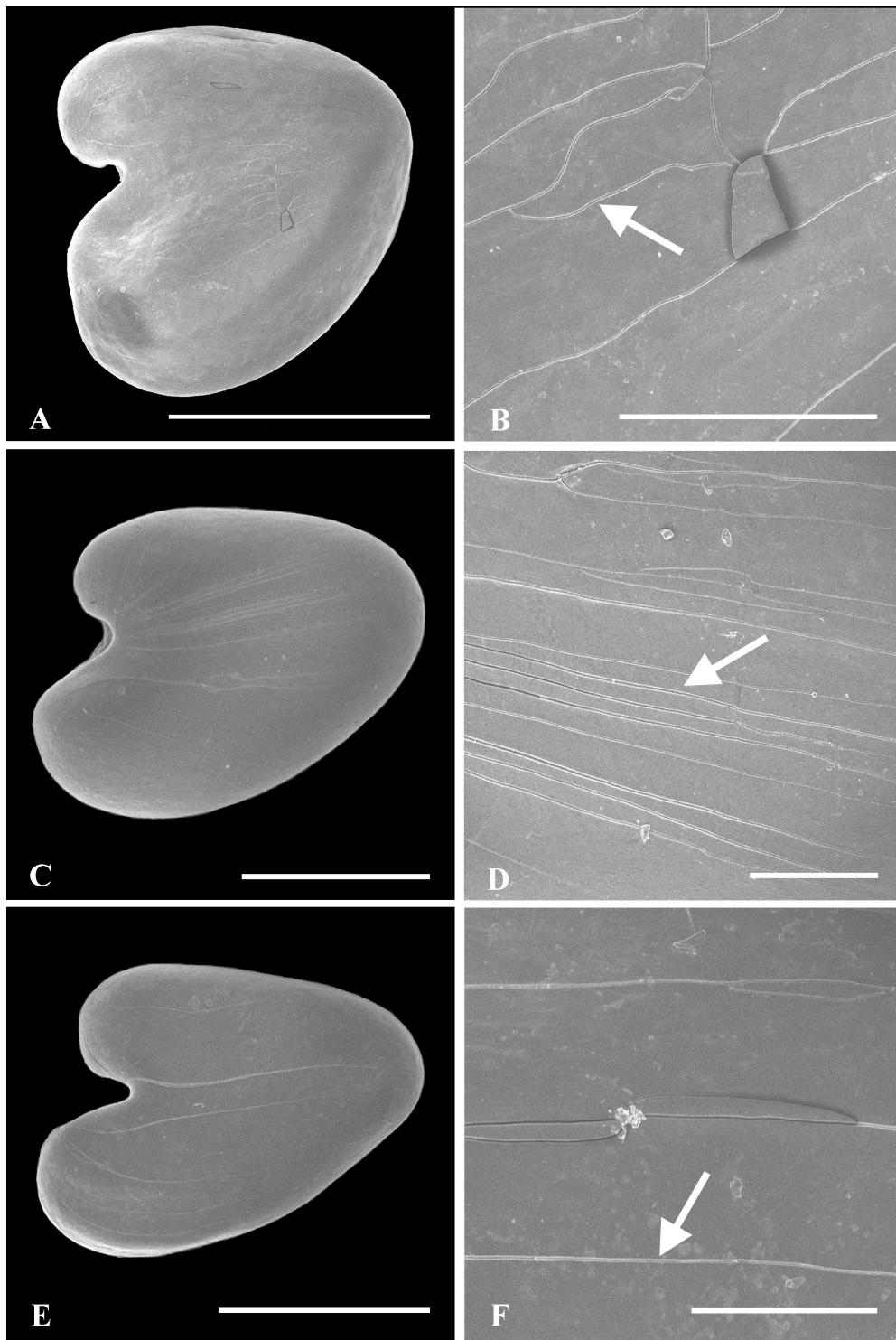


Figure 6. SEM micrographs of *Crotalaria* seeds, Type V: A & B) *C. albida*, C & D) *C. calycina*, E & F) *C. sessiliflora*. Scale bars: 1 mm (A, C, E); 200 µm (B, D, F); white arrows = fracture lines.

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