

# Seed morphology: an addition to the taxonomy of *Tephrosia* (Leguminosae, Papilionoideae, Millettieae) from South America

Rubens Teixeira de Queiroz · Ana Maria Goulart de Azevedo Tozzi · Gwilym Peter Lewis

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**Abstract** Seed morphology was studied using scanning electron microscopy in 13 species, one subspecies and one forma of *Tephrosia* Pers. (Leguminosae, Papilionoideae, Millettieae) occurring in South America. Macromorphological and micromorphological characters were examined, including seed form, colour and size, testa pattern, reticulum anticlinal wall, boundaries of anticlinal wall and hilum form. Crested and simple-reticulate testa patterns were predominant. A foveolate pattern, multifoveolate pattern and subgrooved pattern are all recorded for the first time in this genus. The macromorphological characters displayed continuous variation in shape and size and are thus not significant for species separation. The data obtained in this study together with data from the literature provide additional characters to help classify the genus. A key to the taxa under study is presented.

**Keywords** Classification · Fabaceae · Faboideae · Seed coat · SEM · *Tephrosia*

## Introduction

*Tephrosia* Pers. comprises nearly 350 species (Schrire 2005) and is one of a number of large genera in the family Leguminosae (Geesink 1984). It displays a variable habit, from subshrub to shrub, with imparipinnate leaves, axillary

or terminal pseudoracemes and long, laterally compressed, dehiscent pods containing many seeds. Its geographical distribution is pantropical and also subtropical (Brummitt 1981).

Bentham (1862) divided *Tephrosia* into two sections: *Tephrosia* sect. *Brissonia* (Neck.) DC. and *Tephrosia* sect. *Recueria* Benth. The section *Brissonia* is characterized by a shrubby habit, terminal or axillary (rarely leaf-opposed) inflorescence, by rounded (rarely subulate) calyx teeth and by a glabrous stigma. The section *Recueria* has terminal inflorescences, axillary flowers, subulate calyx teeth, a glabrous style and penicillate (rarely glabrous) stigma. The most recent infrageneric classification was proposed by Brummitt (1981) who, based on the work of Wood (1949), Forbes (1948), Cronquist (1954) and Gillett (1958), divided *Tephrosia* into two subgenera: *Tephrosia* subg. *Tephrosia* and *Tephrosia* subg. *Barbistyla* Brummitt. Subgenus *Tephrosia* is characterized by a glabrous style with trichomes present on the stigma of some species, while subg. *Barbistyla* has trichomes present along the style and stigma. Both subgenera occur in South America. Subgenus *Tephrosia* is represented by *T. adunca* Benth., *T. cinerea* (L.) Pers. f. *cinerea*, *T. cinerea* f. *pseudo-adunca* Hassl., *T. egregia* Sandw., *T. leptostachya* DC., *T. marginata* Hassl., *T. noctiflora* Boj., *T. purpurea* (L.) Pers. subsp. *purpurea*, *T. rufescens* Benth., *T. sessiliflora* (Poir.) Hassl. and *T. senna* Kunth and *Tephrosia* subg. *Barbistyla* by *T. candida* DC., *T. nitens* Benth., *T. sinapou* (Buc'hoz) A. Chev. and *T. vogelii* Hook. f. Taxonomic studies of neotropical species of *Tephrosia* are required because the most recent infrageneric classification of the genus by Brummitt (1981) did not include all 17 of the South American species currently recognized (personal observation, R.T.Q.).

Barthlott (1981) examined the seed surface of 5,000 angiosperm species and 100 gymnosperm species and

R. T. de Queiroz (✉) · A. M. Goulart de Azevedo Tozzi  
Department of Plant Biology, Institute of Biology,  
University of Campinas, PO Box 6109, Campinas,  
SP 13083-970, Brazil  
e-mail: rbotanico@gmail.com

G. P. Lewis  
Herbarium, Library, Art and Archives, Royal Botanic Gardens,  
Kew, Richmond, Surrey TW9 3AB, UK

**Table 1** *Tephrosia* species studied with specimen collector, herbarium and collection site

Taxon	Collector (herbarium)	Collection site
<i>T. adunca</i> Benth.	R.T. Queiroz 1392 (UEC)	BRA. MG. Uberlândia; Parque Estadual do Panga
<i>T. candida</i> DC.	R.D. Ribeiro et al. 656 (RB)	BRA. RJ. Angra dos Reis; Praia do Retiro
<i>T. cinerea</i> (L.) Pers. f. <i>cinerea</i>	A. Allem 9 (RB)	BRA. MT. Corumbá; Fazenda Ianke. (Cerrado)
<i>T. cinerea</i> (L.) Pers. f. <i>cinerea</i>	P. Luetzelburg 27059 (EAC)	BRA. PB. Souza; Agricultural tour of Sao Goncalo. (Caatinga)
<i>T. cinerea</i> f. <i>pseudo-ahunca</i> Hassl.	R.T. Queiroz et al. 1439 (UEC)	BRA. MS. Porto Murтинho; Fazenda das Flores (Chaco)
<i>T. egregia</i> Sandw.	A. Fernandes et al. s.n. (EAC)	BRA. CE. Caucaia; Praia da Pabuba
<i>T. leptostachya</i> DC.	H.S. Irwin et al. 16626 (RB)	BRA. MT. Nova Xavantina
<i>T. marginata</i> Hassl.	N.S. Troncoso 1528 (SI)	ARG. Entre Rios, Salto Grande.
<i>T. nitens</i> Benth.	R.T. Queiroz 1459 (UEC)	BRA. MT. Novo Santo Antônio; Parque Estadual do Araguaia
<i>T. noctiflora</i> Bojer	R.T. Queiroz et al. 1405 (UEC)	BRA. RN. Parnamirim; Jiqui, Fazenda da Enparn
<i>T. purpurea</i> (L.) Pers. subsp. <i>purpurea</i>	P.C. Gadelha Neto 311 (RB)	BRA. PB
<i>T. rufescens</i> Benth.	M.A. Silva et al. 2555 (RB)	BRA. DF. Brasília; Reserva Ecológica do IBGE
<i>T. senna</i> Kunth	H. Alain et al. 31186 (NY)	PER. Ponce; Tuque; in coastal thickets
<i>T. sessiliflora</i> (Poir.) Hassl.	J.J. Wurdack et al. 41161 (RB)	VEN. Bolivar. Salto de Humito
<i>T. sinapou</i> (Buc'hoz) A. Chev.	M.J. Balick et al. 1507 (RB)	BRA. MA. Bom Jardim; Posto indigena de Pindaré
<i>T. vogelii</i> Hook. f.	R.D. Ribeiro s.n. (UEC)	BRA. RJ. Rio de Janeiro

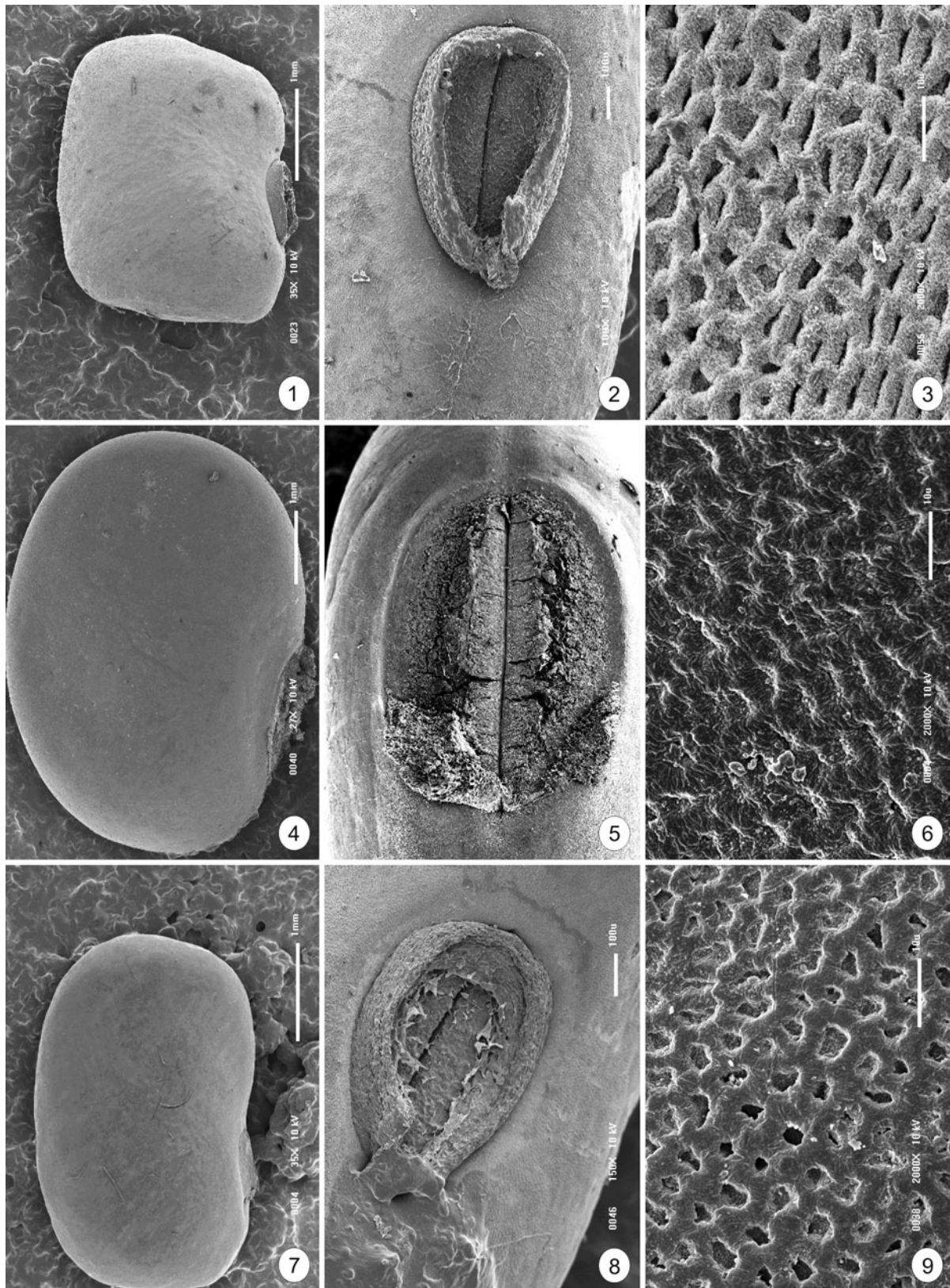
ARG Argentina, RA Brazil (including state abbreviation), PER Peru, VEN Venezuela

**Table 2** Macromorphological seed characters in *Tephrosia* species: size, form, texture and testa colour, hilum position and presence or absence of an aril

Taxon	Length (mm)	Width (mm)	Form	Colour	Testa texture	Speckling	Hilum position	Aril
<i>T. adunca</i>	3.0–4.8	1.8–2.5	Oblong	Ochre	Smooth	Present	Subcentral	Absent
<i>T. candida</i>	4.0–5.0	3.0–4.0	Oval	Ochre	Smooth	Present	Subcentral	Present
<i>T. cinerea</i> f. <i>cinerea</i>	2.5–3.5	1.8–2.2	Oblong	Brown–ferruginous	Smooth	Present	Subcentral	Absent
<i>T. cinerea</i> f. <i>pseudo-ahunca</i>	2.8–3.8	1.2–2.2	Oblong	Ochre	Smooth	Present	Subcentral	Absent
<i>T. egregia</i>	3.0–4.0	2.0–2.2	Rectangular	Ochre	Smooth	Present	Subcentral	Absent
<i>T. leptostachya</i>	3.0	2.0	Oblong–reniform	Ochre	Smooth	Present	Subcentral	Absent
<i>T. marginata</i>	2.0–3.8	2.0–1.2	Rectangular–reniform	Ochre	Smooth	Present	Subcentral	Absent
<i>T. nitens</i>	3.8–4.0	2.5–2.6	Oblong–reniform	Ochre	Smooth	Present	Subcentral	Present
<i>T. noctiflora</i>	3.0–4.0	2.2–2.8	Reniform	Brown	Creased	Absent	Subcentral	Absent
<i>T. purpurea</i> subsp. <i>purpurea</i>	2.9–3.2	2.0–2.3	Oblong–reniform	Brown–ochre	Smooth	Present	Subcentral	Absent
<i>T. rufescens</i>	2.0–3.5	2.0	Rectangular	Ochre	Smooth	Present	Subcentral + central	Absent
<i>T. senna</i>	3.5–4.0	2.0	Reniform	Ochre	Smooth	Present	Subcentral	Absent
<i>T. sessiliflora</i>	3.0–4.8	1.8–2.5	Oval	Black	Smooth	Absent	Subcentral	Present
<i>T. sinapou</i>	3.0–4.0	2.0–2.3	Oblong–reniform	Ochre	Smooth	Present	Subcentral	Absent
<i>T. vogelii</i>	5.0–6.0	2.0–3.0	Oval	Black	Smooth	Absent	Subcentral	Present

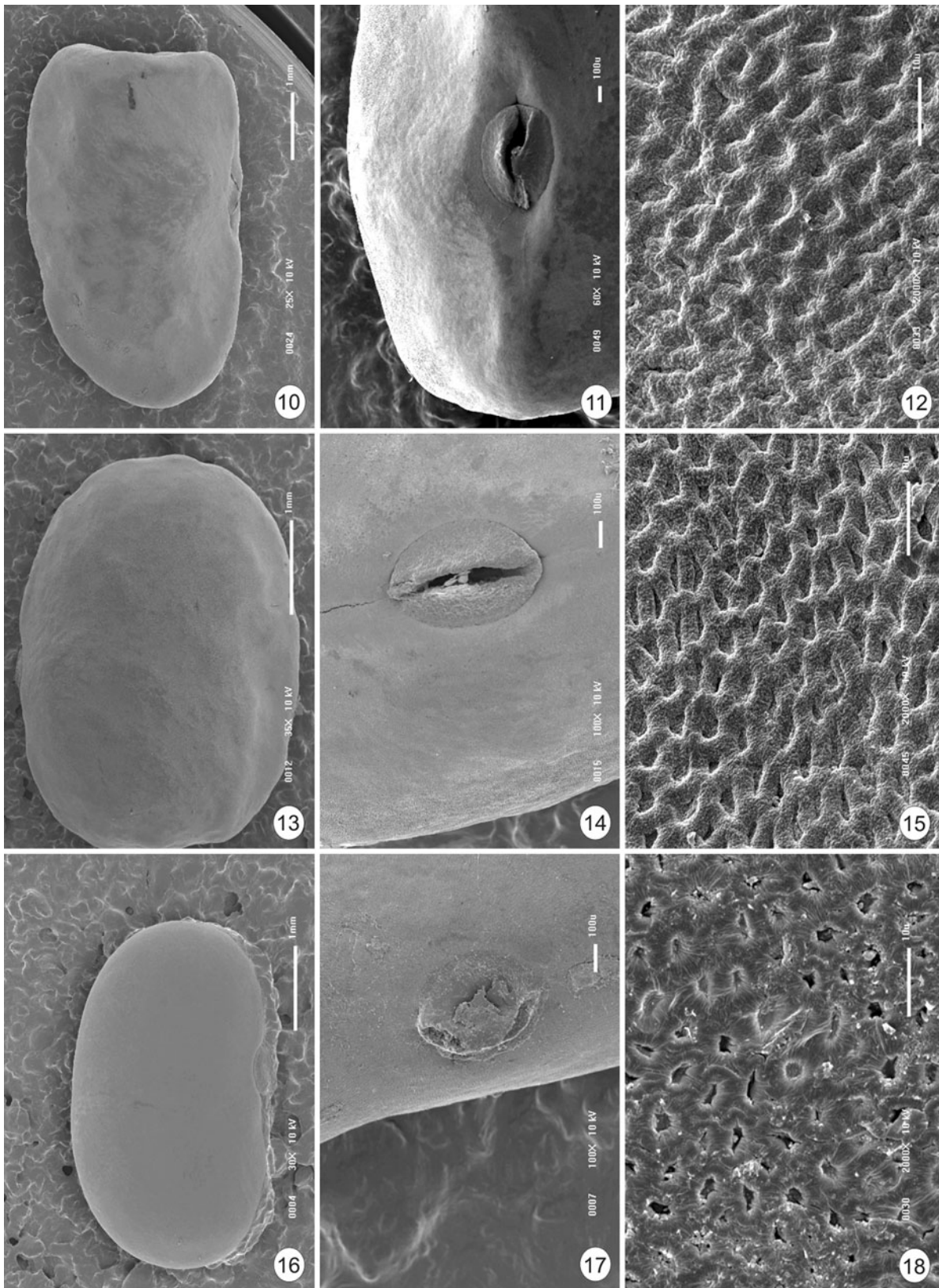
found that scanning electron microscopy (SEM) revealed new structural details. In his study of dicotyledonous seeds, Corner (1976) included 37 species of Leguminosae, including *T. candida* DC., and described a number of

characters important for the taxonomy of the Leguminosae. Kirkbride et al. (2003) described the fruits and seeds of genera of Faboideae (=Papilionoideae) and produced a dichotomous key based on seed structures. The major



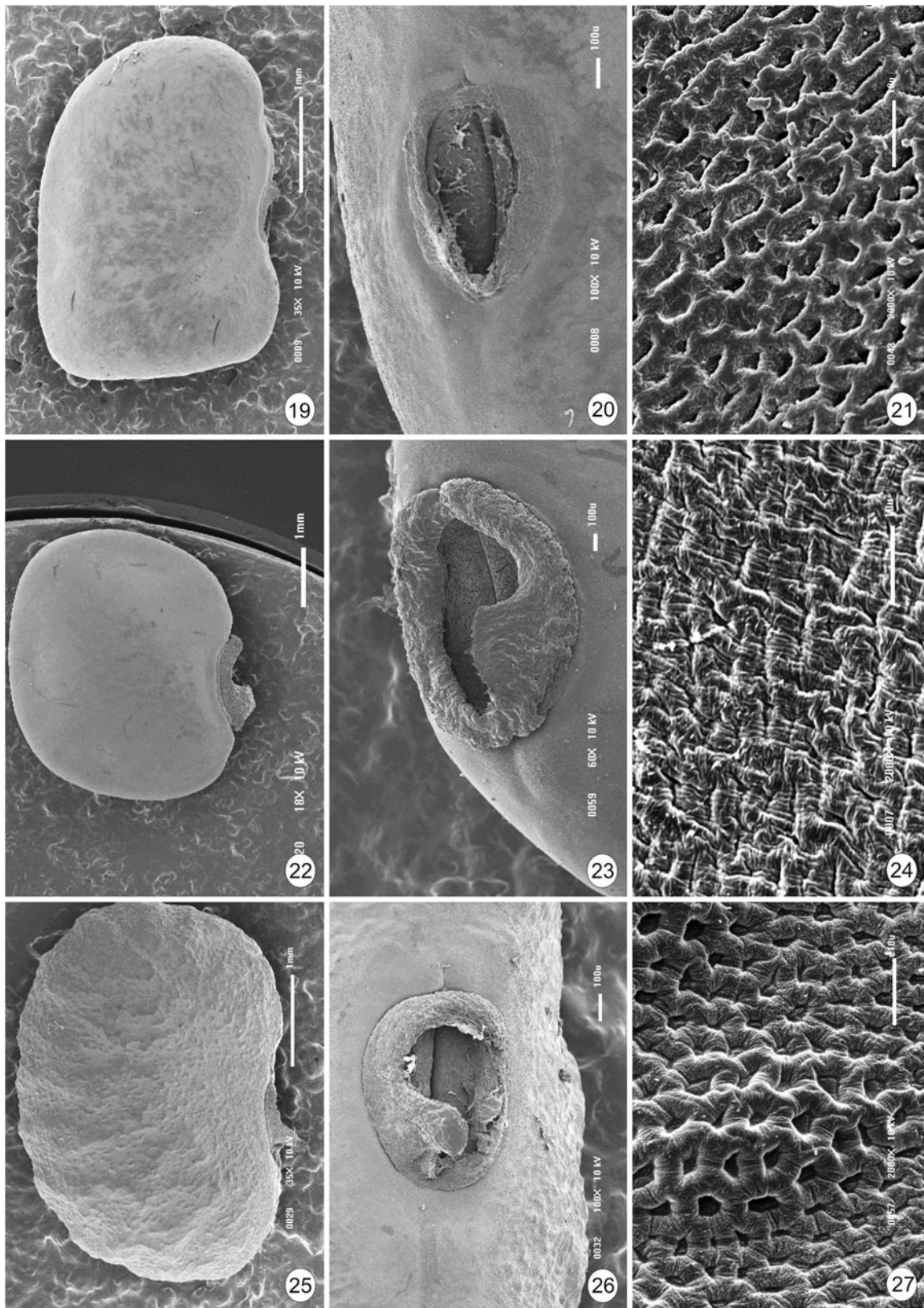
**Figs. 1–9** Seeds of *Tephrosia*: **1–3** *T. adunca* (**1** whole seed side view, **2** circular hilum shape, **3** simple-reticulate testa ornamentation); **4–6** *T. candida* (**4** whole seed side view, **5** elliptical hilum shape, **6** crested testa ornamentation); **7–9** *T. cinerea* f. *cinerea*; (**7** whole seed

side view, **8** circular hilum, **9** simple-reticulate testa ornamentation). *Scale bars* Figs. **1**, **4** and **7** whole seed view, 1 mm; Figs. **2**, **5** and **8** hilum shape, 100 μm; Figs. **3**, **6** and **9** testa ornamentation, 10 μm



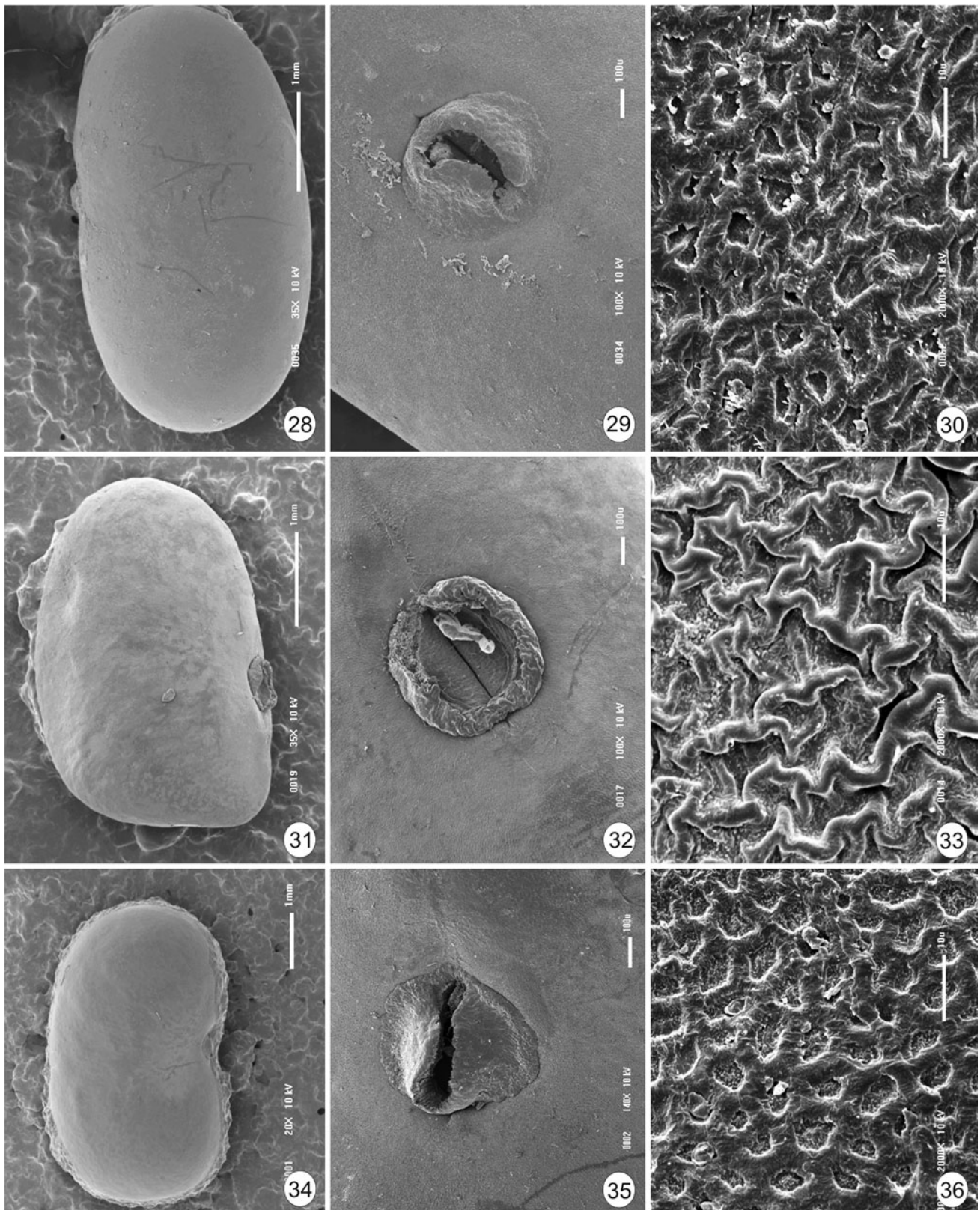
**Figs. 10–18** Seeds of *Tephrosia*: **10–12** *T. cinerea* f. *pseudo-adunca* (**10** whole seed side view, **11** circular hilum shape, **12** simple-reticulate testa ornamentation); **13–15** *T. egregia* (**13** whole seed side view, **14** elliptical hilum shape, **15** simple-reticulate testa ornamentation); **16–18**

*T. leptostachya* (**16** whole seed side view, **17** circular hilum shape, **18** simple-reticulate testa ornamentation). *Scale bars* Figs. **10**, **13** and **16** whole seed view, 1 mm; Figs. **11**, **14** and **17** hilum shape, 100 µm; Figs. **12**, **15** and **18** testa ornamentation, 10 µm



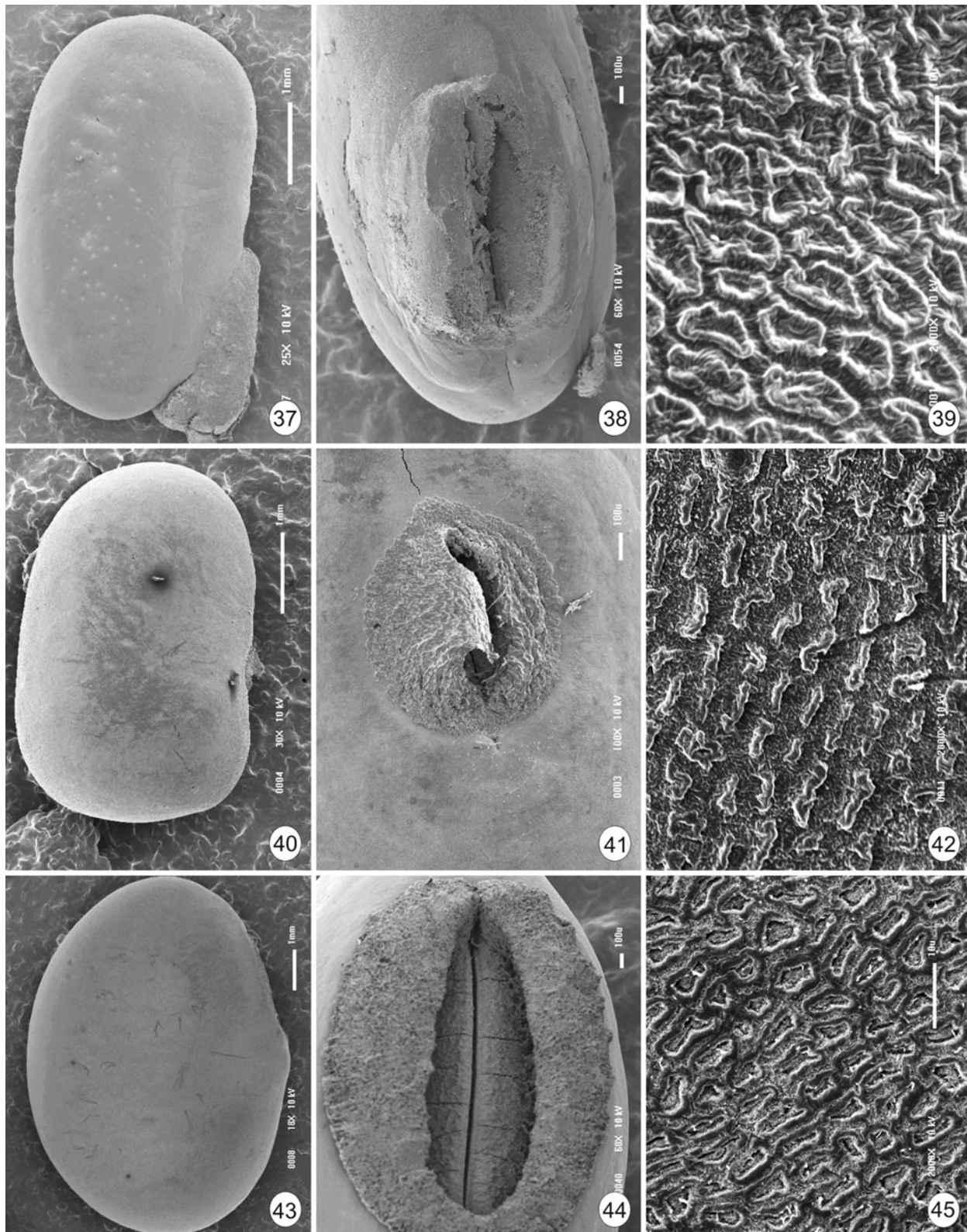
**Figs. 19–27** Seeds of *Tephrosia*: **19–21** *T. marginata* (**19** whole seed side view, **20** oval hilum shape, **21** simple-reticulate testa ornamentation), **22–24** *T. nitens* (**22** whole seed side view, **23** elliptical hilum shape, **24** crested testa ornamentation); **25–27** *T. noctiflora* (**25** whole

seed side view, **26** circular hilum shape, **27** simple-reticulate testa ornamentation). *Scale bars* Figs. **19**, **22** and **25** whole seed view, 1 mm; Figs. **20**, **23** and **26** hilum shape, 100 μm; Figs. **21**, **23** and **27** testa ornamentation, 10 μm



**Figs. 28–36** Seeds of *Tephrosia*: **28–30** *T. purpurea* subsp. *purpurea* (**28** whole seed side view, **29** circular hilum shape, **30** simple-reticulate testa ornamentation); **31–33** *T. rufescens* (**31** whole seed side view, **32** circular hilum shape, **33** simple-reticulate testa

ornamentation); **34–36** *T. senna* (**34** whole seed side view, **35** circular hilum shape, **36** simple-reticulate testa ornamentation). *Scale bars* Figs. **28**, **31** and **34** whole seed view, 1 mm; Figs. **29**, **32** and **35** hilum shape, 100 µm; Figs. **30**, **33** and **36** testa ornamentation, 10 µm



**Figs. 37–45** Seeds of *Tephrosia*: **37–39** *T. sessiliflora* (**37** whole seed side view, **38** elliptical hilum shape, **39** multifoveolate testa ornamentation); **40–42** *T. sinapou* (**40** whole seed side view, **41** circular hilum shape, **42** crested testa ornamentation); **43–45** *T.*

*vogelii* (**43** whole seed side view, **44** elliptical hilum shape, **45** foveolate testa ornamentation). *Scale bars* Figs. **37**, **40**, **43** whole seed view, 1 mm; Figs. **38**, **41**, **44** hilum shape, 100 µm; Figs. **39**, **42**, **45** testa ornamentation, 10 µm

diagnostic characters found in the 50 species of *Tephrosia* analysed by Kirkbride et al. (2003) were the cotyledon with inner face wrinkled, embryonic axis perpendicular to the length of the seed, reticulate testa and hilum within the corona.

Meireles and Tozzi (2008) and Paulino et al. (2010), studying species of *Poecilanthe* and *Indigofera*, respectively, used seed morphology to help solve taxonomic problems. Studies of *Tephrosia* have likewise added new data of taxonomic importance. Bhandari et al. (1985) used seed morphology to delimit nine Indian species of *Tephrosia*. Subba Rao and Shanmukha Rao (1992) used SEM to study the testa patterns of 12 Indian species of *Tephrosia*, from which they obtained the following patterns: simple-reticulate, multireticulate and crested. The recent SEM studies of Al-Ghamdi and Al-Zahrani (2010) in eight species from Saudi Arabia revealed two patterns: simple-reticulate and multireticulate. In addition, these authors drew attention to the organization of the reticulum wall: papillate or wavy. However, these studies did not address the utility of these characters to the infrageneric classification of *Tephrosia*.

The aims of the present study were to describe the macromorphological and micromorphological characters of seeds of neotropical *Tephrosia* species using SEM, and to assess the taxonomic significance of seed characters with regard to the recognition of subgenera and species.

## Materials and methods

Seeds of 13 species, one subspecies and one forma of *Tephrosia* were obtained from specimens collected in the

field or from herbarium specimens, or both (Table 1). Newly collected specimens were deposited in herbarium UEC (Holmgren et al. 1990). Specimens were identified by the first author. In our study, *T. leptostachya* DC. is treated at the specific level, although Brummitt (1968) referred to it as an infraspecific taxon of *T. purpurea* [*T. purpurea* subsp. *leptostachya* (DC.) Brummitt var. *leptostachya*]. We prepared the seed material for SEM study following the procedure of de Castro (2002), and we only used dry seeds. The seeds of each taxon studied were cleaned and mounted directly on a stub and coated with gold using an evaporator (Balzers SCD 050 BAL-TEC). We used a JSM-5800LV (JEOL, Tokyo, Japan) instrument. Testa pattern terminology is based on that of Lersten (1981): substriate (short parallel ridges), simple-reticulate (meshwork of ridges enclosing single cells), simple-foveolate (single cell surrounded by several grooves), multifoveolate (unit of cells surrounded by several grooves) and lophate or crested (short ridges with irregular sides).

## Results

Seed length of the species studied ranged from 2 to 6 mm and width from 1.2 to 4 mm (Table 2). Most species have seeds 2–4 mm long; longer seeds were found in *T. adunca*, *T. candida*, *T. sessiliflora* and *T. vogelii* (Figs. 1, 4, 37, 43). The smallest width was found in *T. cinerea* f. *pseudo-adunca* and *T. marginata* (Figs. 10, 19); the highest in *T. candida* (Fig. 4). Seed shape is oblong (Figs. 1, 7, 10, 16, 22, 28, 40); oval (Figs. 4, 37, 43), rectangular (Figs. 13, 19, 31) or reniform (Figs. 25, 34). Seeds with the lowest

**Table 3** Micromorphological seed characters in species of *Tephrosia* Pers.: testa pattern, reticulum anticlinal wall, boundaries of anticlinal wall and hilum form

Taxon	Testa pattern	Reticulum anticlinal wall	Boundaries of anticlinal wall	Hilum shape
<i>T. adunca</i>	Simple-reticulate	Papillate and uniform	High	Circular
<i>T. candida</i>	Crested	Absent	Absent	Elliptical
<i>T. cinerea</i> f. <i>cinerea</i>	Simple-reticulate	Smooth and uniform	High	Circular
<i>T. cinerea</i> f. <i>pseudo-adunca</i>	Simple-reticulate	Papillate and uniform	High	Circular
<i>T. egregia</i>	Simple-reticulate	Papillate and uniform	High	Elliptical
<i>T. leptostachya</i>	Simple-reticulate	Smooth and wavy	High	Circular
<i>T. marginata</i>	Simple-reticulate	Smooth and uniform	High	Oval
<i>T. nitens</i>	Crested	Absent	Absent	Elliptical
<i>T. noctiflora</i>	Simple-reticulate	Smooth and wavy	High	Circular
<i>T. purpurea</i> subsp. <i>purpurea</i>	Simple-reticulate	Smooth and uniform	High	Circular
<i>T. rufescens</i>	Substriate	Smooth and uniform	High	Circular
<i>T. senna</i>	Simple-reticulate	Smooth and uniform	High	Circular
<i>T. sessiliflora</i>	Multifoveolate	Absent	Channelled	Elliptical
<i>T. sinapou</i>	Crested	Absent	Absent	Circular
<i>T. vogelii</i>	Foveolate	Absent	Channelled	Elliptical



length–width ratio occur in *T. candida* (1.25–1.33) and the highest in *T. vogelii* (2–2.5) (Table 2).

Seed colour is brown, rust coloured or ochre, all with black speckling, or totally black without speckling. Black seeds are found in *T. sessiliflora* and *T. vogelii*. The hilum is located in a central or subcentral position and is circular (Figs. 2, 8, 11, 17, 26, 29, 32, 35, 41), elliptical (Figs. 5, 14, 23, 36, 44) or oval (Fig. 20). An aril is present in the seeds of only four species: *T. candida*, *T. nitens*, *T. sessiliflora* (Fig. 37) and *T. vogelii* (Table 1).

The seed testa is smooth, except in *T. noctiflora*, in which it is creased (Fig. 25; Table 2). Micromorphologically, crested (Figs. 6, 24, 42), foveolar (Fig. 45), multifoveolar (Fig. 39), simple-reticulate (Figs. 3, 9, 12, 15, 18, 21, 27, 30, 36) and substriate (Fig. 33) testa patterns are found, with a smooth (Figs. 9, 21, 30, 36), papillate

(Figs. 3, 12, 15), wavy (Fig. 27) or uniformly reticulate (Figs. 9, 21, 30, 36) anticlinal wall, with the boundaries of the anticlinal walls elevated (Figs. 3, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36) or channelled (Figs. 35, 45) (Table 3).

## Discussion

Although the size and shape of seeds are relatively consistent among the species studied, some characters are diagnostic, e.g. seeds in *T. candida* and *T. vogelii* exceed 4 mm in length (Figs. 4, 43), seeds in *T. candida* exceed 3 mm in width, and seed length in *T. vogelii* is more than double the width, while the ratio is lower in the other species. The substriate testa pattern in *T. rufescens* (Fig. 33), the foveolar pattern in *T. vogelii* (Fig. 45) and

**Table 4** Seed testa pattern in species of *Tephrosia*

Subgenus	Taxon	Testa pattern	Data source <sup>a</sup>
<i>Barbistyla</i>	<i>T. candida</i> DC.	Crested	1, 2
	<i>T. maxima</i> (L.) Pers.	Simple-reticulate	2
	<i>T. nitens</i> Benth.	Crested	1
	<i>T. pulcherrima</i> (Wight ex Baker) Drumm.	Crested	2
	<i>T. sinapou</i> (Buc'hoz) A. Chev.	Crested	1
	<i>T. tinctoria</i> Pers.	Crested	2
	<i>T. vogelii</i> Hook. f.	Foveolate	1
	<i>T. wynaadensis</i> Drumm.	Crested	2
<i>Tephrosia</i>	<i>T. adunca</i> Benth.	Simple-reticulate	1
	<i>T. cinerea</i> (L.) Pers. f. <i>cinerea</i>	Simple-reticulate	1
	<i>T. cinerea</i> f. <i>pseudo-adunca</i> Hassl.	Simple-reticulate	1
	<i>T. desertorum</i> Scheele	Simple-reticulate	3
	<i>T. egregia</i> Sandw.	Simple-reticulate	1
	<i>T. quartiniana</i> Cufod. ex Greuter & Burdet	Simple-reticulate	3
	<i>T. hamiltonii</i> Drumm.	Crested	2
	<i>T. leptostachya</i> DC.	Simple-reticulate	1, 3
	<i>T. marginata</i> Hassl.	Simple-reticulate	1
	<i>T. noctiflora</i> Boj.	Simple-reticulate	1
	<i>T. nubica</i> (Boiss.) Baker	Simple-reticulate	3
	<i>T. pumila</i> (Lam.) Pers.	Simple-reticulate	3
	<i>T. purpurea</i> (L.) Pers. subsp. <i>purpurea</i>	Simple-reticulate	1, 2
	<i>T. purpurea</i> subsp. <i>appolinea</i> (Delile) Hosni & El-Karemy	Simple-reticulate	3
	<i>T. purpurea</i> subsp. <i>leptostachya</i> var. <i>pubescens</i> Baker	Simple-reticulate	3
	<i>T. rufescens</i> Benth.	Substriate	1
	<i>T. senna</i> Kunth	Simple-reticulate	1
	<i>T. sessiliflora</i> (Poir.) Hassl.	Multifoveolate	1
	<i>T. spinosa</i> (L.f.) Pers.	Crested	2
	<i>T. strigosa</i> Sanatapau & Maheshwari	Multireticulate	2
	<i>T. uniflora</i> Pers.	Crested	2
	<i>T. uniflora</i> Pers.	Multireticulate	3
	<i>T. villosa</i> (L.) Pers.	Simple-reticulate	1, 2

<sup>a</sup> 1 This study, 2 Subba Rao and Shanmukha Rao (1992), 3 Al-Ghamdi and Al-Zahrani (2010)

the multifoveolar pattern in *T. sessiliflora* (Fig. 39) are new characters for the genus. The presence of a substrate testa pattern in *T. rufescens* is important because this species is morphologically very similar to *T. adunca* (Fig. 3) and has a similar geographical distribution. The characters listed by Bentham (1862) to separate these two species are essentially limited to the difference in plant stature, the differing number of leaflets, the different plant indumentum and the variation in flower size. In fact, Hassler (1919) considered *T. rufescens* as a variety of *T. adunca*. Our study provides two more characters to differentiate these species: testa pattern (simple-reticulate in *T. adunca*, substrate in *T. rufescens*) and reticulum wall (papillate in *T. adunca*, Fig. 3; smooth and uniform in *T. rufescens*, Fig. 33).

*Tephrosia leptostachya* (Figs. 16, 17, 18) and *T. purpurea* subsp. *purpurea* (Figs. 28, 29, 30) are part of a species complex. Bentham (1862) was the first botanist to highlight the similarity between these two species in Brazil. Brummitt (1968) considered both to belong to one species and recognized subspecies and varieties within *T. purpurea*. In this paper, we treat the two as distinct species, since, notwithstanding the phenotypic similarity in habit, and morphology and colour of the flowers, they are morphologically separable by leaflet form and inflorescence length. The seeds of *T. leptostachya* and *T. purpurea* subsp. *purpurea* are very similar (Figs. 16, 28) but in *T. leptostachya* the reticulum wall is wavy (Fig. 18), while in *T. purpurea* subsp. *purpurea* the wall is uniform (Fig. 30).

Hassler (1919) recognized the taxon *T. cinerea* f. *pseudo-adunca*, differentiated from typical *T. cinerea* by habit, form and size of leaflets, type of indumentum and form of calyx teeth. Apart from the distinct morphology, the typical form has a wide geographical distribution throughout Latin America, while *T. cinerea* f. *pseudo-adunca* has a narrower range in the Chaco in Argentina, Bolivia, Brazil, Paraguay and Uruguay. The testa pattern found in the seeds of these two taxa is the same: simple-reticulate, but forma *pseudo-adunca* has a papillate reticulum wall (Fig. 12), which is not found in *T. cinerea* f. *cinerea* (Fig. 9).

The testa patterns of *T. candida* and *T. purpurea* were studied by Subba Rao and Shanmukha Rao (1992). Our results (namely: oblong seeds with a crested testa pattern, in *T. candida* and reniform seeds, with a simple-reticulate testa pattern, in *T. purpurea*) corroborate their results and indicate that the character states are stable across the geographical range of the two species.

As in the study of Bhandari et al. (1985), we confirm that size, texture, colour, presence or absence of an aril, and seed ornamentation type assist in identifying species. Of the 15 taxa studied by us, *T. adunca*, *T. cinerea* f. *cinerea*, *T. cinerea* f. *pseudo-adunca*, *T. egregia*, *T. leptostachya*, *T. marginata*, *T. noctiflora*, *T. purpurea*

subsp. *purpurea*, *T. rufescens*, *T. sessiliflora* and *T. senna* have a glabrous style, while in *T. candida*, *T. nitens*, *T. sinapou* and *T. vogelii* it is pilose. In the classification of Brummitt (1981), subgenus *Tephrosia* included *T. noctiflora* and *T. sessiliflora*, in addition to the species of section *Recueria* Benth. with glabrous styles, while *Tephrosia* subg. *Barbistyla* included only those species with trichomes present on the style (*T. candida*, *T. nitens*, *T. vogelii* and *T. sinapou*). In section *Brissonia* (Neck.) DC. recognized by Bentham (1862) there are unique seed characteristics, including oblong shape and crested testa pattern in *T. nitens* and *T. sinapou* (Figs. 22, 24, 40, 42), testa pattern lacking speckling, multifoveolate in *T. sessiliflora* (Fig. 39), testa with boundaries of anticlinal wall corrugated, and an aril present in *T. nitens* and *T. sessiliflora* (Figs. 22, 37). In *Tephrosia* sect. *Recueria*, on the other hand, we found the peculiar characteristics of a substrate testa pattern in *T. rufescens* (Fig. 33), a simple-reticulate testa pattern in *T. adunca*, *T. cinerea* and *T. leptostachya* (Figs. 3, 9, 18), and a papillate testa pattern in *T. adunca* (Fig. 3), a uniform reticulum anticlinal wall in *T. cinerea* (Fig. 9), a wavy reticulum anticlinal wall in *T. leptostachya* (Fig. 18) and a central hilum position in all but one species studied (Tables 2, 3).

We also assessed the classification of Brummitt (1981). In *Tephrosia* subg. *Tephrosia* the testa sculpture is predominantly simple-reticulate in *T. adunca*, *T. cinerea* f. *cinerea*, *T. cinerea* f. *pseudo-adunca*, *T. egregia*, *T. leptostachya*, *T. marginata*, *T. noctiflora*, *T. purpurea* subsp. *purpurea* and *T. senna* (Figs. 3, 9, 12, 15, 18, 21, 27, 30, 36). Seeds have the unique characteristics of rectangular shape in *T. egregia* (Fig. 13) or oblong in *T. adunca*, *T. cinerea* f. *cinerea*, *T. cinerea* f. *pseudo-adunca* and *T. purpurea* subsp. *purpurea* (Figs. 1, 7, 10, 28). The testa pattern is multifoveolate in *T. sessiliflora* (Fig. 39), multireticulate or substrate in *T. rufescens* (Fig. 33), and the anticlinal wall is reticulate and papillate in *T. adunca*, *T. cinerea* f. *pseudo-adunca* and *T. egregia* (Figs. 3, 12, 15) or uniform in *T. cinerea* f. *cinerea*, *T. marginata*, *T. purpurea* subsp. *purpurea* and *T. senna* (Figs. 9, 21, 30, 36), with a circular or oval hilum in *T. marginata*. Unique to *Tephrosia* subg. *Barbistyla* is the foveolar testa pattern in *T. vogelii* (Fig. 45) and crested pattern in *T. candida*, *T. nitens* and *T. sinapou* (Figs. 6, 24, 42) (Tables 2, 3).

Subba Rao and Shanmukha Rao (1992) were the first authors to study the micromorphology of *Tephrosia* seeds in 12 species occurring in India. They recorded crested, simple-reticulate, and multireticulate testa patterns (Table 4). Al-Ghamdi and Al-Zahrani (2010) studied seed micromorphology in eight taxa from Saudi Arabia and also observed the multireticulate and simple-reticulate testa patterns (Table 4).

From Table 4, which summarizes data of species from Argentina, Brazil, India and Saudi Arabia, we can see that the characteristics of the testa support previous infrageneric classifications. The two subgenera display fairly characteristic testas, i.e. in *Tephrosia* subg. *Tephrosia* the simple-reticulate ornamentation is predominant, whereas most of the species of *Tephrosia* subg. *Barbistyla* and *Tephrosia* sect. *Brissonia* have a testa with a crested pattern. Based only on the character of the testa pattern, the placement of *T. noctiflora* fits better in *Tephrosia* subg. *Tephrosia* according to the classification proposed by Brummitt (1981), whereas *T. sessiliflora* remains uncertain because of its unique pattern (multifoveolate).

The results provided macromorphological and micro-morphological seed characters which aid identification of the *Tephrosia* species occurring in Latin America.

### Key to the identification of seeds of *Tephrosia* in the neotropics

1. Seed testa with substriate pattern ..... *T. rufescens*
1. Seed testa with a simple-reticulate, foveolate or multifoveolate pattern
  2. Seed testa pattern foveolate
    3. Seed testa pattern simple-foveolate ..... *T. vogelii*
    3. Seed testa pattern multifoveolar ..... *T. sessiliflora*
  2. Seed testa pattern reticulate or crested
    4. Seed testa pattern crested
      5. Seed without aril ..... *T. sinapou*
      5. Seed with aril
        6. Wavy anticlinal wall ..... *T. nitens*
        6. Straight anticlinal wall ..... *T. candida*
  4. Seed testa pattern simple-reticulate
  7. Papillate reticulum
    8. Elliptical hilum in central position ..... *T. egregia*
    8. Circular or oval hilum in subcentral position
      9. Rectangular–reniform seed, oval hilum ..... *T. adunca*
      9. Oblong seed, circular hilum ..... *T. cinerea* f. *pseudo-ahunca*
  7. Smooth reticulum
    10. Reticulum simple, undulating
      11. Seed rugose, brown, without speckling ..... *T. noctiflora*
      11. Seed smooth, ochre coloured, with black speckling ..... *T. leptostachya*
    10. Reticulum uniform, plain
      12. Hilum oval ..... *T. marginata*
      12. Hilum circular
        14. Subcentral hilum ..... *T. cinerea* f. *cinerea*
        14. Central hilum

15. Oblong seeds, length 2.9–3.2 cm ..... *T. purpurea* subsp. *purpurea*
15. Reniform seeds, length 3.5–4.0 mm ..... *T. senna*

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