



The systematic significance of morphological and anatomical variation in fruits of *Crotalaria* and related genera of tribe Crotalarieae (Fabaceae)

MARIANNE M. LE ROUX¹, BEN-ERIK VAN WYK^{1*}, JAMES S. BOATWRIGHT^{1,2} and PATRICIA M. TILNEY¹

¹Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa

²South African National Biodiversity Institute, Compton Herbarium, Private Bag x7, Claremont, 7735, Cape Town, South Africa

Received 28 May 2010; revision 30 September 2010; accepted for publication 7 October 2010

The phylogenetic and taxonomic significance of morphological and anatomical trends in fruits of tribe Crotalarieae has been evaluated, with emphasis on the genus *Crotalaria* and its seemingly distinctive, inflated and balloon-shaped pods. In addition to the normal explosive dehiscence as a means of dispersal, several genera (including *Crotalaria*) show independent evolution of modifications apparently adapted for dispersal by wind, water and gravity. Transverse sections were made of mature pods of 142 species from the 12 currently recognized genera of Crotalarieae. The taxa differ in the orientation of the fibres (related to dehiscence or non-dehiscence), the overall thickness of the fruit wall, the relative proportions of the pericarp layers, the degree of lignification and the presence or absence of trichomes. Three basic pericarp types can be distinguished: type I, with one, two or three zones of various numbers of cell layers of fibres (almost all genera); type II, with a single cell layer of fibres (only in *Rothia*, *Robynsiophyton*, *Lebeckia* and *Lotononis* sections *Listia* and *Leobordea*); and type III, with one zone of several cell layers of gelatinous fibres and multicellular trichomes associated with the endocarp (only in some species of *Calobota* and *Wiborgiella*). Considerable variation was encountered in the tribe, but *Crotalaria* appears to be rather uniform, with type I predominating. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, 165, 84–106.

ADDITIONAL KEYWORDS: anatomy – dehiscence – endocarp – exocarp – fibres – indehiscence – mesocarp – rattlepod – wind-dispersal.

INTRODUCTION

Crotalarieae (Benth.) Hutch. are the largest tribe of papilionoid legumes in Africa (Polhill, 1968; Van Wyk & Schutte, 1995) with *c.* 1204 species (Van Wyk, 2005). The tribe is closely related to Genisteeae Bronn and Podalyrieae Benth., all of which form part of the genistoid alliance (*sensu* Polhill, 1976), more specifically the core genistoids (Crisp, Gilmore & Van Wyk, 2000). The monophyly of Crotalarieae is well supported by molecular, morphological, cytological and

chemical data (Van Wyk & Schutte, 1995; Crisp *et al.*, 2000; Wink & Mohamed, 2003; Boatwright *et al.*, 2008a; Boatwright, Tilney & Van Wyk, 2009). Combined analyses of molecular and morphological data of this tribe indicated that there are three clades and, because of the polyphyly of some of the genera, changes at the generic level resulted in 12 genera being recognized (Boatwright *et al.*, 2008a, 2009). Crotalarieae (Fig. 1) comprise three clades. The 'Cape' clade includes six genera: (1) *Aspalathus* L.; (2) *Wiborgia* Thunb.; (3) *Wiborgiella* Boatwr. & B.-E. van Wyk; (4) *Calobota* Eckl. & Zey.; (5) *Lebeckia* Thunb.; and (6) *Rafnia* Thunb. The poorly known *Lotononis macrocarpa* Eckl. & Zeyh. on its own represents a

*Corresponding author. E-mail: bevanwyk@uj.ac.za

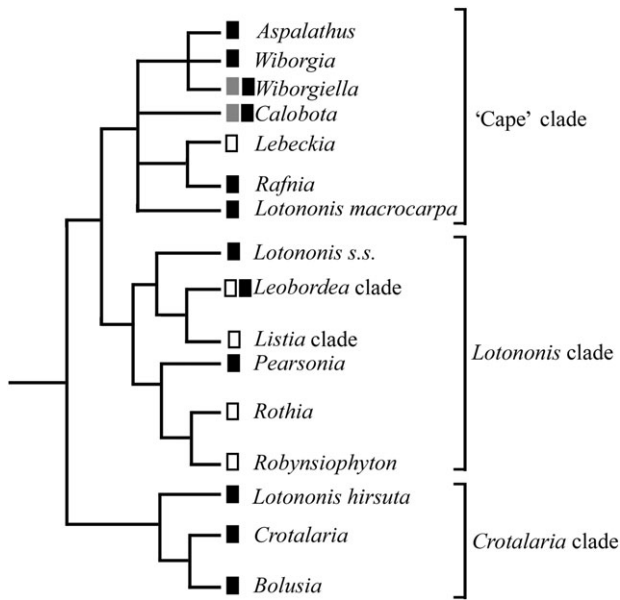


Figure 1. Pericarp structure indicated on a phylogenetic tree for tribe Crotalarieae: type I pericarp (black rectangle); type II pericarp (grey rectangle); type III pericarp (white rectangle).

seventh lineage in this clade. The *Lotononis* clade includes four genera (Boatwright, Wink & Van Wyk, in press): (1) *Lotononis* (DC.) Eckl. & Zeyh.; (2) *Pearsonia* Dümmer; (3) *Robynsiophyton* R.Wilczek; and (4) *Rothia* Pers. The *Crotalaria* clade includes: (1) *Lotononis hirsuta* (Thunb.) D.Dietr.; (2) *Crotalaria* L.; and (3) *Bolusia* Benth. (Boatwright *et al.*, 2008a). All of these lineages have recently been given generic status and the necessary nomenclatural changes have been proposed (Boatwright *et al.*, in press). As the concepts are not yet formalized, we refer to the groups as the *Lotononis hirsuta* clade (genus '*Euchlora*' in Boatwright *et al.*, in press), the *Listia* clade (genus '*Listia*' in Boatwright *et al.*, in press), the *Leobordea* clade (genus '*Leobordea*' in Boatwright *et al.*, in press) and the *Lotononis macrocarpa* clade (genus '*Ezoloba*' in Boatwright *et al.*, in press).

Crotalaria, *Bolusia* and *Lotononis hirsuta* together form the sister group of the remainder of tribe Crotalarieae and include > 700 species, widely distributed across the southern hemisphere. Approximately 500 species are endemic to Africa and Madagascar (Polhill, 1982). *Crotalaria* is commonly known as 'rattlepod' because of the sound produced by the ripe seeds when the inflated fruits are shaken. The generic name is derived from the Greek word *crotalon* (κροτάλου), which means castanet, also referring to this rattling sound. Polhill divided the genus into eight sections based on taximetric analyses (Bisby, 1973; Bisby & Polhill, 1973, Polhill 1982) of morphological, chemical and seed anatomical characters.

Kirkbride, Gunn & Weitzman (2003) published generic descriptions of the fruit morphology and some anatomy for the papilionoid legumes. The pericarp anatomy and dehiscence mechanisms have been described several times for the family (Fahn & Zohary, 1955; Esau, 1962; Fahn, 1967, 1982; Pate & Kuo, 1981). The pericarp consists of an exocarp (single epidermal cell layer), mesocarp (multiple parenchyma and collenchyma cell layers) and endocarp (single or multiple sclerenchyma cell layers with a single inner epidermal cell layer, or multiple parenchyma cell layers with a single epidermal cell layer). Dehiscence is caused by the anisotropic shrinkage of thickened cell walls in the pericarp. The greatest expansion is at right angles to the longitudinal axis of the fruits and microfibrils. When fruits mature, the cell walls dry out and shrink, causing an explosion. Variations of the pericarp structure and modes of dehiscence are numerous (Fahn & Zohary, 1955; Fahn, 1982).

With the clarification of phylogenetic relationships within Crotalarieae (Boatwright *et al.*, 2008a), an opportunity exists to evaluate potentially useful taxonomic characters further. As *Crotalaria* is now known to be one of the early diverging lineages, a comparison of the fruit morphology and anatomy with other genera was expected to yield phylogenetically informative results. This study is aimed at determining the taxonomic value of pericarp anatomical structure at the generic level within Crotalarieae, and at the sectional and species levels, with emphasis on the genus *Crotalaria*. Furthermore, we wished to evaluate the overall pattern in the evolution of fruits in the tribe and explore possible links between the structure of the fruits and the main adaptations to seed dispersal: dehiscence, where the seeds are expelled, or indehiscence, where the whole fruit (diaspore) is dispersed by wind, water or gravity. Anatomical features of dehiscence mechanisms in the tribe were also investigated and compared with those that have previously been reported for legumes in general.

MATERIAL AND METHODS

TAXON SAMPLING

Fruits of 142 species from all 12 genera of Crotalarieae and two of Genisteae were obtained through fieldwork and from specimens from the following herbaria: BOL, JRAU, K, MEL, NBG (including SAM), PRE, UPS and WIND. This sampling represents all or most of the taxonomic diversity in Crotalarieae and also the extremes of the variation in the individual genera. Two species of Genisteae, one of *Dichilus* DC. and one of *Melolobium* Eckl. & Zeyh., were sampled as outgroup taxa. In molecular systematic studies

Figure 2. Morphological variation of fruits in Crotalarieae: A, *Aspalathus linearis* [Van Wyk 3617 (JRAU)]; B, *Wiborgia monoptera* [Schutte 296 (JRAU)]; C, *Wiborgiella leipoldtiana* [Van Wyk et al. 3278 (JRAU)]; D, *Calobota sericea* [Van Wyk et al. 2353 (JRAU)]; E, *C. cuspidosa* [Boatwright et al. 92 (JRAU)]; F, *Lebeckia sepiaria* (L.) Thunb. [Van Wyk et al. 2979 (JRAU)]; G, *L. ambigua* [Van Wyk et al. 2900 (JRAU)]; H, *Rafnia capensis* [Campbell et al. 11 (JRAU)]; I, *Pearsonia sessilifolia* [Van Wyk et al. 3192 (JRAU)]; J, *P. cajanifolia* [Posthumus 1a (JRAU)]; K, *Lotononis densa* [Van Wyk 3122 (JRAU)]; L, *L. globulosa* [Van Wyk 2211 (JRAU)]; M, *L. listii* [Schutte 354 (JRAU)]; N, *L. subulata* [Van Wyk 2884 (JRAU)]; O, *L. macrocarpa* [Schlechter 4925 (BOL)]; P, *L. benthamiana* [Van Wyk 2538 (JRAU)]; Q, *L. hirsuta* [Van Wyk 1338 (JRAU)]; R, *Bolusia amboensis* [Boatwright et al. 248 (WIND)]; S, *Crotalaria lotoides* [Germishuizen 3790 (PRE)]; T, *C. damarensis* [Germishuizen 9247 (PRE)]; U, *C. vasculosa* [De Winter 9460 (PRE)]; V, *C. pisicarpa* [Le Roux et al. 79 (WIND)]; W, *C. longidens* Burtt Davy ex Verdoorn [Le Roux et al. 101 (JRAU)]; X, *C. virgulata* [Van Wyk 3044 (JRAU)]; Y, *C. laburnifolia* [Van Wyk et al. 4334 (JRAU)]. Scale bar, 10 mm.

(Crisp et al., 2000; Boatwright et al., 2008b), these two southern African genera were the earliest divergent lineages in Genisteae. They may show the original character states for Genisteae, avoiding the complication of having to consider further possible modifications higher up in the phylogenetic tree. Voucher specimen information is listed in the Appendix. Author citations for the individual species are also listed in the Appendix.

ANATOMY

Anatomical sections were performed both by hand and with a Porter-Blüm ultramicrotome. For microtome sections, material of 83 species was prepared using a modification of the method of Feder & O'Brien (1968) for embedding in glycol methacrylate (GMA). This involved a final infiltration in GMA for 5 days. Dried material was rehydrated and fixed in formaldehyde-acetic acid-alcohol (FAA; formaldehyde:acetic acid:96% alcohol:water; 10:5:50:35) for 24 h, whereas fresh material was directly fixed in FAA before dehydrating and embedding in GMA. Sections of 3–5 µm were cut and stained using the periodic acid Schiff/toluidine blue (PAS/TB) staining method (Feder & O'Brien, 1968) and mounted. Photographs were taken with a JVC KY-F1030 digital camera. For hand sections, fruit material of 61 species was rehydrated in hot water for approximately 15 min, sectioned with a sharp blade, stained with toluidine blue and scanned for diagnostic characters.

RESULTS

Fruit morphology in tribe Crotalarieae was found to be variable. Figure 2 illustrates some of the variation that was observed in the different genera in terms of size, shape, degree of inflation, presence or absence of a stipe, vestiture, presence or absence of a margin or wing and constrictions between the seeds. Fruits of *Crotalaria* are generally larger than those of most other genera and are typically much inflated or

balloon-shaped (there are a few exceptions, e.g. *C. coursii* M.Peltier, *C. cytisoides* DC., *C. leptocarpa* Balf.f. ssp. *leptocarpa*, *C. leptocarpa* Balf.f. ssp. *contracta* Polhill and *C. linearifoliolata* Chiov., which have laterally compressed fruits), with the base and/or apex rounded and bulging. Similar fruits occur in the related genus *Bolusia* and in *Lotononis hirsuta*. The fruit may rarely also be small in *Crotalaria* (e.g. *C. vasculosa* Graham and *C. pisicarpa* Welw. ex Baker, Fig. 2U and V, respectively) and similar in size to those of other genera in the tribe. *Crotalaria* fruits in general lack adaptations to wind dispersal (except in a few species where the calyx is persistent, e.g. *C. berteroaana* DC., *C. chinensis* L., *C. dubia* Graham and *C. sessiliflora* L.) as seen in other genera (Fig. 3). *Wiborgia*, for example, has fruits with broad wings (Figs 2B and 3E) and similar but narrower wings are found along the upper suture in *Lebeckia meyeriana* Eckl. & Zeyh. (Fig. 3I). Fruits of *Lotononis* section *Digitata* B.-E.van Wyk (Fig. 3A, D) and *L.* section *Synclistus* B.-E.van Wyk (Fig. 3B, C) have small, lightweight, indehiscent fruits with persistent calyces and corollas so that they are easily blown about by the wind. The species of section *Digitata* grow almost exclusively in the cracks of large granite domes, and in this habitat the fruits are highly mobile and easily 'caught' in the cracks (B.-E. van Wyk, pers. observ.). Another adaptation is the 'rolling' fruits of section *Synclistus* (e.g. *L. longicephala* B.-E.van Wyk, Fig. 3B and *L. polycephala* Benth., Fig. 3C). These species occur in bare sandy habitats (mostly inland sand dunes) and the fruits are rolled around by the wind (B.-E. van Wyk, pers. observ.). Similar are the fruits of *Calobota elongata* (Thunb.) Boatwr. & B.-E.van Wyk, also an inhabitant of inland sand dunes. This is the only species of *Calobota* with small, indehiscent, single-seeded fruits enclosed in a persistent calyx and corolla. The similarity with species of *Lotononis* section *Synclistus* is striking. A few species of *Crotalaria* are similar in having small, indehiscent, few-seeded pods that appear to be adapted to dispersal by wind, water or gravity, perhaps with a rolling action.

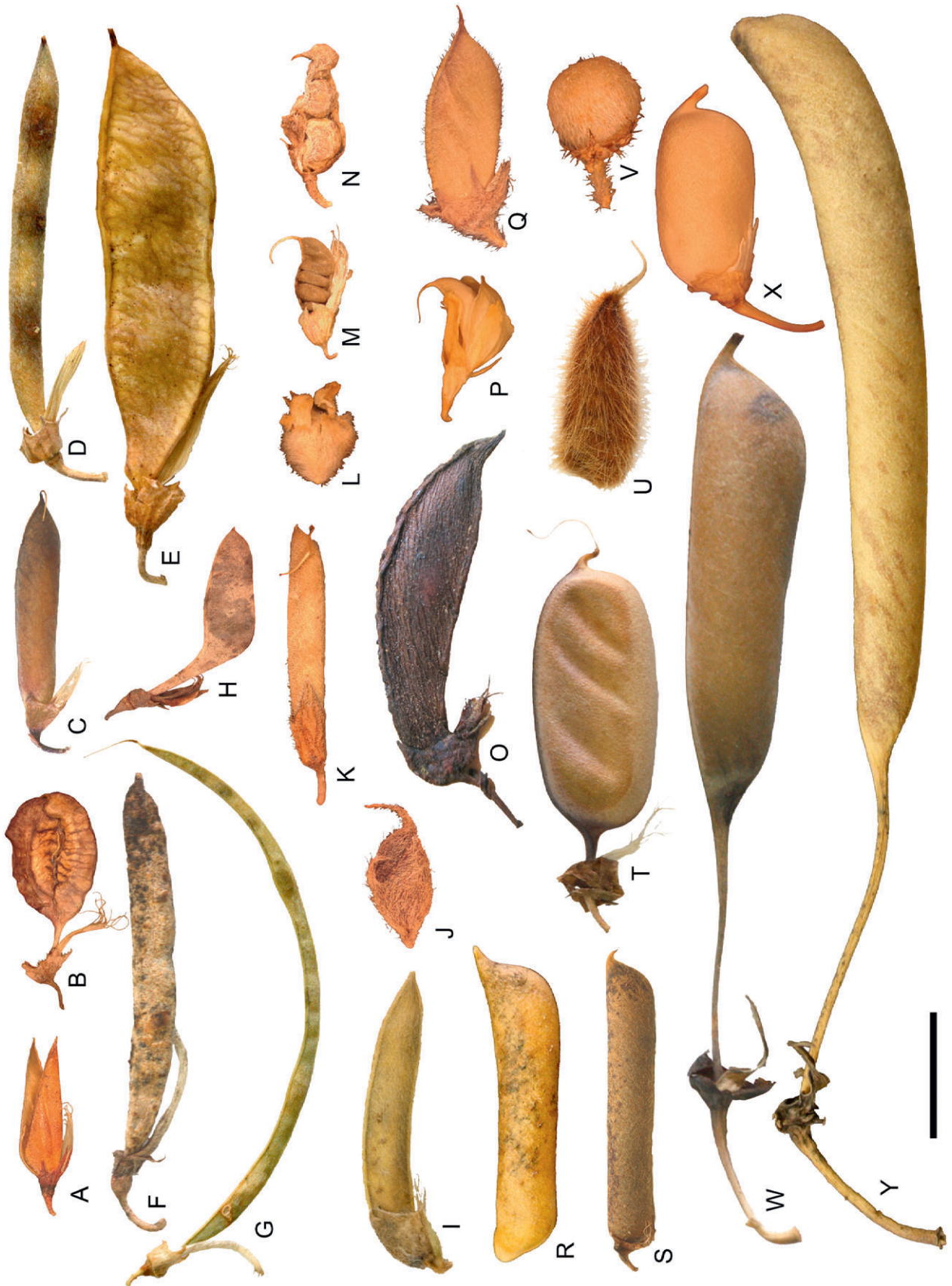


Figure 3. Fruits with different dispersal methods in Crotalariaeae. (A–D) and (H) show a series of three photographs where the fruits are firstly enclosed in persistent calyx and corolla, secondly where the calyx and corolla are removed and thirdly where the fruit is opened. (E–G) and (I) show a series of two photographs where the fruits are firstly closed and secondly opened. A, *Lotononis digitata* [Van Wyk 2341 (JRAU)]; B, *L. longicephala* [Van Wyk 2241 (JRAU)]; C, *L. polycephala* [Van Wyk 2394 (JRAU)]; D, *L. benthamiana* [Van Wyk 2538 (JRAU)]; E, *Wiborgia fusca* [Van Wyk 3213 (JRAU)]; F, *Crotalaria sphaerocarpa* [Le Roux et al. 74 (WIND)]; G, *C. pisicarpa* [Le Roux et al. 78 (WIND)]; H, *Calobota elongata* [Van Wyk 2562b (JRAU)]; I, *Lebeckia meyeriana* [Van Wyk 3351a (JRAU)]. Scale bar, 5 mm.

As *Crotalaria* fruits are typically much inflated, it is of interest to consider the overall pattern in the tribe. The shape of the fruit in transverse section is determined by the orientation of the upper and lower sutures, which can be raised or sunken in various combinations, as shown in Figure 4. In *Crotalaria*, the fruits are often perfectly round in transverse section (Fig. 4O, L) or they may be ellipsoid (Fig. 4H), cordate (Fig. 4K) or somewhat didymous when both the upper and lower sutures are sunken (Fig. 4N). Inflated fruits are also found in other genera of the tribe and are thus not unique to *Crotalaria*. *Bolusia* (Fig. 4Q), *Lotononis hirsuta* (Fig. 4P), *Wiborgiella* and several other species of *Lotononis* have inflated fruits (Fig. 4E, F). In *Crotalaria* section *Hedriocarpae* Wight & Arn., some species have the seeds surrounded by trichomes, which often fill the cavity completely (Fig. 4J). The same is true for species of *Calobota* (Fig. 4C, D). In *Crotalaria* section *Chrysocalycinae* (Benth.) Bak.f. subsection *Glaucæ* (Benth.) Bisby & Polhill and section *Crotalaria* subsection *Longirostres* (Benth.) Polhill, a few species have a line of trichomes inside the fruit along the lower suture. Superficially, the species of *Calobota* (Fig. 4C, D) are similar to some species of *Lebeckia* (Fig. 4A, B) in having inflated, spongy fruits, but these are not homologous: the spongy texture is because of spongy parenchyma in the mesocarp in *Lebeckia*, whereas it is because of a dense layer of endodermal trichomes in *Calobota*.

Diagnostically informative characters of the pericarp that were identified include the number of fibre cell layers in the endocarp, the type of fibres and the presence or absence of multicellular trichomes composing the endocarp cells. Three basic fruit types were identified:

1. Type I – one, two or three zones of various numbers of cell layers of fibres within the endocarp; trichomes (formed from the endocarp cells) occasionally present.
2. Type II – single cell layer of normal fibres within the endocarp; trichomes absent.
3. Type III – one zone of several cell layers of gelatinous fibres; trichomes (formed from the endocarp cells) invariably present.

A short fruit anatomical description for each genus is given below (summarized in Table 1) with an indi-

cation of the classification of the fruit wall type (fruit type classification for all species investigated is listed in the Appendix). The distribution of fruit wall types within the tribe is shown in Figure 1.

GENERIC FRUIT ANATOMICAL DESCRIPTIONS

‘CAPE’ CLADE – TYPE I AND TYPE III FRUIT WALLS ARE PRESENT

Aspalathus: Fruits relatively uniform, thick-walled (Fig. 5A–C). *Exocarp*: Epidermal cells with highly thickened cell walls; mucilage cells absent. *Mesocarp*: Only collenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

Wiborgia: Fruits relatively thin- to thick-walled (Fig. 5D, E). *Exocarp*: Epidermal cells with slightly thickened cell walls; mucilage cells absent. *Mesocarp*: Only parenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres arranged in one direction, parallel to the longitudinal axis of the fruits, or two directions, parallel and perpendicular to the longitudinal axis of the fruits; trichomes absent.

Wiborgiella: Fruits thick-walled (Fig. 5F–I). *Exocarp*: Epidermal cells with slightly to highly thickened cell walls; mucilage cells present or absent. *Mesocarp*: Parenchyma and collenchyma cells present or only collenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres or one zone (except *W. vlokii* Boatwr. & B.-E.van Wyk, with two zones) of several cell layers of gelatinous fibres arranged in one direction, parallel or at 45° angle to the longitudinal axis of the fruits; trichomes mostly absent (but present in *W. inflata* (Bolus) Boatwr. & B.-E.van Wyk and *W. bowieana* (Benth.) Boatwr. & B.-E.van Wyk, Fig. 5G and H, respectively). *Wiborgiella humilis* (Thunb.) Boatwr. & B.-E.van Wyk has exceptionally thin-walled fibre cells (Fig. 5F).

Calobota: Fruits relatively thin- to thick-walled (Fig. 6A–I). *Exocarp*: Epidermal cells with slightly to

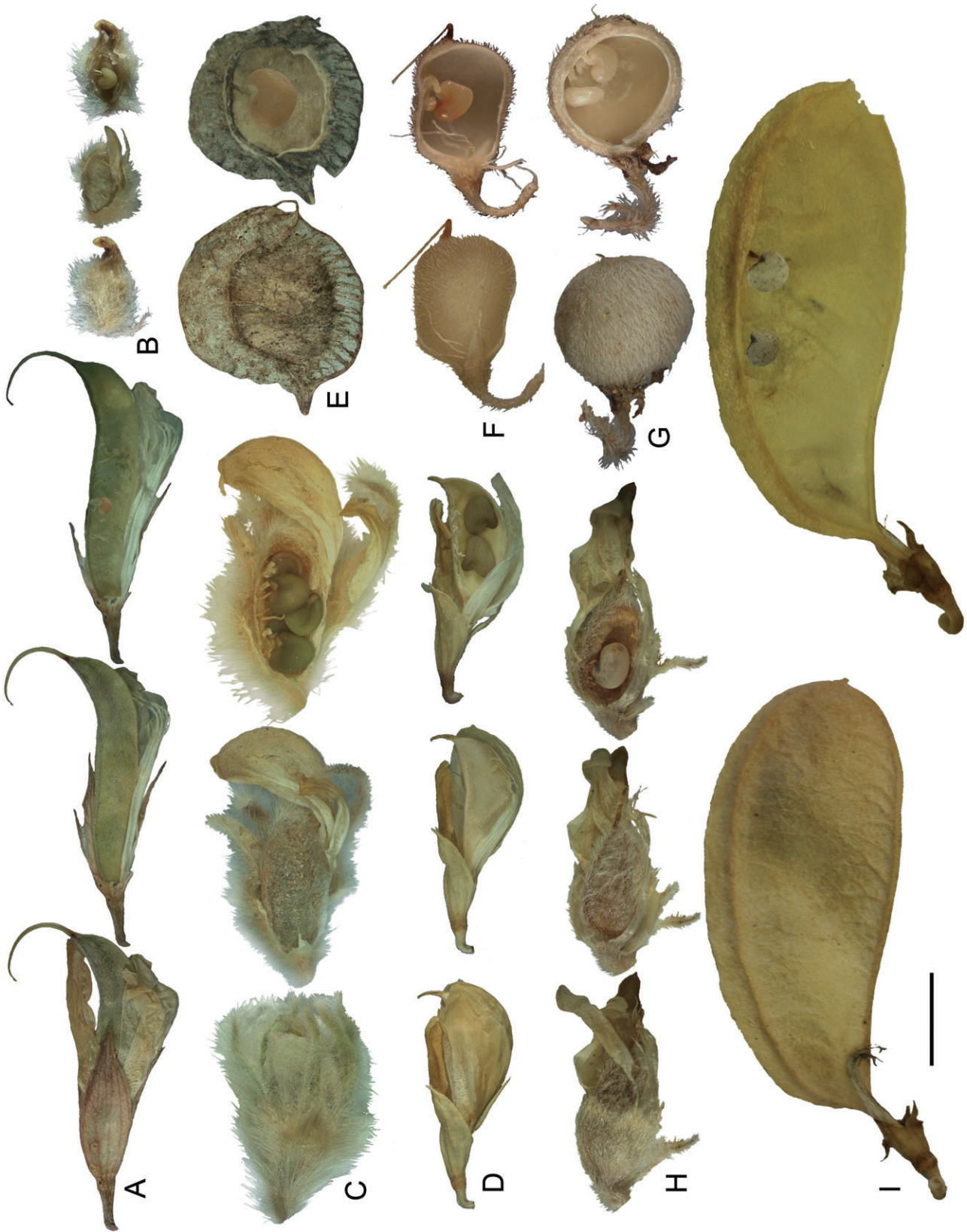




Figure 4. A comparison of inflated fruits in Crotalarieae. A, *Lebeckia brevicarpa* [Le Roux *et al.* 4 (JRAU)]; B, *L. pauciflora* [Le Roux *et al.* 12 (JRAU)]; C, *Calobota cinerea* [Boatwright *et al.* 150 (JRAU)]; D, *C. thunbergii* [Boatwright *et al.* 140 (JRAU)]; E, *Lotononis nutans* [Van Wyk 3442 (JRAU)]; F, *L. pungens* [Vlok 1646 (JRAU)]; G, *Crotalaria laburnifolia* [Van Wyk *et al.* 4333 (JRAU)]; H, *C. damarensis* [Le Roux *et al.* 65 (WIND)]; I, *C. obscura* [Le Roux *et al.* 109 (JRAU)]; J, *C. somalensis* [Gillett 21175 (PRE)]; K, *C. argyraea* [Le Roux *et al.* 82 (WIND)]; L, *C. flavicarinata* [Le Roux *et al.* 72 (WIND)]; M, *C. excisa* [Le Roux *et al.* 108 (JRAU)]; N, *C. recta* [Le Roux 42 (JRAU)]; O, *C. virgulata* [Le Roux *et al.* 38 (JRAU)]; P, *Lotononis hirsuta* [Van Wyk 1338 (JRAU)]; Q, *Bolusia amboensis* [Boatwright *et al.* 248 (WIND)]. Scale bar, 1 mm.

highly thickened cell walls; mucilage cells absent. *Mesocarp*: Parenchyma and collenchyma cells present or only collenchyma cells present. *Endocarp*: One or rarely two zones of various numbers of cell layers of

fibres or one zone of several cell layers of gelatinous fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes only present in fruits with gelatinous fibres.

Table 1. Summary of pericarp characters of *Crotalaria* and all other genera of the tribe Crotalarieae (and two genera of the tribe Genisteae). Authorities for the names of taxa and data for the individual species of each genus are provided in the Appendix. Type I – one, two or three zones of various numbers of cell layers of fibres within the endocarp; trichomes (formed from the endocarp cells) occasionally present. Type II – single cell layer of normal fibres within the endocarp; trichomes absent. Type III – single zone of several cell layers of gelatinous fibres; trichomes (formed from the endocarp cells) invariably present

| Genus and infrageneric group (where available) | Pericarp | Exocarp | | Mesocarp | | Fruit type |
|--|---|---------------------------------|-----------------------------------|-------------------------------------|--------------------------------------|--|
| | Fruit wall thickness – thin (< 140 µm) + thick (> 140 µm) | Cell walls – thin + thick | Mucilage – absent + present | Parenchyma – absent + present | Collenchyma – absent + present | + Type I ++ Type II +++ Type III |
| <i>Aspalathus</i> | + | + | – | – | + | + |
| <i>Bolusia</i> | + | – | – | – | + | + |
| <i>Calobota</i> | + | – | – | –/+ | + | + /+++ |
| <i>Crotalaria</i> | | | | | | |
| Section <i>Calycinae</i> | –/+ | –/+ | –/+ | – | + | + |
| Section | + | –/+ | – | – | + | + |
| <i>Chrysocalycinae</i> | | | | | | |
| Section <i>Crotalaria</i> | + | – | –/+ | – | + | + |
| Section <i>Dispermae</i> | –/+ | – | –/(+) | – | + | + |
| Section <i>Geniculatae</i> | + | – | – | + | + | + |
| Section <i>Grandiflorae</i> | + | + | – | – | + | + |
| Section <i>Hedriocarpae</i> | + | –/+ | – | – | + | + |
| Section <i>Schizostigma</i> | – | – | + | – | + | + |
| <i>Dichilus</i> | + | – | – | + | + | + |
| <i>Lebeckia</i> | + | – | + | + | + | ++ |
| <i>Lebeckia wrightii</i> | + | – | + | – | + | + |
| <i>Lotononis</i> | | | | | | |
| Section <i>Aulacanthus</i> | + | + | – | – | + | + |
| Section | + | | | | | + |
| <i>Buchenroedera</i> | | | | | | |
| Section <i>Digitata</i> | – | | | | | + /++ |
| Section <i>Euchlora</i> | + | + | – | – | + | + |
| Section <i>Krebsia</i> | + | | | | | + |
| Section <i>Leobordea</i> | – | | | | | + |
| Section <i>Leptis</i> | – | | | | | + |
| Section <i>Lipozygis</i> | + | + | – | – | + | + |
| Section <i>Listia</i> | – | – | – | –/+ | + | ++ |
| Section <i>Lotononis</i> | + | | | | | + |
| Section <i>Oxydium</i> | + | + | – | + | + | + |
| Section <i>Polylobium</i> | + | | | | | + |
| Section <i>Synclistus</i> | – | – | – | + | + | ++ |
| <i>Melolobium</i> | + | + | – | – | + | + |
| <i>Pearsonia</i> | + | –/+ | – | – | + | + |
| <i>Rafnia</i> | | | | | | |
| Section <i>Colobotropis</i> | – | + | – | – | + | + |
| Section <i>Rafnia</i> | + | – | – | –/+ | + | + |
| <i>Robynsiophyton</i> | – | – | + | – | + | ++ |
| <i>Rothia</i> | – | – | – | – | + | ++ |
| <i>Wiborgia</i> | + | – | – | + | – | + |
| <i>Wiborgiella</i> | + | – | –/+ | –/+ | + | + /+++ |
| <i>Wiborgiella inflata</i> | + | – | – | + | + | +++ |
| <i>Wiborgiella vlokii</i> | + | – | + | – | + | + |

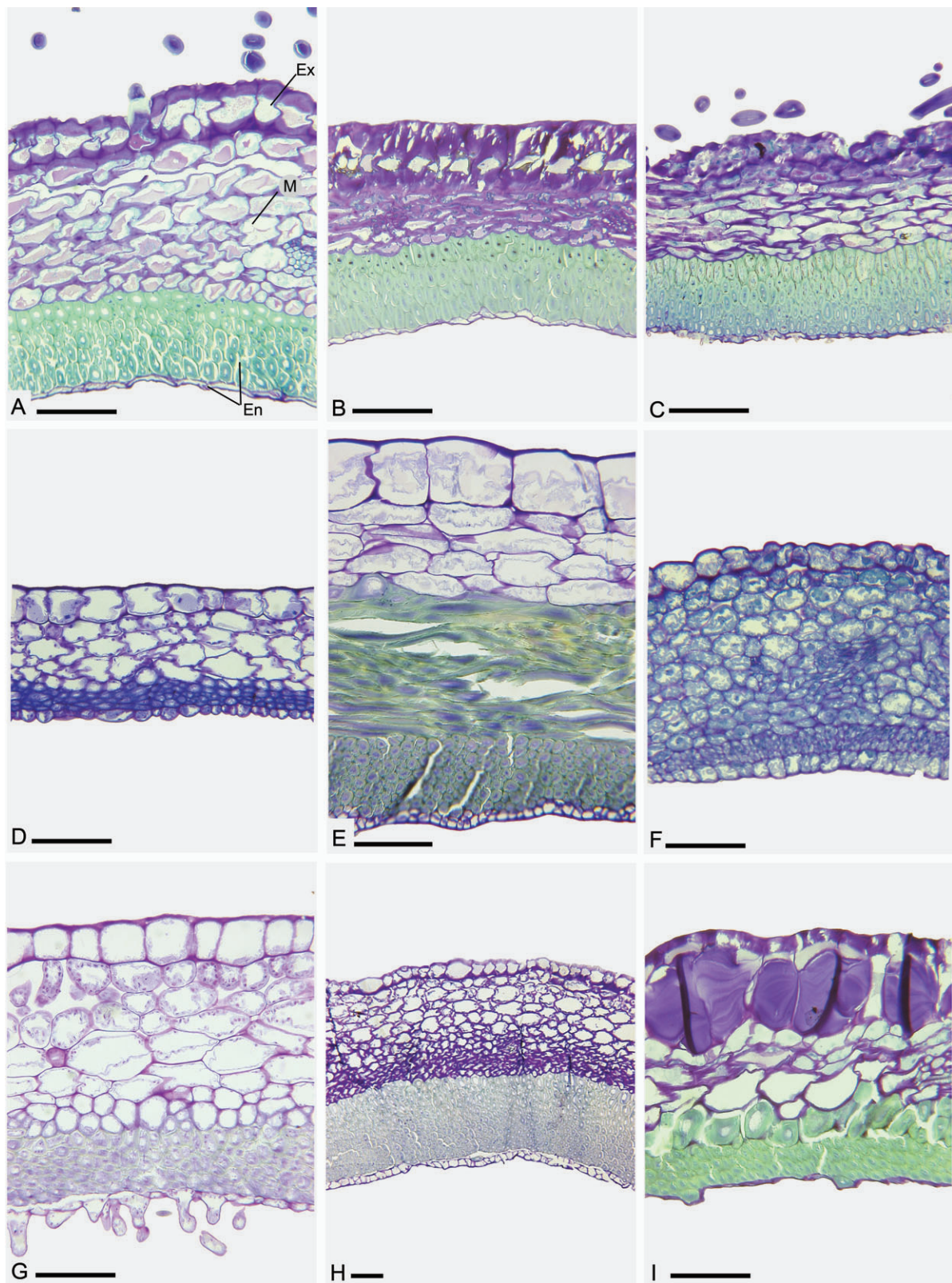


Figure 5. Transverse sections of fruits in Crotalariaeae, all with a type I pericarp: A, *Aspalathus teres* [Van Wyk 1329 (JRAU)]; B, *A. linearis* [Van Wyk 3617 (JRAU)]; C, *A. spinosa* [Van Wyk 2935 (JRAU)]; D, *Wiborgia monopectera* [Boatwright et al. 152 (JRAU)]; E, *W. sericea* [Boatwright et al. 124 (JRAU)]; F, *Wiborgiella humilis* [Boatwright et al. 212 (JRAU)]; G, *W. inflata* [Johns 162 (JRAU)]; H, *W. bowieana* [Streicher s.n. sub Schutte 831 (JRAU)]; I, *W. vlokii* [Vlok 2045 (PRE)]. Pericarp layers: Exocarp (Ex); Mesocarp (M); Endocarp (En). Scale bar, 0.1 mm.

Lebeckia: Fruits thin- to thick-walled (Fig. 7A–C). *Exocarp*: Epidermal cells with slightly thickened cell walls; mucilage cells present. *Mesocarp*: Parenchyma and collenchyma cells present. *Endocarp*: A single cell layer of normal fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent. *L. wrightii* Bolus (Fig. 7C) has a type I pericarp structure. It differs from the other *Lebeckia* spp. in having only collenchyma cells present in the mesocarp and one zone of multiple numbers of cell layers of fibres and trichomes in the endocarp.

Rafnia: Fruits thin- to thick-walled (Fig. 7D, E). *Exocarp*: Epidermal cells with slightly to highly thickened cell walls; mucilage cells absent. *Mesocarp*: Parenchyma and collenchyma cells present or only collenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres arranged in one direction, parallel or at a 45° angle to the longitudinal axis of the fruits; trichomes absent. *Rafnia amplexicaulis* Thunb. has fibre cells that are only slightly lignified (Fig. 7D).

LOTONONIS CLADE – TYPE I AND TYPE II FRUIT WALLS ARE PRESENT

Lotononis: Fruits thin- to thick-walled (Figs 7F–I, 8A–C and 9A). *Exocarp*: Epidermal cells with slightly to highly thickened cell walls; mucilage cells usually absent. *Mesocarp*: Parenchyma and collenchyma cells present or only collenchyma cells present. *Endocarp*: One or rarely two zones of various numbers of cell layers of fibres or a single cell layer of normal fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent. *Lotononis macrocarpa* Eckl. & Zeyh. (Fig. 7F) has slightly lignified fibres and forms part of the ‘Cape’ clade. *Lotononis hirsute* Schinz (Fig. 9A) forms part of the *Crotalaria* clade and has a type I fruit wall structure.

Pearsonia: Fruits thick-walled (Fig. 8D–F). *Exocarp*: Epidermal cells with slightly to highly thickened cell walls; mucilage cells absent. *Mesocarp*: Only collenchyma cells present. *Endocarp*: One zone of various numbers of cell layers of fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

Rothia: Fruits thin-walled (Fig. 8G–H). *Exocarp*: Epidermal cells with slightly thickened cell walls; mucilage cells usually absent. *Mesocarp*: Only collenchyma cells present. *Endocarp*: A single cell layer of normal fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

Robynsiophyton: Fruits thin-walled (Fig. 8I). *Exocarp*: Epidermal cells with slightly thickened cell walls; mucilage cells present. *Mesocarp*: Only collenchyma cells present. *Endocarp*: Usually a single cell layer of normal fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

CROTALARIA CLADE – TYPE I FRUIT WALLS ARE PRESENT

Crotalaria: Fruits usually thick-walled (Figs 9B–I and 10A–F). *Exocarp*: Epidermal cells with slightly to highly thickened cell walls; mucilage cells sometimes present. *Mesocarp*: Parenchyma cells rarely present, collenchyma cells present. *Endocarp*: One, two or three zones of various numbers of cell layers of fibres arranged in one direction, parallel or two directions, parallel and perpendicular to the longitudinal axis of the fruits; trichomes occasionally present.

Bolusia: Fruits thick-walled (Fig. 10G). *Exocarp*: Epidermal cells with somewhat thickened cell walls; mucilage cells absent. *Mesocarp*: Only collenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

GENISTEAE – TYPE I FRUIT WALLS ARE PRESENT

Melolobium: Fruits thick-walled (Fig. 10H). *Exocarp*: Epidermal cells with highly thickened cell walls; mucilage cells absent. *Mesocarp*: Only collenchyma cells present. *Endocarp*: One zone of various numbers of cell layers of fibres arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

Dichilus: Fruits thick-walled (Fig. 10I). *Exocarp*: Epidermal cells with somewhat thickened cell walls; mucilage cells absent. *Mesocarp*: Parenchyma and collenchyma cells present. *Endocarp*: One or two zones of various numbers of cell layers of fibres

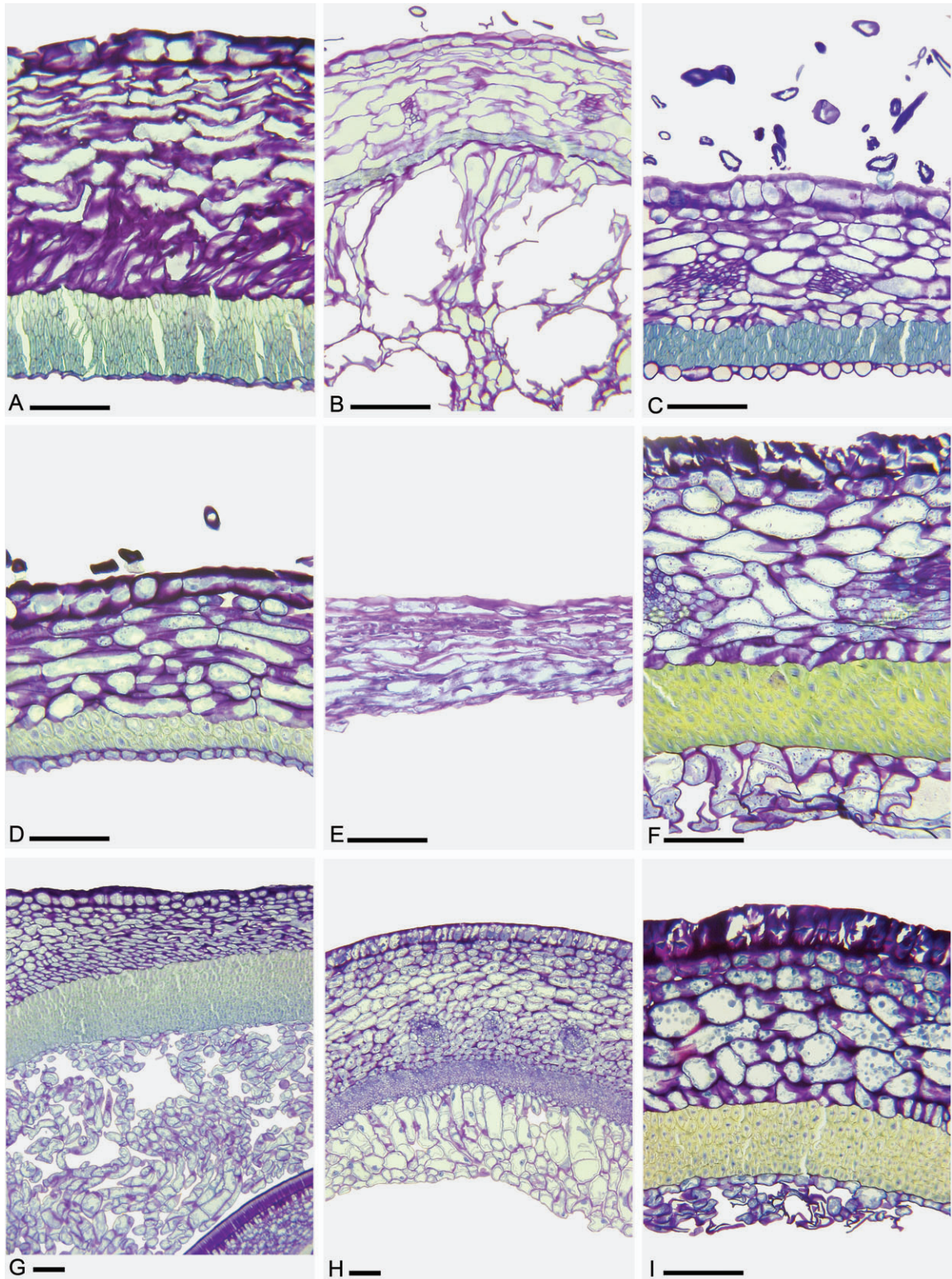


Figure 6. Transverse sections of fruits in Crotalariaeae with a type I pericarp (A–F) and a type III pericarp (G–I): A, *Calobota linearifolia* [Giess *et al.* 6180 (WIND)]; B, *C. elongata* [Van Wyk 2562b (JRAU)]; C, *C. pungens* [Boatwright *et al.* 106 (JRAU)]; D, *C. spinescens* [Boatwright *et al.* 158 (JRAU)]; E, *C. saharae* [Davies 49564 (K)]; F, *C. lotononoides* [Boatwright *et al.* 142 (JRAU)]; G, *C. sericea* [Boatwright *et al.* 151 (JRAU)]; H, *C. cytisoides* [Boatwright *et al.* 114 (JRAU)]; I, *C. halenbergensis* [Boatwright *et al.* 149 (JRAU)]. Scale bar, 0.1 mm.

arranged in one direction, parallel to the longitudinal axis of the fruits; trichomes absent.

DISCUSSION

Fruit morphology is variable and characters sometimes have diagnostic value at the generic level. Examples are the indehiscent, winged, samara-like fruits of *Wiborgia* (Van Wyk, 2005; Boatwright *et al.*, 2009) and the inflated and usually dehiscent fruits of *Crotalaria* (Polhill, 1982). Detailed studies by Polhill (1976) and a taxonomic review by Van Wyk (1991) have shown that genera can usually only be identified by a combination of characters and that fruit characters on their own have limited value. However, the present results have contributed to a better understanding of the relation between form and function in the fruits of *Crotalaria* and related genera.

The anatomy of the pericarp is too conservative to be used diagnostically at the generic level. However, some trends were observed and three basic pericarp types were identified within the tribe. The distribution of these types across the tribe is somewhat congruent with the current phylogenetic analyses, as shown in Figure 1. Note that type I is predominant, type II occurs in three independent lineages (*Lebeckia*, *Leobordea-Listia* and *Rothia-Robynsiophyton*) and type III is found in only two genera (*Calobota* and *Wiborgiella*, albeit only in some species).

'CAPE' CLADE

Aspalathus (Fig. 5A–C), *Wiborgia* (Fig. 5D–F), *Wiborgiella* (Fig. 5G–I), *Rafnia* (Fig. 7D–E) and some species of *Calobota* all have a type I pericarp, and *Lebeckia* spp. (Fig. 7A–C), with the exception of *L. wrightii*, all have a type II pericarp. *Lebeckia wrightii* is a short-lived fireweed with several unusual morphological features, such as stipules, spirally twisted keel petals and black seeds (Le Roux & Van Wyk, 2009). A comparison with its close relative, *L. uniflora* B.-E. van Wyk & M.M. le Roux, may yield interesting results. *Aspalathus* seemingly has a uniform pericarp structure (type I), despite the large number of variable species in the genus. No diagnostic differences were observed among species from different groups within the genus. *Wiborgiella humilis* (Fig. 5F) was transferred to the genus *Wiborgiella* based on molecular data, together with fruit and androecial characters to

support the generic change (Boatwright *et al.*, 2009; Boatwright, Tilney & Van Wyk, 2010). The present fruit anatomical study revealed that there are multiple cell layers of only somewhat lignified cells as opposed to the thick-walled fibres of *Wiborgiella* and *Wiborgia*. Of particular interest is the discovery of an unexpected anatomical difference between *Wiborgiella inflata* and *W. vlokii*, two closely related and anomalous species. They are the only short-lived perennials in the genus and also differ in having gelatinous fibres (elsewhere found only in *Calobota* spp.). Furthermore, *W. vlokii* differs from *W. inflata* in the absence of the endodermal trichomes that are invariably associated with gelatinous fibres in other species investigated. In *Calobota* (Fig. 6A–I) both type I and type III pericarps are found. This is the only other genus in which gelatinous fibres (present in type III) are present and correlates with the two informal groups found within the genus. The one group has thin-walled fruits (type I pericarp) and the second has thick-walled fruits with trichomes associated with the endocarp (type III pericarp), with the exception of *C. elongata* (Thunb.) Boatwr. & B.-E. van Wyk and *C. namibensis* Boatwr. & B.-E. van Wyk, which have type I pericarps. *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E. van Wyk (Fig. 6E) has two cell layers of only somewhat lignified cells and has a type I pericarp.

LOTONONIS CLADE

Recent molecular studies (Boatwright *et al.*, 2008a, in press) have indicated that *Lotononis* is polyphyletic and three clades were identified (Fig. 1): (1) the *Lotononis s.s.* clade (*L.* section *Lotononis* and allies); (2) the *Leobordea* clade [*L.* section *Leobordea* and allies]; and (3) the *Listia* clade (*L.* section *Listia*). These three clades are now considered to be distinct at generic level and formal new circumscriptions were proposed by Boatwright *et al.* (in press), respectively, as '*Lotononis*', '*Leobordea*' and '*Listia*'. The *Lotononis s.s.* clade (Fig. 7G–H) has a type I pericarp, the *Leobordea* clade (Figs 7I and 8A) has both type I and type II pericarps and the *Listia* clade has exclusively type II pericarps (Fig. 8B, C). Sections within the *Leobordea* clade can be recognized using pericarp structure; for example, all species of section *Synclistus* have a type II pericarp. Compared with *Aspalathus*, that has a rather uniform pericarp structure throughout, differences within the *Leobordea* clade are diagnostically

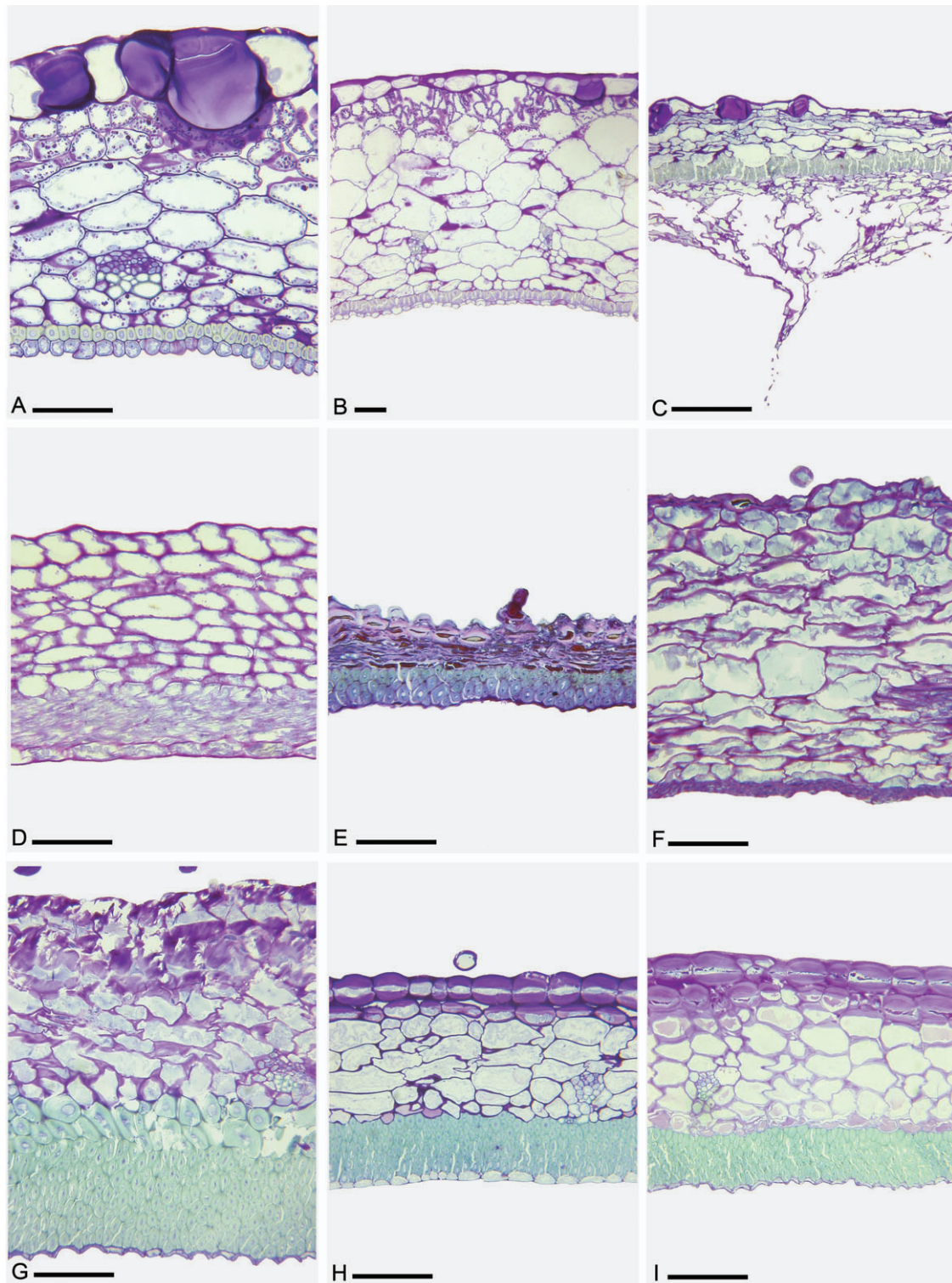


Figure 7. Transverse sections of fruits in Crotonaleae with a type II pericarp (A–B) and a type I pericarp (C–I): A, *Lebeckia contaminata* [Le Roux et al. 16 (JRAU)]; B, *L. pauciflora* [Le Roux et al. 12 (JRAU)]; C, *L. wrightii* [Johns 163 (JRAU)]; D, *Rafnia amplexicaulis* [Campbell et al. 40 (JRAU)]; E, *R. capensis* [Campbell et al. 11 (JRAU)]; F, *Lotononis macrocarpa* [Schlechter 4925 (NBG)]; G, *L. densa* [Van Wyk 3122 (JRAU)]; H, *L. lenticula* [Schutte 300 (JRAU)]; I, *L. eriantha* [Van Wyk 2631b (JRAU)]. Scale bar, 0.1 mm.

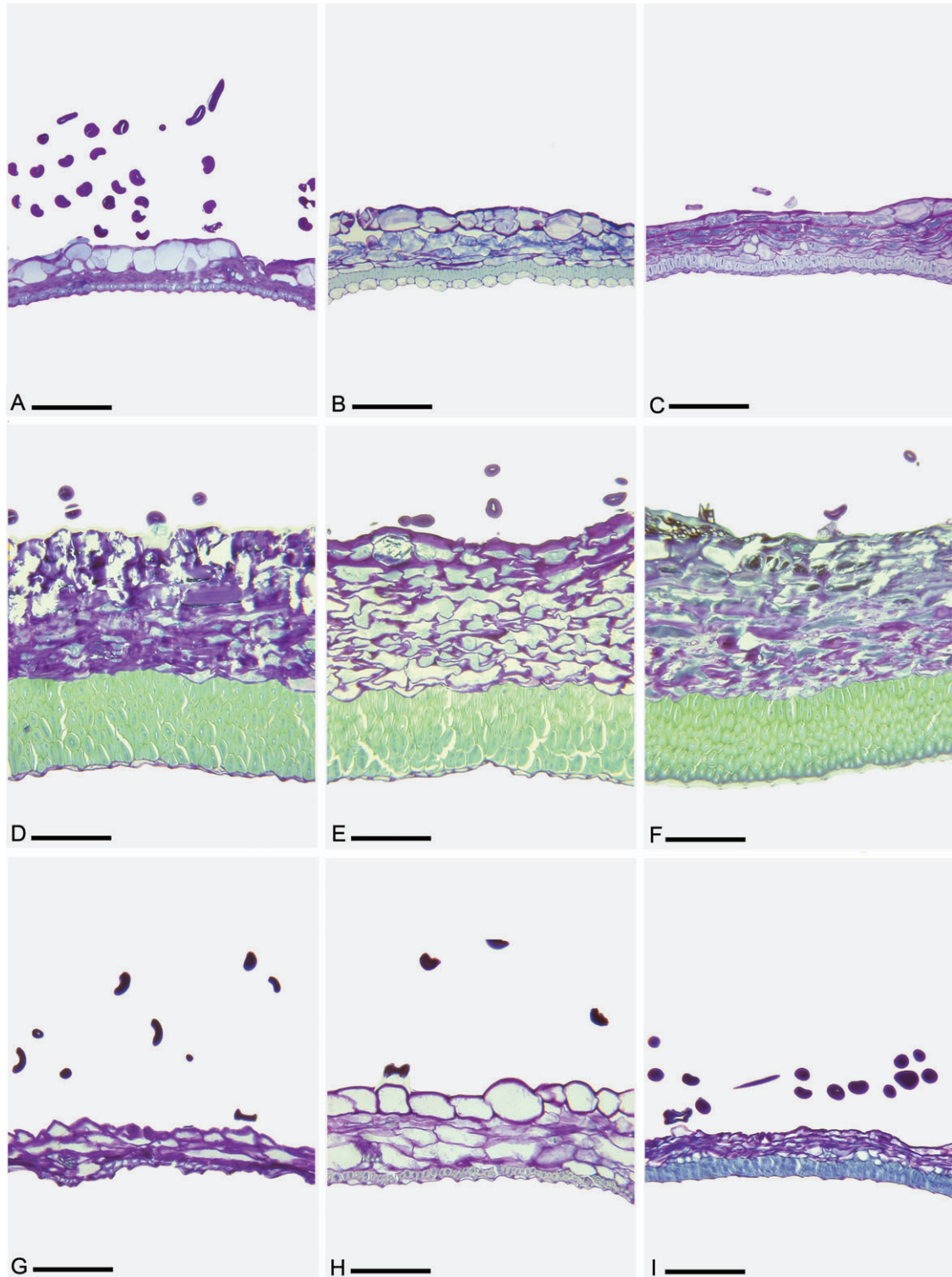


Figure 8. Transverse sections of fruits in Crotalariaeae with a type II pericarp (A–C, G–I) and a type I pericarp (D–F): A, *Lotononis globulosa* [Van Wyk 2211 (JRAU)]; B, *L. listii* [Schutte 354 (JRAU)]; C, *L. subulata* [Van Wyk 2884 (JRAU)]; D, *Pearsonia sessilifolia* [Van Wyk 3192 (JRAU)]; E, *P. cajanifolia* [Posthumus 1a (JRAU)]; F, *P. aristata* [De Castro 346 (JRAU)]; G, *Rothia hirsuta* [Bogdon 2205 (K)]; H, *R. indica* [Latz 16126 (MEL)]; I, *Robynsiophyton vanderystii* [Lisowski 20326 (K)]. Scale bar, 0.1 mm.

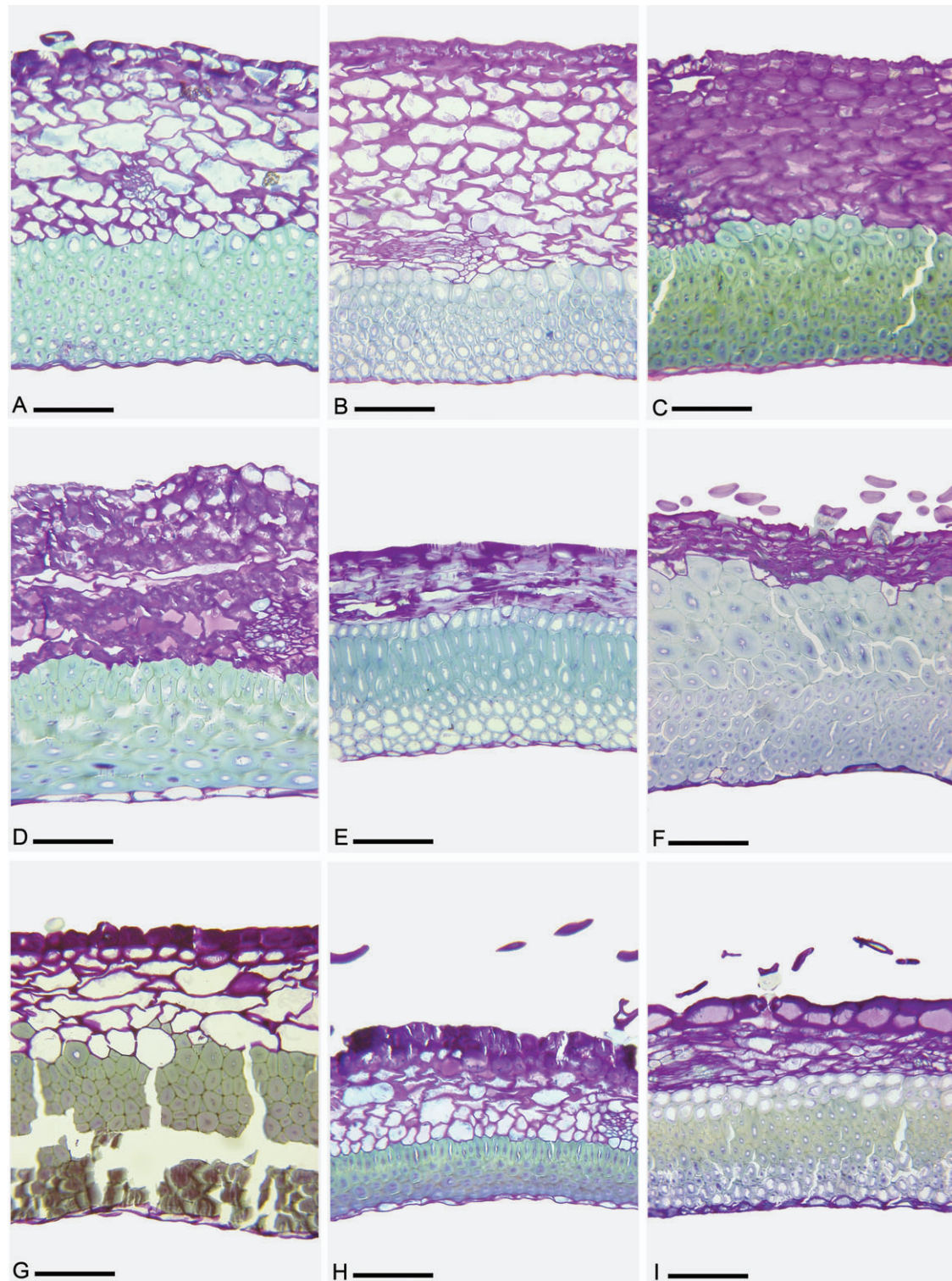


Figure 9. Transverse sections of fruits in Crotalariaeae, all with a type I pericarp: A, *Lotononis hirsuta* [Van Wyk 1338 (JRAU)]; B, *Crotalaria doidgeae* [Viljoen 52 (PRE)]; C, *C. lebeckioides* [Van Wyk 3315 (JRAU)]; D, *C. burkeana* [Nienaber EN 126 (PRE)]; E, *C. natalitia* [Mogg 34335 (JRAU)]; F, *C. dura* [Ward 11910 (PRE)]; G, *C. piscarpa* [Klaasen *et al.* 107 (WIND)]; H, *C. burtii* [Grundy L96 (PRE)]; I, *C. pallida* [Germishuizen 1146 (PRE)]. Scale bar, 0.1 mm.

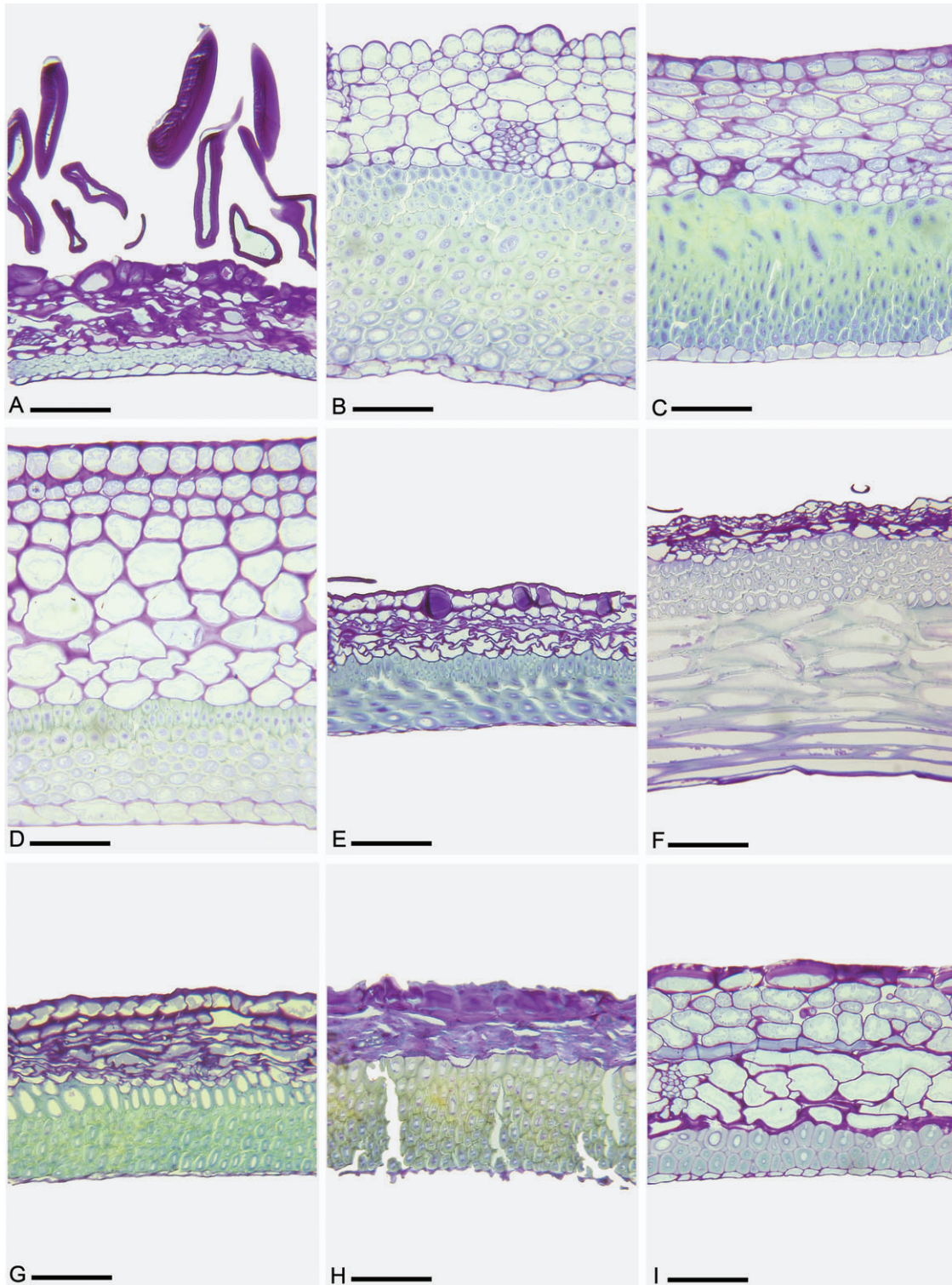


Figure 10. Transverse sections of fruits in Crotonaceae (A–G) and Genisteae (H–I), all with a type I pericarp: A, *Crotalaria vasculosa* [De Winter 9460 (PRE)]; B, *C. virgultalis* [Le Roux 38 (JRAU)]; C, *C. spartioides* [Le Roux et al. 84 (WIND)]; D, *C. recta* [Le Roux 42 (JRAU)]; E, *C. humilis* [Thorne 52437 (NBG)]; F, *C. globifera* [Pienaar 532 (PRE)]; G, *Bolusia amboensis* [Boatwright et al. 248 (WIND)]; H, *Melolobium alpinum* [Schutte 160 (JRAU)]; I, *Dichilus strictus* [Schutte 376 (JRAU)]. Scale bar, 0.1 mm.

useful at an infrageneric level. Species of the *Leobordea* clade are more widely distributed and adaptations to various habitats could account for the observed variation. *Lotononis macrocarpa* (Fig. 7F), an anomalous species of the genus, is placed closer to the 'Cape' group of *Crotalarieae* (Boatwright *et al.*, in press) and has a type I pericarp with multiple cell layers of not highly sclerified cells in the endocarp, which differs from *Lotononis s.s. Rothia* and *Robynsiophyton* have been reported to be closely related to *Pearsonia* (Van Wyk, 1991), which is supported by molecular (Boatwright *et al.*, 2008a) and morphological data (Boatwright, Tilney & Van Wyk, 2008c). A sister relationship between *Rothia* and *Robynsiophyton* (and their generic status, i.e. being distinct from *Pearsonia*), as indicated by Boatwright & Van Wyk (2009) and Boatwright *et al.* (2008a), is here also supported by the fruit anatomy.

CROTALARIA CLADE

The taxa of the earliest diverging clade of *Crotalarieae*, *Lotononis hirsuta* (Fig. 9A), *Crotalaria* (Figs 9B–I and 10A–F) and (3) *Bolusia*, all have a type I pericarp and strongly inflated pods. Molecular and morphological data (Boatwright *et al.*, 2008a, in press) indicated that *L. hirsuta* should be excluded from the genus *Lotononis* and its placement within the *Crotalaria* clade is also supported by the fruit anatomy.

It is difficult to identify apomorphies for the sections within *Crotalaria*. Species from sections *Grandiflorae* (Bak.f.) Polhill and *Geniculatae* Polhill show some uniformity in the pericarp thickness, ratio of the pericarp layers and fibre orientation (Figs 9B, C and 10B, C). These pericarp characters are also similar to those of some individual species from other sections within *Crotalaria* (e.g. *C. recta* Steud. ex A.Rich., Fig. 10D) and other genera, e.g. *Aspalathus* (Fig. 5A–C) and *Pearsonia* (Fig. 8D–F). Other sections in *Crotalaria* display too much variation and it is difficult to find characters to use as apomorphies for specific groups; for example, section *Crotalaria* (Fig. 10C–F) or section *Hedriocarpae* (Figs 9I and 10A). In these cases, fruit characters could be useful diagnostically at the species level.

Fahn & Zohary (1955) considered the presence of multiple cell layers of fibres to be the primitive state in legume fruit and its loss to be derived. The type I pericarp (present in the early diverging lineages of the tribe) therefore represents the most primitive state, whereas the type II, as found in the *Listia* clade, is clearly derived. The plesiomorphic state is also present in *Melolobium* and *Dichilus* (Fig. 10H and I, respectively) of *Genisteae*.

DEHISCENCE

The mechanism of dehiscence and the function of fibre orientation in this process were described by Fahn & Zohary (1955) and several variations of the basic dehiscence model, which can exist within one genus (Fahn & Zohary, 1955; Fahn, 1982). In the type I pericarp, all fibres within a single zone are generally arranged in one direction parallel to the longitudinal axis of the fruits. When the fruits mature, the cells dry out and the cell walls shrink in a perpendicular direction to the longitudinal axis, creating tension and resulting in dehiscence (Fig. 10A–C). Fibres may also be directed at a 45° angle to the longitudinal axis, and the elongated epidermal and hypodermal cells of the exocarp and mesocarp are also orientated at a 45° angle, but in the opposite direction to the fibres. This arrangement of fibres results in the twisting of the valves and the dehiscence of the fruits. Fibre cell layers with different cell wall thicknesses can be arranged in two or more zones. When these cells dry out, each zone creates a different strength of tension, resulting in dehiscence (Fig. 9E, I). Indehiscent fruits appear to have two zones; an outer zone of various numbers of cell layers of fibres oriented parallel to the longitudinal axis and an inner zone of various numbers of cell layers of fibres oriented perpendicular to the longitudinal axis (Figs 9G and 10F).

In the type III pericarp, fibres (all forming one zone) are mostly arranged in a single direction parallel to the longitudinal axis, resulting in dehiscence (Fig. 6G–I). In type II, the fibres are all orientated in the same direction in a single layer of fibres, parallel to the longitudinal axis, and the fruits are sometimes dehiscent.

There are some exceptions to the dehiscence or indehiscence model. Other factors that could influence dehiscence are fruit morphology and additional anatomical features. These modifications were probably necessitated by a need for seed protection and dispersal. Type II pericarp fruits should dehisce, for example, *Lotononis globulosa* B.-E.van Wyk (Fig. 8A), but stay intact. This could be because of the presence of large epidermal cells in the exocarp, which could absorb some of the tension created by the single layer of fibres. *Wiborgia monoptera* E.Mey. is an example of an indehiscent fruit with a type I pericarp. The fruits have multiple layers of fibres orientated at 45° angles to the longitudinal axis and should dehisce; the tension created by the shrinking fibres is possibly absorbed and counteracted by the presence of a wing along the zone of dehiscence.

CONCLUSIONS

Fruit morphology in *Crotalarieae* is diverse and of limited systematic value. However, there are conver-

gent trends in the size, shape and general morphology that can be linked to dispersal and seed protection. In general, the inflated, balloon-shaped fruits typical of *Crotalaria* are a useful diagnostic character and represent the ground plan for further structural modifications seen in other genera of the tribe.

Three different pericarp types are recognized within the tribe: (1) type I, with one, two or three zones of various numbers of cell layers of normal fibres and trichomes occasionally associated with the endocarp; (2) type II, with a single cell layer of normal fibres and (3) type III, with one zone of several cell layers of gelatinous fibres and trichomes associated with the endocarp. The type I pericarp is most widely distributed throughout the tribe. There are some potentially useful generic apomorphies. The lack of monophyly of both *Lebeckia s.l* and *Lotononis s.l.* is supported by the results presented here. Species of *Lebeckia sensu stricto* (Le Roux & Van Wyk, 2009) almost invariably have a type II pericarp, whereas types I and III are present in the segregate genera *Wiborgiella* and *Calobota* (Boatwright *et al.*, 2009, 2010). Species of *Lotononis s.s.* have type I pericarps, whereas the section *Listia* invariably has type II. Although all *Crotalaria* spp. have a type I pericarp, there is some structural variation that may be of value in distinguishing between some of the species. There are no apparent discontinuities between any of the sections.

An example of non-homologous similarity was revealed by comparing pericarp structure of the small, indehiscent, globose and few-seeded fruits (presumably all wind or water dispersed) that are found in species of *Crotalaria*, *Lotononis* section *Synclistus* and one species of *Calobota* (*C. elongata*). These have types I, II and III pericarps, respectively. Also noteworthy is the fact that this seemingly identical dispersal mechanism has evolved independently in each of the three main clades in the tribe.

The pattern of dehiscence and indehiscence in *Crotalaria* and other genera of the tribe seems to conform to the general pattern reported for other legume genera. The orientation of the fibres in the endocarp relative to the longitudinal axis of the fruits is the major determinant, but the presence of different zones of fibres and their orientation relative to one another are also important. Our study has revealed other morphological and anatomical features that may counteract dehiscence, such as the presence of wing tissue along the line of the suture (e.g. *Wiborgia*) or the relative thickness of the single fibre cell layer in relation to the adjoining mesocarp tissue, which seems to be independent of the absolute thickness, or the fibre cell layer (thick in, e.g. *Lebeckia brevicarpa* M.M.le Roux & B.-E.van Wyk, but thin in *Lotononis* section *Synclistus*). Fruit anatomical characters are of potential value not only for new taxonomic interper-

tations but also to deepen our understanding of various morphological and functional adaptations in tribe Crotalarieae.

ACKNOWLEDGEMENTS

The University of Johannesburg and the National Research Foundation are gratefully acknowledged for financial support. The staff and curators of BOL, JRAU, K, MEL, NBG, PRE, UPS and WIND are thanked for their assistance and loan of material.

REFERENCES

- Bisby FA. 1973.** The role of taximetrics in angiosperm taxonomy I. Empirical comparisons of methods using *Crotalaria* L. *New Phytologist* **72**: 699–726.
- Bisby FA, Polhill RM. 1973.** The role of taximetrics in angiosperm taxonomy II. Parallel taximetric and orthodox studies in *Crotalaria* L. *New Phytologist* **72**: 727–742.
- Boatwright JS, Le Roux MM, Wink M, Morozova T, Van Wyk B-E. 2008a.** Phylogenetic relationships of the tribe Crotalarieae (Fabaceae) inferred from DNA sequences and morphology. *Systematic Botany* **33**: 752–761.
- Boatwright JS, Savolainen V, Van Wyk B-E, Schutte-Vlok AL, Forest F, Van der Bank M. 2008b.** Systematic position of the anomalous genus *Cadia* and the phylogeny of the tribe Podalyrieae. *Systematic Botany* **33**: 133–147.
- Boatwright JS, Tilney PM, Van Wyk B-E. 2008c.** A taxonomic revision of the genus *Rothia* (Crotalarieae, Fabaceae). *Australian Systematic Botany* **21**: 422–430.
- Boatwright JS, Tilney PM, Van Wyk B-E. 2009.** The generic concept of *Lebeckia* (Crotalarieae, Fabaceae): reinstatement of the genus *Calobota* and the new genus *Wiborgiella*. *South African Journal of Botany* **75**: 546–556.
- Boatwright JS, Tilney PM, Van Wyk B-E. 2010.** Taxonomy of *Wiborgiella* (Crotalarieae, Fabaceae), a genus endemic to the Greater Cape Region of South Africa. *Systematic Botany* **35**: 325–340.
- Boatwright JS, Van Wyk B-E. 2009.** A revision of the African genus *Robynsiophyton* (Crotalarieae, Fabaceae). *South African Journal of Botany* **75**: 367–370.
- Boatwright JS, Wink M, Van Wyk B-E. in press.** The generic concept of *Lotononis* (Crotalarieae, Fabaceae): reinstatement of the genera *Euchlora*, *Leobordea* and *Listia* and the new genus *Ezoloba*. *Taxon* (in press).
- Crisp MD, Gilmore S, Van Wyk B-E. 2000.** Molecular phylogenetics of the genistoid tribes of papilionoid legumes. In: Herendeen PS, Bruneau A, eds. *Advances in legume systematics* 9. Kew: Royal Botanic Gardens, 249–276.
- Esau K. 1962.** *Anatomy of seed plants*. New York, London: John Wiley and Sons Inc., 316–320.
- Fahn A. 1967.** *Plant anatomy*. Oxford: Pergamon Press, 439–449.
- Fahn A. 1982.** *Plant anatomy*, 3rd edn. Oxford: Pergamon Press, 457–471.

- Fahn A, Zohary M. 1955.** On the pericarpial structure of the legumen, its evolution and relation to dehiscence. *Phytomorphology* **5**: 99–111.
- Feder N, O'Brien TP. 1968.** Plant microtechnique: some principles and new methods. *American Journal of Botany* **55**: 123–142.
- Kirkbride JH Jr, Gunn C, Weitzman AL. 2003.** Fruits and seeds of genera in the subfamily Faboideae (Fabaceae). *Technical Bulletin* **1890**: 1–115, 1030–1055.
- Le Roux MM, Van Wyk B-E. 2009.** A revision of *Lebeckia* sect. *Lebeckia*: the *L. pauciflora* and *L. wrightii* groups (Fabaceae, Crotalariaeae). *South African Journal of Botany* **75**: 83–96.
- Pate JS, Kuo J. 1981.** Anatomical studies of legume pods – a possible tool in taxonomic research. In: Polhill RM, Raven PH, eds. *Advances in legume systematics*, part 2. Kew: Royal Botanic Gardens, 903–912.
- Polhill RM. 1968.** Miscellaneous notes on African species of *Crotalaria* L. II. *Kew Bulletin* **22**: 169–348.
- Polhill RM. 1976.** Genisteeae (Adans.) Benth. and related tribes (Leguminosae). *Botanical Systematics* **1**: 143–368.
- Polhill RM. 1982.** *Crotalaria in Africa and Madagascar*. Rotterdam: AA Balkema Publishers.
- Van Wyk B-E. 1991.** A review of the tribe Crotalariaeae (Fabaceae). *Contributions from the Bolus Herbarium* **13**: 265–288.
- Van Wyk B-E. 2005.** Crotalariaeae. In: Lewis G, Schrire B, Mackinder B, Lock M, eds. *Legumes of the world*. Kew: Royal Botanic Gardens, 273–281.
- Van Wyk B-E, Schutte AL. 1995.** Phylogenetic relationships in the tribes Podalyrieae, Liparieae and Crotalariaeae. In: Crisp M, Doyle J, eds. *Advances in legume systematics* 7. Kew: Royal Botanic Gardens, 283–308.
- Wink M, Mohamed GIA. 2003.** Evolution of chemical defence traits in the Leguminosae: mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the *rbcL* gene. *Biochemical Systematics and Ecology* **31**: 897–917.

APPENDIX
LIST OF FRUIT PERICARP TYPES AND VOUCHER SPECIMENS USED FOR FRUIT ANATOMICAL STUDIES IN THE TRIBE CROTALARIEAE.
THE SPECIES ARE ARRANGED ACCORDING TO THE PHYLOGENY OF BOATWRIGHT *ET AL.* (2008A)

| Species | Infrageneric group (if applicable) | Voucher specimen | Pericarp thickness (mm) | Fruit classification | Dehiscence |
|--|--|-------------------------------------|-------------------------|----------------------|-------------------|
| <i>Aspalathus angustifolia</i> (Lam.) Dahlg. | | <i>Van Wyk 2592</i> (JRAU) | 182 | Type I | Dehiscent |
| <i>Aspalathus bracteata</i> Thunb. | | <i>Van Wyk 914</i> (JRAU) | 169 | Type I | Dehiscent |
| <i>Aspalathus carnososa</i> Berg. | | <i>Van Wyk 3006</i> (JRAU) | 240 | Type I | Dehiscent |
| <i>Aspalathus cf. hirta</i> E.Mey. | | <i>Van Wyk 3295</i> (JRAU) | 353 | Type I | Dehiscent |
| <i>Aspalathus chortophila</i> Eckl. & Zeyh. | | <i>Van Wyk et al. 831</i> (JRAU) | 235 | Type I | Dehiscent |
| <i>Aspalathus intermedia</i> Eckl. & Zeyh. | | <i>Schutte 522</i> (JRAU) | 219 | Type I | Dehiscent |
| <i>Aspalathus juniperina</i> Thunb. subsp. <i>juniperina</i> | | <i>Van Wyk 2756</i> (JRAU) | 339 | Type I | Dehiscent |
| <i>Aspalathus lactea</i> Thunb. subsp. <i>adelphaea</i> (Eckl. & Zeyh.) Dahlg. | | <i>Van Wyk et al. 1564</i> (JRAU) | 266 | Type I | Tardily dehiscent |
| <i>Aspalathus linearis</i> (Burm.f.) Dahlg. | | <i>Van Wyk 3617</i> (JRAU) | 262 | Type I | Dehiscent |
| <i>Aspalathus longifolia</i> Benth. | | <i>Van Wyk 2799</i> (JRAU) | 452 | Type I | Dehiscent |
| <i>Aspalathus pendula</i> Dahlg. | | <i>Van Wyk 3346</i> (JRAU) | 530 | Type I | Dehiscent |
| <i>Aspalathus perfoliata</i> (Lam.) Dahlg. | | <i>Van Wyk 2786</i> (JRAU) | 417 | Type I | Dehiscent |
| <i>Aspalathus spinosa</i> L. | | <i>Van Wyk 2935</i> (JRAU) | 273 | Type I | Dehiscent |
| <i>Aspalathus teres</i> Eckl. & Zeyh. | | <i>Van Wyk et al. 1329</i> (JRAU) | 405 | Type I | Dehiscent |
| <i>Bolusia amboensis</i> (Schinz) Harms | | <i>Boatwright et al. 248</i> (WIND) | 233 | Type I | Dehiscent |
| <i>Calobota angustifolia</i> (E.Mey.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 138</i> (JRAU) | 385 | Type III | Dehiscent |
| <i>Calobota cinerea</i> (E.Mey.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 150</i> (JRAU) | 491 | Type III | Dehiscent |
| <i>Calobota cuspidosa</i> (Burch.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 92</i> (JRAU) | 265 | Type I | Indehiscent |
| <i>Calobota cypoides</i> (Berg.) Eckl. & Zeyh. | | <i>Boatwright et al. 114</i> (JRAU) | 506 | Type III | Dehiscent |
| <i>Calobota elongata</i> (Thunb.) Boatwr. & B.-E.van Wyk | | <i>Van Wyk 2562b</i> (JRAU) | 203 | Type I | Indehiscent |
| <i>Calobota halenbergensis</i> (Merxm. & Schreieb.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 149</i> (JRAU) | 328 | Type III | Dehiscent |
| <i>Calobota linearifolia</i> (E.Mey.) Boatwr. & B.-E.van Wyk | | <i>Gies et al. 6180</i> (WIND) | 270 | Type I | Dehiscent |
| <i>Calobota lotononoides</i> (Schltr.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 142</i> (JRAU) | 397 | Type III | Dehiscent |
| <i>Calobota namibensis</i> Boatwr. & B.-E.van Wyk, ined. | | <i>De Winter et al. 7919</i> (WIND) | | Type I | ?Dehiscent |
| <i>Calobota psiloba</i> (E.Mey.) Boatwr. & B.-E.van Wyk | | <i>Le Roux et al. 20</i> (JRAU) | 291 | Type I | Indehiscent |
| <i>Calobota pungens</i> (Thunb.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 106</i> (JRAU) | 305 | Type I | Indehiscent |
| <i>Calobota saharae</i> (Coss. & Dur.) Boatwr. & B.-E.van Wyk | | <i>Davies 49564</i> (K) | 141 | Type I | Indehiscent |
| <i>Calobota sericea</i> (Thunb.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 151</i> (JRAU) | 479 | Type III | Dehiscent |
| <i>Calobota spinescens</i> (Harv.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 158</i> (JRAU) | 230 | Type I | Tardily dehiscent |
| <i>Crotalaria agatiflora</i> Schweinf. | Section <i>Grandiflorae</i> (Bak.f.) Polhill | <i>Le Roux 45</i> (JRAU) | 531 | Type I | Dehiscent |
| <i>Crotalaria alata</i> Ham. ex D. Don | Section <i>Calycinae</i> Wight & Arn. | <i>Ruairidhore 2628</i> (PRE) | 395 | Type I | Dehiscent |
| <i>Crotalaria alexandri</i> Bak. f. | Section <i>Dispermae</i> Wight & Arn. | <i>Pope 15</i> (PRE) | 117 | Type I | Dehiscent |
| <i>Crotalaria argyrea</i> Welw. ex Bak. | Section <i>Hedriocarpae</i> Wight & Arn. | <i>Van Slageren MSJ/B011</i> (WIND) | 334 | Type I | Indehiscent |
| <i>Crotalaria brachycarpa</i> (Benth.) Burtt Davy ex Verdoorn | Section <i>Crotalaria</i> | <i>Le Roux 49</i> (JRAU) | 771 | Type I | Indehiscent |
| <i>Crotalaria burkeana</i> Benth. | Section <i>Chrysocalycinae</i> (Benth.) Bak.f. | <i>Nienaber EN 126</i> (PRE) | 422 | Type I | Dehiscent |
| <i>Crotalaria burttii</i> Bak.f. | Section <i>Hedriocarpae</i> | <i>Grundy L96</i> (PRE) | 223 | Type I | Tardily dehiscent |
| <i>Crotalaria calycina</i> Schrank | Section <i>Calycinae</i> | <i>De Nevers et al. 3310</i> (PRE) | 359 | Type I | Dehiscent |
| <i>Crotalaria cephalotes</i> Steud. ex A.Rich. | Section <i>Dispermae</i> | <i>Schmidt et al. 1912</i> (PRE) | 338 | Type I | Dehiscent |
| <i>Crotalaria damarensis</i> Engl. | Section <i>Chrysocalycinae</i> | <i>Leippert 4608</i> (WIND) | 488 | Type I | Tardily dehiscent |
| <i>Crotalaria dinteri</i> Schinz | Section <i>Crotalaria</i> | <i>Gies 11637</i> (PRE) | 304 | Type I | Dehiscent |

APPENDIX *Continued*

| Species | Infrageneric group (if applicable) | Voucher specimen | Pericarp thickness (mm) | Fruit classification | Dehiscence |
|---|-------------------------------------|--------------------------------------|-------------------------|----------------------|----------------------------------|
| <i>Crotalaria doidgeae</i> Verdoorn | Section <i>Grandiflorae</i> | Viljoen 52 (PRE) | 437 | Type I | Dehiscent |
| <i>Crotalaria dura</i> Wood & Evans | Section <i>Chrysocalycinae</i> | Ward 11910 (PRE) | 347 | Type I | Indehiscent |
| <i>Crotalaria ephemera</i> Polhill | Section <i>Dispermae</i> | Brooks <i>et al.</i> 93 (PRE) | 183 | Type I | Tardily dehiscent |
| <i>Crotalaria excisa</i> (Thunb.) Bak.f. | Section <i>Crotalaria</i> | <i>Le Roux</i> 108 (JRAU) | 342 | Type I | Dehiscent |
| <i>Crotalaria filicaulis</i> Welw. ex Bak. | Section <i>Dispermae</i> | Teixeira <i>et al.</i> 4463 (PRE) | 207 | Type I | Indehiscent to tardily dehiscent |
| <i>Crotalaria flavicarinata</i> Bak.f. | Section <i>Geniculatae</i> Polhill | <i>Le Roux et al.</i> 72 (WIND) | 330 | Type I | Dehiscent |
| <i>Crotalaria globifera</i> E.Mey. | Section <i>Crotalaria</i> | Pienaar 532 (PRE) | 398 | Type I | Tardily dehiscent |
| <i>Crotalaria goetzei</i> Harms | Section <i>Chrysocalycinae</i> | Thulin 7826 (UPS) | 226 | Type I | Dehiscent |
| <i>Crotalaria griquensis</i> Bolus | Section <i>Crotalaria</i> | Gubb s.n. PRE 825170 (PRE) | 187 | Type I | Indehiscent |
| <i>Crotalaria heidmannii</i> Schinz | Section <i>Geniculatae</i> | <i>Le Roux et al.</i> 69 (WIND) | 502 | Type I | Dehiscent |
| <i>Crotalaria humilis</i> Eckl. & Zeyh. | Section <i>Crotalaria</i> | Thorne 52437 (NBG) | 169 | Type I | Dehiscent |
| <i>Crotalaria hyssopifolia</i> Klotzsch | Section <i>Dispermae</i> | Faulkner 208 (PRE) | 119 | Type I | Tardily dehiscent |
| <i>Crotalaria incana</i> L. | Section <i>Chrysocalycinae</i> | Thulin <i>et al.</i> 9114 (UPS) | 254 | Type I | Tardily dehiscent |
| <i>Crotalaria juncea</i> L. | Section <i>Calycinae</i> | Arnola 9991 (PRE) | 290 | Type I | Dehiscent |
| <i>Crotalaria kirikii</i> Bak. | Section <i>Hedriocarpaceae</i> | Markström <i>et al.</i> MN215A (UPS) | 232 | Type I | Dehiscent |
| <i>Crotalaria laburnifolia</i> L. | Section <i>Grandiflorae</i> | Van Wyk <i>et al.</i> 4333 (JRAU) | 441 | Type I | Dehiscent |
| <i>Crotalaria lanceolata</i> E.Mey. | Section <i>Hedriocarpaceae</i> | <i>Le Roux</i> 37 (JRAU) | 385 | Type I | Dehiscent |
| <i>Crotalaria lebechoides</i> Bond | Section <i>Grandiflorae</i> | Van Wyk 3315 (JRAU) | 397 | Type I | Dehiscent |
| <i>Crotalaria leptocarpa</i> Balf. f. | Section <i>Schizostigma</i> Polhill | Van Wyk <i>et al.</i> 4650 (JRAU) | 397 | Type I | Tardily dehiscent |
| <i>Crotalaria lotoides</i> Benth. | Section <i>Chrysocalycinae</i> | <i>Le Roux</i> 47 (JRAU) | 125 | Type I | Dehiscent |
| <i>Crotalaria mesopontica</i> Taub. | Section <i>Hedriocarpaceae</i> | Ruaburindore 2340 (UPS) | 300 | Type I | Dehiscent |
| <i>Crotalaria montanoi</i> Taub. ex Bak.f. | Section <i>Grandiflorae</i> | <i>Le Roux</i> 95 (JRAU) | 326 | Type I | Dehiscent |
| <i>Crotalaria natalitia</i> Meisner | Section <i>Chrysocalycinae</i> | <i>Le Roux</i> 34335 (JRAU) | 736 | Type I | Dehiscent |
| <i>Crotalaria occidentalis</i> Hepper | Section <i>Calycinae</i> | Mogg 3765 (PRE) | 268 | Type I | Tardily dehiscent |
| <i>Crotalaria orientalis</i> Burtt Davy ex Verdoorn | Section <i>Geniculatae</i> | <i>Le Roux et al.</i> 91 (JRAU) | 123 | Type I | Dehiscent |
| <i>Crotalaria pallida</i> Ait. | Section <i>Hedriocarpaceae</i> | Germshuizen 1146 (PRE) | 449 | Type I | Dehiscent |
| <i>Crotalaria pearsonii</i> Bak.f. | Section <i>Crotalaria</i> | Marloth 12445 (PRE) | 272 | Type I | Dehiscent |
| <i>Crotalaria pisticarpa</i> Welw. ex Bak. | Section <i>Chrysocalycinae</i> | Klaassen <i>et al.</i> 107 (WIND) | 266 | Type I | Dehiscent |
| <i>Crotalaria platysepala</i> Harv. | Section <i>Crotalaria</i> | <i>Le Roux et al.</i> 73 (WIND) | 346 | Type I | Indehiscent |
| <i>Crotalaria prithwitzii</i> Bak.f. | Section <i>Chrysocalycinae</i> | Biggood <i>et al.</i> 3597 (UPS) | 342 | Type I | Tardily dehiscent |
| <i>Crotalaria pseudotenuirama</i> Torre | Section <i>Dispermae</i> | Greenway <i>et al.</i> 11683 (PRE) | 359 | Type I | Indehiscent to tardily dehiscent |
| <i>Crotalaria recta</i> Steud. ex A.Rich. | Section <i>Crotalaria</i> | <i>Le Roux</i> 42 (JRAU) | 218 | Type I | Indehiscent to tardily dehiscent |
| <i>Crotalaria saltiana</i> Andr. | Section <i>Hedriocarpaceae</i> | Hemming 3056 (PRE) | 490 | Type I | Dehiscent |
| <i>Crotalaria somalensis</i> Chiov. | Section <i>Hedriocarpaceae</i> | Gillett 21175 (PRE) | 336 | Type I | Dehiscent |
| <i>Crotalaria spartoides</i> DC. | Section <i>Geniculatae</i> | <i>Le Roux et al.</i> 84 (WIND) | 255 | Type I | Tardily dehiscent |
| <i>Crotalaria spectabilis</i> Robt. | Section <i>Crotalaria</i> | <i>Le Roux et al.</i> 98 (JRAU) | 422 | Type I | Dehiscent |
| <i>Crotalaria sphaerocarpa</i> Perr. ex DC. | Section <i>Geniculatae</i> | <i>Le Roux et al.</i> 74 (WIND) | 596 | Type I | Dehiscent |
| <i>Crotalaria steudneri</i> Schweinf. | Section <i>Hedriocarpaceae</i> | <i>Le Roux</i> 80 (WIND) | 161 | Type I | Tardily dehiscent |
| <i>Crotalaria tenuirama</i> Welw. ex Bak. | Section <i>Dispermae</i> | Teixeira 3.391 (PRE) | 196 | Type I | Dehiscent |
| <i>Crotalaria vasculosa</i> Wall. ex Benth. | Section <i>Hedriocarpaceae</i> | De Winter 9460 (PRE) | 192 | Type I | Dehiscent |
| <i>Crotalaria virgulata</i> Klotzsch | Section <i>Crotalaria</i> | <i>Le Roux</i> 38 (JRAU) | 132 | Type I | Dehiscent |
| <i>Crotalaria xanthochlada</i> Boj. ex Benth. | Section <i>Chrysocalycinae</i> | Hedren <i>et al.</i> 665 (UPS) | 377 | Type I | Dehiscent |
| <i>Dichilus strictus</i> E.Mey. | | Schutte 376 (JRAU) | 295 | Type I | Dehiscent |
| <i>Lebeckia ambigua</i> E.Mey. | | Boatwright <i>et al.</i> 131 (JRAU) | 271 | Type I | Dehiscent |
| <i>Lebeckia brevicarpa</i> M.M.le Roux & B.-E.van Wyk | | <i>Le Roux et al.</i> 4 (JRAU) | 456 | Type II | Tardily dehiscent |
| <i>Lebeckia contaminata</i> (L.) Thunb. | | <i>Le Roux et al.</i> 16 (JRAU) | 886 | Type II | Indehiscent |
| | | | 269 | Type II | Tardily dehiscent |

APPENDIX *Continued*

| Species | Infrageneric group (if applicable) | Voucher specimen | Pericarp thickness (mm) | Fruit classification | Dehiscence |
|---|------------------------------------|--|-------------------------|----------------------|-------------|
| <i>Rafnia amplexicaulis</i> Thunb. | Section <i>Rafnia</i> | <i>Campbell et al. 40</i> (JRAU) | 305 | Type I | Dehiscent |
| <i>Rafnia capensis</i> (L.) Schinz | Section <i>Colobotropis</i> E.Mey. | <i>Campbell et al. 11</i> (JRAU) | 115 | Type I | Dehiscent |
| <i>Rafnia rostrata</i> G.J.Campbell & B.-E.van Wyk | Section <i>Rafnia</i> | <i>Van Wyk 2175</i> (JRAU) | 218 | Type I | Dehiscent |
| <i>Robynsiophyton vanderystii</i> Wilczek | | <i>Lisowski 20326</i> (K) | 79 | Type II | Dehiscent |
| <i>Rathia hirsuta</i> (Guill. & Perr.) Baker | | <i>Bogdan 2205</i> (K) | 109 | Type II | Dehiscent |
| <i>Rathia indica</i> (L.) Druce | | <i>Latz 16126</i> (MEL) | 123 | Type II | Dehiscent |
| <i>Wiborgia monoptera</i> E.Mey. | Subgenus <i>Pterocarpia</i> Dahlg. | <i>Boatwright et al. 152</i> (JRAU) | 180 | Type I | Indehiscent |
| <i>Wiborgia sericea</i> Thunb. | Subgenus <i>Pterocarpia</i> | <i>Boatwright et al. 124</i> (JRAU) | 468 | Type I | Indehiscent |
| <i>Wiborgia tetraptera</i> E.Mey. | Subgenus <i>Pterocarpia</i> | <i>Schutte 737</i> (JRAU) | 225 | Type I | Indehiscent |
| <i>Wiborgiella bouisana</i> (Benth.) Boatwr. & B.-E.van Wyk | | <i>Streicher s.n. sub Schutte 831</i> (JRAU) | 571 | Type I | Dehiscent |
| <i>Wiborgiella humilis</i> (Thunb.) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 212</i> (JRAU) | 296 | Type I | Indehiscent |
| <i>Wiborgiella inflata</i> (H.Bolus) Boatwr. & B.-E.van Wyk | | <i>Johans 162</i> (JRAU) | 462 | Type III | Dehiscent |
| <i>Wiborgiella leipoldiana</i> (Schltr. ex R.Dahlgren) Boatwr. & B.-E.van Wyk | | <i>Boatwright et al. 123</i> (JRAU) | 412 | Type III | Dehiscent |
| <i>Wiborgiella mucronata</i> (Benth.) Boatwr. & B.-E.van Wyk | | <i>Esterhuysen 6880</i> (BOL) | 297 | Type I | Dehiscent |
| <i>Wiborgiella sessilifolia</i> (Eckl. and Zeyh.) Boatwr. & B.-E.van Wyk | | <i>Taylor 4329</i> (PRE) | 375 | Type I | Dehiscent |
| <i>Wiborgiella vlokii</i> Boatwr. & B.-E.van Wyk, ined. | | <i>Vlok 2045</i> (PRE) | 312 | Type I | Dehiscent |