



# Structure and evolution of the pod in *Indigofera* (Fabaceae) reveals a trend towards small thin indehiscent pods

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Pod morphology and anatomy have been studied in 28 species and four varieties of Indian *Indigofera*. Pods of *Indigofera* spp. differ with respect to size, the relative thickness of the fruit wall, the number of sclerenchymatous layers in the endocarp, the presence/absence of hypodermis and trichomes, and the presence of separation tissue. Anatomically, the pericarp is broadly characterized into three types: type I (thin pericarp and three to five sclerenchymatous layers in the endocarp), type II (intermediary pericarp thickness and six to eight sclerenchymatous layers), and type III (thick pericarp and more than eight sclerenchymatous layers). The distribution of these types across the tribe is not congruent with the current phylogenetic analyses. Type III pericarp (present in the early diverging lineages of the tribe) represents the most primitive state, whereas type I and type II pericarps are derived. Fruits of *Indigofera* generally show normal explosive dehiscence as a means of dispersal of seeds, although some species show adaptations for dispersal by wind. In *Indigofera*, dehiscence is caused by a separation layer present at the dorsal and ventral sutures except in some species (*Indigofera hochstetteri*, *Indigofera karnatakana*, *Indigofera glandulosa* var. *sykesii*, and *Indigofera trita* var. *scabra*) in which no separation tissue is present; these species show delayed dehiscence or an indehiscent condition. The indehiscent pod type is considered to be apomorphic. The taxonomic, functional, and evolutionary significance of morphological and anatomical features in fruits of the genus *Indigofera* has been evaluated. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, 176, 260–276.

ADDITIONAL KEYWORDS: anatomy – dehiscence – Leguminosae – morphology.

## INTRODUCTION

*Indigofera* L. (tribe Indigofereae, Fabaceae) includes 700–750 species (Schrire, 2005; Schrire *et al.*, 2009). The genus is pantropical in distribution but the major centres of diversity are in Africa and Madagascar (550 species), the Sino-Himalayan region (105 species), and Australia (50 species); the remaining 45 species occur in the New World (Schrire *et al.*, 2009). In India, the genus is represented by 60 species and 11 varieties, of which 16 species and seven varieties are endemic (Schrire, 1992; Sanjappa, 1995; Chauhan, Pandey & Schaefer, 2013). *Indigofera* spp. are annuals or perennials and habit varies from prostrate

and erect herbs to under-shrubs, robust shrubs, and rarely trees (Fig. 1).

Fruit morphology, anatomy, and dehiscence mechanisms in Fabaceae have been investigated in various studies (Fahn & Zohary, 1955; Esau, 1962; Fahn, 1967, 1982; Pate & Kuo, 1981; Kirkbride, Gunn & Weitzman, 2003). In Fabaceae, the pericarp consists of an exocarp (single or biseriolate epidermal cell layers), mesocarp (multiple parenchyma and collenchymas cell layers), and endocarp (variable number of sclerenchyma cell layers). The mid region of the mesocarp is traversed by vascular strands. The endocarp is septate between seeds and often shows tannin spots. Usually, there is a relationship between the histological structure of the pericarp and the manner of fruit and seed dispersal (Guttenberg, 1971; Fahn & Werker, 1972). When the fruit matures, the two

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**Figure 1.** *Indigofera* species showing habit, leaf types, flower and fruit. A, *Indigofera oblongifolia*. B, *Indigofera trifoliata*. C, *Indigofera astragalina*. D, *Indigofera cassioides*. E, *Indigofera aspalathoides*. F, *Indigofera hendecaphylla*. G, *Indigofera dalzellii*.

valves of the dried legume usually twist. Dehiscence is brought about by the uneven shrinkage of the thickened walls of the pericarp cells. The greatest shrinkage occurs at right angles to the longitudinal axis of the fruit and microfibrils. The cell wall of the mature fruit dries and shrinks causing explosive dehiscence (Fahn & Zohary, 1955; Fahn, 1982).

The mechanism of dehiscence and the function of separation tissue in the endocarp in the process of dehiscence were described by Fahn & Zohary (1955). Buckovic (1952) studied the anatomy of the legume pod and proposed that pod dehiscence was caused by different rates of moisture loss in different tissues, with the rate of loss being much higher in the paren-



chyma cells than in the fibrous cells. Fahn & Zohary (1955) studied the anatomy of the pod, particularly the pericarp structure, of > 100 species of Fabaceae. They recognized 17 types of legume based mainly on the structure of the sclerenchymatous layer of the pericarp. For dehiscence to occur, first the sclerenchymatous cells and/or the cellulose micelles in their cell walls must change orientation and, second, there must be separation tissue present extending into the region of the suture from the inner to the outer epidermis. The change in orientation leads to unequal swelling and shrinking of the cells in different directions, which in turn causes the pod to shatter. According to Roth (1977), this arrangement of fibres is responsible for the helicoidal bending of the valves. In other words, the axis of the bending movement (perpendicular to the long axis of the fibres) is oriented obliquely to the long axis of the fruits and this leads to helicoidal bending. Le Roux *et al.* (2011) concluded that a pattern of dehiscence in which, the orientation of the fibres in the endocarp relative to the longitudinal axis of the fruits is the major determinant for dehiscence to occur. Also, the presence of different zones of fibres and their orientation relative to one another are also important. Yang *et al.* (1990) observed and concluded that pod dehiscence was associated with the degree of mesocarp lignification. Gershon (1961) found that indehiscent species also exhibit decreased lignification of mesocarp cells, and observed that three indehiscent legume species all lacked separation tissues. Fahn & Zohary (1955) described the condition of not having a fibrous region at the sutures, and only thick-walled parenchyma without a separation layer, as most derived and thus considered indehiscence as a derived character in legumes. The species that lacked separation tissue in the pod are considered to be tardily dehiscent or indehiscent and are more derived compared to dehiscent pods.

Because the phylogenetic relationships in Indigoferae are well known (Schrire *et al.*, 2009), an opportunity exists to evaluate other useful taxonomic characters to infer the patterns of evolution. The present study aimed to: (1) to determine the taxonomic value of the morphological and anatomical structures of the pod in Indian *Indigofera*; (2) to trace the evolution of fruit characters in the tribe Indigoferae; and (3) to discuss the role of anatomical features in pod dehiscence and the mechanism involved.

## MATERIAL AND METHODS

### TAXON SAMPLING

Mature pods of 28 species and four varieties representing 32 accessions of *Indigofera* were collected through our field work carried out in different parts of

India. Voucher specimen information is provided with the author citation for these taxa in Table 1. Voucher specimens have been deposited in Delhi University Herbarium.

### POD MORPHOLOGY AND ANATOMY

The length of mature pods (average of ten pods) was measured with the help of a scale. For anatomical studies, pods were fixed in formalin-acetic acid-alcohol and dehydrated through an ethanol series (*sensu* Feder & O'Brien, 1968). Pods were cut into small pieces and were then processed in glycol methacrylate resin 'Basic Resin' infiltration. Sections, 2–3 µm thick, were cut on Microm HM 315 Thermo Scientific microtome and stained with aqueous toluidine blue and mounted in DPX. Photographs were taken with a microscope Primo Star Zeiss with a Canon digital camera (G10 14.7 MP).

### POD ANATOMICAL CHARACTERS AND CHARACTER STATES

The sampled species belong to four clades: the Palaeotropical, Pantropical, Cape, and Tethyan clades of *Indigofera* (*sensu* Schrire *et al.*, 2003, 2009) (Table 1). For character reconstruction, anatomical data were taken from our own observations and from the work of Leite *et al.* (2009) and Dave & Bennet (1990). A total of 41 accessions were included in the present study.

Character states recorded for the pod anatomy include: pod type (I, II, III; coded as states 0, 1, 2, respectively) (Table 2). Phylogenetic trees were constructed using internal transcribed sequence data of the representative species using MrBayes (V. Chauhan & A. K. Pandey, unpubl. data). One hundred most probable trees were retrieved from the TRPROBS file produced in the Bayesian analyses and read into MESQUITE; variation in pod types was examined by ancestral state reconstruction using MESQUITE, version 2.72 (Maddison & Maddison, 2009). Ancestral state reconstruction of characters was carried out using maximum parsimony and maximum likelihood methods. A probability of more than 0.60 for a character compiled from the 100 most probable trees at each node was considered to be the most probable state at that node.

## RESULTS

### POD MORPHOLOGY

Fruits of *Indigofera* are generally linear but pods may be flat, compressed (*Indigofera hochstetteri*), curved [*Indigofera nummularifolia* (L.) Livera ex Alston, *Indigofera constricta* (Thwaites) Trimen,

**Table 1.** Plant accessions used for the anatomical study of *Indigofera* and detailed anatomical description of each plant

Species number	Species names	Voucher	Locality	Trichomes	Perticarp thickness (µm)	Exocarp	Hypodermis	Mesocarp	Endocarp	Dehiscent/ indehiscent pod	Pod length (mm)	Pod types
1	<i>Indigofera aspalaithoides</i> Vahl ex DC.	Chauhan 1468	Madurai Kamraj University Campus, Tamil Nadu	Pubescent	212.51	Epidermis single-layered, thick-walled	Single-layered, thick-walled collenchymatous	4–5 layers, collenchymatous, vasculature present	4–6 layers, sclerenchymatous	Dehiscent pod	13.18	Type II
2	<i>Indigofera astragalina</i> DC.	Chauhan & AKPandey 1448	Tamil Nadu	Pubescent	384.75	Epidermis single-layered, thick-walled	3–5 layers, thick-walled collenchymatous	1st section contains 4–7 layers, collenchymatous tissue, 2nd section contains single-layered, large parenchymatous cells	6–9 layers, sclerenchymatous	Dehiscent pod	13.68	Type III
3	<i>Indigofera atropurpurea</i> Buch.-Ham. ex Hornem	Chauhan & AKPandey 5019	Rajghad, Sirmaur, H.P.	Glabrous	463.12	Epidermis single-layered, thick-walled	2–3 layers, thick-walled, collenchymatous	3–4 layers, narrow collenchymatous cells, mostly idioblasts are present	10–12 layers, sclerenchymatous	Dehiscent pod	40.05	Type III
4	<i>Indigofera barberi</i> Gamble	Chauhan 1472	Megamalai hills, Madurai, Tamil Nadu	Glabrous	171	Epidermis single-layered, thick-walled	Single-layered, collenchymatous	4–5 layers, collenchymatous and parenchymatous, some cells are idioblasts	6–8 layers, sclerenchymatous	Dehiscent pod	10.46	Type II
5	<i>Indigofera cassioides</i> Rottler ex DC.	Chauhan 1474	Megamalai hills, Madurai, Tamil Nadu	Glabrous	328.89	Epidermis single-layered, thick-walled	Epidermis single-layered, thick-walled	6–8 layers, collenchymatous, some cells are idioblasts	6–9 layers, sclerenchymatous	?	26.54	Type III
6	<i>Indigofera colutea</i> (Burm.f.) Merr.	Chauhan 1479	Madurai Kamraj University Campus, Tamil Nadu	Multicellular as well unicellular	123.5	Epidermis single-layered, thick-walled	Single-layered, collenchymatous	2–3 layers, narrow elongated collenchymatous and parenchymatous layer, most cells are idioblasts, vasculature present	4–6 layers, sclerenchymatous	Dehiscent pod	18.29	Type II
7	<i>Indigofera dalzellii</i> Cooke	Chauhan & AKPandey 1431	Masai plateau, Kohlapur, Maharashtra	Pubescent	337.25	Epidermis single-layered, thick-walled	2–3 layers, narrow elongated collenchymatous cells, most cells are idioblasts	8–10 layers collenchymatous tissue, vasculature present	8–11 layers, sclerenchymatous	Dehiscent pod	23.2	Type III
8	<i>Indigofera dosta</i> Buch.-Ham. ex D.Don	Chauhan & AKPandey 5003	Sanghra, Sirmaur, H.P.	Glabrous	361	Epidermis single-layered, thick-walled	Epidermis single-layered, thick-walled	1st section contains 3–4 layers, collenchymatous tissue, 2nd section contains 3–5 layers, elongated, narrow collenchymatous cells, most of them are idioblasts, vascular bundles are present	8–10 layers, sclerenchyma	Dehiscent pod	22.52	Type III
9	<i>Indigofera exilis</i> Grierson & Long	Chauhan & AKPandey 5029	Shimla, H.P.	Glabrous	248.04	Epidermis single-layered, thick-walled	2–3 layers, collenchymatous	4–6 layers, collenchymatous, narrow, elongated cells	Up to 8, sclerenchymatous	Dehiscent pod	30.56	Type II
10	<i>Indigofera gangetica</i> Sanjappa	Chauhan & AKPandey 5030	Shimla, H.P.	Pubescent	192.09	Epidermis single-layered, thin-walled	Epidermis single-layered, thin-walled	1st section contains 3–5 layers, collenchymatous, some cells are idioblasts, 2nd section contains 1–3 layers, narrow elongated parenchymatous tissue, most cells are idioblasts. In this region vascular bundles are present.	7–8 layers, sclerenchymatous tissue	Dehiscent pod	21.65	Type II
11	<i>Indigofera glabra</i> L.	Chauhan & AKPandey 5098	Thummalapalli Uranium mining area, Anantapur Dist., Andhra Pradesh	Glabrous	180.5	Epidermis double-layered, thick-walled	Epidermis double-layered, thick-walled	3–4 layers, narrow cells, collenchymatous, mostly idioblasts are present, vascular bundles are present	5–7 layers, sclerenchymatous tissue	Dehiscent pod	25.44	Type II

Table 1. Continued

Species number	Species names	Voucher	Locality	Trichomes	Pericarp thickness (µm)	Exocarp	Hypodermis	Mesocarp	Endocarp	Dehiscent/ indehiscent pod	Pod length (mm)	Pod types
12	<i>Indigofera glandulosa</i> Wendl. var. <i>sykesii</i> Griffith ex Baker	Chauhan & AKPandey 1442	Belgaon, Karnataka	Pubescent	160.31	Epidermis single-layered, thin-walled		4–6 layers, collenchymatous and parenchymatous, narrow, elongated cells, most cells are idioblasts	4–6 layers, sclerenchymatous	Delayed dehiscent	2.49	Type II
13	<i>Indigofera hebeptala</i> Benth. ex Baker var. <i>glabra</i> Ali	Chauhan 5036	Shimla, H.P.	Glabrous	392.63	Epidermis single-layered, thick-walled		1st section contains 1–2 layers, rounded collenchymatous cells, 2nd section contains 1–2 layers, rounded collenchymatous cells, most cells are idioblasts, 3rd section contains 4–5 layers, narrow elongated parenchymatous cells, vascular bundles present	8–10 layers, sclerenchyma	Dehiscent pod	50.45	Type III
14	<i>Indigofera hebeptala</i> Benth. ex Baker var. <i>hebeptala</i>	Chauhan & AKPandey 5033	Badalta, Sirmaur, H.P.	Glabrous	301	Epidermis single-layered, thick-walled		6–10 layers, collenchymatous, most cells are idioblasts	11–14 layers, sclerenchymatous	Dehiscent pod	50.04	Type III
15	<i>Indigofera heterantha</i> Wallich ex Brandis	Chauhan & AKPandey 5014	Nohradhar, Sirmaur, H.P.	Glabrous	508.91	Epidermis single-layered, thick-walled	4–5 layers, thick-walled collenchymatous	4–8 layers, narrow elongated collenchymatous and parenchymatous, most cells are idioblasts	8–10 layers, sclerenchyma	?	31.86	Type III
16	<i>Indigofera himalayensis</i> Ali	Chauhan 5032	Budgeam, J & K	Glabrous	318.25	Epidermis single-layered, thick-walled	1–2 layers, thick-walled collenchymatous	?	8–10 sclerenchymatous	?	4.02	Type III
17	<i>Indigofera hochstetteri</i> Baker	Chauhan & AKPandey 6002	Hissar Dist., Punjab	Pubescent	47.5	Epidermis single-layered, thick-walled		1st section contains 2–4 layers, collenchymatous, most cells are idioblasts, vasculature present	3–5 layers, sclerenchymatous	Delayed dehiscent	11.56	Type I
18	<i>Indigofera karnatakana</i> Sanjappa	Chauhan & AKPandey 5046	Bannerghatta hills, Karnataka	Glabrous	228	Epidermis single-layered, thick-walled	Double-layered thick-walled collenchymatous	1st section contains 2–3 layers, parenchymatous, 2nd section contains 3–4 layers narrow, collenchymatous most cells are idioblasts	4–6 layers, sclerenchymatous	Delayed dehiscent	22.5	Type II
19	<i>Indigofera karuppiana</i> Palithanam	Chauhan 1465	Kannalli, Nilgiris Dist., Tamil Nadu	Pubescent	184.3	Epidermis single-layered, thick-walled	Single-layered thick-walled collenchymatous	1st section contains 4–5 layers, narrow, elongated parenchymatous tissue, most cells are idioblasts	6–7 layers, sclerenchyma	Dehiscent pod	7.42	Type II
20	<i>Indigofera linifolia</i> (L. f.) Retz. var. <i>campbelli</i> Wight ex Baker	Chauhan & AKPandey 1446	University Campus Garden, Kohlapur, Maharashtra	Pubescent	167.77	Epidermis single-layered, thick-walled	Single-layered, thick-walled collenchymatous	1st section contains 2–3 layers, narrow elongated collenchymatous cells, vasculature is present in this layer, 2nd section contains single-layered rounded collenchymatous cells	3–6 layers, sclerenchymatous	Dehiscent pod	2.1	Type II
21	<i>Indigofera linifolia</i> (L. f.) Retz. var. <i>linifolia</i>	Chauhan & AKPandey 1470	Madurai Kamraj University Campus, Tamil Nadu	Pubescent	202.35	Epidermis single-layered, thick-walled	1–2 layers, collenchymatous	1–2 layers, collenchymatous, some cells are idioblasts	2–6 layers, sclerenchymatous	Dehiscent pod	2.1	Type II
22	<i>Indigofera longitracemosa</i> Boivin ex Baill.	Chauhan 1498	Kalkadu, Tirunelveli Dist., Tamil Nadu		352.45				7–9 layers, sclerenchymatous		15.35	Type III

23	<i>Indigofera oblongifolia</i> Forsk.	Chauhan & AKPandey 5099	Thummalapalli Uranium mining area, Anantapur Dist., Andhra Pradesh	Pubescent	155.13	Epidermis single-layered, thick-walled	Single-layered, collenchymatous	4-8 layers, collenchymatous	3-6 layers, sclerenchymatous	Dehiscent pod	12.42	Type II
24	<i>Indigofera prostrata</i> Willd.	Chauhan & AKPandey 1451	Goa University Campus, Goa	Sparsely pubescent	129.77	Epidermis single-layered, thick-walled	Single-layered, collenchymatous	1-2 layers, narrow, elongated, collenchymatous and parenchymatous, most cells are idioblasts, vascular bundles present	4-6 layers, sclerenchymatous	Dehiscent pod	9.72	Type II
25	<i>Indigofera sessiliflora</i> DC.	Chauhan & AKPandey 6001	Rangard, Jaisalmer Dist., Rajasthan	Pubescent	194.18	Epidermis single-layered, thick-walled	1-2 layers, thick-walled, collenchymatous	4-6 layers, collenchymatous tissue. Vasculature present	4-6 layers, sclerenchymatous	Dehiscent pod	7.86	Type II
26	<i>Indigofera tinctoria</i> L.	Chauhan 1467	Kannali, Tamil Nadu	Glabrous	215.65	Epidermis single-layered, thick-walled	1st section contains 2-3 layers, collenchymatous tissue, 2nd section contains 2-4 layers, narrow large elongated parenchymatous, most cells are idioblasts	6-8 layers, sclerenchymatous	6-8 layers, sclerenchymatous	Dehiscent pod	23.9	Type II
27	<i>Indigofera trifoliata</i> L.	Chauhan & AKPandey 1438	Sugethathi ghat, Kohlapur, Maharashtra	Pubescent	181.45	Epidermis single-layered, thick-walled	Single-layered, thick-walled, collenchymatous	3-4 layers, narrow elongated collenchymatous, most cells are idioblasts	6-8 layers, sclerenchymatous	Dehiscent pod	7.48	Type II
28	<i>Indigofera trifoliata</i> L. var. <i>duthiei</i> (Drumm. ex Naik) Sanjappa	Chauhan & AKPandey 1429	Girgaon, Kohlapur, Maharashtra	Pubescent	123.5	Epidermis single-layered, thick-walled	Single-layered, thick-walled, collenchymatous	3-4 layers, collenchymatous, some cells are idioblasts	4-6 layers, sclerenchymatous	?	7.76	Type II
29	<i>Indigofera trita</i> L. f. var. <i>scabra</i> (Roth) Ali	Chauhan 1460	Marudhamalai hills, Tamil Nadu	Pubescent	181.64	Epidermis single-layered, thick-walled	1-2 layers, thick-walled, collenchymatous	6-8 layers, narrow elongated collenchymatous, vasculature present	4-6 layers, sclerenchymatous	Delayed dehiscent	35.67	Type II
30	<i>Indigofera trita</i> L. f. var. <i>trita</i>	Chauhan 1471	American College, Tamil Nadu	Pubescent	224.39	Epidermis single-layered, thick-walled	1-2 layers, thick-walled, collenchymatous	4-5 layers, collenchymatous and parenchymatous cells	5-7 layers, sclerenchymatous tissue	Dehiscent pod	24.84	Type II
31	<i>Indigofera uniflora</i> Buch.-Ham. ex Roxb.	Chauhan 1459	Madhukkarai hills, Tamil Nadu	Pubescent	95.95	Epidermis single-layered, thick-walled	3 layers, collenchymatous	3 layers, collenchymatous	4-5 layers, sclerenchymatous	?	6.84	Type I
32	<i>Indigofera wightii</i> Graham ex Wight & Arn.	Chauhan 1473	Megamalai hills, Madurai, Tamil Nadu	Pubescent	396.15	Epidermis single-layered, thick-walled	1st section contains 4-6 layers, collenchymatous tissue, 2nd section contains 3-5 layers, elongated large parenchymatous cells Vasculature bundles are present	7-9 layers, sclerenchymatous	7-9 layers, sclerenchymatous	Dehiscent pod	23.18	Type III

**Table 2.** Character matrix showing the character states for all the species used in ancestral state reconstruction in Mesquite

Taxon/character	Pod type	Taxon/character	Pod type
<i>Cyamopsis tetragonoloba</i> FJ769262	2	<i>Indigofera heterantha</i> EU729571	2
<i>Indigastrum argyroides</i> EU729488	2	<i>Indigofera heterotricha</i> EU729535	?
<i>Indigastrum candidissimum</i> EU729489	2	<i>Indigofera himalayensis</i> AF521770	2
<i>Indigastrum costatum</i> AF521716	2	<i>Indigofera hirsuta</i> EU729546	0
<i>Indigastrum fastigiatum</i> EU729490	2	<i>Indigofera hochstetteri</i> EU729656	0
<i>Indigofera dosua</i> AF534790	2	<i>Indigofera karnatakana</i> EU729544	1
<i>Indigofera himalayensis</i> AF534792	2	<i>Indigofera lepedezioides</i> AF521780	1
<i>Indigofera aspalathoides</i> JQ945958	1	<i>Indigofera linifolia</i> EU729629	1
<i>Indigofera astragalina</i> EU729545	2	<i>Indigofera linifolia</i> JQ945964	1
<i>Indigofera astragalina</i> JQ945966	2	<i>Indigofera linnaei</i> EU729670	?
<i>Indigofera atropurpurea</i> EU729575	2	<i>Indigofera linnaei</i> JQ945960	?
<i>Indigofera barberi</i> JQ945961	1	<i>Indigofera longiracemosa</i> AF521786	2
<i>Indigofera bongardiana</i> EU729661	?	<i>Indigofera longiracemosa</i> AY124764	2
<i>Indigofera cassioides</i> EU729573	2	<i>Indigofera microcarpa</i> EU729630	1
<i>Indigofera cassioides</i> JQ945965	2	<i>Indigofera miniata</i> EU729663	?
<i>Indigofera colutea</i> AF521776	1	<i>Indigofera nummulariifolia</i> EU729631	?
<i>Indigofera colutea</i> JQ945956	1	<i>Indigofera oblongifolia</i> AF521778	1
<i>Indigofera concava</i> EU729619	?	<i>Indigofera prostrata</i> JQ945972	1
<i>Indigofera cordifolia</i> AF521741	?	<i>Indigofera schimperii</i> AF274696	?
<i>Indigofera cordifolia</i> JQ945963	?	<i>Indigofera semitrijuga</i> EU729669	?
<i>Indigofera cuneifolia</i> AF521749	?	<i>Indigofera sessiliflora</i> EU729642	1
<i>Indigofera dalzellii</i> AF521793	2	<i>Indigofera spicata</i> EU729671	1
<i>Indigofera dalzellii</i> JQ945962	2	<i>Indigofera squalida</i> EU729633	?
<i>Indigofera denudata</i> EU729617	?	<i>Indigofera suffruticosa</i> AF467051	1
<i>Indigofera dosua</i> AF521771	2	<i>Indigofera tinctoria</i> AF521775	1
<i>Indigofera drepanocarpa</i> EU729632	?	<i>Indigofera trifoliata</i> JQ945971	1
<i>Indigofera gairdnerae</i> EU729507	?	<i>Indigofera trita</i> AF521745	1
<i>Indigofera glabra</i> EU729504	1	<i>Indigofera trita</i> EU729681	1
<i>Indigofera glandulosa</i> EU729634	1	<i>Indigofera truxillensis</i> EU729602	1
<i>Indigofera glandulosa</i> var. <i>skyseii</i> JQ945967	1	<i>Indigofera uniflora</i> JQ945959	0
<i>Indigofera hebeptala</i> AF521768	2	<i>Indigofera wightii</i> EU729519	2
<i>Indigofera hebeptala</i> AF534793	2	<i>Indigofera wightii</i> JQ945957	2

Coding for the character states – pod types: 0, pod type I; 1, pod type II; 2, pod type III.

*Indigofera suffruticosa* Mill., and *Indigofera trita* var. *scabra*) or globose [*Indigofera cordifolia* Heyne ex Roth, *Indigofera linifolia* (Fig. 2). The cross-section of the pod is usually cylindrical, subcylindrical or subtetragonous, except *Indigofera nummulariifolia* in which it is triquetrous. The pods are usually adpressed hairy, except in some species where they are glabrous (*Indigofera atropurpurea*, *Indigofera cassioides*, *Indigofera cedrorum* Dunn, *Indigofera cylindracea* Graham ex Baker, *Indigofera exilis*, *Indigofera glabra*, *Indigofera hamiltonii* Graham ex Duthie & Prain, *Indigofera hebeptala*, *Indigofera himalayensis*, *Indigofera lacei* Craib). The pod is deflexed or spreading or ascending, dehiscent or indehiscent. In some species (*Indigofera barberi*, *Indigofera glandulosa*, *Indigofera karuppiana*, *Indigofera pedicellata* Wight & Arn., *Indigofera prostrata*,

*Indigofera santapau* Sanjappa, *Indigofera thothathrii* Sanjappa, *Indigofera tirunelvelica* Sanjappa), pods have glands on the surface. All the species having gland dotted pods are trifoliolate species. Multicellular, erect, gland-tipped hairs are present on pods in *Indigofera argentea* and *Indigofera colutea*. In some species of *Indigofera*, mature fruits have broad sutures (*Indigofera argentea* Burm.f., *Indigofera arrecta* Hochst. ex A.Rich, *Indigofera bracteata* Graham ex Baker, *I. colutea*, *Indigofera dosua*, *I. suffruticosa*, *Indigofera tirunelvelica*, *Indigofera zollingeriana* Miq.), fruits with obscurely or shortly winged sutures [*Indigofera dalzellii*, *Indigofera glabra*, *Indigofera karuppiana*, *Indigofera prostrata*, *Indigofera thothathrii*, *Indigofera trifoliata* var. *duthieii*], fruits with prominently winged sutures (*Indigofera bracteata* var. *khasiana* Sanjappa, *Indigofera*





**Figure 2.** Morphological variation of fruits of genus *Indigofera*. A, *Indigofera trita* var. *scabra* (1460). B, *Indigofera wightii* (1473). C, *Indigofera cordifolia* (1432). D, *Indigofera linifolia* (1470). E, *Indigofera linnaei* (1469). F, *Indigofera cassioides* (1474). G, *Indigofera karuppiana* (1465). H, *Indigofera hendecaphylla* (1477). I, *Indigofera trifoliata* (1438). J, *Indigofera trita* var. *trita* (1471). K, *Indigofera astragalina* (1448). L, *Indigofera dosua* (1488). M, *Indigofera tinctoria* (1475). N, *Indigofera heterantha* (1485). O, *Indigofera colutea* (1479). P, *Indigofera dalzellii* (1431). Q, *Indigofera aspalathoides* (1468). R, *Indigofera prostrata* (1451). S, *Indigofera glandulosa* (1442). T, *Indigofera barberi* (1472). U, *Indigofera trifoliata* var. *duthiei* (1429). V, *Indigofera uniflora* (1459). W, *Indigofera himachalensis* (1487). Scale bar = 2 mm.

*glandulosa*, *Indigofera trifoliata*) or spiny processes along the sutures (*Indigofera nummulariifolia*).

#### POD ANATOMY

Fruits in *Indigofera* are relatively thin- to thick-walled. Anatomically, the pericarp is distinguishable into exocarp, mesocarp and endocarp.

#### Exocarp

The exocarp generally consists of a single-layered epidermis, except in *I. glabra* where it is biseriate. The exocarp is mostly thick-walled, except *Indigofera gangetica* and *I. glandulosa* var. *sykesii*, in which it is thin-walled. The hypodermis may be present or absent. If present, then it is usually single-layered, but sometimes multiple layers are present (*Indigo-*



*fera astragalina*, *I. atropurpurea*, *Indigofera dalzellii*, *I. exilis*, *Indigofera heterantha* and *Indigofera karnatakana*). The multilayered exocarp is composed of thick-walled collenchymatous cells.

#### Mesocarp

The mesocarp is composed of three or four to eight- to ten-layered collenchymatous tissue but, in some species, parenchymatous tissue is also present (e.g., *Indigofera astragalina*, *Indigofera barberi*, *Indigofera colutea*, *Indigofera gangetica*, *Indigofera glandulosa* var. *sykesii*, *Indigofera hebetata* var. *glabra*, *Indigofera heterantha*, *Indigofera karnatakana*, *Indigofera karuppiana*, *Indigofera prostrata*, *Indigofera tinctoria*, *Indigofera trita* var. *trita*, *Indigofera wightii*). Vascular bundles and most of the idioblasts are present in the mesocarp region.

#### Endocarp

The endocarp is composed of a variable number of sclerenchyma layers, ranging from three to five to ten to 12. The most common endocarp cell layer number in *Indigofera* is six to eight.

On the basis of pericarp thickness and number of sclerenchymatous layers in the endocarp, three basic fruit types can be recognized: pod type I, II, and III. Type I (thin pericarp and three to five sclerenchymatous layers in the endocarp); type II (intermediary pericarp thickness and six to eight sclerenchymatous layers); and type III (thick pericarp and more than eight sclerenchymatous layers) (Figs 3, 4, 5; Table 3).

The anatomical description for the genus is summarized in Table 4 with an indication of the fruit pericarp type (fruit type classification for all species investigated is listed in the Table 1). The distribution of fruit pericarp types in the tribe is shown in Figure 6.

#### POD DEHISCENCE

Both dehiscent and tardily dehiscent pods are present in *Indigofera*, which generally shows normal explosive dehiscence as a mean of dispersal of seeds, although some species show adaptations for dispersal by wind (*I. cordifolia*, *I. linifolia*, *I. glandulosa*, and *I. linnaei* Ali). Some *Indigofera* spp. are similar in having small, light weighted, few seeded, indehiscent or tardily dehiscent fruits with persistent calyces and corollas with the pod embellished with broad sutures, leading to longitudinal fringed wings, appearing to be adapted to dispersal by wind, water or gravity. All these fruit characters indicate a shift to pods playing a role in seed dispersal. The whole pod acts as a diaspore that can easily blown away by wind. In *Indigofera*, pods usually have separation tissue present along the sutures and a variable number of

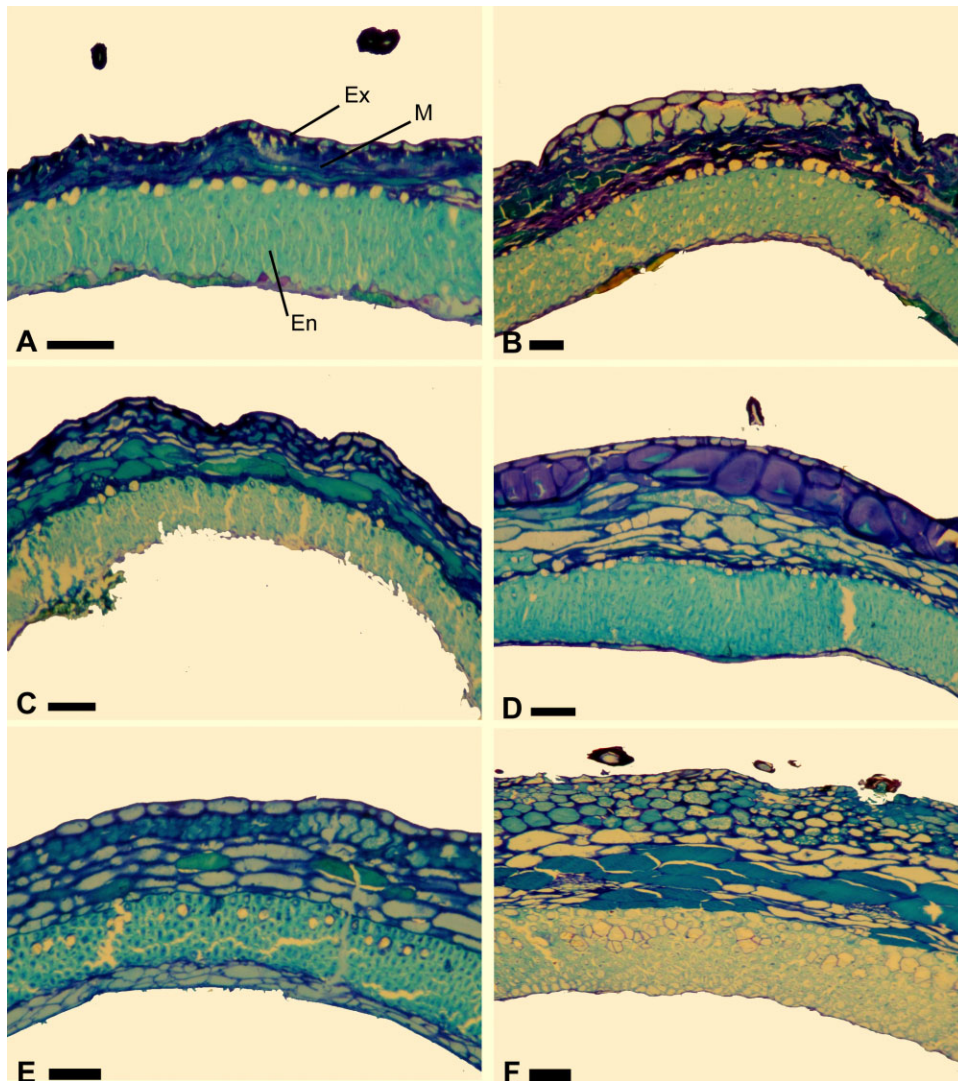
sclerenchymatous layers in the endocarp. These two characteristics are indicative of a dehiscent pod. However, in some taxa (*I. hochstetteri*, *I. karnatakana*, *I. glandulosa* var. *sykesii*, and *I. trita* var. *scabra*), which show a tardily dehiscent or indehiscent condition, no separation tissue is found and there is mostly a low to intermediate number of sclerenchyma layers present in the endocarp. Two Neotropical *Indigofera* spp., *I. microcarpa* Desv. (winged pods) and *I. suffruticosa* (broad sutures but no wings), are tardily dehiscent because there is no separation tissue present (Leite *et al.*, 2009). Roth (1977) reported two anatomical features that appear to reduce the tendency to dehisce in legumes. The first is a longer fruit and the second is a strongly bent fruit. The indehiscent *I. trita* var. *scabra* possesses a long and strongly curved pod.

#### EVOLUTION OF POD ANATOMICAL CHARACTERS

The evolutionary pattern of pod types was inferred from ancestral state reconstruction (Fig. 6), carried out on the 100 most probable trees from Bayesian analyses (TRPROBS file) and the probabilities of a particular ancestral state at specific nodes (Table 5). In the ancestor to *Cyamopsis* DC. + *Indigastrum* Jaub. & Spach + *Indigofera*, however, the two states pod type II and III are present but pod type III probability ( $P = 0.6245$ ) is higher than for other states. In *Indigastrum* and *Cyamopsis*, the pod is of type III. Pod type II was present ( $P = 0.7471$ ) in ancestral *Indigofera*. The common ancestors of clades C ( $P = 0.7311$ ), D ( $P = 0.9784$ ), and E ( $P = 0.9542$ ) have pod type II present, whereas the ancestor of clade F had pod type III ( $P = 0.6033$ ). The transition from pod type II to pod type III takes place at node f. There is a reversal in *I. wightii* (clade E) and *I. dalzellii* (clade C), which have pod type III. In *I. karnatakana* and *I. tinctoria*–*I. truxillensis* Kunth–*I. suffruticosa* (clade F), pod type II has evolved independently from the pod type III condition. In *Indigofera uniflora* (clade E) and *I. hochstetteri* (clade C), pod type I has evolved independently from the pod type II condition and, simultaneously, in *Indigofera hirsuta* L. (clade F), pod type I has evolved independently from pod type III.

#### DISCUSSION

In *Indigofera*, pod morphology is variable and characters have little diagnostic value at the generic level. The genus can be identified by a combination of characters, and fruit characters on their own have limited value (Gillett, 1958; De Kort & Thijsse, 1984; Sanjappa, 1995; Schrire, 1995; Wilson & Rowe, 2004, 2008). An example of nonhomologous similarity was



**Figure 3.** Transverse sections of fruits in *Indigofera*. Paleotropical clade with a type I pericarp (A), type II pericarp (B, C, D, E), and type III pericarp (F). A, *Indigofera uniflora* [Chauhan 1459]. B, *Indigofera glabra* [Chauhan & AKPandey 5098]. C, *Indigofera colutea* [Chauhan 1479]. D, *Indigofera aspalathoides* [Chauhan 1468]. E, *Indigofera barberi* [Chauhan 1472]. F, *Indigofera wightii* [Chauhan 1473]. Scale bar = 50  $\mu$ m.

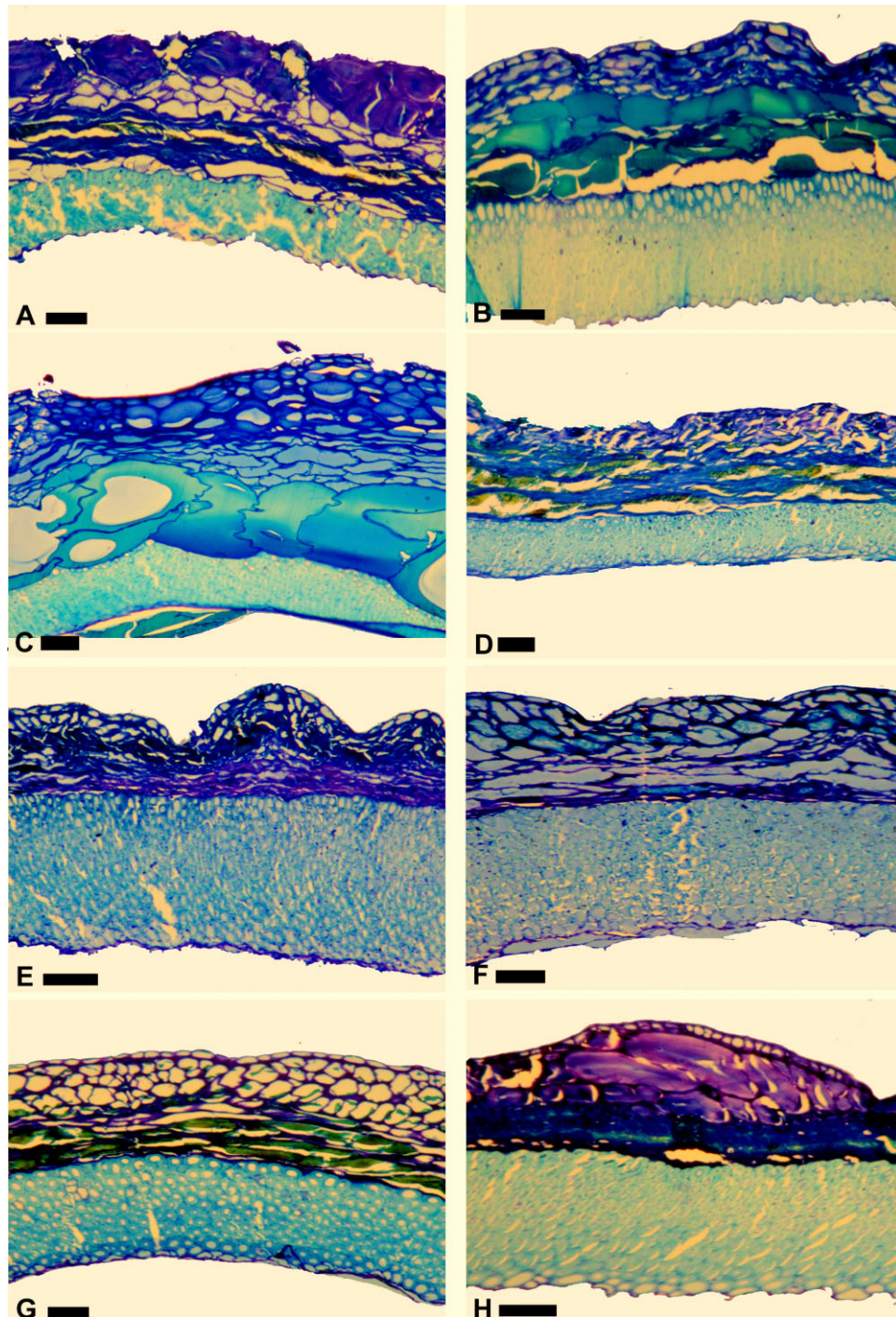
revealed by the presence of flattened and compressed pods that are found in *Indigofera*, *Cyamopsis*, and *Indigastrum*. In *Cyamopsis tetragonoloba* Taub., fruits are longitudinally ridged and septate between seeds.

The anatomy of the pericarp is too conservative to be used diagnostically at the generic level. In *Indigofera glabra*, the exocarp is biseriate, a character also present in the Neotropical species *Indigofera lespedezioides* Kunth (Leite *et al.*, 2009). The exocarp is mostly thin-walled in Neotropical species [*Indigofera campestris* Bong. ex Benth., *I. hirsuta*, *I. lespedezioides*, *I. spicata* Forssk. (introduced to the New World), *I. suffruticosa* and *I. truxillensis* (Leite *et al.*,

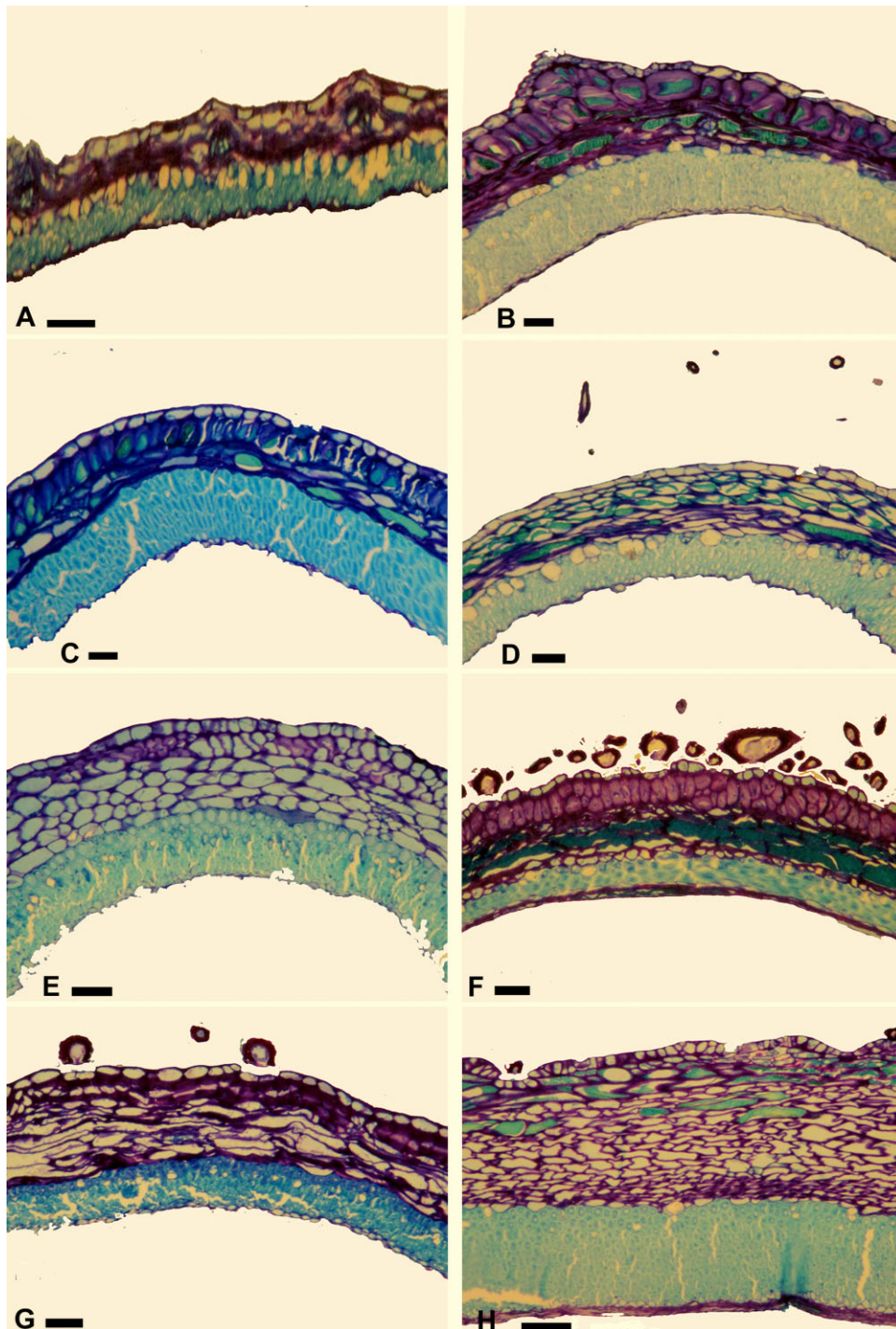
2009)]. However, some trends were observed by us and three basic pericarp types were identified within the tribe. The distribution of these types across the tribe is not congruent with current phylogenetic analyses, as shown in Figure 6.

Our anatomical studies on the *Indigofera* spp. reveal that the genus is closely related to *Cyamopsis*–*Indigastrum* clade of Schrire *et al.* (2003, 2009). All the species that were sampled for the study belong to four clades (Palaeotropical, Pantropical, Cape, and Tethyan clades of *Indigofera* as given by Schrire *et al.*, 2003, 2009). The whole Palaeotropical–Pantropical, Cape, and Tethyan clades form polytomy with each other. Ancestral state reconstructions of pod type





**Figure 4.** Transverse sections of fruits in *Indigofera*. Pantropical clade with a type II pericarp (A, B), and type III pericarp (C, D, E, F, G, H). A, *Indigofera karnatakana* [Chauhan & AKPandey 5046]. B, *Indigofera tinctoria* [Chauhan 1467]. C, *Indigofera astragalina* [Chauhan & AKPandey 1448]. D, *Indigofera heterantha* [Chauhan & AKPandey 5014]. E, *Indigofera hebeptala* var. *hebeptala* [Chauhan & AKPandey 5033]. F, *Indigofera hebeptala* var. *glabra* [Chauhan 5036]. G, *Indigofera dosua* [Chauhan & AKPandey 5003]. H, *Indigofera himalayensis* [Chauhan 5032]. Scale bar = 50  $\mu\text{m}$ .



**Figure 5.** Transverse sections of fruits in *Indigofera*: Tethyan clade with a type I pericarp (A), type II pericarp (B, C, D, E, F, G) and type III pericarp (H). A, *Indigofera hochstetteri* [Chauhan & AKPandey 6002]. B, *Indigofera trifoliata* [Chauhan & AKPandey 1438]. C, *Indigofera prostrata* [Chauhan & AKPandey 1451]. D, *Indigofera glandulosa* var. *sykesii* [Chauhan & AKPandey 1442]. E, *Indigofera trita* var. *trita* [Chauhan 1471]. F, *Indigofera linifolia* [Chauhan & AKPandey 1446]. G, *Indigofera sessiliflora* [Chauhan & AKPandey 6001]. H, *Indigofera dalzellii* [Chauhan & AKPandey 1431]. Scale bar = 50  $\mu$ m.



**Table 3.** Characteristics of the three basic fruit types recognized in the genus *Indigofera*

Pod type	Pericarp thickness	Sclerenchymatous layers	Fruit structure	Distribution in the genus
Type I	Below 100 µm	Three to five layers	Fruits relatively thin-walled, trichomes present. Exocarp: single-layered epidermis, thick-walled. Hypodermis absent. Mesocarp: only collenchymatous cells. Endocarp: three- to five-layered, sclerenchymatous. Delayed dehiscent pod (Figs 3, 5)	Present in <i>Indigofera uniflora</i> and <i>Indigofera hochstetteri</i> .
Type II	100–300 µm	Six to eight layers	Fruits are of intermediary thickness, trichomes absent/present. Exocarp: one- or two-layered epidermis, thin to thick-walled. Hypodermis absent/present, single-layered to two- to three-layered thick-walled collenchymatous. Mesocarp: composed of collenchymatous cells or both collenchymatous and parenchymatous cells. Endocarp: six- to eight-layered, sclerenchymatous. Dehiscent or delayed dehiscent pod (Figs 3, 4, 5)	Predominantly present in <i>Indigofera</i> spp.
Type III	Above 300 µm	More than eight	Fruits relatively thick-walled, trichomes absent/present. Exocarp: epidermis single-layered, thick-walled. Hypodermis absent/present, single to four- to five-layered thick-walled collenchymatous. Mesocarp: comprises collenchymatous cells or both collenchymatous and parenchymatous cells. Endocarp: eight- to 11-layered sclerenchymatous. Dehiscent pod (Figs 3, 4, 5)	Present in <i>Indigofera astragalina</i> , <i>Indigofera atropurpurea</i> , <i>Indigofera cassioides</i> , <i>Indigofera dalzellii</i> , <i>Indigofera dosua</i> , <i>Indigofera hebeptala</i> , <i>Indigofera heterantha</i> , <i>Indigofera himalayensis</i> , <i>Indigofera longiracemosa</i> , and <i>Indigofera wightii</i>

show that the common ancestor of tribe Indigoferae has pod type III. The common ancestor of *Indigofera* had pod type II ( $P = 0.7471$ ) and a change occurred in the ancestor of clade F to pod type III ( $P = 0.6033$ ).

Species of *Indigastrum* and *Cyamopsis* have a type III pericarp, whereas, in *Indigofera*, all three types (I, II, III) are present. Type II is predominant and occurs in the Palaeotropical and Tethyan clades, and independently evolved in *I. karnatakana*, *I. truxillensis*, *I. suffruticosa*, and *I. tinctoria*; type I occurs in three independent lineages (*I. uniflora*, *I. hirsuta*, and *I. hochstetteri*) and type III is observed in *Indigastrum*, *Cyamopsis*, and the pantropical clade with an independent reversal to type III in *I. wightii* and *I. dalzellii*.

The three parameters of the pod (pod size, pericarp thickness, and number of sclerenchyma layers in the endocarp) are correlated. There is a correlation between the size of the pod (length) and the thickness of the endocarp (sclerenchyma tissue) because all the large pods (> 20 mm) have a thick endocarp, small pods (< 10 mm) have a thinner endocarp and inter-

mediate pods (10–20 mm) has intermediate endocarp (e.g. *I. karuppiana*, *I. glabra*, *I. trifoliata*, *I. trita* var. *trita*). Pericarp thickness is directly correlated with number of sclerenchymatous layers in the endocarp.

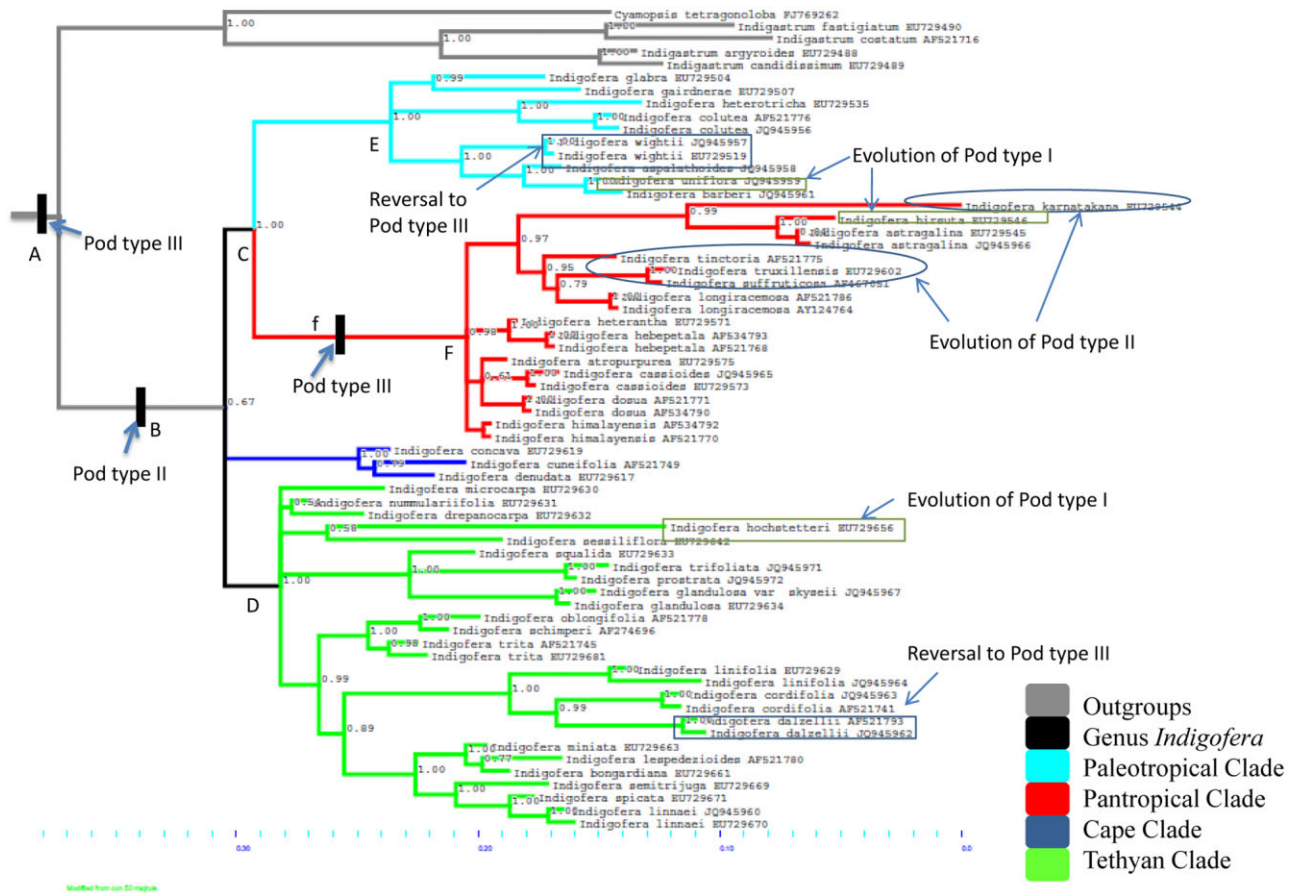
Molecular and morphological data (Schrire *et al.*, 2009) indicated that *Indigastrum parviflorum* (B. Heyne ex Wight & Arn.) Schrire should be excluded from *Indigofera*, although its placement in *Indigastrum* is not supported by fruit morphology or anatomy (V. Chauhan & A. K. Pandey, unpubl. data).

The type III pericarp (present in the early diverging lineages of the tribe) represents the least derived state, whereas type I and II are more derived. Fahn & Zohary (1955) considered the presence of multiple cell layers of sclerenchyma in the endocarp to be the primitive state in legumes and its loss to be derived. Our results also support the earlier findings of Fahn & Zohary (1955), indicating that the small pod, thin pericarp, and fewer sclerenchymatous layers in the endocarp (three to five) (pod type I) and the intermediate pod condition and thin- to thick-walled and five to eight sclerenchymatous layers in the endocarp (pod type II) are more

**Table 4.** Summary of pericarp characters of genus *Indigofera*

Species names	Pericarp thickness in micrometers		Exocarp		Cell walls		Hypodermis		Mesocarp		Endocarp		Dehiscence		Pod types	
	+ < 100 µm	++ 100–300 µm	Number of exocarp layers	-thin	+thick	-absent	+present	-absent	+present	Parenchyma	Collenchyma	+ 3–5 layers, thin	++ 6–8 layers, intermediate	+++ > 8 layers, thick	+Dehiscent pod	Type I/II/III
<i>Indigofera aspalathoides</i> Vahl ex DC.	++	++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera astragalina</i> DC.	+++	+++	One	+	+	+	+	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera atropurpurea</i> Buch.-Ham. ex Hornem	++	++	One	+	+	+	-	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera barberi</i> Gamble	+++	+++	One	+	+	+	+	+	+	+	+	+++	+++	+	+	Type II
<i>Indigofera cassioides</i> Rotler ex DC.	++	++	One	+	+	-	+	+	+	+	+	+++	+++	?	?	Type III
<i>Indigofera colutea</i> (Burm.f.) Merr.	+++	+++	One	+	+	+	+	+	+	+	+	+++	+++	+	+	Type II
<i>Indigofera dalzellii</i> T.Cooke	+++	+++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera dasua</i> Buch.-Ham. ex D.Don	++	++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera exilis</i> Grierson & Long	++	++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type II
<i>Indigofera gangetica</i> Sanjappa	++	++	One	-	+	-	+	+	+	+	+	+++	+++	+	+	Type II
<i>Indigofera glabra</i> L.	++	++	Two	+	+	-	+	+	+	+	+	+++	+++	+	+	Type II
<i>Indigofera glandulosa</i> Wendl. var. <i>sykesii</i> Griffith ex Baker	++	++	One	-	-	-	+	+	+	+	+	+++	+++	-	-	Type II
<i>Indigofera hebeptala</i> Benth. ex Baker var. <i>glabra</i> Ali	+++	+++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera hebeptala</i> Benth. ex Baker var. <i>hebeptala</i>	+++	+++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type III
<i>Indigofera heterantha</i> Wallich ex Brandis	+++	+++	One	+	+	+	+	+	+	+	+	+++	+++	?	?	Type III
<i>Indigofera himalayensis</i> Ali	+++	+++	One	+	+	+	+	+	+	+	+	+++	+++	?	?	Type III
<i>Indigofera hochstetteri</i> Baker	+	+	One	+	+	-	+	+	+	+	+	+	+	-	-	Type I
<i>Indigofera karnatakana</i> Sanjappa	++	++	One	+	+	+	+	+	+	+	+	++	++	-	-	Type II
<i>Indigofera karuppiana</i> Pallithanam	++	++	One	+	+	+	+	+	+	+	+	++	++	+	+	Type II
<i>Indigofera linifolia</i> (L. f.) Retz. var. <i>campbelli</i> Wight ex Baker	++	++	One	+	+	+	+	+	+	+	+	++	++	+	+	Type II
<i>Indigofera linifolia</i> (L. f.) Retz. var. <i>linifolia</i>	+++	+++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera longiracemosa</i> Boivin ex Baill.	++	++	One	+	+	+	-	+	+	+	+	++	++	?	?	Type III
<i>Indigofera oblongifolia</i> Forssk.	++	++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera prostrata</i> Willd.	++	++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera sessiliflora</i> DC.	++	++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera tinctoria</i> L.	++	++	One	+	+	-	+	+	+	+	+	++	++	+	+	Type II
<i>Indigofera trifoliata</i> L.	++	++	One	+	+	+	-	+	+	+	+	++	++	+	+	Type II
<i>Indigofera trifoliata</i> L. var. <i>duthiei</i> (Drumm. ex Naik) Sanjappa	++	++	One	+	+	+	-	+	+	+	+	++	++	?	?	Type II
<i>Indigofera trita</i> L. f. var. <i>scabra</i> (Roth) Ali	++	++	One	+	+	+	-	+	+	+	+	++	++	-	-	Type II
<i>Indigofera trita</i> L. f. var. <i>trita</i>	++	++	One	+	+	-	+	+	+	+	+	++	++	+	+	Type II
<i>Indigofera uniflora</i> Buch.-Ham. ex Roxb.	+	+	One	+	+	-	+	+	+	+	+	+	+	?	?	Type I
<i>Indigofera wightii</i> Graham ex Wight & Arn.	+++	+++	One	+	+	-	+	+	+	+	+	+++	+++	+	+	Type III

Type I, below 100 µm and 3–5 sclerenchymatous layers; type II, 100–300 µm and 3–8 layers; type III, above 300 µm and > 8 layers.



**Figure 6.** Phylogram constructed from 1998 trees in MrBayes. All the clades are labelled. Numbers on the branches indicate the posterior probabilities (pp). All the coloured branches correspond to the four clades formed by Schrire *et al.*, 2009. A black line shows the evolution of the pod type character at specific nodes carried out from the ancestral state reconstruction by parsimony and likelihood methods in MESQUITE. The likelihoods were calculated for the 100 most probable trees in the TRPROBS file in MrBayes. The character states marked show the most probable states at that node.

**Table 5.** Summary of evolution of pod types in tribe Indigoferae

Pod types	Pod type I	Pod type II	Pod type III
A – Recent common ancestor to <i>Cyamopsis</i> + <i>Indigastrum</i> + <i>Indigofera</i> clade	0.0287652 in 100 trees	0.3467892 in 100 trees	<b>0.624524</b> in 100 trees
B – Ancestral <i>Indigofera</i>	0.0077725 in 100 trees	<b>0.747155</b> in 100 trees	0.24512 in 100 trees
C – Ancestral Palaeotropical + Pantropical clade	0.006239 in 100 trees	<b>0.731111</b> in 100 trees	0.262650 in 100 trees
D – Ancestral Tethyan clade	0.00397931 in 100 trees	<b>0.97843279</b> in 100 trees	0.0175879 in 100 trees
E – Ancestral Palaeotropical clade	0.0016906 in 100 trees	<b>0.9542524</b> in 100 trees	0.044057 in 100 trees
F – Ancestral Pantropical clade	0.0049752 in 100 trees	0.3916416 in 100 trees	<b>0.6033832</b> in 100 trees

Maximum likelihood values for reconstruction of pod types (Pod type I, II, III) at specific nodes of the tree (Fig. 6) taken from the 100 most probable trees from the TRPROBS file retrieved from MrBayes.

Probabilities which are more than 0.6 are in bold because they are considered to be the most probable state for that node.

derived compared to the large pod type with thick pericarp and more sclerenchymatous layers in the endocarp (more than eight) (pod type III). It is evident from character reconstruction that an increase in pericarp thickness and number of sclerenchyma layers (pod type III) is probably a plesiomorphic trait of the genus and a decrease in pericarp thickness and number of sclerenchyma layers (pod type II and pod type I) is apomorphic for the tribe.

In *Indigofera*, dehiscence is caused by a separation layer present at the dorsal and ventral sutures except in some species (*I. hochstetteri*, *I. karnatakana*, *I. glandulosa* var. *sykesii*, *I. trita* var. *scabra*) where no separation tissue is present and the species show a tardily dehiscent or an indehiscent condition. The indehiscent pod type is considered to be apomorphic.

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