

Taxonomy and phylogeny of the genus *Crotalaria* (Fabaceae): An overview

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ABSTRACT

Crotalaria (Fabaceae) is the largest legume genus represented by 81 species in India. The existing sectional classification for *Crotalaria* in India is based on morphological characters and is not completely supported by the molecular phylogenetic studies. Molecular systematic studies of the Indian sections *Crotalaria* and *Calycinae* have enabled merging of both these sections into *Calycinae*. Trifoliolate condition in the genus and leaf beak not spirally twisted are plesiomorphies for the genus. Pollen morphology is a poor criterion for infrageneric classification since pollen grains do not show much variability. Chromosome counts for the genus show two basic numbers which are $x=7$ and predominantly $x=8$. Polyploidy is common in the many new world species. *Crotalaria* from the new world seems to be the last colonized and polyploidy is one of the evolutionary strategies for diversification in the New World species. Molecular phylogenetic studies suggest that the sections *Calycinae* and *Crotalaria* are non-monophyletic. The genus *Crotalaria* with 81 species, consisting of 27 endemics (confined to peninsular India) indicates an ongoing rapid evolution and diversification.

Keywords: Fabaceae, *Crotalaria*, taxonomy, chromosome, alkaloids, pollination, pollen morphology, molecular systematics, ITS

INTRODUCTION

The subfamily Papilionoideae (Fabaceae) includes approximately 478 genera and 13,800 species grouped in 28 tribes [1]. Within the Papilionoideae, the “genistoid” clade as a whole, is defined by accumulation of quinolizidine alkaloids and a base chromosome number of $n = 9$ [2-4]. The tribe Crotalarieae is a part of the core genistoid and comprises ca. 1204 species [5]. Generic delimitations within the tribe have changed as a result of morphological, chemotaxonomic [6] and molecular systematic studies [7-10]. Three clades and 16 genera are now recognized in the tribe Crotalarieae (Figure 1). The tribe consists of three genera viz., *Euchlora*, *Bolusia* and *Crotalaria*. *Euchlora* has been reinstated into a separate genus from the previously known *Lotononis hirsuta* which is a non-monophyletic group [7,11].

The genus *Crotalaria* L. (Fabaceae, Papilionoideae) comprises ca. 702 species [12] distributed in tropical and sub-tropical regions of the world (Africa & Madagascar, with 543 species) [12-14], tropical Asia (126 spp.), South America (68 spp.), North America (31 spp.), and India (81 spp.) with little inclusions in Pakistan (11 spp.) and China (42 spp.). The centres of diversity are Eastern and Southern tropical Africa and India with two more centres in Mexico and Brazil [15].

In India, the genus *Crotalaria* is represented by 81 species, of which 27 are endemic. 15 species of *Crotalaria* are listed in the Red Data Book of Indian plants [16-17]. The known causes of

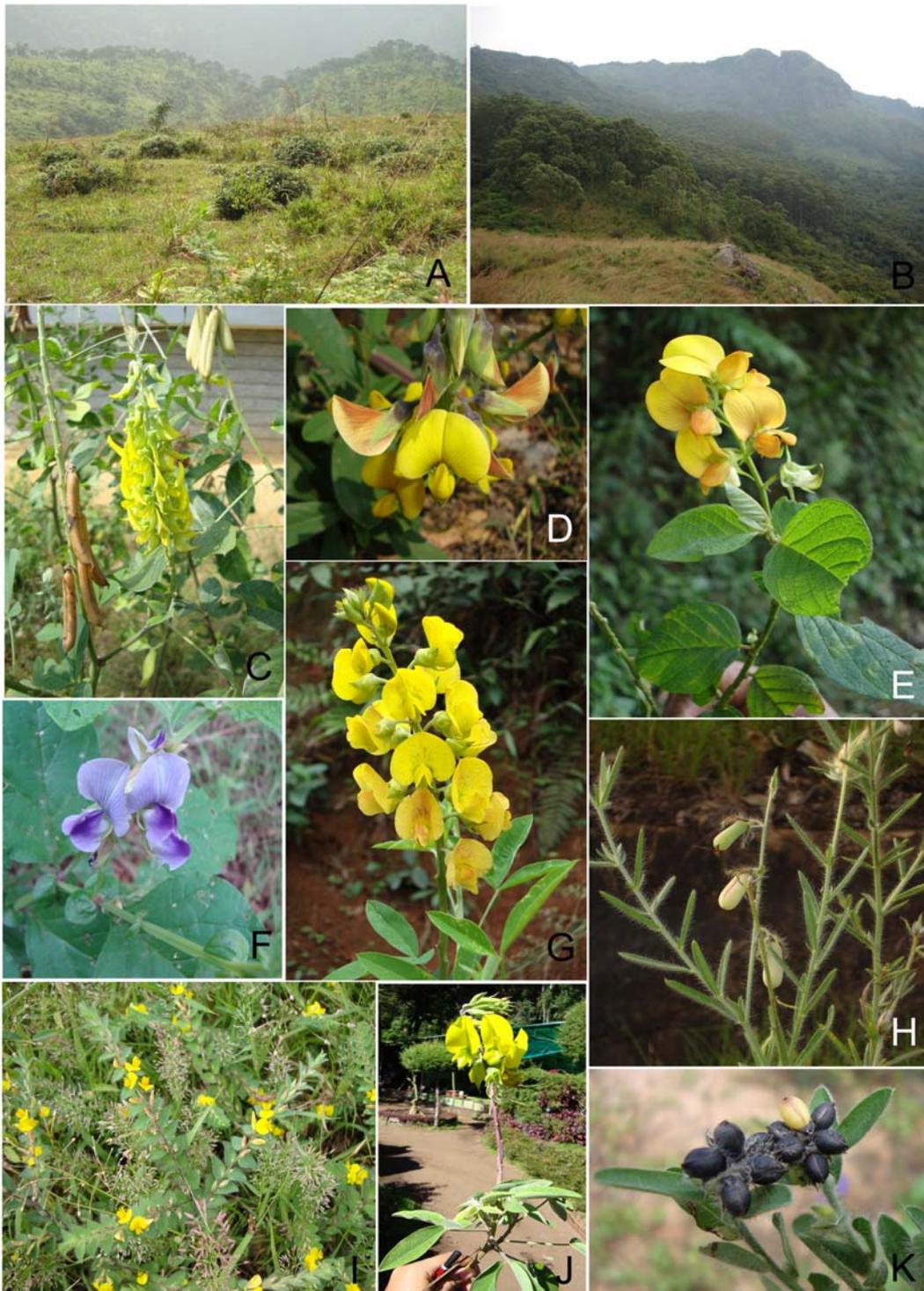


Figure 2. Habit and habitats of *Crotalaria*. A. open grasslands (Pazhani, Tamil Nadu), B. cut slopes (Idukki, Kerala), C. *C. pallida*, D. *C. retusa*, E. *C. semperflorans*, F. *C. verrucosa*, G. *C. micans*, H. *C. mysorensis*, I. *C. hebecarpa*, J. *C. agatiflora*, K. *C. nana*.

The oil has potential application in cosmetic industries. The oil constitutes chiefly three major compounds, geraniol (6.42%), β -ocimene (6.17%) and calamenene (6.09). Our field observations signify that the plant is an excellent colonizer of dry scrub forests and possess cylindrical root nodules which play important role in contributing to the nitrogen economy of scrub forests. The plant is also used as a fuel wood crop and insect repellent in the tribal regions. *Crotalaria* species contain macrocyclic pyrrolizidine alkaloids which are considered as important secondary metabolites largely on account of their biological activities, which include acute hepatotoxic [24], mutagenic [25], carcinogenic [26], teratogenic [27], anticancer [28] and neuroactive properties [29]. Although genus *Crotalaria* has attracted the attention of taxonomists since long, but no attempt has so far been made to review the work done on the systematics and phylogeny of the genus. The objective of the present communication is to review the literature on distribution, taxonomy, chromosome evolution, secondary metabolites, pollen morphology, pollination biology and molecular phylogeny.

TAXONOMY AND INFRAGENERIC CLASSIFICATION

The genus *Crotalaria* was first described by Carolus Linnaeus [30] in the “Species Plantarum” where he enumerated 13 species, viz., *Crotalaria perfoliata*, *C. sagittalis*, *C. juncea*, *C. triflora*, *C. villosa*, *C. verrucosa*, *C. lotifolia*, *C. lunaris*, *C. laburnifolia*, *C. micans*, *C. alba* and *C. quinquefolia*, four of which were native to India. The Indian representatives were *Crotalaria juncea*, *C. verrucosa*, *C. retusa* and *C. quinquefolia*. Out of these, six have been reduced to synonymy and seven still circumscribed as legitimate species. The type of the genus is based on *Crotalaria lotifolia* L. which is now preserved as a lectotype.

The first infrageneric classification of the genus *Crotalaria* was given by Lamarck [31]. He divided the genus into two groups : (a) simple-leaved group and (b) trifoliolate and digitate-leaved group. Later workers [32-34] adopted the previous classifications but [35] erected seven groups I-VII, where he used other characters like petiole length, presence or absence of stipules and their size.

Polhill [13] in his work “Miscellaneous notes on African species of *Crotalaria* L.: II” mentioned the difficulty in finding a natural and workable system. He erected 11 sections and seven subsections based on flower morphology, legume morphology and bracteole shape. Bisby & Polhill [36] together worked on the taxometrics of *Crotalaria* and found that the classification system was largely similar to Polhill [13] but some discrepancies were resolved and it resulted in a workable and unambiguous classification system wherein they recognized eight sections and nine subsections. The sections recognized were: *Grandiflorae*, *Chrysocalycinae*, *Incanae*, *Stipulosae*, *Hedriocarpaceae*, *Geniculatae*, *Schizostigma*, *Calycinae* and *Crotalaria*. This classification was later on followed by Polhill [14], in his book “*Crotalaria* in Africa and Madagascar” which is regarded to be till now the most comprehensive and meticulous description of the genus *Crotalaria* in Africa and Madagascar.

INFRAGENERIC CLASSIFICATION OF INDIAN CROTALARIA

Carolus Linnaeus [30] in his first edition of “Species Plantarum” enumerated 13 species of the genus, but did not give any formal classification. The first classification system with respect to the Indian sub-continent was given by Roxburgh [37] in his “Flora Indica”. He divided the genus into two sections viz., simple leaved and compound leaved. Eight species were included in the compound leaved section and rest in the simple leaved section. He described the genus as having five parted calyx, beaked keel and connate filaments with a fissure at the back which has a circular gap at the base, alternately sagittate anthers and sub-rotund with turgid pods. In his enumeration, he described 17 new species viz., *Crotalaria tenuifolia*, *C. tetragona*, *C. stipulacea*, *C. stricta*, *C.*

montana, *C. fulva*, *C. pulcherrima*, *C. ramosissima*, *C. caespitosa*, *C. prostrata*, *C. alata*, *C. bialata*, *C. orixensis*, *C. cytisoides*, *C. procumbens*, *C. bracteata*, and *C. elliptica*. Wight & Arnott [38], in their Prodromous Florae Peninsulae Indiae Orientalis provided the first dichotomy in classification as that of Roxburgh [37] with some extensions. The first dichotomy was: leaves simple, sessile and shortly petioled and the second one- leaves long-petioled, digitately 3-5 foliolate. Further, under the main leads, they divided the genus into 13 sections giving keys, description and justification of synonymy for many species. The sections were recognized based on nature of stipules, leaf and leaflets, bracts and bracteoles, number of flowers/inflorescence, its position, legume shape, number of seeds/pod, plant surface, calyx length in relation to corolla and vexillum surface. 13 sections established were: *Alatae* Wight & Arn., *Calycinae* Wight & Arn., *Fulvae* Wight & Arn., *Bracteatae* Wight & Arn., *Eriocarpae* Wight & Arn., *Erectae* Wight & Arn., *Diffusae* Wight & Arn., *Sphaerocarphae* Wight & Arn., *Microcarphae* Wight & Arn., *Dispermae* Wight & Arn., *Podocarphae* Wight & Arn., *Hedriocarphae* Wight & Arn. and *Polyphyllae* Wight & Arn. Under the sections *Erectae* and *Diffusae*, he created subsections based on legume surface, leaf stalk, and shape and size of stipules.

Dalzell & Gibson [39] in their “Bombay Flora” did not give any infrageneric classification, the reason being, less number of species (20) wherein they could not find natural groups within the genus since their work mainly focused the Bombay region. Baker [40] in Hooker’s Flora of British India divided the genus into seven sections and three groups. The sections erected by Bentham were: *Arenariae*, *Diffusae*, *Alatae*, *Calycinae*, *Glaucæ*, *Erectae* and *Eriocarpae*. The groups were trifoliolate-dispermae, trifoliolate-polyspermae and multifoliolate. He followed Wight & Arnott [38] largely, but dissolved six section and retained five and newly erected two sections viz., *Arenariae* and *Glaucæ*. The groups, he erected followed the first dichotomy of Wight & Arnott [38]. The sections were recognized based on habit, type of branchlets, nature of leaves, stipules, raceme position, pod surface, shape and its nature. He enumerated 77 species of which two were new additions to the previous work. He provided a key under the sections *Calycinae* and *Eriocarpae* based on pods and inflorescence characters. Cooke [41] in his “Flora of the Presidency of Bombay” gave no sectional classifications and groups. He gave a detailed key for the identification of each species, the first following the leaf nature (leaf simple, trifoliolate and 3-7 foliolate with glabrous pods), probably he did not take into account the previous classification since his work was a regional one.

Fyson [42] in “Flora of Nilgiris and Pulney hill Tops” accepted the sectional classification of the previous workers but somehow did not implement it in his work, probably he did not have many species to enumerate, but he described characteristic features of the genus and variability therein. Fyson [42] mentions about the great differences in general habit which varies from trailing plants, to erect herbs, to large shrubs and small trees. Variation is also observed in the stipules which may be ear-shaped, encircling the axis, prolonged downward as narrow wings or altogether absent. Some species are nearly completely glabrous and others being covered with silky hairs. Combined with these and some other characters, the genus according to Fyson can be easily divided into sections. However, he did not included these features in his work. The constant characters being the inflated pods, corolla form, reniform seeds, monadelphous anthers of two lengths: alternately short and long, wing petals with ridges/puckerings which definitely has connection with the visit of the insects.

Gamble [43] in “Flora of the Presidency of Madras” erected eight sections viz., *Alatae*, *Diffusae*, *Glaucæ*, *Erectae*, *Calycinae*, *Eriocarpae*, *Trifoliolatae* and *Multifoliolatae*, and mainly followed the works of Wight & Arnott [38]. Baker [40] did not propose any new section and retained all the previously recognized names. He gave a detailed key to the species and the sections. Four new species were added to the previous work. Of the 75 species, ten species are reduced to synonymy or to varietal status. Sanjappa [44] enumerated 97 species of *Crotalaria* out of which

four have been reduced to synonymy of various species. Ansari [45] enumerated 90 species (two insufficiently known and one addendum and one new species added subsequently), 1 subspecies, 17 varieties and 2 formae. He made six sections *Calycinae*, *Chrysocalycinae*, *Crotalaria*, *Dispermae*, *Grandiflorae* and *Hedriocarpae* and 12 subsections and used a modified infrageneric classification system of earlier workers [13-14,38,40]. The sectional classification is based on the nature of leaf, form of keel beak, division of calyx, calyx length in relation to corolla, pod surface, nature of stipules, petioles and pod shape.

Humboldt, Bonpland and Kunth [46] described nine new species from the New World. Bentham [47] recognized 32 species from Brazil. Senn [33] documented 31 species from North America. Windler [48], listed 12 unifoliolate species from North America. Flores et al. [49] published notes on Brazilian species of *Crotalaria* section *Calycinae* Wight & Arn. De Munk [50] published 38 species of *Crotalaria* in Malaysia. Holland [51] listed 36 species from Australia and Adema [52] published some notes on Malaysian species of *Crotalaria*. Jianqiang [53] described 42 species of *Crotalaria* occurring in China. Leverett & Woods [54] enumerated seven species of *Crotalaria* from Alabama.

CHROMOSOMAL EVOLUTION: TWO BASE NUMBERS

Windler [48] studied chromosome number for 10 species of North American *Crotalaria* of the section *Calycinae* and stated that the Asian species are ancestral to American species without any chromosomal evidence. Ferreira et al. [55] compared the meiotic behaviour and pollen viability of three species of *Crotalaria*, where they found abnormal meiotic behaviour in *Crotalaria micans*, *C. spectabilis*, and *C. zanzibarica*. The possible reason for this abnormal behaviour is predicted as cytotoxicity which would then have important implications in the evolution and can result in the formation of aneuploids and polyploids. Flores et al. [15] enumerated new chromosome counts for 15 species out of 23 Brazilian species of *Crotalaria*. *Crotalaria incana* L. (*Chrysocalycinae*) has $2n = 14$ and *C. tweediana* (*Calycinae*) Benth. has $2n = 54$ which has a high chromosome count reported for any species in the genus. Two basic numbers were suggested for the species viz., $7x-2$ based on $x=8$ which will be an aneuploid polyploidy and other based on $x = 9$ will be a hexaploid. Predominance of $x=9$ is present in the tribe Crotalarieae [56]. *Crotalaria* from the new world seems to be the last colonized and polyploidy is one of the evolutionary strategies for these species and increase in polyploidy is the trend observed [15]. Southernmost species of *Crotalaria* were examined by Almada et al. [57]. *C. tweediana* $2n=64$ shows highest ploidy level so far recorded. From an evolutionary point of view, decrease in the basic number of chromosome is observed by the phenomenon of dysploidy. All the tetraploids show small chromosomes as compared to diploid species eliminating them from the excess genetic material in the duplicated chromosomes and repetitive DNA [57-58]. This statement is not in conjunct for *C. tweediana* which is expected to have smaller chromosome but has large chromosome. This phenomenon probably works at the tetraploid level. *Crotalaria* species has a chromosomal length (CL) of 1.189, which is the lowest value reported so far for diploids of the genus [57].

Study on chromosome number of Indian *Crotalaria* was initiated by Magoon et al. [59] followed by Patil & Chennaveeraiah [60], Sybenga [61] and Datta & Biswas [62]. Patil & Chennaveeraiah [63] suggested $x = 8$ as base number for the genus, but Datta & Choudhary [64] expressed their doubt for this base number due to the presence of multivalents at the time of microsporogenesis in *C. ferruginea*. Kumar & Subramaniam [65] in their "Chromosome Atlas of Flowering Plants of the Indian subcontinent" enumerated chromosome numbers for 54 species and subsequently more chromosome numbers for other species were added. All the polyploid species of *Crotalaria* are seed fertile which indicates to the survival strategy of these species [66,67]. *Crotalaria* is a dibasic genus with two basic chromosome numbers i.e., $x = 8$ and $x = 7$. Of the two,

$x = 8$ is the predominant one. *Crotalaria polysperma* and *C. medicaginea* have both the races [67,68]. *C. incana* (section *Incanae*), *C. quartiniana* and *C. mauensis* show a base number of $x = 7$ [69]. They evaluated twenty species and noted chromosome stability based on the normal bivalents. Speciation in the genus arose mainly due to the structural changes [70], and gene mutations at the diploid level [69]. Chromosome at pachytene stage in eleven species of *Crotalaria* were studied by Gupta & Gupta [71]. Species of section *Incanae* have predominantly $2n = 14$. Section *Incanae* is derived from $2n = 16$ [72], so the trend moves towards the symmetrical nature of karyotypes. There must have been an increase in the size of the smallest chromosomes in section *Grandiflorae* and to compensate that the reduction in the chromosome number to 14 might have taken place in the section *Incanae* [71]. From evolutionary point of view, it is being accepted that $n = 8$ is the primary base number from which $n = 7$ has evolved [67]. Different viewpoints have been suggested for this reduction. Senn [73] attributed it to aneuploid reduction. Atchinson [74] opined that it is due to loss of a metacentric chromosome which eventually lead to transfer of genetic material to one chromosome making the other susceptible to deletion. This viewpoint has been supported by other workers [70,75]. Nucleolar studies carried out in some species of *Crotalaria* reveal that there is uniformity in nucleolar chromosome number [66,76]. Verma & Raina [69] stated that the nucleolar chromosome number remains one in all other species except *Crotalaria agatiflora* (tree species) whereas Mangotra and Koul [76] record two nucleolar chromosome number.

SECONDARY METABOLITES: THE PYRROLIZIDINE ALKALOIDS

Alkaloids are secondary metabolites that play a vital role in defence, pollination, dispersal etc. Studies on the alkaloids have resulted in clarification of clades in the tribe Crotalariaeae. Data from alkaloids are largely harmonious with the morphological data at the tribal level [77]. Genera included in tribe Crotalariaeae have been classified into four clades viz., Group I: without α -pyridone alkaloids and without esters of alkaloids (*Aspalathus*, *Lebeckia*, *Rafnia*, *Wiborgia* and perhaps *Spartidium*); Group II: specializing in lupanine-type esters (*Pearsonia* and *Rothia*); group III: with macrocyclic pyrrolizidine alkaloids (*Crotalaria* and *Lotononis*); and Group IV: a specialized group with α -pyridone alkaloids (*Argyrobium*, *Dichilus*, *Melolobium* and *Polhillia*) [77].

Both *Crotalaria* and *Bolusia* are sister groups based on morphological characters and included in cape genera. Macrocyclic pyrrolizidine esters in *Lotononis* and *Buchenroedera* revealed a possible relationship with the genus *Crotalaria* and the alkaloid cladogram established it in contrast to the phylogenetic tree based on the morphological characters which states that the *Lotononis* instead of Cape group is nested in the *Lotononis* group [77]. Both these genera share a common ancestry with *Rafnia*, *Aspalathus* and *Lebeckia*. Fletcher et al. [78] studied the hepatotoxic pyrrolizidine alkaloids in 24 *Crotalaria* taxa from Northern Australia. *Crotalaria* species with the highest alkaloid content are: *C. ramosissima*, *C. retusa* var. *retusa*, *C. aridicola* subsp. *densifolia*, *C. crispata*, *C. goreensis*, *C. medicaginea* var. *neglecta*, and *C. novae-hollandiae* subsp. *novae-hollandiae* chemotype. The animal poisoning occurs mainly in cattle, horse (Chillagoe horse disease), chickens and sheep (acute lung damage). The variation in the alkaloid content and alkaloid type was observed in different species of *Crotalaria* [78]. Molecular phylogeny of defence traits in legumes has been studied in detail [79]. Quinolizidine alkaloids are present in genistoid clade, with an exception of *Crotalaria* which has pyrrolizidine alkaloids and which also accumulates isoflavonoids. *Crotalaria* and *Lotononis* derive from the same ancestors. The ancestor accumulates the quinolizidine alkaloid and the genes for producing the biosynthetic enzyme should still be present in *Crotalaria* but turned off completely and partially in *Lotononis*. It is very unlikely that these genes are lost. Accumulation of pyrrolizidine alkaloids seems to be an attainment of a new chemical defence which probably evolved independently [79]. *Crotalaria* seems to be evolved from the same ancestor as of the tribes which produce quinolizidine alkaloids [80].

Secondary metabolites as compared to morphological or molecular sequence data have limited use as taxonomic marker [80]. Chemical analyses provided reasons for the separation of *Hypocalyptus* and *Loddigesia* from the Genisteae proper, and their possible merging with *Crotalaria* in the Crotalarieae. These are the only taxa giving positive leucoanthocyanidin tests, as does *Crotalaria* [81]. Unripe fruit tea extract containing monocrotaline of *Crotalaria retusa* induced a much variable chromosomal aberration in the mouse bone marrow cells when compared to the control mouse. Its clastogenic effectiveness is equivalent to mutagens e.g., cyclophosphamide can act as carcinogenic agent [82]. *Crotalaria* possess risk to human cells which was experimented with the human glial cells and exposure to monocrotaline causes increase in cell DNA damage index, contracted cytoplasm, vimentin destabilization, apoptosis and these indicated to neurological problems [83].

Secondary metabolites are important in classifying the generic position and this has been proved by the strong support for the placement of *Cyclolobium* (Millettieae, Fabaceae) close to Brongniartieae and genistoid tribes and its exclusion from Millettieae based on the presence of mainly quinolizidine alkaloids and DNA marker [84]. Pyrrolizidine alkaloids never co-occur with quinolizidine alkaloids in the same species. The presence of PA in *Lotononis* and *Buchenroedera* provided support for the inclusion of the genus *Buchenroedera* in *Lotononis*.

SYSTEMATIC SIGNIFICANCE OF POLLEN MORPHOLOGY

Pollen morphological studies in the genus *Crotalaria* was initiated by Bakker & Coetzee [85]. Lin & Huang [86] studied the pollen morphology of 18 species of *Crotalaria* in Taiwan and based on exine ornamentation recognized 5 different groups. Overall discrepancy in the pollen morphology of the genus is small, making palynology a poor criterion for infra-generic classification for the *Crotalaria* species found in Taiwan [86]. Palynological studies carried out by Masih et al. [87] for Fabaceae members infer radical variations in the pollen morphology which is helpful for classification at specific and generic levels. Members of Fabaceae examined show triangular pollen grains (in polar view) except *Crotalaria medicaginea* (which was taken as the only representative of the genus) which had circular pollen shape. As compared to other members of Fabaceae, *Crotalaria medicaginea* and *Sophora japonica* had the lowest exine thickness (0.5 µm) recorded [87]. Tectum of *Crotalaria medicaginea* is coarsely reticulate [88].

In India, pollen morphology of some species of *Crotalaria*, viz., *C. striata* L., *C. brownei* Bert. ex DC., *C. hirsuta* Wild., *C. ferruginea* Grah., *C. mysorensis* Roth. and *C. rotundicarinata* E. G. Baker has been carried out [80]. On the basis of P/E (length of the polaraxis and breadth at the equator) ratios, it has been reported that *Crotalaria medicaginea* is phylogenetically related to *C. usaramoensis* and *C. retusa*. *Crotalaria maritime* and *C. retzii* are closely related to *C. juncea* [90]. Pollens are monomorphic, tricolporate and angulaperturate. Datta & Choudhary [89] studied the pollen morphology of 16 species of *Crotalaria* and concluded that the evolution in the genus appears to be divaricate.

Gupta & Gupta [91] studied pollen morphology in 26 diploid *Crotalaria* species and cinched that the pollens were chiefly 3-zonocolporate and rarely 2-zonocolporate, endocolpium was lalongate and rarely lalongate and prolate, sub-prolate or spheroidal in shape and hence confirmed that the genus is fairly consistent in pollen morphology. Gupta & Gupta [71] studied the variation in diploid and induced autotetraploid in the pollen of four species of *Crotalaria* viz., *C. pallida* var. *pallida*, *C. retusa*, *C. juncea* and *C. sericea* and found that pollen grains of colchiploids were larger in size and were 4-colporate (1-7 colporate *C. retusa*) as compared to uniformly 3-colporate grains in the diploids. In colchiploids of *C. juncea*, 4-colporate condition is rare. Colchiploids exhibiting 4-colporate condition infringe the circumscription of Leguminosae and order Rosales [71].

POLLINATION BIOLOGY

Studies carried out on pollination biology on five species of *Crotalaria* viz., *C. juncea*, *C. madurensis*, *C. spectabilis*, *C. retusa* and *C. pallida* show that except *C. juncea* which is a cross pollinator, remaining four species are self pollinated [92]. Pollination in the latter species is ensured by dehiscence of the anthers before anthesis. Even the self pollinated species of *Crotalaria* have yellow and showy flowers which are adaptations for cross pollination. Colour, fragrance, corolla architecture and nectar glands promote the possibility of entomophily [93]. The showy flowers, yellow corolla, the fragrance, and the nectar glands present below the ovary seem to enhance cross pollination, but it has been observed that these characters help in self pollination promoted by the insects by lever mechanism [45]. Two common pollinators, monarch butterfly and bees, seem to be species specific.

MOLECULAR PHYLOGENY

Monophyly of the papilionoids as a family is moderately supported, based on *rbcL* analyses [94]. DNA marker (ITS) based results show a less closer relationship of *Lupinus* with *Crotalaria* [95]. *Crotalaria* belongs to the Genistoid clade which is mainly divided into four separate lineages viz., Northern hemisphere, South African, Australian and Neotropical Australian from where *Crotalariaeae* falls in the South African lineage [4]. The clade has a strong support with *matK* marker [4]. Based on *rbcL* analyses, the genistoid clade comprises the Genistae, *Crotalariaeae*, *Podalyrieae*, *Thermopsidae*, *Euchrestae* and also some *Sophoreae* [96]. At the tribal level viz; *Podalyrieae/Thermopsidae/Sophora flavescens/ Sophora jaubertii* seem to form a monophyletic clade which shares an ancestry with *Genistae* and *Crotalariaeae* when analysed with *rbcL* data set, while with ITS sequence data, they are divided into separate units [97].

Recent molecular systematic studies on the genus *Crotalaria* have thrown light on the sectional and sub-sectional classification [10,12]. Both the studies show that the section *Calycinae* Wight & Arn. and section *Crotalaria* are non-monophyletic and based on the morphological features should be merged to section *Calycinae*. Synapomorphies such as, keel beak pattern, standard petal type, calyx type, callosite and leaf type etc., hold both the sections into one clade. *Crotalaria* and *Bolusia* are sister groups based on morphological characters and molecular analyses [7].

Leaf morphology in *Crotalaria* varies from simple leaves (sections *Calycinae* and *Crotalaria*) to trifoliolate leaves (sections *Chrysocalycinae*, *Dispermae*, *Grandiflorae* and *Hedriocarpae*) and multifoliolate leaves are also present (section *Crotalaria* subsection *Polyphyllae*) with few exceptions in each section. Sequences of the nuclear internal transcribed spacers from species representing all the six recognized sections of Indian *Crotalaria* were subjected to phylogenetic analyses. Ancestral state reconstructions were done for two morphological characters, corolla keel beak, and leaf form. Analyses reveal five major well-supported clades within a monophyletic *Crotalaria* [10]. Clade 1 has members with rostrate-spirally twisted keel beaks while the remaining four clades comprise members with keel beaks rostrate-not spirally twisted. Clade 1 further bifurcates into two clades characterized by differences in leaf morphology: (1a) contains species with simple leaves belonging to section *Calycinae*, section *Crotalaria* sub-section *Crotalaria* and subsection *Bractetae* and multifoliolate leaves belonging to section *Crotalaria* subsection *Polyphyllae*, (1b) contains species with trifoliolate leaves belonging to section *Dispermae*, section *Crotalaria* sub-section *Longirostres*. Sub-clade iii in Clade 1a contains four species complexes, each forming a monophyletic group. Each of the others contains one endemic species and one widespread species that is sympatric with the former (*C. hirta* and *C. mysorensis*; *C. epunctata* and *C. albida*; *C. clarkei* and *C. triquetra*). The study on Indian *Crotalaria* suggests that: (1) all members of section *Calycinae* and most members of section *Crotalaria* form a clade, (2) members

of section *Crotalaria* subsection *Longirostres* and section *Dispermae* form a distinct clade, (3) twisting of the keel beak is an apomorphy within the genus, (4) there is a reversal to keel beak rostrate- not spirally twisted in *C. shevaroyensis* and an independent origin of a rostrate- spirally twisted keel beak in *C. humilis*, and (5) the trifoliolate condition is a plesiomorphy in the genus and the simple leaf condition an apomorphy; there is a single reversal to the trifoliolate condition in *C. orixensis* [10]. The current morphological classification system of Indian *Crotalaria* species includes six sections, but these circumscriptions are not supported by the molecular data.

CONCLUSION

Crotalaria is the largest legume genus with 81 species. The prevailing sectional classification for the genus in India is based on morphological characters and is not seemingly congruent with the molecular phylogenetic studies. The Indian sections *Crotalaria* and *Calycinae* have been merged into section *Calycinae*. Trifoliolate condition in the genus and keel beak not spirally twisted are plesiomorphies for the genus. Pollen morphological studies reveals that the variations are small, making palynology a poor criterion for infra-generic classification. Chromosome counts for the genus have been slightly variable, with two basic numbers $x=7$ and predominantly 8. Polyploidy is common in many new world species. *Crotalaria* from the new world seems to be the last colonized and polyploidy is one of the evolutionary strategies for diversification of these species and increase in polyploidy is the trend observed. Molecular phylogenetic studies suggest that the Indian sections *Calycinae* and *Crotalaria* are non-monophyletic. The genus *Crotalaria* with 81 species, consisting of 27 endemics (confined to peninsular India) indicates an ongoing rapid evolution and diversification.

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