Nordic Journal of Botany 29: 200–203, 2011
doi: 10.1111/j.1756-1051.2010.00920.x,

Subject Editor: Henrik Ærenlund Pedersen. Accepted 12 November 2010

First report of chromosome numbers and karyotypes of two monotypic genera endemic to eastern Asia: *Brachystemma* (Caryophyllaceae) and *Craspedolobium* (Fabaceae)

Jin Xu, Zhi-Jian Yin, Tsuneo Funamoto and Hua Peng

J. Xu, Z.-J. Yin, H. Peng (hpeng@mail.kib.ac.cn), Key Lab. of Biodiversity and Biogeography, Kunming Inst. of Botany, Chinese Academy of Sciences, CN-650204 Kunming, Yunnan, China. JX and ZJY also at: Graduate Univ. of Chinese Academy of Sciences, CN-100049 Beijing, China. – T. Funamoto, Biological Inst., Fundamental Education and Research Center of Pharmaceutical Sciences, Showa Pharmaceutical Univ., 3-chome, Higashi-Tamagawagakuen, Machida City, JP-194-8543 Tokyo, Japan.

The chromosome numbers and karyotypes of *Brachystemma* and *Craspedolobium*, two monotypic genera endemic to eastern Asia, are reported here for the first time. The somatic chromosome numbers are 2n = 40 for *Brachystemma calycinum* and 2n = 22 for *Craspedolobium unijugum*. A karyotype of 2n = 2x = 40 = 28m + 12sm was found in *B. calycinum* and that of 2n = 2x = 22 = 12m + 10sm in *C. unijugum*, both of them have a moderately symmetrical karyotype type 2B and small-sized chromosomes. *Brachystemma* has a unique basic chromosome number in Alsinoideae, which may support its isolated taxonomic position. As do some morphological characters, the basic chromosome number x = 11 suggests that *Craspedolobium* belongs in the Millettioid clade.

The eastern Asiatic flora is characterized by an exceptionally large number of endemic genera, many of them are mono- or oligotypic (Takhtajan 1978), the combined total number of which is 726 (excluding Pteridophytes) (Qian 2001). Two monotypic genera, *Brachystemma* D. Don and *Craspedolobium* Harms, were recently collected during floristic surveys in the Yunnan region (Peng and Wu 1997, Peng 1998, Zhu et al. 2006).

Brachystemma was described by Don (1825) based on specimens collected from Nepal. Mizushima (1955) published a new species *Brachystemma ovatifolium* Mizushima, but subsequently transferred the species to *Stellaria* L. (Mizushima 1966). So *Brachystemma* is still considered as a monotypic genus (Bittrich 1993, Ke 1996, Lu and Gilbert 2001). The type species of the genus is *Brachystemma calycinum* D. Don, an annual herbaceous liana, mainly distributed in southeast Asia, southwest China, and the Himalayas (Ke 1996, Lu and Gilbert 2001). It often climbs among shrubs in open forests or on grasslands along mountain slopes at altitudes from 500 to 2700 m a.s.l. As a folk medicine, *B. calycinum* is used in China for treatment of rheumatism, limb numbness, impotence and foot edema (Wu et al. 1990). Recently, a new cyclic peptide and several new alkaloids have been isolated from this species (Cheng et al. 2002, Wang et al. 2004, Lu et al. 2007), of these two new alkaloids have proved to have inhibitory effects on lymphocyte proliferation (Lu et al. 2007).

Craspedolobium with a single species, *C. schochii*, was described by Harms (1921) based on four gatherings collected from Mengtze and Yunnanfu in the Yunnan Province. Subsequently, Wei and Pedley (2010) found the type specimen of *Millettia unijuga* Gagnepain to be identical to that of *C. schochii*, and they transferred *Millettia unijuga* to *Craspedolobium*. However, *Millettia unijuga* was described by Gagnepain (1913), clearly much earlier than the description of *C. shochii*. Therefore, the epithet "*unijuga*" has priority under the Art. 11.4 of the 'International code of botanical nomenclature' (McNeill et al. 2006), and *Craspedolobium unijugum* (Gagnep) Z. Wei & Pedley is the correct name. *Craspedolobium unijugum* is a woody liana, distributed from southwest China to southeast Asia (Xu et al. 2008, Wei and Pedley 2010). It occurs in sparse woodlands with moist soil along trails at altitudes from 600 to 2400 m a.s.l. In Chinese folk medicine, the plant has been used for promoting blood circulation, removing blood stasis, tonifying the blood, stopping internal bleeding and eliminating dampness (Jiangsu New Medicinal College 1977). In a phytochemical study, Mei et al. (2000) isolated nine phenolic compounds, including a new one, from the 70% acetone extract of aerial parts of *C. unijugum*. Lan (2006) produced a Yeluma Qushi Zhentong medicine liquor for effectively treating rheumatoid arthritis, rheumatic numbness, chronic arthralgia, rheumatic arthralgia and myalgia and traumatic injury, etc. with obvious therapeutic effect. *Craspedolobium unijugum* root is one of five Chinese medicinal materials of this medicine liquor.

Karyological data are of great importance for systematic and evolutionary studies of plants and have been widely used to elucidate inter-familiar and/or generic relationships

and delimitate generic of familiar circumscriptions (Stebbins 1971, Raven 1975, Hong 1990, Tian et al. 2009). To date, the cytological characteristics of the two species here concerned remain unknown. Therefore, we here explore the chromosomal characteristics of the two genera and investigate their possible systematic and evolutionary implication.

Material and methods

The origin of experimental materials of *B. calycinum* and *C. unijugum* are listed in Table 1. Voucher specimens are deposited in KUN.

Root tips from germinated seeds were used for karyological study. The root tips of *B. calycinum* were pretreated in p-dichlorobenzene saturated solution at room temperature for 4 h, while root tips of *C. unijugum* were pretreated in 0.002 mol l^{-1} 8-hydroxyquinoline at room temperature for 4 h, then fixed in Carnoy's fluid (mixture of 95% ethanol and glacial acetic acid in ratio 3:1) at 4°C for at least 30 min. They were macerated in 70% ethanol overnight. After hydrolysis they were put in 1 N HCL at 60°C for 5 min (8 min for *C. unijugum*), then washed in distilled water, and stained with carbol fuchsin for 1 h and squashed for observation.

Karyomorphological observations were made on somatic mitotic metaphase, and measurements of chromosome arms were taken from five well-spread metaphases of five different root tips from each species. Karyotype formulas were derived from measurements of metaphase chromosomes from photomicrographs. The nomenclature used to describe the chromosome morphology follows Levan et al. (1964). The classification of karyotype asymmetry is according to Stebbins (1971).

Results

The metaphase chromosome morphology of the two species studied is shown in Fig. 1–4. The somatic chromosome number was 2n = 40 in *B. calycinum* (Fig. 1), and 2n = 20 in *C. unijugum* (Fig. 2). The length of chromosomes of *B. calycinum* ranged from 2.4 to 1.0 μm with a gradual decrease in length from the longest to the shortest chromosomes (Fig. 3).The total chromosome length was 72.6 μm and average size was 1.82 μm. The karyotype formula of this species is 2n = 2x = 40 = 28m + 12sm (Table 2). The ratio of the longest to the shortest chromosome is 2.34, and the karyotype asymmetry is categorized as type 2B. The length of chromosomes of *C. unijugum* ranged from 3.5 to 1.3 μm with a tri-modal decrease in length from the largest to the shortest chromosomes (Fig. 4). Among the 22 chromosomes, there were clearly recognizable gaps in length between pairs three and four, and between pairs ten and eleven. The total chromosome length is 49.2 μm with an average size of 2.24 μm. The karyotype formula of this species is 2n = 2x = 22 = 12m + 10sm (Table 2). The ratio of the longest to the shortest chromosome is 2.69, and the karyotype asymmetry is of type 2B. The chromosome complements of the two species, both found to be diploid, consisted of median- and submedian-centromeric chromosomes (Fig. 3–4; Table 2).

Discussion

In this study, chromosome numbers and karyotypes of *Brachystemma* and *Craspedolobium* are reported here for the first time. The basic chromosome number of *Brachystemma* and *Craspedolobium* is x = 20 and x = 11, respectively. Both species exhibit a karyotype asymmetry of type 2B, which means that they have moderately symmetrical karyotypes. The average chromosome size of *B. calycinum* is 1.82 μm and that of *C. unijugum* is 2.24 μm. The chromosomes are small-sized.

Bittrich (1993) placed *Brachystemma* in the subfamily Alsinoideae, tribe Alsineae. The basic chromosome number of Alsinoideae have been reported to be x = 6–17 and 19, which is rather variable (Bittrich 1993). *Brachystemma* has a different basic chromosome number of x = 20. McNeill (1962) concluded that the type species *B. calycinum*, has a very isolated taxonomic position, and no affinities with other plants can be suggested unless possibly a remote connection in cotyledon structure with *Arenaria* subgenus *Odontostemma*. This folding over of the cotyledons only occurs in this subgenus and in *Brachystemma*. McNeill also mentioned that it shows highly advanced characters in its specialized inflorescence, scariosus sepals and reduced gynoecium, while it is probably primitive in its *Stellaria*-like habit, though it is a struggling climber. There is no doubt that *Brachystemma* is best regarded as a distinct genus, with general affinity to *Arenaria* L., but having almost certainly followed a separate line of evolution from a very early stage in the development of the Alsinodeae (McNeill 1962). The unique basic chromosome number also suggested that it is a distinct genus. Furthermore, it should be noted that polyploidy is quite common in Alsineae (Bittrich 1993). We infer that *Brachystemma* may have gone through polyploidization from x = 10 to x = 20. Molecular data is lacking for this genus, but it is possible that further molecular phylogenetic studies will help in clarifying the systematic position of *Brachystemma*.

Craspedolobium is morphological similar to some genera from the tribe Millettieae as well as some genera of Phaseoleae. Harms (1921) indicated that its pod is *Millettia*-like (but winged) and *Derris*-like (but dehiscent), its 3-foliolate leaves with asymmetric lateral leaflets suggest a relationship with *Spatholobus* Hasskarl, from which it differs in fruit morphology. Geesink (1984) found that the calyx is hairy inside, as in *Kunstleria* Prain and as in some species of *Spatholobus*, and this genus is intermediate between

Table 1. Original localities, altitudes and voucher specimens used for the current study.

Taxa	Original locality	Altitude (m a.s.l.)	Voucher (KUN)
B. calycinum	China, Yunnan, Gejiu	1130	Peng Hua et al. 6038
C. unijugum	China, Yunnan, Shuangbai	2000	Xu Jin 0701

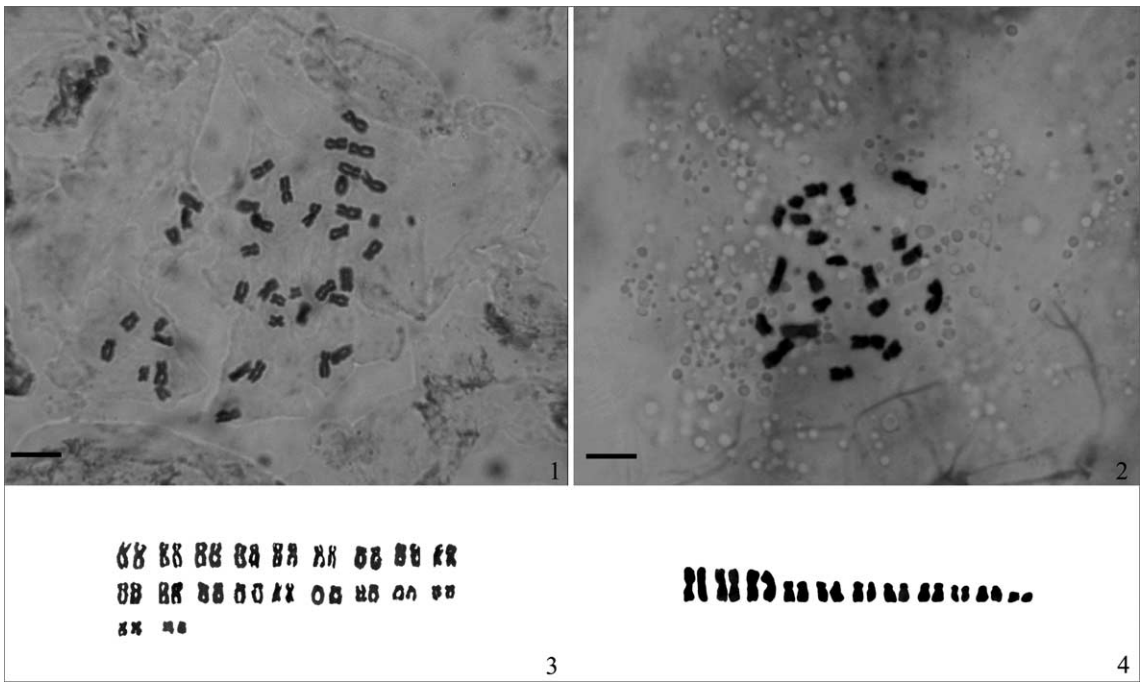

Figure 1–4. Cytological features. (1)–(2) chromosomes of metaphases, (3)–(4) karyotypes. (1), (3) *Brachystemma calycinum*, (2), (4) *Craspedolobium unijugum*. Scale bar = 5 μm.

Phaseoleae–Glycininae and Millettieae. Schrire (2005) placed it in the basal millettioid and phaseoloid group, which may belong either in the Millettioids s.s. or Phaseoleae s.l., or to a clade sister to both these groups. The basic chromosome numbers of *Derris* Loureiro and *Kunstleria* are x = 11 (Goldblatt 1981a, Kumari and Bir 1990), and x = 8, 10, 11, 12, 18 in *Millettia* Wight & Arnott (Goldblatt 1981b). There is no cytological data reported for *Spatholobus*. Therefore, the predominant basic chromosome number is x = 11 for these genera of the *Craspedolobium* affinity circle. *Craspedolobium* could not be differentiated from these genera on basis of the basic chromosome number. Furthermore, either x = 11 or 12 is basic within Millettieae, whereas the basic number for Phaseoleae is almost certainly x = 11 (Goldblatt 1981b). The basic chromosome numbers of the two tribes are stable. From the basic chromosome number of *Craspedolobium*, combined with aforementioned morphologic characters, it is concluded that it does belong in the Millettioid clade (Wojciechowski et al. 2004), which includes all genera of the tribes Millettieae, Abreae, Phaseoleae, and

Table 2. Parameters of mitotic metaphase chromosomes of two species. RL = relative length, AR = arm ratio, T = type of chromosomes classified following Levan et al. (1964), m = median region, sm = submedian region.

Brachystemma calycinum							
No.	RL	AR	T	No.	RL	AR	T
1	6.71	1.25	m	11	5.02	2.06	sm
2	6.39	1.40	m	12	4.84	1.35	m
3	6.18	1.39	m	13	4.73	1.91	sm
4	6.06	1.36	m	14	4.63	1.45	m
5	5.94	1.55	m	15	4.48	2.01	sm
6	5.73	1.40	m	16	4.32	2.02	sm
7	5.54	2.01	sm	17	3.85	1.55	m
8	5.42	1.29	m	18	3.63	1.40	m
9	5.37	1.34	m	19	3.53	1.74	sm
10	5.16	1.25	m	20	2.87	1.28	m
Craspedolobium unijugum							
No.	RL	AR	T	No.	RL	AR	T
1	13.99	1.01	m	7	7.77	2.10	sm
2	13.59	1.11	m	8	7.60	1.81	sm
3	12.79	1.10	m	9	7.07	1.69	m
4	8.47	1.60	m	10	6.89	2.67	sm
5	8.40	1.79	sm	11	5.21	1.36	m
6	8.22	1.73	sm				

Psoraleeae, plus Desmodieae subtribes Desmodiinae and Lespedezinae. In previous molecular phylogenetic studies of Millettieae based on chloroplast *trnK/matK* and nuclear ribosomal ITS data (Hu et al. 2000, 2002), *C. unijugum* has not been included. Thus, further studies are needed to clarify the systematic postion of *Craspedolobium*.

Acknowledgements – We are very grateful to Dr. Chun-Lei Xiang for his valuable comments and help in the preparation of the manuscript, and to David Paterson for his great help in language improvement. Many thanks also to Dr. Peter Goldblatt for his kind help with the literature.

References

Bittrich, V. 1993. Caryophyllaceae. – In: Kubitzki, K. et al. (eds), The families and genera of vascular plants. Vol. 2. Springer, pp. 206–230.

Cheng, Y. X. et al. 2002. Isolation and characterization of Brachystemidines A–E, novel alkaloids from *Brachystemma calycinum*. – J. Nat. Prod. 65: 750–752.

Don, D. 1825. Prodromus florae Nepalensis. – J. Gale, London.

Gagnepain, F. 1913. Especes nouvelles de Millettia. – Notul. Syst. 2: 365–366.

Geesink, R. 1984. Scala Millettiearum. – Leiden Univ. Press.

Goldblatt, P. 1981a. Chromosome numbers in legumes II. – Ann. Miss. Bot. Gard. 68: 551–557.

Goldblatt, P. 1981b. Cytology and the phylogeny of Leguminosae. – In: Polhill, R. M. and Raven, P. H. (eds), Advances in legume systematics, part 2. R. Bot. Gard., pp. 427–464.

Harms, H. 1921. Einige Leguminosen aus China. – In: Fedde, F. (ed.), Fedde Rep. Spec. Nov. Reg. Veg. Fabeckstrasse Press, pp. 133–137.

Hong, D. Y. 1990. Plant cytotaxonomy. –Science Press, Beijing.

Hu, J. M. et al. 2000. Phylogenetic systematics of the tribe Millettieae (Leguminosae) based on chloroplast *trnK/matK* sequences and its implications for evolutionary patterns in Papilionoideae. – Am. J. Bot. 87: 418–430.

Hu, J. M. et al. 2002. Phylogenetic analysis of nuclear ribosomal ITS/5.8S sequences in the tribe Millettieae (Fabaceae): *Poecilanthe–Cyclolobium*, the core Millettieae, and the *Callerya* group. – Syst. Bot. 27: 722–733.

Jiangsu New Medicinal College 1977. Zhongyao Dacidian. – Shanghai Sci. Technol. Press.

Ke, P. 1996. *Brachystemma* D. Don. – In: Tang, C. L. (ed.), Flora Reipubl. Pop. Sin. Vol. 26. Science Press, pp. 251–253.

Kumari, S. and Bir, S. S. 1990. Karyomorphological evolution in Papilionaceae. – J. Cytol. Genet. 25: 173–219.

Lan, Z. 2006. Laportea sinensis medicinal liquor for curing the diseases of rheumatoid arthritis, rheumatic numbness, chronic arthralgia, rheumatic arthralgia and myalgia and traumatic injury. – CN1813949-A.

Levan, A. et al. 1964. Nomenclature for centromeric position on chromosomes. – Hereditas 52: 201–220.

Lu, D. Q. and Gilbert, M. G. 2001. *Brachystemma* D. Don. – In: Wu, Z. Y. et al. (eds), Flora of China. Vol. 6. Science Press, Miss. Bot. Gard. Press, p. 66.

Lu, Q. et al. 2007. Two new alkaloids from *Brachystemma calycinum* and their inhibitory effects on lymphocyte proliferation. – Chem. Biodiv. 4: 2948–2952.

McNeill, J. 1962. Taxonomic studies in the Alsinoideae. I. Generic and infra-generic groups. – Notes R. Bot. Gard. Edinburgh 24: 79–155.

McNeill, J. et al. 2006. International code of botanical nomenclature, Vienna code. – Reg. Veg. 146, ARG Gantner Verlag.

Mei, S. X. et al. 2000. Constituents of *Craspedolobium schochii*. – Fitoterapia 71: 623–626.

Mizushima, M. 1955. A new species from Nepal collected by S. Nakao in 1953. – Acta Phytotax. Geobot. 16: 42–43.

Mizushima, M. 1966. Novelties in the Himalayan Cucurbitaceae and Caryophyllaceae. – J. Jap. Bot. 41: 259.

Peng, H. 1998. The seed plants from Mt Wuliangshan in the south–central Yunnan, China. – Yunnan Sci. Technol. Press.

Peng, H. and Wu, Z. Y. 1997. The preliminary floristical study on mid-montane humid evergreen broad-leaved forest in Mt Wuliangshan. – Acta Bot. Yunnan. 20: 12–22.

Qian, H. 2001. A comparison of generic endemism of vascular plants between east Asia and North America. – Int. J. Plant Sci. 162: 191–199.

Raven, P. H. 1975. The bases of angiosperm phylogeny: cytology. – Ann. Miss. Bot. Gard. 62: 724–764.

Schrire, B. D. 2005. Tribe Milletieae. – In: Lewis, G. P. et al. (eds), Legumes of the World. R. Bot. Gard. Kew, pp. 367–387.

Stebbins, G. L. 1971. Chromosomal evolution in higher plants. – Edward Arnold.

Takhtajan, A. 1978. Floristic regions of the World. – Soviet Sciences Press, in Russian.

Tian, X. M. et al. 2009. Karyological studies of *Parapteropyrum* and *Atraphaxis* (Polygonaceae). – Caryologia 62: 261–266.

Wang, C. et al. 2004. Study on the spatial structure of brachystemin C, a new cyclic peptide from *Brachystemma calycinum*. – J. Mol. Struct. 688: 67–71.

Wei, Z. and Pedley, L. 2010. *Craspedolobium* Harms. – In: Wu, Z. Y. et al. (eds), Flora of China. Vol. 10. Science Press, Miss. Bot. Gard. Press, p. 189.

Wojciechowski, M. F. et al. 2004. A phylogeny of legumes (Leguminosae) based on analysis of the plastid *matK* gene resolves many well-supported subclades within the family. – Am. J. Bot. 91: 1846–1862.

Wu, Z. Y. et al. 1990. A compendium of New China herbal medicine. Vol. 3. – Shanghai Sci. Technol. Press.

Xu, J. et al. 2008. Distribution change of *Craspedolobium schochii* and unacceptability of its endemism to China. – J. Trop. Subtrop. Bot. 16: 266–270.

Zhu, H. et al. 2006. Geological history, flora, and vegetation of Xishuangbanna, southern Yunnan, China. – Biotropica 38: 310–317.