Karyological Studies of 20 Populations of *Psammosilene tunicoides*

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Summary *Psammosilene tunicoides* W. C. Wu & C. Y. Wu, belonging to the monotypic genus *Psammosilene* in the family Caryophyllaceae, is an endangered and endemic plant to China. A karyological study was carried out on 20 populations of *P. tunicoides* across its distribution region. All populations of the *P. tunicoides* studied are diploid ($2n=2x=28$), and the basic chromosome number was confirmed to be $x=14$. Various chromosome morphologies were revealed, but didn't show clear geographical pattern. *P. tunicoides* is initially placed in *Silene* L., but *P. tunicoides* is different from *Silene* L. in the basic chromosome number of $x=10$ and 12. The results of this study support the view that *P. tunicoides* is turned into the independent genus from *Silene* L. Combining the available chromosome studies of *Saponaria* L. and *Psammosilene tunicoides*, the two species have relatively consistent chromosome diversification with a basic number of 14, and most species of the *Saponaria* L. contain diploid individuals, which suggested that there are certain phylogenetic relationships between *Psammosilene tunicoides* and *Saponaria* L.

Key words *Psammosilene tunicoides*, Chromosome, Karyotype, Caryophyllaceae.

Psammosilene tunicoides W. C. Wu & C. Y. Wu is an endemic Chinese species, distributed within an extremely limited range only in Yunnan Province, Sichuan Province, and Guizhou Province in Southwest China at an altitude range of 2000–3800 m (Lu *et al.* 2001). Based on our recent field surveys, its area of occupancy has been on a continuing decline. As the species is undoubtedly facing high risk of extinction, it has been ranked as the second most endangered species in China and is subject to national protection. Based on the species categories of the International Union for Conservation of Nature and Natural Resources (IUCN), *P. tunicoides* has been listed as a gradually endangered plant (Dai *et al.* 2007). This species is one of the most important Chinese traditional herbs and one of the ingredients of Yunnan Baiyao, which has been used in Tibetan medicine, Naxi medicine, and Yi medicine to treat rheumatism pain for a long time (Lan 1976). A series of bioactive compounds of saponines have been extracted from this herb (Zhong *et al.* 2003). It has received wide attention in the last few decades due to its remarkable pharmacological effects, particularly in bruises effects (Yang *et al.* 2008), analgesic and anti-inflammatory properties (Song 1981), and antibacterial and antifungal activity (Miao and Cai 2010). At present, *P. tunicoides* has been widely used to make sprays and cataplasm in China (Jin and Tan 2005). More than 10 t of dry materials of this species are used by medical companies every year in China. The tremendous demand of *P. tunicoides* has caused a serious reduction in native populations due to over-harvest.

P. tunicoides belonging to the monotypic genus *Psammosilene* in the Caryophyllaceae, where the whole *Psammosilene* genus contains only one species, is distributed uniquely in southwestern China, particularly its Yunnan region (Lu *et al.* 2001). When its specimen was collected in Yun-

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Table 1. Localities and vouchers of plant samples examined with the results of chromosome analysis: karyotype formula (KF); karyotype asymmetry (KA); arm ratio = sum of the long arms/sum of the short arms; T.C.% = sum of the long arms/total haploid length $\times 100\%$.

Species	Locality no.	Localities and altitudes (m)	Voucher	2n/basic no./ ploidy level	KF	KA	AI	Arm ratio	T.C.%
<i>Psammosilene tunicoides</i>	1	China, Yunnan, Gejiu; 2100	GJ090916	28/14/2x	8m+20sm	2B	0.86	1.89	61.95
	2	China, Guizhou, Weining; 2400	WN120919	28/14/2x	22m+6sm	2A	0.89	1.48	59.69
	3	China, Yunnan, Xuanwei; 2100	XW090908	28/14/2x	18m+10sm	2A	0.96	1.49	59.88
	4	China, Yunnan, Huize; 2750	HZ101009	28/14/2x	18m+10sm	2B	1.35	1.53	60.49
	5	China, Yunnan, Dongchuan; 2840	DC100908	28/14/2x	18m+10sm	2A	0.99	1.56	60.87
	6	China, Yunnan, Xundian; 1990	XD100926	28/14/2x	14m+14sm	2A	0.89	1.62	61.87
	7	China, Yunnan, Kunming; 2320	KM090928	28/14/2x	18m+10sm	2B	1.07	1.66	60.87
	8	China, Yunnan, Fumin; 2380	FM100922	28/14/2x	20m+8sm	2B	1.16	1.75	63.58
	9	China, Yunnan, Wuding; 2640	WD100916	28/14/2x	22m+6sm	2A	0.89	1.53	60.52
	10	China, Yunnan, Baoshan; 2050	BS100929	28/14/2x	20m+8sm	2B	1.13	1.65	62.27
	11	China, Yunnan, Eryuan; 2070	EY100908	28/14/2x	18m+10sm	2A	1.32	1.63	65.36
	12	China, Yunnan, Jianchuan; 2560	JC100908	28/14/2x	22m+6sm	2B	0.84	1.42	58.62
	13	China, Yunnan, Heqing; 2200	HQ100906	28/14/2x	20m+8sm	2A	1.13	1.52	60.28
	14	China, Yunnan, Lijiang; 2460	LJ090829	28/14/2x	12m+16sm	2A	1.02	1.78	64.01
	15	China, Yunnan, Ninglang; 2470	NJ101008	28/14/2x	14m+14sm	2A	0.76	1.49	63.77
	16	China, Sichuan, Yanyuan; 2360	YY120906	28/14/2x	24m+4sm	2B	1.08	1.40	58.33
	17	China, Sichuan, Muli; 2550	ML110923	28/14/2x	12m+16sm	2B	1.22	1.76	63.78
	18	China, Sichuan, Daocheng; 3260	DC120912	28/14/2x	18m+10sm	2B	0.96	1.63	61.92
	19	China, Sichuan, Deirong; 3180	DR110916	28/14/2x	16m+12sm	2A	0.84	1.61	61.69
	20	China, Yunnan, Zhongdian; 3230	ZD110908	28/14/2x	16m+12sm	2A	0.82	1.63	62.04

Fig. 1. Sampling localities of *Psammosilene tunicoides*. The numbers are the same as those in Table 1.

nan Lijiang by Englishman Forrest in 1906, this species was identified as a new species belonging to *Silene* L. by the Botanical Garden in Berlin, Germany, and it was published named *Silene cryptantha* Diels in Volume 5 of the Edinburgh Botanic Garden Notes in 1912. Later Wu Yunzhen and Wu Zhengyi collected the specimen of this species in Yunnan Kunming. Because they studied and found that the anatomic structures of this species, especially the anatomic structures of the flowers and fruits, distinguished it from the plants of *Silene* L., they classified this species into the monotypic genus *Psammosilene* (Wu and Wu 1945), which has been accepted by their successors (Kunming Institute of Botany, Chinese Academy of Sciences 1984, 1995, Hou 1998, Lu *et al.* 2001). The monotypic genus or family is classified as a key concern, and priority is given for the conservation of the taxa internationally to protect their irreplaceable character in the systematization or genetic features. So *P. tunicoides* has a high conservation value. On the other hand, *P. tunicoides* has a special system location, which has great value for system evolution of Silenoideae and even Caryophyllaceae, especially the systematic relationship research of Trib. Diantheae Pax.

In this study, we carried out karyological studies on *P. tunicoides* across its distribution region to detect geographical chromosome morphological variation. Chromosome number and karyology of *P. tunicoides* were studied, the results of which will provide cytology theoretical basis for taxonomic status of *P. tunicoides*.

Materials and methods

There are a total of 20 populations of *P. tunicoides* included in this study (Table 1). The sampling localities are shown in Fig. 1; the numbers on the map are the same as the sampling numbers in Table 1. Voucher specimens are deposited at Yunnan University of Traditional Chinese Medicine, Kunming, China.

Somatic chromosomes were studied using root meristems from introduced plants. Actively growing root tips were cut off and pretreated with *p*-dichlorobenzene at room temperature for 2 h, then fixed in fresh Carnoy's fluid (absolute ethanol:glacial acetic acid=3:1) at 4°C for *ca.* 30 min. Before staining, the materials were hydrolyzed with 1N HCL for 10 s at 60°C. Finally, samples were stained with a drop of carbol fuchsin and squashed. Permanent slides were made using the neutral balsam as the mounting medium. The best metaphase plates were photographed with a digital camera (LEICA DM1000) mounted on a LEICA DFC490 microscope. The nomenclature used for the description of the chromosome morphology followed Levan *et al.* (1964). Karyotype asymmetry was determined by the categories of Stebbins (1971). The asymmetry index value (abbreviation AI, $AI = (CV_{CL} \times CV_{CI}) / 100$, CV_{CL} expressing the relative variation in chromosome length, CV_{CI} expressing the relative variation in centromeric index) was determined according to Paszko (2006). The centromeric terminalization value (abbreviation T.C., $T.C.\% = \text{sum of the long arms} / \text{total haploid length} \times 100\%$) was determined according to Arano (1963).

Results

All 20 populations of this species are diploid ($2n=2x=28$). Metaphase chromosomes of 20 populations of *P. tunicoides* are shown in Fig. 2. Detailed descriptions of the cytological features of each population are as follows.

All populations are diploid with eight different karyotype formulae (Table 1). Among them, $2n=2x=18m+10sm$ (Fig. 2C–E, G, K, R) is the main karyotype formulae. Others are $2n=2x=20m+8sm$ (Fig. 2H, J, M), $22m+6sm$ (Fig. 2B, I, L), $12m+16sm$ (Fig. 2N, Q), $14m+14sm$ (Fig. 2F, O), $16m+12sm$ (Fig. 2S, T), $8m+20sm$ (Fig. 2A), and $24m+4sm$ (Fig. 2P). Their arm ratios (sum of the long arms/sum of the short arms) are from 1.40 to 1.89. The asymmetry of the karyotype is categorized as 2A and 2B, accounting for 55% and 45%, respectively. Values of T.C.% of over 60% are observed for 16 populations, while they are 59.69%, 59.88%, 58.62%, and 58.33% for the remaining four populations from Weining, Guizhou; Xuanwei, Yunnan; Jianchuan, Yunnan; and Yanyuan, Sichuan, respectively.

The chromosome numbers of 132 *Silene* and *Saponaria* taxa are available in chromosome number databases (Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/ipcn.html>). The taxa names and chromosome numbers are listed in Table 2; the distribution information and references were not included to save space. The chromosome number of 12 *Silene* and *Saponaria* species distributed in China are reported (marked by “*” on taxa names).

Discussion

Karyotype diversity of P. tunicoides

All 20 studied *P. tunicoides* populations are diploid, which is largely consistent with the previous studies by Pan *et al.* (2004) and basic chromosome number ($x=14$). Chromosome morphology varied among different populations and eight different karyotype formulae were detected. Among them, the main karyotype formulae is $2n=2x=18m+10sm$ with a percentage of 30.00%. However, the variation of karyotype formula, karyotype asymmetry, and T.C.% values did not show a clear geographical pattern. All populations of *P. tunicoides* have a karyotype asymmetry

Fig. 2. Mitotic metaphase of *Psammosilene tunicoides* (A–T): A) Gejiu, Yunnan; B) Weining, Guizhou; C) Xuanwei, Yunnan; D) Huize, Yunnan; E) Dongchuan, Yunnan; F) Xundian, Yunnan; G) Kunming, Yunnan; H) Fumin, Yunnan; I) Wuding, Yunnan; J) Baoshan, Yunnan; K) Eryuan, Yunnan; L) Jianchuan, Yunnan; M) Heqing, Yunnan; N) Lijiang, Yunnan; O) Ninglang, Yunnan; P) Yanyuan, Sichuan; Q) Muli, Sichuan; R) Daocheng, Sichuan; S) Deirong, Sichuan; T) Zhongdian, Yunnan. Bar=10 μ m.

Table 2. Chromosome numbers of 132 *Silene* and *Saponaria* taxa from chromosome number databases (Missouri Botanical Garden).

Taxon	2n	Taxon	2n
<i>Silene alba</i> (Mill.) E. H. Krause.*	24	<i>Silene depressa</i> Bieb.	24
<i>Silene alba</i> (Mill.) E. H. Krause subsp. <i>divaricata</i> Reichb.	24	<i>Silene dichotoma</i> Blackburn.	24
<i>Silene araratica</i> Boiss.	24	<i>Silene dichotoma</i> Blackburn. subsp. <i>racemosa</i> Blackburn.	24
<i>Silene araxina</i> Trautv.	24	<i>Silene dioica</i> (L.) Clairv.*	24
<i>Silene arguta</i> Fenzl.	48	<i>Silene doganii</i> A. Duran & Y. Menemen.	24
<i>Silene aucheriana</i> Boiss.	24	<i>Silene duralii</i> Y. Bağcı.	24
<i>Silene avromana</i> Boiss.	24	<i>Silene eremicana</i> Boiss.	48
<i>Silene behen</i> L.	24	<i>Silene eremitica</i> Boiss.	24
<i>Silene bellidifolia</i> Blackburn and Morton.	24	<i>Silene eretica</i> L.	24
<i>Silene boryi</i> Boiss.	24, 48	<i>Silene erysimifolia</i> Stapf.	48
<i>Silene bupleuroides</i> L.	24	<i>Silene fabaria</i> (L.) Sm.	24
<i>Silene caesarea</i> Boiss.	24	<i>Silene firma</i> Sieb. et Zucc.	46
<i>Silene capensis</i> Oth.	24	<i>Silene fortunei</i> Vis.	24
<i>Silene capitellata</i> Nitzsch.	24	<i>Silene fruticosa</i> L.	24
<i>Silene cappadocica</i> Boiss. & Heldr.	48	<i>Silene gallica</i> L.*	24
<i>Silene capraria</i> Sommier.	24	<i>Silene gigantea</i> L.	24
<i>Silene caryophylloides</i> (Poir) Oth. subsp. <i>subulata</i> Coode et Cullen.	24	<i>Silene gigantea</i> L. var. <i>incana</i> Chowdh.	24
<i>Silene caucasica</i> (Bunge) Boiss.	24	<i>Silene goniocaula</i> Boiss.	48
<i>Silene chlorifolia</i> SM.	24	<i>Silene goulimyi</i> Turill.	24
<i>Silene ciliata</i> Pourr.	24, 36, 48, 72, 96, 120, 196	<i>Silene graeca</i> Boiss. & Spruner.	24
<i>Silene claviformis</i> (Torr. & Frém.) P. H. Raven.	24	<i>Silene guntensis</i> Fedtsch.	48
<i>Silene colorata</i> Blackburn and Morton.	24	<i>Silene gynodioica</i> Ghanazfar.	48
<i>Silene colorata</i> Poiret.	24	<i>Silene hifacensis</i> Rouy ex Willk.	24
<i>Silene commelinifolia</i> Boiss.	24	<i>Silene hirticalyx</i> Boiss.	72
<i>Silene commelinifolia</i> Boiss. var. <i>isophylla</i> Bornm.	24	<i>Silene holzmannii</i> Boiss.	24
<i>Silene commelinifolia</i> Boiss. var. <i>ovatifolia</i> Melzh.	24	<i>Silene inaperta</i> Blackburn.	24
<i>Silene compacta</i> Fischer. ex Hornem.	24	<i>Silene isaurica</i> Contandr. et Quezel.	24
<i>Silene confertiflora</i> Chowdh.	24	<i>Silene italica</i> (L.) Perso.	24
<i>Silene conica</i> L.	20	<i>Silene lacera</i> (Stev.) Sims Schönswetter & Tribsch Iter Georgicum.	18
<i>Silene conica</i> L. subsp. <i>subconica</i> var. <i>grisebachii</i> .	20	<i>Silene laeta</i> Blackburn.	24
<i>Silene conoidea</i> L.*	20	<i>Silene lasiantha</i> C. Koch.	24
<i>Silene cythnia</i> (Halacsy) Walters.	24	<i>Silene latifolia</i> Brit et Rendle.*	24
<i>Silene damboldtiana</i> Greuter and Melzh.	24	<i>Silene laxa</i> Boiss. & Kotschy.	24
<i>Silene davidii</i> (Franchet) Oxelman & Lidén.*	48	<i>Silene longipetala</i> Vent.	24
		<i>Silene lucida</i> Chowdhuri.	48

Table 2. Continued.

Taxon	2n	Taxon	2n
<i>Silene lycanica</i> Chavdh.	24	<i>Silene ruprechtii</i> Schischk.	24
<i>Silene manissadjianii</i> Freyn.	24	<i>Silene salsuginea</i> Hub.-Mor.	24
<i>Silene maritima</i> With.	24	<i>Silene samia</i> Melzheimer. & Christodoulak.	24
<i>Silene marschallii</i> Meyer	24	<i>Silene sartorii</i> Boiss. & Heldr.	20
<i>Silene meyeri</i> Fenzl ex Buhse subsp. <i>persica</i> Kunth.	24	<i>Silene saxatilis</i> Sims.	24
<i>Silene meyeri</i> Fenzl ex Buhse.	24	<i>Silene scabriflora</i> Brot Characteristics.	24
<i>Silene microphylla</i> Boiss.	48	<i>Silene scouleri</i> Hook	60
<i>Silene neglecta</i> Weihe.	24	<i>Silene sedoides</i> Poiret.	24
<i>Silene nepalensis</i> Majumdar*	24	<i>Silene shahrudensis</i> Rech.	24
<i>Silene nigrescens</i> (Edgeworth) Majumdar*	48	<i>Silene sicula</i> Ucria.	24
<i>Silene nizvana</i> Melzh.	24	<i>Silene sisianica</i> Boiss. & Buhse.	24
<i>Silene noctiflora</i> L.*	24	<i>Silene skorpilii</i> Vel.	24
<i>Silene nocturna</i> L.	24, 36	<i>Silene spargulifolia</i> (Willd) Bieb.	24
<i>Silene nodulosa</i> Viv.	24	<i>Silene spinescens</i> Sm.	24
<i>Silene nurensis</i> Boiss.	24	<i>Silene stapfii</i> Melzh.	24
<i>Silene nutans</i> Heredity (Edinb).	24	<i>Silene subconica</i> Friv.	20
<i>Silene odontopetala</i> Fenzl.	30	<i>Silene supina</i> M. Bieb.	24, 48
<i>Silene olympica</i> Melzh.	24	<i>Silene swertiifolia</i> Boiss.	24
<i>Silene olympica</i> Boiss.	24	<i>Silene tenella</i> (Pall.) DC.	24
<i>Silene orientaltimongolica</i> Kozhevnikov.*	24	<i>Silene trachyphylla</i> Franchet.*	24
<i>Silene otites</i> Wibel.	24	<i>Silene urvillei</i> Dum.-Urville.	24
<i>Silene paphlagonica</i> Bornm.	24	<i>Silene vallesia</i> L.	24, 48
<i>Silene paradoxa</i> L.	24	<i>Silene viridiflora</i> L.	24
<i>Silene patula</i> Blackburn.	24	<i>Silene viscaria</i> Bornm.	24
<i>Silene peduncularis</i> Boiss.	24	<i>Silene viscosa</i> (L.) Pers.	24
<i>Silene pendula</i> L.*	24	<i>Silene vulgaris</i> (Moench) Garcke.	24
<i>Silene persica</i> Boiss.	24	<i>Silene vulgaris</i> subsp. <i>commutata</i> Pau.	24
<i>Silene propinqua</i> Fisch. & C. A. Mey.	40	<i>Silene vulgaris</i> subsp. <i>macrocarpa</i> Turrill.	48
<i>Silene pseudancheriana</i> Melzh.	24, 48	<i>Silene vulgaris</i> subsp. <i>maritima</i> (Heldr.) Hayek.	48
<i>Silene pungens</i> Boiss.	24	<i>Silene yetii</i> Bocquet*	72
<i>Silene rhynchocarpa</i> Melzh.	24	<i>Saponaria officinalis</i> L.	28
<i>Silene rubella</i> Larsen.	24		

*. *Silene* species distributed in China

of type 2A and 2B with arm ratio less than 2.00, indicating that all populations have relatively low interchromosomal asymmetry and a monomodal karyotype. There are a few points that need to be mentioned. The southernmost population of Gejiu (Yunnan) has the special karyotype formula, $2n=2x=8m+20sm$, and the highest arm ratio. Population Yanyuan (Sichuan) also has a unique karyotype formula, $2n=2x=24m+4sm$, and the lowest arm ratio and T.C.%.

Taxonomic status of P. tunicoides

P. tunicoides was first identified as a new species belonging to *Silene* L. in 1912. There are many reports about chromosome studies of the genus *Silene*; the chromosome number is mostly $2n=24$ with a few cases of $2n=48$ or $2n=20$, and the basic chromosome number is $x=12$ or $x=10$ (Luque and Diaz Lifante 1991, Nersesian and Goukasian 1995, Runemark 1996), but chromosome number $2n=28$ is not yet reported. Therefore the results of this study support the view that *Psammosilene tunicoides* is turned into an independent genus from *Silene* L. The genus *Saponaria* L. contains ca. 30 species only distributed in the Mediterranean. One species of *Saponaria* L. is introduced and cultivated in China. Chromosome information on the *Saponaria* genus showed that the chromosome number of *Saponaria* genus is $2n=24$ (Munn 1997). From the number of chromosomes, there are certain phylogenetic relationships between *Psammosilene tunicoides* and *Saponaria* L.

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