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广义报春苣苔属（苦苣苔科）的染色体新计数及其分类学意义*

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摘要：报道了广义报春苣苔属 14 种 3 变种和 7 个未定名种的染色体数目，并对其近缘属——广义石山苣苔属的 3 个种也进行了细胞学研究。结合最近的分类处理和系统发育假设，对所得结果和以前发表的染色体数据进行了综合分析，结果表明：广义报春苣苔属的染色体数目为 $2n=36$，基数为 $x=18$，表现出高度稳定性。染色体相对较小，以中间着丝粒和亚中间着丝粒染色体为主。尽管一些类群的染色体大小之间存在微小差异，但是染色体形态的均一性和染色体数目的一致性有力地支持分子系统学的研究结果。另外，相同的染色体数目及相似的染色体形态也表明广义报春苣苔属和广义石山苣苔属的亲缘关系较近，与分子系统学的结果一致。

关键词：染色体数目；染色体形态；广义石山苣苔属；广义报春苣苔属；分类处理
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New Chromosome Counts and Their Taxonomic Implications in *Primulina sensu lato* (Gesneriaceae)

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Abstract: Chromosome numbers can be helpful in understanding the taxonomy and phylogenetic patterns within plant groups. *Primulina sensu lato* (*s.l.*), a genus that has recently been redefined and expanded to include all species of Chiritopsis, two of Wentsaiboea and all of section Gibbosaccus of Chirita, has not been well documented cytologically. In the present study we determined the chromosome numbers of fourteen species, three varieties and seven undescribed taxa of *Primulina s.l.* using conventional rapid squash techniques. In addition, three species of its closest ally, *Petrocodon sensu lato* (*s.l.*), were also investigated cytologically. With the exception of *Primulina tabacum*, all counts are new. A high stability of chromosome number, $2n=36$, based on $x=18$, was recorded in *Primulina s.l.* Chromosomes were relatively small in size, with mostly metacentrics and submetacentrics predominating. Although slight variation in chromosome size occurs among taxa, the overall homogeneity of chromosome morphology

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and pattern of basic chromosome number conforms with the results of molecular phylogenetic studies, which strongly support the monophyly of *Primulina s. l.*, and also recent taxonomic treatment. Interestingly, the possession of the same chromosome numbers together with similar chromosome morphology, indicates that *Primulina s. l.* and *Petrocodon s. l.* are closely related, which is consistent with the molecular phylogeny of these genera.

Key words: Chromosome numbers; Chromosome morphology; *Petrocodon sensu lato*; *Primulina sensu lato*; Taxonomic treatments

Primulina sensu stricto (s. s.), belonging to Tribe Didymocarpeae (Gesneriaceae, Cyrtandroideae), was established formally by Hance (1883) with a single species, *Primulina tabacum* Hance. However, two recent molecular studies of phylogenetic relationships of *Chirita* D. Don and associated genera resulted in some taxonomic changes (Wang *et al.*, 2011; Weber *et al.*, 2011a). The monotypic *Primulina tabacum*, two species of *Wentsaiboea* D. Fang & D. H. Qin and all of *Chiritopsis* W.T. Wang were nested in a well-supported clade mainly with species of *Chirita* sect. *Gibbosaccus* C.B. Clarke (Wang *et al.*, 2011; Weber *et al.*, 2011a). As a result, the previous taxonomic treatments were critically re-evaluated and new taxonomic combinations were made to reflect evolutionary relationships, which brought all species of this group into the synonymy of *Primulina sensu lato* (*s. l.*). As newly defined, *Primulina s. l.* represents now a large genus of Old World Gesneriaceae, comprising approximately 130 + species, characterized by acaulescent, perennial plants mainly with opposite decussate leaves in whirls of three (Wang *et al.*, 1998; Li and Wang, 2004; Wei *et al.*, 2010; Wang *et al.*, 2011; Weber *et al.*, 2011a; Liu *et al.*, 2011; Hong *et al.*, 2012; Huang *et al.*, 2012; Li *et al.*, 2012; Wen *et al.*, 2012a, b, c, d; Wu *et al.*, 2012a; Wu *et al.*, 2012b; Xu *et al.*, 2012a, b). All the species occur predominately in limestone areas of western and southern China, with about a dozen species extending into or being endemic to Vietnam. The Guangxi Province in China is perhaps the center of species diversity for this genus, with high levels of species richness and many endemism (Wang *et al.*, 1998; Li and Wang, 2004; Wei *et al.*, 2010; Weber *et al.*, 2011a).

Although in recent years molecular data has become the center of numerous phylogenetic investigations, cytological data still play an important role in increasing our understanding of the genome evolution when seen in the light of molecular phylogenies (Weber and Burtt, 1998; Jong and Möller, 2000; Liu *et al.*, 2001; Liu, 2004; Liu and Yang, 2011). In Gesneriaceae, the cytologically investigated taxa frequently possess small chromosomes, ranging from less than 1 to 2 μm, and as a result few karyotypes have been published so far, mainly in Chinese taxa (Wang *et al.*, 1998; Wang and Gu, 1999; Zhou *et al.*, 2004; Yang *et al.*, 2012) and two for African taxa (Möller and Kiehn, 2004). However, even the establishment of ploidy levels or the exact chromosome numbers can be of taxonomic significance, since these data can be very useful in supporting taxonomic decisions. For instance in the dissection of *Didymocarpus* Wall., a genus originally composed of over 250 species but split into three different genera, *Didymocarpus* s. s., *Hovanella* A. Weber & B. L. Burtt and *Henckelia* Spreng. (Weber and Burtt, 1998). In addition to morphological and biogeographical data, they used differences in chromosome number and chromosome morphology to support their decisions (Möller and Kiehn, 2004). Another example is the genus *Streptocarpus* Lindl. Based on vegetative morphology, this genus was divided into two subgenera, *Streptocarpella* Fritsch and *Streptocarpus* (Hilliard and Burtt, 1971). Jong and Möller (2000) found that there was a strong correlation between basic number and growth form in the two subgenera of the genus on the African mainland.

Despite the potential taxonomic importance of chromosomal data, the newly defined *Primulina s. l.*

is poorly known cytologically. The first chromosome number in this genus was determined for *P. dryas* (=*Chirita sinensis*) with $2n=36$ (Ratter and Prentice, 1964). During the following forty years, no further numbers were added. Until recently, due mainly to the work of Christie *et al.* (2012) and Liu *et al.* (2012), the number of counts increased significantly. To date, chromosome data are available for 59 species out of *ca.* 130+ species recognized in *Primulina s. l.*. The earlier investigations indicated that the somatic chromosome numbers for *Primulina s. l.* are highly conservative, with $2n=36$ being prevalent, apart from two dubious counts and one polyploidy event (Cao *et al.*, 2003; Zhou *et al.*, 2004; Christie *et al.*, 2012; Liu *et al.*, 2012; Yang *et al.*, 2012). However, except for *P. tabacum*, all the available counts are for species previously assigned to *Chirita* sect. *Gibbosaccus*, while those of erstwhile *Wentsaiboea* and *Chiritopsis* had not yet been studied cytologically to date. Thus, the present survey was undertaken to contribute more chromosomal data. Additionally, three species of *Petrocodon sensu lato* (*s. l.*) were included in this study for comparison of the chromosomal characteristics between these closely related genera. *Petrocodon* has also recently been remodeled taxonomically (Weber *et al.*, 2011b) and is the closest relative of *Primulina s. l.* (Möller *et al.*, 2011), and the chromosome count in this genus is only available for *P. hancei*, with $2n=20$ (Cao *et al.*, 2003).

Materials and methods

The species with both current and previously applied names, origin information, and voucher specimens are listed in Table 1. Plants of 24 taxa of *Primulina s. l.* were collected from wild populations growing on limestone karsts mainly of Guangxi Province, with a few from Guangdong and Hainan Provinces of China, and cultivated in pots in the greenhouse of the Guangxi Institute of Botany of Chinese Academy of Sciences in Guilin, China. Actively growing root tips were harvested and pretreated with distilled water at 0 ℃ for 24 h before being fixed in Farmer's solution (absolute alcohol: glacial acetic acid 3∶1) at 4 ℃ for at least 30 min, then stored in 70% aqueous ethanol until studied. After being macerated in a 1∶1 mixture of 1 mol·L^{-1} HCl and 45% acetic acid at 60 ℃ for 3 minutes, the root tips were rinsed in distilled water, stained, and squashed in Carbol Fuchsin. The best metaphase plates were photographed using an Olympus BX51 microscope (Tokyo, Japan) with an Olympus DP71 camera attachment. The chromosome numbers were counted in at least ten cells with well-spread chromosomes from five different root tips from one plant for each species. The chromosome lengths were measured from at least three metaphase cells using the software package Image J (Media Cybernetics, Bethesda, USA). For the species treatment and generic circumscription, we followed Li and Wang (2004) and Weber *et al.* (2011a, b). Voucher specimens are deposited in the herbarium at the Guangxi Institute of Botany (IBK), Guangxi Zhuangzu Autonomous Region and Chinese Academy of Sciences.

Results

Chromosome numbers were obtained for 24 taxa of *Primulina s. l.* (including eight previously assigned to *Chiritopsis*, six of *Chirita* sect. *Gibbosaccus*, two of *Wentsaiboea*, along with *Primulina tabacum* and seven undescribed taxa), as well as three species of *Petrocodon s. l.* (two formerly in *Lagarosolen* and one in *Didymocarpus*) (Table 1). All of these are illustrated in Figs.1−2 to show chromosome morphology and size. The *Primulina* taxa studied are highly uniform in chromosome number, all with $2n=36$, a number also observed in three species of *Petrocodon s. l.*. Except for the confirmatory count for *Primulina tabacum*, all the counts are presented here for the first time. One or two chromosome satellites were observed in ten *Primulina* taxa, with one satellite being predominant and occurring in eight taxa, whereas just a single satellite was found in one *Petrocodon* species. The chromosome size in both genera

Table 1 The new and old names of the taxa investigated in this study, together with the locality, voucher specimens, somatic chromosome numbers, and satellite numbers

New names used here	Names as previously applied	Locality	Voucher specimen	2*n*	Number of satellites maximally detected
Primulina s.l.					
P.bicolor (W.T. Wang) Mich. Möller & A. Weber	*Chirita bicolor* W.T. Wang	Qingyuan, Guangdong	Kang-YD02-1	36	
P.bipinnatifida (W.T. Wang) Y.Z. Wang	*Chiritopsis bipinnatifida* W.T. Wang	Lingui, Guangxi	P054	36	1
P.cordifolia (D. Fang & W.T. Wang) Y.Z. Wang	*Cs.cordifolia* D. Fang & W.T. Wang	Liujiang, Guangxi	P051	36	2
P.glandulosa var. *yangshuoensis* (F. Wen, Yue Wang & Q.X. Zhang) Mich. Möller & A. Weber	*Cs.glandulosa* D. Fang, L. Zeng & D.H. Qin var. *yangshuoensis* F. Wen, Yue Wang & Q.X. Zhang	Yangshuo, Guangxi	P081	36	1
P.heterotricha (Merr.) Y.Z. Wang	*C.heterotricha* Merr.	Changjiang, Hainan	Kang-HNCJ01	36	
P.lobulata (W.T. Wang) Mich. Möller & A. Weber	*Cs.lobulata* W.T. Wang	Qingyuan, Guangdong	Kang-YD02-2	36	
P.luochengensis (Yan Liu & W.B. Xu) Mich. Möller & A. Weber	*Wentsaiboea luochengensis* Yan Liu & W.B. Xu	Luocheng, Guangxi	P046	36	
P.luzhaiensis (Yan Liu, Y.S. Huang & W.B. Xu) Mich. Möller & A. Weber	*C.luzhaiensis* Yan Liu, Y.S. Huang & W.B. Xu	Yongfu, Guangxi	P111	36	1
P.pteropoda (W.T. Wang) Y.Z. Wang	*C.pteropoda* W.T. Wang	Baoting, Hainan	Kang-HNBT01	36	
P.renifolia (D. Fang & D.H. Qin) Y.Z. Wang	*W.renifolia* D. Fang & D.H. Qin	Duan, Guangxi	P232	36	
P.repanda (W.T. Wang) Y.Z. Wang	*Cs.repanda* W.T. Wang	Donglan, Guangxi	P027	36	2
P.repanda var. *guilinensis* (W.T. Wang) Mich. Möller & A. Weber	*Cs.repanda* var. *guilinensis* W.T. Wang	Guilin, Guangxi	P128	36	
P.ronganensis (D. Fang & Y.G. Wei) Mich. Möller & A. Weber	*C.ronganensis* D. Fang & Y.G. Wei	Rongan, Guangxi	P039	36	
P. sp.nov.1		Luoding, Guangdong	Kang-GDLD01	36	
P. sp.nov.2		Yangjiang, Guangdong	Kang-YC03	36	1
P. sp.nov.3		Longshan, Hunan	Kang-GXPL05	36	
P. sp.nov.4		Guilin, Guangxi	Kang-HNLS06	36	
P. sp.nov.5		Zhaoqing, Guangdong	Kang-GDHJ01	36	1
P. sp.nov.6		Yingde, Guangdong	P123	36	1
P. sp.nov.7		Zhaoqing, Guangdong	Kang-GDHJ02	36	
P.subulata (W.T. Wang) Mich. Möller & A. Weber	*Cs.subulata* W.T. Wang	Yunfu, Guangdong	Kang-GDYA01	36	1
P.subulata var. *yangchunensis* (W.T. Wang) Mich. Möller & A. Weber	*Cs.subulata* var. *yangchunensis* W.T. Wang	Yangchun, Guangxi	P128	36	
P.tabacum Hance		Renhua, Guangdong	Kang-DXS06	36	
P.villosissima (W.T. Wang) Mich. Möller & A. Weber	*C.villosissima* W.T. Wang	Zhaoqing, Guangdong	Kang-QXY01	36	1
Petrocodon s.l.					
P.niveolanosus (Y.G. Wei, Yan Liu & F. Wen) Y.G. Wei & Mich. Möller	*Didymocarpus niveolanosus* Wei, Liu & Wen	Jingxi, Guangxi	Liu12517	36	
P.jingxiensis (Y.G. Wei, Yan Liu & F. Wen) Y.G. Wei & Mich. Möller	*Lagarosolen jingxiensis* Wei, Liu & Wen	Jingxi, Guangxi	Liu12518	36	1
P.hechiensis (Yan Liu, H.S. Gao & W.B. Xu) A.Weber & Mich. Möller	*L. hechiensis* Liu, Gao & Xu	Hechi, Guangxi	Liu12512	36	

Note: *Cs*, *Chiritopsis*; *C*, *Chirita*

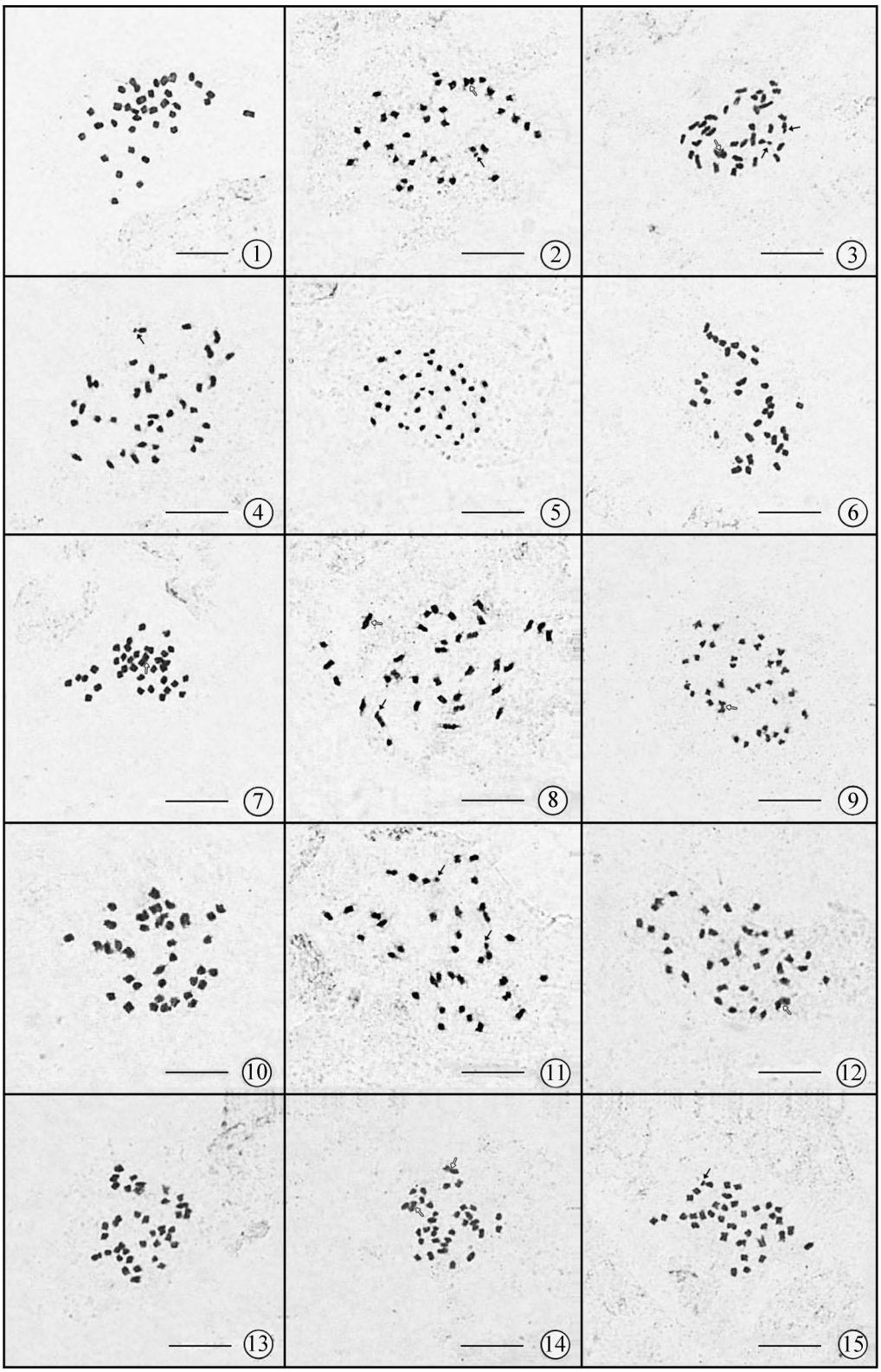

Fig. 1　1–15. Somatic chromosomes of *Primulina s.l.*. 1. *P. bicolor*; 2. *P. bipinnatifida*; 3. *P. cordifolia*; 4. *P. glandulosa* var. *yangshuoensis*; 5. *P. heterotricha*; 6. *P. lobulata*; 7 *P. luochengensis*; 8. *P. luzhaiensis*; 9. *P. pteropoda*; 10. *P. renifolia*; 11. *P. repanda*; 12. *P. repanda* var. *guilinensis*; 13. *P. ronganensis*; 14. *P.* sp. nov. 1; 15. *P.* sp. nov. 2. Open arrows, overlapping/touching chromosomes; solid arrows, satellites. Bar = 10 μm

is small according to the classification of Lima-defaria (1980). The taxa in *Primulina s.l.*, previously assigned to *Chiritopsis*, *Chirita* sect. *Gibbosaccus* and *Wentsaiboea*, show sight variation in chromosome size, mostly falling into the range of 0.6 to 2.4 μm, while those in *Petrocodon s.l.* possess relatively large chromosomes, with lengths of 1.3 to 3.3 μm. Although in both genera the small chromosomes don't allow a detailed karyotype analysis, it was possible to observe from the photographs of the well-spread and somewhat larger chromosomes that the karyotypes are symmetrical and very similar to one another, consisting mainly of metacentric and submetacentric chromosomes (Fig.1: 13; Fig.2: 3, 10).

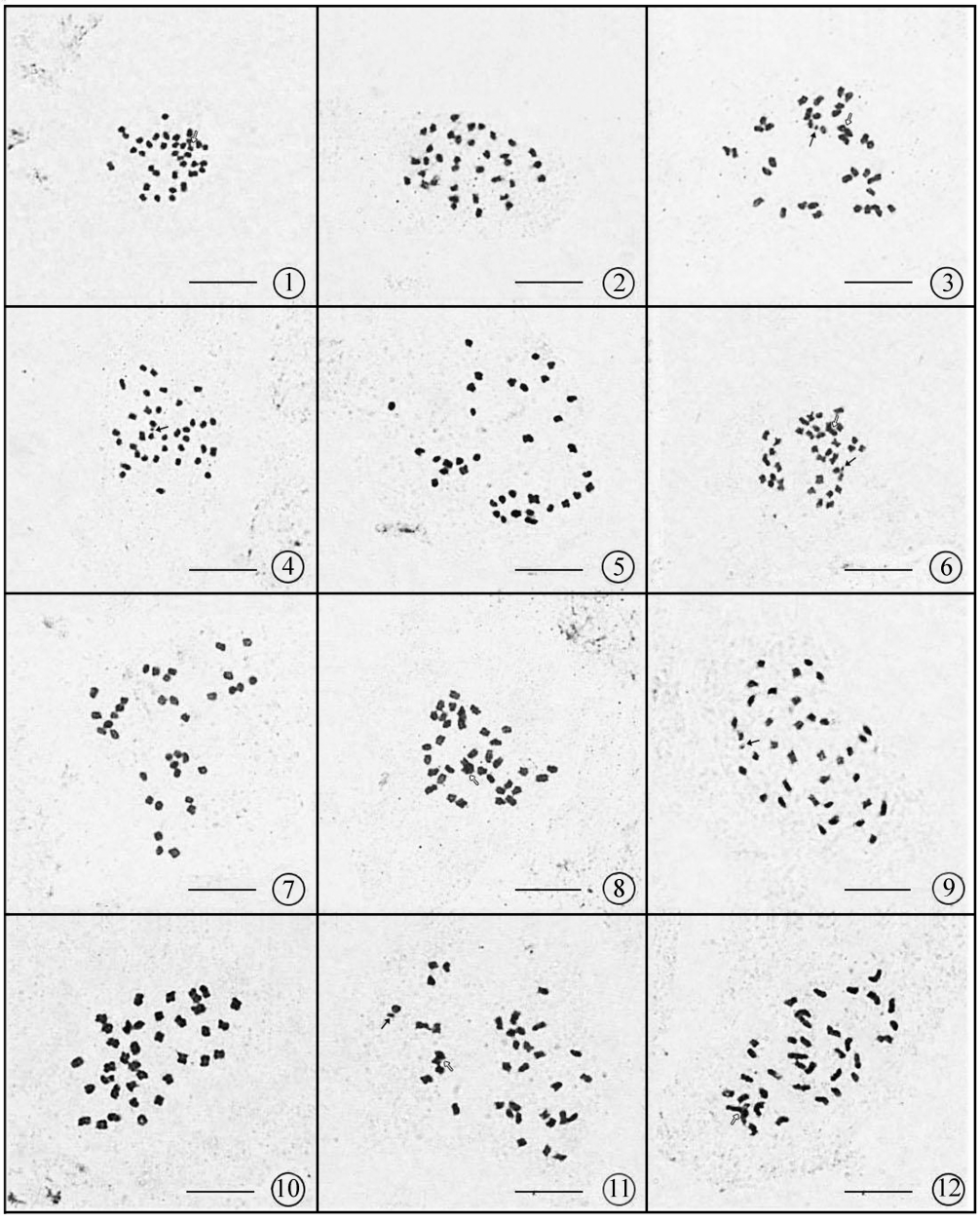

Fig. 2 1–12. Somatic chromosomes of *Primulina s.l.* and *Petrocodon s.l.*. 1. *Primulina* sp. nov. 3; 2. *P.* sp. nov. 4; 3. *P.* sp. nov. 5; 4. *P.* sp. nov. 6; 5. *P.* sp. nov. 7; 6. *P. subulata*; 7. *P. subulata* var. *yangchunensis*; 8. *P. tabacum*; 9. *P. villosissima*; 10. *Petrocodon hechiensis*; 11. *Pet. jingxiensis*; 12. *Pet. niveolanosus*. Open arrows, overlapping/touching chromosomes; solid arrows, satellites. Bar = 10 μm

Discussion

The present study was carried out to survey extensively the chromosome numbers in *Primulina s. l.*, with samples spanning all transferred genera and covering most of the morphological range. Chromosome numbers were obtained for eight taxa previously assigned to *Chiritopsis*, six of *Chirita* sect. *Gibbosaccus* and two of *Wentsaiboea*, as well as the monotype species *P. tabacum* and seven undescribed taxa. Exception for *P. tabacum*, all the taxa were counted for the first time. The taxa examined have the same chromosome number of $2n=36$, and the number obtained for *P. tabacum* by Christie *et al.* (2012) and Yang *et al.* (2012) were confirmed here. Chromosome numbers we obtained, together with previous reports of another 59 species (Ratter and Prentice, 1964; Cao *et al.*, 2003; Zhou *et al.*, 2004; Christie *et al.*, 2012; Liu *et al.*, 2012; Yang *et al.*, 2012), has provided us with reliable chromosome numbers for 76 of the ca. 130+ total species currently recognized in this genus. With the exception of *P. tamiana* (B. L. Burtt) Mich. Möller & A. Weber ($2n=32$ in Christie *et al.* 2012) and *P. longgangensis* (W. T. Wang) Y. Z. Wang ($2n=28$ in Cao *et al.* 2003 and $2n=72$ in Christie *et al.* 2012), the somatic chromosome number of *Primulina s. l.* is highly conserved, with $2n=36$. It should be noted that, although *P. tamiana* has been placed in *Primulina s. l.* (Weber *et al.*, 2011a), the preliminary molecular data suggest that the species might not belong to this genus (Christie *et al.* 2012). As for *P. longgangensis*, the count in Cao *et al.* (2003) is dubious since the figure shows a poorly reproduced or prepared prometaphase, while the counts of $2n=72$ in Christie *et al.* (2012) and $2n=36$ in Liu *et al.* (2012) indicate that this species might be a polyploidy with two ploidy levels. This would make *Primulina* uniformly $2n=36$ with one polyploidy. As all the *Primulina* species currently recognized are morphologically very similar to each other, we can postulate that the basic chromosome number for the genus is $x=18$, as well as by Liu *et al.* (2012). This is supported by the fact that its closest ally, *Petrocodon s. l.*, has predominantly also $2n=36$ chromosomes (see below).

In Gesneriaceae, small chromosome size frequently prevents detailed analysis of mitotic metaphase nuclei beyond mere establishment of chromosome numbers (Möller and Kiehn, 2004). A very similar situation was observed in *Primulina s. l.*. The chromosomes of taxa previously assigned to *Chirita* sect. *Gibbosaccus*, *Chiritopsis*, and *Wentsaiboea* as well as the monotypic *P. tabacum* have similar sizes, most falling in the range of 0.6 to 2.4 μm. Only slight variations occur among all the taxa where chromosomal data are available, including previously reported data (e.g. Christie *et al.*, 2012; Liu *et al.*, 2012; Yang *et al.*, 2012). The occurrence of chromosome satellites is also a common phenomenon in *Primulina s. l.* (Christie *et al.*, 2012; Liu *et al.*, 2012; Yang *et al.*, 2012). Of the 76 species with available chromosomal data, 23 species possess at least one or two satellites. They are relatively small in size, frequently residing on the ends of the larger chromosomes. Therefore, it is necessary to exercise great care when exceptionally large satellites are present, for satellites may tend to detach and incorrect counts can be the result of such unrecognized such 'satellites'. From well-spread metaphase chromosomes, the complements are most often consisting of small-sized metacentrics and submetacentrics. If we disregard the differences in the presence or absence and number of satellites, taxa of *Primulina s. l.* are quite similar with respect to chromosome morphology, albeit with slight variations in chromosome size.

Up to now, the available chromosome counts of *Primulina s. l.* have covered 57% species of *Chirita* sect. *Gibbosaccus*, 53% of *Chiritopsis* and 100% of *Wentsaiboea*, including their type species, *P. drays* (=*Chirita sinensis*), *P. repanda* (=*Chiritopsis repanda*) and *P. renifolia* (=*Wentsaiboea renifolia*). As a whole, the high uniformity of chromosome numbers and similarity of chromosome morphology are congruent with the results of molecular phylogenetic studies (Wang *et al.*, 2011; Weber *et al.*, 2011a), strong-

ly support the monophyly of *Primulina s. l.* as currently constructed and the taxonomic decision of transferring all species of *Chiritopsis* and two of *Wentsaiboea* to the originally monotypic *Primulina*, together with all of *Chirita* sect. *Gibbosaccus*.

On the other hand, the close relationship of *Primulina s. l.* and *Petrocodon s. l.* revealed by Möller *et al.* (2009, 2011), is supported by the chromosomal data we obtained here. Three species of *Petrocodon s. l.* possess the same somatic chromosome number of $2n=36$ as *Primulina s. l.*, and their chromosome morphology are also very similar to each other, with metacentrics and submetacentrics predominating. It is noteworthy that the chromosomes of taxa in *Petrocodon s. l.* appear to be larger than those of *Primulina s. l.*, which somewhat supports their status as two independent genera. Nevertheless, further investigations are very desirable on this genus, particularly since another species in the extended *Petrocodon s. l.* has been counted with $2n=20$ chromosomes (Cao *et al.*, 2003)

As aforementioned, a high stable somatic chromosome number $2n=36$ appears to be predominant in *Primulina s. l.*. Christie *et al.* (2012) argued that at first this number might be a straightforward duplication from an ancestor with $2n=18$ chromosomes. However, Christie *et al.* (2012) further argued that, given that members of other clades around *Primulina s. l.* include genera with high somatic chromosome numbers, e.g., *Loxostigma* C. B. Clarke ($2n=34$), *Hemiboea* C. B. Clarke ($2n=32$, 36), *Petrocosmea* Oliv. ($2n=34$), and *Oreocharis* Benth. ($2n=34$) (Möller *et al.*, 2002 onwards), chromosome duplication in *Primulina s. l.* might have occurred long before the diversification of this genus. Our findings that some species of the closest ally, *Petrocodon s. l.*, shared the same chromosome number with *Primulina s. l.* supported their deduction. Although at present there are insufficient data to speculate on the age of the genus *Primulina s. l.*, it may be relatively young given the uniformity in its chromosome numbers and chromosome morphology. However, much more work remains to be done.

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