

NEW CHROMOSOME COUNTS IN OLD WORLD GESNERIACEAE: NUMBERS FOR SPECIES HITHERTO REGARDED AS *CHIRITA*, AND THEIR SYSTEMATIC AND EVOLUTIONARY SIGNIFICANCE

F. CHRISTIE, S. BARBER & M. MÖLLER

Chromosome numbers were determined in 23 accessions representing 21 species hitherto belonging to *Chirita* (Gesneriaceae), a genus that has recently been remodelled and split into five different genera: *Damrongia*, *Henckelia*, *Liebigia*, *Microchirita* and *Primulina*. The previously monotypic *Primulina tabacum* was also investigated. Counts for 19 species were new, two were confirmatory and two gave different numbers from previously published counts. The results here, together with previously published cytological data for the erstwhile genus *Chirita*, were analysed in the light of the taxonomic revision of the genus and published phylogenetic data. *Chirita* was originally highly heterogeneous in chromosome numbers, including seven different somatic numbers, $2n = 8, 18, 20, 28, 32, 34$ and 36 . Among the five remodelled genera, *Henckelia* was found to be as equally heterogeneous as the erstwhile *Chirita*, *Microchirita* included only two chromosome numbers, $2n = 18$ and 34 , the three species of *Damrongia* were uniform with $2n = 18$, while species belonging to the extended *Primulina* showed only one basic number, $x = 18$, with 15 samples being diploid, and one being tetraploid. In the light of recent phylogenetic studies, polyploid as well as dysploid changes appear to have shaped the genomes of the newly defined genera *Henckelia*, *Microchirita* and, to a lesser degree, *Primulina*.

Keywords. *Chirita*, cytology, *Damrongia*, dysploidy, genome evolution, *Henckelia*, *Liebigia*, *Microchirita*, polyploidy, *Primulina*, somatic chromosome numbers.

INTRODUCTION

Chromosome numbers in the genus *Chirita* D. Don (Gesneriaceae), as defined until recently, exhibited a high level of diversity (Kiehn *et al.*, 1998; Möller *et al.*, 2002 onwards), showing a wide range of basic numbers ($x = 4, 8, 9, 10, 14, 17$) (Kiehn *et al.*, 1998) and an even higher range of haploid numbers ($n = 4, 9, 10, 14, 16, 17, 18$) (Möller *et al.*, 2002 onwards; Möller & Kiehn, 2003).

The genus has long been recognised as taxonomically problematic with regard to delimitation, homogeneity and subdivision despite two traditional revisions (Wood,

1974; Wang, 1985a, 1985b). Recently, *Chirita* has been the subject of two further studies, this time supported by molecular data and phylogenetic approaches (Wang *et al.*, 2011; Weber *et al.*, 2011), to bring the taxonomy in line with evolutionary relationships. The latter work presents a complete listing of the necessary generic changes, resulting in the elimination of the genus name *Chirita* with all former species now sunken into synonymy in one of the five emerging genera: (i) *Henckelia* Spreng. with a radically altered delimitation (see Weber *et al.*, 2011) and the synonymisation of most of *Chirita* sect. *Chirita* (including the type species) and the monotypic *Hemiboeopsis* W.T.Wang; (ii) *Damrongia* Kerr ex Craib which includes several species previously in *Chirita* sect. *Chirita*; (iii) *Liebigia* Endl. which resulted from raising *Chirita* sect. *Liebigia* (Endl.) C.B.Clarke back to generic rank; (iv) *Microchirita* (C.B.Clarke) Yin Z.Wang which resulted from raising *Chirita* sect. *Microchirita* C.B.Clarke to generic rank; (v) *Primulina* Hance, previously monotypic but now greatly expanded to include all of *Chirita* sect. *Gibbosaccus* C.B.Clarke, together with all of *Chiritopsis* W.T.Wang and two species of *Wentsaiboea* D.Fang & D.H.Qin (Weber *et al.*, 2011).

These systematic rearrangements raise important issues: whether this new taxonomic framework has led to cytologically more uniform taxonomic units, and whether any of these units show a characteristic basic or somatic chromosome number. To address these issues we obtained the chromosome numbers of 21 species hitherto ascribed to *Chirita* and of *Primulina tabacum* Hance, and extracted published chromosome numbers from the RBGE WebCyte database (Möller *et al.*, 2002 onwards) and published literature (Lu *et al.*, 2002; Cao *et al.*, 2003) for *Chirita* as previously defined. We then analysed the results in the light of the new taxonomic framework and phylogenetic hypotheses for the erstwhile genus *Chirita*.

MATERIALS AND METHODS

Material

Roots were taken from plants growing in the living research collection at the Royal Botanic Garden Edinburgh (RBGE). We sampled 23 accessions of 21 species of *Chirita*, as previously applied, and *Primulina tabacum* (Table 1). Voucher specimens are deposited at E. To maximise the cytological success rate, leaf or stem cuttings were cultivated in perlite in propagators, and roots harvested after about 6 weeks. The growing conditions were a base heat of 20°C, 24°C ambient air temperature and a high humidity between 80% and 100%. The cuttings were watered regularly, usually once every one to two days, to stimulate a rapid and continuous root growth essential for mitotic divisions.

Methods

The cytological methods followed Jong & Möller (2000). In brief, root tips were pre-treated either in saturated aqueous 1-bromonaphthalene or in 0.002 M

8-hydroxyquinoline in the dark for 4 hours at room temperature. The roots were then fixed in freshly prepared Farmer's fluid (3 parts ethanol : 1 part glacial acetic acid). They were hydrolysed for 30 minutes in 5 M HCl at room temperature, then stained with fresh Feulgen reagent prepared according to Fox (1969) for 2 hours in the dark. The stained material was softened in an enzyme 1:1 mixture of 4% pectinase (Sigma 2401) and 4% cellulase (BDH or Calbiochem 21947) at 36°C for 30 minutes. Root tips were squashed in an acetocarmine counterstain (0.4%). Counterstaining reduces the likelihood of fading in permanent slides. These were prepared following a modified quick-freeze method (Conger & Fairchild, 1953; Jong, 1997). Chromosome lengths were determined from 3–5 metaphase cells.

Images were captured using Zeiss AxioVision rel.4.7 and AxioCam MRc5 cameras mounted on both AxioSkop and AxioPhot brightfield microscopes (Zeiss, Welwyn Garden City, UK).

RESULTS AND DISCUSSION

In the present cytological investigation we obtained 18 new counts for species previously assigned to *Chirita*, confirmed two previously published counts and added new somatic chromosome numbers for two previously investigated species. We also obtained the first count for the hitherto monotypic *Primulina tabacum* (Table 1).

The chromosome counts obtained here revealed seven somatic numbers among the samples analysed ($2n = 8, 18, 22, 32, 34, 36, 72$). These covered all but three ($2n = 20, 28, 54$) numbers counted in previous studies for the genus *Chirita* as previously defined (Table 2). The results are given under their new systematic names and in alphabetic order.

Damrongia

Both *Damrongia fulva* (Barnett) D.J.Middleton & A.Weber and *D. purpureolineata* Kerr ex Craib were counted with $2n = 18$. These were new counts for the species. The two species had similar-sized chromosomes with 1.0 to 1.4 and 1.3 to 1.7 μm lengths, respectively, and their karyotype consisted of mostly metacentric chromosomes (Fig. 1A–C).

Henckelia

For *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller we determined $2n = 18$ for this accession from Guangxi, China (Fig. 1D). Its chromosomes were relatively uniform, between 1.3 and 1.4 μm in length. The same number was obtained previously by Ratter (1963), as *Chirita anachoreta* Hance. No locality information was given for this widespread species (China: Yunnan, Guangxi, Hunan, Guangdong; Taiwan; Burma; Thailand; Laos; Vietnam).

TABLE 1. *Primulina tabacum* and species of *Chirita* investigated cytologically in the present study, with RBGE accession, voucher and collection numbers, locality information and somatic chromosome numbers. For species previously belonging to *Chirita*, the old and new names are given

New names used here	Names as previously applied	RBGE Living Plant acc. no.	Cytology voucher no. at E ^a	Field collection no. ^a	Wild collection locality	2n	
						This study	Previous
<i>Damrongia</i>							
<i>D. fulva</i> (Barnett) D.J.Middleton & A.Weber	<i>Chirita fulva</i> Barnett	20101237-A	MMOG 57	<i>D. Middleton</i> 5393	Thailand, Nakhon Si Thammarat	18	new
<i>D. purpureolineata</i> Kerr ex Craib	<i>C. purpureolineata</i> (Kerr ex Craib) D.Wood	20092178-A	MMOG 64	<i>P. Triboun</i> s.n.	Thailand, Lamphun prov.	18	new
<i>Henckelia</i>							
<i>H. anachoreta</i> (Hance) D.J. Middleton & Mich.Möller	<i>C. anachoreta</i> Hance	20080129-A	MMOG 68	MMO 06-877	China, Guangxi, Gui Ping	18	conf.
<i>H. dielsii</i> (Borza) D.J.Middleton & Mich.Möller	<i>C. dielsii</i> (Borza) B.L.Burt	20010590-B	MMOG 69	<i>S.B. &</i> <i>M. Wilson</i> s.n.	China, Yunnan	22	18, 20
<i>H. monantha</i> (W.T.Wang) D.J.Middleton & Mich.Möller	<i>C. monantha</i> W.T.Wang	20070716-A	MMOG 65	ex cult. HBV	China [Hunan]	34	new
<i>H. pumila</i> (D.Don) A.Dietr.	<i>C. pumila</i> D.Don	20041526-A	20041526-A ^b	MMO 04-431	Thailand, Chiang Mai	8	conf.
<i>H. pumila</i> (D.Don) A.Dietr.	<i>C. pumila</i> D.Don	20090613-A	MMOG 16	MMO 06-978	China, Yunnan, Jinping	8	conf.

TABLE 1. (Cont'd)

<i>Primulina</i>							
<i>P. aff. balansae</i> (Drake) Mich.Möller & A.Weber	<i>C. aff. balansae</i> Drake	20022508-B	CULTE No. 15739	NVFDE 111	Vietnam, Cao Bang	36	new
<i>P. bogneriana</i> (B.L.Burttt) Mich.Möller & A.Weber	<i>C. bogneriana</i> B.L.Burttt	19990640-B	CULTE No. 15740	ex MBG	Vietnam, Halong Bay	36	new
<i>P. bogneriana</i> (B.L.Burttt) Mich.Möller & A.Weber	<i>C. bogneriana</i> B.L.Burttt	20070715-A	MMOG 58	ex cult. HBV (ex MBG)	Vietnam, Halong Bay	36	new
<i>P. flavimaculata</i> (W.T.Wang) Mich. Möller & A.Weber	<i>C. flavimaculata</i> W.T.Wang	19951204-B	19951204-B ^b	ex Beijing Inst. Bot.	China [Guangxi/Guizhou]	36	new
<i>P. hedyotidea</i> (Chun) Yin Z.Wang	<i>C. hedyotidea</i> Chun	20081060-A	MMOG 60	MMO 05-757A	China, Guangxi, Longzhou	36	new
<i>P. liboensis</i> (W.T.Wang & D.Y.Chen) Mich.Möller & A.Weber	<i>C. liboensis</i> W.T.Wang & D.Y.Chen	20080201-A	MMOG 56	MMO 07-1098	China, Guangxi, Nan Dang	36	new
<i>P. liguliformis</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. liguliformis</i> W.T.Wang	20080197-A	MMOG 54	MMO 07-1080	China, Guangxi, Hechi city	36	new
<i>P. longgangensis</i> (W.T.Wang) Yin Z.Wang	<i>C. longgangensis</i> W.T.Wang	19951202-B	MMOG 62	ex Beijing Inst. Bot.	China, Guangxi, Longgang	72	28
<i>P. lutea</i> (Yan Liu & Y.G.Wei) Mich.Möller & A.Weber	<i>C. lutea</i> Yan Liu & Y.G.Wei	20080180-A	MMOG 53	MMO 06-909	China, Guangxi, He Zhou city	36	new
<i>P. minutimaculata</i> (D.Fang & W.T.Wang) Mich.Möller & A.Weber	<i>C. minutimaculata</i> D.Fang & W.T.Wang	20022513-A	—	NVFDE 133	Vietnam, Cao Bang	36	new
<i>P. obtusidentata</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. obtusidentata</i> W.T.Wang	20040687	MMOG 59	MMO 03-351	China, Guizhou, Jiangkou	36	new

TABLE 1. (Cont'd)

New names used here	Names as previously applied	RBGE		Field collection no. ^a	Wild collection locality	2n	
		Living Plant acc. no.	Cytology voucher no. at E ^a			This study	Previous
<i>P. napoensis</i> (Z.Yu Li) Mich. Möller & A.Weber	<i>C. napoensis</i> Z.Yu Li	20080226-A	MMOG 55	MMO 07-1121	China, Guangxi, Jingxi	36	new
<i>P. spadiciformis</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. spadiciformis</i> W.T.Wang	19951205-A	19951205-A ^b	ex Smithsonian	China [Guangxi, Wuming]	36	new
<i>P. spinulosa</i> (D.Fang & W.T.Wang) Yin Z.Wang	<i>C. spinulosa</i> D.Fang & W.T.Wang	19982294-A	19982294-A ^b	<i>Y.G. Wei</i> s.n.	China, Guangxi, Fusui Xian	36	new
<i>P. subrhomboidea</i> (W.T.Wang) Yin Z.Wang	<i>C. subrhomboidea</i> W.T.Wang	19961905-A	MMOG 63	<i>I. La Croix</i> s.n.	China [Guangxi]	36	new
<i>P. tamiana</i> (B.L.Burt) Mich.Möller & A.Weber	<i>C. tamiana</i> B.L.Burt	19981743-A	CULTE No. 15738	SVE 01/114	Vietnam, Vinh Phu	32	new
<i>Primulina tabacum</i> Hance		19951540-A	19951540-A	<i>Q.J. Xie & C.X. Ye</i> s.n.	China, Guangdong, Lian river	36	new

^aMMOG – Michael Möller Garden voucher, RBGE; CULTE – cultivated RBGE; MMO – Michael Möller, RBGE; NVFDE – Northern Vietnam First Darwin Expedition; SVE – Soviet-Vietnam Expedition (Liberec Botanic Garden, Czech Republic & St Petersburg Botanic Garden); HBV – Vienna University Botanic Garden; MBG – Munich Botanic Garden; conf. – confirmatory count.

^bRBGE Living Plant acc. no. used as cytology voucher no.

TABLE 2. Previously published chromosome counts of *Chirita* species with collection information, locality information, results of chromosome counts and references

Names as previously applied	Collection no. (voucher dep.)	Locality [distribution]	<i>n</i>	<i>2n</i>	Reference
<i>Chirita anachoreta</i> Hance	[C3718 cult. RBGE] (E)	Wild origin	—	18	Ratter (1963)
<i>C. asperifolia</i> (Blume) B.L.Burtt	[cult. Cornell University]	[Indonesia; Sumatra, Java] wild origin	14	—	Lee (1962)
<i>C. asperifolia</i> (Blume) B.L.Burtt	[C4032 cult. RBGE] (E) ex Bailey Hortorium 59-2259	[Indonesia; Sumatra, Java] not of known wild origin	16	32	Ratter & Prentice (1967)
<i>C. bifolia</i> D.Don	<i>Vasudevan</i> 2582	India, Bowali	12II+4I*	—	Vasudevan (1976)
<i>C. bimaculata</i> D.Wood	[C5927 cult. RBGE] (E) from seed of <i>Burtt</i> 5611	Thailand, wild origin	17	—	Milne (1975)
<i>C. caliginosa</i> C.B.Clarke	<i>Weber & Anthonysamy</i> 870521-1/2 (WU)	Malaysia, Perak, Kinta distr.	9	18	Kiehn <i>et al.</i> (1998)
<i>C. caliginosa</i> C.B.Clarke	[C4283 cult. RBGE] (E)	[Peninsular Malaysia, Malaysian Borneo] wild origin	9	—	Ratter & Prentice (1967)
<i>C. dielsii</i> (Borza) B.L.Burtt	<i>Yuan-Xue Lu</i> 984001	China, Yunnan, Jingdong, Wenbu	—	18	Lu <i>et al.</i> (2002)
<i>C. dielsii</i> (Borza) B.L.Burtt	<i>Z.J. Gu</i> 011013 (KUN)	China, Yunnan, Jingdong	—	20	Zhou <i>et al.</i> (2004)
<i>C. eburnea</i> Hance	<i>Z.J. Gu</i> 02501 (KUN)	China, Guizhou, Bijie	—	36	Zhou <i>et al.</i> (2004)
<i>C. hamosa</i> R.Br.	<i>Kurzweil</i> HK 445 (WU)	Thailand, Chiang Mai prov.	—	34–36	Kiehn <i>et al.</i> (1998)
<i>C. hamosa</i> R.Br.	[C8032 cult. RBGE] (E) from seed of <i>Panigrahi</i> 12231	India, Allahabad	17	—	Milne (1975)
<i>C. involucrata</i> Craib	<i>Weber</i> 870510-1/2 (WU)	Malaysia, Pahang, Lipis distr.	—	18	Kiehn <i>et al.</i> (1998)
<i>C. involucrata</i> Craib	[C8251 cult. RBGE] (E) from seed of <i>Bogner</i> 424	Thailand, Luang Suan	9	—	Milne (1975)

TABLE 2. (Cont'd)

Names as previously applied	Collection no. (voucher dep.)	Locality [distribution]	<i>n</i>	<i>2n</i>	Reference
<i>C. lacunosa</i> (Hook.f.) B.L.Burt	Weber 870510-1/8 (WU)	Malaysia, Pahang, Lipis distr.	—	18	Kiehn <i>et al.</i> (1998)
<i>C. lavandulacea</i> Stapf	[C3795 cult. RBGE] (E)	[Vietnam] wild origin	—	34	Ratter & Prentice (1964)
<i>C. lavandulacea</i> Stapf	[cult. Cornell University] (BH)	Vietnam	—	34	Rogers (1954)
<i>C. lavandulacea</i> Stapf	—	Vietnam, not of known wild origin	18	—	Sugiura (1938)
<i>C. lavandulacea</i> Stapf	—	Vietnam, not of known wild origin	18	—	Sugiura (1940)
<i>C. longgangensis</i> W.T.Wang	Ming Cao 0414 (IBK)	China, Guangxi, Longzhou	—	28	Cao <i>et al.</i> (2003)
<i>C. macrophylla</i> Wall.	Yuan-Xue Lu 4020	China, Yunnan, Tongbiguan, Yingjiang	—	18	Lu <i>et al.</i> (2002)
<i>C. macrophylla</i> Wall.	[C4221 cult. RBGE] (E) <i>I. Smitinand</i> S7639	[India, Nepal, Bhutan, China, Myanmar, Thailand, Vietnam] wild origin	9	18	Ratter & Prentice (1967)
<i>C. micromusa</i> B.L.Burt	[C5927 cult. RBGE] (E)	Thailand, wild origin	17	—	Ratter & Milne (1970)
<i>C. moonii</i> Gardner	Kiehn MK 900829-1/3 (PTBG, WU)	Sri Lanka	—	18	Kiehn & Lorence (1996)
<i>C. pumila</i> D.Don	[C3679 cult. RBGE] (E) ex Palmengarten Frankfurt/Main	Himalaya	4	—	Ratter (1963)
<i>C. pumila</i> D.Don	Vasudevan 2581	India, Uttaranchal, Nainital	4II	—	Vasudevan (1976)

TABLE 2. (Cont'd)

<i>C. rupestris</i> Ridl.	[C8252 cult. RBGE] (E) from seed of <i>Bogner</i> 434	Thailand, Chun Phan	9	—	Milne (1975) as <i>C. caerulea</i>
<i>C. sericea</i> Ridl.	—	Peninsular Malaysia	—	18	Hellmayr (1989)
<i>C. sericea</i> Ridl.	<i>Weber</i> 860818-1/1 (WU)	Peninsular Malaysia, Perak, Kinta distr.	—	18	Kiehn <i>et al.</i> (1998)
<i>C. sericea</i> Ridl.	[cult. RBGE] (E)	Peninsular Malaysia, wild origin	—	18	Ratter (1963)
<i>C. sinensis</i> Lindl.	[C4051 cult. RBGE] (E)	China, wild origin not known	—	36	Ratter & Prentice (1964)
<i>C. speciosa</i> Kurz	[cult. Bailey Hortorium at Cornell University]	[India, Myanmar, China, Vietnam, Thailand] not of known wild origin	—	18	Fussell (1958)
<i>C. speciosa</i> Kurz	<i>Yuan-Xue Lu</i> 4013	China, Yunnan, Tongbiguan, Yingjiang	—	18	Lu <i>et al.</i> (2002)
<i>C. speciosa</i> Kurz	[C3768 cult. RBGE] (E) from seed of <i>Forrest</i> 26522	Myanmar, hills around Kan-kuei	—	18	Ratter (1963)
<i>C. urticifolia</i> Buch.- Ham. ex D. Don	[C2673 cult. RBGE] (E) ex Messrs Ghose	India, Darjeeling	—	34	Ratter & Prentice (1964)
<i>C. walkerae</i> Gardner	<i>Theobald & Grupe</i> 2357 (E) [C8457 cult. RBGE]	Sri Lanka, wild material	9	—	Milne (1975)
<i>C. zeylanica</i> Hook.	[C4694 cult. RBGE] (E) from seed of <i>H.E.</i> <i>Moore</i> 9036	Sri Lanka, Hunnasgiriya, 25 miles E of Kandy	10	—	Ratter & Prentice (1967)
<i>C. sp.</i>	[C8246 cult. RBGE] (E)	Thailand, wild origin	9	—	Milne (1975)

*I – univalents, II – bivalents.

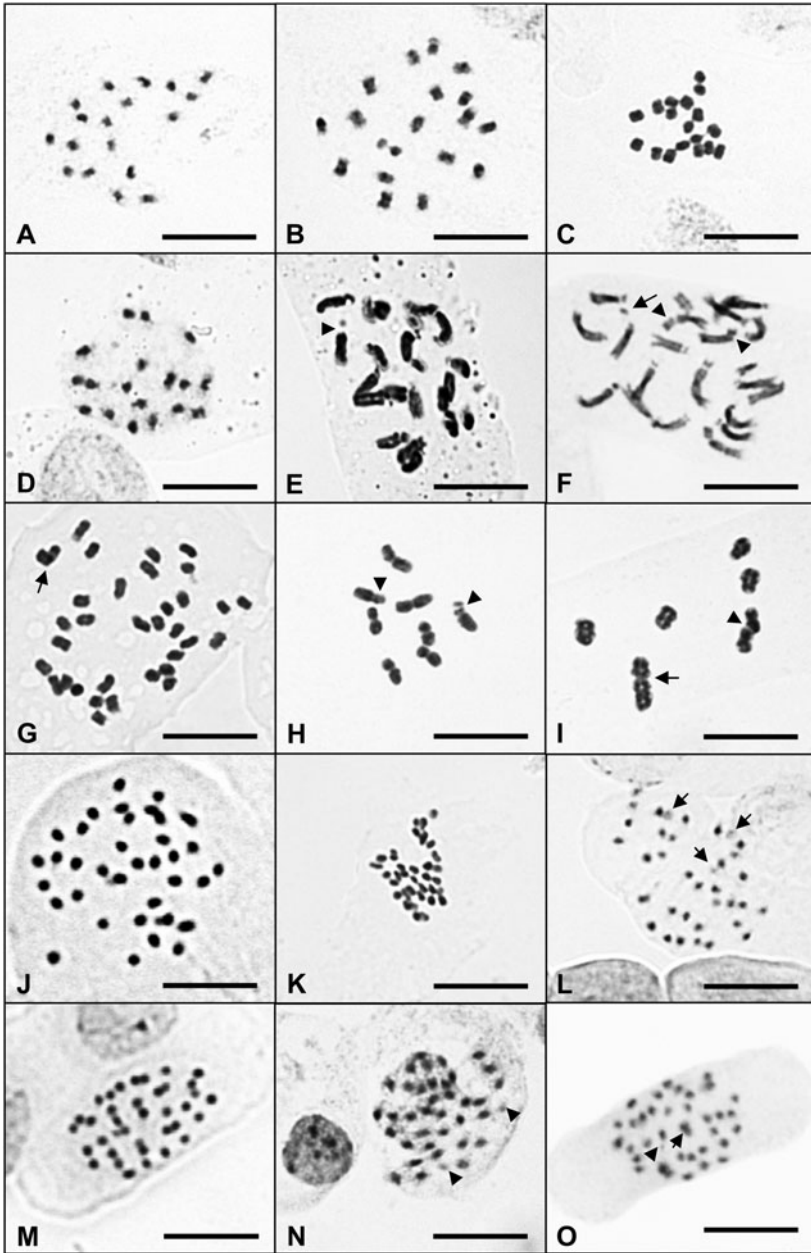


FIG. 1. Somatic metaphase chromosome numbers of species of *Chirita*, as defined previously, cytologically investigated in the present study, arranged by current name. A: *Damrongia fulva* prometaphase, $2n = 18$; B: *D. purpureolineata* prometaphase, $2n = 18$; C: *D. purpureolineata* metaphase, $2n = 18$; D: *Henckelia anachoreta* late prometaphase, $2n = 18$; E: *H. dielsii* metaphase, $2n = 22$, arrowhead indicates stalked satellite NOR; F: *H. dielsii* prometaphase, $2n = 22$, arrow indicates satellite NOR, arrowheads indicate satellite NOR

Henckelia dielsii (Borza) D.J.Middleton & Mich.Möller was relatively unambiguously counted here with $2n = 22$ (Fig. 1E,F). The chromosomes were comparatively large, 2.5 to 4.4 μm . Previously, the somatic chromosome number of this species was determined with $2n = 18$ (Lu *et al.*, 2002) and $2n = 20$ (Zhou *et al.*, 2004), for both as *Chirita dielsii* (Borza) B.L.Burt. The count by Lu *et al.* (2002) appears somewhat unconvincing, since, even considering the presence of the large satellites, the published metaphase image appears to show more than 18 chromosomes. The count by Zhou *et al.* (2004) shows two pairs of chromosomes with satellites, in one the satellite being particularly large. In our preparations most chromosomes appear telocentric and acrocentric in late prometaphase chromosomes, with some submetacentric and one pair of distinctly small chromosomes. The latter show centromeres and are thus unlikely to be homologous to the large satellites in Zhou *et al.* (2004). Furthermore, the nucleolus organiser region (NOR) chromosomes with a large and small satellite can be observed in our preparation (Fig. 1F), confirming the observations of Zhou *et al.* (2004) in this respect. Therefore, *Henckelia dielsii* appears to possess a variable number of chromosomes. It occurs together with *Henckelia pumila* (D.Don) A.Dietr. in Jingdong, Yunnan, China, where the material of *H. dielsii* for both Zhou *et al.* (2004) and the present study was collected. The two species hybridise in nature (M. Möller, pers. obs.), and this may explain the variable chromosome count in *Henckelia dielsii*. The two species have similar chromosome sizes, but differ not only in chromosome number but greatly in their chromosome morphology, with *Henckelia pumila* having mostly symmetrical (i.e. metacentric) chromosomes while *H. dielsii* has predominantly highly asymmetrical ones (i.e. telo- and acrocentrics) (Fig. 1E,F). To fully understand this intriguing relationship between these two species in Jingdong more analyses are needed.

For *Henckelia monantha* (W.T.Wang) D.J.Middleton & Mich.Möller the somatic number of chromosomes obtained was $2n = 34$, a new count for this species (Fig. 1G). The chromosomes were mostly metacentric, of elongated shape, and quite large, with lengths of 1.4 to 2.4 μm . This number has been observed in several other species of *Chirita*, as previously applied, in section *Microchirita*, such as *C. bimaculata* D.Wood [now *Microchirita bimaculata* (D.Wood) A.Weber & D.J.Middleton] (Milne, 1975), *C. hamosa* R.Br. [now *Microchirita hamosa* (R.Br.) Yin Z.Wang] (Milne, 1975),

with short stalk; G: *H. monantha* metaphase, $2n = 34$, arrow indicates two overlapping chromosomes; H: *H. pumila* from Thailand metaphase, $2n = 8$, arrowheads indicate NOR chromosomes; I: *H. pumila* from China, late metaphase, $2n = 8$, arrow indicates two touching chromosomes, arrowhead indicates two slightly overlapping chromosomes; J: *Primulina* aff. *balansae* metaphase, $2n = 36$; K: *P. bogneriana* from Munich Botanic Garden metaphase, $2n = 36$; L: *P. bogneriana* from Vienna University Botanic Garden metaphase, $2n = 36$, arrows indicate chromosomes out of focus plane; M: *P. flavimaculata* metaphase, $2n = 36$; N: *P. hedyotideae* late prometaphase, $2n = 36$, arrowheads indicate satellite NORs; O: *P. liboensis* early metaphase, arrow indicates two touching chromosomes, arrowhead indicates satellite NOR, $2n = 36$. Scale bars 10 μm .

C. lavandulacea Stapf [now *Microchirita lavandulacea* (Stapf) Yin Z.Wang] (Ratter & Prentice, 1964) and *C. micromusa* B.L.Burt [now *Microchirita micromusa* (B.L.Burt) A.Weber & D.J.Middleton] (Ratter & Milne, 1970). This number has also been found in *Chirita urticifolia* Buch.-Ham. ex D.Don [now *Henckelia urticifolia* (Buch.-Ham. ex D.Don.) A.Dietr.] (Ratter & Prentice, 1964), the type of *Chirita* and hence *Chirita* sect. *Chirita* to which *H. monantha* belonged. Thus, $2n = 34$ occurs in both erstwhile sections *Chirita* and *Microchirita* (see below).

The lowest number found here was $2n = 8$ for *Henckelia pumila* (Table 1, Fig. 1H,I), confirming earlier counts by Ratter (1963) and Vasudevan (1976), in both as *Chirita pumila* D.Don. The chromosomes were relatively large with lengths of 2.7 to 4.1 μm . The chromosome complement consisted of three pairs of metacentrics and one pair of sub-metacentrics; the latter possessed a subterminal NOR (Fig. 1H, arrow-heads). With the new counts here, this species now has four counts from different geographic areas, the Himalayas (Ratter, 1963, with no further geographic details), the Himalayan foothills in NW India (Vasudevan, 1976), S Yunnan in SW China (MMO 06-978, Fig. 1H) and Chiang Mai in N Thailand (MMO 04-431, Fig. 1I). This species has a very wide distribution range (India, Nepal, Bhutan, China, Myanmar, Vietnam, Thailand), and the samples analysed cytologically span its entire geographic range. So far all counts have been $2n = 8$, suggesting a highly conservative genome evolution in terms of trends in chromosome number changes, or a rapid spread of the species across its distribution range. The difference in chromosome morphology in Fig. 1H and 1I is most likely due to the latter having progressed further in mitosis and having perhaps over-condensed. To ascertain whether there exist karyotype variations between the samples more detailed analyses are required.

Primulina

For 14 species of *Primulina*, previously belonging to *Chirita* sect. *Gibbosaccus*, the newly determined chromosome number was $2n = 36$ (Table 1, Figs 1J–O, 2A,C–I). Their chromosomes were small, mostly between 0.7 and 1.6 μm long, except for *Primulina* aff. *balansae* (Drake) Mich.Möller & A.Weber with 1.0 to 2.0 μm , and mostly round to oval in shape. In some preparations one (Fig. 1O) or two (Figs 1N, 2F,G) terminal NORs with, sometimes short (Fig. 2G), satellite stalks were observed. A somatic number of $2n = 36$ has only previously been observed for two other species, *Primulina eburnea* (Hance) Yin Z.Wang (Zhou *et al.*, 2004), as *Chirita eburnea* Hance, and *P. dryas* (Dunn) Mich.Möller & A.Weber (Ratter & Prentice, 1964), as *Chirita sinensis* Lindl. Both species also belonged to *Chirita* sect. *Gibbosaccus*.

The highest chromosome count in the present study was obtained for *Primulina longgangensis* (W.T.Wang) Yin Z.Wang with $2n = 72$ (Fig. 2B), with chromosome sizes between 0.8 and 1.1 μm . This species was investigated previously by Cao *et al.* (2003), as *Chirita longgangensis* W.T.Wang, who reported $2n = 28$ chromosomes. Though the plants originated from different localities, from Longgang, Guangxi in

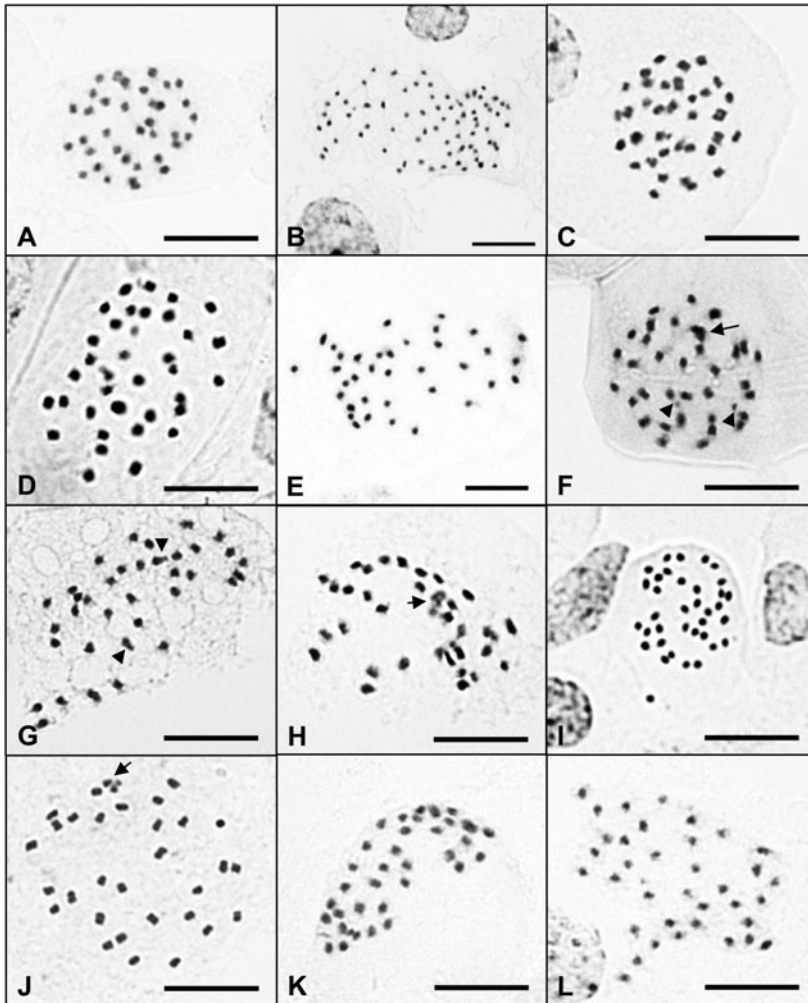


FIG. 2. Somatic metaphase chromosome numbers of species of *Chirita*, as defined previously, and *Primulina tabacum* cytologically investigated in the present study, arranged by current name. A: *Primulina liguliformis* prometaphase, $2n = 36$; B: *P. longgangensis* early metaphase, $2n = 72$; C: *P. lutea* prometaphase, $2n = 36$; D: *P. minutimaculata* metaphase, $2n = 36$; E: *P. napoensis* late prometaphase, $2n = 36$; F: *P. obtusidentata* late prometaphase, $2n = 36$, arrow indicates three overlapping chromosomes, arrowheads indicate satellite NORs; G: *P. subrhomboidea* late prometaphase, $2n = 36$, arrowheads indicate NORs; H: *P. spadiciformis* prometaphase, $2n = 36$, arrow indicates three overlapping chromosomes; I: *P. spinulosa* metaphase, $2n = 36$; J: *P. tamiana* metaphase, $2n = 32$, arrow indicates two overlapping chromosomes; K: *P. tabacum* prometaphase, $2n = 36$; L: *P. tabacum* burst prometaphase, $2n = 36$. Scale bars 10 μm .

the present study and Longzhou, Guangxi in Cao *et al.* (2003), this high discrepancy is surprising, particularly the apparent difference in basic numbers, 18 and 14, respectively. Judging from the published image in Cao *et al.* (2003) it is difficult to

see how the number of chromosomes was determined, since the figure shows a poorly reproduced or prepared prometaphase. A basic number of $x = 14$ is unusual among species previously in *Chirita*, and has been counted only once before, for *C. bifolia* D.Don (Vasudevan, 1976). *Chirita bifolia*, however, is now placed in *Henckelia*, as *H. bifolia* (D.Don) A.Dietr. (Table 3). Compared to other counts in the genus *Primulina*, $2n = 72$ appears to represent a polyploid number, with a basic number of $x = 9$ or $x = 18$, and would equate to an octoploid or tetraploid condition (see below).

Primulina tamiana (B.L.Burt) Mich.Möller & A.Weber has $2n = 32$ chromosomes (Fig. 2J). This species mostly has metacentric chromosomes 1.1 to 1.5 μm in length which are rectangular in shape, unlike the other species in this genus. This somatic number has been counted only once before, for *Liebigia speciosa* (Blume) Endl. (Ratter & Prentice, 1967), as *Chirita asperifolia* (Blume) B.L.Burt. However, *Chirita asperifolia* has also been counted with $2n = 28$ (Lee, 1962).

Primulina tabacum was also counted with $2n = 36$ chromosomes (Fig. 2K,L), like most species in this newly defined genus. Its chromosomes were 0.8 to 1.4 μm in length, which is as small as those of the *Chirita* species which previously belonged to *Chirita* sect. *Gibbosaccus*.

Diversity across Chirita as previously defined

From all previous and new counts of species previously placed in *Chirita*, a high diversity in somatic numbers, with $2n = 8, 18, 20, 28, 32, 34, 36$ at the species level and additional numbers of $2n = 22$ and 72 in species with variable chromosome numbers (Tables 2 and 4), can be observed. Such a high variation in somatic numbers is not unprecedented in the Gesneriaceae and has also been observed for example in the genus *Didymocarpus* Wall. ($2n = 20, 22, 24, 26, 28, 32, 36$) (Kiehn *et al.*, 1998; Möller & Kiehn, 2003).

However, this was before *Chirita* was redefined (Wang *et al.*, 2011; Weber *et al.*, 2011), and any speculation on the relationships between these numbers has to be discussed in the new taxonomic framework (Table 3).

Diversity within the new taxonomic framework

The split of *Chirita* into the five independent genera *Damrongia*, *Henckelia*, *Liebigia*, *Microchirita* and *Primulina* necessitates a new look at their genome diversity and changes in chromosome numbers (Table 3).

Of the six species listed in *Damrongia* (Weber *et al.*, 2011), three have now been counted (*D. fulva*, *D. lacunosa* (Hook.f.) D.J.Middleton & A.Weber and *D. purpureolineata*), all with $2n = 18$ (Table 3).

More than 50 species of *Henckelia sensu* Weber & Burt (1998) have been counted (Kiehn *et al.*, 1998). However, only two of these belong to *Henckelia sensu* Weber *et al.* (2011), the rest to species in *Loxocarpus* R.Br. and *Codonoboea* Ridl. (see Weber *et al.*,

TABLE 3. Summary of chromosome counts in species previously included in *Chirita*, with the type species of each currently recognised genus indicated (underlined)

New names	Names as previously applied	2n
<i>Damrongia fulva</i> (Barnett) D.J.Middleton & A.Weber	<i>C. fulva</i> Barnett	18*
<i>Damrongia lacunosa</i> (Hook.f.) D.J.Middleton & A.Weber	<i>C. lacunosa</i> (Hook.f.) B.L.Burtt	18
<u><i>Damrongia purpureolineata</i></u> Kerr ex Craib	<i>C. purpureolineata</i> (Kerr ex Craib) D.Wood	18*
<i>Henckelia anachoreta</i> (Hance) D.J.Middleton & Mich.Möller	<i>C. anachoreta</i> Hance	18*
<i>Henckelia bifolia</i> (D.Don) A.Dietr.	<i>C. bifolia</i> D.Don	28
<i>Henckelia communis</i> (Gardner) D.J.Middleton & Mich.Möller	<i>C. zeylanica</i> Hook.	20
<i>Henckelia dielsii</i> (Borza) D.J.Middleton & Mich.Möller	<i>C. dielsii</i> (Borza) B.L.Burtt	18, 20, 22*
<i>Henckelia floccosa</i> (Thwaites) A.Weber & B.L.Burtt		32 ^a
<i>Henckelia grandifolia</i> A.Dietr.	<i>C. macrophylla</i> Wall.	18
<u><i>Henckelia incana</i></u> (Vahl) Spreng.		54 ^b , ±90 ^c
<i>Henckelia innominata</i> (B.L.Burtt) A.Weber & B.L.Burtt		32 ^c
<i>Henckelia monantha</i> (W.T.Wang) D.J.Middleton & Mich.Möller	<i>C. monantha</i> W.T.Wang	34*
<i>Henckelia moonii</i> (Gardner) D.J.Middleton & Mich.Möller	<i>C. moonii</i> Gardner	18
<i>Henckelia pumila</i> (D.Don) A.Dietr.	<i>C. pumila</i> D.Don	8*
<i>Henckelia speciosa</i> (Kurz) D.J.Middleton & Mich.Möller	<i>C. speciosa</i> Kurz	18
<i>Henckelia urticifolia</i> (D.Don) A.Dietr.	<i>C. urticifolia</i> Buch.-Ham. ex D.Don	34
<i>Henckelia walkerae</i> (Gardner) D.J.Middleton & Mich.Möller	<i>C. walkerae</i> Gardner	18
<u><i>Liebigia speciosa</i></u> (Blume) Endl.	<i>C. asperifolia</i> (Blume) B.L.Burtt	28, 32
<i>Microchirita bimaculata</i> (D.Wood) A.Weber & D.J.Middleton	<i>C. bimaculata</i> D.Wood	34
<i>Microchirita caliginosa</i> (C.B.Clarke) Yin Z.Wang	<i>C. caliginosa</i> C.B.Clarke	18
<u><i>Microchirita hamosa</i></u> (R.Br.) Yin Z.Wang	<i>C. hamosa</i> R.Br.	34
<i>Microchirita involucrata</i> (Craib) Yin Z.Wang	<i>C. involucrata</i> Craib	18
<i>Microchirita lavandulacea</i> (Stapf) Yin Z.Wang	<i>C. lavandulacea</i> Stapf	34, 36
<i>Microchirita micromusa</i> (B.L.Burtt) A.Weber & D.J.Middleton	<i>C. micromusa</i> B.L.Burtt	34

TABLE 3. (Cont'd)

New names	Names as previously applied	2n
<i>Microchirita rupestris</i> (Ridl.) A.Weber & Rafidah	<i>C. rupestris</i> Ridl.	18
<i>Microchirita sericea</i> (Ridl.) A.Weber & Rafidah	<i>C. sericea</i> Ridl.	18
<i>Primulina</i> aff. <i>balansae</i> (Drake) Mich.Möller & A.Weber	<i>C. aff. balansae</i> Drake	36*
<i>Primulina bogneriana</i> (B.L.Burt) Mich.Möller & A.Weber	<i>C. bogneriana</i> B.L.Burt	36*
<i>Primulina dryas</i> (Dunn) Mich. Möller & A.Weber	<i>C. sinensis</i> Lindl.	36
<i>Primulina eburnea</i> (Hance) Yin Z.Wang	<i>C. eburnea</i> Hance	36
<i>Primulina flavimaculata</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. flavimaculata</i> W.T.Wang	36*
<i>Primulina hedyotidea</i> (Chun) Yin Z.Wang	<i>C. hedyotidea</i> Chun	36*
<i>Primulina liboensis</i> (W.T.Wang & D.Y.Chen) Mich.Möller & A.Weber	<i>C. liboensis</i> W.T.Wang & D.Y.Chen	36*
<i>Primulina liguliformis</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. liguliformis</i> W.T.Wang	36*
<i>Primulina longgangensis</i> (W.T.Wang) Yin Z.Wang	<i>C. longgangensis</i> W.T.Wang	28, 72*
<i>Primulina lutea</i> (Yan Liu & Y.G.Wei) Mich.Möller & A.Weber	<i>C. lutea</i> Yan Liu & Y.G.Wei	36*
<i>Primulina minutimaculata</i> (D.Fang & W.T.Wang) Yin Z.Wang	<i>C. minutimaculata</i> D.Fang & W.T.Wang	36*
<i>Primulina napoensis</i> (Z.Yu Li) Mich.Möller & A.Weber	<i>C. napoensis</i> Z.Yu Li	36*
<i>Primulina obtusidentata</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. obtusidentata</i> W.T.Wang	36*
<i>Primulina spadiceiformis</i> (W.T.Wang) Mich.Möller & A.Weber	<i>C. spadiceiformis</i> W.T.Wang	36*
<i>Primulina spinulosa</i> (D.Fang & W.T.Wang) Yin Z.Wang	<i>C. spinulosa</i> D.Fang & W.T.Wang	36*
<i>Primulina subrhomboidea</i> (W.T.Wang) Yin Z.Wang	<i>C. subrhomboidea</i> W.T.Wang	36*
<i>Primulina tabacum</i> Hance		36*
<i>Primulina tamiana</i> (B.L.Burt) Mich.Möller & A.Weber	<i>C. tamiana</i> B.L.Burt	32*
	<i>Chirita</i> sp. C.8246	18

^aMilne (1975), ^bThathachar (1942), ^cRatter & Prentice (1967).

*Counted in the present study.

TABLE 4. Summary of chromosome counts for *Chirita*, as previously applied, and for genera with species previously included in *Chirita*. In square brackets is the number of new species' counts obtained in the present paper; in round brackets is chromosome variation in species with variable chromosome numbers. Predominant numbers in bold

	<i>2n</i>							Classes	Variable species	Counted/spp.
	8	18	20	28	32	34	36			
<i>Chirita</i> ^a	1	12 [2]	1	1	1[1]	5[1]	12 [11]	7	5[2]	38/80–140 ^a
<i>Damrongia</i>	–	3	–	–	–	–	–	1	–	3/6 ^b
<i>Henckelia</i>	1	5	1	1	2	2	–	6	2(18, 20, 22), (54, ±90)	14/56 ^b
<i>Liebigia</i>	–	–	–	–	–	–	–	–	1(28, 32)	1/12 ^b
<i>Microchirita</i>	–	4	–	–	–	3	–	2	1(34, 36)	8/18 ^b
<i>Primulina</i>	–	–	–	–	1	–	16	2	1(28, 72)	18/129 ^b
Sum	1	12	1	1	3	5	16	7	5	44/221 ^b

^aAccording to Weber (2004).

^bAccording to Weber *et al.* (2011).

2011; Kiew & Lim, 2011). The species now removed from *Henckelia* are highly uniform in their chromosome number, with $2n = 18$ (Kiehn *et al.*, 1998). The newly defined genus *Henckelia sensu* Weber *et al.* (2011) is, however, now extremely diverse, with nine different somatic numbers observed, though three of these, $2n = 22, 54, \pm 90$, involve species with variable counts. This is still quite remarkably variable, given that of the 56 species now in the genus only 14 have been cytologically investigated (Table 4).

Only one chromosome count is available as yet for the 12 species of *Liebigia*, *L. speciosa* with two different counts of $2n = 28$ and 32 chromosomes (Table 3). More work is required here.

In *Microchirita* only two somatic numbers, $2n = 18$ and 34, have been observed, and in similar proportions (Table 4). However, only about half the *Microchirita* species have been counted, and more data are needed before a final pattern may emerge. There is some geographic pattern, with species with $2n = 18$ occurring in Peninsular Malaysia and S Thailand, while species with $2n = 34$ have a more northerly distribution in C and N Thailand, Vietnam and S China.

It is interesting that the newly defined genus *Primulina*, previously monotypic and now the largest among the five genera with about 130 species, appears to possess the most conserved chromosome number, with 16 out of 18 species analysed possessing $2n = 36$ (excluding the odd count of $2n = 28$ for *P. longgangensis*, see above). Our count of $2n = 72$ for this species would fit a polyploidy based on $x = 9$ or 18 (see below). Only *Chirita tamiana*, with $2n = 32$, seems to truly differ from the other *Primulina* species. This is an odd species that has been placed in *Primulina* (Weber *et al.*, 2011), though preliminary molecular data suggest that the species might not belong in this genus (M. Möller, unpublished data). This would make *Primulina* uniformly $2n = 36$ with one polyploid.

Evolution of chromosome numbers in the new taxonomic framework

Using recent comprehensive molecular phylogenetic analyses (Möller *et al.*, 2009, 2011; Weber *et al.*, 2011) we can infer genome evolution within the newly established genera that show variation in chromosome numbers, namely *Henckelia*, *Microchirita* and *Primulina*. This will then enable an investigation of putative polyploidy series (e.g. $x = 9, 18, 27, 36$ and $4, [8], 16$) and the origin of the lowest count among Gesneriaceae with $x = 4$.

Using parts of the phylogeny in Weber *et al.* (2011) where the topology is well supported, we can investigate the evolution of chromosome numbers of the species and genera investigated. We find that in *Henckelia* s.l. (Fig. 3) it would appear that $2n = 18$ is ancestral, and polyploidisation in *H. urticifolia* is accompanied by a reduction in the number from a somatic $2n = 18$ to a basic number of $x = 17$. However, on the whole, *Henckelia* s.l. is too heterogeneous and undersampled (14 out of 56 species counted) to enable definite conclusions to be drawn about the extensive genome rearrangements that must have occurred within this clade. It is interesting that the Gesneriaceae species with the lowest chromosome number, *Henckelia pumila*, with $2n = 8$, is in this clade, with *H. dielsii* and *H. anachoreta* as sister with $2n = 18$, and *H. bifolia* with $2n = 28$. If the ancestral state in this area of the phylogeny is indeed $2n = 18$, as discussed above, extensive Robertsonian fusion events (e.g. Hair & Beuzenberg, 1958; Jones, 1974; John & Freeman, 1975) must have occurred in this species to result in a reduction from a haploid complement of $x = 9$ to $x = 4$, while fission events or genome duplication and fusion events must have occurred to give $2n = 28$ for *Henckelia bifolia*. Thus, no obvious ascending or descending series are seemingly present. Further cytogenetic studies in these species, perhaps involving chromatin level determinations (e.g. Gregory, 2005; Leitch *et al.*, 2005; Siljak-Yakovlev *et al.*, 2008) and the use of fluorescent chromosome probes

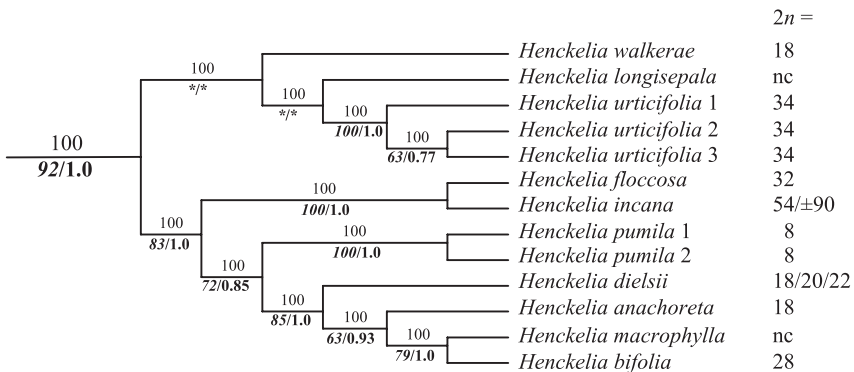


FIG. 3. The *Henckelia* clade (modified from Weber *et al.*, 2011), with somatic chromosome numbers indicated. nc = not counted. Numbers above branches are majority rule consensus proportions; numbers below branches are branch support values, in italics to the left as bootstrap values, and to the right as Bayesian posterior probabilities.

(e.g. Kasai *et al.*, 2000) may shed light on these processes that shaped the karyotypes in this cytologically highly interesting clade.

In the phylogeny in Weber *et al.* (2011) *Microchirita* is divided into two sub-clades (Fig. 4), one with species possessing $2n = 34$ chromosomes, the other with species having exclusively $2n = 18$ for those counted and included in the phylogeny. The genome evolution in this clade has seemingly involved only one change in chromosome number, and at present it is difficult to ascertain in which direction the change occurred.

In *Primulina* only one basic number, $x = 18$, has been found (excluding the somewhat dubious count of $2n = 28$ and $2n = 32$ for *P. tamiana*, see above), with one polyploidy event. This does not enable any great inferences on chromosome evolution in the genus. Even though 18 out of the around 130 species have been counted now, only six of these are included in the phylogeny (Fig. 5). More counts are necessary to confirm whether $2n = 36$ is the prevailing and ancestral number and to determine the degree of polyploidy and levels of polyploidy within this genus. Perhaps it is polyploidy that aided the high diversification and speciation of this genus and the evolution of the many species found in the limestone areas of SW China.

Genome evolution among genera

Given that the five genera with species previously included in *Chirita* are scattered widely across the Old World Gesneriaceae it is conceivable that their genome evolution, in terms of changes in chromosome number, is tightly linked to the evolution of a large portion of the advanced didymocarpoid Gesneriaceae. However, the weakly supported backbone topology of the phylogenetic tree in Weber *et al.* (2011) currently precludes deeper discussions of evolutionary events concerning genome relationships at this level. Nonetheless, the distribution of chromosome numbers on this tree suggests that $2n = 18$ has been conserved in parts of the tree

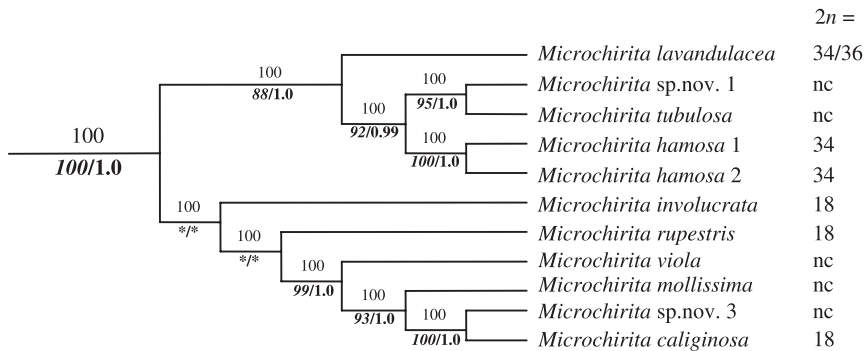


FIG. 4. The *Microchirita* clade (modified from Weber *et al.*, 2011), with somatic chromosome numbers indicated. nc = not counted. Numbers along branches as in Fig. 3.

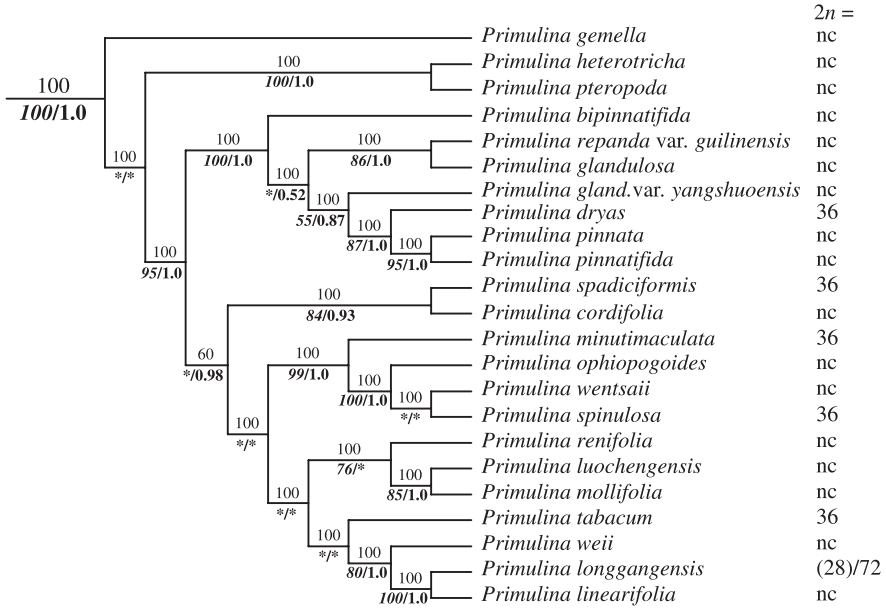


FIG. 5. The *Primulina* clade (modified from Weber *et al.*, 2011), with somatic chromosome numbers indicated. nc = not counted. Numbers along branches as in Fig. 3.

involving some genera hitherto included in *Chirita*, namely *Damrongia*, *Microchirita* and *Henckelia*. The $2n = 36$ count for *Primulina* at first seems a straightforward duplication from an ancestor with $2n = 18$ chromosomes. The clade closest to this genus (with high statistical support) until recently included eight genera that have now all been synonymised into *Petrocodon* Hance (Weber *et al.*, 2011). From this clade a count is only available for *Petrocodon hancei* (Hemsl.) A. Weber & Mich. Möller, as *Didymocarpus hancei* Hemsl., with $2n = 20$ (Cao *et al.*, 2003). The ancestral chromosome state for *Primulina* or *Petrocodon* is, therefore, inconclusive and further cytological studies are necessary, particularly in the expanded genus *Petrocodon*. In the phylogeny in Weber *et al.* (2011) members of other clades around *Primulina* include genera with high somatic chromosome numbers, such as *Anna* ($2n = 34$), *Hemiboea* C.B. Clarke ($2n = 32, 36$), *Loxostigma* C.B. Clarke ($2n = 34$), *Lysionotus* D. Don ($2n = 30, 32$), *Petrocosmea* Oliv. (mostly $2n = 34$) (from Möller *et al.*, 2002 onwards), and *Pseudochirita* W.T. Wang ($2n = 28$) (Cao *et al.*, 2003), and in the neighbouring clade *Aeschynanthus* Jack ($2n = 28, 30, 32$), *Agalmyla* Blume ($2n = 32$), *Cyrtandra* J.R. Forst. & G. Forst. (mostly $2n = 34$), and *Oreocharis* Benth. ($2n = 34$) (Möller *et al.*, 2002 onwards). Thus it seems likely that the duplication in *Primulina* occurred long before the differentiation of this genus. However, much more work is needed on the advanced didymocarpoid Gesneriaceae, both molecularly to obtain a stable hypothesis of genus relationships, and cytologically to allow a full discussion on the genome rearrangements over time to take place.

The systematic remodelling of the genus *Chirita*, while bringing the taxonomy greatly in line with molecular phylogenetic relationships, has not completely resulted in genera with uniform chromosome numbers. *Henckelia* is as equally heterogeneous as the erstwhile *Chirita* while, on the other hand, the *Damrongia* and *Primulina* species so far analysed show highly conserved numbers. *Microchirita* includes two numbers but more taxa need to be investigated. It appears that, although some of the genera to emerge from *Chirita* are fairly uniform in chromosome number, the previous diversity in *Chirita* has mostly simply been transferred to *Henckelia* in its new circumscription. Polyploid and dysploid changes seem to mark the genome evolution of *Henckelia*, *Microchirita* and *Primulina*. On comparison of chromosome counts to the recently published phylogenetic hypotheses, no ascending or descending chromosome number series have been observed, but rather a patchwork of evolutionary increases and decreases in chromosome numbers. Though some insight into the evolution of the genomes through changes in chromosome numbers of the genera has been gained, much more work remains to be done.

ACKNOWLEDGEMENTS

We acknowledge and greatly appreciate A. Ensell, L. Galloway and S. Scott for their care of the Living Collections at the Royal Botanic Garden Edinburgh. We would like to thank D. J. Middleton for the collection and provision of living plant material of *Damrongia* species, and Prof. Y. G. Wei for supporting field collections in Guangxi. We would also like to thank the Percy Sladen Memorial Fund and the Royal Botanic Garden Edinburgh Expedition Fund for supporting the fieldwork of M.M. The Royal Botanic Garden Edinburgh is funded by the Rural and Environment Science and Analytical Services division (RESAS) in the Scottish Government.

REFERENCES

- CAO, L. M., CAO, M., TANG, X. L. & WEI, Y. G. (2003). Chromosome numbers of 4 species in the Gesneriaceae from Guangxi. *Guihaia* 23(4): 331–333.
- CONGER, A. D. & FAIRCHILD, L. M. (1953). A quick-freeze method for making smear slides permanent. *Stain Tech.* 28: 281–283.
- FOX, D. P. (1969). Some characteristics of the cold hydrolysis technique for staining plant tissues by the Feulgen reaction. *J. Histo. Cytochem.* 17: 226.
- FUSSELL, C. P. (1958). Chromosome numbers in the Gesneriaceae. *Baileya* 6: 117–125.
- GREGORY, T. R. (2005). The C-value enigma in plants and animals: a review of parallels and an appeal for partnership. *Ann. Bot.* 95: 133–146.
- HAIR, T. B. & BEUZENBERG, E. J. (1958). Chromosomal evolution in the Podocarpaceae. *Nature* 181: 1584–1586.
- HELLMAYR, E. (1989). Chromosomenzählungen an Blütenpflanzen der Malaiischen Halbinsel. 5. *Österreichisches Botanikertreffen in Innsbruck 25.–28. Mai 1989*. Innsbruck.
- JOHN, B. & FREEMAN, M. (1975). Causes and consequences of Robertsonian exchange. *Chromosoma* 52: 123–136.

- JONES, K. (1974). Chromosome evolution by Robertsonian translocation in *Gibasis* (Commelinaceae). *Chromosoma* 45: 353–368.
- JONG, K. (1997). *A Laboratory Manual of Cytological Techniques*. Edinburgh: Royal Botanic Garden Edinburgh.
- JONG, K. & MÖLLER, M. (2000). New chromosome counts in *Streptocarpus* (Gesneriaceae) from Madagascar and the Comoro Islands and their taxonomic significance. *Pl. Syst. Evol.* 224: 173–182.
- KASAI, F., TAKAHASHI, E. I., KOYAMA, K., TERAO, K., SUTO, Y., TOKUNAGA, K. ET AL. (2000). Comparative FISH mapping of the ancestral fusion point of human chromosome 2. *Chromosome Res.* 8: 727–735.
- KIEHN, M. & LORENCE, D. H. (1996). Chromosome counts on angiosperms cultivated at the National Tropical Botanical Garden, Kaua'i, Hawai'i. *Pacific Sci.* 50: 317–323.
- KIEHN, M., HELLMAYR, E. & WEBER, A. (1998). Chromosome numbers of Malayan and other paleotropical Gesneriaceae. I. Tribe Didymocarpeae. *Beitr. Biol. Pflanzen* 70: 407–444.
- KIEW, R. & LIM, C. L. (2011). Names and new combinations for Peninsular Malaysian species of *Codonoboea* Ridl. (Gesneriaceae). *Gard. Bull. Singapore* 62(2): 253–275.
- LEE, R. E. (1962). Chromosome numbers in the Gesneriaceae. *Baileya* 10: 33–45.
- LEITCH, I. J., SOLTIS, D. E., SOLTIS, P. S. & BENNETT, M. D. (2005). Evolution of DNA amounts across land plants (Embryophyta). *Ann. Bot.* 95: 207–217.
- LU, Y. X., SUN, X. F., ZHOU, Q. X. & GU, Z. J. (2002). Chromosome numbers in ten species in the Gesneriaceae from Yunnan. *Acta Bot. Yunnan.* 24: 377–382.
- MILNE, C. (1975). Chromosome numbers in the Gesneriaceae: V. *Notes Roy. Bot. Gard. Edinburgh* 33: 523–525.
- MÖLLER, M. & KIEHN, M. (2003). A synopsis of cytological studies in Gesneriaceae. *Edinburgh J. Bot.* 60(3): 425–447.
- MÖLLER, M., PULLAN, M., KIEHN, M. & SKOG, L. E. (2002 onwards). RBGE WebCyte – Gesneriaceae cytology database. <http://elmer.rbge.org.uk/webcyte/webcyte.php>
- MÖLLER, M., PFOSSER, M., JANG, C. G., MAYER, V., CLARK, A., HOLLINGSWORTH, M. L. ET AL. (2009). A preliminary phylogeny of the 'didymocarpoid Gesneriaceae' based on three molecular data sets: Incongruence with available tribal classifications. *Amer. J. Bot.* 96: 989–1010.
- MÖLLER, M., FORREST, A., WIE, Y. G. & WEBER, A. (2011). A molecular phylogenetic assessment of the advanced Asiatic and Malesian didymocarpoid Gesneriaceae with focus on non-monophyletic and monotypic genera. *Pl. Syst. Evol.* 292: 223–248.
- RATTER, J. A. (1963). Some chromosome numbers in the Gesneriaceae. *Notes Roy. Bot. Gard. Edinburgh* 24: 221–229.
- RATTER, J. A. & MILNE, C. (1970). Chromosome numbers in the Gesneriaceae: IV. *Notes Roy. Bot. Gard. Edinburgh* 30: 183–187.
- RATTER, J. A. & PRENTICE, H. T. (1964). Chromosome numbers in the Gesneriaceae: II. *Notes Roy. Bot. Gard. Edinburgh* 25: 303–307.
- RATTER, J. A. & PRENTICE, H. T. (1967). Chromosome numbers in the Gesneriaceae: III. *Notes Roy. Bot. Gard. Edinburgh* 27: 205–209.
- ROGERS, O. M. (1954). Some chromosome counts in the Gesneriaceae. *Baileya* 2: 14–18.
- SILJAK-YAKOVLEV, S., STEVANOVIC, V., TOMASEVIC, M., BROWN, S. C. & STEVANOVIC, B. (2008). Genome size variation and polyploidy in the resurrection plant genus *Ramonda*: Cytogeography of living fossils. *Environ. Exp. Bot.* 62: 101–112.
- SUGIURA, T. (1938). A list of chromosome numbers in angiospermous plants V. *Proc. Imp. Acad. (Tokyo)* 14: 391–392.
- SUGIURA, T. (1940). Studies on the chromosome numbers in higher plants. V. *Cytologia* 10: 363–370.

- THATHACHAR, T. (1942). Studies in Gesneriaceae, gametogenesis and embryogeny of *Didymocarpus tomentosa* Wt. *J. Indian Bot. Soc.* 21: 185–193.
- VASUDEVAN, K. N. (1976). Contribution to the cytotaxonomy and cyto geography of the flora of the western Himalayas (with an attempt to compare it with the flora of the Alps). Part III. *Ber. Schweiz. Bot. Ges.* 86: 152–203.
- WANG, W. T. (1985a). A revision of the genus *Chirita* in China (I). *Bull. Bot. Res., Harbin* 5(2): 71–97.
- WANG, W. T. (1985b). A revision of the genus *Chirita* in China (II). *Bull. Bot. Res., Harbin* 5(3): 37–86.
- WANG, Y. Z., MAO, R. B., LIU, Y., LI, J. M., DONG, Y., LI, Z. Y. ET AL. (2011). Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments. *J. Syst. Evol.* 49: 50–64.
- WEBER, A. (2004). Gesneriaceae. In: KUBITZKI, K. (ed.) *The Families and Genera of Vascular Plants*, vol. VII: *Dicotyledons – Lamiales (except Acanthaceae including Avicenniaceae)* (vol. ed. J. W. KADEREIT), pp. 63–158. Berlin & Heidelberg: Springer.
- WEBER, A. & BURTT, B. L. (1998). Remodelling of *Didymocarpus* and associated genera (Gesneriaceae). *Beitr. Biol. Pflanzen* 70: 293–363.
- WEBER, A., MIDDLETON, D. J., FORREST, A., KIEW, R., LIM, C. L., RAFIDAH, A. R. ET AL. (2011). Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae). *Taxon* 60: 767–790.
- WOOD, D. (1974). A revision of *Chirita* (Gesneriaceae). *Notes Roy. Bot. Gard. Edinburgh* 33: 123–205.
- ZHOU, P., GU, Z. J. & MÖLLER, M. (2004). New chromosome counts and nuclear characteristics for some members of Gesneriaceae subfamily Cyrtandroideae from China and Vietnam. *Edinburgh J. Bot.* 60: 449–466.

Received 26 September 2011; accepted for publication 21 February 2012