EDINBURGH JOURNAL OF BOTANY 71 (3): 297–308 (2014)

doi:10.1017/S0960428614000158

NEW CHROMOSOME COUNTS OF *BERBERIS* L. (BERBERIDACEAE) SUGGEST THAT POLYPLOIDY DOES NOT PLAY A SIGNIFICANT ROLE IN THE DIVERSIFICATION OF THE GENUS IN THE NEPAL HIMALAYA

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Chromosomes were counted for nine of the 24 taxa of *Berberis* (Berberidaceae) from Nepal, five of which were counted for the first time. The results show that all these species have somatic chromosome numbers of $2n = 28$. The absence of polyploids suggests that polyploidy may not have been a major driver of speciation and diversification of *Berberis* in the Nepal Himalaya.

Keywords. Berberidaceae, *Berberis*, chromosomes, Himalaya, Nepal.

Introduction

The importance of cytological data for the study of evolution and diversification of plant species has long been recognised (Stebbins, 1971; Stace, 2000). Furthermore, polyploidy is considered to be one of the major forces behind speciation in higher plants (Stebbins, 1971; Soltis & Soltis, 1995), and it has been estimated that 50–70% of angiosperm species are polyploids or have a polyploid origin (Stebbins, 1971; Grant, 1981; Masterson, 1994). It has previously been suggested that polyploidy is one way by which plants can adapt to harsh climates at high altitudes (Janaki Ammal, 1950; Yoshida, 2006) and therefore they are expected to be more frequent at high altitudes than low altitudes. The Nepal Himalaya exhibits extreme elevation ranges within very short distances so the occurrence of polyploids might be expected at higher altitudes. Indeed, polyploidy has been shown to play an important role in the speciation and diversification of some Himalayan taxa, for example *Sedum* L., *Rhododendron* L. and *Anaphalis* L. (Janaki Ammal, 1950; Wakabayashi & Ohba, 1999; Meng *et al.*, 2010).

The Berberidaceae include about 12 herbaceous and two woody genera (*Berberis s.l.* which now includes *Mahonia* Nutt., and *Nandina* Thunb.) (Mabberley, 2008). Using character compatibility analysis, Meacham (1980) recognised four major groups within the family and these were supported by chromosome numbers and fruit characters. An *ndhF*, *rbcL* and chloroplast DNA restriction site analysis recognised

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four chromosomal clades in Berberidaceae (x = 6, 7, 8 and 10) (Kim & Jansen, 1996, 1998; Kim *et al.*, 2004). *Berberis s.l.* and *Ranzania* T.Ito form a clade with x = 7 (Kim & Jansen, 1998; Kim *et al.*, 2004). *Ranzania* only has $2n$ = 14, while all chromosome counts for *Berberis* are $2n$ = 28 and 56 (Bottini *et al.*, 1997, 1999, 2000; Rounsaville & Ranney, 2010). From the distribution of somatic chromosome numbers in Berberidaceae (Kim & Jansen, 1998), the ancestral number appears to be $2n$ = 14 for the *Berberis* & *Ranzania* clade. Since meiotic counts in *Berberis* indicate the absence of multivalents, the genus could be regarded as a genus of tetraploid origin, that has undergone diploidisation and can be described as a diploid with a base number of x = 14.

The Argentinean species of simple-leaved *Berberis* have been relatively well studied cytologically (Bottini *et al.*, 1997, 1999, 2000), and most are diploid with $2n$ = 28 chromosomes. Higher ploidy levels were reported only for *Berberis buxifolia* Lam. and *B. heterophylla* Juss., both of which had $2n$ = 56 (Bottini *et al.*, 1997).

The chromosome numbers of Nepalese species of simple-leaved *Berberis* species are almost completely unknown. Of the 21 simple-leaved species recognised in Nepal (Adhikari *et al.*, 2012), chromosome counts have previously been published for only one species (*Berberis asiatica* Roxb. ex DC.) from a plant of Nepalese origin (Malla *et al.*, 1975). The published chromosome counts of other Nepalese species, *Berberis aristata* DC., *B. angulosa* Wall. ex Hook.f. & Thomson, *B. concinna* Hook.f. and *B. wallichiana* DC., were based either on plants of Indian origin, or on cultivated specimens of unknown origin. Of the Nepalese compound-leaved species, one species, *Berberis napaulensis* (DC.) Laferr., has been counted so far ($2n$ = 28; Sharma, 1970). The published chromosome numbers for Nepalese species of *Berberis* and some closely related species from the Himalayan region are shown in Table 1.

The objective of this paper was to determine the chromosome numbers of Nepalese species of *Berberis* to find out whether polyploidy was involved in the evolution and diversification of simple-leaved species of *Berberis* in Nepal.

Material and Methods

Chromosomes were counted in roots harvested from plants growing in the living collections at the Royal Botanic Garden Edinburgh. Prior to the roots being harvested, plants were re-potted with new compost and transferred to an environmentally controlled glasshouse (average day temperature 18°C and night temperature 16°C) to stimulate root growth.

Two pre-treatments were tested to prevent the formation of mitotic spindles (Jong, 1997): α-bromo-naphthalene for 3 hours at room temperature and 0.002M 8-hydroxyquinoline for 5 hours at 4°C. 8-hydroxyquinoline gave better results than α-bromo-naphthalene. Root tips were then fixed with freshly prepared Farmer's Fluid (3 : 1, absolute ethanol : glacial acetic acid) and kept at 4°C overnight.

They were stained with Feulgen reagent freshly prepared according to Fox (1969; see Jong, 1997). Before squashing, the root tips were softened in a 1 : 1 enzyme mixture

TABLE 1. List of published chromosome numbers for Nepalese *Berberis* and closely related species from the Himalaya with information on origin and references

Taxon	Chromosome no.		Ploidy level	Origin	Primary references	Secondary references, database	Methods
	n	2*n*					
B. angulosa Wall. ex Hook.f. & Thomson		28	Diploid	Not known	Giffen (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. angulosa Wall. ex Hook.f. & Thomson	14			Not known	Sharma (1970). Original not seen	IPCNR (1975–1978), TROPICOS	NA
B. aristata DC.		28	Diploid	Not known	Giffen (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. aristata DC.	14		Diploid	Not known	Sandhu & Mann (1988)	IPCNR (1988–1989), TROPICOS	NA
B. aristata DC.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. aristata DC.	14		Diploid	Not known	Mehra (1976)	IPCNR (1975–1978), TROPICOS	NA
B. aristata DC.	14		Diploid	India: West Himalayas	Mehra & Sareen (1969)	IPCNR (1967–1971)	NA
B. aristata DC.	14		Diploid	Not known	Mehra & Sareen (1973)	IPCNR (1973/1974)	NA
B. asiatica Roxb. ex DC.	14		Diploid	India: Central	Singhal & Gill (1984)	IPCNR (1986–1987), TROPICOS	NA
B. asiatica Roxb. ex DC.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. asiatica Roxb. ex DC.	14		Diploid	Nepal: Godawari	Malla *et al.* (1975)	IPCNR (1975–1978), TROPICOS	NA
B. chitria Lindl.	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. chitria Lindl. var. *chitria*	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979–1981), Khatoon & Ali (1993), TROPICOS	NA

TABLE 1. (*Cont'd*)

Taxon	Chromosome no.		Ploidy level	Origin	Primary references	Secondary references, database	Methods
	n	2*n*					
B. chitria var. *occidentalis* Ahrendt	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979–1981), TROPICOS	NA
B. chitria var. *occidentalis* Ahrendt	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. concinna Hook.f.		28	Diploid	Not known	Giffen (1936)	Bolkhovskikh *et al.* (1969)	Root tip
B. coriaria Royle	14		Diploid	Not known	Sandhu & Mann (1988)	IPCNR (1988–1989), TROPICOS	NA
B. lycium Royle	14		Diploid	Not known	Giffen (1936)	Bolkhovskikh *et al.* (1969)	PMC
B. lycium Royle	14		Diploid	India: Kashmir Himalaya	Jee *et al.* (1989)	IPCNR (1990–1991), TROPICOS	PMC
B. lycium Royle	14		Diploid	Not known	Sandhu & Mann (1988)	IPCNR (1988–1989), TROPICOS	NA
B. lycium Royle var. *lycium*	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979–1981), Khatoon & Ali (1993), TROPICOS	NA
B. lycium Royle var. *lycium*	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. lycium Royle var. *subfascicularis* Ahrendt	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979–1981), TROPICOS	NA
B. lycium Royle var. *subfascicularis* Ahrendt	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. napaulensis (DC.) Laferr.		28	Diploid	Not known	Sharma (1970). Original not seen.	IPCNR (1975–1978)	NA

TABLE 1. (*Cont'd*)

B. napaulensis (DC.) Laferr.		28	Diploid	Nepal: Godawari	Malla *et al.* (1974)	IPCNR (1973–1974)	NA
B. orthobotrys Bienert ex Aitch. subsp. *orthobotrys*	24		Hypotetraploid	Pakistan, Thalle	Khatoon (1991) (PhD thesis). Original not seen	Khatoon & Ali (1993), TROPICOS	NA
B. ulicina Hook.f. & Thomson	14		Diploid	India: Kashmir Himalaya	Jee *et al.* (1989)	IPCNR (1990–1991), TROPICOS	PMC
B. umbellata Wall. ex G.Don	14		Diploid	India: Uttar Pradesh	Singhal *et al.* (1980)	IPCNR (1979–1981), Khatoon & Ali (1993), TROPICOS	NA
B. umbellata Wall. ex G.Don	14		Diploid	India: West Himalayas	Bir & Thakur (1984)	IPCNR (1986–1987), TROPICOS	NA
B. umbellata Wall. ex G.Don	14		Diploid	Not known	Gill *et al.* (1984)	IPCNR (1984–1985)	NA
B. wallichiana DC.	14		Diploid	Not known	Sharma (1970). Original not seen	IPCNR (1975–1978), TROPICOS	NA

IPCNR: Index to Plant Chromosome Number Report. NA: Not available. PMC: Pollen mother cell. TROPICOS: www.tropicos.org.

of 4% cellulase and 4% pectinase for 30 minutes at 35°C. Each root tip meristem was excised onto a slide with a few drops of 0.04% acetocarmine as counter stain, and permanent slides were prepared using the vapour exchange method (Jong, 1997). Chromosomes were observed with a Zeiss Axioskop brightfield microscope and images were taken at different magnifications using a digital AxioCam MRc5 camera and AxioVision Rel. 4.7 (Zeiss, Welwyn Garden City, UK). Chromosomes were counted in at least five cells to confirm the number.

Results and Discussion

Chromosome counts from the present study are listed in Table 2. All chromosome numbers found in this study were $2n = 28$. This is the first report of the chromosome numbers of nine taxa of *Berberis* from the Nepal Himalaya and, of these, five (*B. concinna* var. *extensiflora*, *B. jaeschkeana* var. *usteriana*, *B. mucrifolia*, *B. pendryi* and *B. thomsoniana*) were counted for the first time.

1. ***Berberis angulosa*** Wall. ex Hook.f. & Thomson

Berberis angulosa has the somatic chromosome number of $2n = 28$ (Fig. 1A). Large satellites were prominent in metaphasic stages of mitotic divisions (arrows). This report of a chromosome number of $2n = 28$ for *Berberis angulosa* from Nepal is consistent with previous reports by Giffen (1936), though the present work is the first report of chromosome numbers for this species from a plant from Nepal.

2. ***Berberis aristata*** DC.

Root tips from two individuals of the same accession were examined and both had chromosome numbers of $2n = 28$ (Fig. 1B, C). Two satellite chromosomes were prominent in all spreads (arrows). In Fig. 1C, the chromosomes are in prometaphase and the satellites have been detached during slide preparation (arrows). Most chromosomes appear metacentric. The present count of $2n = 28$ agrees with previous reports by Giffen (1936), Mehra & Sareen (1969, 1973), Mehra (1976), Gill *et al.* (1984) and Sandhu & Mann (1988). This is the first report of the chromosome number of this species from a locality in Nepal.

3. ***Berberis concinna*** var. ***concinna*** Hook.f.

The present count (Fig. 1D) confirms the previous count of $2n = 28$ by Giffen (1936). Large satellites were found in the late prometaphase (arrows) that shows mostly metacentric chromosomes. This is the first chromosome count of this species from a locality in Nepal.

TABLE 2. Chromosomes counts of *Berberis* taxa obtained in this study

Taxon	Accession	Voucher information	Collection details	2*n*	Fig.
B. angulosa Wall. ex Hook.f. & Thomson	20071214A	EL 126	Nepal: Rasuwa, near Cholangpati	28	1A
B. aristata DC.	20071210A	EL 122	Nepal: Rasuwa, Dovan	28	1B
B. aristata DC.	20071210B	EL 122	Nepal: Rasuwa, Dovan	28	1C
B. concinna Hook.f. var. *concinna*	20071213	EL 125	Nepal: Rasuwa, near Cholangpati	28	1D
B. concinna Hook.f. var. *extensiflora* Ahrendt	20071208A	EA 105	Nepal: Myagdi, Ghorepani	28	1E
B. jaeschkeana C.K.Schneid. var. *usteriana* C.K.Schneid.	20071203B	EA 49	Nepal: Mustang, Kaligandaki	28	1F
B. mucrifolia Ahrendt	20071201A	EA 40	Nepal: Mustang, Kaligandaki	28	1G
B. pendryi Bh.Adhikari	20071200C	EA 36	Nepal: Mustang, Muktinath	28	1H
B. thomsoniana C.K.Schneid.	20071212A	EL 124	Nepal: Rasuwa, near Chandanbari	28	1I
B. thomsoniana C.K.Schneid.	20071212B	EL 124	Nepal: Rasuwa, near Chandanbari	28	1J
B. wallichiana DC.	20071211A	EL 123	Nepal: Rasuwa, above Deurali	28	1K
B. wallichiana DC.	20071211B	EL 123	Nepal: Rasuwa, above Deurali	28	1L

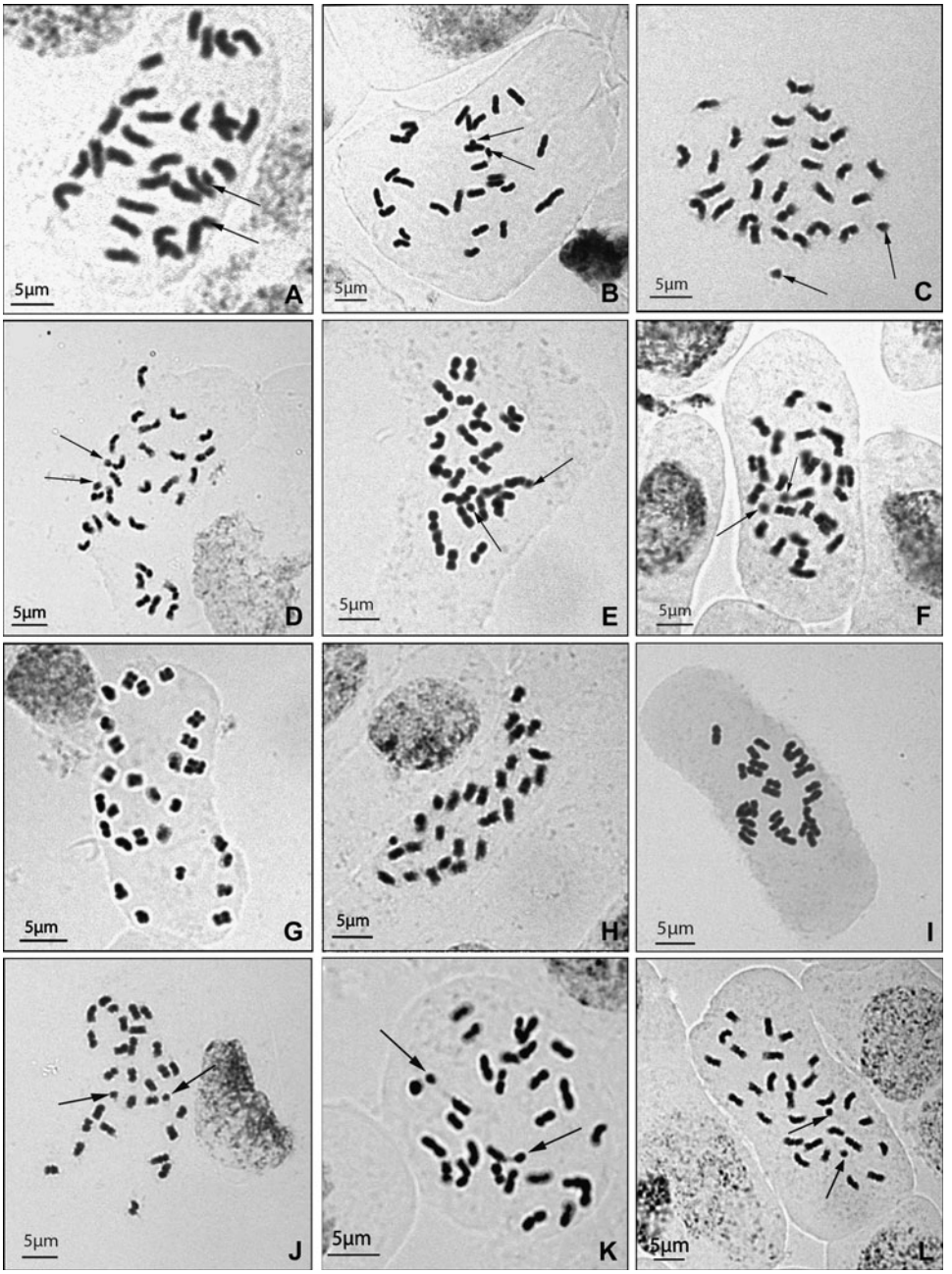

FIG. 1. Mitotic chromosomes of *Berberis*. A. *B. angulosa* (20071214A), metaphase, 2*n* = 28; B. *B. aristata* (20071210A), metaphase, 2*n* = 28; C. *B. aristata* (20071210B), prometaphase, 2*n* = 28; D. *B. concinna* var. *concinna* (20071213), late prometaphase, 2*n* = 28; E. *B. concinna* var. *extensiflora* (20071208A), metaphase, 2*n* = 28; F. *B. jaeschkeana* var. *usteriana* (20071203B), prometaphase, 2*n* = 28; G. *B. mucrifolia* (20071201A), metaphase, 2*n* = 28; H. *B. pendryi*

4. ***Berberis concinna*** var. ***extensiflora*** Ahrendt

A somatic chromosome number of $2n = 28$ was counted for this species (Fig. 1E). Large satellites are prominent in Fig. 1E (arrows). This is the first report of the chromosome number of *Berberis concinna* var. *extensiflora.*

5. ***Berberis jaeschkeana*** var. ***usteriana*** C.K.Schneid.

Root tips from two individuals of the same accession were examined and both had a somatic chromosome number of $2n = 28$. Satellites were prominent in prometaphase spreads (Fig. 1F, arrows). Most chromosomes appear metacentric. This is the first report of the chromosome number of *Berberis jaeschkeana*.

6. ***Berberis mucrifolia*** Ahrendt

A somatic chromosome number of $2n = 28$ was counted (Fig. 1G). The chromosomes showed mostly median centromeres. This is the first report of the chromosome number of this species.

7. ***Berberis pendryi*** Bh.Adhikari

The somatic chromosome number counted was $2n = 28$ (Fig. 1H), and this is the first report of the chromosome number of this species.

8. ***Berberis thomsoniana*** C.K.Schneid.

Root tips of two individuals from the same accession were examined and both had the diploid chromosome number of $2n = 28$ (Fig. 1I, J). Satellites were prominent in prometaphase chromosomes (Fig. 1J, arrows) but not in metaphase chromosomes (Fig. 1I). The chromosomes were mostly metacentric. This is the first report of the chromosome number of *Berberis thomsoniana*.

9. ***Berberis wallichiana*** DC.

Root tips of two individuals from the same accession were examined and both had a somatic chromosome number of $2n = 28$. Satellites were prominent in both prometaphasic and metaphasic chromosomes (Fig. 1K, L, arrows). Satellite stalks were clearly visible in the metaphasic chromosomes (Fig. 1K, arrows). This count agrees with a previous count by Sharma (1970), and is the first count of this species from Nepal.

The species of *Berberis* examined during this study grow between 2500 and 4500 m altitude in a variety of environmental conditions and all were found to possess

(20071200C), prometaphase, $2n = 28$; I. *B. thomsoniana* (20071212A), metaphase, $2n = 28$; J. *B. thomsoniana* (20071212B), prometaphase, $2n = 28$; K. *B. wallichiana* (20071211A), metaphase, $2n = 28$; L. *B. wallichiana* (20071211B), prometaphase, $2n = 28$. Arrows indicate satellites.

$2n = 28$ chromosomes. Rounsaville & Ranney (2010) also found that polyploidy was not common among wild and cultivated taxa of *Berberis s.l.*, and found only one naturally occurring polyploid, the compound-leaved North American taxon *B. nervosa* Pursh, among 124 samples in 70 taxa. Khatoon & Ali (1993) reported a hypotetraploid, *Berberis orthobotrys* Bienert ex Aitch., from the Himalayan foothills in Pakistan but their original work could not be consulted so it is difficult to speculate on the origin or the consistency across *B. orthobotrys* of the aneuploid number of $n = 24$ reported there. Although polyploids seem to play an important role in speciation and diversification in some high altitude taxa (Janaki Ammal, 1950; Wakabayashi & Ohba, 1999; Meng *et al.*, 2010), *Berberis* does not seem to follow this trend. This would agree with the findings of Wakabayashi & Ohba (1988) in *Saxifraga*, which has similarly low numbers of polyploids, suggesting that polyploidy may not be an important factor in species diversification in the alpine flora of the Nepal Himalaya (Ohba, 1988; Wakabayashi & Ohba, 1988).

Examining only nine taxa out of 24 cannot rule out the presence of polyploids among Nepalese *Berberis*, however, and further studies in other taxa are needed to clarify the role of polyploidy in the speciation and diversification of Himalayan plants.

Acknowledgements

We should like to thank Kwiton Jong for his help and advice. Thanks are also due to David Knott for his help with the living collection at the Royal Botanic Garden Edinburgh, and Clare Morter who looked after the *Berberis* living collection in the glasshouse. This study formed part of the first author's PhD project and was funded by the University of Edinburgh, the Royal Horticultural Society and the Royal Botanic Garden Edinburgh. The Royal Botanic Garden Edinburgh is supported by the Rural and Environment Science and Analytical Services division (RESAS) in the Scottish Government.

References

Adhikari, B., Pendry, C. A., Pennington, R. T. & Milne, R. I. (2012). A revision of *Berberis s.s.* (Berberidaceae) in Nepal. *Edinburgh J. Bot.* 69(3): 447–522. DOI: 10.1017/S0960428612000261.

Bir, S. S. & Thakur, H. (1984). SOCGI plant chromosome number reports II. *J. Cytol. Genet.* 19: 114–115.

Bolkhovskikh, Z., Grif, V., Matvejeva, T. & Zakharyeva, O. (1969). *Khromosomnye chisla tsvetkovykh rastenii* [Chromosome numbers of flowering plants]. Leningrad: Izdatel'stvo 'Nauka' Leningradskoe Otdelenyi.

Bottini, M. C. J., Greizerstein, E. J. & Poggio, L. (1997). Numeros chromosomicos y contenido de adn de cuatro especies patagonicas del genero *Berberis* (Berberidaceae). *Bol. Soc. Argent. Bot.* 32(3–4): 235–239.

Bottini, M. C. J., Greizerstein, E. J. & Poggio, L. (1999). Ploidy levels and their relationships with the rainfall in several populations of Patagonian species of *Berberis* L. *Caryologia* 52: 75–80.

BOTTINI, M. C. J., GREIZERSTEIN, E. J., AULICINO, M. B. & POGGIO, L. (2000). Relationships among genome size, environmental conditions and geographical distribution in natural populations of NW Patagonian species of *Berberis* L. (Berberidaceae). *Ann. Bot.* 86(3): 565–573.

FOX, D. P. (1969). Some characteristics of the cold hydrolysis technique for staining plant tissue by the Feulgen reaction. *J. Histochem. Cytochem.* 17(4): 266–272.

GIFFEN, M. H. (1936). The chromosomes numbers of *Berberis*. *Trans. Roy. Soc. S. Afr.* 24: 203–206.

GILL, B. S., BIR, S. S. & SINGHAL, V. K. (1984). *Cytological Studies in Some Western Himalayan Wood Species II. Polypetalae*. Delhi: Puja Publishers.

GRANT, V. (1981). *Plant Speciation*. New York: Columbia University Press.

JANAKI AMMAL, E. K. (1950). Polyploidy in the genus *Rhododendron*. *The Rhododendron Year Book* 5: 92–98.

JEE, V., DHAR, U. & KACHROO, P. (1989). Cytogeography of some endemic taxa of Kashmir Himalaya. *Proc. Indian Natl. Sci. Acad., B* 55: 177–184.

JONG, K. (1997). *Laboratory Manual of Plant Cytological Techniques*. Edinburgh: Royal Botanic Garden Edinburgh.

KHATOON, S. (1991). *Polyploidy in the flora of Pakistan – an analytical study*. PhD thesis, Department of Botany, University of Karachi, Karachi, Pakistan.

KHATOON, S. & ALI, S. I. (1993). *Chromosome Atlas of the Angiosperms of Pakistan*. Karachi, Pakistan: Department of Botany, University of Karachi.

KIM, Y.-D. & JANSEN, R. K. (1996). Phylogenetic implications of *rbcL* and ITS sequence variation in the Berberidaceae. *Syst. Bot.* 21(3): 381–396.

KIM, Y.-D. & JANSEN, R. K. (1998). Chloroplast DNA restriction site variation and phylogeny of the Berberidaceae. *Amer. J. Bot.* 85(12): 1766–1778.

KIM, Y.-D., KIM, S.-H., KIM, C. H. & JANSEN, R. K. (2004). Phylogeny of Berberidaceae based on sequences of the chloroplast gene *ndhF*. *Biochem. Syst. Ecol.* 32(3): 291–301.

MABBERLEY, D. J. (2008). *The Plant-Book*. Cambridge: Cambridge University Press.

MALLA, S. B., BHATTARAI, S., SHRESTHA, M. & SINGH, M. P. (1974). In: IOPB chromosome number reports XLVI. *Taxon* 23: 801–812.

MALLA, S. B., BHATTARAI, S., GORKHALI, M. & SINGH, M. P. (1975). In: IOPB chromosome number reports XLIX. *Taxon* 24: 501–516.

MASTERSON, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264(5157): 421–424.

MEACHAM, C. A. (1980). Phylogeny of the Berberidaceae with an evaluation of classification. *Syst. Bot.* 5(2): 149–172.

MEHRA, P. N. (1976). *Cytology of Himalayan Hardwoods*. Calcutta: Sree Saraswaty Press.

MEHRA, P. N. & SAREEN, T. S. (1969). In: IOPB chromosome number reports XXII. *Taxon* 18: 433–442.

MEHRA, P. N. & SAREEN, T. S. (1973). Cytology of some Himalayan trees. *Silvae Genet.* 22: 66–70.

MENG, Y., SUN, H., YANG, Y.-P. & NIE, Z.-L. (2010). Polyploidy and new chromosome counts in *Anaphalis* (Asteraceae: Gnaphalieae) from the Qinghai-Tibet Plateau of China. *J. Syst. Evol.* 48(1): 58–64.

OHBA, H. (1988). The alpine flora of the Nepal Himalayas: An introductory note. In: OHBA, H. & MALLA, S. B. (eds) *The Himalayan Plants*, pp. 19–46. Tokyo: University of Tokyo Press.

ROUNSAVILLE, T. J. & RANNEY, T. G. (2010). Ploidy levels and genome sizes of *Berberis* L. and *Mahonia* Nutt. Species, hybrids, and cultivars. *Hortscience* 45(7): 1029–1033.

SANDHU, P. S. & MANN, S. K. (1988). SOCGI plant chromosome number reports VII. *J. Cytol. Genet.* 23: 219–228.
SHARMA, A. K. (1970). Annual report, 1967–1968. *Res. Bull. Univ. Calcutta Cytogenetics Lab.* 2: 1–50.
SINGHAL, V. K. & GILL, B. S. (1984). SOCGI plant chromosome number reports II. *J. Cytol. Genet.* 19: 115–117.
SINGHAL, V. K., GILL, B. S. & BIR, S. S. (1980). In: IOPB chromosome number reports LXVII. *Taxon* 29: 355–357.
SOLTIS, D. E. & SOLTIS, P. S. (1995). The dynamic nature of polyploid genomes. *Proc. Natl. Acad. Sci. U.S.A.* 92(18): 8089–8091.
STACE, C. A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon* 49(3): 451–477.
STEBBINS, G. L. (1971). *Chromosomal Evolution in Higher Plants*. London: Edward Arnold (Publishers) Ltd.
WAKABAYASHI, M. & OHBA, H. (1988). Cytotaxonomic study of the Himalayan *Saxifraga*. In: OHBA, H. & MALLA, S. B. (eds) *The Himalayan Plants* I, pp. 71–89. Tokyo: University of Tokyo Press.
WAKABAYASHI, M. & OHBA, H. (1999). Chromosome numbers of seven species of *Sedum* and *Sinocrassula indica* (Crassulaceae) in East Himalaya. *J. Jap. Bot.* 74: 228–235.
YOSHIDA, T. (2006). Geobotany of the Himalaya I. *Newsl. Himalayan Bot.* 37: 1–24.

Received 25 November 2013; accepted for publication 4 June 2014