



Chromosome numbers of *Berberis* Sect. *Wallichianae* from Taiwan: a new basis for taxonomic and evolutionary implications

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ABSTRACT: As has long been known *Berberis* Sect. *Wallichianae* in Taiwan evidence a high species diversity all of which are endemic to the island. Though cytological data has been used to understand adaptive speciation for certain *Berberis* species elsewhere, little is still known about the chromosome numbers of Taiwanese *Berberis*. In this study, the chromosomes of eleven Taiwanese *Berberis* Sect. *Wallichianae* were counted for the first time and were all determined to be diploids ($2n = 28$). This result not only reconfirms previous studies elsewhere which have concluded that polyploidization may not be a dominant evolutionary process in *Berberis* but also suggests that geographic isolation or other extrinsic factors may play a more important role on the rapid speciation of *Berberis* in Taiwan.

KEY WORDS: *Berberis*, High mountain flora, Polyploidy, Rapid species diversification, Taiwan

INTRODUCTION

Berberis L. is the largest genus of Berberidaceae Jussieu, comprising some 400 unifoliolate-leaved species of shrubs and small trees (Ahrendt, 1961; Yu and Chung, 2017). Most species of the genus occur in North Temperate regions and in the South American Andean spine. Although the genus is one of the most diverse temperate woody taxa of the Northern Hemisphere (Frodin, 2004), little is known about what evolutionary mechanisms have triggered such high diversity.

Cytology is of importance for the systematics of Berberidaceae (Kim and Jansen, 1996; Wang *et al.*, 2007). In particular, chromosome number has been taken as a strong support for the establishment of the infrafamilial classification (Loconte, 1993; Wang *et al.*, 2007). With chromosome base number of $x = 7$, *Ranzania*, T. Ito, *Berberis* and *Mahonia* Nutt. comprise the largest subfamily of Berberidaceae, Berberidoideae Eaton (Loconte, 1993; Wang *et al.*, 2007). However, while some studies have further presumed *Berberis* and *Mahonia* have an ancient tetraploid origin, the two genera are usually described as base number of $x = 14$ (Sodagar *et al.*, 2012; Adhikari *et al.*, 2014). When compared with other genera of Berberidaceae, it is also noteworthy that only a few *Berberis* species have been investigated to establish chromosome numbers and other cytological data (Adhikari *et al.*, 2014; Rice *et al.*, 2015). Such limited cytological data may have implications for the validity of current taxonomy and its application in related disciplines such as pharmacy and horticulture.

Berberis Sect. *Wallichianae* C.K. Schneid. comprises some 114 species, representing one of the most diversified *Berberis* group endemic to East and

Tropical Asia. (Harber, per. com.; Chamberlain and Hu, 1985). Unlike the other *Berberis* in the Old World, the group is usually characterized by the evergreen habit, which can be also seen in the *Berberis* found in the South American Andes (Schneider, 1939, 1942; Ahrendt, 1941, 1961; Chamberlain and Hu, 1985; Harber, 2012). Because of its high diversity and endemism, the section has earned itself an ideal study group for understanding the speciation of *Berberis*. In Taiwan, *B.* Sect. *Wallichianae* occurs at mid to high elevations (Fig. 1) and has long perplexed botanists by its variable morphology as well as its convoluted taxonomic history (Yu and Chung, 2014). While the most recent revision recognizes 11 endemic species from Taiwan, the island has not only become an important center of diversity of the section but also is one of few geographic regions that is host to wholly endemic species across the section's natural range (Harber, per. com.; Yu and Chung, 2014). Interestingly Taiwanese *B.* Sect. *Wallichianae* can be divided into two clades each corresponding to different sepal morphology, but which together formed a monophyletic group together with *B. barandana* S. Vidal endemic to the Philippines. This group is further connected to all sampled Chinese *B.* Sect. *Wallichianae* taxa by a relatively long branch whereas all terminal branches within it are short, suggesting a rapid speciation event may have occurred (Yu and Chung, 2014).

The rapid bursts of *Berberis* species in Taiwan could have been triggered and maintained by extrinsic factors (e.g., climatic and geological history or colonization of new ecozone; Vamosi and Vamosi, 2011) or by intrinsic factors, such as genetic architecture rearrangement and changes of ploidy levels (Schluter, 2000; Glor, 2010) or

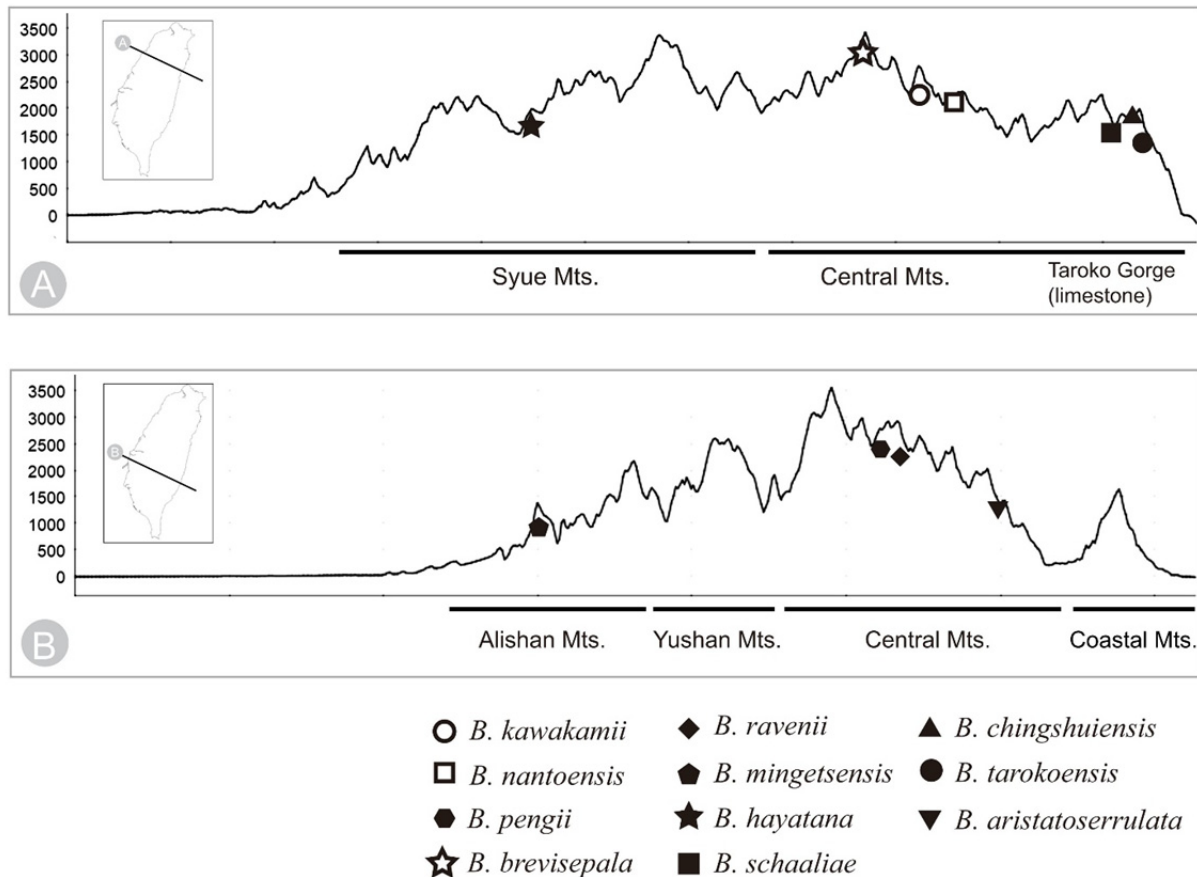


Fig. 1. Elevational distribution of samples of the 11 *Berberis* Sect. *Wallichianae* species of Taiwan used in present study. **A.** Showing the locations of species which occur in the northern part of Taiwan while **B.** showing species found in the southern part of Taiwan. Each species is denoted by different kinds of polygons.

by the interplay of both factors. However, as suggested by previous studies, polyploidization, which impedes mating or fertilization between species over a short time span, is one of the major driving force for rapid plant diversification (Aversano *et al.*, 2012; Madlung, 2013). Polyploids are also well documented to have the potential to colonize new habitats that pose challenges to their diploid parents (e.g., high-altitude environments) (Madlung, 2013; Mas de Xaxars *et al.*, 2016). For *Berberis* Sect. *Wallichianae*, its evergreen counterparts in South America have been demonstrated to show a correlation between habitat types and ploidy/ genome size, implying the cytological variation may be related to the speciation of *Berberis* (Bottini *et al.*, 1999, 2000). In contrast, based on CCDB v.1.45 (Chromosome counts database version, <http://ccdb.tau.ac.il/>), hitherto information has been available for only ten *B.* Sect. *Wallichianae* species and they are all diploids. This uniformity in chromosome number suggests that polyploidy may not have been a major driver of speciation for the group. Concerning about the difference shown here, this study, therefore, aims to further improve our understanding of the role that

ploidy level may play in the evolution of *Berberis* by examining the chromosome numbers of the rapid diversified Sect. *Wallichianae* lineages of Taiwan.

MATERIALS AND METHODS

Of eleven Taiwanese *Berberis* Sect. *Wallichianae* species, the chromosome numbers were determined from root tips of greenhouse accessions collected from wild (Table 1). The roots were pretreated in 2 mM 8-hydroxyquinolene for 5 hours at 18°C, fixed in absolute ethanol and glacial acetic acid mixture (3:1) at room temperature for 16 hours, and then were transferred to 1 N HCl for 8 minutes at 60°C. The chromosomes were stained with leuco-basic fuchsin solution for 1 hour in the dark and softened in 1% pectinase for 1 hour at room temperature. The root tip meristem was squashed in a few drops of 45% acetic acid. Chromosomes were observed with a Zeiss AxioImager microscope and counted for at least three cells from two root tips to determine the chromosome numbers.

**Table 1.** Chromosome numbers and voucher information of eleven endemic *Berberis* Sect. *Wallichianae* of Taiwan.

Taxon	Voucher information	Collection details	2n	Fig.
<i>Berberis aristatoserrulata</i>	Y.-L.Huang015 (HAST)	Hualien: Patuolushan	28	2A
<i>Berberis brevisepala</i>	Y.-L.Huang006 (HAST)	Hualien: Luentaiwenshan	28	2B
<i>Berberis chingshuiensis</i>	Y.-L.Huang142 (HAST)	Hualien: Chingshuishan	28	2C
<i>Berberis hayatana</i>	Y.-L.Huang021 (HAST)	Yilan: Jancing Historic Trail	28	2D
<i>Berberis kawakamii</i>	Y.-L.Huang001 (HAST)	Nantou: Batongguan Historic Trail	28	3A
<i>Berberis mingetsensis</i>	Y.-L.Huang004 (HAST)	Chiayi: Mienyueh Spur Line	28	3B
<i>Berberis nantoensis</i>	Y.-L.Huang003 (HAST)	Hualien: Little Qilai Trail	28	3C
<i>Berberis pengii</i>	Y.-L.Huang013 (HAST)	Pingtung: Mt. Peitawushan, Kuaikushanchuan	28	3D
<i>Berberis ravenii</i>	Y.-L.Huang010 (HAST)	Pingtung: Mt. Peitawushan, Kuaikushanchuang	28	4A
<i>Berberis schaaliae</i>	Y.-L.Huang117 (HAST)	Hualien: Heping Logging Road	28	4B
<i>Berberis tarokoensis</i>	Y.-L.Huang007 (HAST)	Hualien: Yanhai Logging Road	28	4C

RESULTS AND DISCUSSION

Chromosome numbers of all *Berberis* Sect. *Wallichianae* of Taiwan are $2n = 28$ (Figs. 2, 3, 4; Table 1). This is the first report of the chromosome numbers of *Berberis* in Taiwan.

1. *Berberis aristatoserrulata* Hayata

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage (Fig. 2A), and most chromosomes appear metacentric. Large satellites are prominent as shown by arrows.

2. *Berberis brevisepala* Hayata

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage (Fig. 2B). The chromosomes were mostly metacentric with large satellites as shown by arrows.

3. *Berberis chingshuiensis* T. Shimizu

The somatic chromosome number counted was $2n = 28$ in the prometaphasic stage (Fig. 2C). Satellites were prominent as indicated by arrows.

4. *Berberis hayatana* Mizush.

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage (Fig. 2D). The chromosomes were mostly metacentric with large satellites as shown by arrows.

5. *Berberis kawakamii* Hayata

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stages (Fig. 3A), and the chromosomes appear metacentric.

6. *Berberis mingetsensis* Hayata

The somatic chromosome number counted was $2n = 28$ in the metaphasic stage (Fig. 3B).

7. *Berberis nantoensis* C.K. Schneid.

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage with satellites as shown by arrows (3C).

8. *Berberis pengii* C.C. Yu & K.F. Chung

The somatic chromosome number counted was $2n = 28$ in the metaphasic stage (Fig. 3D) and most chromosomes appear metacentric.

9. *Berberis ravenii* C.C. Yu & K.F. Chung

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage and

satellites are shown by arrows (4A).

10. *Berberis schaaliae* C.C. Yu & K.F. Chung

A somatic chromosome number of $2n = 28$ was counted for the species in the metaphasic stage (Fig. 4B). The chromosomes were mostly metacentric with satellites as shown by arrows.

11. *Berberis tarokoensis* S.Y. Lu & Y.P. Yang

The somatic chromosome number counted was of $2n = 28$ for the species in the metaphasic stage (Fig. 4C). The chromosomes were mostly metacentric with satellites as shown by arrows.

Our results show that all *Berberis* sect. *Wallichianae* species of Taiwan are diploids with $2n = 28$, supporting the viewpoint of Adhikari *et al.* (2014) that polyploidization may not be a common phenomenon in *Berberis*.

Although polyploidization has been assumed to be common in high-mountain flora (Petit and Thompson, 1999; Rieseberg and Willis, 2007; Pachschröll *et al.*, 2015), it is not evidenced by present case of *Berberis* Sect. *Wallichianae* in Taiwan. In fact, there are only a few cases demonstrating the relationship between polyploids and high-mountain plant radiation (Madlung, 2013; Mas de Xaxars *et al.*, 2016). Specifically, Brochmann *et al.* (2004) and te Beest *et al.* (2012) have argued that although polyploid may have the ability to colonize new habitats or harsh environments, it is seldom to find its relevance to the cold-hardiness or to the adaptation to high-altitude environment. Despite inhabiting different elevations (Fig. 1), there is no relationship between habitat types and cytological data in Taiwanese *B.* Sect. *Wallichianae*, and this stands in contrast to the finding of Bottini *et al.* (1999, 2000) for South American evergreen *Berberis*. However, as the chromosome database of the section is still relatively poor (less than 20% of estimated diversity), it still needs further investigation to determine the role that polyploidization may play in the diversification of *B.* Sect. *Wallichianae*.

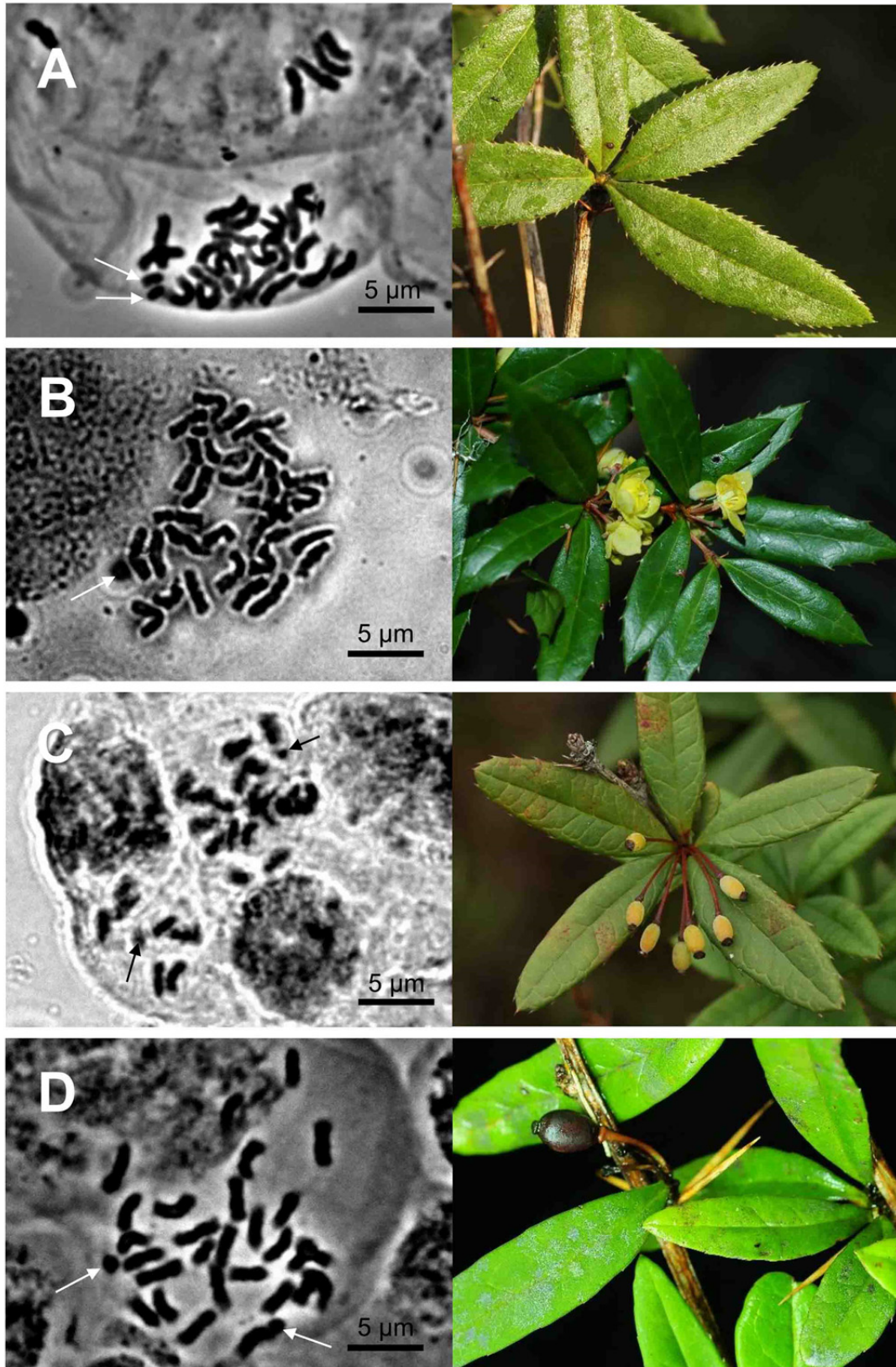


Fig. 2. Chromosomes of *Berberis* Sect. *Wallichianae* of Taiwan with arrows indicating satellites (left side). The gross morphology of *Berberis* Sect. *Wallichianae* of Taiwan (right side). **A:** *B. aristatoserrulata* Hayata. **B:** *B. brevisepala* Hayata. **C:** *B. chingshuiensis* T. Shimizu. **D:** *B. hayatana* Mizush.

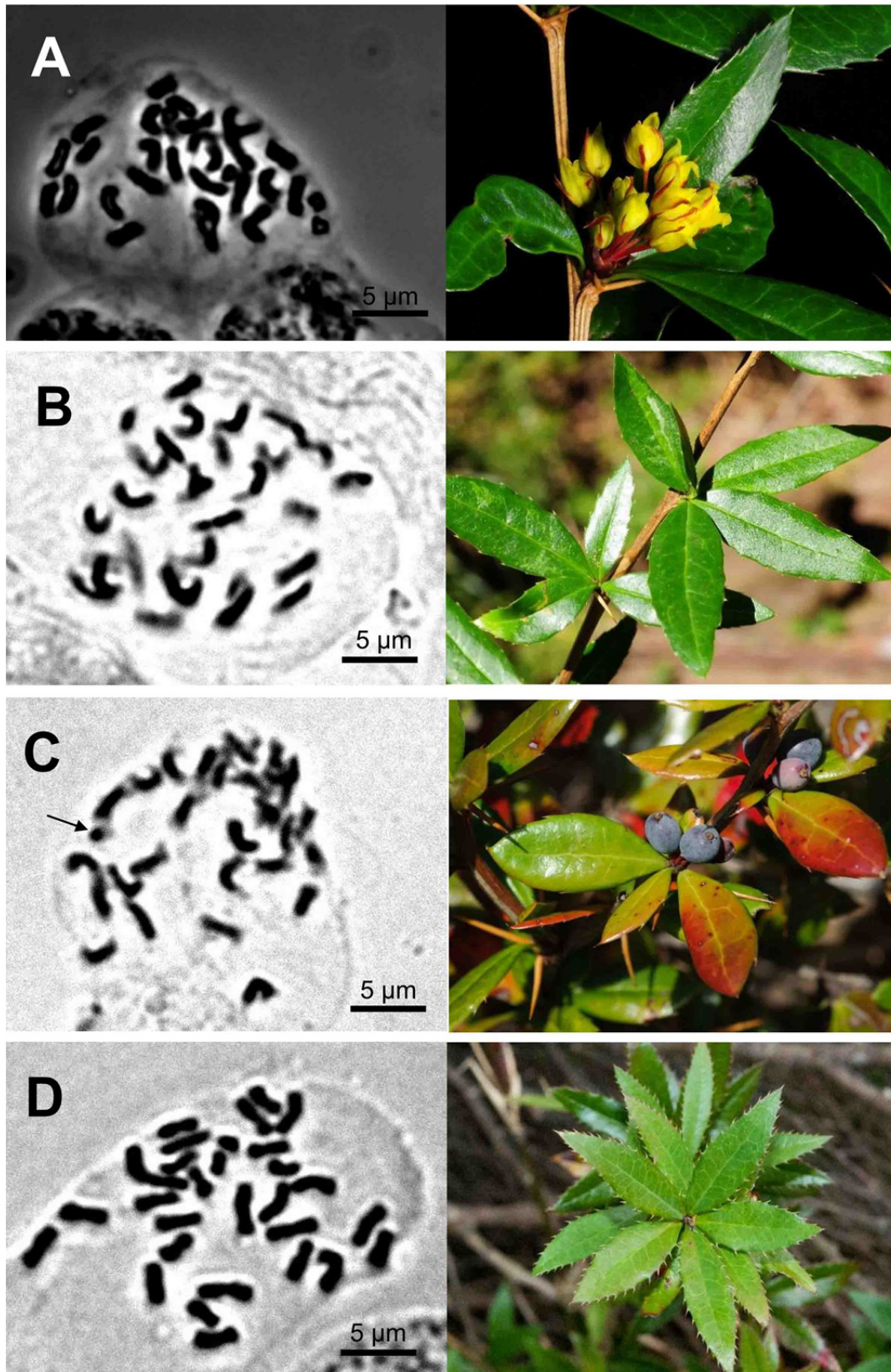


Fig. 3. Chromosomes of *Berberis* Sect. *Wallichianae* of Taiwan with arrows indicating satellites (left side). The gross morphology of *Berberis* Sect. *Wallichianae* of Taiwan (right side). **A:** *B. kawakamii* Hayata. **B:** *B. mingetsensis* Hayata. **C:** *B. nantoensis* C.K. Schneid. **D:** *B. pengii* C.C. Yu & K.F. Chung.

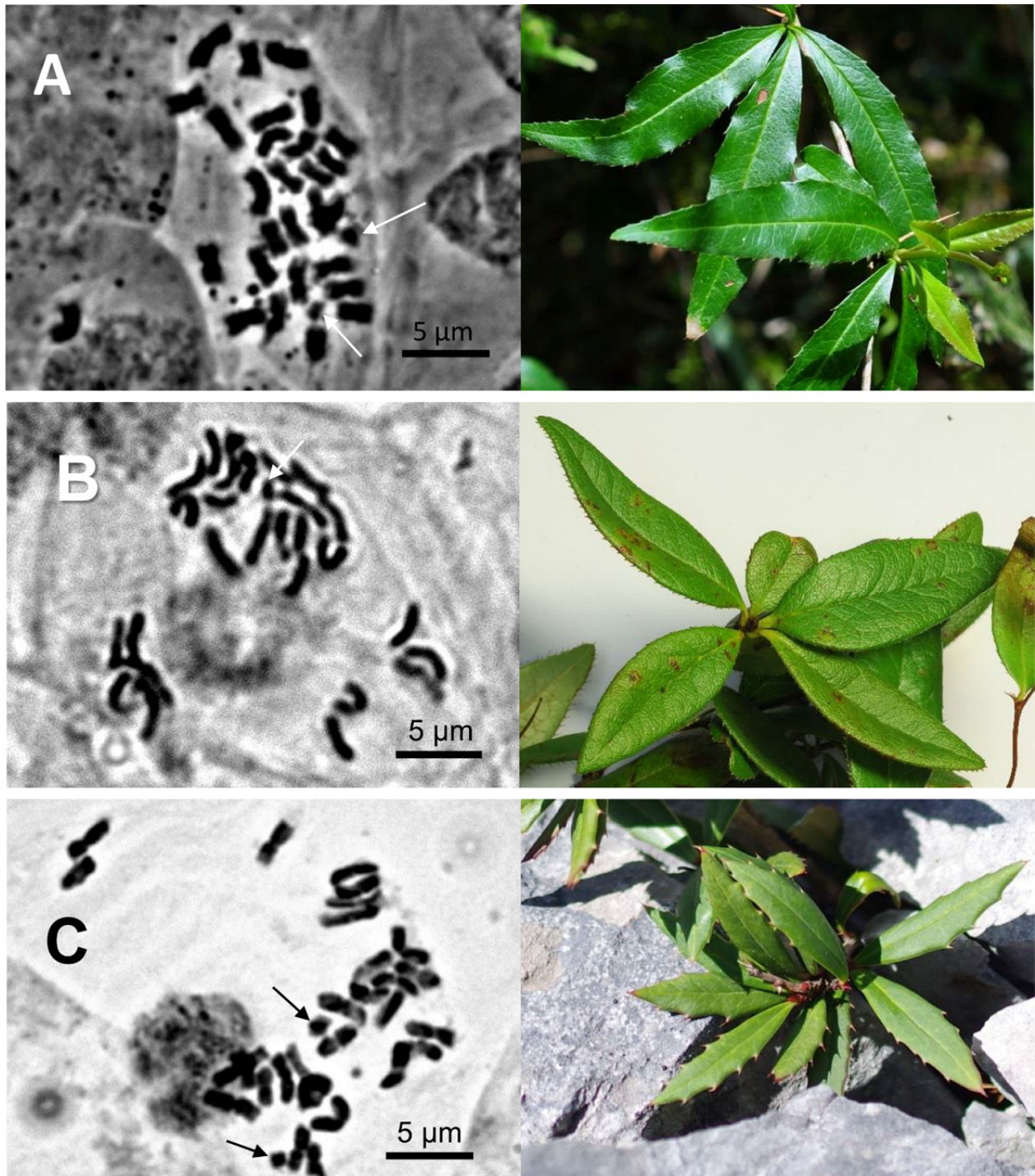


Fig. 4. Chromosomes of *Berberis* Sect. *Wallichianae* of Taiwan with arrows indicating satellites (left side). The gross morphology of *Berberis* Sect. *Wallichianae* of Taiwan (right side). **A:** *B. ravenii* C.C. Yu & K.F. Chung. **B:** *B. schaaliae* C.C. Yu & K.F. Chung. **C:** *B. tarokoensis* S.Y. Lu & Y.P. Yang.

In addition to *Berberis*, *Euphrasia* Linnaeus and *Gentiana* Linnaeus are the other two prominent genera that are thought to have produced rapid species diversification in the high mountains of Taiwan (Chen, 2001; Wu *et al.*, 2005; Favre *et al.*, 2016). Again, there is no polyploidization found on either genera. Wu

(2009) showed that all endemic *Euphrasia* species of Taiwan are diploids and Wang *et al.* (2009) has found that dysploidy is more common in Taiwanese *Gentiana* than polyploidy. While most species of the three genera have rather restricted distributions, we hence hypothesize that, instead of polyploidy, plant



diversification in the high mountain systems of Taiwan may be influenced by certain extrinsic factors rather than intrinsic process (Rieseberg and Willis, 2007; Lowry *et al.*, 2008).

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