

## Cytotaxonomy of *Begonia rubropunctata* and *B. purpureofolia* (Begoniaceae)

MASASHI NAKATA<sup>1\*</sup>, KAIYUN GUAN<sup>2</sup>, JINGXIU LI<sup>2</sup>, YUANXUE LU<sup>2</sup> and HONGZHE LI<sup>2</sup>

<sup>1</sup>Botanic Gardens of Toyama, 42 Kamikutsuwada, Fuchu-machi, Toyama 939-2713, Japan

<sup>2</sup>Kunming Botanical Garden, Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan 650204, China

Received January 2007; accepted for publication August 2007

The chromosome numbers of four Chinese *Begonia* species were counted for the first time as follows:  $2n = 22$  in *B. rubropunctata*,  $2n = 18$  in *B. purpureofolia*,  $2n = 44$  in *B. pedatifida*, and  $2n = 22$  in *B. villifolia*. The first two species had relatively large chromosomes with early condensed chromatin in the distal and interstitial or proximal regions, whereas the last two had small chromosomes with early condensed chromatin in the proximal regions. Although they have been reduced in the literature to synonyms of *B. pedatifida* and *B. villifolia*, respectively, *B. rubropunctata* and *B. purpureofolia* should be treated as distinct species because of the differences in chromosome numbers and other chromosome features. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, **155**, 513–517.

**ADDITIONAL KEYWORDS:** Chinese flora – chromosome numbers – early condensed chromatin – interphase nucleus – Yunnan.

### INTRODUCTION

*Begonia* L. (Begoniaceae) is a pantropical genus with a few species extending to warm temperate zones. It contains about 1400 species classified into 63 sections (Doorenbos, Sosef & de Wilde, 1998). More than 130 species occur in China, mostly south of the Yangtze Valley, especially in south-eastern Yunnan and south-western Guangxi Provinces (Ku, 1999). The cytological knowledge on Chinese *Begonia* is very limited, except for Taiwanese taxa, all of which have been studied in detail (Peng, Chen & Yen, 1988; Peng & Chen, 1991; Peng & Sue, 2000; Oginuma & Peng, 2002). Recently, chromosome numbers of *Begonia* spp. have been reported, mainly of species from Yunnan Province, China, which were cultivated in Kunming Botanical Garden (Tian *et al.*, 2002; Nakata *et al.*, 2003; Li, Guan & Ma, 2005). However, further cytological studies on *Begonia* spp. from the Chinese

mainland are still desirable to aid in the understanding of their taxonomic relationships.

*Begonia rubropunctata* S.H.Huang et Y.M.Shui (Fig. 1) and *B. purpureofolia* S.H.Huang et Y.M.Shui (Fig. 2) have been described by Huang & Shui (1994) based on specimens collected in southern Yunnan Province, China. However, Ku (1999) treated them as synonyms of *B. pedatifida* Lévl. (Fig. 3) and *B. villifolia* Irmsch. (Fig. 4), respectively. As part of our cytological studies on Chinese *Begonia*, Section *Platycentrum*, we have observed the chromosomes of the four species; our findings, which are discussed taxonomically, are reported here.

### MATERIAL AND METHODS

The plants used in this study were cultivated in the conservation glasshouse of Kunming Botanical Garden. Voucher specimens prepared from living collections are preserved at the Kunming Institute of Botany (KUN), and duplicates have been placed at the Botanic Gardens of Toyama (TYM).

\*Corresponding author. E-mail: nakata@bgtym.org



**Figures 1–4.** Four studied *Begonia* species in Kunming Botanical Garden. Fig. 1. *B. rubropunctata*. Fig. 2. *B. purpureo-folia*. Fig. 3. *B. pedatifida*. Fig. 4. *B. villifolia*. Scales in the pots indicate 1 cm.

Chromosome observations were made from root tip meristems. Fresh root tips, 5 mm in length, were pretreated with 2 mM 8-hydroxyquinoline aqueous solution at 12–14 °C for 8 h and fixed with Farmer's fixative (99.5% ethanol–glacial acetic acid, 3 : 1) at 5 °C for a minimum of 20 h. The fixed root tips were macerated in 1 M hydrochloric acid at 60 °C for 5 min, immediately rinsed with water at room temperature for 2–5 min, and stained with 1% acetic orcein (synthetic; Tokyo Kasei Kogyo, Co., Tokyo, Japan) at room temperature (20–25 °C) for 15–20 h. Squashes were made, and chromosomes at interphase, prophase, prometaphase, and metaphase were observed and photographed using an Olympus microscope.

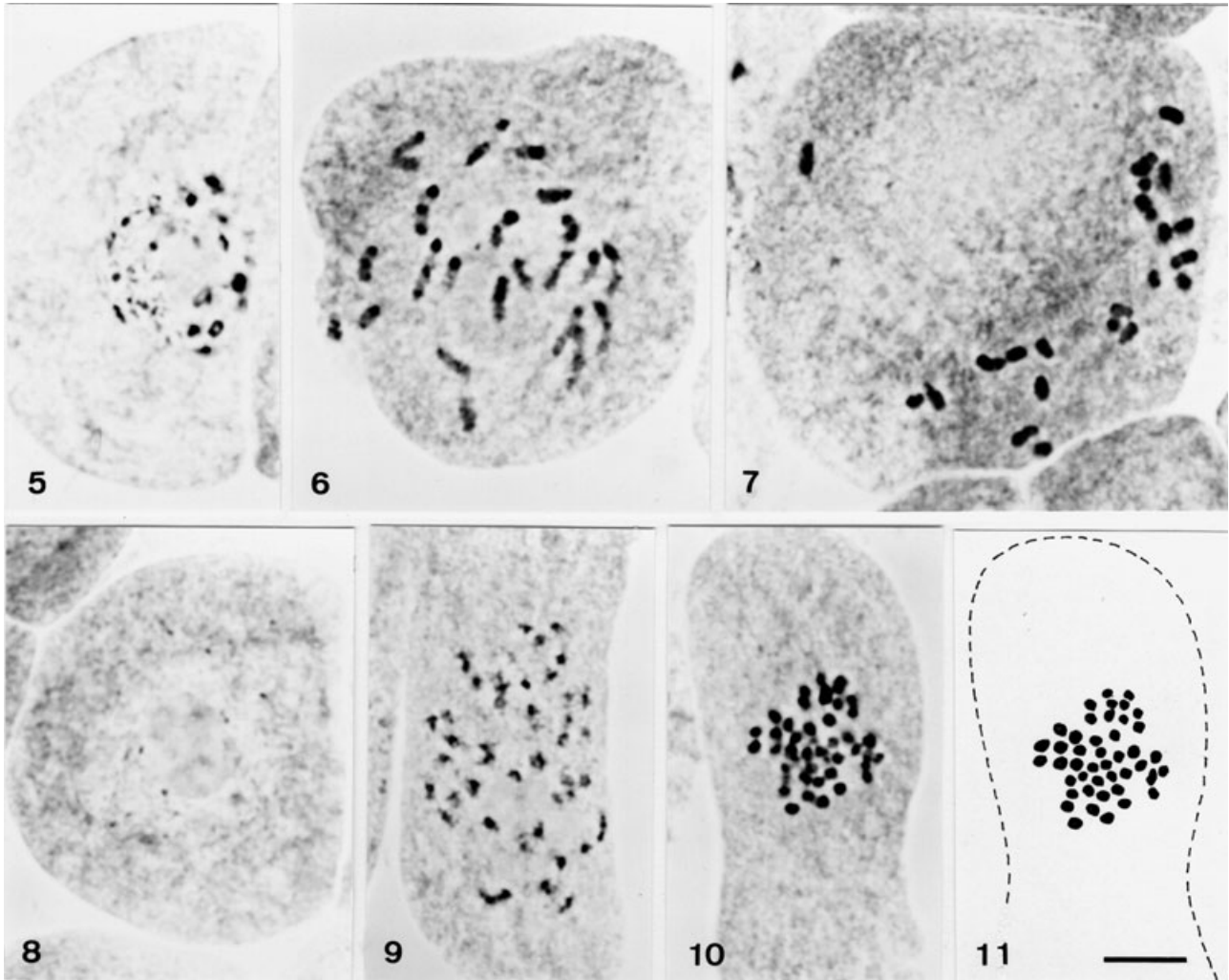
The description of chromosome morphology followed Tanaka (1971, 1977).

## RESULTS AND DISCUSSION

### *BEGONIA RUBROPUNCTATA*

Three plants collected at the type locality, Xishuangbanna, Yunnan Province, were observed (Vouchers: *Guan & Nakata* 200204-1, 200204-2, *Lu & Nakata* 27034, 27037).

The interphase nucleus had round- or rod-shaped, large chromocentres, less than 1 µm in length (Fig. 5), and was categorized as the complex chromocentre



**Figures 5–11.** Mitotic chromosomes of *Begonia*. Figs 5–7. *B. rubropunctata*. Fig. 5. Interphase nucleus. Fig. 6. Prometaphase chromosomes ( $2n = 22$ ). Fig. 7. Metaphase chromosomes ( $2n = 22$ ). Figs 8–11. *B. pedatifida*. Fig. 8. Interphase nucleus. Fig. 9. Prometaphase chromosomes. Fig. 10. Metaphase chromosomes ( $2n = 44$ ). Fig. 11. Explanatory drawing of Fig. 10 showing  $2n = 44$  chromosomes. Scale bar, 5  $\mu\text{m}$ .

type (Tanaka, 1971, 1977). The chromosome number of  $2n = 22$  was counted at prometaphase (Fig. 6) from 11 cells and at metaphase (Fig. 7) from eight cells. At prophase and prometaphase, early condensed chromosome segments were observed in the distal regions and also in the interstitial or proximal regions of many chromosomes (Fig. 6). The chromosomes at metaphase were relatively large amongst the four species studied, and were 1–2  $\mu\text{m}$  in length (Fig. 7).

#### *BEGONIA PEDATIFIDA*

A plant collected in 1998 in Sichuan Province was observed (Vouchers: Guan & Nakata 200203-1, 200203-2).

The interphase nucleus had small chromocentres, less than 0.5  $\mu\text{m}$  in length (Fig. 8), and was catego-

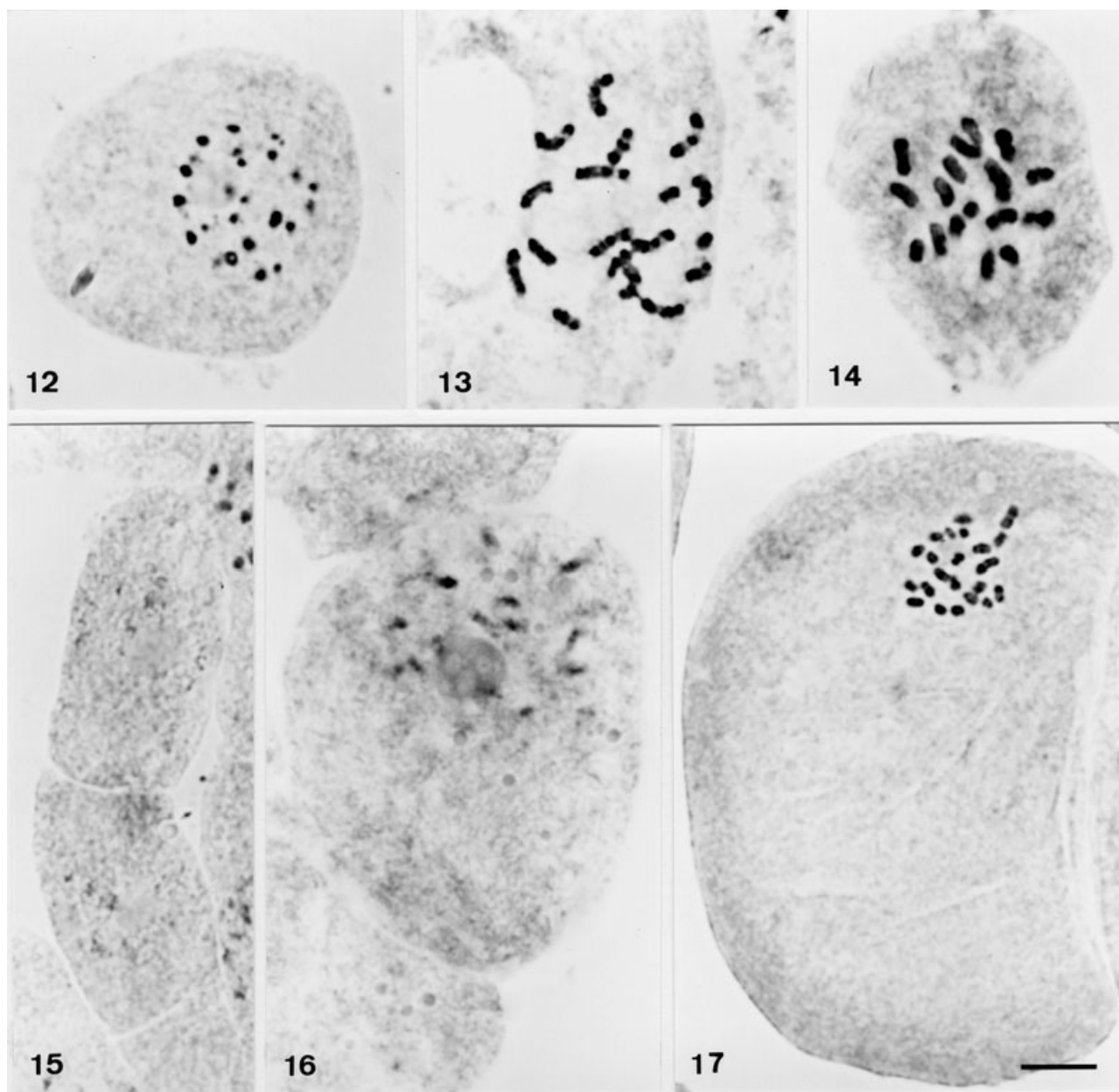
rized as the simple chromocentre type (Tanaka, 1971, 1977). At prophase and prometaphase, early condensed chromosome segments were observed in the proximal regions of the chromosomes (Fig. 9). The chromosome number  $2n = 44$  was counted at metaphase (Figs 10, 11) from 13 cells. Because the chromosome number of  $2n = 22$  is the most frequent in Chinese *Begonia* (Nakata *et al.*, 2003), *B. pedatifida* is presumed to be a tetraploid. The chromosomes at metaphase were small, less than 1  $\mu\text{m}$  in length.

#### *BEGONIA PURPUREOFOLIA*

A plant collected in 1997 in Mengzi Xian, Yunnan Province (Vouchers: Guan & Nakata 200202-1, 200202-2) was observed.

The interphase nucleus had round-shaped, large chromocentres, 0.5–1  $\mu\text{m}$  in length (Fig. 12), and





**Figures 12–17.** Mitotic chromosomes of *Begonia*. Figs 12–14. *B. purpureofolia*. Fig. 12. Interphase nucleus. Fig. 13. Prometaphase chromosomes ( $2n = 18$ ). Fig. 14. Metaphase chromosomes ( $2n = 18$ ). Figs 15–17. *B. villifolia*. Fig. 15. Interphase nuclei. Fig. 16. Prophase chromosomes. Fig. 17. Metaphase chromosomes ( $2n = 22$ ). Scale bar, 5  $\mu\text{m}$ .

was categorized as the complex chromocentre type (Tanaka, 1971, 1977). The chromosome number  $2n = 18$  was counted at prometaphase (Fig. 13) from three cells and at metaphase (Fig. 14) from five cells. At prophase and prometaphase, early condensed chromosome segments were observed in the distal regions and also in the interstitial or proximal regions of many chromosomes (Fig. 13). The chromosomes at metaphase were the largest amongst the four species

studied, and were 1–3  $\mu\text{m}$  in length. Thus, the chromosome morphology of this species is similar to that of *B. rubropunctata*, although the chromosome number is different.

#### *BEGONIA VILLIFOLIA*

A plant collected in 1996 in Malipo Xian, Yunnan Province and one collected in 1997 at Mt. Laojun,

Maguan, Yunnan Province (Vouchers: *Guan and Nakata* 200201-1, 200201-2) were observed.

The interphase nucleus had small chromocentres, less than 0.5 µm in length (Fig. 15), and was categorized as the simple chromocentre type (Tanaka, 1971, 1977). At prophase and prometaphase, early condensed chromosome segments were observed in the proximal regions of the chromosomes (Fig. 16). In both plants, the chromosome number  $2n = 22$  was counted at metaphase (Fig. 17). The chromosomes at metaphase were small, less than 1 µm in length. *Begonia villifolia* is similar to *B. pedatifida* in chromosome morphology, although their chromosome numbers are quite different, *B. villifolia* being diploid and *B. pedatifida* tetraploid.

### CYTOTAXONOMIC REMARKS

Huang & Shui (1994) described *B. rubropunctata* as distinguishable from *B. pedatifida* by its nearly glabrous leaves and stems spotted with reddish-purple. However, Ku (1999) considered that *B. rubropunctata* was conspecific with *B. pedatifida*. We have shown that *B. rubropunctata* ( $2n = 22$ ) is quite different from *B. pedatifida* ( $2n = 44$ ), not only in chromosome number but also in other chromosome features described above. *Begonia rubropunctata* should therefore be treated as a separate species, at least from the cytotaxonomic viewpoint.

*Begonia purpureofolia* was described by Huang & Shui (1994) as being similar to *B. villifolia*, but differing in its smaller flowers, reddish anthers, purple-coloured abaxial leaf surface, etc. Ku (1999) simply reduced *B. purpureofolia* to synonymy under *B. villifolia*. *Begonia purpureofolia* ( $2n = 18$ ) is quite different from *B. pedatifida* ( $2n = 22$ ), not only in chromosome number but also in other chromosome features, and so should be treated as a distinct species.

As the chromosomes of *Begonia* are small in size and difficult to stain by conventional methods (cf. Legro & Doorenbos, 1969; Sarkar, 1989), cytological knowledge of Chinese *Begonia* is very limited. By using improved methods, however, significant reports of chromosomes have been presented for Chinese taxa of *Begonia* in recent years (e.g. Oginuma & Peng, 2002; Tian *et al.*, 2002; Nakata *et al.*, 2003; Li *et al.*, 2005). Thus, chromosome cytology is now contributing strongly to the clarification of taxonomic relationships amongst the Chinese taxa of this genus.

### ACKNOWLEDGEMENTS

We thank Dr P. E. Brandham, Royal Botanic Gardens, Kew, for critical revision of the manuscript, and

Dr S. Kurokawa, former director of the Botanic Gardens of Toyama, for helpful comments on an earlier version of the manuscript. We also thank Mr B. Wen, Xishuangbanna Botanic Garden, for his help in collecting material. This study was partly supported financially by the Local Authorities International Cooperation Promotion Project ('Model Project'), the Council of Local Authorities for International Relations (CLAIR) of Japan, and the Science Foundation of Yunnan Province, No. 2001 (0060M).

### REFERENCES

- Doorenbos J, Sosef MSM, de Wilde JJFE. 1998. The sections of *Begonia*, including descriptions, keys and species lists. (Studies in Begoniaceae VI.) *Wageningen Agricultural University Papers* **98**: 1–266.
- Huang SH, Shui YM. 1994. New taxa of *Begonia* from Yunnan. *Acta Botanica Yunnanica* **16**: 333–342 (in Chinese with Latin description).
- Ku TC. 1999. Begoniaceae. In: Ku TC, ed. *Flora Reipublicae Popularis Sinicae Tomus*, Vol. 52. Beijing: Science Press, 126–269 (in Chinese).
- Legro RAH, Doorenbos J. 1969. Chromosome numbers in *Begonia*. *Netherlands Journal of Agricultural Science* **17**: 189–202.
- Li HZ, Guan KY, Ma H. 2005. Chromosome numbers of five species of *Begonia* from China. *Acta Botanica Yunnanica* **27**: 92–94 (in Chinese with English abstract).
- Nakata M, Guan KY, Godo T, Lu YX, Li JX. 2003. Cytological studies on Chinese *Begonia* (Begoniaceae) I. Chromosome numbers of 17 taxa of *Begonia* collected in 2001 field studies in Yunnan. *Bulletin of the Botanic Gardens of Toyama* **8**: 1–16.
- Oginuma K, Peng CI. 2002. Karyomorphology of Taiwanese *Begonia* (Begoniaceae): taxonomic implications. *Journal of Plant Research* **115**: 225–235.
- Peng CI, Chen YK. 1991. Hybridity and parentage of *Begonia buimontana* (Begoniaceae) from Taiwan. *Annals of the Missouri Botanical Garden* **78**: 995–1001.
- Peng CI, Chen YK, Yen HF. 1988. *Begonia ravenii* (Begoniaceae), a new species from Taiwan. *Botanical Bulletin of the Academia Sinica* **29**: 217–222.
- Peng CI, Sue CY. 2000. *Begonia* × *taipeiensis* (Begoniaceae), a new natural hybrid in Taiwan. *Botanical Bulletin of the Academia Sinica* **41**: 151–158.
- Sarkar AK. 1989. Taxonomy of *Begonia* L. (Begoniaceae) as judged through cytology. *Feddes Repertorium* **100**: 241–250.
- Tanaka R. 1971. Types of resting nuclei in Orchidaceae. *Botanical Magazine, Tokyo* **84**: 118–122.
- Tanaka R. 1977. Recent karyotype studies. In: Ogawa K, Kurozumi K, Koike S, Sato S, eds. *Plant cytology (Shokubutsu Saibougaku)*. Tokyo: Asakurashoten, 293–326 (in Japanese).
- Tian DK, Guan KY, Zhou QX, Gu ZJ. 2002. Chromosome numbers of eight taxa of *Begonia* from Yunnan. *Acta Botanica Yunnanica* **24**: 245–249 (in Chinese with English abstract).