Cytological Study of *Begonia* Sect. *Baryandra* (Begoniaceae)

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Summary *Begonia* sect. *Baryandra* is distributed mainly in the Philippines with a few species in Borneo and New Guinea. Previous chromosome information of sect. *Baryandra* has restricted to *ca.* 16 species and their karyotypes have never been investigated. Here we report the cytological investigations of 26 species of sect. *Baryandra*, including five karyotype analyses that are the first report in sect. *Baryandra*, 24 species were reported cytologically for the first time, and two species were re-examined. Incorporating previous and present studies, chromosome numbers of 40 of the 78 species in sect. *Baryandra* are now available. Except for some intraspecific variant counts of $2n=26$ and 44, and a tetraploid number of $2n=56$, 39 species have the chromosome number of either $2n=28$ or 30. The karyotypic formula of the three investigated species is uniform as $2n=28=10m+18sm(st)$, and two as $2n=30=8m+22sm(st)$. Based on molecular phylogenetic relationships, ancestral state reconstruction suggests that $2n=30$ is the most likely ancestral chromosome number of sect. *Baryandra*, with $2n=28$ a derived number. Based on our and previous studies, the chromosome evolution in *Begonia* sect. *Baryandra* is discussed.

Keywords Chromosome number, Karyotype, *Begonia* sect. *Baryandra*.

Begonia L. is the fourth largest genus of angiosperms of *ca.* 2,000 described species pan-tropically distributed in Africa, America, and Asia (Hughes *et al.* 2015 onward: <https://padme.rbge.org.uk/Begonia/home>, Moonlight *et al.* 2018). Based on phylogenetic analyses of extensive taxon sampling, Moonlight *et al.* (2018) revised Doorenbos *et al.* (1998)'s classic work and proposed a new infrageneric classification of 70 sections. Subsequently, section *Flocciferae* N.Krishna & Pradeep was published (Krishna *et al.* 2020), bringing the total number of sections in *Begonia* to 71. Asia harbors the highest species diversity of *Begonia*, with more than 1,000 species placed in 20 sections (Hughes *et al.* 2015 onward: <https://padme.rbge.org.uk/Begonia/home>, Moonlight *et al.* 2018). Among the 20 Asian *Begonia* sections, the circumscription of sect. *Baryandra* A.DC. has undergone the most changes. Originally, *Begonia* sect. *Baryandra* was a monotypic section with only *B. oxyperma* A.DC. (Doorenbos *et al.* 1998). However, molecular phylogenetic analyses showed that *B. oxyperma* was nested within a strongly supported clade comprising

Philippine and Bornean species previously in sect. *Diploclinium* (Lindl.) A.DC. (Hughes 2008, Hughes *et al.* 2011, Rubite 2012, Rubite *et al.* 2013). Because species in this clade are phylogenetically and morphologically coherent, sect. *Baryandra* was expanded to include all known 48 Philippine and Bornean species of sect. *Diploclinium* (Rubite *et al.* 2013). Subsequent botanical explorations and taxonomic studies revealed additional diversity of sect. *Baryandra* across the Philippines (e.g., Rubite *et al.* 2014, 2015, 2018, 2020, Tandang *et al.* 2016, Peng *et al.* 2017, Hughes *et al.* 2018, Magtoto *et al.* 2018, Calaramo *et al.* 2019, Pranada *et al.* 2019, Ang *et al.* 2020, Bustamante *et al.* 2020, Rule *et al.* 2020), drastically increasing the section to 78 species up to date (Hughes *et al.* 2015 onward: <https://padme.rbge.org.uk/Begonia/home>).

Although chromosome cytology has only been investigated in 16 species of *Begonia* sect. *Baryandra*, a great variation in chromosome numbers of $2n=26$, 28, 30, 32, 44 and 56 has been reported (Legro and Doorenbos 1969, 1971, Doorenbos *et al.* 1998, Kokubugata and Madulid 2000, Oginuma and Peng 2002, Hughes *et al.* 2011, Nakamura *et al.* 2013, Rubite *et al.* 2014, 2015, Peng *et al.* 2015a, 2017), suggesting that chromosomal changes could have been the major driving force for the

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generation of high species diversity in the section. However, because their chromosomes are small and generally short ($< 2\ \mu\text{m}$ long), karyotypes of sect. *Baryandra* have never been reported.

In this paper, we report the first karyotypes of *Begonia* sect. *Baryandra* in five species. We also report the first chromosome counts for 24 species and new chromosome counts of two species reported earlier. Based on the present results and previous chromosome reports for a total of 40 species, the chromosome evolution in *Begonia* sect. *Baryandra* is also discussed.

Materials and methods

The species/accessions of *Begonia* sect. *Baryandra* investigated along with their voucher information are listed in Table 1. The sectional classification of *Begonia* followed Rubite *et al.* (2013) and Moonlight *et al.* (2018). Somatic chromosomes were examined using root tips. Methods of pretreatment, fixation, maceration, and staining for chromosome observations were described elsewhere (Kono *et al.* 2020). Categories of chromosome morphology on the basis of the position of centromeres followed Levan *et al.* (1964).

We performed ancestral state reconstruction to infer the ancestral chromosome number of *Begonia* sect. *Baryandra*. First, the phylogenetic tree was reconstruct-

ed based on four chloroplast noncoding regions (*ndhA* intron, *ndhF-rpl32* spacer, *rpl32-trnL* spacer, and *trnC-trnD* region) from Hughes *et al.* (2015), including 50 species (70 samples) of sect. *Baryandra* and five species as outgroups to represent other Asian *Begonia* sections (*Coelocentrum*, *Flocciferae*, *Haagea*, and *Jackia*). All sequences were downloaded from NCBI. Each dataset was aligned using MAFFT v7.45 (Katoh and Standley 2013), respectively, and four aligned matrices were concatenated using AMAS (Borowiec 2016) for phylogenetic analyses. The maximum likelihood (ML) phylogenetic tree was reconstructed using RAxML v8.2.4 (Stamatakis 2014) based on the GTR+GAMMA model with 100 searches for the best tree and 1,000 bootstrap replicates to assess clade support.

Chromosome numbers were scored based on the references and results of the current study as showed in Table 2. Ancestral character states were reconstructed using maximum parsimony (MP) and ML methods implemented in Mesquite v3.5 (Maddison and Maddison 2010: <http://mesquiteproject.org>). In the MP reconstruction, any state change of character was assumed unordered. For the ML analysis, the Mk1 model (Markov k-state 1 parameter model) (Lewis 2001) was selected with equal probability for any character state change. Ancestral character reconstructions were mapped on the ML tree.

Table 1. Studied taxa and their collection data in *Begonia* sect. *Baryandra*.

Taxon	Collection ¹⁾	Chromosome number (2n)
<i>Begonia acuminatissima</i> Merr.	Philippines, Rubite 506	30
<i>B. anisoptera</i> Merr.	Philippines, Rubite 479	30
<i>B. biliranensis</i> Merr.	Philippines, Rubite 308	30
<i>B. camiguinensis</i> Elmer	Philippines, Peng 23853	28
<i>B. castilloi</i> Merr.	Philippines, Rubite 92	30
<i>B. cleopatrae</i> Coyle	Philippines, Peng 22548	30
<i>B. droseroides</i> C. I Peng, Rubite & C. W. Lin	Philippines, Peng 23418	28
<i>B. elmeri</i> Merr.	Philippines, Rubite 319	28
<i>B. elnidoensis</i> C. I Peng, Rubite & C. W. Lin	Philippines, Peng 23508	30
<i>B. gabaldonensis</i> Rubite, C. I Peng & C. W. Lin	Philippines, Peng 23356	28
<i>B. gitingensis</i> Elmer	Philippines, Rubite 255, Peng 20850	30
<i>B. hernandioides</i> Merr.	Philippines, Rubite 106, Peng 22517	28
<i>B. klemmei</i> Merr. ²⁾	Philippines, Rubite 182	30
<i>B. longiscapa</i> Warb.	Philippines, Rubite 289, 309	30
<i>B. luzonensis</i> Warb.	Philippines, Rubite 420	30
<i>B. manillensis</i> A. DC.	Philippines, Peng 23255	30
<i>B. mindorensis</i> Merr.	Philippines, Peng 23456	28
<i>B. neopurpurea</i> L. B. Sm. & Wassh.	Philippines, GKP 71	28
<i>B. nigritarum</i> (Kamel) Steud.	Philippines, Peng 23858	30 ³⁾
<i>B. obtusifolia</i> Merr.	Philippines, Peng 23828	30
<i>B. oxysperma</i> A. DC.	Philippines, Rubite 213, Peng 23015	30 ³⁾
<i>B. rubiteae</i> M. Hughes	Philippines, Rubite 346	30
<i>B. rufipila</i> Merr.	Philippines, Rubite 265	30
<i>B. tayabensis</i> Merr.	Philippines, Rubite 360	28
<i>B. trichochila</i> Warb.	Philippines, Peng 20764	30
<i>B. wadei</i> Merr. & Quisumb.	Philippines, Rubite 356	30

¹⁾ All collections are kept at HAST. ²⁾ Micrograph is not shown. ³⁾ Chromosome number disagreed with previous report

Table 2. Chromosome data in *Begonia* sect. *Baryandra*.

Taxon	2n	Reference
<i>Begonia acuminatissima</i> Merr.	30	Present study
<i>B. anisoptera</i> Merr.	30	Present study
<i>B. biliranensis</i> Merr.	30	Present study
<i>B. blancii</i> M. Hughes & C. I Peng	30	Hughes <i>et al.</i> (2011)
<i>B. camiguinensis</i> Elmer	28	Present study
<i>B. castilloi</i> Merr.	30	Present study
<i>B. chingipengii</i> Rubite	28	Rubite <i>et al.</i> (2014)
<i>B. cleopatrae</i> Coyle	30	Present study
<i>B. culasiensis</i> C. I Peng, Rubite, C. W. Lin & K. F. Chung	30	Peng <i>et al.</i> (2017)
<i>B. droseroides</i> C. I Peng, Rubite & C. W. Lin	28	Present study
<i>B. elmeri</i> Merr.	28	Present study
<i>B. elnidoensis</i> C. I Peng, Rubite & C. W. Lin	30	Present study
<i>B. fenicis</i> Merr.	26	Oginuma and Peng (2002)
	28	Nakamura <i>et al.</i> (2013)
	56	Kokubugata and Madulid (2001)
<i>B. gabaldonensis</i> Rubite, C. I Peng & C. W. Lin	28	Present study
<i>B. gitingensis</i> Elmer	30	Present study
<i>B. gueritziana</i> Gibbs	30	Hughes <i>et al.</i> (2011)
<i>B. hernandioides</i> Merr.	28	Present study
<i>B. hughesii</i> R. Rubite & C. I Peng	30	Rubite <i>et al.</i> (2015)
<i>B. klemmei</i> Merr.	30	Present study
<i>B. longiscapa</i> Warb.	30	Present study
<i>B. luzonensis</i> Warb.	30	Present study
<i>B. manillensis</i> A. DC.	30	Present study
<i>B. merrilliana</i> C. I Peng, Rubite, C. W. Lin & K. F. Chung	28	Peng <i>et al.</i> (2017)
<i>B. mindorensis</i> Merr.	28	Present study
<i>B. moneta</i> C. I Peng, Rimi & C. W. Lin	30	Peng <i>et al.</i> (2015a)
<i>B. neopurpurea</i> L. B. Sm. & Wassh.	28	Present study
<i>B. nigritarum</i> (Kamel) Steud.	30	Present study
	44	Doorenbos <i>et al.</i> (1998)
<i>B. obtusifolia</i> Merr.	30	Present study
<i>B. oxysperma</i> A. DC.	ca.28	Doorenbos <i>et al.</i> (1998)
	30	Present study
<i>B. rubiteae</i> M. Hughes	30	Present study
<i>B. rufipila</i> Merr.	30	Present study
<i>B. subnummularifolia</i> Merr.	32	Legro and Doorenbos (1971)
<i>B. suborbiculata</i> Merr.	30	Hughes <i>et al.</i> (2011)
<i>B. sykakiengii</i> Rubite, C. I Peng, C. W. Lin & K. F. Chung	28	Peng <i>et al.</i> (2017)
<i>B. tagbanua</i> M. Huges, C. I Peng & R. Rubite	30	Rubite <i>et al.</i> (2015)
<i>B. tandangii</i> C. I Peng & Rubite	28	Nakamura <i>et al.</i> (2013)
<i>B. tayabensis</i> Merr.	28	Present study
<i>B. taraw</i> C. I Peng, R. Rubite & M. Hughes	28	Rubite <i>et al.</i> (2015)
<i>B. trichochila</i> Warb.	30	Present study
<i>B. wadei</i> Merr. & Quisumb.	30	Present study

Results

The chromosomal cytology of the following 25 species of *Begonia* sect. *Baryandra* was studied for the first time. Additionally, karyotypes of five species were analyzed, which are the first reports in sect. *Baryandra*, *B. acuminatissima*, *B. camiguinensis*, *B. elmeri*, *B. gabaldonensis* and *B. obtusifolia*.

The chromosome number of *B. camiguinensis* Elmer at mitotic metaphase is $2n=28$ (Fig. 1a, b, k). The size of chromosomes is bimodal: the longest pair of chromosomes is about $1.6\mu\text{m}$ long, and the others are about $0.9\text{--}1.3\mu\text{m}$ long. Among the 28 chromosomes, ten chromosomes (Nos. 1–10 in Fig. 1k) have centromeres at median position (m-chromosome) and the other 18 chromosomes (Nos. 11–28 in Fig. 1k) have centromeres

at submedian or subterminal position (sm- and st-chromosome). Ten m-chromosomes are small in size of about 0.9 to $1.1\mu\text{m}$ long. Eight sm(st)-chromosomes (Nos. 11–14, 17, 18, 23, 24 in Fig. 1k) have the secondary constriction and/or small constriction (SC-chromosome). Satellite chromosomes are not observed (Fig. 1a, b, k).

The chromosome number of *B. elmeri* Merr. at mitotic metaphase is $2n=28$ (Fig. 1c, d, l). The size of chromosomes varies gradually: the longest pair of chromosomes is about $1.4\mu\text{m}$ long, and the shortest pair is about $0.9\mu\text{m}$ long. Among the 28 chromosomes, ten chromosomes (Nos. 1–10 in Fig. 1l) have centromeres at the median position and the other 18 chromosomes (Nos. 11–28 in Fig. 1l) have centromeres at the submedian or subterminal position. Ten m-chromosomes are small in size of about 0.9 to $1.2\mu\text{m}$ long. The SC-chromosomes

Fig. 1. Somatic chromosomes at metaphase of five *Begonia* species in sect. *Baryandra*. (a and b) *B. camiguinensis* ($2n=28$). (c and d) *B. elmeri* ($2n=28$). (e and f) *B. gabaldonensis* ($2n=28$). (g and h) *B. acuminatissima* ($2n=30$). (i and j) *B. obtusifolia*. b, d, f, h, and j are drawings of a, c, e, g, and i, respectively. k, l, m, n, and o are chromosome arrangement of respective b, d, f, h, and j based on the karyotypic formulae. Scale bar in a–j = $5\mu\text{m}$ and in k–o = $2\mu\text{m}$.

are observed in eight sm(st)-chromosomes (Nos. 11–14, 15–18 in Fig. 1l). Satellite chromosomes are not observed (Fig. 1c, d, l).

The chromosome number of *B. gabaldonensis* Rubite, C.I. Peng & C. W. Lin at mitotic metaphase is $2n=28$ (Fig. 1e, f, m). The size of chromosomes varies gradually: the longest pair of chromosomes is about $1.1\mu\text{m}$ long, and the shortest pair is about $0.8\mu\text{m}$ long. Among the 28 chromosomes, ten chromosomes (Nos. 1–10 in Fig. 1m) have centromeres at the median position and the other 18 chromosomes (Nos. 11–28 in Fig. 1m) have centromeres at the submedian or subterminal position. Ten m-chromosomes are small in size of about 0.8 to $1.1\mu\text{m}$ long. The SC-chromosomes are observed in six sm(st)-chromosomes (Nos. 11–14 and 17, 18 in Fig. 1m). Satellite chromosomes are not observed (Fig. 1e, f, m).

The chromosome number of *B. acuminatissima* Merr.

at mitotic metaphase is $2n=30$ (Fig. 1g, h, n). The size of chromosomes is bimodal: the longest pair of chromosomes is about $1.6\mu\text{m}$ long, and the others are about 0.8 – $1.3\mu\text{m}$ long. Among the 30 chromosomes, eight chromosomes (Nos. 1–8 in Fig. 1n) have centromeres at the median position and the other 22 chromosomes (Nos. 9–30 in Fig. 1n) have centromeres at the submedian or subterminal position. Eight m-chromosomes are small in size of about 0.9 to $1.3\mu\text{m}$ long. The SC-chromosomes are observed in eight sm(st)-chromosomes (Nos. 9–12, 19, 20 and 27, 28 in Fig. 1n). Satellite chromosomes are not observed (Fig. 1g, h, n).

The chromosome number of *B. obtusifolia* Merr. at mitotic metaphase is $2n=30$ (Fig. 1i, j, o). The size of chromosomes varies gradually: the longest pair of chromosomes is about $1.4\mu\text{m}$ long, and the shortest pair is $0.9\mu\text{m}$ long. Among the 30 chromosomes, eight chro-

Fig. 2. Somatic chromosomes at metaphase of ten *Begonia* species in sect. *Baryandra*. (a) *B. droseroides* ($2n=28$). (b) *B. hernandioides* ($2n=28$). (c) *B. mindorensis* ($2n=28$). (d) *B. neopurpurea* ($2n=28$). (e) *B. tayabensis* ($2n=28$). (f) *B. tayabensis* ($2n=30$). (g) *B. biliranensis* ($2n=30$). (h) *B. castilloi* ($2n=30$). (i) *B. cleopatrae* ($2n=30$). (j) *B. elnidoensis* ($2n=30$). Scale bar=5 μ m.

mosomes (Nos. 1–8 in Fig. 1o) have centromeres at the median position and the other 22 chromosomes (Nos. 9–30 in Fig. 1o) have centromeres at the submedian or subterminal position. Eight m-chromosomes are small in size of about 1.0 to 1.1 μ m long. The SC-chromosomes are observed in eight sm(st)-chromosomes (Nos. 9, 10, 15, 16, and 21–24 in Fig. 1o). Satellite chromosomes are not observed (Fig. 1i, j o).

The chromosome number of $2n=28$ was counted for the first time in five species of *B. droseroides* C. I Peng, Rubite & C. W. Lin (Fig. 2a), *B. hernandioides* Merr. (Fig. 2b), *B. mindorensis* Merr. (Fig. 2c), *B. neopurpurea* L. B. Sm. & Wassh. (Fig. 2d) and *B. tayabensis* Merr. (Fig. 2e). The chromosome size of all five species with $2n=28$ varies from about 1.0 to 2.0 μ m long, and the longest pair of chromosomes has centromeres at the submedian position, but the others are uncertain with respect to exact positions of centromeres. The chromosome

number of $2n=30$ was counted for the first time in 16 species of *B. anisoptera* Merr. (Fig. 2f), *B. biliranensis* Merr. (Fig. 2g), *B. castilloi* Merr. (Fig. 2h), *B. cleopatrae* Coyle (Fig. 2i), *B. elnidoensis* C. I Peng & Rubite & C. W. Lin (Fig. 2j), *B. gitingensis* Elmer (Fig. 3a), *B. longiscapa* Warb. (Fig. 3b), *B. luzonensis* Warb. (Fig. 3c), *B. manillensis* A DC. (Fig. 3d), *B. rubiteae* M. Hughes (Fig. 3e), *B. rufipila* Merr. (Fig. 3f), *B. trichochila* Warb. (Fig. 3g), *B. wadei* Merr. & Quisumb. (Fig. 3h), *B. nigritarum* (Kamel) Steud. (Fig. 3i), *B. oxysperma* A.DC. (Fig. 3j), and *B. klemmei* Merr. without the micrograph. The chromosome size of all 15 species varies from about 1.0 to 2.0 μ m long, and the longest pair of chromosomes has centromeres at the submedian position, but the others are uncertain with respect to exact positions of centromeres.

The chromosome number of $2n=30$ was counted for *B. nigritarum* (Fig. 3i) and *B. oxysperma* (Fig. 3j), which disagreed with $2n=44$ and $2n=ca.28$ reported previously

Fig. 3. Somatic chromosomes at metaphase of ten *Begonia* species in sect. *Baryandra* with $2n=30$. (a) *B. gitingensis*. (b) *B. longiscapa*. (c) *B. luzonensis*. (d) *B. manillensis*. (e) *B. rubiteae*. (f) *B. rufipila*. (g) *B. trichochila*. (h) *B. wadei*. (i) *B. nigritarum*. (j) *B. oxysperma*. Scale bar = 5 μ m.

in Doorenbos *et al.* (1998).

Based on ancestral character state reconstruction using both MP and ML, $2n=30$ is inferred as the most likely ancestral chromosome number of sect. *Baryandra* (Fig. 4).

Discussion

By adding the results of the present study, the reported chromosome numbers in *Begonia* sect. *Baryandra* are increased to 40 species or 51.3% of the 78 described species in the section. Among the 40 species, the chromosome numbers of all but *B. subnummularifolia* Merr. ($2n=32$; Legro and Doorenbos 1971) are either $2n=28$ or $2n=30$ (Table 2), though disagreements were found between present and previous studies. Specifically, our study shows $2n=30$ for *B. nigritarum*, while the species was reported to be $2n=44$ by Doorenbos *et al.* (1998) without further details. For *B. oxysperma*, the type species of sect. *Baryandra*, Doorenbos *et al.* (1998) report-

ed $2n=28$ with some uncertainty, while our study counts $2n=30$. However, because most previous studies such as Doorenbos *et al.* (1998) did not provide micrographs, it is hard to judge the nature of those discrepant chromosome counts and reconfirmations are needed for cases like *B. nigritarum* and *B. oxysperma*. For the Bornean species *B. subnummularifolia*, Legro and Doorenbos (1971) stated that "they have not been able to find sufficient cells showing distinct metaphase chromosomes which could be counted without any doubt," therefore, $2n=32$ of the species also need reconfirmation. Indeed, because of the technical challenge of *Begonia* cytology, it is highly possible that incorrect chromosome counts could have been reported. For instance, in a previous study, Oginuma and Peng (2002) reported $2n=26$ in *B. fenicis*; however, after close scrutiny of the micrograph and drawing, they (*i.e.*, the second and third author of the manuscript) conclude that $2n=28$ should be the correct chromosome number for the species. Nevertheless, intraspecific chromosomal variation does occur in sect.

Fig. 4. Inference of ancestral chromosome number of *Begonia* sect. *Baryandra*. The phylogenetic tree was reconstructed based on four chloroplast noncoding regions (*ndhA* intron, and *ndhF-rpl32*, *rpl32-trnL*, and *trnC-trnD* intergenic spacers) of Hughes *et al.* (2015). a) ML and b) MP.

Baryandra as a tetraploid count of $2n=56$ was reported for *B. fenicis* by Kokubugata and Madulid (2000).

Amongst the 190 *Begonia* species investigated by Legro and Doorenbos (1969, 1971, 1973), $2n=22$, 28, and 38 were found to be the most common chromosome numbers. Among the 20 Asian sections, $2n=28$ was reported in three sections: 14 species in sect. *Baryandra* (Nakamura *et al.* 2013, Rubite *et al.* 2014, 2015, Peng *et al.* 2017, present study), one species each in sections *Petermannia* (Piton 1962) and *Reichenheimia* (Li *et al.* 2005). The chromosome number $2n=30$, on the other hand, was reported in 86 species of seven sections: 24 species in sect. *Baryandra* (Hughes *et al.* 2011, Rubite *et al.* 2015, Peng *et al.* 2017, present study), 56 species in sect. *Coelocentrum* (Doorenbos *et al.* 1998, Ku *et al.* 2004, 2006, 2008, Liu *et al.* 2005, 2007, 2020, Fang *et al.* 2006, Peng *et al.* 2005, 2007, 2008a, b, 2012, 2013, 2014, 2015b, c, Li *et al.* 2016, Han *et al.* 2018, Kono *et al.* 2020), two species in *Petermannia* (Legro and Doorenbos 1969, 1971, 1973), and one species each

in sections *Diploclinium* (Tian *et al.* 2002), *Haagea* (Legro and Doorenbos 1971, Doorenbos *et al.* 1998), *Jackia* (Legro and Doorenbos 1969, Doorenbos *et al.* 1998) and *Reichenheimia* (Sharma and Bhattacharyya 1957). It appears that $2n=30$ is the most common number in Asian *Begonia*.

Given $2n=28$ and 30 are the most dominant chromosome numbers in sect. *Baryandra*, either $2n=28$ or $2n=30$ could be the ancestral chromosome number in the section. Molecular phylogenetic studies have indicated that sect. *Baryandra* is monophyletic (Hughes *et al.* 2011, 2015, Rubite *et al.* 2013, Moonlight *et al.* 2018), and sister to the clade of sections *Bractebegonia*, *Jackia*, *Petermannia*, and *Symbegonia* (Moonlight *et al.* 2018). Among them, $2n=30$ was reported in both sections *Jackia* and *Petermannia* (Legro and Doorenbos 1969, 1973, Doorenbos *et al.* 1998), and $2n=28$ was only reported in sect. *Petermannia* (Piton 1962). When chromosome numbers of both $2n=28$ and $2n=30$ are mapped on a phylogenetic tree of the clade of sect. *Baryandra*

(Hughes *et al.* 2015) (Fig. 4), both MP and ML analyses indicates that $2n=30$ is the ancestral chromosome number of sect. *Baryandra* and $2n=28$ is the derived one.

Karyotype analyses and chromosome evolution

In *Begonia*, karyotypes were previously only known from 18 species in three Asian sections *Coelocentrum*, *Diploclinium*, and *Platycentrum* (Oginuma and Peng 2002, Peng *et al.* 2014, 2015b, Kono *et al.* 2020). In the present study, karyotypes of five species were analyzed, presenting the first report in sect. *Baryandra*. All three species with $2n=28$ showed the uniform karyotype of $2n=28=10m+18sm(st)$, and two species with $2n=30$ also showed uniform karyotype of $2n=30=8m+22sm(st)$. Among 18sm(st)-chromosomes of $2n=28$, 6–8 SC-chromosomes were observed, accounting for 21–29% in the respective chromosome complements. Among 22 sm(st)-chromosomes of $2n=30$, eight SC-chromosomes were observed, accounting for 27% of the respective chromosome complements. The presence of 21–29% and 27% of SC-chromosomes in sect. *Baryandra* suggests that additional chromosomal changes have played an important role in generating the high species diversity in sect. *Baryandra* same as sect. *Coelocentrum* (Kono *et al.* 2020). Based on molecular phylogenetic relationships (Rubite *et al.* 2013, Hughes *et al.* 2015, Moonlight *et al.* 2018) $2n=30$ was inferred as the ancestral chromosome number by Mesquite (Fig. 4), while $2n=28$ is the derived chromosome number. It suggests that four small-sized sm(st)-chromosomes of $2n=30$ resulted by chromosome fusion to a pair of m-chromosomes. Consequently, the chromosome number decreased from $2n=30$ to $2n=28$, and its karyotype was changed from $2n=30=8m+22sm(st)$ to $2n=28=10m+18sm(st)$.

The karyotype $2n=30=8m+22sm(st)$ for two species of sect. *Baryandra* resembles to that of species in sect. *Coelocentrum* (Kono *et al.* 2020), therefore, it is difficult to distinguish species between sect. *Baryandra* and *Coelocentrum*, cytologically.

Rubite *et al.* (2013) transferred 32 Filipino and Bornean species from sect. *Diploclinium* classified by Doorenbos *et al.* (1998) to sect. *Baryandra*. Among 32 species, chromosome data available from the present study and previous reports show that all five species of *B. camiguinensis*, *B. elmeri*, *B. fenicis*, *B. hernandoides* and *B. mindorensis* have $2n=28$, and all 14 species of *B. acuminatissima*, *B. anisoptera*, *B. biliranensis*, *B. castilloi*, *B. gitingensis*, *B. longiscapa*, *B. luzonensis*, *B. manillensis*, *B. nigritarum*, *B. obtusifolia*, *B. rufipila*, *B. suborbiculata*, *B. trichochila* and *B. wadei* have $2n=30$. Thus, we could support the hypothesis proposed by Rubite *et al.* (2013), cytologically.

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