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## NEW CHROMOSOME COUNTS AND NUCLEAR CHARACTERISTICS FOR SOME MEMBERS OF *GESNERIACEAE* SUBFAMILY *CYRTANDROIDEAE* FROM CHINA AND VIETNAM

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The morphological characteristics of mitotic interphase, prophase and metaphase nuclei were analysed for 21 taxa in 14 genera from three different tribes of subfamily *Cyrtandroideae* (*Gesneriaceae*) from Asia. Interphase nuclei were classified into three categories: (1) complex chromocentres, as in *Ancylostemon*, *Briggsia*, *Briggsiopsis*, *Chirita*, *Loxostigma*, *Lysionotus*, *Oreocharis*, *Petrocosmea*, *Raphiocarpus* and *Whytockia*, (2) simple chromocentres, as in *Paraboea* and *Rhabdothamnopsis*, and (3) round prochromosomes, as in *Anna* and *Didymocarpus*. Species with complex chromocentres seem to be most common among all three tribes. The data further indicate that tribe *Didymocarpeae* is cytologically much more heterogeneous than other tribes, which accords with the diversity in morphological characters. Three types of chromosome condensation pattern during mitotic prophase were observed, here described as interstitial, gradient and proximal. Except for *Ancylostemon*, exhibiting a proximal type, and *Lysionotus* and *Whytockia* possessing a gradient type, all other species analysed to date exhibit the interstitial type. Our study also reports the chromosome numbers of 12 species in 10 genera of subfamily *Cyrtandroideae* from China and Vietnam; 11 of these counts are new.

*Keywords.* China, chromosome numbers, condensation pattern, *Cyrtandroideae*, *Gesneriaceae*, interphase, metaphase, mitosis, prophase, Vietnam.

### INTRODUCTION

The *Gesneriaceae*, according to the classification of Burt & Wiehler (1995), are divided into three subfamilies, of which the *Cyrtandroideae*, being the largest, has attracted most attention. Cytological studies in *Gesneriaceae* have been carried out over a number of decades, and have mainly focused on diversity in chromosome number (especially basic number) and chromosome size (Ratter, 1975; Skog, 1984), and satellites (Kiehn *et al.*, 1998). Until recently, however, evolutionary relationships among these genera were unclear and authors generally listed names in alphabetical order. Moreover, in subfamily *Cyrtandroideae*, only c.17% of species and c.50% of genera have been analysed cytologically to date (Möller & Kiehn, 2004) and thus few conclusions on chromosome evolution in the subfamily can be drawn.

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Detailed cytological analyses can provide data useful for various aspects of taxonomy, such as the classification of segregating species – e.g. *Moussonia* Regel from *Kohleria* Regel (Wiehler, 1975); *Eucodonia* Hanst. from *Achimenes* Pers. (Wiehler, 1976) – or in reassessing morphologically difficult groups. Weber & Burt (1998b) used cytological information to support a new delimitation of *Didymocarpus* Wall.; Jong & Möller (2000) applied metaphase data in *Streptocarpus* Lindl. at subgeneric level and found a striking conformity between basic number and molecular phylogeny.

Earlier observations have shown that the distribution pattern of chromatin in the interphase nucleus and mitotic prophase is stable within species (Okada & Tamura, 1979). On the other hand, interphase nuclei in mitotic cells can show large variation in chromatin morphology between species (Tischler, 1934). There is also evidence that the distribution pattern of chromatin in interphase nuclei is characteristic of natural groups; Tanaka (1971) used interphase nuclear morphology to postulate a phylogenetic tree for the *Orchidaceae*, and Yang (1993) drew on such data when discussing the systematic positions of *Asteropyrum* J.R. Drumm & Hutchinson and *Calathodes* Hook.f. & Thoms. in *Ranunculaceae*. Interphase nuclear analysis has also been applied in other families, such as *Caprifoliaceae* s. str. (Zhang *et al.*, 2002), *Onagraceae* (Kurabayashi *et al.*, 1962), woody polycarpous plants (Okada, 1975), *Lauraceae* (Okada & Tanaka, 1975) and *Ranunculaceae* (Okada & Tamura, 1979). Only recently have such studies been carried out in *Gesneriaceae*, including analyses of condensation patterns (the thickening and shortening of chromosomes during mitotic prophase and interphase). Wang *et al.* (1998) showed no variation in resting nuclei and mitotic prophase characteristics among *Whytockia* W.W. Sm. species, but Wang & Gu (1999) found variation in interphase and prophase nuclei among four species in the three genera of subfamily *Cyrtandroideae* analysed.

In the present study we report the chromosome numbers of 12 *Gesneriaceae* species from China and Vietnam, present an analysis of their mitotic prophase and interphase characteristics, and discuss our results in combination with data previously published in the light of the current tribal taxonomy of the subfamily.

#### MATERIALS AND METHODS

The material studied and details of voucher specimens are shown in Table 1, which also includes authorities for all species. The plants investigated were collected from wild populations in Yunnan Province, China, and mostly cultivated in the Botanical Garden of Kunming Institute of Botany (KUN), though some are in cultivation at the Royal Botanic Garden Edinburgh (E). Material of a few species (*Anna submontana*, *Raphiocarpus petelotii*, *Loxostigma griffithii*) was prepared according to Jong & Möller (2000), but the majority were prepared as follows. Root tips were pretreated with 0.002 mol/l 8-hydroxyquinoline for 1.5 hours at about 20°C, and fixed in Farmer's solution (3 parts ethanol : 1 part glacial acetic acid) at 4°C for 30 minutes. After maceration in a mixture of 1 N hydrochloric acid and 45% acetic

TABLE 1. *Gesneriaceae* taxa analysed for interphase, prophase and metaphase characters, with details of localities and voucher specimen numbers

| Taxon   | Voucher specimen        | Locality                | Interphase nuclei | Condensation behaviour | $2n/$ chromosome shape | Data source      |
|---|-------------------------|-------------------------|-------------------|------------------------|------------------------|------------------|
| <b>Tribe Didymocarpeae</b>                              |                         |                         |                   |                        |                        |                  |
| <i>Ancylostemon aureus</i> (Franch.) B.L. Burtt         | Y.Z. Wang 96002 (PE)    | China: Yunnan, Binchuan | C                 | P                      | 34/r                   | Wang & Gu (1999) |
| <i>Anna mollifolia</i> (W.T. Wang) W.T. Wang & K.Y. Pan | 99706 (KUN)             | China: Yunnan, Malipo   | R                 | I                      | 34*/r                  | This study       |
| <i>A. submontana</i> Pellegr.                           | MMO 01-85; 20020451 (E) | China: Yunnan, Maguan   | R                 | I                      | 34*/r                  | This study       |
| <i>Briggsia muscicola</i> Craib                         | 011022 (KUN)            | China: Yunnan, Xiping   | C                 | I                      | 34*/r                  | This study       |
| <i>Briggsiopsis delavayi</i> (Franch.) K.Y. Pan         | Y.Z. Wang 96053 (PE)    | China: Sichuan, Leibo   | C                 | I                      | 34/r                   | Wang & Gu (1999) |
| <i>Chirita dielsii</i> (Borza) B.L. Burtt               | 011013 (KUN)            | China: Yunnan, Jingdong | C                 | I                      | 20/r                   | This study       |
| <i>C. eburnea</i> Hance                                 | 02501 (KUN)             | China: Guizhou, Bijie   | C                 | I                      | 36*/g                  | This study       |
| <i>Didymocarpus purpureobracteatus</i> W.W. Sm.         | 991106 (KUN)            | China: Yunnan, Pingbian | R                 | I                      | 28*/g                  | This study       |
| <i>Oreocharis henryana</i> Oliv.                        | 0191401 (KUN)           | China: Yunnan, Huize    | C                 | I                      | 34*/r                  | This study       |
| <i>Paraboea barbatipes</i> K.Y. Pan                     | 99902 (KUN)             | China: Yunnan, Malipo   | S                 | I                      | 36*/g                  | This study       |
| <i>Petrocosmea sericea</i> C.Y. Wu ex H.W. Li           | 991104 (KUN)            | China: Yunnan, Xichou   | C                 | I                      | 34*/r                  | This study       |

TABLE 1. (Cont'd)

| Taxon  | Voucher specimen                  | Locality                       | Interphase nuclei | Condensation behaviour | $2n/$ chromosome shape | Data source               |
|--|-----------------------------------|--------------------------------|-------------------|------------------------|------------------------|---------------------------|
| <i>Raphiocarpus petelotii</i> (Pellegr.) B.L. Burtt                    | GCH29/2081; 19982405 (E)          | Vietnam: Lao Cai               | C                 | I                      | 32*/r                  | This study                |
| <i>Rhabdohammopsis sinensis</i> Hemsl.                                 | 00504 (KUN)                       | China: Yunnan, Malipo          | S                 | I                      | 36*/g                  | This study                |
| <b>Tribe Trichosporaceae</b>   |                                   |                                |                   |                        |                        |                           |
| <i>Loxostigma griffithii</i> (Wight) C.B. Clarke                       | GSE96 7668; 19962309 (E)          | China: Yunnan, Nujiang Lisu AP | C                 | I                      | 34*/r                  | This study                |
| <i>Lysionotus carnosus</i> Hemsl.                                      | Y.Z. Wang 92088 (PE)              | China: Yunnan, Hekou           | S-C               | G                      | 30/r                   | Wang & Gu (1999)          |
| <i>L. serratus</i> D. Don var. <i>pterocaulis</i> C.Y. Wu ex W.T. Wang | Y.Z. Wang 92078 (PE)              | China: Yunnan, Hekou           | S-C               | G                      | 32/r                   | Wang & Gu (1999)          |
| <b>Tribe Epithemateae</b>  |                                   |                                |                   |                        |                        |                           |
| <i>Whytockia bijiensis</i> Y.Z. Wang & Z.Y. Li                         | Y.Z. Wang 94002 (PE), 95001 (KUN) | China: Guizhou, Bijie          | C                 | G                      | 18/r                   | Wang <i>et al.</i> (1998) |
| <i>W. hekounensis</i> Y.Z. Wang  | Y.Z. Wang 93008 (PE)              | China: Yunnan, Hekou           | C                 | G                      | 18/r                   | Wang <i>et al.</i> (1998) |
| <i>W. purpurascens</i> Y.Z. Wang                                       | Y.Z. Wang 93082 (PE)              | China: Yunnan, Hekou           | C                 | G                      | 18/r                   | Wang <i>et al.</i> (1998) |
| <i>W. tsiangiana</i> (Hand.-Mazz.) A. Weber var. <i>tsiangiana</i>     | Y.Z. Wang 94001 (PE)              | China: Guizhou, Jiangkou       | C                 | G                      | 18/r                   | Wang <i>et al.</i> (1998) |

TABLE 1. (Cont'd)

| Taxon                              | Voucher specimen | Locality        | Interphase nuclei | Condensation behaviour | $2n/$ chromosome shape | Data source               |
|------------------------------------|------------------|-----------------|-------------------|------------------------|------------------------|---------------------------|
| <i>W. tsiangiana</i> (Hand.-Mazz.) | Y.Z. Wang        | China: Sichuan, | C                 | G                      | 18/r                   | Wang <i>et al.</i> (1998) |
| A. Weber var. <i>wilsonii</i>      | 93026 (PE)       | Leibo           |                   |                        |                        |                           |
| A. Weber                           |                  |                 |                   |                        |                        |                           |

*Abbreviations.* Voucher specimens: PE = Peking; E = Royal Botanic Garden Edinburgh; KUN = Kunming Institute of Botany. Interphase nuclei: C = complex chromosome type; R = round chromosome type; S = simple chromosome type; G = gradient type; I = interstitial type; G = gradient type. Chromosome shape: r = rod-shaped; g = globular.

\* New chromosome counts.

acid at 60°C for 40 s, the material was stained with 1% aceto-orcein, and then squashed for cytological observation.

Karyotype images were automatically generated using the Ikaros software package (Imaging Associates).

Representative interphase nuclei were selected from undisturbed cells with large round nuclei. Prophase cells were selected where the hetero- and euchromatin distribution could be most clearly distinguished. The classification of interphase nuclei and the description of condensation pattern of chromosomes during mitotic prophase follows Tanaka (1971).

In the interphase nuclei of species with **complex chromocentres**, numerous darkly stained chromocentres (more than the number of chromosomes) can be seen, occasionally showing aggregation. They form several larger heteropycnotic blocks (blocks of DNA that are out-of-phase with respect to the coiling cycle) varying in size and number.

In the **simple chromocentre** category the interphase nuclei display a few darkly stained chromocentres (fewer than the chromosome number). The chromocentres show an irregular rough surface in outline.

In the **prochromosome** category, round heteropycnotic bodies of almost equal size occur in the interphase nuclei. The number of these bodies corresponds to the number of chromosomes with heterochromatic segments, and they are thus termed prochromosomes. A very clear distinction between prochromosomes and diffused chromatin is apparent.

The condensation pattern in which deeply stained segments are distributed discontinuously along the chromatin is termed interstitial. Other types reported in the literature are commented on in the discussion.

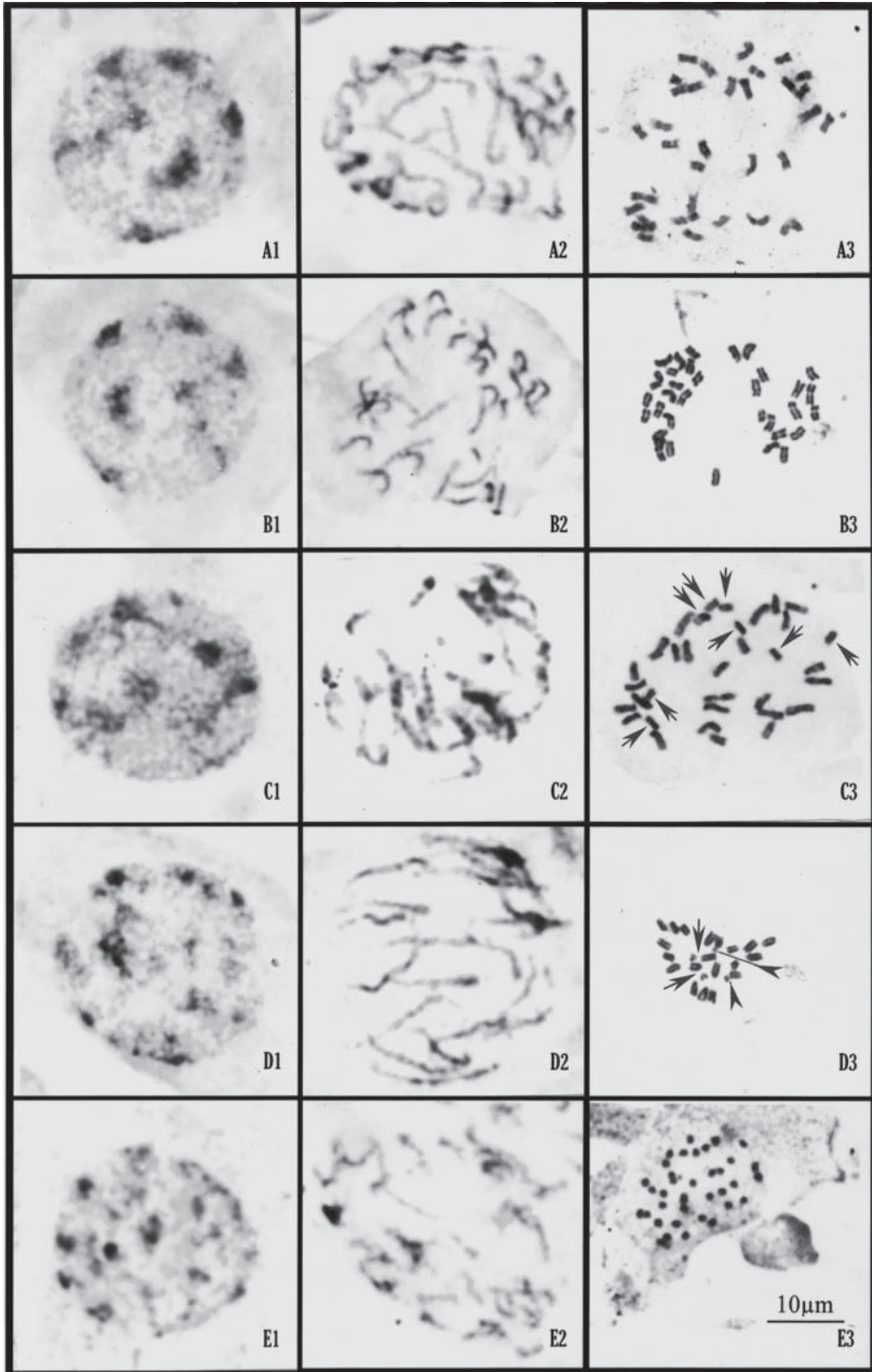
## RESULTS

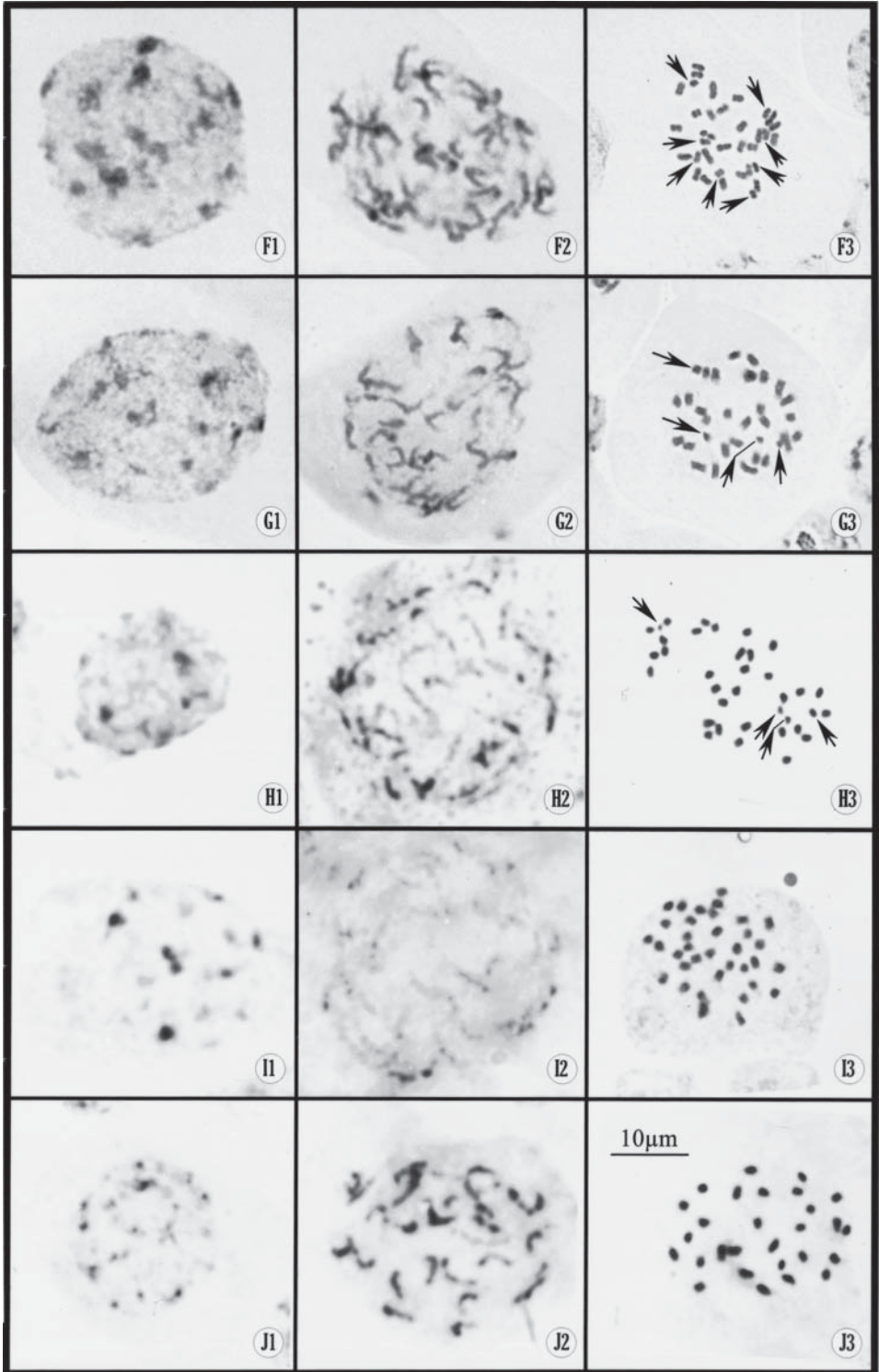
The observations are listed in Table 1. Eleven counts are new and one is of a new accession of *Chirita dielsii*, a species for which a published result already exists (Lu *et al.*, 2002). The categories of interphase nuclei in the 10 genera studied are given in Table 1. The rest are derived from published information.

### *Interphase nuclei*

The taxa analysed here fall into three categories:

- 1 Complex chromocentres: *Briggsia muscicola*, *Chirita dielsii*, *C. eburnea*, *Oreocharis henryana*, *Petrocosmea sericea*, *Loxostigma griffithii*, *Raphiocarpus petelotii*; Fig. 1: A1–G1;
- 2 Simple chromocentres: *Paraboea barbatipes* and *Rhabdothamnopsis sinensis*; Fig. 1: H1, I1; and
- 3 Round prochromosomes: *Anna mollifolia*, *A. submontana*, *Didymocarpus purpureobracteatus*; Fig. 1: J1–L1.







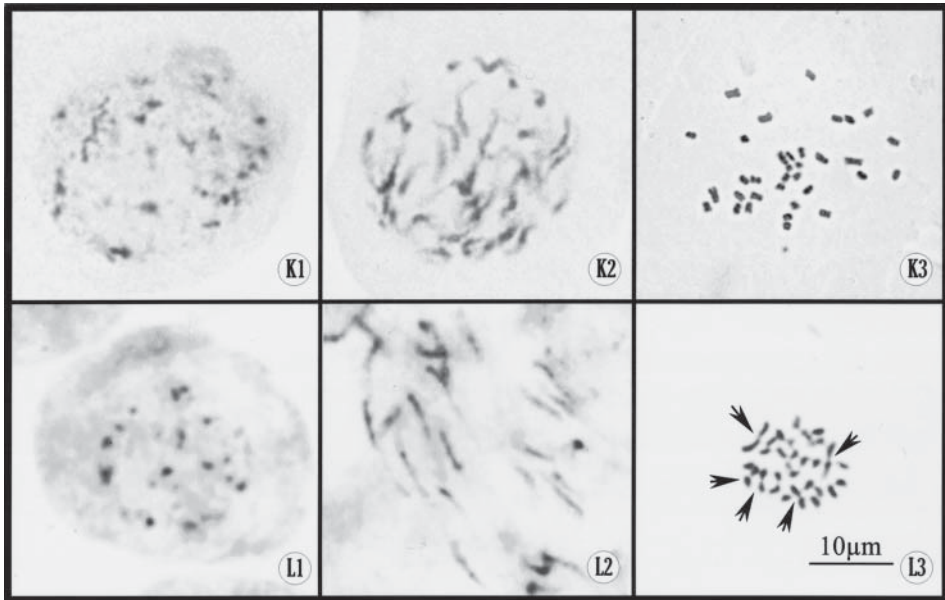


FIG. 1. Karyomorphology of Asian *Gesneriaceae* species: column 1 = interphase nuclei; column 2 = prophase nuclei; column 3 = metaphase spreads. A–G: Complex chromocentres: A, *Oreocharis henryana*,  $2n=34$ ; B, *Briggsia muscicola*,  $2n=34$ ; C, *Petrocosmea sericea*,  $2n=34$  (arrows indicate small chromosomes); D, *Chirita dielsii*,  $2n=20$  (arrows indicate satellite chromosomes); E, *Chirita eburnea*,  $2n=36$ ; F, *Loxostigma griffithii*,  $2n=34$  (arrows indicate small chromosomes); G, *Raphiocarpus petelotii*,  $2n=32$  (arrows indicate small chromosomes). H & I: Simple chromocentres: H, *Paraboea barbatipes*,  $2n=36$  (arrows indicate small chromosomes); I, *Rhabdothamnopsis sinensis*,  $2n=36$ . J–L: Round prochromosomes: J, *Didymocarpus purpureobracteatus*,  $2n=28$ ; K, *Anna submontana*,  $2n=34$ ; L, *Anna mollifolia*,  $2n=34$  (arrows indicate touching chromosomes).

#### *Prophase nuclei*

All taxa analysed show interstitial chromatin condensation (Fig. 1: A2–L2).

#### *Metaphase chromosomes*

Great variation in somatic chromosome number, shape and size was observed in the genera analysed.

For *Chirita* D. Don, two different somatic numbers were encountered,  $2n=20$  for *C. dielsii* and  $2n=36$  for *C. eburnea*. The chromosomes of the former are c.1.4–3.4µm long, mainly acrocentric, with two pairs of chromosomes showing terminal satellites (Fig. 1: D3, arrowed), while *C. eburnea* has nearly twice as many, smaller, globular chromosomes (c.0.8–1.2µm) showing slight size variation (Fig. 1: E3).

*Didymocarpus purpureobracteatus* has  $2n=28$  rod-shaped chromosomes (Fig. 1: J3) with some size variation (0.8–2.4µm).

*Raphiocarpus petelotii* ( $2n=32$ ) has a relatively greater range of sizes of rod-shaped chromosomes, from 1.8 to 3.9µm (Fig. 1: G3). This may be partly due to

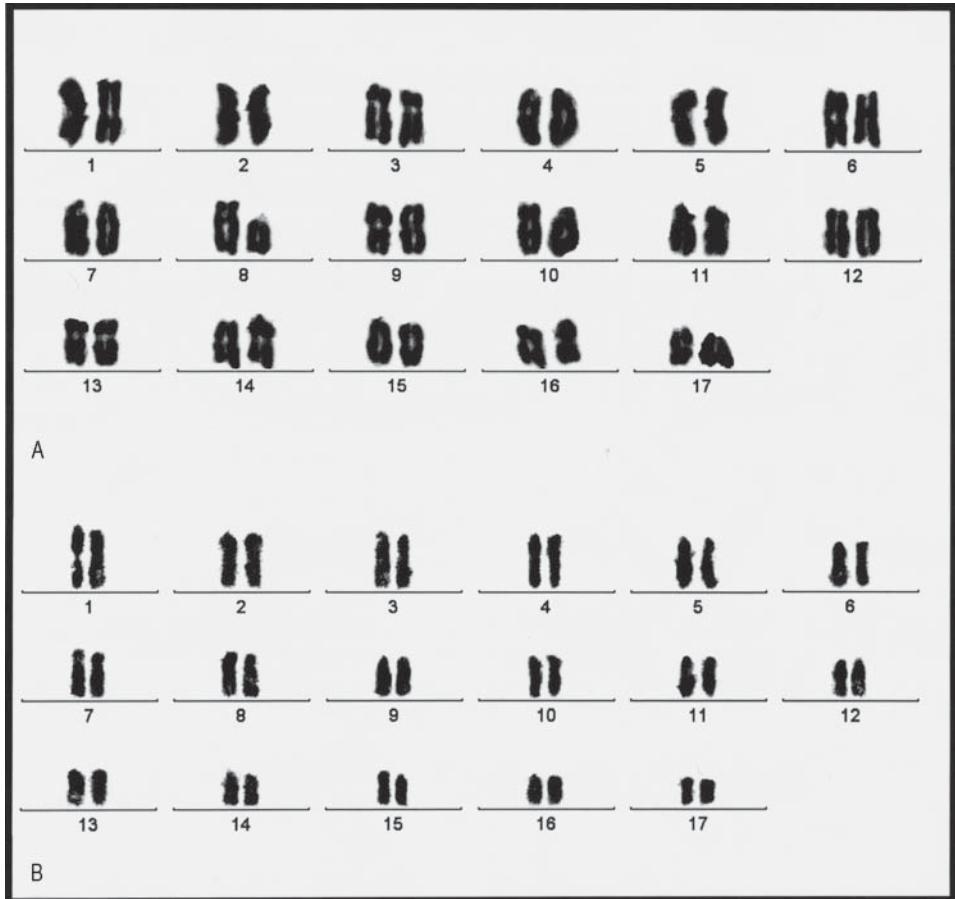


FIG. 2. Karyomorphology of Asian *Gesneriaceae* species: A, karyotype of *Briggsia muscicola*; B, karyotype of *Petrocosmea sericea*.

the late pro-metaphase analysed, with chromosomes not yet fully contracted. Two chromosome pairs were noticeably smaller and dot-like.

For most species investigated a somatic chromosome number of  $2n=34$  was observed. The two *Anna* Pellegr. species have similar chromosome complements. Those of *A. submontana* are rod-shaped,  $0.8\text{--}1.9\mu\text{m}$  long, mainly metacentric and submetacentric with three larger pairs (Fig. 1: K3); in *A. mollifolia* the chromosomes are also rod-shaped, and  $c.0.8\text{--}2.4\mu\text{m}$  long (Fig. 1: L3). *Briggsia muscicola* possesses larger, metacentric to acrocentric, rod-shaped chromosomes ( $c.1.4\text{--}2.8\mu\text{m}$ ), with three smaller pairs (Fig. 1: B3; Fig. 2: A). *Oreocharis henryana* ( $2n=34$ ) has chromosomes similar in shape to *B. muscicola* although they are longer overall ( $c.2.2\text{--}4\mu\text{m}$ ), which may be explained by the fact that late pro-metaphase was analysed (Fig. 1: A3); the centromere positions are clearly visible. However, this species appears to possess more metacentric chromosomes than *B. muscicola*. *Petrocosmea sericea* ( $2n=34$ ) shows, with *Oreocharis* Benth., the largest chromosomes, reaching

c.4 $\mu$ m, and also a great variation in size, with at least four pairs of smaller chromosomes at c.1.6 $\mu$ m (Fig. 1: C3, arrowed; Fig. 2: B). *Loxostigma griffithii* ( $2n=34$ ) has rod-shaped, mainly metacentric to submetacentric chromosomes c.1.2–2.2 $\mu$ m long, with four smaller pairs (Fig. 1: F3, arrowed).

*Paraboea barbatipes* ( $2n=36$ ) has chromosomes of a similar globular shape to *Didymocarpus*, with a size range of 1.8–2.4 $\mu$ m. At least two pairs are significantly smaller (Fig. 1: H3, arrowed). *Rhabdothamnopsis sinensis* ( $2n=36$ ) has chromosomes similar to *Paraboea barbatipes*, which are globular and c.1.1–1.3 $\mu$ m long (Fig. 1: I3).

## DISCUSSION

Our own data on 12 species of tribe *Didymocarpeae* were supplemented by published data on five genera from China reported by Wang *et al.* (1998) and Wang & Gu (1999). These included two genera from the *Didymocarpeae*, one from the *Trichosporeae* and one from the *Epithemateae*. Their chromosome numbers and other characters are summarized in Table 1.

In the following discussion cytological characters will be discussed in the interphase categories observed to date.

### 1. Complex chromocentres

Most *Gesneriaceae* fall into this category, which includes taxa from three tribes of the Old World subfamily *Cyrtandroideae*: *Didymocarpeae*, *Trichosporeae* and *Epithemateae*. The somatic chromosome numbers range from  $2n=18$  to  $2n=34$ , the majority of *Didymocarpeae* having  $2n=34$ . Most species with complex chromocentres have rod-shaped chromosomes, except for *Chirita eburnea*.

#### *Briggsia*

Ratter & Prentice (1964) reported  $2n=68$  for *Briggsia muscicola*, and Ratter (1963)  $2n=34$  for *B. aurantiaca* B.L. Burt. In our study we also obtained  $2n=34$  for *B. muscicola*. If we regard  $2n=34$  as the diploid state in this genus, then  $2n=68$  might be a tetraploid derivative. The plant with  $2n=68$  was, however, not of wild origin and it is feasible that the tetraploid form was derived in cultivation. A similar situation may have occurred in *Streptocarpus thompsonii* R. Br. where a single accession of unknown garden origin was shown to be tetraploid (Jong & Möller, 2000). On the other hand, natural polyploids are known in some *Gesneriaceae* genera including *Aeschynanthus* Jack (Kiehn & Weber, 1998; Rashid *et al.*, 2001; Lu *et al.*, 2002), *Columnnea* L. (Morley, 1967) and *Streptocarpus* (Jong & Möller, 2000).

#### *Chirita*

This is a cytologically heterogeneous genus with a range of basic numbers including  $x=4, 7, 9, 10$  and  $17$  (Burt & Wiehler, 1995). Although sect. *Gibbosaccus* is larger than the other sections, proportionally fewer species are cytologically known.

*Chirita eburnea* which also belongs to sect. *Gibbosaccus* has the same haploid number ( $x=18$ ) as another section member, *C. sinensis* Lindl. (Ratter & Prentice, 1964). *Chirita lacunosa* (Hook.f.) B.L. Burtt of section *Chirita* has  $2n=18$  (Kiehn *et al.*, 1998). *Chirita dielsii* (section *Chirita*) has  $2n=20$ , although as Lu *et al.* (2002) reported  $2n=18$  for this species there is some confusion about the exact number. Careful observation of several clearly countable metaphase spreads gave us  $2n=20$  for *C. dielsii*, and the published images by Lu *et al.* (2002) do not allow an unambiguous determination of the chromosome number. Thus, to determine whether this species shows variation in chromosome number requires further work. The chromosomes of *C. dielsii* (1.4–3.4 $\mu\text{m}$ ) were much larger than those of *C. eburnea* (0.8–1.2 $\mu\text{m}$ ). The size of *C. eburnea* chromosomes is similar to those of the Malayan species in this genus analysed by Kiehn *et al.* (1998). In *C. dielsii* there are a few medium-sized chromosomes and four distinct satellite chromosomes, which could not be seen in *C. eburnea* due to their small size.

A chromosome number of  $2n=18$  is common in *Chirita* and occurs in all three sections, suggesting that this may be ancestral. However, only about a fifth of the species have been investigated, and the variation in basic number (see above) may indicate that the genus includes different evolutionary lineages. This is also suggested by preliminary molecular data (M. Pfosser *et al.*, pers. comm.).

#### *Oreocharis*

Only two of the 28 currently recognized species of *Oreocharis* have been investigated. *Oreocharis aurea* Dunn has, like *O. henryana* reported here,  $2n=34$  (Lu *et al.*, 2002). The chromosomes are quite large for the *Cyrtandroideae*. Preliminary molecular analyses suggest affinities with *Briggsia* Craib and *Ancylostemon* Craib (M. Pfosser *et al.*, pers. comm.); both are also  $2n=34$  with similar rod-like chromosomes (Wang & Gu, 1999).

#### *Petrocosmea*

Five out of c.25 species of *Petrocosmea* Oliv. have been investigated cytologically, and for all but one a somatic number of  $2n=34$  has been established (Fussell, 1958; Ratter, 1963; Ratter & Prentice, 1967; Skog & Oliver, 1988; L.E. Skog, pers. comm.). Our count for *P. sericea* agrees with the majority of counts for this genus. Lu *et al.* (2002) reported a chromosome number of  $2n=32$  for *P. barbata* Craib, a new number for the genus.

#### *Loxostigma*

Our count of  $2n=34$  is the first for the genus, the only member of tribe *Trichosporeae* investigated by us. Among other genera in this tribe basic numbers of  $x=14$ , 15 and 16 are widespread, but  $x=17$  in *L. griffithii* is unique. Evidence from

preliminary molecular analyses places *Loxostigma* close to genera in other tribes with  $x=17$ , such as *Petrocosmea* (M. Pfosser *et al.*, pers. comm.). This may indicate that the current tribal circumscription of *Trichosporeae* does not reflect a natural group, and that seed appendages, the main uniting character of the tribe, may have evolved several times independently.

### *Raphiocarpus*

The present count of  $2n=32$  is the first for this genus. Since the re-delimitation of *Didissandra* C.B. Clarke by Weber & Burt (1998a), *Raphiocarpus* includes all the species from China and Vietnam formerly in *Didissandra*. The species now in *Raphiocarpus* are insufficiently known and may not represent a natural group (Weber & Burt, 1998a). There is little cytological evidence linking *Didissandra* with *Raphiocarpus*, and the only count for the former, *D. frutescens* C.B. Clarke, shows  $2n=20$  (Kiehn *et al.*, 1998). More species in both genera have to be analysed cytologically to clarify genus delimitations.

### *Ancylostemon*, *Briggsiopsis*, *Lysionotus* and *Whytockia*

Wang & Gu (1999) showed complex chromocentres to be present in *Ancylostemon aureus* and *Briggsiopsis delavayi* (both in tribe *Didymocarpeae*), and also in *Lysionotus serratus* var. *pterocaulis* and *L. carnosa* (in tribe *Trichosporeae*). Wang *et al.* (1998) also found complex chromocentres in *Whytockia bijiensis*, *W. hekounensis*, *W. purpurascens* and *W. tsiangiana* in tribe *Epithemateae*, and all the above species were reported to have rod-shaped chromosomes.

## 2. Simple chromocentres

Two genera, both in tribe *Didymocarpeae*, possess simple chromocentres; they both have  $2n=36$  and globular chromosomes.

### *Rhabdothamnopsis*

*Rhabdothamnopsis sinensis* ( $2n=36$ ), the only species in the genus, is endemic to South China (Yunnan, Sichuan, Guizhou), growing in dense forests, along streams in forested areas, and in thickets along roadsides at altitudes of 1600–2200(–4600)m (Li, 1996). Franchet described it independently as *Streptocarpus chinensis* Franch. (Wang *et al.*, 1992; Wang, Pan *et al.*, 1998), as it has the tubular flowers and twisted fruit characteristic of that genus. Apart from being disjunct from the rest of *Streptocarpus*, which is mainly African and Madagascan, the flowers are axillary and the twiggy and shrubby habit is quite distinctive. With a basic number of  $x=18$  *Rhabdothamnopsis* Hemsl. differs from *Streptocarpus* which has  $x=15, 16$  and multiples thereof (Jong & Möller, 2000). Further, molecular data unequivocally

place *Rhabdothamnopsis* in a monophyletic clade of Asian species, with predominantly twisted fruits, including *Paraboea* Ridl. and *Ornithoboea* C.B. Clarke, and not with *Streptocarpus* which forms a monophyletic sister group (Möller *et al.*, 1999). It seems that cytology, rather than morphology, reflects phylogenetic relationships in this group. Thus, it seems prudent to retain *Rhabdothamnopsis* as a monotypic genus.

### *Paraboea*

Previous counts in *Paraboea* have included  $n=9$ , 16, 17 and 18 (Kiehn *et al.*, 1998). Our count of  $2n=36$  for *P. barbatipes* falls well in line with the majority of counts for this genus. Only a small minority (14%) of the 87 species recognized in *Paraboea* have been counted (Möller, unpublished data), and it is too early to speculate further on possible groupings within the genus, or its affinities within tribe *Didymocarpeae*. We found *P. barbatipes* to have significantly larger chromosomes than in previously published accounts (M. Kiehn, pers. comm.). This may be due to the different tissues, pretreatment or staining techniques used by us (root tips, 8-hydroxyquinoline and aceto-orcein) and by Kiehn *et al.* (1998) (flower buds, no pretreatment and Feulgen). More comparative data are needed.

### 3. Round prochromosomes

Four species from two genera in tribe *Didymocarpeae* were investigated and placed in this category. The chromosome numbers of species in this category range from  $2n=28$  to  $2n=34$ . All but one species, *Didymocarpus purpureobracteatus*, have rod-shaped chromosomes.

### *Anna*

Burt & Wiehler (1995) placed *Anna* in tribe *Didymocarpeae* whereas Wang *et al.* (1992) assigned it to tribe *Trichosporeae*, on account of the characteristic prominent seed appendages. Ontogenetic analysis of seed development is required to ascertain the degree of affinity between *Anna* and other members of tribe *Trichosporeae*. Such data are not yet available for *Anna* (Christie & Mendum, 2002). Chromosome number seems to be constant in the genus, as both *A. mollifolia* and *A. submontana* (two out of the three species in the genus) have  $2n=34$  chromosomes, as distinct from other genera in tribe *Trichosporeae* which range through  $2n=28$ , 30, 32, 60, 64 and 96, with the majority having  $2n=32$  (Ratter & Prentice, 1967; Wang & Gu, 1999; Rashid *et al.*, 2001). *Anna* shares  $2n=34$  with *Briggsia*, *Oreocharis* and *Petrocosmea*, though the latter three are in a different interphase nucleus class. A basic number of  $x=17$  is very common across tribe *Didymocarpeae* and it is probable that *Anna* belongs there rather than in the *Trichosporeae*.

*Didymocarpus*

This genus now contains around 80 species (Weber & Burtt, 1998b) of which less than a fifth have been investigated cytologically. It includes species with a range of basic chromosome numbers of  $x=11, 12, 14, 16$  and  $18$ , with the majority having  $x=11$  and  $14$  (Kiehn *et al.*, 1998). Our count of  $2n=28$  for *D. purpureobracteatus* suggests affinities with *D. cordata* Wall. and *D. insulsus* Craib (Kiehn *et al.*, 1998). Compared to the closely related genus *Chirita*, we find that the chromosome morphology of the two genera shows some anomalies, *C. eburnea* chromosomes being more similar to *Didymocarpus* than to *C. dielsii*. This may not be surprising (see remarks on *Chirita* below) but more data are needed to understand the relationship between the two genera.

The African genus *Saintpaulia* H. Wendl. is also reported to have a similar interphase nucleus, described by Sera & Karasawa (1984) as possessing 'rod-shaped' prochromosomes.

**Correlation between nuclear characters and current tribal arrangements**

At present there appears to be little correlation between chromosome number or morphology and interphase or prophase characters. Interestingly, while all *Whytockia* species appear very uniform with respect to chromosome number and morphology, the two *Chirita* species differ considerably in both number and morphology. *Chirita* is now thought to be an assemblage of old phylogenetic lineages (M. Pfosser *et al.*, pers. comm.) and may not represent a natural genus.

The characters of resting interphase nuclei also show no consistent differences between the tribes as defined at present. Sera & Karasawa (1984) and Sera (1992) found intermediates between rod prochromosomes and round prochromosomes in the African genus *Saintpaulia*, which is so far unique in subfamily *Cyrtandroideae*. But complex chromocentres appear to be the most widespread character among the three tribes investigated here. However, a regrouping of genera according to similarities in interphase nucleus characters would not be tenable; complex chromocentres, for example, occur in all tribes, including *Epithemateae*. While the other tribes may not be natural, this tribe is clearly a natural, monophyletic lineage (Mayer *et al.*, 2003). The fact that complex chromocentres occur in all tribes analysed so far may indicate that they are an ancestral feature in Old World *Gesneriaceae* from which the simple chromocentres and prochromosomes have evolved.

Three different types of condensation pattern in prophase nuclei were observed. In tribe *Epithemateae* only the gradient type was observed, although only one of the seven genera in this tribe was analysed. In this type there is little or no differentiation between early and late condensing segments during prometaphase. Species in tribes *Didymocarpeae* and *Trichosporeae* contain two condensation types, but the interstitial type is by far the most common in the former tribe, and outside tribe

*Didymocarpeae*, only *Loxostigma* of tribe *Trichosporeae* possesses the interstitial type. This is unlikely to be the result of parallel evolution, but may reflect phylogeny, as molecular data suggest a close relationship between *Loxostigma* and other taxa from tribe *Didymocarpeae* with an interstitial condensation pattern and  $2n=34$ , as in *Anna*, *Briggsia* and *Petrocosmea* (M. Pfosser *et al.*, pers. comm.). Of the species studied by us only *Ancylostemon aureus* showed a proximal chromatin condensation pattern in the prochromosomal nuclei, with proximally early and distally late condensing chromatin. *Saintpaulia* is the only other genus reported with this type of condensation pattern (Sera & Karasawa, 1984).

From molecular phylogenetic data it is likely that the current tribal arrangement in this family does not reflect natural lineages but is artificial (Wang & Li, 1998; M. Pfosser *et al.*, pers. comm.). Although an outline phylogenetic hypothesis for Old World *Gesneriaceae* is being developed, there are at present too few karyomorphological data available. The fact that the only African genus so far investigated, *Saintpaulia*, shows unique or rarely observed characters highlights the necessity to include a geographically much wider range of samples. To understand chromosome evolution within this group of plants fully, more data, cytological and also molecular phylogenetic, are required. However, our initial study demonstrates the great diversity in nuclear characters among species and also their potential use in the systematic classification of Old World *Gesneriaceae*. This is an exciting research area that needs to make full use of developments in chromosome fluorescent *in-situ* hybridization, allowing detailed genome analyses at nucleotide level.

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