

## Cytotaxonomical Studies of the Orchidaceae from Vanuatu and its Adjacent Regions: I

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Our 1996 and 1997 Botanical Expeditions to Vanuatu and its neighboring regions provided more than 300 collections of living materials of the Orchidaceae. Living plant collections are essential for studies related to biodiversity such as systematics using both morphological and molecular characters, cytology, developmental biology, genetics, conservation, and others. We are using these collections for reexamination of orchid flora in the regions (Yukawa and Hashimoto 1998), molecular systematics (Yukawa *et al.* in press), and micropropagation for purposes of conservation (Konishi *et al.* unpublished).

In series of this study, we intend to investigate chromosomal characters of representative orchid taxa in Vanuatu and its adjacent Pacific islands, because few reports such as Jones *et al.* (1982), Lim (1985a, 1985b, 1985c), and Ishida *et al.* (1992) cover the cytotaxonomical information of the Orchidaceae in these islands.

### Materials and Methods

Table 1 shows the materials used in this study. Further data of each collection are provided by either of Hashimoto *et al.* (1998), Konishi *et al.* (1998) and Yukawa (1998). Voucher specimens and permanent slides are deposited in TNS. Root tips were harvested from the plants in cultivation and pretreated in 2mM 8-hydroxyquinoline at 20°C for two hours. Subsequently, they were fixed in 45% acetic acid at 4°C for ten minutes. They were macerated in a mixture 1N hydrochloric acid and 45% acetic acid (2 : 1) at 60°C for ten minutes, followed by 2% acetic orcein staining. The squash method was applied to investigate chromosomes at mitotic metaphase.

Table 1. Chromosome numbers of the materials observed

| Species   | Origin                  | Collection number | Chromosome number (2n) |
|---|-------------------------|-------------------|------------------------|
| <i>Coelogyne macdonaldii</i> F. Muell. & Kraenzl. | Vanuatu, Espiritu Santo | Yukawa 97-2167    | 40                     |
| <i>Dendrobium mohlianum</i> Reichb. f.            | Vanuatu, Espiritu Santo | Yukawa 97-2074    | 38                     |
| <i>Dendrobium morrisonii</i> Schltr.              | Vanuatu, Espiritu Santo | Hashimoto 88      | 38                     |
| <i>Dendrobium polysema</i> Schltr.                | Vanuatu, Espiritu Santo | Yukawa 97-2122    | 38                     |
| <i>Malaxis</i> aff. <i>brevidentata</i>           | Vanuatu, Espiritu Santo | Yukawa 97-2066    | 36                     |
| <i>Robiquetia bertholdii</i> (Reichb. f.) Schltr. | Vanuatu, Efate          | Yukawa 97-2007    | 38                     |
| <i>Pseuderia smithiana</i> C. Schweinf.           | Fiji, Viti Levu         | Sugimura 4302     | 40                     |

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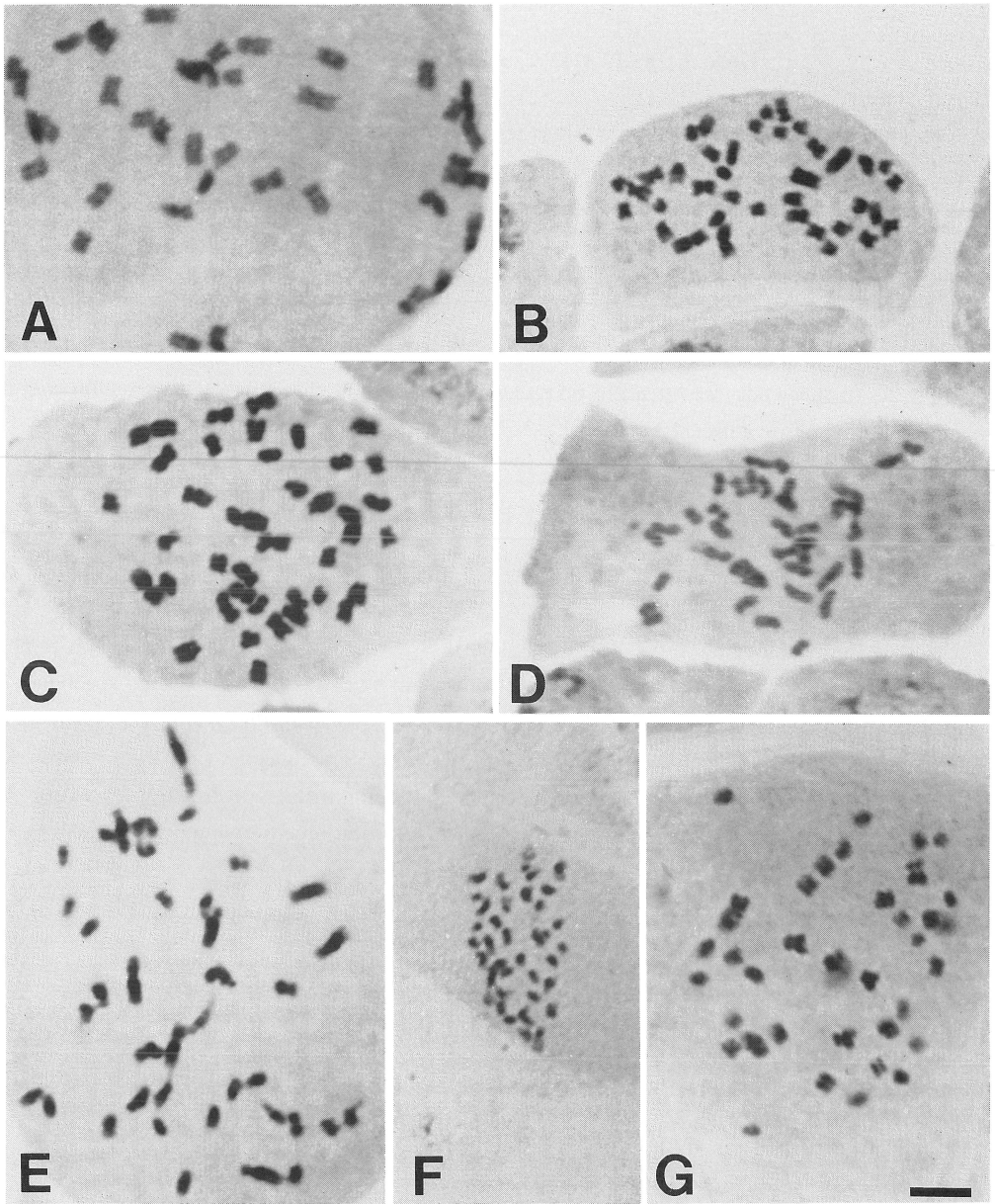


Fig. 1. Orcein-stained chromosomes at mitotic metaphase of seven species of the Orchidaceae. A. *Coelogyne macdonaldii*. B. *Dendrobium mohlianum*. C. *Dendrobium morrisonii*. D. *Dendrobium polysema*. E. *Malaxis* aff. *brevidentata*. F. *Robiquetia bertholdii*. G. *Pseuderia smithiana*. Bar = 10  $\mu$ m.

### Results and Discussion

1. *Coelogyne macdonaldii* F. Muell. and Kraenzl.,  $2n=40$  (Fig. 1A).

Lim (1985c) counted  $2n=40$  for the material from Vanuatu under a later synonymous name *C. lamellata* Rolfe. The prevailing chromosome number of this genus is  $2n=40$  (e.g. Tanaka 1964; Mehra and Kashyap 1978), but  $2n=38$  (e.g. Mehra and Kashyap 1978; Li and Chen 1989) and  $2n=44$  (e.g. Sharma 1970) were also observed. Previous counts for the species of section *Speciosae* to which the present species belongs are  $2n=40$  for *C. beccarii* Reichb. f., *C. fragrans* Schltr., and *C. macdonaldii* (Lim 1985c) and  $2n=40-44$  for *C. eberhardtii* Gagnep. (Chardard 1963).

2. *Dendrobium mohlianum* Reichb. f.,  $2n=38$  (Figs. 1B and 2).

The number  $2n=38$  coincides with the previous count of this species (Jones *et al.* 1982) as well as records of other species in section *Calyptrochilus*.

3. *Dendrobium morrisonii* Schltr.,  $2n=38$  (Figs. 1C and 3).

This species, a member of section *Pedilonum*, has not been cytologically studied. As suggested by leaf surface micromorphology (Yukawa *et al.* 1991) and DNA phylogeny (Yukawa unpublished), section *Pedilonum* is not a monophyletic group and this argument is in line with heterogeneous chromosomal features of this section which comprises the  $2n=38$  and  $2n=40$  species. Our result indicates that this species, together with species such as *D. alaticaulinum* van Royen, *D. bracteosum* Reichb. f., *D. bullenianum* Reichb. f., *D. calcaratum* A. Rich., *D. capituliflorum* Rolfe, *D. goldschmidtianum* Kraenzl., *D. pseudoglomeratum* Reeve & Woods, and *D. smillieae* F. Muell., constitutes a  $2n=38$  clade in section *Pedilonum*; this clade is also characterized by a long, fusiform stem, a dense, many-flowered inflorescence, and a simple, undifferentiated labellum with a transverse callus.

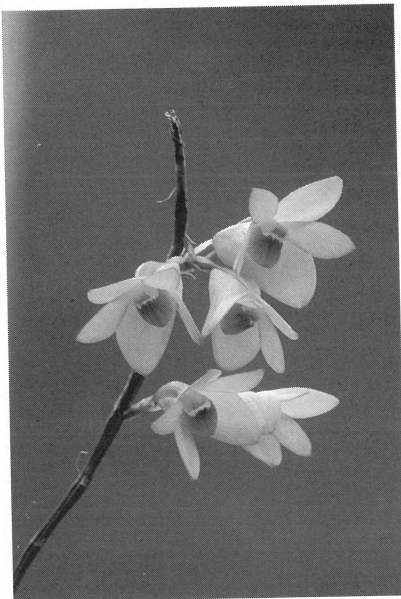


Fig. 2. *Dendrobium mohlianum* in cultivation,  $\times$ ca. 1.0. Yukawa 97-2167.



Fig. 3. *Dendrobium morrisonii* in cultivation,  $\times$ ca. 1.2. Hashimoto 88.

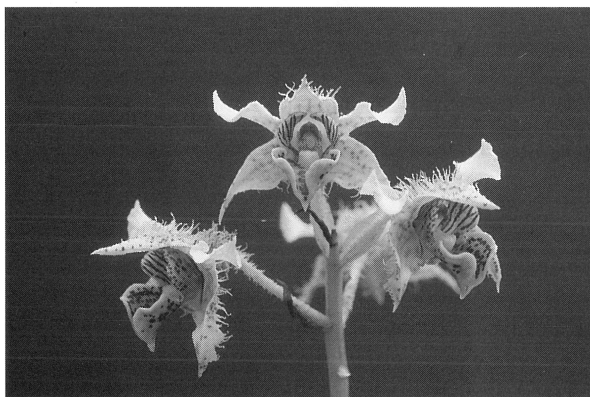


Fig. 4. *Dendrobium polysema* in cultivation,  $\times$ ca.0,7. Yukawa 97-2122.

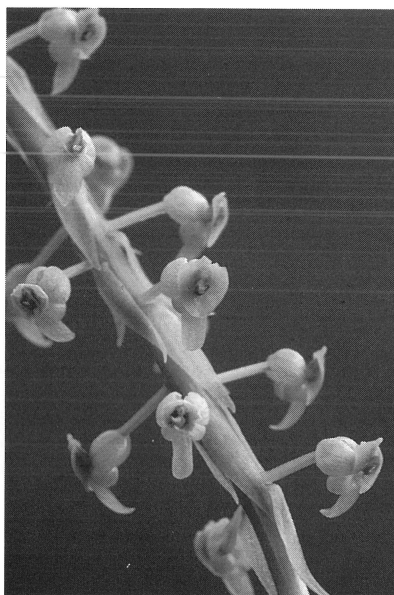


Fig. 5. *Malaxis* aff. *brevidentata* in cultivation,  $\times$ ca. 1,9. Yukawa 97-2066.

4. *Dendrobium polysema* Schltr.,  $2n=38$  (Figs. 1D and 4).

Although Jones *et al.* (1982) reported that two collections of this species from Papua New Guinea have  $2n=38+B_s?$ , we failed to find any B chromosomes. *D. macrophyllum* A. Rich., a closely related species to *D. polysema*, also shows a similar intraspecific variation:  $2n=38$  (Philippines material, Hashimoto 1987; Vanuatu material, Jones *et al.* 1982);  $2n=38+2f$  (Papua New Guinea material, Lim 1985b);  $2n=40$  (under a synonymous name *D. musciferum* Schltr., Hashimoto 1982). Coexistence of  $2n=38$  and  $2n=40$  within a single species is also known from several other *Dendrobium* species (e.g. Sau and Sharma 1983).

5. *Malaxis* aff. *brevidentata* C. Schweinf.,  $2n=36$  (Figs. 1E and 5).

Various somatic chromosome numbers have been reported for this genus such as 26, 28, 30, 36, 38, 42 and 60. It is likely that aneuploid and polyploid series play an important role on the evolution of this genus.  $2n=36$  was also recorded in *M. acuminata* D. Don (under a synonymous name *Microstylis wallichii* Lindl., Vij *et al.* 1981) and its closely related species, *M. hahajimensis* A. Kobayashi (Ono 1977; Ono and Masuda 1981). The present species may have a close relationship with other  $2n=36$  species of the genus.

6. *Robiquetia bertholdii* (Reichb. f.) Schltr.,  $2n=38$  (Fig. 1F).

We confirmed the count of Lim (1985c) where he treated this species as *R. minus* (Reichb. f.) Garay, a later synonym. All the counts hitherto for the genus *Robiquetia* show  $2n=38$ , which is also prevalent among the members of other genera of tribe Vandeeae.

7. *Pseuderia smithiana* C. Schweinf.,  $2n=40$  (Fig. 1G).

This is the first cytological record for the genus *Pseuderia*. The genus has been treated as a member of tribe Dendrobieae; Yukawa *et al.* (1993, 1996), however, elucidated that *Pseuderia* clearly belongs to tribe Podochileae, subtribe Podochilinae based on variations of chloroplast DNA restriction sites and sequences. Previously,  $2n=38$  and 40 were counted for the species in subtribe Podochilinae and our observation is concordant with the placement of the genus *Pseuderia* in subtribe Podochilinae.

### Summary

Mitotic chromosomes of seven taxa from Vanuatu and its adjacent regions were examined by the standard acetic orcein staining method. *Coelogyne macdonaldii* showed the chromosome number of  $2n=40$ ; three species of the genus *Dendrobium* studied showed that of  $2n=38$ ; *Malaxis* aff. *brevidentata* showed that of  $2n=36$ ; *Robiquetia bertholdii* showed that of  $2n=38$ ; and *Pseuderia smithiana* showed that of  $2n=40$ . The chromosome number of the genus *Pseuderia* was reported for the first time in the present study.

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