

Chromosome analysis in *Psychmorchis pusilla* (L.) Dodson & Dressier: the smallest chromosome number known in Orchidaceae

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Abstract — The neotropical genus *Psychmorchis* Dodson & Dressier has the smallest chromosome numbers registered for the orchid family. The previous counts in the genus are $n=5$, 7 for *P. pusilla* (L.) Dodson & Dressier and $n=7$ for *P. glossomystax* (Rchb. f.) Dodson & Dressier. In the present work meiosis and mitosis were analysed in two natural populations of *P. pusilla* and an *in vitro* cultivated sample. The chromosome analysis also included staining with the fluorochromes chromomycin A₃ (CMA) and 4'-6-diamidino-2-phenylindole (DAPI). All individuals analysed showed a somatic complement of 12 chromosomes. The meiotic analysis revealed regular behaviour with formation of six bivalents in the natural populations. The C-banding plus Giemsa staining revealed small centromeric bands, while the staining with fluorochromes did not show any differentially bright region. The chromosome stability in the materials studied suggests that chromosome numbers previously reported for the species may be incorrect. The reduction of $n=7$ to 6 , by Robertsonian translocation, is the mechanism that would best explain the origin of the karyotype in *P. pusilla* and suggests that $x=7$ is the most likely base number in the Oncidiinae.

Key words: C-banding, CMA/DAPI, Cytogenetics, Oncidiinae, Orchidaceae, *Psychmorchis*.

INTRODUCTION

The genus *Psychmorchis* was segregated from *Oncidium* by DODSON and DRESSLER (1972) from the section *Iridifolia* (*sensu* KRANZLIN 1922), characterised basically by lack of a pseudo-bulb, monopodial growth, and leaves laterally flattened and equitant. It includes five species distributed in Brazil and other countries of Tropical America (PABST and DUNGS 1977). *Psychmorchis pusilla* (L.) Dodson & Dressier is the species type of the genus and is equally widely distributed in the Neotropics.

The chromosome numbers reported for the genus are $n=5$ (DODSON 1957a,b; SINOTO 1962; KUGUST 1966) and $n=7$ (KUCUS 1966) in *P. pusilla* and $n=7$ for *P. glossomystax* (Rchb. f.) Dodson & Dressier (KUCUS 1966).

The Orchidaceae generally show high chromosome numbers, with $n=19$, 20 being the most frequent (JONES 1974). The chromosome

numbers reported for *Psychmorchis* are the smallest known in orchids and may represent the remaining of the original diploid stock of the family. However, there is neither a clear karyotype analysis nor a good photographic documentation of the chromosomes of *P. pusilla*. Therefore, a detailed analysis of this karyotype may contribute to a better understanding of karyotype evolution in subtribe Oncidiinae and orchids in general. The objective of the present work was to review the karyotype of this species, as well as to evaluate the importance of its chromosome number in the cytogenetics of Oncidiinae. The chromosome number was investigated in somatic and meiotic cells. The karyotype was described in terms of chromosome complement length, C-banding pattern and chromosome reacting with the fluorochromes chromomycin A₃ (CMA) and 4'-6-diamidino-2-phenylindole (DAPI). These fluorochromes preferentially stain GC-rich or AT-rich DNA sequences, respectively, and the nucleolus organiser region, which stains positively with CMA in most plant species (SCHWEIZER 1976; MORAWETZ 1986).

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MATERIALS AND METHODS

Plants collected in Belem do Para (State of Para) and Camocim do Sao Felix (State of Pernambuco) were cultivated in the experimental garden of the Department of Botany, at the Federal University of Pernambuco. Another sample cultivated *in vitro*, also collected originally in Belem do Para, was kindly supplied by Dr. Gilberto Kerbauy, from Bioscience Institute at Sao Paulo University. Samples of these materials were deposited in Herbarium Vasconcelos Sobrinho (PEUFR) of the Federal Rural University of Pernambuco and Herbarium of Sao Paulo University (SPF). For chromosome analyses, flowers buds were sectioned longitudinally and subsequently pretreated with 8-hydroxyquinoline 0.002M for 24 hours at 4 °C. The sections were then fixed in Carnoy 3:1 (ethanol/acetic acid) for a period of 3 to 24 hours and stored at -20 °C in the fixative.

For conventional chromosome analysis, the material was hydrolysed in 5 N hydrochloric acid for 30 minutes and stained with haematoxylin at 1% (GuERRA 1999). For C-banding, flower buds were digested in a cellulase (2%) and pectinase (20%) solution at 37 °C for 2 hours, then squashed on microscopic slides in 45 % acetic acid and aged for two days at room temperature. The slides were subsequently treated with 45% acetic acid at 60 °C for 10 minutes, followed by 5% barium hydroxide at room temperature for 10 minutes, 2XSSC at 60 °C for 80 minutes, and stained with 3% Giemsa prior to mounting in Entellan (SCHWARZACHER *et al.* 1980). For the staining with fluorochromes, the buds were digested in enzyme solution and squashed as described previously, aged for three days, stained with CMA 0.5 mg/ml, for 60 minutes, counterstained with distamycin 0.2 mg/ml for 30 minutes, and stained with DAPI 2 g/ml for 30 minutes (SCHWEIZER 1976). Measurement of the chromosome size was made directly from the enlarged pictures of five very dispersed metaphases stained with haematoxylin.

RESULTS

The somatic complement in each of the three samples of *P. pusilla* exhibited twelve chromosomes, of which four pairs were metacentrics and two acrocentrics (Fig. 1a), with size varying from 4.87 to 3.14 μ m. In several prophase cells the proximal chromosome segments were strongly stained, probably due to the early condensation of this region. In inter-phase the chromatin was more or less equally distributed, with dot-like chromocentres (Fig. 1b). Meiotic analysis of individuals from Per-

nambuco and Para revealed regular behaviour of the six bivalents (Fig. 1c). In pollen mitosis six chromosomes were observed. Cells treated with C-banding presented only dot-like centromeric bands in all chromosomes (Fig. 1d). In some cells the pericentromeric segments were more stained than the euchromatin. The staining with fluorochromes did not show any distinctly bright segment not even in the short arm of the smallest chromosome pair, where the NOR seemed to be located. However, in some cells the pericentromeric segments were brighter with CMA and rather homogeneously stained with DAPI (Fig. 1e, f). These brighter segments seemed to correspond to the early condensed regions of the conventionally stained prometaphase chromosomes. The idiogram in Fig. 2 summarises these data.

DISCUSSION

The detailed chromosome morphology of *P. pusilla*, as many other Orchidaceae, is difficult to visualise. JONES and DAKER (1968) observed that removal of the most external tissue of the root tips improves the contraction and visualisation of chromosome morphology. In the flower buds of *Psychomorchis*, however, it did not work. Nevertheless, many metaphase plates with $2n=12$ or $n=6$ were observed. The present record differs clearly from previous counts of $2n=10$ (DODSON 1957a, b; SINOTO 1962) and $2n=14$ (KUCUS 1966). The disagreement may be due to chromosome number variation or mistake in the previous counts. The record of DODSON (1957a,b) was based on an anaphase with little definition of the exact chromosome number, whereas SINOTO (1962, 1969) and KU-GUST (1966) did not present any photographic documentation of the analysed material.

The low chromosome number found in *Psychomorchis* has been considered as a possible base number of the subtribe Oncidiinae and of Orchidaceae in general, since it is the smallest known number in the family. DODSON (1957b) considered that $n=5$ could be the base number for Orchidaceae, but because $n=10$ and $n=20$ were more frequent in the family, he suggested $x=10$ as the most probable base number. SINOTO (1962), considering the chromosome number variation in Oncidiinae, admitted two base numbers, $x=5$ and $x=7$. CHARANASRI and

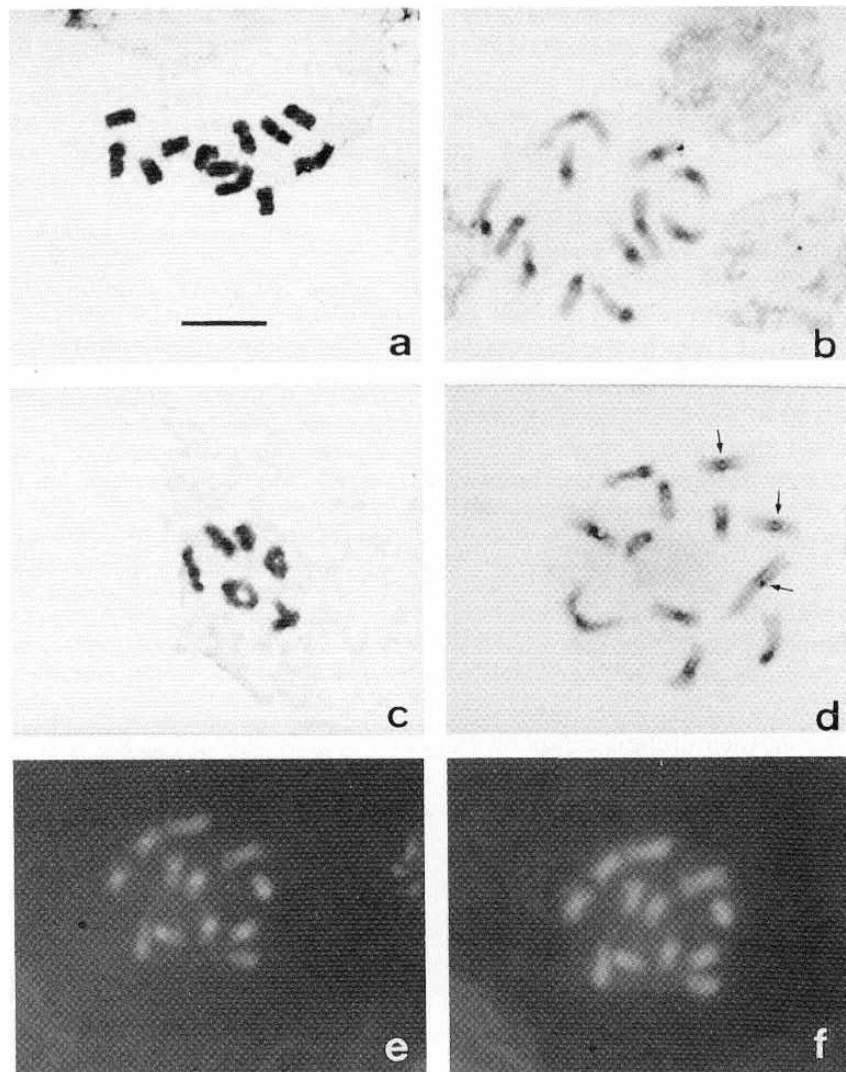


Fig. 1 — Mitotic and meiotic chromosomes of *Psygmorchis pusilla*. a. Mitotic metaphase with eight metacentrics and four acrocentric chromosomes, b. Mitotic prometaphase showing pericentromeric segment deeply stained, c. Diakinesis with six bivalents, d. Mitotic prophase displaying dot-like C-bands (arrows) and pericentromeric regions slightly more stained, e-f. Mitotic prometaphase stained with CMA (e) and DAPI (f). Bar represents 10 μ m.

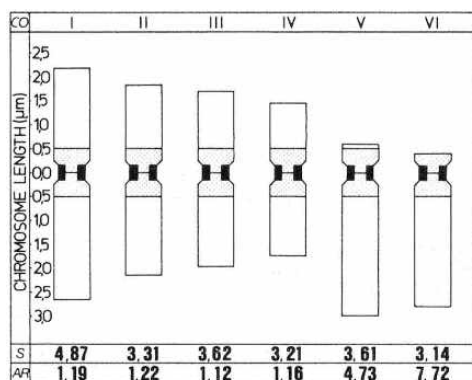


Fig. 2 — Idiogram of *Psygmorchis pusilla* with C-bands (solid blocks) and early prophase condensed regions (dotted).

KAMEMOTO (1975), based on a large amount of data, considered that *Oncidium* and its allies genera had the basic number $x=7$. More recently, Chase and collaborators (CHASE 1986; CHASE and OLMSTEAD 1988; CHASE and PALMER 1992) defended the hypothesis that the subtribe Oncidiinae has a high original base number ($x=30$ or $x=28$), which was reduced by descending dysploidy until $n=5$, 7 in *Psygmorchis*, constituting one of the largest dysploid series known in plants. However, in *Lockartia*, a genus of Oncidiinae previously placed in a separate subtribe (see CHASE and PIPPEN 1988), there are species with $n=28$ and $n=7$ (CHARA-NASRI and KAMEMOTO 1975; GARAY 1963). The

occurrence of two long dysploid series make the hypothesis of $x=30$, 28 rather improbable. Furthermore, the chromosome number variation observed in Oncidiinae is clearly indicative of a polyploid series based in $x=7$ (GUERRA, in press). Therefore, $x=7$ seems to be the most probable base number for Oncidiinae.

The karyotype with $n=6$ in *P. pusilla* most likely results from descending dysploidy of an ancestral complement with $x=7$. The lack of distal heterochromatic blocks in the karyotype of this species hinders recognition of the mechanism involved in the numeric reduction. However, the morphometric analysis and the C-banding pattern may suggest a Robertsonian translocation of two small acrocentric chromosomes into the largest chromosome pair.

The meiotic normality and the constancy in chromosome number in the populations of Pernambuco and Para, separated by more than a thousand kilometres and strong ecological barriers, suggest that $n=6$ is well established in the species. It is possible that the numbers reported by previous authors ($n=5$, 7) are not correct, since stable numeric polymorphisms at the intraspecific level, except polyploidy, are rare in Orchidaceae (see e. g. JONES and DAKER 1968; CHARANASRI *et al.* 1973; CHARANASRI and KAMEMOTO 1975). Incorrect chromosome counts are frequent in the literature, leading sometimes to cytotaxonomic misinterpretations (MERXMULLER 1970; GUERRA, in press). JONES (1974) drew special attention to those cases in which "chromosome numbers varies with authors rather than with taxon", as in *Psymorchis*. The chromosome number of *P. glossomistax* also needs confirmation, since this species seems to be derived from a natural hybrid with *P. pusilla* (VAN DER PIJL and DODSON 1966; PABST and DUNGS 1977).

Acknowledgements. — The authors greatly thank Prof. William Sanders for revision of english version, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support.

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