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***Ponthieva orchioides* SCHLECHTER (*Orchidaceae* - *Spiranθοideae*):
in vitro-propagation, culture and chromosome number**

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Abstract: DEUTSCH G. (2002): *Ponthieva orchioides* SCHLECHTER (*Orchidaceae-Spiranθοideae*): in vitro-propagation, culture and chromosome number. — *Stapfia* **80**: 327-331.

Fully developed seeds of *Ponthieva orchioides* SCHLECHTER (*Orchidaceae-Spiranθοideae*) were surface sterilized, sown on asymbiotic media and kept in darkness. After five weeks germination occurred and after one year of in vitro-cultivation, it was possible to establish young seedlings in mineral soil. After four years of cultivation in soil, first flowers appeared. The somatic chromosome number ($2n = 46$) was counted from mitotic metaphases in the ovary of young buds and is reported here for the first time for this species. Similar karyotypes and equal chromosome numbers in subtribe *Spiranθinae* and in the genus *Ponthieva* from subtribe *Cranichidinae* suggest that these subtribes could be closely related. Since selfing of flowers failed, self-sterility is assumed.

Zusammenfassung: DEUTSCH G. (2002): *Ponthieva orchioides* SCHLECHTER (*Orchidaceae-Spiranθοideae*): in vitro-Anzucht, Kultur und Chromosomenzahl. — *Stapfia* **80**: 327-331.

Reife Samen von *Ponthieva orchioides* SCHLECHTER (*Orchidaceae-Spiranθοideae*) wurden oberflächensterilisiert, auf asymbiotischem Medium ausgesät und dunkel aufgestellt. 5 Wochen nach der Aussaat keimten die Samen und nach etwa einem Jahr in vitro Kultur konnten die Sämlinge in mineralischem Substrat etabliert werden. Nach etwa 4 Jahren Erdkultur entwickelten sich erste Blütenstände. Die Chromosomenzahl ($2n = 46$) aus mitotischen Metaphasen junger Samenanlagen, konnte, erstmals für diese Art, ermittelt werden. Nachdem die Subtribus *Spiranθinae* und die Gattung *Ponthieva* (Subtribus *Cranichidinae*) den gleichen Karyotyp und die gleiche Chromosomenzahl aufweisen, dürften beide Subtriben näher miteinander verwandt sein. Da die Selbstung der Blüten fehlschlug, könnte Selbststerilität vorliegen.

Key words: *Ponthieva*, *Orchidaceae*, *Spiranθοideae*, *Cranichidinae*, in vitro-propagation, protocorm, chromosome number, self-sterility.

Introduction

Ponthieva orchioides SCHLECHTER is a terrestrial orchid belonging to the subfamily *Spiranθοideae*, subtribe *Cranichidinae*. The genus *Ponthieva* R. BR., which was named after the merchant Henri de PONTHEU, consists of about 50 species native to tropical America (MABBERLY 1997: 578) with its main distribution in the Andes.

Data on the propagation of members from this genus are rather rare. Only BAKER et al. (1987) reported on the germination of seeds from closed capsules of North Ameri-

can *Ponthieva racemosa*. No data about further growth or establishment of plants in soil were given.

Little is known about the karyology among members of subtribe *Cranichidinae* and especially of genus *Ponthieva*, since MARTÍNEZ (1985: 142-143) has investigated only one species, *Ponthieva mandonii*.

Material and Methods

In vitro-culture: Karl Robatsch collected ripe capsules of *Ponthieva orchioides*, during his excursion to Venezuela in 1996. Seeds were pre-treated in 2% H₂SO₄ for 2 minutes, then sterilized in 1% Sodium hypochlorite (NaOCl) for 5 min. Exact sterilization time was possible using a sterilization apparatus (DEUTSCH 2001). Seeds were sown on plates with modified SM media (MALMGREN 1992) and kept in darkness at 20°C until germination occurred. After germination, protocorms were sub-cultured on the same media every 2-3 months and kept under light conditions (12h) at 20°C.

Seedling establishment and further culture: After the seedlings had produced the first roots and leaves, they were planted in pots either in a mixture of Seramis and rotted bark (2:1), or in the same mixture with limestone. Pots were covered with plastic bags during the first weeks of culture, to increase humidity. Adult plants were cultivated under semi-shadow conditions at room temperature with a short resting phase with less watering.

Chromosome counts: Buds of *Ponthieva orchioides* were fixed in a mixture of alcohol-chloroform-acetic acid (5:3:1) and stored at -18°C until examination. Mitotic metaphases in young ovules were examined after staining with Carmin-acetic acid using a Reichert Polyvar microscope.

Results

After five weeks about 70% of the seeds germinated readily by rupturing of the testa and producing rhizoids. Four months later, protocorms of about 5-7 mm size produced the first roots and two months later, leaves were visible. Through regular sub-cultivation, the plants grew fast and each plant produced 2-3 fleshy roots of about 2-3.5 cm length and 3 mm in diameter and most of them produced a small rosette of 2-3 leaves of about 1-1.5 cm length. At this stage, about one year after germination, young plants were established in soil.

Because growing conditions were unknown, seedlings were potted either in slightly acidic soil or in soil with a small amount of limestone added. Both mixtures contained a high percentage of mineral compounds. Dying back of leaves and rapid loss of seedlings indicated, that alkaline soil is not suitable for cultivation of *Ponthieva*.

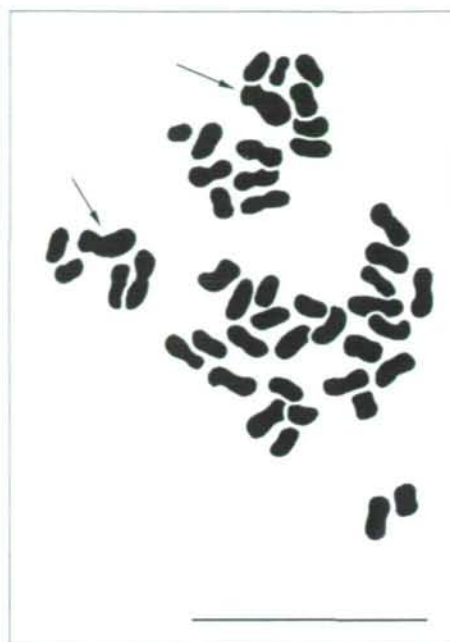


Fig. 2: Mitotic chromosomes of *Ponthieva orchioides* SCHLECHTER. $2n = 46$. Arrows indicate the two larger submeta-centric chromosomes (scale bar = 10 μm).

Fig. 1: *Ponthieva orchioides* SCHLECHTER, detail of inflorescence. Photograph taken 23.3.2001 (scale bar = 0.5 cm).

Only plants potted or re-potted in acidic soil survived and produced new leaves, forming a small rosette of about 4-5 narrow leaves. Plants were kept in a short resting phase of about two months, with less watering, after which they produced fresh leaves and inflorescences. After four years of cultivation first flowers appeared in February 2001 on an 18 cm tall inflorescence.

The small white flowers were non-resupinate and about 0.6 cm in diameter (Fig. 1). The two asymmetric petals stuck together and formed a broad labellum-like structure at the front, whereas the true labellum was rather small and three-lobed, with two broader lateral parts and a short small median part (Fig. 1), which gave the flower a resupinate appearance at first sight.

Since both individuals flowered at different times no cross-pollination was possible. No fruit set was observed on selfed flowers.

The chromosome number was determined with $2n = 46$ (Fig. 2). Two individuals were counted. Chromosomes were small with marked variation in size, the smallest being 1 μm and the largest 2.3 μm in length. Two submetacentric chromosomes were a little bit larger than the rest (Fig. 2).

Discussion

The chromosome number of *Ponthieva orchioides* is $2n = 46$, which is the same as that counted in *Ponthieva mandonii* by MARTÍNEZ (1985). This chromosome number is rather rare in *Orchidaceae*. It has been found only in *Spiranthisinae* and *Cranichidinae* (MARTÍNEZ 1985). MARTÍNEZ also recorded two acrocentric chromosomes, which were larger than the rest. Most of the *Spiranthisinae* and also *Ponthieva mandonii* possess this typical karyotype with one large pair of chromosomes (MARTÍNEZ 1985). In *Ponthieva orchioides* one pair of larger submetacentric chromosomes could also be observed, whereas the rest of the chromosomes differed in sizes. Similar karyotypes and the same chromosome numbers suggest that subtribes *Spiranthisinae* and *Cranichidinae* could be closely related.

For *Ponthieva orchioides*, self-sterility is suggested. No seed set was observed after selfing of flowers. Most species of *Orchidaceae* are self-compatible. Only for Australian *Cryptostylis* (STOUTAMIRE 1975, DEUTSCH, unpublished data) and for some *Oncidium* species self-sterility is reported (DRESSLER 1996: 122).

BAKER et al. (1987) reported germination of *Ponthieva racemosa* seeds obtained from closed capsules after eight weeks with 8-82% viability, depending on media composition. Protocorm development was only possible on VACIN & WENT media and on KNUDSON media, but only the first stages of protocorm development could be observed (BAKER et al. 1987: 82). Although ripe seeds of *Ponthieva orchioides* were used in our experiments, they germinated readily after about five weeks with a viability of about 70%. In *Ponthieva orchioides* development was rather fast. First roots were visible just after about four months and after one year, plants were successfully established in soil.

Knowledge of these conditions, which are necessary for germination and natural growth, especially of terrestrial orchids could be of great value for future conservation projects of endangered terrestrial orchid species.

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