

Gametophyte development and sporophyte formation in *Lygodium cubense* Kunth (Schizaeaceae, Pteridophyta).

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INTRODUCTION

Lygodium cubense Kunth. is a climbing fern endemic in Cuba. It is found mainly in open and sunny conditions at forest edges and in roadside vegetation. Although gametophytes of other species of the genus e.g. *L. japonicum* (Thunb.) Sw. have already been studied (Bauke, 1878a; Clarke, 1936; Klebs, 1916/17; Manabe & al., 1987; Momose, 1949; Raghavan, 1980; Raghavan & Huckaby, 1980), the biology or reproduction of *L. cubense* has not been investigated so far. The present study deals with the gametophyte development from spore germination to maturity and sporophyte formation.

MATERIAL AND METHODS

Spore material of *Lygodium cubense* was collected from fertile fronds according to Chiou & Farrar (1994) in Soroa, Pinar del Rio, Cuba in November 1996 and kept at room temperature. A voucher specimen is deposited in the Herbarium Johannes Bisse at the National Botanical Garden in Habana (HAJB, No. 73854).

Spores were sown without any pre-treatment on peat moss and cultivated under semi-sterile conditions at $(22 \pm 1)^\circ\text{C}$ and c. 3000 Lux for 10 hours per day. Spore germination, gametophyte and sporophyte development were monitored, and their successive stages documented to highlight species-specific peculiarities.

General observations were carried out with a binocular equipped with a camera (Wild Photomakroskop M400, Wild Heerbrugg). For scanning electron microscopy, gametophytes were critical point dried (Emtech K850), sputter coated with gold-palladium (Emtech Low Voltage Cool Sputter Coater K550), and studied in a scanning electron microscope (SEM, Type Philips 515).

RESULTS

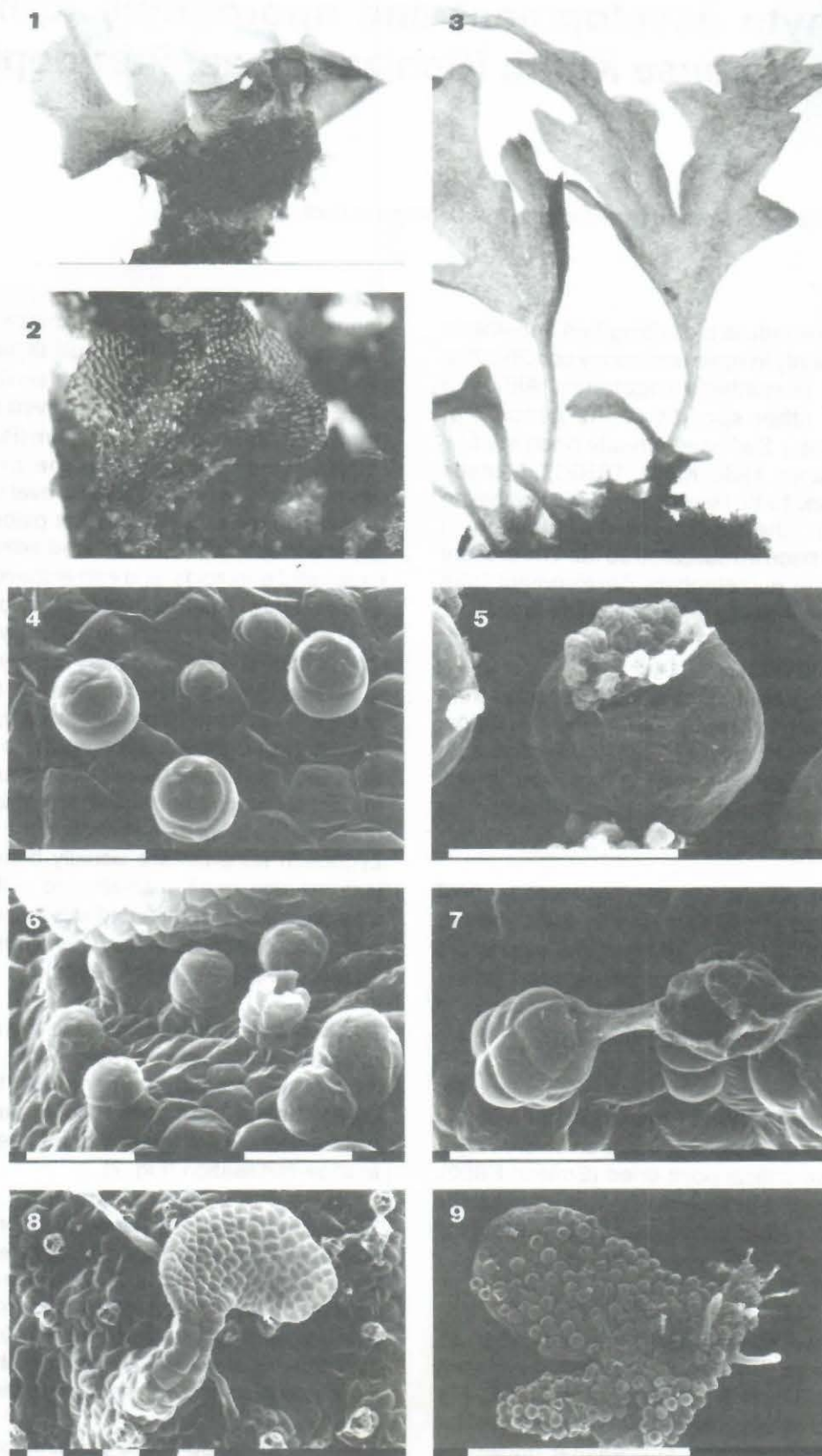
Lygodium cubense spores are light brown, trilet, tetrahedral-globose, verrucate and approximately $53 \times 127 \mu\text{m}$ in size (Tryon & Lygardon, 1991: 105, Fig. 28.5-28.6; personal observations). The germination of the spores occurred particularly successive under the conditions stated above within 11-15 days with a maximum rate at the second and third day. The percentage of germination was about 80-100%.

The so far investigated gametophytes (prothallia) of the genus *Lygodium* show great similarities. They are two-lobed and develop a thick multi-layered midrib. Young prothallia of *L. cubense* exhibit a similar unequal growth of the two lobes (Fig. 2) as observed in *L. japonicum* by Bauke (1878a) and in *L. circinatum* (Burm.) Sw. by Twiss (1910). During maturation the prothallia become symmetrically two-lobed with an oval midrib (Fig. 1). The thallus surface and margins are glabrous except of the lower surface, which is covered with long hyaline and multi-cellular rhizoids, and with antheridia and archegonia when mature. Antheridia consist of one basal cell, one ring cell, and a round cap cell (Fig. 4) which divides during maturation. The two cap cells burst at the central seam to release the spermatozoids (Fig. 5). Corresponding observations have been described by Bauke (1876) for antheridia of *L. japonicum*, contrary to Clarke (1936) who described the antheridia of *L. japonicum* as multi-cellular. In shaded places antheridia are also produced on the upper surface of the thallus. The gametophytes of *Lygodium cubense* are usually bisexual but in dense cultures also small heart-shaped prothallia occur which may be covered completely with antheridia (Fig. 9). This was also observed in *L. japonicum* by Bauke (1876; 1878a; 1878b) and Prantl (1881).

The archegonia are confined to the midrib towards the meristem surrounded by antheridia. The archegonium neck is cylindrical (Fig. 6) and bent towards the middle part of the thallus. It consists of three rows, each of four cells which are triangular in the uppermost row splitting to allow fertilisation (Fig. 7).

The cultures were observed for more than 12 months. The first sexually formed sporophyte appeared after 40 days emerging from the archegonium (Fig. 8). After approximately 80 days every second gametophyte had started sporophyte formation. Already very young sporophytes show the typical branching which leads to the characteristic climbing form of the fronds (Fig. 3).

Contrary to the 80-100% germination rate observed for fresh spores, rates were lower for stored spores. The spore samples were stored at room temperature, refrigerated at $+4^\circ\text{C}$ and at -25°C in a deep-freezer.



Figs. 1-9. *Lygodium cubense* Kunth. 1: mature prothallium (5x). 2: young prothallium (9x). 3: young sporophytes (5x). 4-5: antheridia with spermatozoids (white bar: 0,1mm). 6-7: archegonia (white bar: 0,1mm). 8: archegonium with emerging sporophyte (white bar: 0,1mm). 9: male prothallium with antheridia. (white bar: 1mm).

After 6 and 12 months of storage germination rates were about 50%. No more germination occurred after 24 months. This indicates a complete viability loss within two years independent on the type of storage.

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