

## Embryology and seedling development in *Syngonanthus rufipes* Silveira (Eriocaulaceae)

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### Abstract

Seed and germination of *Syngonanthus rufipes* Silveira are studied to clarify the infra- and intrafamilial relationships of the family. The characteristic surface of the seed of *Syngonanthus rufipes* Silveira is formed by remnants of the anticlinal walls of the inner layer of the outer integument. The outermost layer degenerates. The seed is provided with a reduced operculum. This is the first record of an operculum in Eriocaulaceae. In earlier studies of species of *Eriocaulon*, *Paepalanthus*, *Leiothrix* and *Syngonanthus* (MONTEIRO-SCANAVACCA & MAZZONI 1978; HOHENDORFF, unpublished; RAMASWAMY & AREKAL 1981; RAMASWAMY & AREKAL 1982;) the seed of Eriocaulaceae is described as inoperculate. The presence of a reduced operculum in *Syngonanthus* is of great taxonomic importance and supports the assumption of a close relationship between Xyridaceae and Eriocaulaceae. Furthermore it supports the concept of *Syngonanthus* being closer to the phylogenetic base of Eriocaulaceae than *Eriocaulon*. While a close relationship between Eriocaulaceae and Xyridaceae is assumed in many classifications, *Syngonanthus* and its relatives (mainly *Rondonanthus*) are generally seen as derived genera within Eriocaulaceae. It is supposed that the presence of an operculum is always correlated with the lack of the primary root. The present study supports the concept of HENSOLD & GIULIETTI (1991) that *Rondonanthus* is closer to the base of Eriocaulaceae than *Eriocaulon*. Further studies in *Rondonanthus* and *Syngonanthus* (probably sect. *Thysanocephalus*) have to prove whether the occurrence of an operculum is more widespread in Eriocaulaceae than generally assumed.

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### Zusammenfassung

Samen und Samenkeimung von *Syngonanthus rufipes* Silveira werden mit der Zielsetzung untersucht, neue Fakten für den Anschluß und die Gliederung der Eriocaulaceen zu finden. Die Samenoberfläche der untersuchten Art ist durch charakteristische Reste, der antiklinalen Zellwände der inneren Schicht des äußeren Integumentes gekennzeichnet. Die äußere Schicht des äußeren Integumentes degeneriert. Der Same von *S. rufipes* weist ein reduziertes Operculum auf. Dies ist der erste Nachweis eines Operculums bei Eriocaulaceen. In früheren Untersuchungen an *Eriocaulon*, *Paepalanthus*, *Leiothrix*, und *Syngonanthus* (MONTEIRO-SCANAVACCA & MAZZONI 1978; HOHENDORFF, unveröffentlicht; RAMASWAMY & AREKAL 1981; RAMASWAMY & AREKAL 1982;) wird der Same immer als inoperculat beschrieben. Der Nachweis eines Operculums bei *Syngonanthus* ist von großer taxonomischer Bedeutung und stützt die Vermutung, daß eine enge Verwandtschaft von Eriocaulaceen und Xyridaceen besteht. Weiterhin wird dadurch nahegelegt, daß *Syngonanthus* der phylogenetische Wurzel der Eriocaulaceen näher ist als *Eriocaulon*. Während eine enge Verwandtschaft von Eriocaulaceen und Xyridaceen in vielen Arbeiten vermutet wird, wurde bisher allgemein angenommen, daß *Eriocaulon* die ursprünglichste Gruppe innerhalb der Eriocaulaceen darstellt. In der vorliegenden Arbeit wird weiterhin vermutet, daß das Vorkommen eines Operculums immer zwangsläufig mit dem Fehlen der Primärwurzel verbunden ist. Die Untersuchungen stützen die Auffassung von HENSOLD & GIULIETTI (1991), daß *Rondonanthus* der Basis der Eriocaulaceen wesentlich näher ist als *Eriocaulon*. Weitere Untersuchungen an *Rondonanthus* und *Syngonanthus* (vermutlich die Sect. *Thysanocephalus*) müssen erweisen, ob das Vorhandensein eines Operculums bei den Eriocaulaceen möglicherweise weiter verbreitet ist als bisher vermutet wurde.

### Introduction

*Syngonanthus rufipes* Silv. grows in the so-called "campos rupestres" of Brazil, a special type of open grassland in the "Cadeia do Espinhaço", a mountain chain passing Minas Gerais and Bahia.

Species of *Syngonanthus* and other Eriocaulaceae are known in Brazil as "semprevivas" and are exported dried in large quantities as ornamentals. The inflorescences of some species of *Syngonanthus* are also used as "artificial flowers" to decorate juvenile Cactaceae for commerce. The material for trade is mostly collected in natural sites before the seeds are dispersed. Therefore several species have to be regarded as endangered.

Eriocaulaceae are easily to be recognized because of their Asteraceae-like scapose inflorescences. The vegetative parts often form graslike rosettes. Reproduction is generally by seeds, but some of the perennial species show also a pronounced vegetative reproduction by profliferating heads.

The embryology of Eriocaulaceae has previously been studied by MONTEIRO-SCANAVACCA & MAZZONI et al. (1978; *Leiothrix*), AREKAL & RAMASWAMY (1980; *Eriocaulon*), RAMASWAMY & AREKAL (1982; *Eriocau-*

lon). According to DAHLGREN et al. (1985), the little differentiated embryo, which develops according to the asterad-type, can be seen as a characteristic of the whole family. Studies of the seedcoat structure have been carried out by GIULIETTI et al. (1988) and revealed seed coat structures as useful and important diagnostic characters. The vegetative anatomy of many other species of Eriocaulaceae has been studied by SCATENA (1990) in order to clarify the phylogeny of Eriocaulaceae.

In the past, *Eriocaulon* and *Mesanthemum* were seen as the most primitive genera of Eriocaulaceae. This was doubted recently, and HENSOLD & GIULIETTI (1991) suggested *Rondonanthus* as the most primitive genus within the family. According to STÜTZEL (1990) bifid commissural stigmas are the primitive condition within Eriocaulaceae, which would also exclude *Eriocaulon* and *Mesanthemum* from being the most primitive genera. The present study tries to clarify this problem on the basis of embryological studies and studies of the seedling development. In addition to their taxonomic relevance, investigations on embryology including seedling development are also important to facilitate cultivation of Eriocaulaceae.

### Material and methods

The material of *Syngonanthus rufipes* Silveira was collected in the "Serra do Cipó" (Município Santana do Riacho, Minas Gerais) along the road which connects Lagoa Santana and Conceição do Mato Dentro. A herbarium specimen is deposited at the Herbarium of the Instituto de Biociências, Universidade de São Paulo (FSC 10913, SPF). To study the germination, the seeds have been sterilized and disseminated on agar supplied with the macro- and micronutrients according to LINSMAIER & SKOOG (1965). For anatomical study, samples were fixated in FAA and dehydrated with butanol according to JOHANSEN (1940) and infiltrated with paraffin. Serial sections were made using a rotation microtome and stained with fuchsine and astra blue.

### Results

The seeds of *S. rufipes* Silv. are oblong, the walls of the inner layer of the outer integument form characteristic longitudinal ribs (fig. 1). The outermost layer degenerates (as usual in most Eriocaulaceae). In longitudinal sections, the reduced embryo attached to the starchy endosperm can be seen (fig. 11). The seedcoat is formed by quadrangular cells with thin cellwalls. The cells are filled with a dark brown substance, probably phenolic substances (fig. 14). The maior part of the seed consists of the starchy endosperm. In the distal part of the seed under the micropyle the operculum can be seen. The operculum is formed by the inner integument. The mature embryo is undifferentiated or little differentiated, bell-shaped with the narrow end towards the micropyle. In the embryo

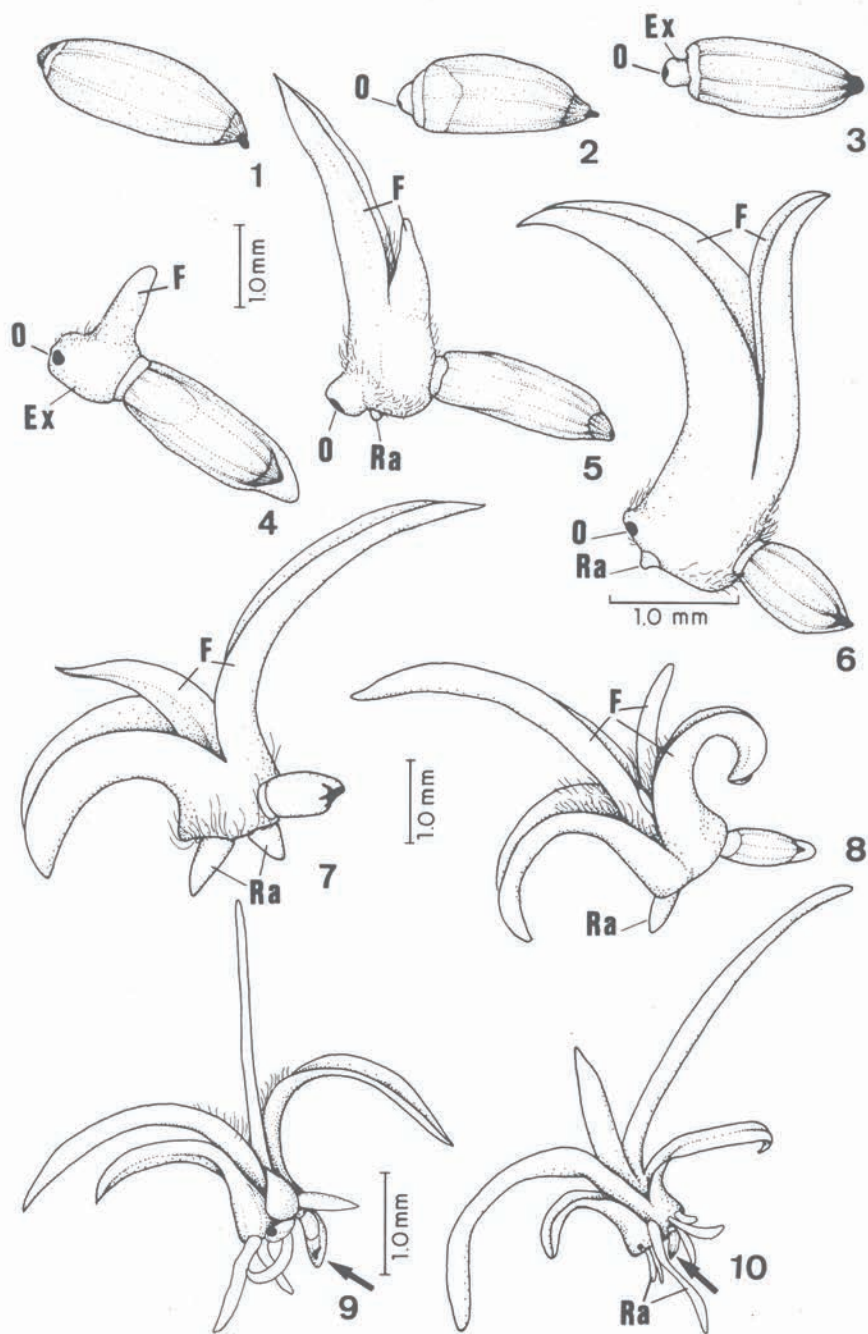


Fig. 1 - 10: Morphology of seed and seedling development of *Syngonanthus rufipes*. 1 - seed; 2 & 3 - seed 3 - 5 days after sawing; 4 & 5 - seed 12 days after sawing; 6 - 8 - plantlets 20 days after sawing; 9 & 10 - plantlets 27 days after sawing, the arrow indicates the seedcoat. (Ex - embryo axis; F - leaves; O - operculum; Ra - adventitious root)

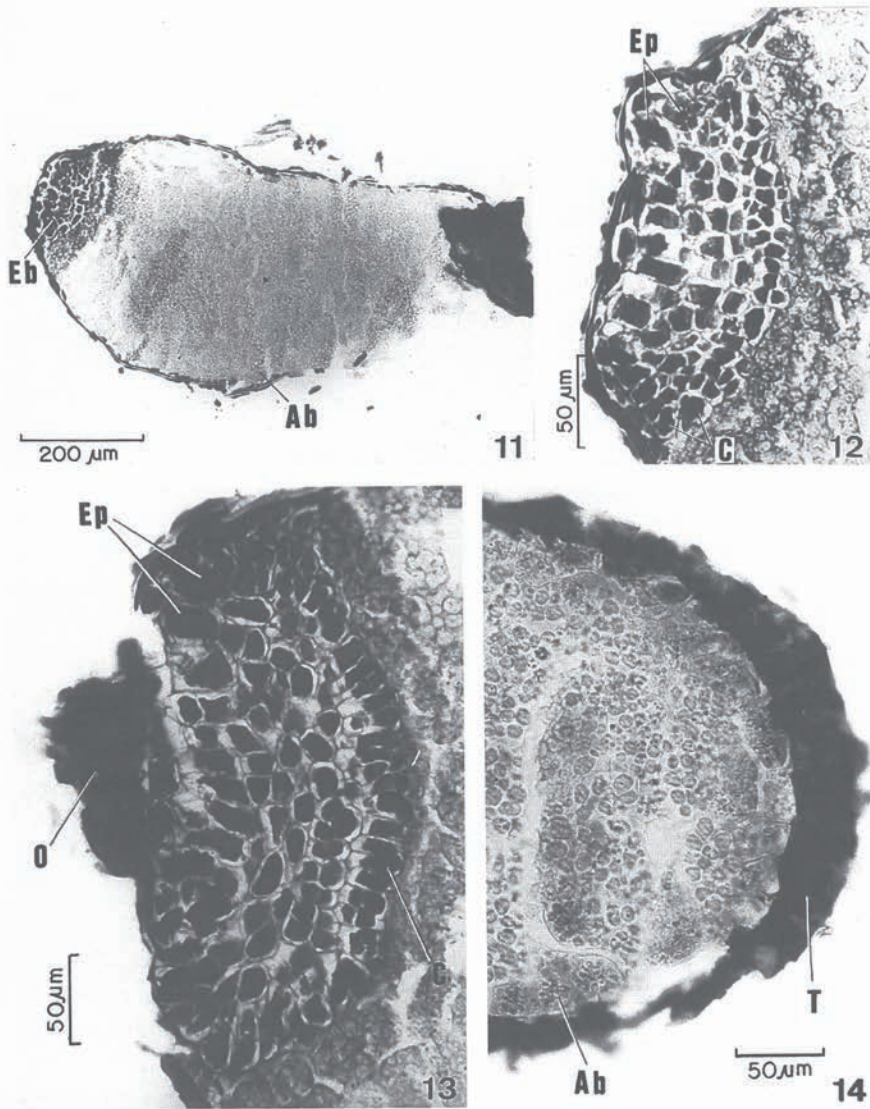


Fig. 11 - 14: seed anatomy of *Syngonanthus rufipes*. 11 - longitudinal section of the seed. 12 - embryo. 13 - operculum and embryo. 14 - posterior part of the seed. (Ab - starchy endosperm; C - area of the reduced cotyledo; Eb - embryo; Ep - epicotyledonar area; O - operculum; T - seedcoat)

two regions can be distinguished by the colour (not visible in black and white photographs) and the shape of the cells. The region which is opposite the micropyle and attached to the endosperm consists of elongated cells and represents the reduced haustorial cotyledon.

Between the third and fifth day after dissemination the germination becomes apparent (fig. 2, 3). The first part of the embryo which becomes visible is not the primary root, but the undifferentiated axis of the embryo (fig. 3, Ex) with the operculum at its distal end. After 12 days, the first leaf becomes visible (fig. 4) and the first adventitious root appears. The primary root is not formed at all. After about 20 days further leaves and adventitious roots appear. the cotyledon remains small and stays inside the seed during the whole germination process.

During germination, the cotyledonar area grows and pushes the embryo through the micropyle. In fig. 16 the operculum can be seen. Below the operculum (indicated by an arrow) one can see a group of epidermal and subepidermal cells in a position, where the primary root should be expected. The other epidermal cells near the operculum are elongated and papillose and possibly have an absorptive function similar to root hairs. They might be homologous to the hairs at the base of the hypocotyl in *Xyris*.

In the further development of the seedling, the differentiation of the first adventitious root can be seen in fig. 17, 18 (Ra). The region below the operculum now consists of dark cells which can be interpreted as the last remnants of the undeveloped primary root (Rp). The first green leaves as well as further adventitious roots develop from the axis of the embryo, which becomes now more differentiated and shows the vegetative apex and the first procambium strand (fig. 17, 18 Pr).

### Discussion

The study of seeds and germination of *Syngonanthus rufipes* shows several facts of taxonomic and ecologic importance. The embryo is very little differentiated and the cotyledon consists only of one or two rows of cells attached to the endosperm. This coincides with the observations reported by RAMASWAMY et al. (1981) and others and seems to be characteristic for the family as a whole. It is a marked difference to *Xyris*, where the cotyledon is differentiated into a haustorial part and a basal assimilatoric part. Another important fact is the development of an undifferentiated or little differentiated embryo axis during germination. This has also been reported by HARE (1950) for *Eriocaulon septangulare*. In *Xyris* the germination seems to start in the same way which shows some similarity to the situation in Orchidaceae, where a so-called "pro-

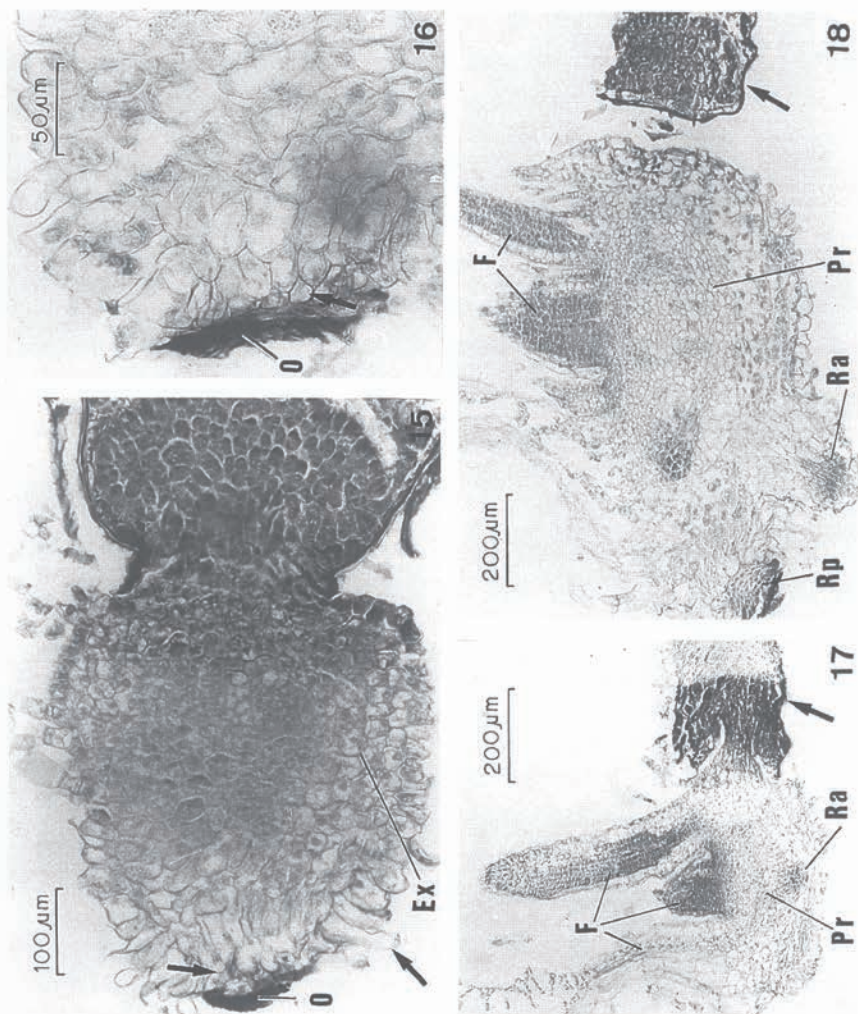


Fig. 15 - 18: Longitudinal sections of germinating seeds and young seedlings of *Syngonanthus rufipes*. 15 - seedling at the state of fig. 3, 16 - detail of the degenerated radicle. 17 - 18 stages of the seedling development corresponding to fig. 5 and 6, arrows indicate the seedcoat. (Ep - epicotyledonar area; Ex - embryo axis; F - leaves; O - operculum; Ra - adventitious root; Rp - degenerated radicle; Pr - procambium)

tocorm" develops first. The typical cormophytic organisation develops relatively late after germination. Before germination, the embryo shows no shoot apex and no root apex. In this paper, we use the term embryo axis proposed by RAMASWAMY et al. (1981). From this embryo axis the leaves and the adventitious roots develop in the further steps after germination.

While the reduction of the embryo to a degree in which all characteristics of cormophytic organisation are missing may have developed in several groups independently (e.g. Orchids), the presence of an operculum seems to be of great taxonomic importance. An operculum has never been reported in Eriocaulaceae before, despite the detailed studies of embryology and germination by HARE (1950) on *Eriocaulon septangulare* RAMASWAMY et al. (1981) on *E. robusto-brownianum* and on *Leiothrix fluitans* by MONTEIRO SCANAVACCA & MAZZONI (1978).

Probably the operculum reported here for *Syngonanthus rufipes* corresponds to a structure called "caruncula" in *Leiothrix fluitans* by MONTEIRO SCANAVACCA & MAZZONI. This caruncula is a prominent thickening of the inner integument in the micropylar region. If it is sklerified, such a caruncula might function like an operculum, because the germinating seedling cannot penetrate the endostome and has to push away the caruncula as a whole like an operculum.

The presence of an operculum in Eriocaulaceae might be a further synapomorphy for Xyridaceae and Eriocaulaceae. It is of some importance, that the operculum in Eriocaulaceae is obviously absent in *Eriocaulon* and present in a primitive group of *Syngonanthus*. If Xyridaceae are accepted as the sistergroup of Eriocaulaceae, this would be another fact to support the concept of HENSOLD (1991) stating that not *Eriocaulon*, but the syngonanthoid genus *Rondonanthus* might be closest to the base of Eriocaulaceae.

Finally, the fact that the primary root does not develop in *Syngonanthus* seems to be of phylogenetic importance. According to YAMASHITA (1983, 1991), in most angiosperms the first root is of exogenous origin and the radicle is always exogenous. According to RAMASWAMY et al. (1981) the seedling of *Eriocaulon robusto-brownianum* "lacks a true radicle" and the first root to appear (called "primary root" by them) seems to be of endogenous origin and develops after the first two folious leaves have been formed. The lack of the radicle in *Syngonanthus* is probably a consequence of the presence of the operculum. A root apex covered with a hard structure like an operculum will not be able to penetrate the soil. The root cap of terrestrial roots is always mucilaginous. Probably the first root in *Eriocaulon* has to be interpreted as the first lateral root and



not as the radicle. If this interpretation is accepted the development of the seedling would be uniform within the Eriocaulaceae and the lack of a primary root in *Eriocaulon* could be interpreted as reminiscence of an operculum which has been lost during evolution. The suggested correlation of the presence of an operculum and the lack of a primary root appears to be clear from the functional aspect and leads to consistent phylogenetic interpretations. Therefore this statement needs further investigation including other taxa with reduced or lacking primary root, e.g. grasses.

Our study on embryology and seedling development in Eriocaulaceae has revealed some important new facts for the interpretation of the phylogeny of the family and their related groups. But from the study also new questions arise, which need further investigation. Thus the homologies between the seedlings of Eriocaulaceae and Xyridaceae are still uncertain. Based on his study on gynoeceal nectaries and pollenmorphology, STÜTZEL (1990) assumes the Xyridaceae Abolbodoideae to be a sistergroup for Eriocaulaceae. But most data on embryology and seedling development are only available for *Xyris* and are still lacking for *Abolboda*. Comparative studies of the genera *Abolboda* (Xyridaceae), *Syngonanthus* and *Rondonanthus* (Eriocaulaceae) are needed to verify the phylogenetic relationships based on the results of the present study. Additional studies must include also other families of the Commelinales to make sure, that the characters used here are no plesiomorphies.

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