ISSN 2526-1746

JOURNAL OF
GEOESPATIAL MODELLING

UNVEILING THE ENIGMA OF LIVERWORTS IN CANGAS: INTRACAPSULAR GERMINATION AND LIPID RESERVES IN TWO *CHEILOLEJEUNEA* (SPRUCE) STEPH. (LEJEUNEACEAE) SPECIES

Pablo Oliveira Santos[1]*; Mateus Fernandes Oliveira[1]; Orvalina Augusta Teixeira[1], Adaíses Simone Maciel-Silva[1]

[1]Federal University of Minas Gerais, UFMG. Institute of Biological Sciences. Belo Horizonte/MG, Brazil.
*pablo.santos.professor@gmail.com

Key words:
Endospory; reproduction, adaptive strategies.

ABSTRACT

Intracapsular germination of spores can occur in the three phyla: Bryophyta, Marchantiophyta, and Anthocerothophyta. This strategy allows for the protected development of young plants during the early stages. However, knowledge is scarce on the germination and establishment of the species *Cheilolejeunea xanthocarpa* (Lehm. & Lindenb.) Malombe and *Cheilolejeunea discoidea* (Lehm. & Lindenb.) Kachroo & R.M. Schust, making it difficult to understand their life history and ecology. Therefore, the goal of this study was to record the intracapsular germination of these two liverwort species found in areas of Cangas. Three colonies of *C. xanthocarpa* and *C. discoidea* were collected in Cangas, in the Serra da Calçada Natural Monument, Minas Gerais. The samples were collected and cultivated on climatized room. After one week, the mature sporophytes were analyzed, and the released spores were stained with Sudan Black and Sudan Red to identify lipid reserves. During the analysis, different stages of development of the sporophytes were observed. The colonies showed a significant amount of mature sporophytes, containing sporelings with multiple cells, lipid reserves, and rhizoids. These lipid reserves found in spores and sporelings indicate an energy reserve for the establishment of juvenile gametophytes. These characteristics are advantageous for the effective establishment of plants on tree trunks and branches. Our understanding is that the larger spores make it difficult to disperse by wind over long distances. The discovery of intracapsular germination and lipid reserves in the protonemas of these species raises questions about the adaptive strategies of these plants for establishment under stressful conditions. This information is important for understanding the reproductive biology of liverworts and their ecology. Furthermore, considering the diversity of bryophyte species in Brazil and the lack of studies on the reproductive biology of these species, this study contributes to filling knowledge gaps in these plants.

INTRODUCTION

The intracapsular germination of bryophyte spores, mostly also endosporic (i.e., development of the initial gametophyte within the exospore), is a recognized characteristic in liverworts, mosses, and hornworts (SCHUSTER, 1988; KÜRSCHNER, 2004; SCHUETTE and RENZAGLIA, 2010; ALFAYATE et al., 2013; SILVA-E-COSTA and LUIZI-PONZO, 2019).

It is considered an important strategy for many epiphytic taxa, helping to avoid frequent desiccation of plants in this type of niche (SCHUETTE and RENZAGLIA, 2010). In ferruginous rocky outcrops, locally called Cangas, the vegetation presents adaptations to deal with frequent stresses such as high solar radiation, low humidity, and strong winds (JACOBI et al., 2007; JACOBI and CARMO, 2008). Adaptations such as intracapsular germination of spores, as well as endospory, favor the establishment of epiphytic bryophytes, as young plants are protected from the external environment in their first stages of development (SILVA-E-COSTA and LUIZI-PONZO, 2019; PEÑALOZA-BOJACÁ et al., 2023).

Among the liverwort species commonly found in Cangas's habitats are species of the genera *Cheilolejeunea* (Spruce) Steph., *Frullania* Raddi., *Lejeunea* Lib., and *Metzgeria* Raddi (OLIVEIRA et al., 2021). Asexual reproduction through buds and deciduous parts is common in many species of bryophytes in Cangas, but liverworts are very efficient in completing their sexual cycle and forming sporophytes and spores (PEÑALOZA-BOJACÁ et al., 2018). From this perspective, the present study aimed to record the intracapsular germination of two liverwort species commonly found in Cangas: *Cheilolejeunea xanthocarpa* (Lehm. & Lindenb.) Malombe and *C. discoidea* (Lehm. & Lindenb.) Kachroo & R.M. Schust.

The spores of these species, as well as many others in the Lejeuneaceae family, do not have precise data on their germination and establishment, making many studies on the life history and ecology of these species difficult. In the bryological literature, it is said that all members of the Lejeuneaceae family have intracapsular germination (SCHUSTER, 1988), but this information is superficial, and little is known about the autoecology of each species, especially in the tropics.

Furthermore, since newly germinated spores, still within the exospores, can experience months of desiccation until the plants are completely established on the substrate, we investigated the presence of lipid reserve in spores.

MATERIAL AND METHODS

Three colonies of both species of *Cheilolejeunea* were collected in April 2019. The samples were on living trunks located in the Cangas of the Serra da Calçada Natural Monument, Minas Gerais (20°05'43"S and 43°58'59"W).

Using a spatula and pocket knife, the colonies were removed from the substrate and stored in airtight plastic bags. In the laboratory, the plants were kept moist and acclimatized under cultivation room conditions (temperature between 16.2°C - 22.7°C, photoperiod of 12 hours, and light intensity of approximately 24 $\mu mol.m^{-2}.s^{-1}$). Some plant branches were removed from their colonies and observed under a stereomicroscope and optical microscope to confirm the species, following Gradstein et al. (2001) and references therein.

After one week, the plants were analyzed under a stereomicroscope to detect mature sporophytes; these were kept at room temperature overnight and analyzed the following day to record probable intracapsular germination (i.e., within the sporangium). Under a stereomicroscope, the plants

were photographed to record the exact moment of sporangia dehiscence. Spores released from newly dehiscent sporangia were analyzed on slides under an optical microscope. To identify lipid reserves, the spores were stained with Sudan Black and Sudan Red following the methodology proposed by Lison (1960) and Jensen (1962), respectively.

RESULT AND DISCUSSION

We observed different stages of development of the sporophytes of *Cheilolejeunea xanthocarpa* and *C. discoidea*, including the perianth (i.e., the organ of leaf origin that surrounds the archegonia; **Fig. 1A**), mature sporophytes (**Fig. 1B-D**, indicated by white arrows), and open capsules (**Fig. 1B** and **1D**, indicated by red arrows).

Both *C. xanthocarpa* and *C. discoidea* colonies had many mature sporophytes, which opened full of newly germinated spores (i.e., sporelings). Still within the sporangia, sporelings with more than three cells were observed, with a large central lipid reserve and the presence of rhizoids (**Fig. 1E-G**). Lipid reserves were confirmed in the protonemas (newly sporelings) of both species (**Fig. 1H-K**), indicating that these diaspores have an energy reserve for the establishment of juvenile gametophytes. These lipid reserves have already been found in moss spores (a related group of liverworts), where the hypothesis that they serve as an energy reserve just like the endosperm of seeds was proposed (GELLERMAN, 1972).

When studying the life history of organisms, conflicting demands are very common. As an example, a consequence of producing larger offspring is reducing the ability to disperse over long distances. In fact, the sporelings of the two liverwort species studied, as well as others common in Cangas, are at least two to three times larger than the spores considered small in bryological literature (e.g., 25 μm; DURING, 1979), which probably makes it difficult to disperse by wind over long distances.

On the other hand, within the Canga environment, the observed strategies of intracapsular germination, endospory, and lipid reserves of the protonemas of liverwort species, of the genus *Cheilolejeunea*, must be extremely advantageous for the effective establishment of new plants on trunks and branches of Canga trees and bushes.

CONCLUSION

This study is important for understanding aspects of the reproductive biology of liverworts, revealing some of their ecology and life history. Determining that *Cheilolejeunea xanthocarpa* and *C. discoidea*, collected in Cangas, present intracapsular germination, and that their protonemas have lipid reserves raises hypotheses about the adaptive strategies for the establishment of these epiphytic liverworts under stressful conditions.

Since there is a great diversity of bryophyte species in Brazil, studies on the reproductive biology of these plants are extremely necessary as many species remain unexplored.

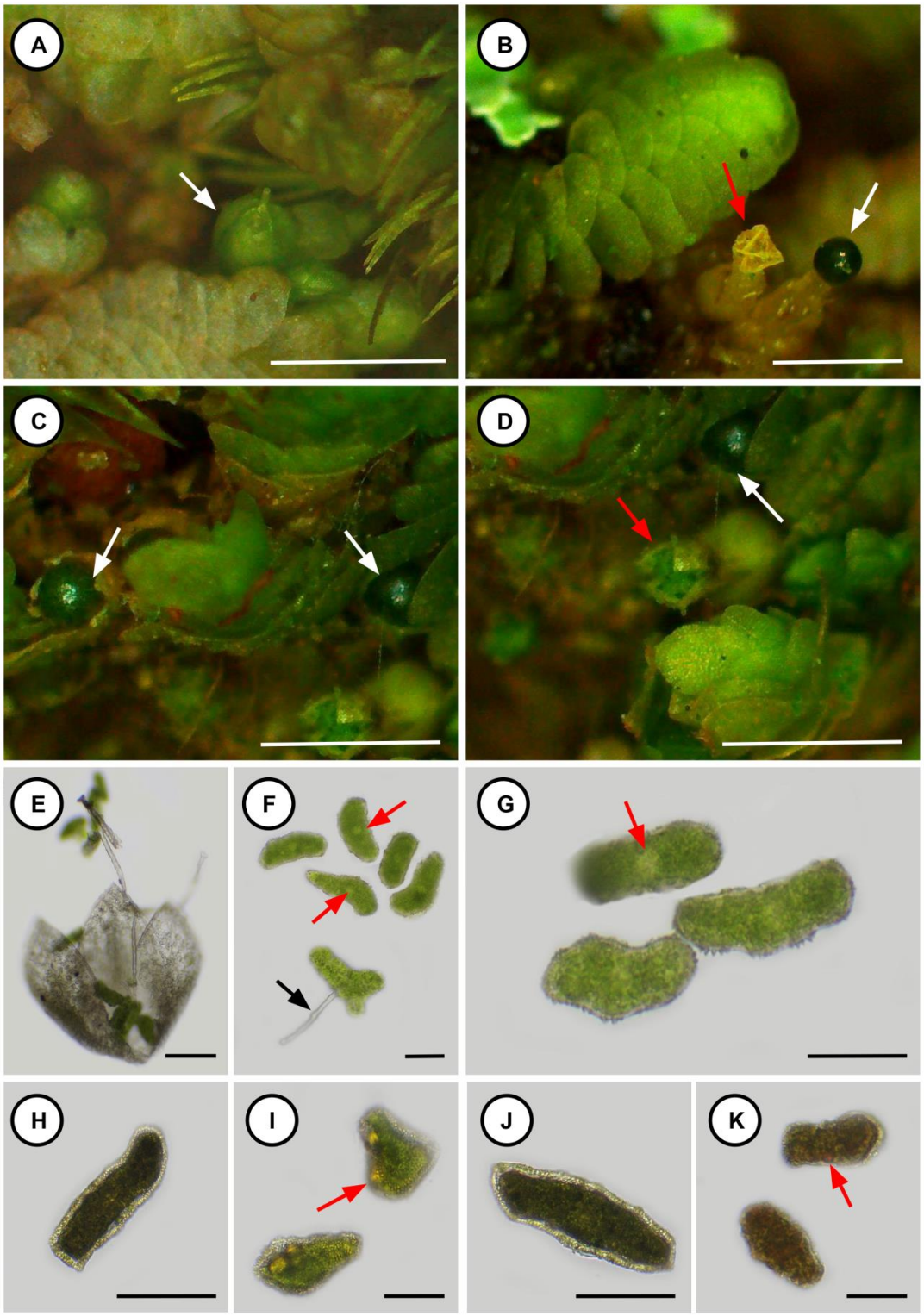

Figure 1. Sporophytes and protonemas of *Cheilolejeunea xanthocarpa* and *C. discoidea*. **A**- Perianth (arrow) of *C. xanthocarpa;* **B**- *C. xanthocarpa* sporophytes (red arrows = dehiscent sporangium; white arrow = unopened sporangium); **C-** *C. discoidea* sporophytes (white arrow = unopened sporangium); **D-** Many spores from a recently opened sporangium (red arrow) of *C. discoidea*; **E-** Sporangium with four valves and elateria of *C. xanthocarpa*; **F-** Spores extracted from an unopened sporangium (red arrows indicate a large oil body) and protonema collected from an open sporangium, with rhizoids (black arrow) of *C. xanthocarpa*; **G-** Spores extracted from an unopened sporangium (red arrows indicate a large oily body) of *C. discoidea*; **H-** *C. xanthocarpa* spore stained with Sudan Black (note some black microbodies); **I-** Spores of *C. xanthocarpa* stained Sudan Red (note some large, yellow bodies); **J-** *C. discoidea* spore stained with Sudan Black (note some black microbodies); **K-** Spores of *C. discoidea* stained Sudan Red (note some large bodies, orange in color). White scale bar = 2 mm; black scale bar = 25µm.

ACKNOWLEDGMENTS

The authors would like to thank FAPEMIG (00395-14) and CNPQ (459764/2014-4) for their financial support.

REFERENCES

Alfayate, C.; Ron, E.; Estébanez, B.; Pérez-Batista, M.Á. Mature spores of four pleurocarpous mosses in the Canary Islands: ultrastructure and early germination stages. **The Bryologist**, v. 116, n. 2, p. 97-112, 2013.

During, H. J. Life strategies of bryophytes: a preliminary review. **Lindbergia**, p. 2-18, 1979.

Gellerman, J. L.; Anderson, W. H.; Schlenk, H. Highly unsaturated lipids of *Mnium*, *Polytrichum*, *Marchantia*, and *Matteuccia*. **Bryologist**, p. 550-557, 1972.

Gradstein, S. R.; Churchill, S.P.; Salazar-Allen, N. Guide to the bryophytes of tropical America. **Memoirs-New York Botanical Garden**, 2001.

Jacobi, C. M.; Carmo, F. F. The contribution of ironstone outcrops to plant diversity in the Iron Quadrangle, a threatened Brazilian landscape. **AMBIO: A Journal of the Human Environment**, v. 37, n. 4, p. 324-326, 2008.

Jacobi, C. M.; Carmo, F. F. Plant communities on ironstone outcrops: a diverse and endangered Brazilian ecosystem. **Biodiversity and Conservation**, v. 16, p. 2185-2200, 2007.

Jensen, W. A. Botanical histochemistry. **WH Freeman and Company, San Francisco**, 1962.

Kürschner, H. Intracapsular spore germination in *Brachymenium leptophyllum* (Mull. Hal.) A. Jaeger (Bryaceae, Bryopsida) - an achorous strategy. **Nova Hedwigia**, p. 447-451, 2004.

Lison, L. Histochemie et cytochemie animales: principles et méthodes. Paris: Gaulthier Villars, 1960.

Oliveira, B. A.; De Oliveira, M. F.; Maciel-Silva, A. S. What can bryophyte diversity on Cangas (ironstone outcrops) teach us? **Journal of Vegetation Science**, v. 32, n. 3, p. e13029, 2021.

Peñaloza-Bojacá, G. F.; De Oliveira, B. A.; Araújo, C. A. T.; Fantecelle, L.B.; Maciel-Silva, A. S. Bryophyte reproduction on ironstone outcrops: delicate plants in harsh environments. **Flora**, v. 238, p. 155-161, 2018.

Peñaloza-Bojacá, G. F.; Vilas-Boas, T.; Villarreal A, J. C.; Maciel-Silva, A. S. Differential Effects of Desiccation on Hornworts with Contrasting Life Histories in Tropical Montane Forests: A Functional Trait—Based Perspective. **Forests**, v. 14, n. 2, p. 255, 2023.

Schuette, S.; Renzaglia, K. S. Development of multicellular spores in the hornwort genus *Dendroceros* (Dendrocerotaceae, Anthoceroto phyta) and the occurrence of endospory in Bryophytes. **Nova Hedwigia**, v. 91, n. 3, p. 301, 2010.

Schuster, R. M. Ecology, reproductive biology and dispersal of Hepaticae in the tropics. **The Journal of the Hattori Botanical Laboratory**, v. 64, p. 237-269, 1988.

Silva-e-Costa, J. C.; Luizi-Ponzo, A. P. Spores of *Plagiochila* (Dumort.) Dumort.: the taxonomic relevance of morphology and ultrastructure. **Acta Botanica Brasilica**, v. 33, p. 391-404, 2019.