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ADVANCING OUR UNDERSTANDING OF THE REPRODUCTIVE BIOLOGY OF *BRACHYMENIUM CONSIMILE* (MITT.) A. JAEGER (BRYACEAE, BRYOPHYTA)

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ABSTRACT

Bryophytes offer valuable insights into plant life histories, encompassing diverse reproductive systems. However, comprehensive data on tropical species, especially those inhabiting rare and threatened environments like ferruginous rocky outcrops, remain scarce. Here, we investigate the reproductive biology of *Brachymenium consimile* (Mitt.) A. Jaeger, with a focus on its sexual system. Our investigation reveals a wide spectrum of sexual expressions in gametophytes, signifying a polyoicous sexual system, thereby challenging prior dioicous classifications. These findings enrich our understanding of bryophyte reproduction, underscoring the imperative for further research in this domain.

INTRODUCTION

Bryophytes, a taxonomic group characterized by shared traits as the predominance of the gametophyte phase in their life cycle, poikilohydria, and reproduction via spores and asexual diaspores (GLIME, 2017), represent exceptional models for investigating plant life histories (MACIEL-SILVA et al., 2013). Within this group, which encompasses mosses, liverworts, and hornworts, various sexual systems are observed: (I) monoicous, where both male and female structures coexist on a single gametophyte; (II) dioicous, in which distinct sexes are found on separate gametophytes; and (III) polyoicous, where a single species manifests both sexual systems (MACIEL-SILVA and PÔRTO, 2014).

Mosses, constituting the most diverse subset within bryophytes (COX et al., 2014), exhibit a notable prevalence of dioicous taxa, comprising over half of all documented species (WYATT, 1982). Intriguingly, the Bryaceae family serves as an example where all three sexual systems coexist, as evident in genera like *Ptychostomum* Hornsch., *Rosulabryum* J.R. Spence, and *Brachymenium* Schwägr. (SPENCE, 2014), where species can exhibit monoicous, dioicous, or polyoicous reproductive strategies. However, comprehensive information regarding the reproductive biology of tropical bryophyte species, particularly those inhabiting rare and threatened environments, remains notably deficient.

In the specific case of *Brachymenium consimile* (Mitt.) A. Jaeger, the sexual system of this epiphytic plant, typically found in fragmented forest patches within ferruginous rocky outcrops, locally called *Cangas* in Brazil (OLIVEIRA et al., 2021), remains shrouded in uncertainty. Existing literature on the genus *Brachymenium* presents inconsistent descriptions of this species. While some authors exclusively designate it as dioicous (OCHI, 1980; SPENCE, 2014; CANESTRARO and PERALTA, 2022), there are accounts of it being either dioicous (ALLEN, 2002) or autoicous (a form of monoicous in which archegonia and antheridia occur on separate branches of the same gametophyte; MACIEL-SILVA and PÔRTO, 2014).

Hence, the main goal of this study was to elucidate the reproductive biology of the moss *B. consimile*. Specifically, we aimed to determine its sexual system, investigate the presence of sexual dimorphism, quantify the spore production per sporangium, and explore the occurrence of anisospory (characterized by spores exhibiting a bimodal size distribution, where smaller spores develop into male gametophytes; MOGENSEN, 1981).

MATERIAL AND METHODS

In May 2018, three colonies of *B. consimile* were collected from a *Canga* in the Serra da Gandarela National Park, Minas Gerais, Brazil (20°05’13”S and 43°41’11”W). These colonies were situated on different phorophytes and were approximately two meters apart. The collection was carried out meticulously using a spatula and a knife to carefully detach the colonies from living tree trunks. Subsequently, the collected specimens were placed in paper bags for transportation.

Upon arrival at the laboratory, the plant material underwent examination under both a stereomicroscope and an optical microscope to confirm the species, following OCHI (1980) and ALLEN (2002). Specimens from each colony were then deposited in the BHCB herbarium (vouchers 194777, 194780, and 194781).

To determine the sexual system, we analyzed one hundred gametophytes from each colony under a stereomicroscope and an optical microscope. This analysis aimed to document sexual expression, including the presence of archegonia and/or sporophytes (♀), antheridia (♂), or both (♀ and ♂). Additionally, we measured the length of each plant to investigate the possibility of dimorphism.

For estimating the number of spores produced per sporangium, we selected four mature sporophytes from each sample and employed the method described by MACIEL-SILVA et al. (2014). This method involved thoroughly macerating each capsule in a droplet of distilled water (0.2 mL) using a glass rod on a glass slide. A sample of this homogenized mixture of spores and water was collected using a Pasteur pipette and added to a Neubauer counting chamber. Spores were counted in four repetitions of 0.0001 mL, allowing us to estimate the number of spores in each capsule.

Furthermore, we examined another sample under an optical microscope equipped with an ocular micrometer to record the largest diameter of at least 100 fresh spores per sporangium. Data processing and analysis were performed using Statistica software, version 7.0 (STATSOFT, 2004).

RESULTS AND DISCUSSION

Among the 300 observed gametophytes, our analysis revealed the presence of female plants (47%), male plants (12%), bisexual plants (29%), and plants with no sexual expression (12%). These findings strongly suggest that *B. consimile* possesses a polyoicous sexual system (**Figure 1A**), as opposed to a strictly dioicous or monoicous one. Furthermore, among the gametophytes with sporophytes (205), 61% were female, and 39% were bisexual. This observation underscores the significant influence of sexual systems on the reproductive parameters within the life history of various organisms (LONGTON, 1992).

In bryophytes, monoicous species are recognized to exhibit higher sexual expression and reproductive success compared to dioicous species, mainly because of the closer proximity of sexes (LONGTON, 1992; MACIEL-SILVA and VÁLIO, 2011). Conversely, dioicous species benefit from cross-fertilization, despite generally having lower reproductive success (BISANG et al., 2004).

For polyoicous species like *B. consimile*, our study addresses a substantial gap in knowledge concerning their reproductive biology (HOCK et al., 2009), emphasizing the necessity of such investigations to enhance our understanding of the functioning, dynamics, and ecological significance of this particular reproductive system.

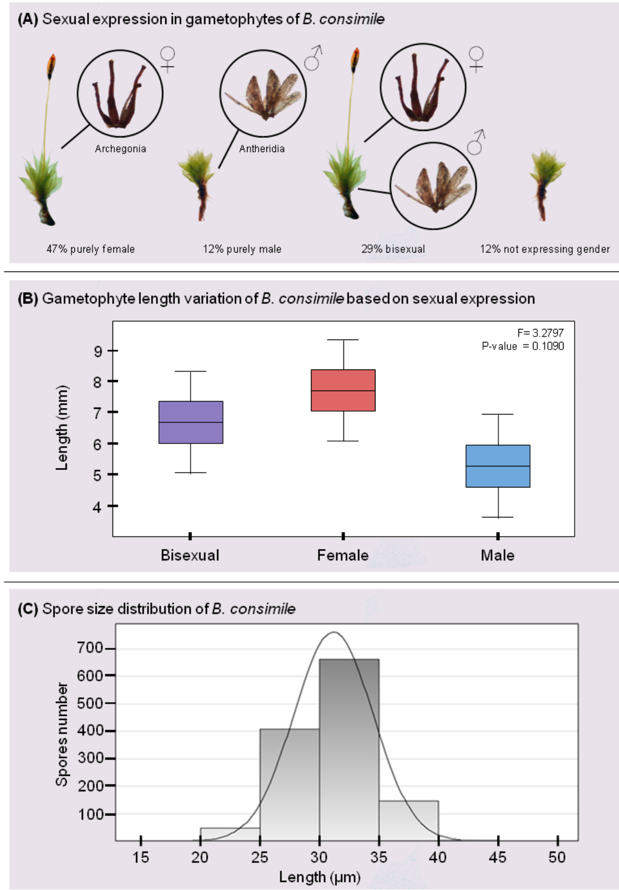

Figure 1. Insights into the reproductive biology of *Brachymenium consimile*. (A) Sexual expression in gametophytes. (B) Gametophyte length variation based on sexual expression. (C) Spore size distribution.

Regarding sexual dimorphism, while female and bisexual plants exhibited slightly higher average sizes (7.6mm *vs.* 6.7mm, respectively) compared to male plants (5.7mm), statistical analysis did not reveal a significant difference (**Figure 1B**).

Based on our data, we cannot definitively conclude the presence of clear dimorphism among the various sexual forms of *B. consimile*. Nevertheless, it's possible that such a pattern may exist in nature, as demonstrated in other mosses (e.g., NEWTON, 1971; SANTOS et al., 2018), provided a more extensive sampling of colonies and plants (exceeding 300) is conducted.

Finally, it's noteworthy the spores of this species are considered large, with an average size of 31µm, categorizing them as "large" (i.e., >25µm, DURING, 1979). Our analysis did not reveal distinct size classes among the spores (**Figure 1C**), which ranged from 18 to 45µm. On average, each sporangium contained an estimated 96,250 spores, with a minimum count of 60,000 and a maximum count of 235,000. The size and abundance of spores are critical aspects of the reproductive strategies employed by bryophytes (JOENJE and DURING, 1977). Even larger spores, such as those of *B. consimile*, are capable of dispersal over long distances through wind transport or by attaching to animals (FRAHM, 2009).

CONCLUSIONS

The novel insights obtained from this study regarding the reproductive biology of *B. consimile* represent a significant contribution to our comprehension of the understudied polyoicous sexual system within bryophytes. Furthermore, the confirmation of polyoicy in this species has raised intriguing new questions: (I) What is the significance of the coexistence and the relative contributions of different sexual states in the maintenance of *B. consimile* populations? (II) Is there temporal variation in sexual expression (female, male, and bisexual) within *B. consimile* populations?

Future investigations in this domain of bryology are imperative not only to address these specific inquiries but also to elucidate broader patterns pertaining to polyoicy in bryophytes.

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