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WAGNER LUIZ DOS SANTOS

ECOLOGIA DE MUSGOS ACROCÁRPICOS TROPICAIS: REPRODUÇÃO, DISTRIBUIÇÃO E ECOFISIOLOGIA

ECOLOGY OF TROPICAL ACROCARPOUS MOSSES: REPRODUCTION, DISTRIBUTION, AND ECOPHYSIOLOGY

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Orientador: Dr Fábio Pinheiro
Coorientadora: Dra Kátia Cavalcanti Pôrto
Coorientadora: Dra Juçara Bordin

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COMISSÃO EXAMINADORA

Prof.(a) Dr.(a). Kátia Cavalcanti Pôrto

Prof.(a) Dr(a). David Nicholas McLetchie

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Prof.(a) Dr(a). Wanessa Vieira Silva Menezes Batista

Prof.(a) Dr(a). Maria do Carmo Estanislau Amaral

Os membros da Comissão Examinadora acima assinaram a Ata de Defesa, que se encontra no processo de vida acadêmica do aluno.

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RESUMO

A base desta tese abrange uma exploração de múltiplos aspectos da biologia reprodutiva, distribuição e ecofisiologia de várias espécies de briófitas, com ênfase particular em musgos acrocárpicos tropicais. Na primeira parte deste estudo, nosso objetivo foi investigar uma variedade de características reprodutivas em diferentes espécies, fornecendo insights abrangentes sobre a reprodução de briófitas. Esta seção incluiu uma análise da expressão sexual, alocação reprodutiva, razão sexual, densa dependência, demanda conflitante e custos reprodutivos. O Capítulo 1 destacou a formação de esporófitos em *Weissia jamaicensis* (Mitt.) Grout, expondo os custos associados à produção de ramos e à sobrevivência das brotações. O Capítulo 2 explorou o musgo *Fissidens flaccidus* Mitt., revelando uma grande densa dependência na expressão sexual masculina. O Capítulo 3 destacou-se sobre os distintos padrões de alocação reprodutiva dentro de sistemas sexuais monoicos, especialmente gonioautoicos, cladautoicos e rizautoicos. No Capítulo 4, uma revisão abrangente enfatizou a necessidade crítica de padronização na quantificação da alocação reprodutiva em briófitas e destacou a adoção de terminologia padronizada em estudos futuros. Em conjunto, esses estudos enriquecem a compreensão da biologia reprodutiva de musgos acrocárpicos tropicais e destacam a importância da padronização metodológica e consistência terminológica neste campo de pesquisa.

A segunda parte desta tese investiga a distribuição das espécies de *Fissidens*, examinando a intricada relação entre seus sistemas sexuais e características funcionais. Esta parte compreende dois capítulos que investigam a ligação entre sistemas sexuais e distribuição de briófitas. O Capítulo 5 analisa as características funcionais de diferentes sistemas sexuais na Mata Atlântica, revelando variações significativas que indicam diversas estratégias reprodutivas, influenciando a adaptação e sobrevivência das espécies neste ecossistema. O Capítulo 6 utiliza uma ferramenta de modelagem de distribuição de espécies para prever como os sistemas sexuais das espécies de *Fissidens* se relacionam com a adequação e tolerância aos impactos do aquecimento global. Os resultados mostram que diferentes sistemas sexuais exibem taxas distintas de adequação e tolerância a esses efeitos, sugerindo capacidades variadas de lidar com as mudanças climáticas causadas pelo aquecimento global. Esses estudos têm implicações para a ecologia, evolução, conservação e gestão das espécies de *Fissidens* diante das mudanças ambientais.

A terceira e última parte desta tese se concentra em estudos ecofisiológicos realizados na Universidade de Nevada Las Vegas (UNLV), com foco em *Bryum argenteum* Hedw, uma espécie de musgo amplamente distribuída em todo o mundo. Foi feita uma comparação entre genótipos da Mata Atlântica (uma floresta úmida) e da Caatinga (savana) para discernir possíveis diferenças. Três capítulos destacam os principais resultados desta pesquisa: O Capítulo 7 fornece observações de campo sobre a expressão sexual em *B. arge*nteum, revelando uma frequência menor de expressão sexual em plantas masculinas em comparação com plantas femininas, evidenciando a Hipótese do “Macho Tímido”. O Capítulo 8 esclarece distinções fenológicas entre genótipos masculinos e femininos, destacando diferenças no desenvolvimento fenológico e nas respostas fisiológicas. O Capítulo 9 investiga a influência da fenofase (estágios específicos do desenvolvimento vegetal) na tolerância de indivíduos de *B. argenteum* à dessecação, enfatizando o impacto significativo das fases de desenvolvimento na capacidade da espécie de suportar a dessecação. Essas descobertas contribuem para nossa compreensão da ecofisiologia e biologia reprodutiva de *B. argenteum* e lançam luz sobre os potenciais efeitos das condições ambientais em seu desempenho em diferentes ecossistemas florestais.

ABSTRACT

The foundation of this thesis encompasses a multiple aspects of exploration of the reproductive biology, distribution, and ecophysiology of various bryophyte species, with a particular emphasis on tropical acrocarpous mosses. In the initial part of this study, our objective was to delve into an array of reproductive traits across different species, offering comprehensive insights into bryophyte reproduction. This section encompassed an examination of sexual expression, reproductive allocation, sex ratios, dependency densities, trade-offs, and reproductive costs. The Chapter 1 highlighted the formation of sporophytes in *Weissia jamaicensis* (Mitt.) Grout, exposing the associated costs linked to ramet production and shoot survival. Chapter 2 delved into the moss *Fissidens flaccidus* Mitt., unveiling a profound dependence on male sexual expression. Chapter 3 concerned on the distinct patterns of reproductive allocation within monoicous sexual systems, particularly gonioautoicous, cladautoicous, and rhizautoicous. In Chapter 4, a comprehensive review underscored the critical need for standardization in quantifying reproductive allocation in bryophytes and emphasized the adoption of standardized terminology in future studies. Collectively, these discoveries enrich our understanding of the reproductive biology of tropical acrocarpous mosses and underscore the significance of methodological standardization and terminological consistency in this field of research.

The second segment of this thesis delves into the distribution of *Fissidens* species, scrutinizing the intricate relationship between their sexual systems and functional traits. This part comprises two chapters that elucidate the nexus between sexual systems and bryophyte distribution. Chapter 5 scrutinizes the functional traits of different sexual systems in the Atlantic Forest, revealing noteworthy variations that indicate diverse reproductive strategies, ultimately influencing species adaptation and survival within this ecosystem. Chapter 6 employs species distribution modeling to predict how the sexual systems of *Fissidens* species are related to suitability and tolerance to the impacts of global warming. The results highlight distinct rates of suitability and tolerance among different sexual systems, suggesting varying capacities for coping with climate change due to global warming. These findings carry implications for the ecology, evolution, conservation, and management of *Fissidens* species in the face of changing environmental conditions.

The third and final section of this thesis is dedicated to ecophysiological studies conducted at the University of Nevada Las Vegas (UNLV), focusing on *Bryum argenteum* Hedw, a widely distributed moss species. A comparative analysis was undertaken between

genotypes from the Atlantic Forest (a wet forest) and the Caatinga (a dry forest) to discern potential differences. Three chapters encapsulate the key outcomes of this research: Chapter 7 provides field observations on sex expression in B. argenteum, revealing a lower frequency of sex expression in male plants compared to female plants, supporting the "Shy Male" Hypothesis. Chapter 8 elucidates phenological distinctions between male and female genotypes, highlighting differences in phenological development and physiological responses. Chapter 9 investigates the influence of phenophase on desiccation tolerance, emphasizing the significant impact of developmental stages on the moss species' ability to withstand desiccation. These findings advance our comprehension of the ecophysiology and reproductive biology of *B. argenteum* while shedding light on the potential effects of environmental conditions on its performance in diverse forest ecosystems.

SUMMARY

INTRODUCTION

A brief introduction to ecology and life-history theory

The word "Ökologie" derives from the combination of the Greek terms "oikos", which means house, and "logos", which means "study" (Schwarz and Jax, 2011). It is defined as: the scientific study of the interactions that determine the distribution and abundance of organisms (Krebs 2014). This definition relates what most studies concerning ecology try to explain, the relationship of organisms with their abundance and distribution in the ecosystem (Collins and Glenn, 1991; Ehrlén and Morris, 2015). Abundance means how many organisms occur in each area, and distribution means how these organisms are distributed in the ecosystem (Krebs, 2014).

Ecology is governed by several theories, which are defined by Marquet et al. (2014) as "*An integrated and hierarchical set of empirical hypotheses that together explain a significant fraction of scientific observations*". Theory reduces the apparent complexity of nature because it selects the essential features of a system, provides abstract characterizations, and makes predictions for as-yet-unobserved phenomena that additional data can be used to test (Ulanowicz, 2001; Marquet et al., 2014). Approximately, there are approximately 70 ecological theories, however many of them are not efficient to explain nature (May and McLean, 2007; Marquet et al., 2014).

The ecological theory with the most mentions on the Web of Knowledg is the life history theory, whose name says it is associated with the life history of organisms (Marquet et al., 2014). Basically, the life history is the timeline of events in the life of an organism and is defined in terms of life history traits calculated among individuals within a population (Morris, 2009). In this way, life history theory can be understood as a "*collection of ideas proposed to explain both the diversity of life history patterns and the mechanisms by which natural selection has shaped life history traits"*(Morris, 2009).

Life history theory predicts that every resource produced by an organism is allocated to its different vital functions (Oli and Coulson, 2016). These vital functions are classified as: maintenance, growth, and reproduction (Morris, 2009). Maintenance includes activities such as water regulation, maintenance of photosynthetic systems, production of secondary metabolites, structures to reduce predation, and resistance against competitor (Thornely, 1970; Rungsung et al., 2015). Growth refers to the increase in the biomass of vegetative structures, which, ultimately, will give support to reproductive organs (Agren, 1985; Ingestad and Lund, 1986; Barrett, 2015). The reproductive cycle includes the production of gametangia, gametes and production of offspring (Reekie and Bazzaz, 2002; Block et al., 2009).

Darwin (1877) was the pioneer in studies on the theory of life history. In one of his first studies on the subject, he reported that in hermaphrodite flowers, there was resource compensation between the sexual functions. Thus, he reported that in these flowers, the plants that presented large stamens had poorly developed pistils, and the same was observed in flowers that presented developed pistils and had small stamens. Thus, he asserted that the resource was competed between the sexual functions, and that this observation was termed the Law of Compensation (Darwin, 1877). However, these days, the Law of Compensation is referred to as trade-off (Charlesworth and Charlesworth, 1981).

The trade-offs that occur in organisms are extremely important for their development, reproduction, and dispersal (Stearns, 1989; Liu et al., 2009). However, when he is strongly affected by a certain trait, this can lead to problems at population levels (Thompson and Eckert, 2004). For example, when some trait generates a lack of resources for reproduction in each organism, there is an absence of reproduction and dispersion, consequently limiting its habitat (Santos et al., 2022). In addition, it may reduce genetic diversity and consequently leave populations with less tolerance to environmental phenomena (Thompson and Eckert, 2004; Liu et al., 2009; Zhang et al., 2021).

Bryophytes

Bryophytes are a group of small land plants known for their ability to survive in humid and shady environments (Mishler, 2003; Harris et al., 2020). Comprising mosses, liverworts, and hornworts, bryophytes are characterized by their lack of true vascular tissues, such as xylem and phloem, which are found in more complex plants (Shaw and Goffinet, 2000). Due to this absence, they generally do not grow very tall, and often form green mats on damp soil, tree trunks, or rocks (Shaw and Goffinet, 2000, Batista et al., 2018, 2021). Their reproductive structures consist of male and female gametangia, which produce gametes that unite to form sporophyte and spores, which germinate to give rise to the next generations (Maciel-Silva and Pôrto, 2014). Furthermore, bryophytes are divided into two groups based on their growth habits: acrocarpous, which produce sporophytes at the tips of their stems, and pleurocarpous, which produce sporophytes on the sides of their stems (Shaw and Goffinet, 2000). Despite their relative structural simplicity, bryophytes play a significant ecological role by helping to retain moisture, prevent soil erosion and cycle nutrients in diverse ecosystems (Smith, 2012).

Bryophytes have a significant historical importance in the context of the evolution of terrestrial plants, representing an intermediate stage in the transition from aquatic to terrestrial life (Mishler and Churchill, 1985; Shaw et al., 2011). Their lack of conducting tissues limits their size and height, but also provides them with remarkable adaptation to life in moist environments, where many other plants struggle to thrive (Shaw and Goffinet, 2000; Mishler, 2003; Oliver, 2005). Additionally, these plants play a vital role in ecosystems as pioneers in degraded areas, contributing to soil formation and providing habitat for various microorganisms (Cao et al., 2020). Although they are often overshadowed by more structurally complex plants, bryophytes play a fundamental role in global ecology and in understanding plant evolution.

Reproductive biology in bryophytes: patterns and processes

Unlike most tracheophytes, bryophytes have a reproductive cycle in which the lasting phase is haploid (gametophyte) (Maciel-Silva and Pôrto, 2014). Therefore, for sexual reproduction, bryophytes need to express their sexes in the gametophytes (Shaw and Goffinet, 2000). The reproductive structures of bryophytes are produced in structures called gametoecia. Gametoecia are modified leafy structures that protect the gametangia, with the male-carrying gametangia being called perigonia and the female-carrying ones being referred to as perichaetia (Glime 2022). The gametangia are the structures that produce gametes, with antheridia being the gametangia that produce the male gametes, known as sperm cells, which are flagellated (Haig, 2016). Archegonia produce the female gametes, known as eggs, unlike antheridia that produce thousands of sperm cells per structure, each archegonium produces only one egg (Maciel-Silva and Pôrto, 2014; Haig, 2016). Thus, with the maturation of the gametangia, in the presence of water, the male gametes swim to the egg and fertilize it. Although the presence of water is a limiting factor for fertilization, there are reports of insects aiding in the fertilization of bryophytes (Cronberg, 2012). With the fertilization of the egg, the sporophyte is formed, which constitutes the diploid phase of the bryophyte life cycle (Maciel-Silva and Pôrto, 2014; Haig, 2016). Upon sporophyte formation, spores are produced in the sporophyte's capsule, and under favorable conditions, they are released into the ecosystem, germinate, form a protonema, and initiate the reproductive cycle of bryophytes (Glime 2022).

The reproduction of bryophytes is strongly linked to their sexual systems (Stark et al., 2000; Bisang and Ehrlén, 2002; dos Santos et al., 2023). When both male and female reproductive structures are formed on the same plant, they are referred to as monoicous, while species where the reproductive structures are found on different plants, they are called dioicous (Wyatt, 1985). Bryophytes have distinct sexual system terminology compared to tracheophytes due to the production of reproductive structures in the haploid phase (Santos et al., 2022). These sexual systems, monoicous and dioicous, are classified based on the formation of reproductive structures on the gametophyte, resulting in a gradient of reproductive structures (Wyatt, 1985; Maciel-Silva and Pôrto, 2014).

Monoicous systems are classified as follows:

- *Synoicous* – male and female gametangia found mixed in the same gametophyte.

- *Paroicous* – male and female gametangia found in the same gametophyte, with male gametangia adjacent to female gametangia.

- *Gonioautoicous* – apical perichaetia and axillar perigonia.

- *Cladautoicous* – male and female branches attached within a common shoot.

- *Rhizautoicous* – a shoot with perigonium attached by rhizoids to a shoot with perichaetia.

Dioicous systems are characterized by:

- *Dioicous* – shoots with separate male and female gametangia (Fig.1).

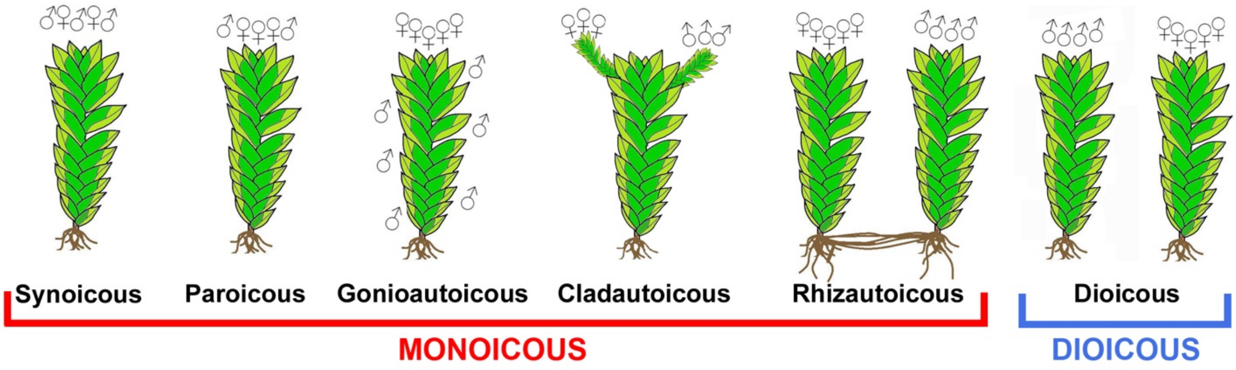

Fig. 1 - Sexual systems of monoicous and dioicous bryophytes.

Some predictions are associated with sexual systems. For instance, Stark and Brinda (2013) suggests that reproductive allocation (the proportion of resources allocated to reproduction) in the male function increases as sexes become more separated in sexual systems. Thus, reproductive allocation in the male function relative to the female function is lower in the synoicous system and increases towards the dioicous system. The authors raised this question based on analogies with flowering plants, primarily guided by the findings of Bergh and Verboom (2011). In the rhizautoicous system, Stark and Delgadillo (2001) propose that the separation of male and female gametangia into different shoots can influence various aspects. Firstly, the presence of sexes in different branches might consider the species functionally dioicous. Secondly, since the production of gametangia occurs in separate branches, and branches undergo constant somatic mutations, they become genetically distinct, whereas in synoicous and paroicous systems, this is less likely to occur. Additionally, the distance between male and female branches promotes high chance of cross-fertilization, as these branches tend to come into closer proximity with other individuals. Finally, being a monoicous system commonly presents a higher sex expression than dioicous species, consequently increasing the chance of fertilization compared to dioicous species.

The sex expression in dioicous bryophyte species is a topic of great interest for bryologists studying reproduction (Söderström and Gunnarsson, 2003). This is due to the recurring lack of male sex expression (Bisang and Hedenäs, 2005; Jong et al., 2018). Several studies have attempted to explain the reasons behind the lack of sex expression, yet a consensus on why male plants fail to express their sex has not been reached (McLetchie, 1992; Bowker et al., 2000; McLetchie and Puterbaugh, 2000; McLetchie et al., 2001; Bisang and Hedenäs, 2005; Stark et al., 2010). Pioneering investigations into explaining the lack of male sex expression in dioicous species, Stark et al. (2000) associated the lack of male sex expression with reproductive cost. These authors hypothesized that the sex that is more costly (requiring greater resource allocation for reproduction) is expressed in lower quantities in populations. In this study, the authors used the species *Syntrichia caninervis* Mitt., which in a previous study exhibited heavily female-biased populations. The authors supported the hypothesis, as reproductive allocation was significantly higher in male plants compared to female plants. This same hypothesis was tested for the dioicous moss *Pseudocalliergon trifarium* Loeske by Bisang et al. (2006). However, the results did not support the hypothesis, as in this species, the resource allocated to reproduction was greater in the female function, and the number of branches expressing sex was higher in female plants. Another hypothesis investigated to explain the lack of male sex expression is the "shy male hypothesis" (Stark et al., 2010). In this hypothesis, the prediction is that the number of male and female plants expressed in populations is equivalent, yet a higher number of female plants is observed because male plants fail in sex expression. However, all studies that tested this hypothesis either did not support it or only provided limited evidence for the hypothesis (Bisang and Hedenäs, 2013; Ekwealor et al., 2017, 2022).

Sex expression is strongly associated with the sex ratio of bryophyte populations (Glime 2022). However, most studies have indicated populations with a skewed sex ratio towards the female sex (McLetchie, 1992; McLetchie et al., 2001; Bisang and Hedenäs, 2005; Bisang et al., 2006, 2017; Stark et al., 2009, 2010; Ekwealor et al., 2022), while a smaller proportion report species with populations biased towards the male sex (Shaw et al., 1993; Alvarenga et al., 2013; Holá et al., 2014). The sex ratio is extremely important as it directly influences the population dynamics of species. The reasons for skewed populations are diverse and can be related to various ontogenetic stages of plant life (McLetchie, 1992; Horsley et al., 2011; Holá et al., 2014). The sex ratio of bryophytes is expected to be balanced during spore formation, as each meiotic division produces a pair of spores, one male and one female (Newton, 1972; Bisang et al., 2017). This has been the consensus among studies that have investigated the sex ratio of spores up to now (Haig, 2016). However, when populations reach adulthood (expressing sex), the sex ratio is strongly skewed towards the female sex.

Distribution and diversity of bryophytes

World distribution of bryophytes

Bryophytes have a wide global distribution, being found in a variety of environments and climates (Patiño and Vanderpoorten, 2018). Its most recurrent found in moist and shaded habitats, which leads them to thrive in temperate, tropical, and boreal regions. In tropical regions, bryophytes are often found in humid tropical forests, where abundant moisture and shade provide a favorable environment for their growth. However, they are also found in extremely arid places such as deserts and dry forests. In temperate and boreal areas, these plants can be found in a variety of locations, from coniferous forests to swamps, wetlands, and deserts.

The distribution of bryophytes is also influenced by the availability of water and suitable substrates. They are often found in places where water is abundant, such as streambanks, lakeshores, and high-humidity areas (Acebey et al., 2003; Batista et al., 2018). Furthermore, these plants are capable of colonizing various substrates, including soil, rocks, tree trunks, leaves, and even artificial surfaces like building walls (Barbosa-Oliveira, 2006; Alvarenga et al., 2013; Batista et al., 2018). Their ability to adapt to different types of habitats is one of the factors contributing to their global distribution (Shaw and Goffinet, 2000). In summary, bryophytes are found in a wide variety of ecosystems around the world, playing important ecological roles and contributing to biodiversity in many terrestrial environments.

Taxonomic and functional diversity

The diversity of bryophytes has often been assessed based on species counts. In this context, tropical regions stand out as true diversity hotspots (Acebey et al., 2003; Bryological, 2011). Among these regions, the neotropical area deserves special mention, as it exhibits a remarkable abundance of bryophyte species, many of which are endemic to this ecosystem (Delgadillo, 1995). However, in recent years, research on bryophyte diversity has evolved beyond mere consideration of taxonomic diversity. There is now a growing focus on assessing the functional diversity of these organisms (Ah-Peng et al., 2014; Henriques et al., 2017; Souza et al., 2020). Functional diversity refers to the variety of functions and roles played by different species in an ecosystem. It goes beyond simple species counts and focuses on the various ways in which organisms interact with the environment and with each other to perform specific functions that are crucial for the ecosystem's function.

The first study that clearly examined functional diversity in bryophytes was conducted by Henriques et al. (2017), focusing on a trait-based functional diversity approach to understand bryophyte diversity patterns on the Azores Islands. This study fills a gap because, despite their essential biogeochemical roles in ecosystems, bryophytes have not been widely investigated from a functional perspective. The study focuses on Terceira Island, where it examines multivariate variations in richness and composition of functional traits along an altitudinal gradient. The results reveal significant differences in trait composition between coastal and mountain communities, indicating adaptive responses to changes in environmental conditions. The study highlights the potential impact of future climate changes on the functional diversity of bryophytes on Terceira Island and suggests comparisons with similar protocols applied on other islands. The second study, led by Souza et al. (2020), explores the relationship between functional traits and the distribution of epiphytic bryophytes in an Atlantic Forest fragment. This study emphasizes how trait-based approaches can reveal bryophyte responses to environmental changes. Six traits related to water relationships and light tolerance were examined, with lobe size in liverworts selected for morphometric analyses. The results highlight that, along vertical and horizontal gradients, the functional trait composition varied considerably, while functional diversity remained relatively stable. Traits related to water storage and protection against excessive light were more common in the canopy and at the fragment's edges. This study underscores the importance of functional composition across environmental gradients and emphasizes the significance of liverwort lobes as a significant trait explaining bryophyte adaptive strategies. Both studies emphasize how assessing the functional diversity of bryophytes can provide valuable insights into their responses to environmental changes, highlighting the importance of considering not only taxonomic diversity but also the essential functions and interactions these organisms play in ecosystems.

In this regard, these two prominent studies in the field stand out for their pioneering nature and for enriching our understanding of the roles of traits and the functional diversity of bryophytes, which play a crucial role in ecosystem functioning. Furthermore, functional traits can directly or indirectly influence the physiology of organisms.

Phenological development, ecophysiology, and desiccation tolerance

The concepts of phenological development, ecophysiology, and desiccation tolerance are intrinsically interconnected in the context of bryophytes. This is noteworthy, considering that phenological development, which encompasses observable and cyclical changes in the growth and maturation phases of living organisms, is directly influenced by exposure to desiccation (Horsley et al., 2011; dos Santos et al., 2020). Desiccation, characterized by the loss of moisture when the relative humidity falls below 50%, essentially impacts the physiology of these organisms (Beckett, 2003; Alpert, 2006). Exploring these themes becomes crucial to understand how organisms manage to persist in ecosystems, even in the face of factors that limit their development.

Among the research that explored such interconnections, a series of species have been meticulously monitored regarding their phenological development, revealing notable distinctions between the sexes in various cases (Glime 2022). Even more intriguing, among these investigations, those that delineated such discrepancies indicated a marked inclination towards advantages favoring the female sex (Stark and McLetchie, 2006; Horsley et al., 2011;

Santos et al., 2022). However, it is worth noting that, to date, no research has been dedicated to analyzing the phenophase relationships of gametophytes in conjunction with development and desiccation tolerance. These findings are highly significant not only for understanding these associations but perhaps for reaching a consensus on the pattern of sex ratio and sexual bias in the studied species, as in other biological traits of the species.

Thesis Status

Based on this theoretical foundation, this thesis is dedicated to the investigation of the biology of tropical acrocarpous mosses, aiming to fill knowledge gaps related to the reproduction, distribution, and eco-physiology of the studied species. For better organization, the thesis has been structured into three distinct parts: the first part focuses on reproduction, comprising four chapters; the second part centers on distribution, with two chapters; and finally, the third part deals with eco-physiology, consisting of three chapters.

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Chapter 1

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An overview of reproductive allocation and reproductive costs in bryophytes: challenges and prospects

Wagner Luiz dos Santos[1,3]; Kátia Cavalcanti Pôrto[2]; Fábio Pinheiro[1]

[1] *Biology Institute, Department of Plant Biology, University of Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil*

[2] *Biosciences Center, Department of Botany, Federal University of Pernambuco, Moraes Rego Av., s/n, Recife, PE 50670-901, Brazil*

[3] Corresponding author: wag.lsantos1@gmail.com

Wagner Luiz dos Santos: http://orcid.org/0000-0002-4434-2542
Kátia Cavalcanti Pôrto: http://orcid.org/0000-0002-1861-0216
Fábio Pinheiro: http://orcid.org/0000-0003-3243-2652

Abstract

Reproductive allocation and cost play a crucial role in the survival of organisms, but research on these traits in bryophytes has been limited and inconsistent. To address this, we conducted a literature review focusing on bryophyte studies. Our goal was to clarify inconsistencies and explore reproductive allocation and cost concepts, as well as current trends in bryophyte reproduction. We examined different approaches and highlighted advantages and limitations. We emphasized five key topics: the importance of understanding these concepts, the significance of bryophytes as model organisms, historical research, terminological and methodological inconsistencies, sexual dimorphism and reproductive allocation, and measurement methods. Furthermore, we provided insights into future perspectives. Based on our findings, we advocate for standardized quantification of reproductive allocation and consistent terminology. Standardization would enhance comparability and synthesis of results, ultimately advancing our understanding of reproductive allocation and cost of reproduction in bryophytes.

Keywords: Biomass Allocation, Liverworts, Moss, Reproductive Effort, Reproductive Investment, Trade-off

Introduction

Life history theory, an approach that investigates the timing of key events in an organism's life cycle, predicts that every resource produced by an organism is allocated to its different vital functions (Oli and Coulson, 2016), namely, maintenance, growth, and reproduction (Morris, 2009). In plants, maintenance includes activities such as water regulation, maintenance of photosynthetic systems, production of secondary metabolites, and production of structures to reduce predation and to provide resistance against competitors (Thornely, 1970;

Rungsung *et al.*, 2015). Growth refers to the increase in the biomass of vegetative structures, which, ultimately, will give support to reproductive organs (Agren, 1985; Ingestad and Lund, 1986; Barrett, 2015). The reproductive cycle includes the production of gametangia, gametes, and offspring (Reekie and Bazzaz, 2002; Block *et al.*, 2009).

The amount of an organism's resources that is invested in reproduction is called reproductive allocation (RA) (Reekie and Bazzaz, 2002). This resource can be carbon, energy, and even water (Reekie and Bazzaz, 1987; Delph *et al.*, 1996; Ehrlén *et al.*, 2000). However, the quantification of biomass, measured in absolute (reproductive biomass) or relative terms (proportion of resource allocated to reproduction or RA), is the most frequent method used in studies addressing the theme. When a large part of the resources available for an organism is allocated to reproduction, the other functions (growth and homeostasis) receive a decreased amount of such resources (Stearns, 1976, 1989). However, if the amount of resource varies over time, especially in highly seasonal environments, an adjustment in resource allocation trade-offs may be necessary (Stark, 2002; Maciel-Silva and De Oliveira, 2016). The partition of the acquired resources between multiple traits has been reported since Darwin's time. In his book "The different forms of flowers on plants of the same species", Darwin (1877) described the resource limitation as the *Law of Compensation*. He postulated that when a resource was not allocated to one specific trait, it was naturally reallocated to another. As an example, he used the morphology of monoecious flowers. He reported that plants that were greater pistils (i.e. greater allocation of resources to this trait) had the smallest stamens. Currently, this condition is referred to as a trade-off (Charlesworth and Morgan, 1991).

Trade-offs can be governed by ecological, environmental, and evolutionary forces like natural selection (Stearns, 1976, 1989, 2000). According to Wilson (1983), natural selection should favor the RA pattern that promotes the highest fitness of individuals. Thus, we should expect a variation in RA related to several factors such as sex, age, and environmental conditions where the organisms are found. However, variations in RA strategies may be due to genetic or/and phenotypic factors (Smith-gill, 1983). For example, greater individuals have higher fitness in RA than smaller ones. Resource levels allocated for basic maintenance can be extremely high (Tuomi *et al.*, 1983). Thus, an organism that allocates large amounts of resources to reproduction and growth (without a trade-off) may be excluded from the population by natural selection unless plenty of resources are available (Wilson, 1983).

When the reproductive cycle of a plant begins, an important change in the pattern of allocation of resources occurs (Karlsson and Méndez, 2005). Various structures begin to develop and grow, going through different phenophases. Metabolic energy and nutrients are required for the formation of reproductive structures (gametangia, gametes, and offspring) (Thornely, 1970). When there is fertilization, the reproductive cost (RC) tends to be greater for female plants, as they need to allocate resources not only to the production of eggs (pre-zygotic investment) but also offspring (post-zygotic investment) (Obeso, 2002; Karlsson and Méndez, 2005).

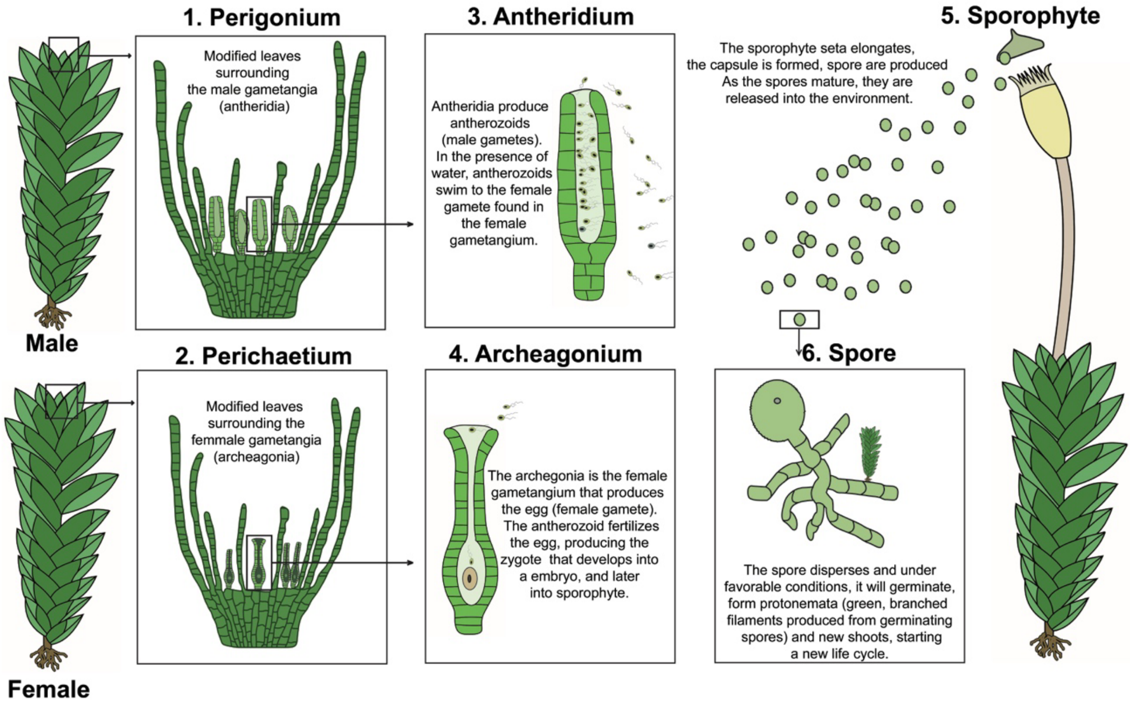
Reproductive cost is defined by Obeso (2002) as the "losses in the potential future reproductive success caused by current investments in reproduction". In flowering plants, RC has been largely investigated and shown to vary among sexes and across environments and sexual systems (Delph, 1999; Reekie and Bazzaz, 2002; Harris and Pannell, 2008). Sexual systems in plants refer to the organization and distribution of reproductive structures, such as male and female organs, in a plant (Charlesworth 2002), and it is strongly related to RA and RC in plants (Charlesworth 2002, Obeso 2002). The influence of sexual systems on RC is widely observed in plants in the most diverse ways (Obeso, 2002; Karlsson and Méndez, 2005; Rydgren *et al.*, 2010; Santos *et al.*, 2018). For example, variations in RA within a sexual system across different ecosystems or levels of disturbance can indicate how sexual performance responds to environmental changes (Abrahamson and Gadgil, 1973; Hickman, 1977). Further, cases of sexual systems in which there is a pattern of greater RA to the male or female sexual function may suggest that the RC for one of the sexes is greater (Cruden and Lyon, 1985), with consequences at individual and populations levels.

Bryophytes as model organisms to understand RA and RC

Bryophytes (mosses, liverworts, and hornworts) are a monophyletic group of plants comprising the simplest plants on Earth living in terrestrial environments (Harris *et al.*, 2021). Because bryophytes do not have lignin in their cells, they are smaller in size than tracheophytes and cannot control water loss (Shaw and Goffinet, 2000). As for the reproduction of bryophytes, they present an alternation of generations in which the sporophyte (diploid phase) is supported and nourished by the gametophyte (haploid phase) (Haig, 2016). Gametophytes in bryophytes can be thallose or leafy (Shaw and Goffinet, 2000). All mosses are leafy, liverworts can be thallose or leafy, and hornworts are exclusively thallose (Glime, 2017a). Bryophyte reproduction depends on the formation of male gametangia (antheridia) that produce male gametes (antherozoids) and female gametangia (archegonia) that produce female gametes (eggs) (Haig, 2016). Gametangia are formed in structures called gametoecia (modified leaves that protect the gametangia). Gametoecia producing antheridia and archegonia are called perigonia and perichaetia, respectively (Shaw and Goffinet, 2000). When antherozoids are viable, they swim in the water film and fertilize the eggs, forming sporophytes (Haig, 2016) (Fig. 1).

Fig 1 – Scheme of the reproductive cycle of a dioicous moss. The male gametoecium (perigonium) is the structure that produces and protects the male gametangia (antheridia). Antheridia produce male gametes (antherozoids), which are flagellated and swim to the female gametoecia (perichaetia) to fertilize the female gamete (egg) found within the female gametangia (archegonia). With the fertilization of the egg, cell divisions occur to form the sporophyte, which produces the spores. In the environment, under favorable conditions, the spores germinate and form new plants, starting a new life cycle.

As in angiosperms, bryophyte species may produce male and female reproductive organs in the same individual or in separate individuals. These species are respectively known as monoecious and dioecious in angiosperms, but in bryophytes they are called monoicous and dioicous because the reproductive structures are produced in the haploid phase (Wyatt, 1985; Maciel-Silva and Pôrto, 2014) The monoicous and dioicous sexual systems of bryophytes present subdivisions according to variations in the position of the gametoecia in the gametophyte (Fig. 2) (Wyatt, 1985; Maciel-Silva and Pôrto, 2014a). This diversity of sexual systems provides an opportunity to experimentally investigate the effect of the distance between the sexes on reproduction.



Modified leaves surrounding the male gametangia (antheridia)

Antheridia produce antherozoids (male gametes). In the presence of water, antherozoids swim to the female gamete found in the female gametangium.

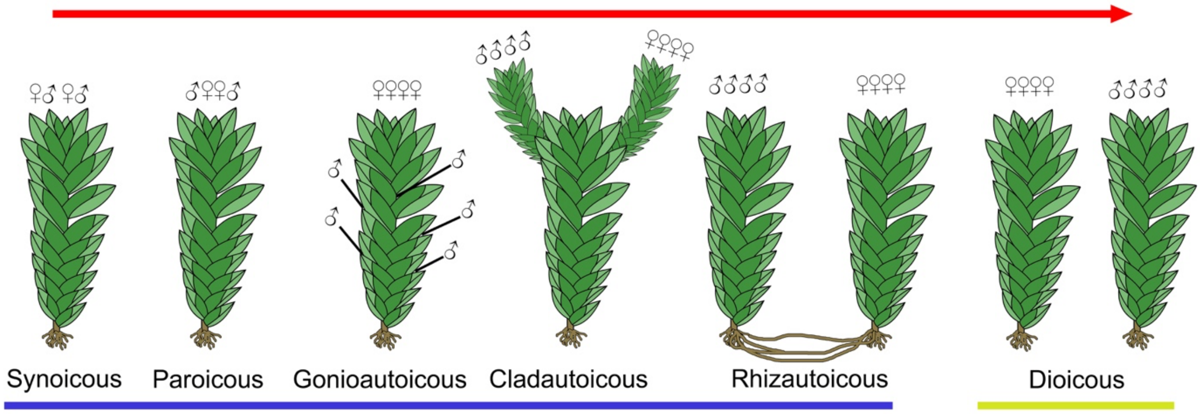
The sporophyte seta elongates, the capsule is formed, spore are produced As the spores mature, they are released into the environment.

Modified leaves surrounding the femmale gametangia (archeagonia)

The archegonia is the female gametangium that produces the egg (female gamete). The antherozoid fertilizes the egg, producing the zygote that develops into a embryo, and later into sporophyte.

The spore disperses and under favorable conditions, it will germinate, form protonemata (green, branched filaments produced from germinating spores) and new shoots, starting a new life cycle.

Fig 2 – Sexual systems of bryophytes. The distance between sexes increases from left to right. Synoicous – male and female gametangia mixed in the same gametoecia; Paroicous – male and female gametangia in the same gametoecia, but male gametangia are clustered and adjacent to female gametangia; Gonioautoicous – female gametoecia in the apex of the ramet and male



gametoecia in the axil of the ramet; Cladautoicous – male and female gametoecia on separate branches of a principal ramet; Rhizautoicous – male and female gametoecia on separate ramets, however, the ramets are attached by rhizoids; Dioicous – male and female gametoecia in different plants.

Bryophytes have proven to be powerful models for understanding complex processes in plants (Wyatt, 1985; Wood *et al.*, 2000; Müller *et al.*, 2016). Some aspects that make bryophytes excellent model organisms for the study of RA and RC are: [1] the high potential for clonal propagation (Rydgren and Økland, 2003; Maciel-Silva, 2017) that facilitates the quantification of parameters such as RA and RC, since fitness is quantified as the growth rate of a clonal population (Stearns, 2000); [2] the small size of the plants and their reproductive structures that allows the quantification of the biomass of entire vegetative and reproductive parts, which is virtually unfeasible for trees, for example; [3] the small size of the plants associated with high cloning and regeneration rates that facilitates experimental studies in which the plant and its development can be manipulated; and [4] the great morphological diversity associated with sexual and asexual reproduction (Frey and Kürschner, 2011; Maciel-Silva and Pôrto, 2014b). Thus, bryophytes encompass several characteristics that facilitate the experimental analysis and interpretation of data related to RA and RC in plants. Notwithstanding the usefulness of these plants, bryophytes have only rarely been to investigate the ecology and evolution of RA and the trade-offs involved in this process.

Bryophytes have a much faster life cycle compared to angiosperms (Oliveira and Porto, 2001; MacIel-Silva and Marques Válio, 2011; dos Santos *et al.*, 2020). For example, in several species of flowering plants, it takes several years for the first reproduction to occur (Munguía-Rosas *et al.*, 2011; Grogan and Schulze, 2012). In contrast, some species of bryophytes complete their reproductive cycle within a few months, especially tropical species (Glime, 2017b). Besides this advantage, the small size of bryophytes and the diversity of substrates they colonize allow their manipulation in the field and in the laboratory, which is impossible for many angiosperms (Stark *et al.*, 2009; Stark and Brinda, 2013; Santos *et al.*, 2022).

History of the study of RA and RC in bryophytes

The first studies that quantified RA in bryophytes were carried out with moss species from the polar region (Convey and Smith, 1993; Convey, 1994a, 1994b). They used the terminology "reproductive investment" and "reproductive effort" to refer to post-zygotic RA. Convey and Smith (1993) quantified the RA in several ways, including the measurement of the biomass of the reproductive and vegetative structures. The same was done by Convey (1994a) to compare the RA of moss species in different ecosystems in arctic regions. He observed that the pattern of RA was different among species, highlighting the diversity of reproductive strategies found in bryophytes. Finally, Convey (1994b) associated physiological aspects to RA to verify whether environmental variations could cause changes in RA in moss species from a maritime Antarctic locality. He reported a weak correlation between net photosynthesis and RA, suggesting that these species had a high and regular investment in sporophyte production. However, the quantification of RA in these studies is somewhat misleading, since RA was calculated as a ratio of reproductive to vegetative structures and not as the proportional biomass allocated to reproductive structures. Thus, these studies do not portray the true RA of the studied species.

Kimmerer (1994) quantified the RA to sporophytes in two bryophyte species, *Dicranum flagellare* Hedw. and *Tetraphis pellucida* Hedw., in temperate forest. Similarly to the studies mentioned above, RA was quantified as the ratio of reproductive to vegetative biomass. In this study, the author also investigated the allocation to asexual reproduction. The species differed in their reproductive characteristics: both had asexual reproduction, but *D. flagellare* showed a predominance of asexual reproduction, while *T. pellucida* produced asexual propagules at a lower intensity. *Dicranum flagellare* invested significantly more biomass in asexual reproduction, while the overall RA to sexual reproduction of *T. pellucida* was greater than that of *D. flagellare*. Furthermore, branches of *D. flagellare* germinated significantly faster than gemmae of *T. pellucida*. Thus, this was the first study to show that RA to sexual reproduction limits the resources available for vegetative reproduction. These results suggested that reproduction could entail a RC, although the way in which RA was quantified does not inform the real RA of the species studied.

González-Mancebo and During (1997) investigated the RA in nine species of mosses from the perspective of life forms. The authors applied two different ways to quantify the RA and compared them between acrocarpous and pleurocarpous life forms, which are, respectively, forms in which the sporophytes are produced at the apex of the stem/main branch of upright shoots and forms in which sporophytes are produced on short, specialized lateral branches or buds of typically prostrate shoots, forming freely branched mats (Glime and Chavoutier, 2017). Reproductive allocation, which they referred to as reproductive effort, was calculated per area and per individual. In this study, the true RA was quantified for the first time for bryophytes. The authors investigated questions exploring the interplay between RA and life history traits such as: (1) Do short-lived acrocarpous mosses have greater reproductive effort than perennials? and (2) Is shoot size correlated with sporophyte weight, or is sporophyte weight relatively flexible, with reproductive mass independent of gametophyte size? The results showed that the RA per area did not differ significantly between the two groups. In the six acrocarpous species, the RA to sporophytes per sporophyte-bearing shoot was generally quite low compared to the pleurocarpous species. The two measures of RA (area and individual) produced different results and apparently reflect different trade-offs. The lower RA in the acrocarpous species conveys the idea that the production of several branches in pleurocarpous mosses results in a greater amount of resource for the formation of sporophytes because a greater amount of resources is produced and can be allocated to sexual reproduction (sporophyte formation). Furthermore, it was observed that the biomass of the sporophytes was positively correlated with branch size in some species and negatively correlated in others. This finding may give an idea of the earliest steps of plant reproduction on terrestrial ecosystems.

The studies until then had focused only on the quantification of RA. Then, for the first time, Ehrlén *et al.* (2000) quantified the RC in the dioicous moss *Dicranum polysetum* Sw. The authors tested the hypothesis that RA to sporophyte production may imply costs, such as reduced performance of gametophytes in the later steps of the life cycle. Sporophyte production was investigated in three different ways: (1) by examining the variation in sporophyte production in unmanipulated sporophytic shoots; (2) by comparing sporophytic and non-sporophytic shoots (not carrying perichaetia); and (3) by comparing control sporophytic shoots with shoots whose reproductive sinks were experimentally removed. They estimated that 74.8% of the total carbon investment during the growth interval went to sexual structures in sporophyte-producing shoots. Branches that aborted all sporophytes had significantly higher growth rates than those that formed sporophytes. The difference in apical vegetative growth between control branches and branches in which sexual reproduction was manipulated was mainly due to different length increments, since biomass did not differ significantly. Thus, post-zygotic RC was quantified for the first time in a bryophyte and it was demonstrated that sporophyte maturation competes for resources, affecting the future development of new branches in *D. polysetum.* The RC in *D. polysetum* was further investigated by Bisang and Ehrlén (2002) using a slightly different approach. In this case, the authors proposed in a new experiment a way to quantify RA that considers only the photosynthetic portions of the plants. Making use of innate markers for growth intervals, precisely the position of gynoecia in *D. polysetum*, the authors dissected the individuals of this moss according to growth intervals, quantifying the sporophytes and perichaetia from previous years. They removed the brown portions of the gametophytes, leaving only the photosynthetically active parts to be weighed. This is because the brown parts can be dead, and even if they are alive (when they can regenerate), they do not influence the production of resources for reproduction. They found that the RA in plants that formed sporophytes was 16% while in those that did not form sporophytes was 1.3%. The authors also found several results that suggest the existence of RC: (1) the reproductive investment was inversely proportional to annual segment biomass prior to sporophyte initiation; (2) sporophyte formation was negatively related to annual sprouting segment and innovation size; (3) sporophyte formation reduced the probability of new perichaetia initiation; (4) investments in innovations and reproductive structures were negatively correlated; and (5) the mean mass per sporophyte + perichaetium decreased with the increase in the number of mature sporophytes per perichaetium. Thus, this study deepened the knowledge about the RC in the studied species. Furthermore, they showed that forming sporophytes entails a greater RC than forming perichaetia.

Using a population approach to investigate the relationship between sex ratio and RA, Stark *et al.* (2000) studied the moss *Syntrichia caninervis* Mitt., a species that has strongly female biased populations (Bowker *et al.*, 2000). They investigated the ‘cost of sex hypothesis’, which predicts that “the sex that is more expensive (the one that requires more energy) is the rarest in populations”. Thus, they investigated the assumptions that male sexual expression is more expensive than female sexual expression and that sexual reproduction resource is limited. This study was innovative because it was the first to quantify RA at the pre-zygotic level in both sexes (male and female) in addition RA to sporophytes at a population level in bryophytes. Reproductive biomass was quantified by removing perichaetial and perginonial leaves and weighing sets of gametangia. The authors reported that the reproductive costs in the studied populations were lower to the female than to the male function. Furthermore, they discussed resource limitation and observed a relationship between sporophyte production and population density. This study was very relevant for a better understanding of sex ratios in bryophytes. However, in the same vein of Stark *et al.* (2000), Bisang *et al.* (2006) investigated the possible association of RC with sex ratios in *Pseudocalliergon trifarium* Loeske. This species, like *S. caninervis*, has populations strongly biased towards the female sex and low fertilization rates. However, the findings contradicted the proposals of Stark *et al.* (2000), since the RA in *P. trifarium* was higher to the female function than to the male function. The RC did not influence the sex ratios in *P. trifarium*. Thus, with this result, it became clear that the two species behave differently in terms of RC and reproductive traits.

Until this point, RA had been quantified in relation to sexual reproduction (perigonia, perichaetia, and sporophytes). McLetchie and Puterbaugh (2000) quantified, for the first time, the RA to asexual reproduction and also for the first time in a liverwort. The authors estimated the biomass of the gemmae produced using the formula: $= \frac{\text{Reproductive mass}}{\text{Reproductive mass+Vegetative mass}}$. Male plants allocated proportionally more biomass to gemmae than females. In turn, females grew faster and produced more meristematic tips, which, in a way, influenced the population parameters. Another species of liverwort whose RA has been investigated is *Lophozia silvicola* Buch. Laaka-Lindberg (2001) investigated possible relationships between RA to sexual and asexual structures and RC in *L. silvicola*. Higher RA to sexual structures was inversely proportional to the number of gemmae and associated with lower the investment in growth. Female plants had higher RA to sexual structures, but these results must be analyzed with caution because RA was estimated by weighing female plants with pre- and post-zygotic structures altogether. Thus, it is not possible to establish whether vegetative growth and gemmae production are negatively related with pre- or with post-zygotic RA.

To investigate long-term reproductive costs, Rydgren and Økland (2002) followed a population of the dioicous moss species *Hylocomium splendens* (Hedw.) Schimp. for five years. They divided the population into two subcategories: female plants with and without sporophytes. Their findings showed that population growth was more influenced by female plants that did not form sporophytes than by those which did form sporophytes. The sub-population that did not form sporophytes had a higher branching rates, greater survival, and greater size development. In another study, the authors investigated the short-term RC in the species, demonstrating that sporophyte formation induced significant costs in terms of less favorable size development of new segments, lower branching frequency, and greater risk of not producing new annual segments (Rydgren and Økland 2003). Furthermore, they claimed that the costliest phase of sporophyte development was the last one (when the capsule expands and matures and spores are produced), because a large amount of resources is consumed in this phase. The RC was also reported in the tropical species *Crossomitrium patrisiae* (Brid.) Müll.

Hal. (Alvarenga *et al.*, 2016). In this study, the researchers observed lower growth rates of new branches in the colonies where sporophytes were formed. Thus, in the later three studies mentioned, the costs brought about by RA to sporophytes was detected using different types of measurements, although RA was not measured in any of them.

In the attempt to understand why the distribution of *Pogonatum dentatum* (Brid.) Brid. had expanded (spreading from the mountains down to the lowlands) in Fennoscandia, Hassel and Söderström (2003) quantified several reproductive traits of the species. The results of the studies showed that male plants were smaller than the female ones and matured in the second year of life and that female mountain plants produced sporophytes only in the third year of life, while lowland plants formed sporophytes in the second year. Despite this difference in the formation of sporophytes in female plants, the RA does did not differ between the two areas. The authors also mentioned that the establishment of populations by spores was more common in lowland areas, because in the mountain region, there were few spores available in the soil. The associations between life history traits and RA (referred to as reproductive effort) in the species *Pogonatum aloides* (Hedw.) P. Beauv., *Polytrichum commune* Hedw., and *Polytrichum juniperinum* Hedw. were explored by Hedderson and Longton (2008). The authors transplanted plants in their respective environments between upland and lowland localities. They observed that there were differences in life history traits and that some traits were genetically determined, although the environment and phenotypic plasticity were also significant contributors to the observed variation. In addition, the transplants indicated divergences in the plasticity regarding male reproductive effort and investment in vegetative shoots by female plants among populations. The RA to female and male sexual functions was respectively 35% and 50% in *P. aloides* (35% and 50%), 18% and 6.7% in *P. commune* (18% and 6.7%), and 18% and 35% in *P. juniperinum*. (18% and 35%).

The RC in the widespread moss *Pterygoneurum ovatum* (Hedw.) Dixon was investigated by Stark *et al.* (2009) to test the hypothesis that a trade-off exists between current sexual reproduction and subsequent clonal regeneration and that reproduction and regeneration are resource limited. After the experimental removal of leaves and sporophytes in early stages of development (embryo) from gametophytes, the following findings of the study indicated the existence of RC in the species: [1] shoots whose sporophytes were removed presented a higher protonemal growth rate; [2] natural abortion of sporophytes presented a positive correlation with time to protonemal emergence; [3] shoots whose leaves were removed showed lower probability of sporophyte maturation in relation to control shoots with intact leaves. In the same vein, evidence of RC was found in the moss *Weissia jamaicensis* (Mitt.). The relative and absolute biomass of reproductive structures of *Weissia jamaicensis* were quantified, among other reproductive traits, by Santos *et al.* (2022). Relative biomass was higher in sporophytic shoots, followed by male and female non-sporophytic shoots. Absolute biomass also varied among the three categories of shoots. Reproductive cost was indicated by a negative correlation between regeneration rate and RA: plants that allocated more resources to reproduction had lower regeneration rates. These results suggest that RA influences reproductive traits. Since sexual reproduction is more expensive, the more expensive sex tends to have lower regeneration rates, what may consequently lead to biases in population sex ratios.

As mentioned in the studies by Stark *et al.* (2009) and Santos *et al.* (2022), some species may show patterns that contradict the expectations. For example, Horsley *et al.* (2011) investigated the reproductive traits the dioicous moss *Bryum argenteum* Hedw. in cultivation, specifically sex-specific patterns in parameters related to the life-history traits of the species. Among other objectives, they investigated whether there were sex-specific patterns of pre-zygotic RI (evaluating the production and biomass of gametangial structures). They found that male and female plants showed different development patterns. Male plants showed higher rates of sexual expression than female plants, while female plants were greater in size. Male plants showed a significantly higher RA than female plants. This result was contrary to what was expected according to the life history theory, because although male plants had a RA that was approximately 24 times higher than that of female plants, they presented faster sex expression and higher rates of sexual expression.

Brzyski *et al.* (2014) quantified the RA and its impact on the population genetic diversity of the liverwort *Marchantia inflexa* Nees & Mont. in natural (rivers) and disturbed (roadsides) habitats. Reproductive allocation was quantified based on the number of gemmae-producing cups. This was, thus, an indirect measure of RA. The source environment had a considerable impact on the responses of male and female plants. Roadside-collected female plants had higher growth and asexual reproduction than river-collected female plants, while males showed the reversed pattern, although these differences were not significant. An indirect method of quantification of RA was also used by Pereira *et al.* (2016). They assessed the RA in six sympatric species of Calymperaceae by counting the number of gametangia. They observed that plants that reproduced asexually (by gemmae) had a lower number of gametangia. Shortlidge *et al.* (2017) investigated the effects of global warming on the moss *Polytrichastrum alpinum* (Hedw.) G.L. Sm. using open-top chambers to follow the development of the species. They demonstrated that passive warming reduces the stress and shifts the reproductive effort (measured as the number of sporophytes produced) of the species. Although passive warming did not increase sporophyte production in *P. alpinum*, it increased gametangia production in both males and females, suggesting that warming may alleviate the environmental constraints on reproductive effort. Although Brzyski et al. (2014), Pereira et al. (2016), and Shortlidge et al. (2017) claimed to have quantified the RA, they actually quantified the (sexual or asexual) reproductive structures.

Stark and Brinda (2013) studied the reproductive parameters, including the pre-zygotic RA for the first time in a monoicous moss, the rhizautoicous moss *Aloina bifrons* (De Not.) Delgad. They observed that the cultured plants were protandrous in gametangial development and in time to maturation, but mature gametes were present at the same time. Male plants were smaller in size and presented greater RA ((which was calculated by area) than female plants. In addition, the authors created ideal conditions for fertilization, but fertilized females aborted the sporophytes, suggesting self-incompatibility. In this study, the authors hypothesized that RA to the male function increases as the distance between sexes increases, and this is expected to be seen across the range of sexual systems existing in bryophytes (See fig 2). The first tropical moss to have the RA quantified was the rhizautoicous moss *Fissidens flaccidus* Mitt. Santos *et al.* (2018) quantified the RA in terms of absolute and relative biomass allocation and investigated the existence of sexual dimorphism in the species. They showed that male ramets had a higher RA, even exceeding the allocation to sporophytes. Furthermore, the analyses showed that there was a trade-off between relative RA and vegetative growth. Thus, these results suggest that there is a RC in the male function because ramets that presented greater allocation to the sexual reproduction grew less. Furthering the study of the relationship between RA and the population ecology of *F. flaccidus*, Santos *et al.* (2023) observed that the formation of male plants was dense density dependent. The number of male plants did not influence the fertilization rates of the populations, since the large RA to the male function ensured fertilization even when male individuals were few in the populations. Thus, the compiled data on RA in this rhizautoicous species suggest the functionality of this system as a dioicous system, as proposed by Stark and Brinda (2013). Finally, more recently, in the attempt to test the pattern of RA across the sexual systems of bryophytes proposed by Stark and Brinda (2013), Santos *et al.* (2023b) compared the RA of rhizautoicous, cladautoicous, and gonioautoicous species of the genus *Fissidens*. Among the main findings, the rhizautoicous species behaved like a dioicous species (greater allocation to the male function), while the other systems showed no sex-specific differences in RA.

Reproductive allocation and RC have been studied in different families (Fig. 3A) and genera (Fig. 3B). However, as presented in this section, RA has been quantified in various ways

(Table 1), leading to significant methodological inconsistencies among the studies. Thus, in the next section of this article, we will discuss the methodological and terminological issues associated with the study of RA and RC in bryophytes.

Terminological inconsistencies and methodological problems

Studies carried out with higher plants in general present several terminologies related to RA and RC (Table 2). It is not different in studies with bryophytes. As shown in the previous section, many terminologies have been used, and sometimes incorrectly. For example, some authors have used the terms reproductive effort, reproductive investment, and biomass production to refer to RA (Table 3). This imprecise application of different terms for the same concept contributes to confuse and mislead the conclusions in the studies. The term reproductive investment has been the most used, followed by RA, reproductive effort, and biomass allocation (Table 3).

Tab 1 – Compilation of results from studies that quantified reproductive allocation in bryophytes. Absolute reproductive allocation is the biomass of reproductive structures. Relative reproductive allocation is the proportion of resources allocated to reproduction. Mean values per species and sex are presented. ♂ = male sexual structures (perigonia or antheridia), ♀ = female pre-zygotic structures (perichaetia or archegonia), and ⚥ = post-zygotic reproductive structures (sporophytes). Note that: a = reproductive allocation was quantified by area in mm², and b = pre- and post-zygotic structures were weighed together.

Species	Absolute Reproductive Allocation (Biomass)			Relative Reproductive Allocation (%)			**Reference**
Mosses	♂	♀	⚥	♂	♀	⚥	
Aloina bifrons (De Not.) Delgad.	204 mg mm^{-2}	24 mg mm^{-2}	_	_	_	_	Stark and Brinda (2013)
Andreaea gainii Cardot	_	_	26.4 µg	_	_	0.24	Convey and Smith (1993)
Andreaea regularis Müll. Hal.	_	_	27.3 µg	_	_	0.31	Convey and Smith (1993)
Andreaea regularis Müll. Hal.	_	_	27.3 µg	_	_	0.31	Convey (1994b)
Andreaea regularis Müll. Hal.	_	_	26.8 µg	_	_	0.31	Convey (1994b)
Andreaea regularis Müll. Hal.	_	_	54.3 µg	_	_	0.37	Convey (1994b)
Bartramia patens Brid.	_	_	439.9 µg	_	_	0.56	Convey and Smith (1993)
Bartramia patens Brid.	_	_	439.9 µg	_	_	0.61	Convey (1994b)
Bartramia patens Brid.	_	_	1338 µg	_	_	0.49	Convey (1994b)
Bartramia patens Brid.	_	_	694.3 µg	_	_	0.52	Convey (1994b)
Brachythecium rutabulum (Hedw.) Schimp.	_	_	286 mg cm^{-2}	_	_	0.056a	González-Mancebo and During (1997)
Bryum argenteum Hedw.	26.5 mg mm^{-2}	11.36 mg mm^{-2}	_	_	_		Horsley et al. (2011)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb.	_	_	637 µg	_	_	2.65	Convey and Smith (1993)

Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb.	_	_	673 µg	_	_	2.73	Convey (1994b)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb.	_	_	201.4 µg	_	_	0.99	Convey (1994b)
Bryum pseudotriquetrum (Hedw.) G. Gaertn., B. Mey. & Scherb.	_	_	1541 µg	_	_	2.73	Convey (1994b)
Dicranoweisia antarctica (Müll. Hal.) Kindb.	_	_	130 µg	_	_	0.69	Convey and Smith (1993)
Dicranoweisia antarctica (Müll. Hal.) Kindb.	_	_	130 µg	_	_	0.74	Convey (1994b)
Dicranoweisia antarctica (Müll. Hal.) Kindb.	_	_	159.9 µg	_	_	0.61	Convey (1994b)
Dicranoweisia cirrata (Hedw.) Lindb. ex Milde	_	_	106 mg cm^{-2}	_	_	0.021a	González-Mancebo and During (1997)
Dicranoweisia cirrata (Hedw.) Lindb. ex Milde	_	_	0.034 mg	_	_	0.034	González-Mancebo and During (1997)
Dicranoweisia grimmiacea (Müll. Hal.) Broth.	_	_	147.6 µg	_	_	0.48	Convey and Smith (1993)
Dicranoweisia grimmiacea (Müll. Hal.) Broth.	_	_	147.6 µg	_	_	0.5	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth.	_	_	206.8 µg	_	_	0.29	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth.	_	_	94.4 µg	_	_	0.36	Convey (1994b)
Dicranoweisia grimmiacea (Müll. Hal.) Broth.	_	_	206.4 µg	_	_	0.43	Convey (1994b)
Dicranum flagellare Hedw.	_	_	12.2 µg	_	_	_	Kimmerer (1994)
Dicranum polysetum Sw.	_	_	_	_	_	74.8	Ehrlén et al. (2000)
Dicranum polysetum Sw.	_	_	_	_	1.3	16	Bisang and Ehrlén (2002)
Encalypta patagonica Broth.	_	_	265.7 µg	_	_	1.24	Convey and Smith (1993)
Fissidens flaccidus Mitt.	7.8 µg	11.69 µg	29.9 µg	35.15	12.61	25.4	Santos et al. (2018)
Fissidens flaccidus Mitt.	0.0046 mg	0.0070 mg	0.0223 mg	30.32	11.88	30.49	Santos et al. (2023a)
Fissidens pseudoplurisetus Bordin, Pursell & O. Yano	0.0090 µg	0.0087 µg	_	1.60	7.42	_	Santos et al. (2023b)
Fissidens scariosus Mitt.	0.0024 µg	0.0123 µg	_	27.36	16.84	_	Santos et al. (2023b)

Fissidens weirii Mitt.	0.0050 µg	0.0081 µg	_	4.41	6.93	_	Santos et al. (2023b)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	127.8 µg	_	_	1.47	Convey (1994b)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	329.8 µg	_	_	1.32	Convey (1994b)
Hennediella austrogeorgica (Cardot) Blockeel	_	_	207.3 µg	_	_	1.48	Convey (1994b)
Homalia trichomanoides (Hedw.) Brid.	_	_	292 mg cm^{-2}	_	_	0.04a	González-Mancebo and During (1997)
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	300.4 µg	_	_	0.92	Convey and Smith (1993)
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	300.4 µg	_	_	0.98	Convey (1994b)
Muelleriella crassifolia (Hook. f. & Wilson) Dusén	_	_	326.8 µg	_	_	0.92	Convey (1994b)
Orthotrichum affine Brid.	_	_	188 mg cm^{-2}	_	_	0.122a	González-Mancebo and During (1997)
Orthotrichum affine Brid.	_	_	0.123 mg	_	_	0.15	González-Mancebo and During (1997)
Orthotrichum diaphanum Schrad. ex Brid.	_	_	117 mg cm^{-2}	_	_	0.087a	González-Mancebo and During (1997)
Orthotrichum diaphanum Schrad. ex Brid.	_	_	0.036 mg	_	_	0.16	González-Mancebo and During (1997)
Orthotrichum pulchellum Brunt.	_	_	273 mg cm^{-2}	_	_	0.089a	González-Mancebo and During (1997)
Orthotrichum pulchellum Brunt.	_	_	0.042 mg	_	_	0.12	González-Mancebo and During (1997)
Orthotrichum striatum Hedw.	_	_	250 mg cm^{-2}	_	_	0.026a	González-Mancebo and During (1997)
Orthotrichum striatum Hedw.	_	_	0.059 mg	_	_	0.04	González-Mancebo and During (1997)
Pogonatum aloides (Hedw.) P. Beauv.	_	_	_	50.17	35.13	_	Hedderson and Longton (2008)
Pogonatum aloides (Hedw.) P. Beauv.	_	_	_	48.77	34.34	_	Hedderson and Longton (2008)
Pogonatum aloides (Hedw.) P. Beauv.	_	_	_	53.46	36.13	_	Hedderson and Longton (2008)
Pogonatum dentatum (Brid.) Brid.	_	_	_	_	_	0.34	Hassel and Söderström (2003)
Polytrichum alpinum Hedw.	_	_	2636 µg	_	_	0.32	Convey (1994b)

Polytrichum commune Hedw.	_	_	_		6.6	18.36	_	Hedderson and Longton (2008)
Polytrichum commune Hedw.	_	_	_		6.7	12.33	_	Hedderson and Longton (2008)
Polytrichum commune Hedw.	_	_	_		6.7	21.41	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw.	_	_	_		36.19	21.45	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw.	_	_	_		34.73	22.8	_	Hedderson and Longton (2008)
Polytrichum juniperinum Hedw.	_	_	_		23.69	14.03	_	Hedderson and Longton (2008)
Pottia austrogeorgica Cardot	_	_	127.8 µg		_	_	1.38	Convey and Smith (1993)
Pottia heimii (Hedw.) Hampe	_	_	280.6 µg		_	_	1.99	Convey and Smith (1993)
Pseudocalliergon trifarium (F. Weber & D. Mohr) Loeske	121.15 µg	174.24 µg	_		8.60	11.20	_	Bisang et. al. (2006)
Pterygoneurum ovatum (Hedw.) Dixon	_	_	124.4 µg		_	_	2.09	Convey and Smith (1993)
Rhynchostegium confertum (Dicks.) Schimp.	_	_	129 mg cm^{-2}		_	_	0.069a	González-Mancebo and During (1997)
Schistidium antarctici (Cardot) L.I. Savicz & Smirnova	_	_	90.6 µg		_	_	0.67	Convey and Smith (1993)
Syntrichia caninervis Mitt.	17.1 µg	2.7 µg	_		_	_	_	Stark et al. (2000)
Tetraphis pellucida Hedw.	_	_	54.2 µg		_	_	_	Kimmerer (1994)
Tortula saxicola Cardot	_	_	168.2 µg		_	_	1.48	Convey and Smith (1993)
Weissia jamaicensis (Mitt.) Grout.	0.0629 mg	0.0479 mg	0.148 mg		15.28	6.74	21.56	Santos et al. (2022a)
Liverworts	♀	♂	⚥		♀	♂	⚥	
Lophozia silvicola Buch.	4.8 µg	83.3 µg[b]	_	_	_	_	_	Laaka-Lindberg (2001)
Marchantia inflexa Nees et Mont.	0.0292 mg	0.0092 mg	_	_	_	_	_	McLetchie and Puterbaugh (2000)

Tab 2 – Terminology used in studies to refer to reproductive allocation and reproductive cost in plants along with the definitions used by the authors.

Terminology	Theme	Summary	References
Direct costs	Reproductive cost	Costs of reproduction during the same breeding season	(Obeso, 2002)
Short-term costs	Reproductive cost	Reduction of growth or reproduction in the next breeding season	(Rydgren and Okland, 2003)
Long-term costs	Reproductive cost	Have short-term implications in the production process, that is, used in a short production interval.	(Nicotra, 1999)
Physiological costs	Reproductive cost	Physiological trade-offs derived from reproductive allocation	(Karlsson, 1994)
Relative somatic costs	Reproductive cost	Differences in vegetative growth rate associated with differences in reproductive allocation	(Tuomi *et al*., 1983)
Absolute reproductive allocation	Reproductive allocation	Biomass allocated in reproduction in terms absolute or relative reproductive allocation	(Harris and Pannell, 2008)
Relative reproductive allocation	Reproductive allocation	Proportion of resource allocated to reproduction	(Karlsson and Méndez, 2005)
Reproductive investment	Reproductive allocation	Resource allocated to reproduction (quantified by area or by shoot)	(Stark and Brinda, 2013; Santos *et al*., 2018)
Reproductive effort	Reproductive allocation	Resource allocated to reproduction	(Reekie and Bazzaz, 2002)
Sex allocation	Reproductive allocation	Biomass allocated to reproduction	(Laaka-Lindberg, 2001)

Tab 3 – Compilation of studies that quantified reproductive allocation (RA) and/or reproductive cost (RC) in bryophytes and respective terminologies adopted in the study, variable measured for estimation of RA, form of reported results, bryophyte group to which the species belongs, and life phase (pre- or post-zygotic) in which the reproductive parameters were quantified.

Paper	**RA terminology**	**RC terminology**	**RA measurement**	**Bryophyte group**	**Phase**
(Alvarenga *et al.*, 2016)	-	Reproductive cost	-	Moss	Post
(Bisang and Ehrlén, 2002)	Reproductive effort	Reproductive cost	Biomass (%)	Moss	Pre and Post
(Bisang *et al.* 2006)	Reproductive effort	Reproductive cost	Biomass (%)	Moss	Pre
(Brzyski *et al.*, 2014)	Reproductive allocation	-	Count of gemmae-producing cups	Liverwort	Asexual
(Convey and Smith, 1993)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Convey, 1994a)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Convey, 1994b)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Ehrlén *et al.*, 2000)	Reproductive investment	Reproductive cost	Biomass (%)	Moss	Post
(González-Mancebo and During, 1997)	Reproductive effort	-	Biomass (%)	Moss	Post
(Hassel and Söderström, 2003)	Reproductive investment	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Hedderson and Longton, 2008)	Reproductive effort	-	Biomass (%)	Moss	Pre and Post
(Horsley *et al.*, 2011)	Reproductive investment	-	Biomass (gametangia)	Moss	Pre
(Kimmerer, 1994)	Biomass allocation	-	Biomass (ratio reproductive to vegetative biomass)	Moss	Post
(Laaka-Lindberg, 2001)	Biomass allocation	-	Biomass (%)	Liverwort	Pre and Post

(McLetchie and Puterbaugh, 2000)	Biomass allocation	-	Biomass (%)	Liverwort	Asexual
(Pereira *et al.*, 2016)	Reproductive allocation	-	Count of gametangia	Moss	Pre
(Rydgren and Økland, 2002)	-	Ultimate cost	-	Moss	Post
(Rydgren and Økland, 2003)	-	Short-term costs	-	Moss	Post
(Santos *et al.*, 2018)	Reproductive investment	-	Biomass (%)	Moss	Pre and Post
(Santos *et al.,* 2022a)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre and Post
(Santos *et al.,* 2023a)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre and Post
(Santos *et al.,* 2023b)	Reproductive allocation	Reproductive cost	Biomass (%)	Moss	Pre
(Shortlidge *et al.,* 2017)	Reproductive effort	-	Count of sporophytes	Moss	Post
(Stark and Brinda, 2013)	Reproductive investment	-	Biomass per area	Moss	Pre
(Stark *et al.*, 2000)	Reproductive allocation	-	Biomass per area	Moss	Pre and Post

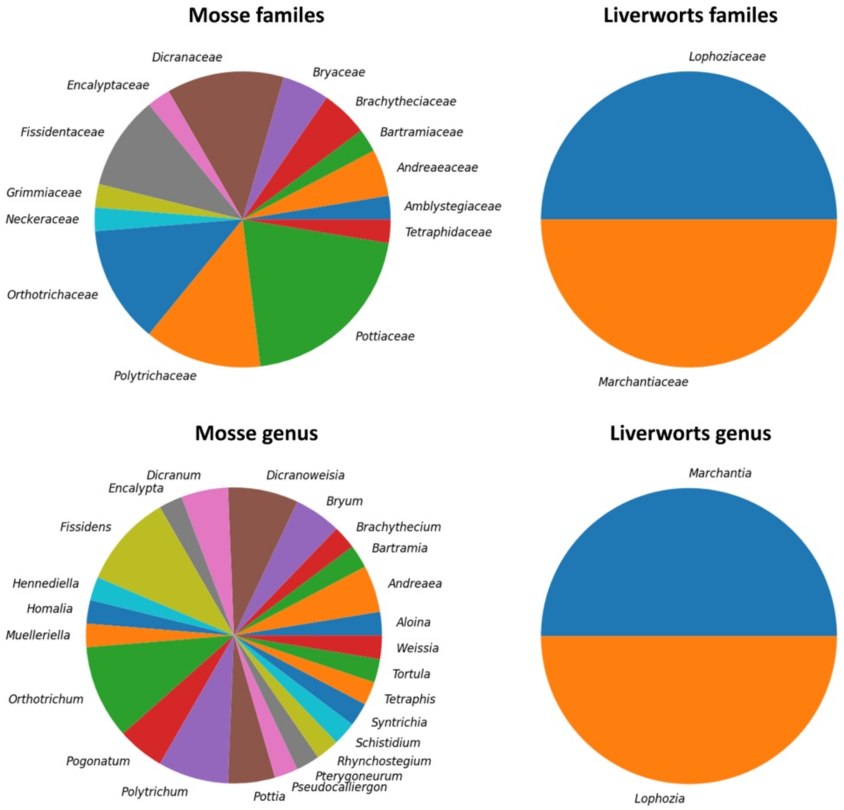


Fig 3 – Number of liverwort and moss species per family (A) and genus (B) whose reproductive allocation or reproductive cost has been investigated.

In a review of RA, Karlsson and Méndez (2005) mentioned that the most adequate term is RA (proportion of resources destined to reproduction). Terminological inconsistencies regarding RC, in turn, are discussed by Obeso (2002). Another problem found in the investigation of RA, is the way it has been quantified. In the previous section, we showed that RA has been measured in different ways, either counting or weighing reproductive structures. Of the 25 studies that investigated RA in bryophytes, 50% correctly quantified this parameter (Tab. 3). Among the other studies, most quantified RA by calculating the ratio of reproductive to vegetative biomass, and the others counted the reproductive structures (Tab. 3). Not only standardizing the terminology but also the methodology for quantification is a necessity in the field of investigation of RA. Terminological and methodological variations preclude the correct understanding of the works, the comparisons of the results of multiple studies, and consequently the understanding of ecological processes and mechanisms of the species investigated.

As for the measurement of RC, its quantification cannot be standardized, since this variable can be reflected in several ways and at several stages of the life of the organisms. For example, Bisang and Ehrlén (2002) found RC in terms of reduced growth of new branches in *D. polysetum* while Stark *et al.* (2009) found a cost in terms of lower regeneration of asexual propagules in *Pterygoneurum ovatum* (Hedw.) Dix. Therefore, there is no single correct way of quantifying RC.

Relationship between sexual dimorphism and RA

Sexual dimorphism is defined as any physiological or morphological differences related to sex (Delph, 1999). These differences are often related to RA. In morphological terms, female plants are often larger than male plants (Delph, 1999) because they need to support the offspring (Lewin, 1988). Thus, a large vegetative biomass is necessary for the development and maintenance of the offspring.

In bryophytes, sexual dimorphism has been reported in mosses and liverworts, and in some cases, this morphological variation has been attributed to reproduction. Some species have larger male plants (Laaka-Lindberg, 2001; Rydgren and Økland, 2002), others have larger female plants (Shaw *et al.*, 1993; McLetchie and Puterbaugh, 2000; Laaka-Lindberg, 2001, 2005; Stark *et al.*, 2001; McDaniel, 2005; Horsley *et al.*, 2011; Stark and Brinda, 2013; Santos *et al.*, 2018), and others show little sexual dimorphism in size (Shaw *et al.*, 1999; Stark *et al.*, 2001; Bisang *et al.*, 2006; Santos *et al.*, 2022). Furthermore, sexual dimorphism has been observed at various ontogenetic stages, such as in cases of anisospory (sexual dimorphism in the spore size) (Sérgio *et al.*, 2020).

Morphological sexual dimorphism is associated with RA because allocation of resources to reproduction limits the resources available for growth. Thus, when RA is consistently larger in one of the two sex functions in a given species, the lower amount of resources left for vegetative growth results in smaller sizes in the plants of the sex with greater RA. For example, in the liverwort *L. silvicola*, Laaka-Lindberg (2001) reported that RA led to sexual dimorphism in the species: the sex that allocated more resources to reproduction had smaller vegetative development. The influence of RA on sex-specific morphological traits has been demonstrated in many other studies (Table 4).

Tab 4 – Trade-offs: Type 1 = Sexual reproduction vs. vegetative growth; Type 2 = Asexual reproduction vs. vegetative growth; Type 3 = Sexual reproduction vs. asexual reproduction; Not observed (no conflicting demand detected).

Species	**Sexual system**	**RA**	**Sexual dimorphism**	**Trade-off**	**Reference**
Aloina bifrons (De Not.) Delgad.	Rhizautoicous	F < M	F > M	Type 2	Stark and Brinda (2013)
Anastrophyllum hellerianum (Nees ex Lindenb.) R.M.Schust.	Dioicous	-	F > M	Type 2	Pohjamo and Laaka-Lindberg (2004)
Bryum agenteum Hedw.	Dioicous	F < M	F > M	Type 1	Horsley *et al.* (2011)
Ceratodon purpureos (Hedw.) Brid.	Dioicous	F < M	F < M	Type 1	Shaw *et al.* (1999)
Crossomitrium patrisiae (Brid.) Müll. Hal.	Dioicous	-	F = M*	Type 1	Alvarenga *et al.* (2013, 2016)
Dicranum polysetum Sw.	Dioicous	F < E	F > M	Type 1	Bisang *et al*. (2002)
Fissidens flaccidus Mitt.	Rhizautoicous	F < M	F > M	Type 1, 2 and 3	Santos *et al*. (2018, 2023a)
Fissidens scariosus Mitt.	Rhizautoicous	F < M	F > M	Type 2	Santos *et al*. (2023b)
Hylocomium splendens (Hedw.) Schimp.	Dioicous	-	F > M	Type 1	Rydgren and Økland (2002)
Lophozia silvicola H.Buch.	Dioicous	F > M	F < M	Type 1 and 3	Laaka-Lindberg (2001)
Marchantia inflexa Nees & Mont.	Dioicous	F > MG	F < M	Type 4	McLetchie and Puterbaugh (2000)
Pseudocalliergon trifarium (F. Weber & D. Mohr) Loeske.	Dioicous	F > M	F = M*	Type 1	Bisang *et al*. (2006)
Sphaerocarpus texanus Austin	Dioicous	-	F > M	Type 2	McLetchie (2001).
Syntrichia carninervis Mitt.	Dioicous	F = M	F > M	-	Stark *et al*. (2001)
Weissia jamaisencis	Dioicous	F < M	F > M	Type 2	Santos *et al*. (2022)

Methods used to measure reproductive allocation in bryophytes

The quantification of RA is not a trivial process. It requires attention from the selection of ramets to the weighing of branches and reproductive structures. Since inconsistencies in the weighting of bryophytes for the quantification of RA are observed, we propose eight steps for standardization of the method, namely: (1) ramet selection; (2) cleaning; (3) removal of brown parts; (4) detachment of reproductive structures; (5) storage of vegetative and reproductive structures; (6) drying; (7) weighing, and (8) calculation of RA.

1 – Ramet selection:

To quantify RA, it is necessary to sort the material and select the ramets to be weighed. The selected sex-expressing ramets need to be viable (current growth cycle).The color and appearance are key characteristics to ensure that the plant analyzed corresponds to an actively growing ramet in the current growth cycle. Ramets of the current growth cycle are normally green and do not look brittle. Ramets are classified into two groups according to the phase of reproduction: (1) pre-zygotic (not fertilized), that is, ramets with antheridia and/or archegonia; and (2) post-zygotic (fertilized, that is, with sporophytes). The selection of sporophytic ramets must meet some requirements: (1) the ideal phenophase must be 'intact operculum' (Stark, 1985, 2002; Santos *et al.*, 2020); (2) the operculum, capsule and seta must be intact; and (3) aborted sporophytes (brown and withered) should not be selected.

2 – Ramet cleaning

Each ramet must be cleaned separately and any residual substrate must be removed from the plant. Otherwise, tiny residues, such as soil, leaf remnants, insect exoskeletons and so on will affect the estimate reproductive or vegetative biomass, leading to misinterpretations of the data (Fig 4 – A and B).

3 – Removal of brown parts

Once the ramets are clean, it is necessary to remove the brown parts (bottom of the gametophyte) (Fig 4 – C). As Bisang *et al.* (2006) remark, although the lower brown portion of the gametophytes may be alive (when they can regenerate), they do not influence the RA because they are not photosynthetically active.

4 – Detachment of reproductive structures

After the ramets have been selected and cleaned and the lower brown portion has been removed, the process of detaching the reproductive structures begins. Here we will discuss how to detach pre- and post-zygotic reproductive structures. In pre-zygotic ramets, only gametoecial leaves and the male and female gametangia, that is, the gametoecia, must be detached from the plant (Fig 4 – D). This step must be done with great care because when the reproductive structures are detached, gametangia may come off and be lost. The manipulation of post-zygotic ramets and sporophytes, in turn, is easier. To detach the sporophyte, the gametophyte must be held on one side and the sporophyte must be gently plucked at the base using tweezers (Fig 4 – E, F). For further analysis, the calyptra should be removed from the sporophyte, since this structure is a remnant of female gametangium (Fig 4 – G).

5 – Storage of vegetative and reproductive structures

The vegetative (gametophytes) and reproductive (gametoecia and sporophytes) structures must be packed in small paper envelopes to be dried. When placing the structures in small paper envelopes, it is advisable to protect the envelopes with aluminum foil so as to avoid the risk of them to open and release the structures. In this step, it is important not to press the envelopes while the structures are being placed in them because the reproductive structures are small and may be squashed and lost. At this stage, it is important to label the envelopes describing the vegetative structures and the respective reproductive structures stored in each one of them.

6 – Drying

Drying the plants is an important step because this process will eliminate all the water from the plant tissue and allow the measurement of the real biomass of the structures. Thus, each envelope must be placed in an oven for 72 h at 70 °C. This temperature is efficient for mosses, but a longer drying time may be necessary for other groups, as for example for complex thalloid liverworts such as Marchantiaceae and Ricciaceae, due to the complexity of their tissues. Structures can also be dried in desiccation chambers, but in this case, more time will be required for proper drying.

7 – Weighing

Once the structures are dry, they must be weighed. For this, a microanalytical or preferably an ultramicroanalytical balance should be used. the high sensitivity of ultramicroanalytical balances (0.1µg) make them ideal for weighing gametangia, providing greater accuracy in the quantification of biomass. As mentioned earlier, some studies quantified RA as the biomass of gametangia by weighing pre-zygotic structures. However, Stark *et al.* (2009) argue that the biomass of the gametoecia, and not only of the gametangia, need to be weighted because the gametoecial leaves protect the gametangia and help in the dispersion and or capture of the gametes. Therefore, the biomass of gametoecia must be weighed for the quantification of RA. Sporophytes and ramets must be weighed on a microbalance or ultramicrobalance.

8 – Calculation of RA

After weighing the reproductive and vegetative structures, the RA can be calculated in two ways: (1) Absolute RA – absolute biomass of reproductive structures; (2) relative RA – proportion of the total biomass of the ramet (vegetative plus reproductive parts) that corresponds to reproductive structures.

By following the steps proposed here, it is possible to know the real amount of resources allocated to reproduction in absolute and relative terms, and this standardization will allow comparisons among different studies.

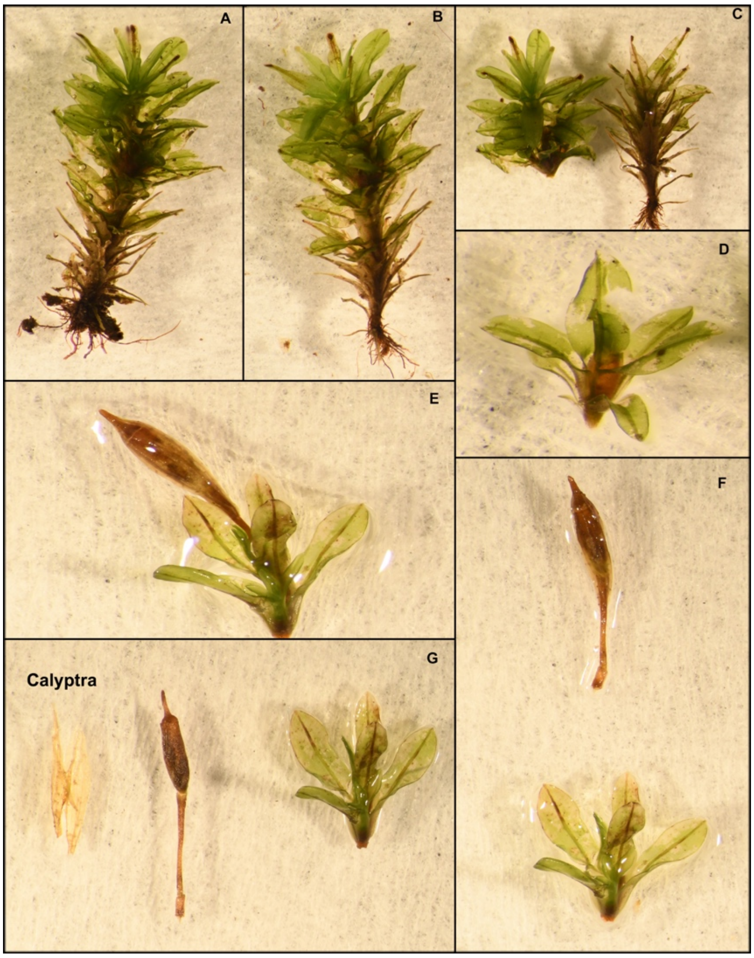


Fig 4 – *Calymperes palisotii* Schwaegr. individuals used as a model for the schematic representation of the steps for quantification of reproductive allocation in bryophytes. A - dirty ramets before cleaning; B – ramet after being cleaned with distilled water; C – separation of the brown portion (bottom part of the ramet) from the green, photosynthetic -portion (upper part of the ramet); D - selection of a gametoecium (a “perigonium” is being used here as an example); E - sporophytic female ramet with attached sporophyte; F - sporophytic female ramet with detached sporophyte; G - perigonium with detached antheridia; H - sporophyte with closed operculum and detached calyptra.

Perspectives in the studies of RA and RC in bryophytes

The synthesis of concepts in RA and RC of bryophytes presented in previous sections provides a base for new and experienced scientists interested in the field. In the next section, we will point out some topics that still need to be addressed in order to deepen the knowledge of RA and RC in bryophytes.

The simple morphological and functional characteristics of bryophytes, which were practically the first plants to successfully colonize terrestrial environments, are favorable for the study of RA and RC, allowing the implementation of experiments and the measurement of the total pool of resources.

As stressed earlier in this study, the varied forms of estimation of RA and RC in bryophytes complicate the comparisons among the studies published so far. The most appropriate ways to quantify RA and RC in bryophytes were presented. We will conclude this article by sigesting some promising areas for future research on RA and RC in bryophytes.

1 - Sexual systems

The variation of RA and RC across sexual systems is a topic that still deserves to be explored in bryophytes. Most moss and liverwort species studied so far have a dioicous sexual system. And as far as the monoicous system is concerned, only the rhizautoicous system (which is considered functionally dioicous) has been investigated. The investigation of RA in cosexual monoicous systems is fundamental to understand possible patterns and implications for the reproduction of these systems. Stark and Brinda (2013) predicted that larger distance between the sexes should increase the relative RA to the male function. If this prediction is confirmed in future studies, this could potentially explain several patterns related to the reproductive biology of bryophytes, such as, for example, the absence of sporophytes in many dioicous mosses. The only study carried out with cosexual monoicous species was the one performed with species of the genus *Fissidens*. New data from other families may shed further light on the patterns of RA in bryophyte species.

2 - Sexual lability

Sexual lability has been reported in some species of bryophytes (Korpelainen, 1998). Thus, understanding the pattern of lability of the species is important to understand their reproduction and population dynamics. Therefore, we emphasize the importance of investigating the relationship between labile sex expression and RA, because lability can be a strategy to ensure reproduction in bryophyte populations. An important question to be explored is whether the RA of genotypes exhibiting sexual lability follows the same pattern of RA as genotypes that do not exhibit liabilit**y.** When genotypes that change sex present greater RA to the new sex, this may indicate that enhancing that sexual function is important for maintaining the populations of this species.

3 – Genomics

No molecular biology studies related to sexual systems in bryophytes have been conducted so far. Thus, we highlight the importance of looking into this theme. We suggest investigating the transcriptome (complete set of transcripts of a given organism) to find out which genes are expressed for sexual expression. Also, it is important to investigate whether the intensity of sexual expression affects the lability or the pattern of RA in the species, since several physiological aspects controlled by gene expression can influence the biology of organisms.

4 – Reproductive cost

As defined earlier, RC is the effect of current reproductive effort on future reproductive success. However, most studies that investigated RC did not quantify RA. In some cases, RA was estimated in order to provide information on RC. Thus, the correct estimation of RA is essential to measure the RC. Further, the RC could be investigated in the context of other parameters besides future reproductive success, as for example, in terms of tolerance to abiotic factors, photosynthetic rates, and even morphological differences.

5 – Ecosystems

Terrestrial ecosystems have a great diversity of climatic characteristics, such as temperature, precipitation, solar radiation, and so forth. It is very important to know how certain species behave in these ecosystems. Some bryophyte species are cosmopolitan, occurring in all continents. One example is *B. argenteum* (Longton, 1981; Castetter *et al.*, 2019). This species is found naturally occurring in the most diverse terrestrial ecosystems, from the polar region to deserts, tropical forests, savannas, and temperate forests. It has been proven that the environment can influence the reproduction of mosses, as reported by Bisang *et al.* (2020) in the moss *Drepanocladus lycopodioides* (Brid.) Warnst., whose sexual expression varies according to region and environment in wetlands. Thus, studies investigating how resources are allocated in different environments are essential to understand the reproductive biology of the species.

Concluding remarks and perspectives

It is clear that RA and RC in bryophytes are not trivial topics and require further research for the clarification of ecological patterns and processes. To date, studies have been conducted in temperate forests in Europe and the US, deserts in the US, tropical forests in South America, and the Arctic region (Fig. 5). Thus, there is a gap in studies in different ecosystems such as tropical wetlands, savannas, and dry forests. Furthermore, measuring the RA following the steps proposed in the topic "*Methods used to measure reproductive allocation in bryophytes*" will provide absolute and relative RA data and even RA per square area that can be straightforwardly compared among multiple studies.

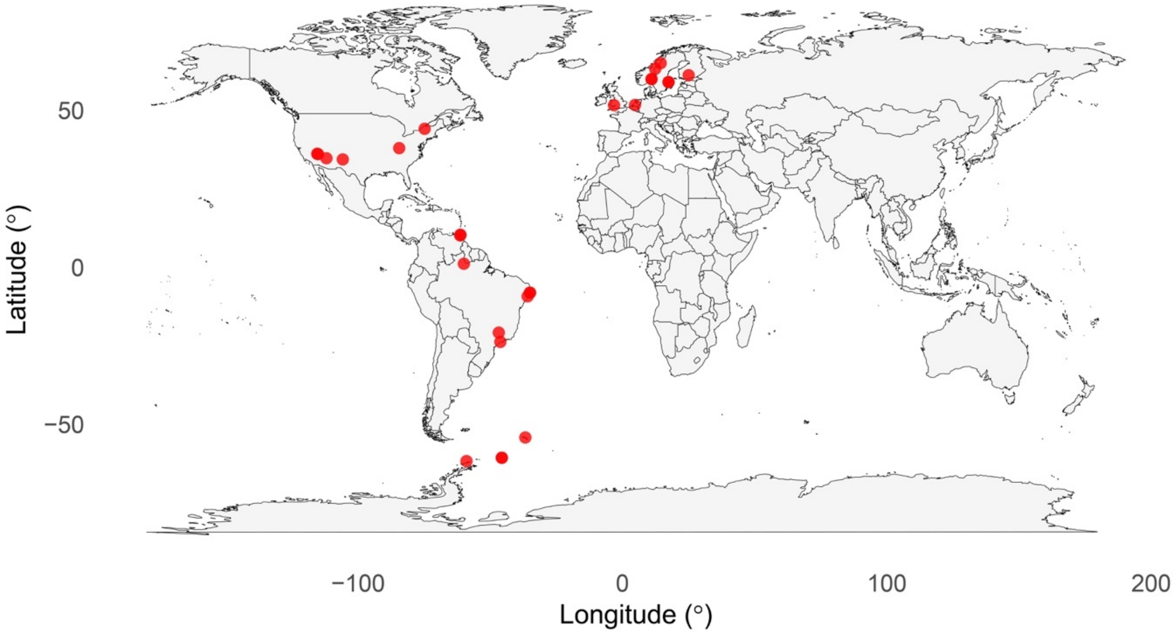


Fig 5 – Map showing localities in the world where studies on reproductive allocation and reproductive cost of bryophytes have been conducted.

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Author Contributions

Wagner Luiz dos Santos proposed the idea for the article, and he initially drafted the manuscript. Fabio Pinheiro and Kátia Cavalcanti Pôrto provided valuable feedback on earlier versions of the manuscript. Wagner Luiz dos Santos conducted the final editing. All authors carefully reviewed and approved the final manuscript. Additionally, all authors contributed to the conception and design of the study.

Declarations

Research involving Human Participants: Was not applicable

Informed Consent: Was not applicable

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Legends

Figures

Fig 1 – Scheme of the reproductive cycle of a dioicous moss. The male gametoecium (perigonium) is the structure that produces and protects the male gametangia (antheridia). Antheridia produce male gametes (antherozoids), which are flagellated and swim to the female gametoecia (perichaetia) to fertilize the female gamete (egg) found within the female gametangia (archegonia). With the fertilization of the egg, cell divisions occur to form the sporophyte, which produces the spores. In the environment, under favorable conditions, the spores germinate and form new plants, starting a new life cycle.

Fig 2 – Sexual systems of bryophytes. The distance between sexes increases from left to right. Synoicous – male and female gametangia mixed in the same gametoecia; Paroicous – male and female gametangia in the same gametoecia, but male gametangia are clustered and adjacent to female gametangia; Gonioautoicous – female gametoecia in the apex of the ramet and male gametoecia in the axil of the ramet; Cladautoicous – male and female gametoecia on separate branches of a principal ramet; Rhizautoicous – male and female gametoecia on separate ramets, however, the ramets are attached by rhizoids; Dioicous – male and female gametoecia in different plants.

Fig 3 – Number of liverwort and moss species per family (A) and genus (B) whose reproductive allocation or reproductive cost has been investigated.

Fig 4 – *Calymperes palisotii* Schwaegr. individuals used as a model for the schematic representation of the steps for quantification of reproductive allocation in bryophytes. A - dirty ramets before cleaning; B – ramet after being cleaned with distilled water; C – separation of the brown portion (bottom part of the ramet) from the green, photosynthetic -portion (upper part of the ramet); D - selection of a gametoecium (a "perigonium" is being used here as an example); E - sporophytic female ramet with attached sporophyte; F - sporophytic female ramet with detached sporophyte; G - perigonium with detached antheridia; H - sporophyte with closed operculum and detached calyptra.

Fig 5 – Map showing localities in the world where studies on reproductive allocation and reproductive cost of bryophytes have been conducted.

Tables

Tab 1 – Compilation of results from studies that quantified reproductive allocation in bryophytes. Absolute reproductive allocation is the biomass of reproductive structures. Relative reproductive allocation is the proportion of resources allocated to reproduction. Mean values per species and sex are presented. ♂ = male sexual structures (perigonia or antheridia), ♀ = female pre-zygotic structures (perichaetia or archegonia), and ⚥ = post-zygotic reproductive structures (sporophytes). Note that: ^{a} = reproductive allocation was quantified by area in mm^{2}, and ^{b} = pre- and post-zygotic structures were weighed together.

Tab 2 – Terminology used in studies to refer to reproductive allocation and reproductive cost in plants along with the definitions used by the authors.

Tab 3 – Compilation of studies that quantified reproductive allocation (RA) and/or reproductive cost (RC) in bryophytes and respective terminologies adopted in the study, variable measured for estimation of RA, form of reported results, bryophyte group to which the species belongs, and life phase (pre- or post-zygotic) in which the reproductive parameters were quantified.

Tab 4 – Trade-offs: Type 1 = Sexual reproduction vs. vegetative growth; Type 2 = Asexual reproduction vs. vegetative growth; Type 3 = Sexual reproduction vs. asexual reproduction; Not observed (no conflicting demand detected).

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Chapter 2

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Sex-specific differences in reproductive life-history traits of the moss *Weissia jamaicensis*

Wagner L. Santos[1*]*; Katia C. Pôrto*[2]*; Fabio Pinheiro*[1]

[1]Biology Institute, Department of Plant Biology, University of Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil
[2]Biosciences Center, Department of Botany, Federal University of Pernambuco, Moraes Rego Av., s/n, Recife, PE 50670-901, Brazil
*Correspondence
Wagner L. Santos, Biology Institute, Department of Plant Biology, State University of Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil.
Email: wag.lsantos1@gmail.com

ABSTRACT

Premise: We investigated sex-specific differences in the life-history traits of a metapopulation of the dioicous moss *Weissia jamaicensis*. Field observations revealed high rates of fertilization, which is uncommon for most dioicous bryophytes. We raised four hypotheses associated with the way the reproductive traits are related to the fertilization rate in this metapopulation.

Methods: We sampled 10 patches of the metapopulation and quantified sexual expression, sex ratio, reproductive success, and reproductive allocation. The ramets were classified as male, non-sporophytic female, sporophytic female, or non-sex-expressing. Thirty ramets from each of the categories expressing sex were placed for regeneration to test the effect of reproductive allocation on this trait.

Results: We found greater expression of the female function in all patches, implying a female bias in the metapopulation. The number of male ramets was variable in each patch and did not affect reproductive success. At the prezygotic level, the allocation of resources to the male function was higher. However, the large allocation of resources to sporophyte development in sporophytic females, which exceeded allocations at prezygotic levels, was related to the higher mortality rate of these ramets, suggesting reproductive cost.

Conclusions: The prezygotic ramets that allocated the greatest amount of resources to reproduction expressed sex less frequently, biasing the sex ratio toward the sex that allocated the least amount of resources to reproduction. Overall, the ramets that allocated the greatest amount of resources to reproduction had the lowest regeneration rate, suggesting reproductive cost.

Keywords: bryophyte ecology, cost of reproduction, fertilization rate, metapopulation, regeneration, reproductive allocation, reproductive success, sex ratio, sexual expression, trade-off.

INTRODUCTION

Life history is the timeline of key events in the life cycle of an organism, defined in terms of life-history traits calculated among individuals in a population (Oli and Coulson, 2016). The life-history theory predicts that every resource available to an organism is directed to its vital functions, namely growth, reproduction, maintenance, and defense (Delph, 1999). The allocation of resources in these different functions usually induces trade-offs (Stearns, 1976; Delph et al., 1996), which are the costs paid when a beneficial change in one trait resulting from resource allocation is linked to a detrimental change in another (Stearns, 1989; Obeso, 2002).

Among trade-offs, those that involve reproduction are defined as sexual reproductive costs. The reproductive cost has been the object of several studies due to its complex effects on vegetative development and future performance of organisms (Stearns, 1989; Ehrlén et al., 2000; Stark et al., 2000; Obeso, 2002). The reproductive cost has been found to be different for the female and male function, and this brings consequences to population parameters such as sexual expression, sex ratio, and reproductive success (Stark et al., 2000). According to the theory of reproductive cost, the more expensive sex—the one in which the formation of sex organs requires more energy—is expected to appear in smaller quantities in populations, originating a sexual bias toward the less expensive sex (Bowker et al., 2000; Stark et al., 2000; Obeso, 2002).

Bryophytes are a monophyletic group of land plants (Harris et al., 2020) that comprises the mosses, liverworts, and hornworts. Due to the simple architecture of their vegetative and reproductive organs, bryophytes are ideal models to test ecological theories concerning resource allocation in different vital functions (Vanderpoorten and Goffinet, 2009). Many traits can be easily recorded in bryophyte populations, increasing the explanatory power of ecological studies, for the following reasons. First, since fitness is measured as the growth rate of a population composed of identical phenotypes (Stearns, 2000), and bryophytes have a high rate of clonal growth, these plants can be used as an excellent model to quantify fitness. Second, due to the typical regenerative potential of gametophytes, the reproductive cost incurred by the survival of this life stage can be easily quantified (Stark et al., 2007). And third, given the small size of bryophytes, the total biomass of reproductive and vegetative structures can be determined to quantify the trade-off between the reproductive and the vegetative function (Ehrlén et al., 2000; Bisang et al., 2006).

Studies investigating different life-history features of bryophytes suggest that the sexual systems (dioicy and monoicy) are associated with the reproductive traits of each species (Glime and Bisang, 2017a). For example, species with separate sexes—that is, rhizautoicous (i.e., monoicous species in which antheridia and archegonia are produced in different ramets connected through rhizoids) and dioicous species—show greater reproductive allocation to the male function (Stark and Brinda, 2013; dos Santos et al., 2018). Besides, populations of bryophytes with separate sexes present fewer sex-expressing male ramets, lower rates of sexual expression, and a trend toward lower reproductive success (Horsley et al., 2011; dos Santos et al., 2020). On the other hand, populations of cosexual monoicous species present higher rates of sexual expression, shorter distances between the sexes, and greater fertilization rates (de Oliveira and Pôrto, 1998; Pôrto and de Oliveira, 2002; dos Santos et al., 2020). However, some species of the two sexual systems—dioicous and rhizautoicous—have been observed to deviate from the expected pattern (e.g., dioicous species with male biased populations; Alvarenga et al., 2013; Holá et al., 2014).

Several studies have found unbalanced sex ratios in unisexual species of different land plants (Field et al., 2013; Segalla et al., 2021). In unisexual bryophytes, the rarity or absence of male sex expression is recurrently observed (Bisang and Hedenäs, 2005). One potential explanation for this phenomenon is the reproductive cost, since resource allocation to male sex expression is often more expensive than that to the female function (Stark et al., 2000). Reproductive allocation is often calculated in absolute (biomass) and relative (proportion of resources allocated to reproduction) levels (Obeso, 2002), but other metrics are also used, such as the number of gametangia produced and the density of gametoecia per square area (Stark and Brinda, 2013; Pereira et al., 2016). Trade-offs are usually quantified on the basis of reproductive allocation, since it is this parameter that reveals the real amount of resources allocated to reproduction (Laaka-Lindberg, 2001; dos Santos et al., 2018).

Weissia jamaicensis (Mitt.) Grout. is a dioicous moss and, as expected for dioicous species, sporophytes are recurrently absent in the field (Müller et al., 2018). However, we found a metapopulation of `with unexpectedly high levels of sporophyte formation. Thus, in order to investigate the underlying causes of such an exceptional condition, we investigated the following hypotheses, which suggest situations that increase the chances of fertilization: (1) the sex ratios of the metapopulation and within each colony (patch) are not biased (1:1); (2) there is a high rate of sexual expression among males, which enhances the reproductive success because the presence of more male ramets expressing sex increases the amount of gametes for fertilization; (3) reproductive allocation is higher in the male than in the prezygotic female function, such that the greater amount of resources allocated to the male function generates a greater amount of male gametes; and (4) reproduction and regeneration of ramets trade off each other, such that greater reproductive allocation is associated with lower regeneration rates of ramets.

MATERIALS AND METHODS

Study site

The study was conducted in a remnant of Cerrado s.s. in the municipality of Itaú de Minas, state of Minas Gerais, Brazil (20°46′20.8″S, 46°45′09.9″W). The weather in Itaú de Minas is tropically hot and dry according to Koppen classification, with an average monthly temperature of 23°C (Alvares et al., 2013) (INMET, https://portal.inmet.gov.br). The rainy season occurs in November–February (spring–summer), and December and January are the rainiest months (INMET).

Study species and sampling

Weissia jamaicensis is a dioicous moss found in Africa, North America, and the Neotropics, recurrently without sporophytes (Müller et al., 2018). In Brazil, *W. jamaicensis* is found on roadside rocks and riverbanks, in altitudes of 100–800 m, in Savanna and Atlantic Rainforest (Costa, 2015) (Figure 1).

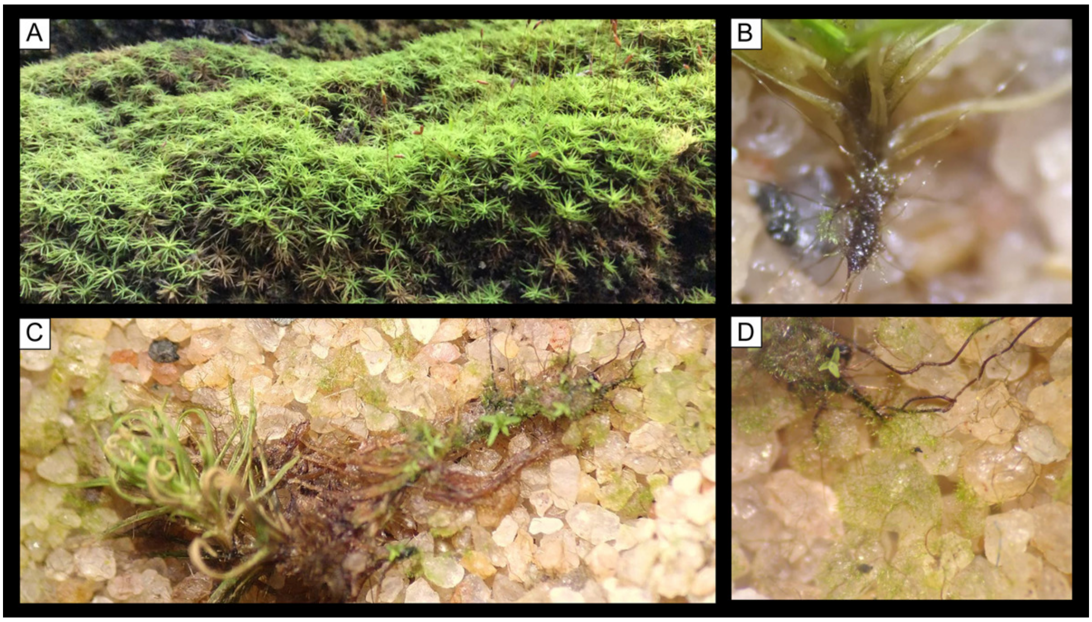

Figure 1 - (A) *Weissia jamaicensis* in its natural habitat. (B) Protonema being formed at the base of the stem. (C) Ramets with protonemata and new ramets being formed. (D) Protonemata and rhizoids with a juvenile ramet.

The metapopulation of *W. jamaicensis* selected for this study was found in a rural area of the municipality of Itaú de Minas with an approximate size of 20 × 15 m. The plants were growing in patches on a thin layer of sandy soil above a limestone rock. In July 2020, one

12 × 12 cm sample was collected from each of ten of these patches. To extract the maximum amount of information about reproductive parameters, samples were collected randomly with a minimum sampling distance of one meter. The samples were collected by placing a 12 × 12 cm germination box on the colony and pressing it against the ground to remove the plants (Appendix **S1**).

Sexual expression, sex ratio, reproductive success

In the laboratory, each box was divided into nine quadrants of 4 × 4 cm, and five of these quadrants (the central one and the four of the corners) were selected for harvesting ramets (Appendix **S1**). Ten ramets were collected in each of the five quadrants, totaling 50 ramets per box. Thus, 50 ramets were collected from each patch, for 500 in total. These ramets were used to quantify sexual expression, sex ratio, and reproductive success.

To quantify sexual expression, each ramet was classified into one of the following sexual conditions: (a) male—ramet with perigonia; (b) non-sporophytic female—ramet with perichaetia, without sporophytes; (c) sporophytic female—ramet with sporophytes; and (d) non-sex-expressing—ramet without sexual expression. Sexual expression and sex ratios (Segalla et al., **2021**) were calculated for the metapopulation (all patches) and for each patch. Sexual expression was calculated as the proportion of ramets expressing sex (male, non-sporophytic female, and sporophytic female), and sex ratios were calculated on the basis of numbers of male and female ramets (calculation of sex ratios in this study is based on ramets expressing sex). Reproductive success was calculated as the proportion of female ramets that produced sporophytes.

Reproductive allocation

To quantify reproductive allocation, 30 male, 30 non-sporophytic, and 30 sporophytic female ramets were randomly selected. The methodology proposed by Bisang and Ehrlén (**2002**) was followed: each ramet was cleaned and the biomass of brown portions of the gametophytes was removed, leaving only the photosynthetically active parts to be weighed. This is because the brown parts may be dead, and even if they are alive (when they can regenerate), they do not affect the allocation of resources destined for reproduction. Thus, the reproductive parts (perigonia, perichaetia, and sporophytes) were separated from the ramets, placed in small paper envelopes wrapped in aluminum foil, and dried in an oven for 72 h at 70°C.

After drying, each ramet and its respective reproductive structures were weighed on an ultramicrobalance (SE2 ultramicrobalance; Sartorius, Goettingen, Germany; precision ± 0.1 µg). The vegetative biomass and the absolute and proportional reproductive biomass (absolute reproductive allocation [ARA] and relative reproductive allocation [RRA], respectively) were measured.

Regeneration

To investigate the sex-specific regeneration rates in *W. jamaicensis*, 30 male, 30 non-sporophytic female, and 30 sporophytic female ramets were selected. Each male and non-sporophytic female ramet had the gametoecia excised and the number of gametangia counted. Then, each ramet was cleaned with 10% sodium hypochlorite solution for 20 s and sterile mineral water. After drying at room temperature, each ramet was placed in a Petri dish with sand from the collection site and each Petri dish was hydrated weekly with 10 mL of water collected in a waterfall next to the collection site. The Petri dishes were kept in a greenhouse at room temperature and 50% shade and monitored weekly for 7 wk. The following parameters were quantified for each ramet: week in which protonema formation started; week in which the protonema formed the first ramets; and total number of ramets produced.

Statistical analyses

The rate of sexual expression was calculated for each patch and the metapopulation. Generalized linear models (GLMs) were applied to determine whether sexual expression varied according to patch and sex. First, a full model was created in which the response variable was sexual expression and the predictor variables were the patch, the numbers of male and female ramets, and the interaction between these variables. A null model was created to test whether the observed variation occurred at random. Since the response variable was a proportion, the binomial family was used in the model. The models were compared using the chi-square test, and since the observed difference was significant ($P < 0.001$), the null model was rejected and the full model was adopted to obtain our answers. Interactions were not significant and, therefore, excluded from the model. The full model was run to investigate which of the variables were important for sexual expression. The model was subjected to a residual analysis to assess the adequacy of the error distribution, and subdispersion was found. Thus, the distribution family was changed to quasibinomial so that subdispersion was addressed.

To investigate our first hypothesis of nonbiased sex ratios, a matrix with the numbers of male and female (sporophytic and non-sporophytic) ramets per patch was created and then the chi-square test was applied to analyze whether there was sex bias in each patch and in the metapopulation.

To test our second hypothesis, GLMs were used to investigate whether greater numbers of male ramets induced greater reproductive success. Two models were created: a full model with reproductive success as response variable and number of male ramets and sex ratio as predictor variables, and a null model with reproductive success as response variable to test whether the probability of reproductive success was explained by chance (i.e., not influenced by any of the factors). The logit link function and a binomial distribution were used in both models. The chi-square test was applied to compare the models and, since a significant difference was found, the null model was rejected and the full model was used. The summary of the first model was checked to determine whether the predictor variables and their interactions influenced sexual expression in *W. jamaicensis*. The model was submitted to a residual analysis to assess the adequacy of the error distribution, and overdispersion was detected; thus, a quasibinomial distribution was used. To test our third hypothesis of greater male than prezygotic female reproductive allocation, the three categories of ramets studied had their absolute and relative reproductive allocation compared through the Kruskal-Wallis test with post hoc Dunn's test with Bonferroni correction, since data were not normally distributed even after transformations.

The numbers of gametangia produced by male and non-sporophytic female ramets were compared using a *t*-test; for that, all the assumptions were analyzed. The numbers of gametoecia were also compared between male and non-sporophytic female ramets using the Mann-Whitney *U*-test, since data were not normally distributed even after transformations. The absolute and relative reproductive allocation differed between male, non-sporophytic female, and sporophytic female ramets, being smaller in non-sporophytic female ramets and greater in sporophytic female ramets. Thus, the following life-history traits were compared between ramets with low (non-sporophytic female ramets), medium (male ramets), and high (sporophytic female ramets) reproductive allocation: (1) start of protonema formation, (2) formation of the first ramet, and (3) total number of ramets produced. The Kruskal-Wallis test with post hoc Dunn's test with Bonferroni correction was used to this end because the variables presented nonparametric distribution even after transformations.

RESULTS

Sexual expression, sex ratio, and reproductive success

The rate of sexual expression in the metapopulation was 57.2% (Table 1), and only three of the ten patches analyzed presented sexual expression below 50%. Sexual expression rates varied nonsignificantly across patches, but the proportion of male and female ramets varied significantly between patches (Table 2). The interaction term between sex and patch was not significant.

Sex ratios, which are based on sexually expressing ramets, were skewed toward the female sex in seven of the 10 patches, toward males in two patches, and balanced in one patch (Table 1). The metapopulation was female biased, with a sex ratio of 1.83♀:1♂ (Table 1).

Reproductive success was not significantly influenced by the variables tested in the GLM (Table 3). The number of male ramets, the sex ratios, and the interaction between these variables showed no influence on the number of fertilized female ramets in the patches of the metapopulation.

There was a significant difference between male and prezygotic female reproductive allocation ($t = -7.83$, $df = 58$, $P < 0.001$): male ramets produced a greater number of gametangia and biomass of gametoecia than female ramets ($U = 240$, $P < 0.001$).

The vegetative biomass and the absolute and proportional reproductive biomass differed between sexes. Sporophytic and non-sporophytic female ramets had higher average vegetative biomass, and a significant difference was observed only between these two categories of ramets ($\chi 2 = 14.509$, $df = 2$, $P < 0.001$; Figure 2A). Absolute reproductive allocation differed between the three categories of ramets ($\chi 2 = 65.278$, $df = 2$, $P < 0.001$; Figure 2B). Finally, the relative reproductive allocation differed between the three categories ($\chi 2 = 45.628$, $df = 2$, $P < 0.001$; Figure 2C). The inversely proportional relationship between relative reproductive allocation and survival of ramets supports the existence of reproductive cost (Figure 3).

Table 1. Numbers of male, non-sporophytic female, and sporophytic female ramets, absolute and proportional numbers of sex-expressing ramets, and sex ratio per patch. Significance indicates occurrence of sexual bias (^{ns}nonsignificant; *$P < 0.05$, ***$P < 0.01$, ***$P < 0.001$).

	Category of ramets				**Expressing sex ramets**		**Population parameters**		
Patch	**Male**	**Non-sporophytic female**	**Sporophytic Female**	**Non sex-expressing**	**Total female**	**Total male**	**Sexual expression (%)**	**Sex ratio (♀/♂)**	**Sex Characterization**
1	8	18	7	17	25	8	66	3.13:1**	Female biased
2	2	6	4	38	10	2	24	5.00:1*	Female biased
3	30	0	12	8	12	30	84	0.40:1**	Male biased
4	1	6	23	20	29	1	60	29.00:1***	Female biased
5	0	6	0	44	6	0	12	–	Female biased
6	4	3	12	31	15	4	38	3.75:1*	Female biased
7	34	0	8	8	8	34	84	0.24:1***	Male biased
8	13	9	15	13	24	13	74	1.85:1$^{n.s.}$	Unbiased
9	0	12	24	14	36	0	72	–	Female biased
10	9	5	15	21	20	9	58	2.22:1*	Female biased
Metapopulation	**101**	**65**	**120**	**214**	**185**	**101**	**57.2**	**1.83:1*** **	**Female biased**

Table 2. Generalized linear model effects of sex and patch on sexual expression without interaction term.

	Response Variable (Sexual Expression)
Patches	−0.003 (0.010) t = −0.260 p = 0.804
Female ramet	0.091 (0.004) t = 25.906 p < 0.001
Male ramet	0.096 (0.003) t = 32.129 p < 0.001
Constant	−2.328 (0.092) t = −25.193 p < 0.001
Observations	10
Residual Deviance	0.449 (df = 6)
Null Deviance	120.567 (df = 9)

Table 3. Generalized linear model effects of number of male ramets, sex ratio, and their interaction on the reproductive success of the studied metapopulation.

	Response Variable (Reproductive success)
Male	0.257 (0.197) t = 1.306 p = 0.240
Sex ratio	0.159 (0.096) t = 1.667 p = 0.147
Male: Sex ratio	−0.135 (0.099) t = −1.372 p = 0.220
Constant	0.410 (0.545) t = 0.753 p = 0.481
Observations	10
Residual Deviance	23.082 (df = 6)
Null Deviance	52.097 (df = 9)
Note:	*p<0.1; **p<0.05; ***p<0.01

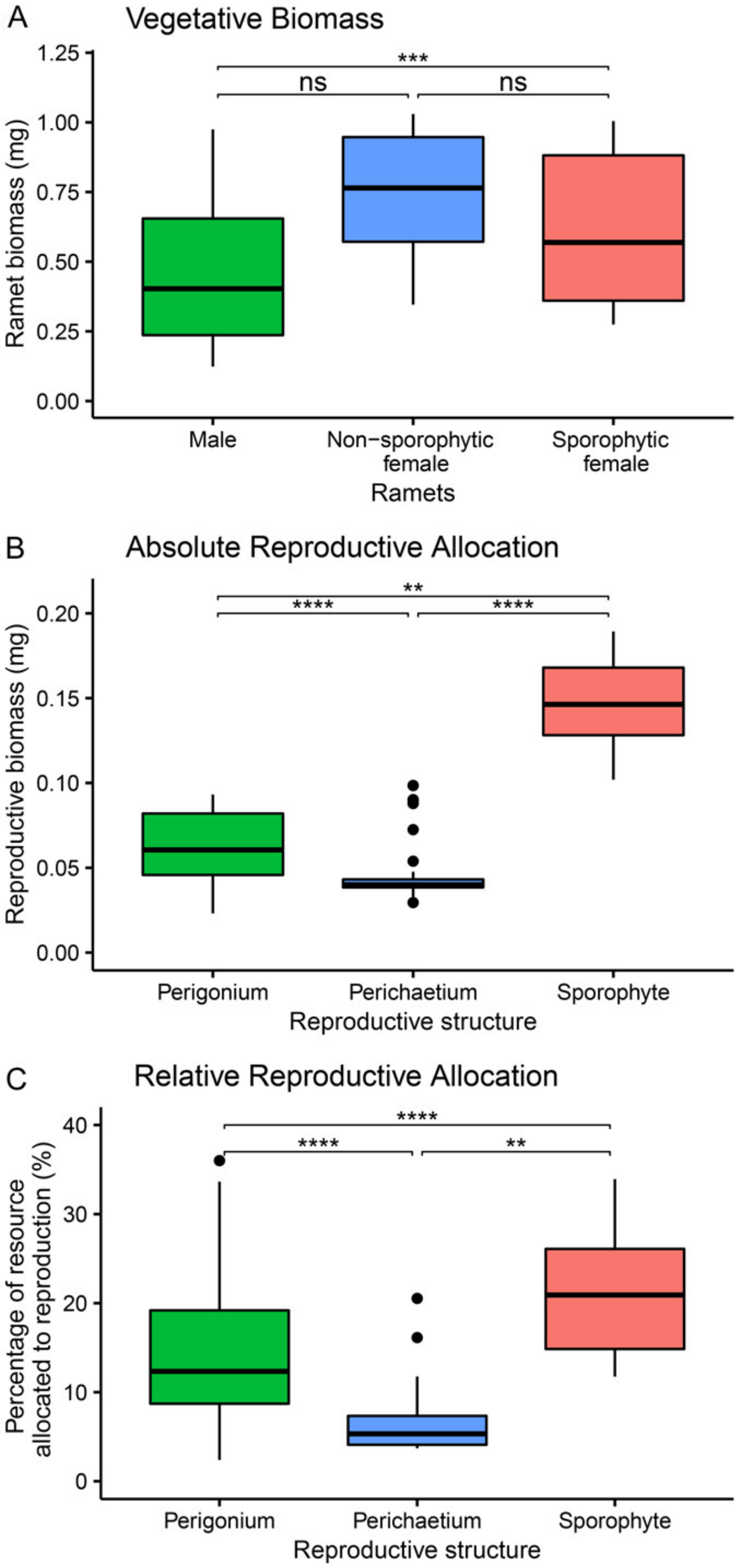

Figure 2 - (A) Vegetative biomass, (B) absolute reproductive allocation, and (C) relative reproductive allocation of male, non-sporophytic female, and sporophytic female ramets of *Weissia jamaicensis* (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ **** $P < 0.0001$; ^{ns} nonsignificant).

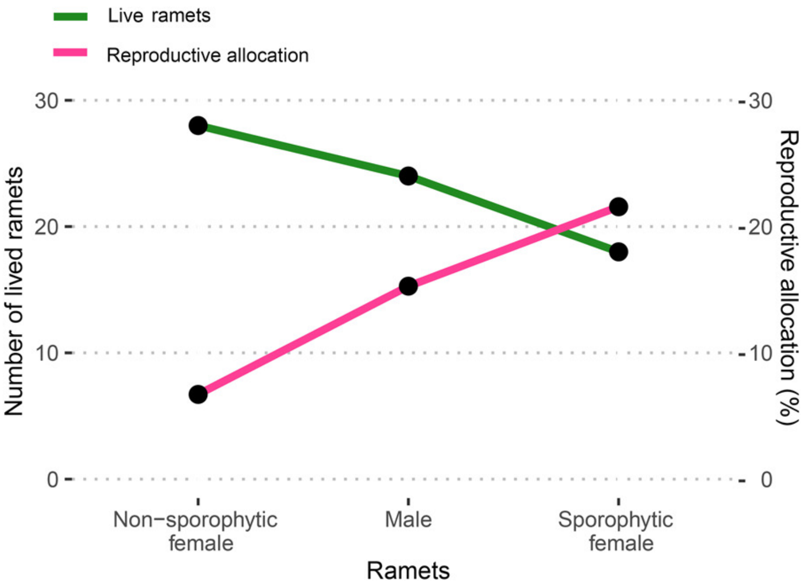

Figure 3 - Illustration of the trade-off between relative reproductive allocation and numbers of live male, sporophytic female, and non-sporophytic female ramets of *Weissia jamaicensis*.

The initiation of protonema formation did not differ significantly between sexes ($\chi^2 = 4.61$, df = 2, $P = 0.10$; Table **4**), indicating that, regardless of sex, when conditions for regeneration are established, protonemata will form in due time. No sex-specific differences were observed in the formation of the first ramets either ($\chi^2 = 3.77$, df = 2, $P = 0.15$; Table **4**). In turn, the number of ramets produced after seven weeks of the experiment varied between sexes ($\chi^2 = 20.88$, df = 2, $P < 0.001$; Table **4**), and the ramets that allocated a larger amount of resources in reproduction presented lower regeneration rates, suggesting reproductive cost.

Table 4. Descriptive statistics (means ± SD) of the life-history traits initiation of protonema formation, start of ramet formation, and number ramets formed (superscript letters signify the parameters of significance; matching letters indicate equal averages) and results of Kruskal-Wallis test.

	Ramet category			Kruskal-Wallis		
	Male	Non-sporophytic female	Sporophytic female			
Variables	(X ± SD)	(X ± SD)	(X ± SD)	X^2	df	p
Initiation of protonema formation	0.80 ± 0.40^a	0.93 ± 0.25^a	0.70 ± 0.65^a	4.61	2	0.10
Beginning of ramet production	3 ± 1.76^a	3.66 ± 1.02^a	2.56 ± 2.22^a	3.77	2	0.15
Ramets produced	3.40 ± 2.02^a	4.66 ± 1.74^b	2.23 ± 1.27^c	20.88	2	0.001

DISCUSSION

Dioicy is commonly associated with a suite of life histories and reproductive traits that may involve genetic, demographic, and ecological costs (Barrett et al., 2010; Renner, 2014). For instance, the obligate outcrossing mechanism imposed by dioicy means that when potential mates of the opposite sex are absent, individuals will remain effectively sterile (Käfer et al., 2017). Populations of dioicous mosses often show a sexual bias that contributes to decrease the levels of fertilization, mainly when compared to monoicous species (Longton, 1992). In *W. jamaicensis*, high levels of fertilization were found despite the significant female bias found in most sampled patches, contradicting the expected patterns for dioicous species showing strong sexual bias (Bisang and Hedenäs, 2005). We found that reproductive allocation was higher in males than in prezygotic females, and this negatively affected the growth of male ramets. Thus, our results revealed a trade-off in allocation associated with sex-differential reproductive costs (Freeman et al., 1997; Zhang et al., 2014). The combination of detailed data from field observations with the regeneration rates obtained in the greenhouse experiment in this study provides information not only about the impacts of sexual bias in the reproduction of mosses, but also about the origin of the specialization and competitive ability in resource allocation between sexes.

Sex-specific variation in reproductive traits and its effects on population parameters

Our results showed that sexual expression did not vary across patches in the study metapopulation, but sex expression varied between sexes. In dioicous mosses, female sex expression is observed more frequently than male sex expression (Glime and Bisang, 2017a). The "shy male hypothesis," which predicts that male ramets fail to sexually express, has been proposed and tested in some studies in the attempt to explain this pattern. Stark et al. (2010) tested it with the dioicous moss *Bryum argenteum* Hedw. They quantified the sexual expression rates in sex-expressing ramets and non-sex-expressing ramets grown until sexual expression. They observed that >80% of the ramets that expressed sex were female and that male expression was rarer in xeric sites. Furthermore, the authors observed that the calculated sex ratio of the non-expressing ramets did not differ from the sex ratio of those expressing sex. The same pattern was found for the moss *Drepanocladus lycopodioides* (Brid.) Warnst. by Bisang and Hedenäs (2013), in which the sex ratio was calculated using sex-specific molecular markers. These authors found a female-skewed sex ratio in the population of 2.6:1, so that the shy male hypothesis was refuted for this species.

To further test the shy male hypothesis, it is essential to think about spore sex ratios. These ratios have been examined so far in only a few species of bryophytes through cytological evidence and spore germination until sexual maturity (Glime and Bisang, 2017a). According to Glime and Bisang (2017b), in dioicous bryophyte species, at the beginning of meiosis, the sporocyte (the cell that gives rise to spores) presents a set of chromosomes containing a male chromosome and a set containing a female chromosome. Thus, if the process occurs normally during meiosis, one sporocyte will produce four daughter cells—two female and two male—consequently generating a 1:1 ratio. Among the species in which spore sex ratios have been quantified through germination, the liverwort *Sphaerocarpos texanus* Austin. had a sex ratio of 1:1, but higher mortality rates of male spores caused populations to become female biased (McLetchie, 1992). Likewise, the spore sex ratio of the dioicous moss *Ceratodon purpureus* (Hedw.) Brid. did not differ significantly from 1:1, but sexual dimorphism in life-history traits was observed (Shaw and Gaughan, 1993; Shaw and Beer, 1999). Norrell et al. (2014) reported a variation in sporophyte production and spore viability for *C. purpureus* that could bias the sex ratio of the population. Finally, more recently, Bisang et al. (2017) investigated the spore sex ratio in the dioicous moss *D. lycopodioides* and also observed a balanced sex ratio (1:1), suggesting that the sexual dimorphism during the formation of the ramets favors a female bias. Furthermore, Eppley et al. (2018) demonstrated that female-biased ramet ratios in *C. purpureus* were established in the gametophytic phase, during protonemal or ramet formation. Based on the above considerations, we can entertain the possibility that biotic (spore mortality, germination, and growth rate) and abiotic (availability of nutrients and harshness of the habitat) factors may differentially affect the development of male spores of *W. jamaicensis*, leading to female-biased sex ratios. However, conclusive evidence for this hypothesis depends on further investigation.

It is logical to think that the functional sex ratio influences the reproductive success because more male ramets imply a greater number of antherozoids for fertilization. Our results refuted our first and second hypotheses: the sex ratios were not balanced and there was no higher male sex expression in the metapopulation. Yet reproductive success was high despite the fact that male ramets were less abundant than female ramets in most patches and in the metapopulation. Two possible reasons for this are that (1) the male ramets were homogeneously distributed in the population, such that they were always close to female ramets, resulting in high fertilization rates; and (2) the antherozoids dispersed over longer distances than typically assumed, favored by moisture availability/water flow or even microarthropod dispersal. We consider the second reason particularly plausible because male ramets were absent in some patches where sporophyte formation was observed. Also, the number of male ramets did not significantly influence the reproductive success. It has been suggested that the segregation of sexes (Wyatt, 1977) and the availability of male ramets (Hugonnot et al., 2014) in dioicous mosses lead to lower fertilization rates, but our results did not support either of these assumptions in the studied metapopulation. Similarly to what we observed in *W. jamaicensis*, the distance from male ramets did not influence fertilization rates in two species of Pottiaceae, *Hyophila involuta* (Hook.) Jaeg. and *Barbula agraria* Hedw. (de Oliveira and Pôrto, 2005), or in the liverwort *Marchantia polymorpha* L. (McAdam et al., 2021). In the latter case, all female plants were fertilized. The authors concluded that these results were explained by the amount of antherozoids produced and their ability to "swim" around by flagella. However, in the species *Dicranum flagellare* Hedw., Kimmerer (1994) observed that the fertilization rate was influenced by population density: an optimal density enhanced the production of sporophytes, and when the density deviated upward or downward from the expected optimum, the fertilization rate decreased. Therefore, it can be suggested that the density of ramets in *W. jamaicensis* may affect fertilization rate.

Higher male reproductive allocation leads to greater reproductive success

Regarding the reproductive allocation by male and prezygotic females, *W. jamaicensis* followed the pattern for dioicous mosses of greater allocation to the male function. Thus, our third hypothesis was corroborated. This is likely related to the goal of enhancing reproductive success (dos Santos et al., 2020). Male ramets were also less abundant in the studied metapopulation, skewing the sex ratio toward females, which is in line with the reproductive cost theory (Obeso, 2002), in that the most expensive sex is found in less quantity. However, we suggest that this greater allocation of resources to the male function may reflect a strategy to save resources, because a single male plant is capable of fertilizing several female plants. If male plants had high rates of sexual expression and high reproductive allocation, there would be an excess of male gametes in relation to female gametes to be fertilized. Stark et al. (2000) found that the male reproductive allocation in *Syntrichia caninervis* Mitt., the model plant of the family Pottiaceae used in several of their studies, was more costly than the female prezygotic reproductive allocation, and they found evidence of higher reproductive cost for the male function in several ontogenetic stages, in terms of leaf regeneration (Stark and McLetchie, 2006), population parameters (Stark et al., 2001, 2004), and tolerance to abiotic stresses (Stark et al., 2005; Stark and McLetchie, 2006).

Another aspect that we can associate with the results on reproductive allocation and reproductive cost at the prezygotic level is the production of new ramets. Our results showed that regeneration rates did not differ between male and non-sporophytic female ramets in terms of time for onset of protonema formation and of ramet formation. However, the total number of ramets produced was higher among females (Figure 2C). Thus, the greater regeneration rates of female ramets may explain the skewed sex ratios. This is in line with the findings of Stark et al. (2004), who quantified leaf regeneration rates according to leaf age and sex in *S. caninervis* and found no differences in the time until protonemal emergence, linear extension of the protonemata, or rate of biomass accumulation. Nevertheless, those authors concluded that the faster proliferation of female ramets may help explain the rarity of males in this species. Following the same line of reasoning, we consider it very likely that female ramets of *W. jamaicensis* are more frequent in the studied metapopulation due to their higher regeneration rates.

Offspring costs impact the life of sporophytic female ramets

It is reasonable to think that postzygotic reproductive costs exceed prezygotic costs (Glime and Bisang, 2017a). The large number of sporophytic female ramets that died after reproduction and that consequently generated the lowest number of new ramets may be indicative of the cost of sporophyte production. The first bryophyte species to have the cost of sporophyte production estimated was the moss *Dicranum polysetum* Sw. (Ehrlén et al., 2000). The higher biomass allocated to the vegetative function in plants that aborted all the sporophytes in this species suggested that sporophyte formation led to lower vegetative growth. A negative correlation between the development of mature sporophytes and new annual segments was also detected in *D. polysetum* (Bisang and Ehrlén, 2002). Similarly, in our study, greater reproductive allocation was associated with lower post-reproduction development (regeneration), indicating sex-specific differences in the life-history traits of *W. jamaicensis*.

CONCLUSIONS

Despite the consistent female bias observed in the metapopulation studied, most patches showed high levels of fertilization. In each patch, the number of male ramets did not influence the formation of sporophytes, suggesting that female bias has a positive effect in the reproduction of *W. jamaicensis*. Additionally, reproductive cost was indicated by the fact that reproductive allocation was inversely related to regeneration rates (Haig, **2016**). Since only one metapopulation was investigated here, our results should be interpreted with caution. Future studies should focus on (1) identifying the sex of ramets using molecular methods so as to quantify the genetic sex ratio of the populations and not only the sex ratio of sex-expressing ramets; (2) analyzing whether the different sexual conditions of the ramets show different tolerance to environmental factors such as temperature and water availability, in order to investigate whether female ramets are more tolerant and consequently better competitors, justifying their greater abundance in the population; and (3) investigating whether population density can influence population parameters (sex ratios, sexual expression, and reproductive success).

AUTHOR CONTRIBUTIONS

W.L.S. originally formulated the idea, developed the methodology, and conducted fieldwork. W.L.S., K.C.P., and F.P. collaborated in imaging analysis. W.L.S. performed the statistical analyses and wrote the manuscript. K.C.P. and F.P. corrected and discussed the manuscript.

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Chapter 3

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Life-history traits and density dependency in metapopulations of a tropical moss: a monoicous species that is almost dioicous

Wagner Luiz dos Santos[1]; Juçara Bordin[2]; Kátia Cavalcanti Pôrto[3]; Fábio Pinheiro[1]

[1] Department of Plant Biology, University of Campinas, Rua Monteiro Lobato., 250, Barão Geraldo, Campinas, São Paulo, Brazil

[2] State University of Rio Grande do Sul, Machado de Assis, 1456, RS 95520-000, Brazil.

[3] Department of Botany, Federal University of Pernambuco, Moraes Rego Av., s/n, University City, Recife, Pernambuco, Brazil

Corresponding author: wag.lsantos1@gmail.com

This paper deserves to be honored as a Highlighted Student Paper:

This study has the potential to be a Highlighted Student Paper because most of the ideas, hypotheses, and questions were conceived by me during the period of pandemic restrictions and because the findings are unprecedented considering the data available in the literature for monoicous bryophytes. In this article, we investigated the biology and ecology of a tropical moss species with a monoecious sexual system, *Fissidens flaccidus*, which becomes functionally dioecious due to sex segregation. Several reproductive traits found in *F. flaccidus* such as female-biased sex ratio, low male sexual expression, and higher male reproductive allocation are not common in monoicous species. Our data indicate that *F. flaccidus* behaves like a dioecious species, expanding our view regarding the diversity of reproductive strategies found in bryophytes.

Declaration of authorship: WLS and KCP conceived the ideas. WLS, KCP, and FP designed the methodology. WLS conducted field work supported by KCP. WLS, FP, KCP and JB analyzed the data. WLS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ABSTRACT

Life-history traits, such as reproductive allocation, sexual expression, sex ratio, and reproductive success, are central aspects of a species' ecology and evolution. For example, bias in male and female sex expression may play a large role in determining the viability of populations in the face of environmental pressures, such as population fragmentation, climate change and habitat occupancy. Thus, in this study, we investigated reproductive traits in 10 meta-populations of *Fissidens flaccidus* Mitt. From each meta-population, 30 patches were randomly selected, and 1 cm^2 samples were collected form each patch. A total of 20,173 ramets were analyzed and classified into male, non-sporophytic female, sporophytic female, and non-sex expressing. In addition, population density in each patch was quantified. Our results showed that relative reproductive allocation in perigonia and sporophytes is greater than perichaetia. Trade-off between sexual relative reproductive allocation and asexual gemma production was observed, suggesting an important role of female ramets in asexual reproduction. The number of male ramets does not influence the reproductive success observed in each patch, and ramet density may induce male sex expression. Thus, we concluded that reproductive allocation in male function is efficient, since fewer male ramets can assure a considerable reproductive success. Furthermore, our results suggest that there may be a habitat preference between the sexes, since male ramets are found in patches with high density and mostly below female ramets, suggesting an avoidance of direct sunlight by male ramets.

Keywords: Reproductive allocation, Sexual dimorphism, Sex ratio, Sexual expression,· Trade-off.

INTRODUCTION

Life history is a schedule of key events in an organism's life cycle, and is usually defined in terms of life-history traits averaged across individuals within a population (Oli and Coulson 2016). This life history theory asserts that vital functions, such as growth, reproduction, maintenance and defense, compete for limited resources obtained by an organism (Delph 1999). It is often observed that the allocation of resources in different traits of the organism's life history induces trade-offs (Stearns 1976; Delph et al. 1996). Such trade-offs represent the costs paid when the available resource is allocated differentially among traits (Stearns 1989; Obeso 2002). For example, in the aquatic monocot *Butomus umbellatus* L., plants that are pollinated show a significant reduction in clonal bulbil (Thompson and Eckert 2004). Thus, trade-offs are commonly observed in reproductive traits, showing a crucial influence in the demography and maintenance of populations (Rydgren and Okland 2003; Horsley et al. 2011).

Reproductive traits, such as sex expression (proportion of individuals that are expressing sex) and sex ratio (proportion of females and male in a population), are important variables influencing the reproductive performance of population (Glime and Bisang 2017b). In this context, many biotic or abiotic factors can affect development and reproductive traits. For instance, some species need high levels of humidity to express sexuality, as during the rainy season, while others do not (Maciel-Silva et al. 2012; Maciel-Silva and De Oliveira 2016). Sexual reproduction plays an important role in maintaining species, and in some cases, population density is a determining factor of reproductive performance. Density dependence has been recurrently reported in species of plants and animals (Hanski 1990; Gunton and Kunin 2009). Furthermore, sexual systems which are defined by Leonard (2018) as "pattern of gender allocation that characterizes a species", have also been linked to the reproductive success of populations, for example in *Fissidens scarious* Mitt., and *Fissidens submarginatus* Bruch., which have reproductive traits differently associated with sexual systems (Santos et al. 2020).

In plants, the sexual system is classified in relation to the distribution (distance) between reproductive structures (Bergh and Verboom 2011). Species that have male and female reproductive structures on the same plant are classified as monoecious, and dioecious species that have reproductive structures on separate plants. However, in bryophytes, as the reproductive structures are produced in the haploid phase, these systems are called monoicous and dioicous (Glime and Bisang 2017a).

The simple architecture of vegetative and reproductive organs of bryophytes makes them excellent models for ecological studies (Harris et al. 2020). Indeed, bryophytes are considered models to understand the ecology and evolution of sexual systems (Suzuki et al. 2018; Harris et al. 2020), reproductive allocation (Stark and Brinda 2013; Santos et al. 2022), and reproductive cost (Bisang and Ehrlén 2002; Rydgren and Økland 2002). Evidence suggests that reproductive allocation is strongly related to sexual systems in bryophytes. Sexual systems of bryophytes present a gradient of distance between the sexes, and the more distant the sexes, the greater is the relative reproductive allocation (proportion of resource allocated to reproduction) in the male function is expected (Stark and Brinda 2013). Reproductive allocation may also change over different reproductive seasons, mainly on functionally dioecious plants, in which sex expression may change following environmental variation (Glime and Bisang 2017a). Indeed, reproductive allocation may be more responsive to different habitat conditions in functionally dioicous bryophytes (rhizautoicous system which male and female ramet are spatially separated, however attached only by rhizoids) (Stark and Brinda 2013; Santos et al. 2018) than in dioicous species (Alvarenga et al. 2013). Given the importance of dioecious systems in the promotion and maintenance of genetic diversity of plants (Renner and Ricklefs 1995; Barrett 2002), studies on functionally dioecious species may clarify the role of habitat heterogeneity in the sex expression (Stehlik et al. 2008), including reproductive allocation.

In this study, we quantified the reproductive allocation (absolute and relative), and the following reproductive traits: sexual expression, sex ratio, reproductive success, and population density for a monoicous species that has similarities with dioicy. *Fissidens flaccidus* Mitt. is a species of moss with a rhizautoicous sexual system that reproduces sexually and asexually (by clavate gemma in stem tissues). The rhizautoicous system presents individualized male and female ramets that are connected, at least initially, by rhizoids. This sexual system, therefore, functionally resembles the dioicous system, since the ramets presumably do not compete for resources for their development and formation of reproductive structures. In this context, we investigate the following questions: First, is male relative reproductive allocation greater than female? This is the usual pattern found in dioicous mosses for pre-fertilization allocation (Stark and Brinda 2013), and rhizautoicous species have segregation of sexual functions (functionally dioicous). Therefore, we expect the reproductive allocation at the prezygotic level to be greater in male sexual function compared to female sexual function. Second, is there a trade-off between sexual and asexual reproduction? Since, according to life history theory, the resources available to individuals are finite, and these resources are subject to competition among different life history features or phases (Oli and Coulson 2016). Therefore, we expect that ramets that have higher relative reproductive allocation will produce less gemmae. Third, is the number of male ramets a determinant greater reproductive success for females? Since the greater the quantity of male ramets expressing sex, the greater the quantity of male gametes and consequently the chance of fertilization of the female gametes. Thus, we expect that the meta-populations that have the highest number of males will, consequently, have greater reproductive success. Fourth, is meta-population density related to the sexual expression of ramets? As density dependence effects are recurrent in many animals and plants, we expect that meta-population density influences the reproduction of the species.

MATERIALS AND METHODS

Study species, study site, and sampling

Fissidens flaccidus Mitt. is a monoicous acrocarpous moss with rhizautoicous sexual system. The rhizautoicous system has male ramets, those that have perigonia (modified leaves enclosing antheridia) and female ramets, those that have perichaetia (modified leaves enclosing archegonia) connected by rhizoids. *F. flaccidus* is distributed in the Neotropical region, Africa, New Guinea, and Australia (Pursell 2007). In Brazil, the species has a wide distribution, so that they are found in all Brazilian phytogeographic domains (Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Wetland) (Bordin and Yano 2013; Flora do Brasil 2021). *F. flaccidus* produces sporophytes in the rainy season, in addition to presenting clavate gemmae in ramets (Santos et al. 2018).

Sampling was carried out in July 2021 in the gardens of the Biosciences Center of the Federal University of Pernambuco (Recife, Brazil). According to Alvares et al. (2013), Recife has a tropical, hot, and humid climate according to the Köppen classification, with an average monthly temperature of 23 °C. The rainy season occurs in the autumn–winter period (March–August), with June–July being the rainiest months (Coutinho et al. 1998). The Biosciences Center has isolated gardens, in which meta-populations of *F. flaccidus* are often found. Thus, we selected 10 gardens to collect the material. The size of the gardens where the samples were collected ranged from 144m^2 (12 × 12 m) to 400m^2 (20 × 20 m), and each garden presented a minimum distance of 10 m. The sizes of the patches varied considerably, since some patches had few ramets while others had an area larger than 900cm^2 (30 × 30 cm). In each metapopulation, 30 patches were randomly selected. In each selected patch, samples of 1 × 1 cm were collected, totaling 30 cm^{-2} per meta-population. The samples were placed in small Petri dishes to preserve the structures in the ramets.

In the laboratory, the samples were analyzed to confirm the species identification. The ramets were then quantified for each sample and classified into the following categories: (1) male—ramets with perigonia; (2) non-sporophytic female—ramets with perichaetia; (3) sporophytic female—ramet with sporophyte, and (4) non-sex expressing ramet—without gametoecia or sporophyte.

Reproductive allocation and gemma production

To quantify reproductive allocation and gemma production, 50 ramets were randomly selected from each of the following categories: non-sporophytic female; sporophytic female, and non-sex expressing, and 48 male ramets (male ramet density was lower in meta-populations of *F. flaccidus*) for the entire study. These collections were performed in meta-populations where male ramets had already been found. Since, where male ramets were found, the other categories of ramets were always found. For each ramet, the gemmae were separated of ramets and counted. Posteriorly gametangia were counted without destroying gametoecia. *Fissidens* simple structure and distichous leaf arrangement makes it possible to quantify gametangia without destroying gametoecia. After, ramets were washed with distilled water. Then, the perigonia, perichaetia and sporophytes were extracted and stored in small paper envelopes together with it respective ramets.

Envelopes with the ramets and reproductive structures were then covered with aluminum foil and placed to dry in an oven for 72H at 70 °C. Once removed from the oven, the envelopes were placed in a box sealed with silica gel. Then, reproductive structures (perigonia, perichaetia and sporophytes), and ramets were weighed. To carry out the weighing, an ultra-micro-analytical balance model SE2 ultra-micro-balance, of Sartorius, Goettingen, Germany, precision of 0.1 µg was used.

After weighing, the reproductive allocation was quantified as absolute and relative. Absolute reproductive allocation was quantified by the biomass of the reproductive structures (perigonia, perichaetia, and sporophyte). Relative reproductive allocation was quantified as the proportion of biomass allocated to reproduction. We follow the formula: : $\mathrm{RA} = \frac{RB}{RB+VB}$ where

RA is relative reproductive allocation, RB is reproductive biomass, and VB is ramet biomass (McLetchie and Puterbaugh 2000).

Reproductive traits

Sex expression, sex ratio, reproductive success, and meta-population density

After transformation (log), the data were not normally distributed, and non-parametric analyses were used. The data used was without the transformation. The Kruskal–Wallis test was used to compare ramet biomass, absolute reproductive allocation, relative reproductive allocation and gemma production between different sexual conditions. To compare gametangia of male and non-sporophytic female ramets, we applied a Wilcoxon test.

Once it was clear that gemma production varied with sex expression and sex, we tested possible trade-offs between the different functions. To test possible trade-off among reproductive traits (sexual *versus* asexual), we used Generalized Linear Model (GLM). First, we tested if gemma production is related to ramet biomass and absolute reproductive allocation. To answer this question, we created two models, a full model in which the amount of gemmae produced as response variable and the ramet biomass and the absolute reproductive allocation as predictor variables, with a Poisson distribution along with a model null, which tests the randomness of the data. Then a comparison was carried out between the two models with chi-square test. Since there were no differences between the models, no significance was observed. Even so, we checked the full model summary to see the results.

The second trade-off among reproductive traits was also tested with a GLM. We created two models, one being a full, with response variable as gamma production and predictor variables as relative reproductive allocation and ramet biomass and a null model. Since the chi-square test between null and full model was significantly different, we excluded the null model and analyzed the full model. The model summary was then analyzed for data interpretation, and interactions were tested. The distribution applied in the model was Poisson; since under-dispersion was observed, the distribution was changed to quasi-Poisson.

Reproductive traits

First, sexual expression was analyzed. The normality of the variable was tested, and non-parametric tests were applied, given the non-normality of the data. Thus, the expression rate between meta-populations was compared using the Kruskal–Wallis test. The sex ratio was quantified by the ratio of female ramets (sum of sporophytic and non-sporophytic ramets) divided by the number of males. The Chi-square test was applied to confirm the sexual bias of the meta-population. We applied the Kruskal–Wallis test to compare reproductive success between meta-populations. Metapopulation density was reported by density graphs.

Once we observed that the fertilization rate was higher in samples with high density and with greater numbers of male ramets, we applied a GLM to determine if the amount of male ramets and the density of the samples influence reproductive success. We created a full model, where the response variable was the reproductive success, and the predictor variables were the amount of male ramets and the sample density. Thus, full model was compared with null model. For both models, the distribution was binomial and compared via the chi-square test. Since the result was significant, the null model was excluded. The dispersion of the data was checked, and since it was over-dispersion, the model distribution was changed to quasi-binomial. The model was analyzed using a test of variance via the *F* test.

The GLM was also applied to determine if meta-population density influences sex expression on male ramets. For that, two models were created, namely: full model, having as response variable the amount of male ramets and as predictor variable the density of samples, and null model. For both models, the distribution was Poisson. The full and null models were compared via chi-square test. Once the test was significant, the null model was excluded, and the full model was used to analyze the data. Data dispersion was checked and neither over-dispersion nor under-dispersion was observed.

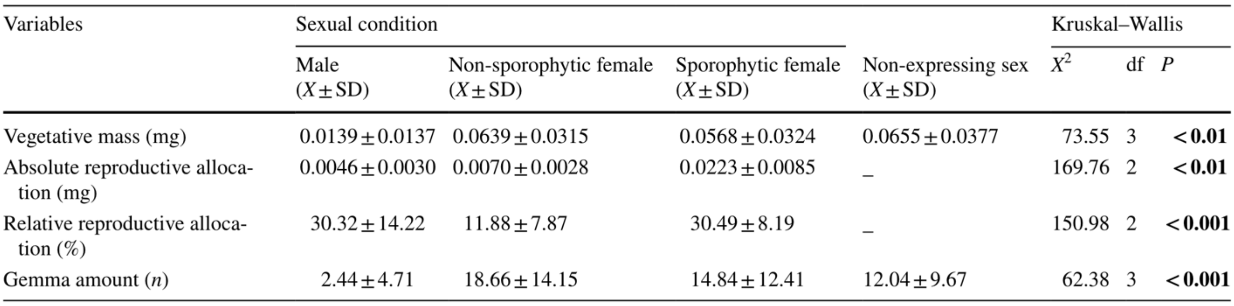
The analyses were performed using the RStudio software version 1.4.1717 (RStudio Team 2021). The Multicomp package (Hothorn et al. 2016) was used to perform the post analysis of the GLMs. The Package ‘dunn.test’ (Dinno and Dinno 2017) was used to perform Dunn's test as a post hoc analysis in the Kruskal–Wallis test. The ggplot2 (Wickham et al. 2016) package was used to make the maps.

Results

Reproductive allocation and gemmae production

The male ramets had lower vegetative biomass compared to other ramets category (non-sex expressing, sporophytic and non-sporophytic females) (Table 1), which in turn did not differ from each other ($X^2 = 73.55$, df = 3, $P < 0.0001$) (Fig. 1. a). Allocation at the prezygotic level (perigonia and perichaetia) did not differ from each other (Fig. 1b). However, the absolute allocation of the sporophyte was significantly higher ($X^2 = 169.85$, df = 3, $P < 0.0001$) (Table 1). Relative reproductive allocation was similar between male plants and female sporophytic plants (Table 1), whereas non-sporophytic females had a significantly lower relative reproductive allocation than male and sporophyte plants ($X^2 = 151.26$, df = 3, $P < 0.0001$) (Fig. 1c). Gemma production was significantly lower in male plants ($X^2 = 62.38$, df = 3, $P = 0.0001$), whereas the other ramet categories did not differ from one another (Fig. 1d). Male and female ramets produced similar numbers of gametangia ($W = 948.5$, $P = 0.10$) (Table 1).

Table 1 Mean and standard deviation of ramet mass, absolute reproductive allocation, relative reproductive allocation, and gemma production



Variables	Sexual condition				Kruskal–Wallis		
	Male ($X \pm$ SD)	Non-sporophytic female ($X \pm$ SD)	Sporophytic female ($X \pm$ SD)	Non-expressing sex ($X \pm$ SD)	X^2	df	P
Vegetative mass (mg)	0.0139 ± 0.0137	0.0639 ± 0.0315	0.0568 ± 0.0324	0.0655 ± 0.0377	73.55	3	**< 0.01**
Absolute reproductive allocation (mg)	0.0046 ± 0.0030	0.0070 ± 0.0028	0.0223 ± 0.0085	_	169.76	2	**< 0.01**
Relative reproductive allocation (%)	30.32 ± 14.22	11.88 ± 7.87	30.49 ± 8.19	_	150.98	2	**< 0.001**
Gemma amount (n)	2.44 ± 4.71	18.66 ± 14.15	14.84 ± 12.41	12.04 ± 9.67	62.38	3	**< 0.001**

Regarding the trade-off between gemma production and the ramet and reproductive biomass variables, the full and null GLM models did not show significant differences, suggesting the model's non-significance; the summary of the full model indicated no association between the variables. Thus, ramet biomass and absolute reproductive allocation do not show any association (Table 2). On the other hand, the model that tested whether gemma production is associated to relative reproductive allocation and ramet biomass was significant. According to the results, those plants that allocate a greater amount of resource to relative reproductive allocation, have a lower gemma production (Fig. 2). Furthermore, the model showed significance in the interactions between relative reproductive allocation and vegetative biomass, suggesting that the smaller the growth and relative reproductive allocation, the greater the gemma production (Table 3).

Fig. 1

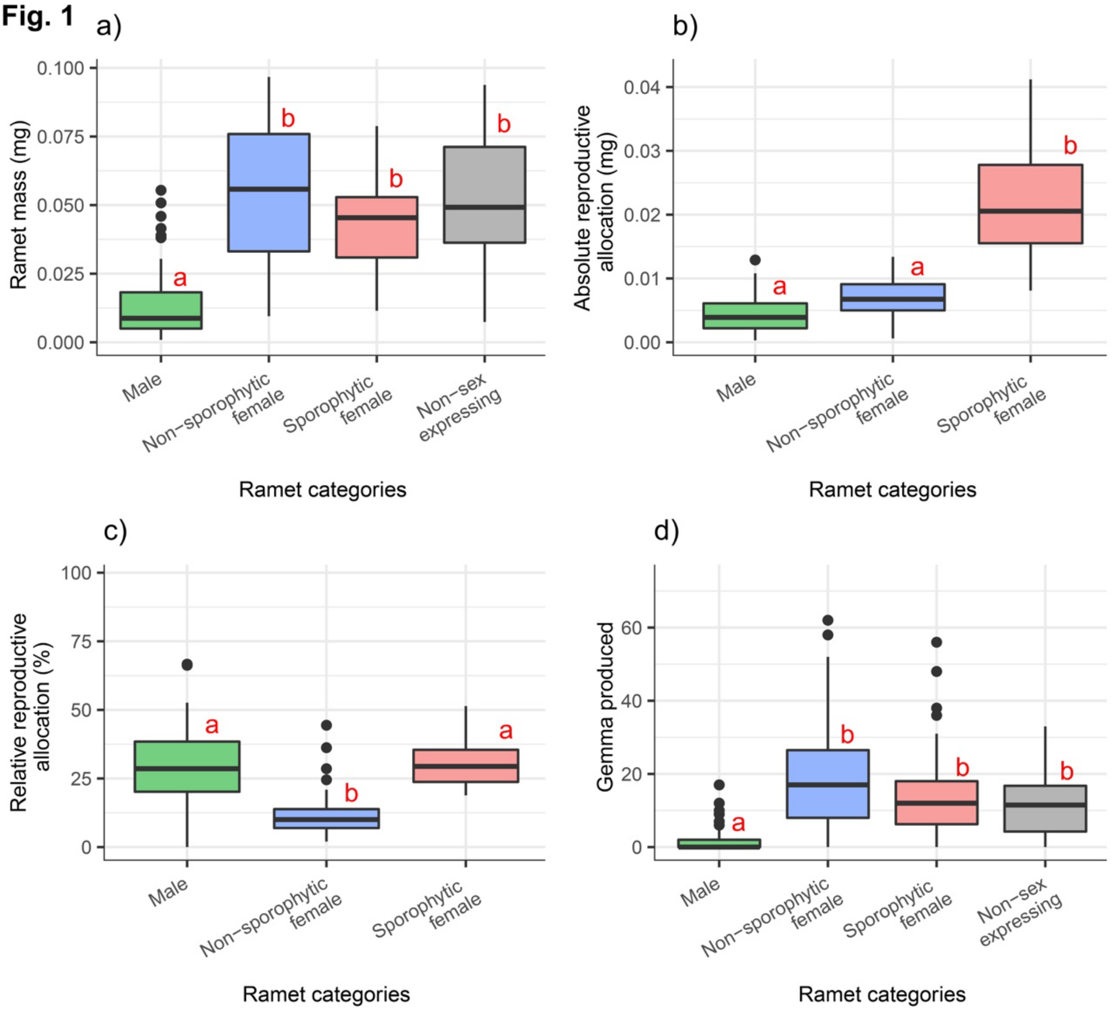


Fig. 1 Graphs reporting a ramets biomass, b absolute reproductive allocation, c relative reproductive allocation and d amount of gemma produced. The letters indicate the significance parameters

Table 2 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM) – Gemma production		
	Df	**Deviance**
Gametangia biomass	1	20.10$^{n.s}$
Ramet biomass	1	2.42$^{n.s}$
Observations		197
Akaike Information Criterion		NA
Residual Deviance		2506.5 (Df = 194)
Null Deviance		2529 (Df = 196)

Response variable (gemma production), predictor variables (gametangia biomass, and ramet biomass
$^{n.s.}$not significant

Fig. 2

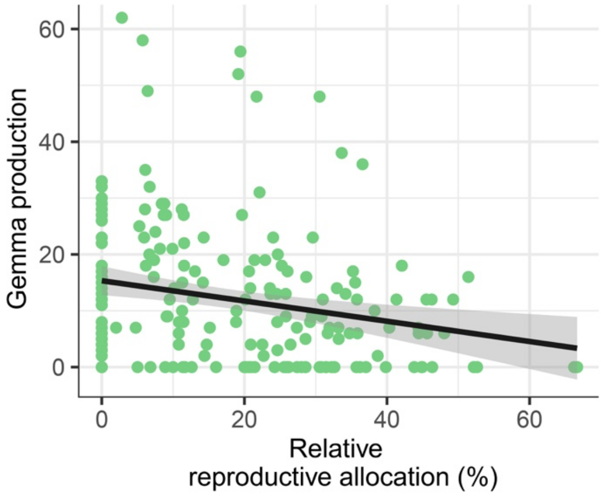


Fig. 2 Scatterplot of trade-off between relative sexual reproductive allocation (% ramet biomass) and gemma production (number ramet/cm^2)

Table 3 Results of Generalized Linear Models (GLM). Response variable (gemma production), predictor variables (Relative reproductive allocation, and ramet biomass)

Generalized Linear Models (GLM) – Gemma production		
	Df	**Deviance**
Relative reproductive allocation	1	20.10**
Vegetative biomass	1	117.44$^{n.s}$
Reproductive relative allocation *versus* Vegetative biomass	1	215.24***
Observations		197
Akaike Information Criterion		NA
Residual Deviance		2176.2 (Df = 193)
Null Deviance		2529.0 (Df = 196)

**p < 0.01 ***p = 0.001 n.s Not significant

Reproductive traits

The results indicated that sexual expression is roughly equivalent among meta-populations ($X^2 = 252.11$, df = 218, $P = 0.05$) (Table 4). Metapopulations 2, 3, 7, and 10 had the highest sexual expression proportion (Fig. 3). While meta-population 6, has a no sex expression. Regarding sex ratio, all meta-populations showed a bias toward females (Table 4). However, the exact sex ratio was quantified for meta-populations 1, 3, 5, 7 and 10, as they were the only meta-populations that presented male ramets. Thus, the analyses showed that all meta-populations that expressed sex were biased toward females (Supplementary tab 1).

Metapopulation	Ramets				Mean of reproductive traits to samples				Absolute reproductive traits to metapopulation			
	Male	Non-sporophytic female	Sporophytic female	Non Expressing sex	Sex expression X ± SD	Sexual proportion ♀:♂	Reproductive success per sample X ± SD	Density per sample 1 x 1 cm X ± SD	Sex expression	Sexual proportion ♀:♂	Reproductive success	Metapopulation Density 30 x 30 cm
1	29	304	648	2747	28.25 ± 18.76	32.14:1	69.70 ± 25.25	124.3 ± 30.31	26.31	32.83:1 ***	68.07	3728
2	0	530	0	1071	31.91 ± 12.60	-	0	31.91 ± 13.33	33.10	- ***	0	1601
3	83	263	704	1852	37.31 ± 13.14	11.70:1	69.66 ± 18.20	69.66 ± 22.98	36.18	11.65:1 ***	72.80	2902
4	0	127	0	767	12.26 ± 13.02	-	0	29.80 ± 8.79	14.21	-	0	894
5	35	348	481	2810	26.46 ± 16.87	23.69:1	55.05 ± 32.26	122.46 ± 37.08	23.52	23.69:1 ***	58.02	3674
6	0	0	0	1182	0	-	0	39.40 ± 9.86	0.00	- ***	0	1182
7	74	269	745	1945	35.60 ± 11.18	13.70:1	69.90 ± 22.01	101.10 ± 26.87	35.87	13.70:1 ***	73.47	3033
8	0	250	0	827	21.96 ± 9.94	-	0	35.90 ± 9.65	23.21	- ***	0	1077
9	0	216	0	819	19.82 ± 13.85	-	0	34.5 ± 6.76	20.87	- ***	0	1035
10	14	229	162	642	38.40 ± 27.08	27.93:1	21.74 ± 37.16	35.56 ± 9.13	38.68	27.93:1 ***	41.43	1047

Fig. 3

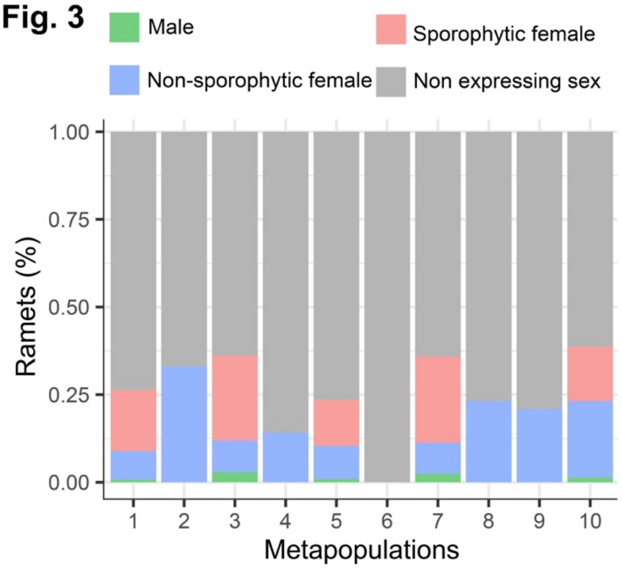


Fig. 3 Sexual expression of rhizautoicous moss *Fissidens flaccidus*. Percentage of ramets male, non-sporophytic female, sporophytic female, and non-sex expressing

Reproductive success (proportion of females that formed sporophytes) did not differ between meta-populations that presented sporophytes ($X^2 = 108.46$, df = 91, $P = 0.09$) (Fig. 4). The meta-populations that showed greater reproductive success (1, 3, 5 and 7) presented higher meta-population density (Fig. 5). While meta-population 10 showed lower density and a lower tendency in the production of sporophytes. Suggesting that meta-population density can influence the reproductive success of populations.

Fig. 4

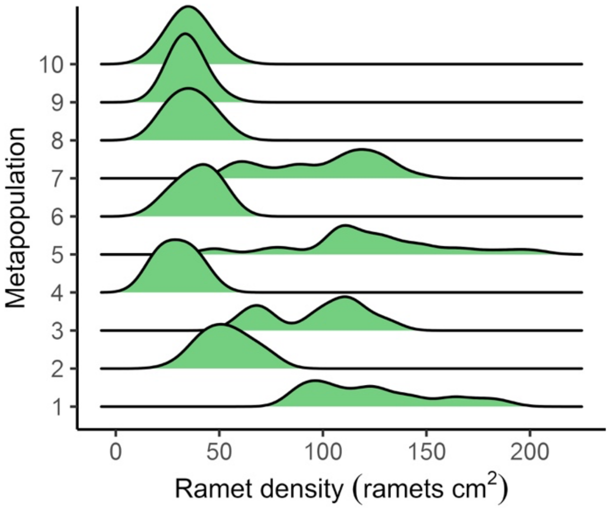


Fig. 4 Density graphics show ramet density per meta-population

Fig. 5

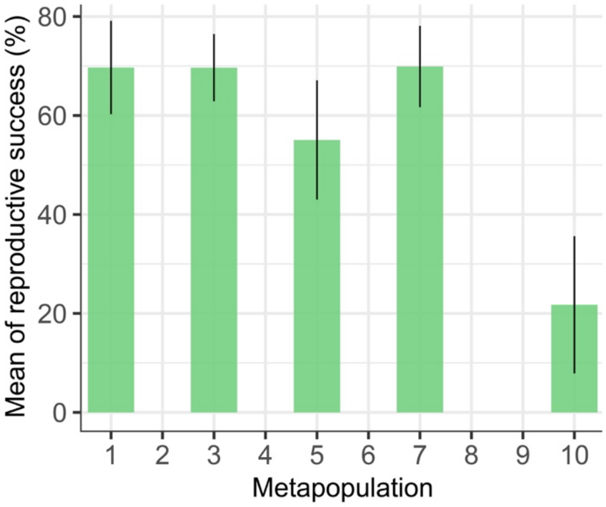


Fig. 5 Bar plot represents reproductive success by meta-population with error bar

Regarding the GLM result that we investigated whether reproductive success is higher in samples with high density and with a greater amount of male ramet, it did not show any significant result (Table 5). On the other hand, the model that was applied to investigate if population density entails male sex expression, presented a significance (Table 6). This model showed that population density is an important factor to male ramets express their sex.

Table 5 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM) Response variable as reproductive success		
	Df	**Deviance**
Male ramets	1	0.0026 $^{n.s}$
Total ramets	1	11.5135$^{n.s}$
Observations		300
Akaike Information Criterion		NA
Residual Deviance		283.31 (Df = 27)
Null Deviance		294.82 (Df = 29)

Response variable (reproductive success), predictor variables (Male ramets, and total ramets) $^{n.s}$.Not significant

Table 6 Results of Generalized Linear Models (GLM)

Generalized Linear Models (GLM) Response variable as male sexual expression		
	Df	**Deviance**
Populational density	1	298***
Observations		300
Log Likelihood		-1,356.703
Akaike Information Criterion		NA
Residual Deviance		644.59 (Df = 298)
Null Deviance		877.09 (Df = 299)

Response variable (male sexual expression), predictor variables (Population density) ***p < 0.0001

DISCUSSION

Sexual systems differ in morphological and reproductive attributes, which confer different mechanisms for each sex. However, the variation in the spatial distance between the sexes can characterize the reproductive allocation and meta-population dynamics of the species. Our main findings indicated that: (1) reproductive allocation is higher in male function; (2) there is a trade-off between sexual and asexual reproduction and (3) that density is a limiting factor in male sexual expression (the greater the population density, the greater the sexual expression of male ramets). These results indicate a similarity in reproductive patterns observed recurrently in dioicous bryophytes species. Thus, we can state that rhizautoicous sexual systems are monoicous sexual systems that behave like a dioicous system. Since in this sexual system, male and female ramets are individualized and connected only by rhizoids, that is, each ramet has a resource intended only for a sexual function, male or female, as in dioicous species. Since the only function of the rhizoids is to make the adhesion of the ramets to the substrate.

The high relative reproductive allocation in the male function reflects a trade-off

Gender differences were not observed in the absolute reproductive allocation in the prezygotic phases (perigonia and perichaetia formation). On the other hand, for relative reproductive allocation, male ramets had a significantly higher mean. This pattern of greater reproductive allocation in male function compared to females is observed in most dioicous species and some rhizautoicous species (Stark et al. 2000; Horsley et al. 2011; Stark and Brinda 2013; Santos et al. 2018). Stark and Brinda (2013) noted that, as the distances between males and females increase, the greater is the relative reproductive allocation in male function. Thus, in rhizautoicous sexual systems where male and female ramet connections dissolve, plants can be considered functionally dioicous. It is expected that the larger the distance between the male and female ramets, the chances of fertilization decline. Indeed, results reported by several studies confirm this expectation (Glime and Bisang 2017b). Thus, we can infer that this pattern (greater sexual relative reproductive allocation in male function) may be interpreted as an evolutionary strategy that increases the chance of fertilization in sexual systems showing large distances between sexual functions.

Male ramet biomass is lower than other ramet categories (non-sex expressing, sporophytic, and non-sporophytic female). This result has been related to this same species (Santos et al. 2018), and a strong sexual dimorphism was measured. The lower male ramet biomass may be related to reproductive cost using relative reproductive allocation. According to Obeso (2002), there are many definitions of reproductive cost, among them, direct cost is defined as somatic costs of reproduction during the current reproductive season. Therefore, if a direct cost is related to the current reproductive season, a trade-off between growth and reproduction is expected. Indeed, male ramets have a much smaller biomass than females, since the proportion of resources allocated to perigonia is relatively high and these resources are not available for growth. In this context, phenological observations can provide clues to the effect of reproductive allocation on species biology. For instance, Santos et al. (2020) reported on a study where the phenology of two species of *Fissidens* with different sexual systems was observed, namely: *Fissidens scarious* Mitt.—rhizautoicous; and *Fissidens submarginatus* Bruch.—gonioautoicous. The authors observed that in the species with the rhizautoicous sexual system, protandry occurred, as they allocate more resources to reproduction, compared to the gonioautoicous (with male and female sex structures produced distally along single ramets) species. In addition, the sexual system that presented the highest reproductive allocation (rhizautoicous) was more seasonal. While the gonioautoicous system presented antheridia production throughout the year, however, in those ramets that formed sporophytes, a greater amount of aborted antheridia was found, suggesting reproductive cost.

Reproductive allocation competes for resources with asexual reproduction

The results showed that reproductive allocation for sexual reproduction (*i.e.*, resource allocated to the formation of perigonia, perichaetia, and sporophytes) entails fewer resources for gemma production. Trade-offs occur because environments are variable, often seasonal, and the plants are sessile organisms, they must adjust to these conditions. In this context, trade-offs can be interpreted as adjustments to environmental variations (Liu et al. 2009). A clear example of a trade-off between sexual and asexual reproduction was reported in a liverwort by Laaka-Lindberg (2001) in the species *Lophozia silvicola* H. Buch, where the author found female plants allocating more resources to sexual reproduction compared to male plants. On the other hand, the amount of gemma was higher in plants that did not express sex, and the average increased from males to females. The latter finding makes it clear that sexual and asexual reproduction competes for resources produced by the plant. Nonetheless, this trade-off relationship between sexual and sexual functions has been reported recurrently in bryophytes (Kimmerer 1991; Bisang and Ehrlén 2002; Fuselier and Mcletchie 2002; Rydgren and Okland 2003; McLetchie and Stark 2006; Hedderson and Longton 2008; Stark et al. 2009; Horsley et al. 2011).

Metapopulation density can influence the reproductive fitness of meta-populations

Our results showed that the density of male ramets is not a factor increasing reproductive success in meta-populations of *F. flaccidus*. Thus, our findings indicate that the large reproductive allocation directed at male sexual function is efficient in bringing about the reproductive success of the studied meta-populations. Reese (1984) showed that in dioicous *Syrrhopodon texanus* Sull. male plants are very rare. However, when males are found, there are also females bearing sporophytes, similar to our results. Regarding sex ratio in dioicous bryophytes, commonly populations are found with female bias. For instance, Stark (2002) reports that 2/3rd of the studied dioicous species ($N = 30$) have populations with a female bias, 5 with a male bias, and 5 without a sexual bias. In the same sense, Bisang and Hedenäs (2005) show that approximately 80% of analyzed species ($N = 103$) presented a female bias. However, although less studied, rhizautoicous species tend to express a female ramet bias, namely: *Atrichum undulatum* (Hedw.) P. Beauv., *Tortula muralis* Hedw. (Longton and Miles 1982),*Weissia controversa* Nees & Hornsch. (Anderson and Lemmon 1972), and *Fissidens scarious* Mitt. (Santos et al. 2020). Deviating from this expected pattern is the moss *Aloina bifrons* (De Not.) Delgad. (Stark and Brinda 2013).

Metapopulation density was associated with male sexual expression induction, that is, the denser the meta-population, the greater the chance of male ramets expressing sex. Male reproductive allocation in dioicous and functional dioicous species, have been related to phenology since it is more costly. According to phenological patterns, usually in those plants where there is greater reproductive allocation in a function, they are developed first. Indeed, protandry is commonly observed in these plants with greater reproductive allocation in male function. For example, in *Aloina bifrons* (De Not.) Delgad., Stark and Brinda (2013) showed that the development of the male plants preceded the female ramets and, at the end of the experiment, a greater amount of male ramets was observed. Similar to the findings by Santos et al. (2020), however, the number of female ramets was higher in rhizautoicous *Fissidens scarious*. Thus, we can suggest that meta-population density may create alternative microhabitat that favors male ramets' expression, which may take advantage from specific conditions created by higher or lower densities. However, we emphasize that other factors may be influencing the results found. In addition, more studies focusing on understanding the effects of density on sexual expression need to be carried out.

The preference for microhabitat in relation to sex has been reported in some bryophyte studies. For example, Bowker et al. (2000) found habitat preferences by sex in the widely distributed desert dioicous moss *Syntrichia caninervis* Mitt. in populations from the Mojave Desert in Nevada (USA). It was observed that male plants tend to establish and persist in shade, compared to female plants that are found across a span of shaded and open microhabitats. In the same sense, Cameron and Wyatt (1990) reported for the monoicous species *Splachnum ampullaceum* Hedw., *Splachnum sphaericum* Hedw., and dioicous *Splachnum rubrum* Hedw. that low light intensity and other factors favor the formation of male ramets. Thus, an explanation that population density is associated with male sexual expression is suggested, since the greater density of patches leads to a favorable microclimate for the ramets to express the male sex. Furthermore, the male ramets have a reduced size that are mostly protected near the female plants. Thus, more studies need to be carried out with functionally dioicous species to know if male ramets show preference for habitat, as our findings only suggest this association.

Thus, we can conclude that the studied meta-populations present characteristics of dioicous species such as greater reproductive allocation in the male sexual function. In addition, the reproductive allocation in the male function is sufficient to generate greater reproductive success. Since the number of male ramets is not positively correlated with the fertilization rate, meta-populations that have few male ramets have the potential to induce greater reproductive success as well as meta-populations that have many male ramets. Finally, our results suggest a possible preference for habitat in relation to male ramets, since these are always present in meta-populations that present higher density. Thus, we emphasize that the system investigated in this study (rhizautoicous) is strongly plastic, since the same ramet that forms perigonia can form perichaetia when it regenerates. Resembling to the moss *Atrichum undulatum* (Hedw.) P. Beauv., in which the branches have the potential to produce male and female gametangia, but each one in different seasons. Furthermore, considering that the climate change scenario will certainly impact the reproduction bryophytes (Shortlidge et al. 2017), the rhizautoicous system can be considered an excellent sexual system to investigate the effect of climate change, since it may have identical genotypes for different sexes (Stearns 1989).

Supplementary Information

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Author contribution statement WLS and KCP: conceived the ideas. WLS, KCP, and FP: designed the methodology. WLS: conducted field work supported by KCP. WLS, FP, KCP and JB: analyzed the data. WLS: led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations Conflict of interest The authors declare that they have no conflicts of interest.)

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

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Chapter 4

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The spatial arrangement of sexes is related to reproductive allocation in mosses: a comparative study of reproductive allocation in three different monoicous sexual systems

Wagner Luiz dos Santos[1] , Kátia Cavalcanti Pôrto[2] , Juçara Bordin[3] , Fábio Pinheiro[1],* and Irene Bisang[4]

[1] Department of Plant Biology, University of Campinas, Rua Monteiro Lobato 255, Barão Geraldo, Campinas, São Paulo, Brazil
[2] Department of Botany, Federal University of Pernambuco, Avenida Moraes Rego, s/n, University City, Recife, Pernambuco, Brazil
[3] State University of Rio Grande do Sul, Machado de Assis, 1456, RS 95520-000, Brasil
[4] Department of Botany, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden * For correspondence. E-mail biopin@unicamp.br

ABSTRACT

Background and Aims

We examined the relationship between reproductive allocation and vegetative growth in three monoicous sexual systems of bryophytes. The sexual systems show a gradient of increasing distance between the sexes, from gonioautoicous to cladautoicous to rhizautoicous. Here, we investigated the following two hypotheses: (1) reproductive allocation differs between sexes and sexual systems, and male reproductive allocation increases with increasing distance between male and female gametangia; and (2) reproductive allocation is negatively related to vegetative growth.

Methods

We sampled the three sexual systems, represented by three moss species of the genus *Fissidens* in the Atlantic Forest of Southeastern Brazil. Ramets were washed in the laboratory; the reproductive structures were detached from the vegetative ramets and sorted regarding sex and individual, dried at 70 °C for 72 h, and weighed in an ultramicrobalance. We calculated the mean reproductive and vegetative mass and reproductive allocation and used generalized linear models to test our predictions.

Key Results

Reproductive allocation differed between species and sexes. It was higher in the rhizautoicous than in the cladautoicous and gonioautoicous species. Mean reproductive allocation was greater in males than in females of the rhizautoicous species, greater in females than males of the cladautoicous species, and did not differ between the sexes in the gonioautoicous species. Estimates of reproductive and vegetative mass were positively related in females of the rhizautoicous species. Vegetative mass was not related to reproductive allocation in the gonioautoicous species, but negatively related to reproductive allocation in the male and female branchlets of the cladautoicous species and in the female ramets of the rhizautoicous species.

Conclusions

The reproductive allocation patterns differ between the rhizautoicous species and the ‘truly’ monoicous species, with shorter intersexual distances, which implies that our hypotheses were supported only in part. We suggest that the hypotheses should be reformulated and tested further by comparing ‘truly’ monoicous species with dioicous species and by including other genera.

Key words: Cladautoicous, *Fissidens pseudoplurisetus*, *Fissidens scariosus*, *Fissidens weirii*, gonioautoicous, monoicy, reproductive allocation, reproductive mass, reproductive investment, reproductive trade-off, rhizautoicous, sexual systems.

INRTRODUCTION

Life-history theory predicts that the three essential functions of life, namely reproduction, growth and defence, compete for resources (Delph, 1999; Oli and Coulson, 2016). Thus, understanding the patterns of resource allocation to different functions in organisms is crucial to understanding their life-history traits (Charlesworth and Morgan, 1991; Söderström and Gunnarsson, 2003). Reproductive allocation (RA) is commonly quantified as the proportion of the total dry mass allocated to reproductive structures (Ashman, 1994; Karlsson and Méndez, 2005; Suzuki, 2005). Following the 'principle of allocation', which deals with the allocation of resources that are available in limited amounts, allocation to one function should have negative consequences for other functions (Karlsson and Méndez, 2005). This is commonly referred to as 'trade-offs', which eventually influence the performance of populations (Charlesworth and Morgan, 1991). Plant allocation patterns have a genetic component and are influenced by plant traits (e.g. size) and environmental factors, such as competition, environmental stress or stand density (Rice *et al.*, 2001; Karlsson and Méndez, 2005; Friedman *et al.*, 2020).

Studies of RA in plants have a long history and have been focused mainly on angiosperms (Darwin, 1877; Fox, 1993; Chiang and Lin, 2001; Suzuki, 2005; Hautier *et al.*, 2009; Zhang *et al.*, 2021). In bryophytes, RA has attracted attention only in recent years. The diversity of sexual systems exhibited by bryophytes (Wyatt, 1985) coupled with their relatively simple structure, largely lacking storage organs, makes these plants suitable organisms in which to understand the trade-offs involving RA in plants (Convey and Smith, 1993; Convey, 1994*a*, *b*; González-Mancebo and During, 1997; Ehrlén *et al.*, 2000; Stark *et al.*, 2000; Laaka-Lindberg, 2001; Bisang and Ehrlén, 2002; Horsley *et al.*, 2011; Stark and Brinda, 2013; Santos *et al.*, 2018, 2022*a*, *b*).

Unlike seed plants, the bryophyte life cycle is dominated by the haploid phase, and the bryophyte reproductive structures are formed on the gametophyte (Parihar, 1963). Thus, a distinction is generally made between sex expression in the diploid sporophyte phase of angiosperms (monoecious, dioecious) and sex expression in the haploid gametophyte phase of bryophytes (monoicous, dioicous) (Glime and Bisang, 2017). More than half of all bryophyte species in the world are dioicous [i.e. they carry male (antheridia) and female (archegonia) sexual structures on separate individuals], and the rest are monoicous, with male and female sexual structures on the same individual (Fig. 1; and see below in the subsection '*Studied* *species, reproductive structures and sample collection*') (Parihar, 1963; Wyatt, 1982; Bisang and Hedenäs, 2005). The diploid sporophyte develops on the female gametophyte from a zygote after fertilization (Fig. 1). Fertilization can fail owing to lack of water, spatial distance between the male and female reproductive organs, or unsuitable environmental conditions (e.g. Bisang *et al.*, 2004; Haig, 2016; Hedenäs and Bisang, 2019). This implies that RA in female bryophytes occurs both before (archegonia and related structures) and after (sporophyte) fertilization and might be restricted to the former if fertilization fails. In male bryophytes, however, RA is always restricted to the pre-fertilization (prezygotic) stage. Different spatial positions of male and female gametangia are found in the monoicous sexual system, with increasing distance between sexual organs from the synoicous to the rhizautoicous arrangement (Fig. 1). The few studies conducted to date on RA in monoicous bryophytes have investigated species with a rhizautoicous sexual system (Stark and Brinda, 2013; Santos *et al.*, 2018, 2022*b*).

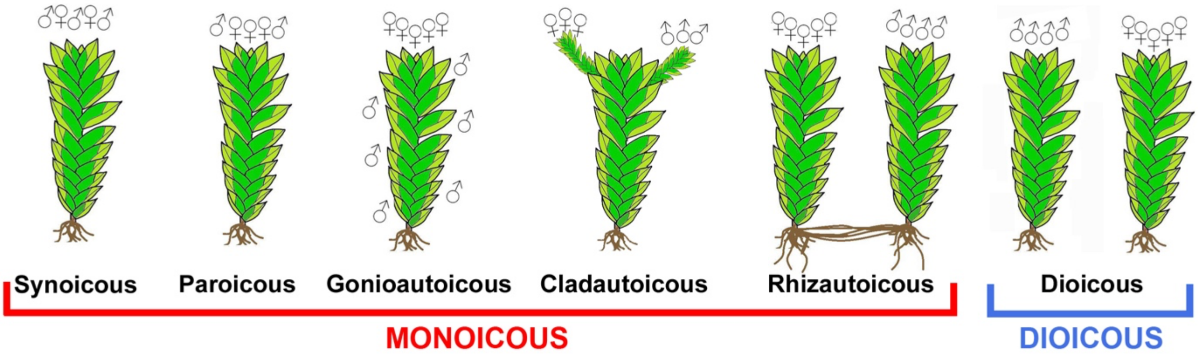

Fig. 1 Schematic drawing of the sexual systems of bryophytes. Definitions: cladautoicy, perigonia and perichaetia on separate branches but both branches connected to one main branch; dioicy, male and female gametoecia on different plants; gonioautoicy, terminal perichaetia and axillary perigonia in the same ramet; paroicy, antheridia adjacent to archegonia in the same gametoecia; rhizautoicy, male ramet (with perigonia) attached to the female ramet (with perichaetia) by rhizoids; and synoicy, antheridia and archegonia mixed in the same gametoecia. Definitions are adapted from Wyatt (1985) and Stark and Brinda (2013).

Sexual systems affect mating success in plants in many ways (Longton and Schuster, 1983; Bergh and Verboom, 2011; Stark and Brinda, 2013; Santos *et al.*, 2020). For instance, according to Longton and Schuster (1983), outcrossing relative to self-fertilization is expected to increase as the distance between antheridia and archegonia increases in self-compatible monoicous bryophyte species (i.e. from the synoicous to the rhizautoicous mating system). In flowering plants, Bergh and Verboom (2011) suggested that the prezygotic allocation to female function in hermaphroditic species should increase proportionally to the degree of self-fertilization. Given that higher selfing rates in bryophyte species with short intersexual distances seem plausible, Stark and Brinda (2013) applied the notion of hermaphrodite flowering plants of Bergh and Verboom (2011) to bryophytes, namely that prezygotic RA to the male relative to the female function should increase with decreasing selfing (i.e. along the axis from synoicy to dioicy). Stark and Brinda (2013) found a higher RA per culture surface area and per shoot in males than in females in the rhizautoicous *Aloina bifrons* (De Not.) Delgad., yet they reported that their postulation was inconsistent with the allocation pattern observed in three other rhizautoicous species. Later, Santos *et al*. (2018) found that male RA exceeded female RA on a shoot basis in the rhizautoicous *Fissidens flaccidus* Mitt.

In this study, we test the prediction postulated by Stark and Brinda (2013) in three *Fissidens* species. This is a suitable genus in which to investigate the theme because it comprises species with all sexual systems observed in bryophytes, thus encompassing the whole range of intersexual distances (Fig. 1). We compare RA in *Fissidens scariosus* Mitt. (rhizautoicous), *Fissidens pseudoplurisetus* Bordin, Pursell & O. Yano (cladautoicous) and *Fissidens weirii* Mitt. (gonioautoicous) and investigate the possible relationships between RA and vegetative growth. We test the following hypotheses: (1) prezygiotic RA differs between sexes and sexual systems, and male RA increases with increasing distance between male and female gametangia (Fig. 2); and (2) the investment in reproductive structures, measured as absolute values and proportional values relative to vegetative mass, is negatively related to vegetative growth (Fig. 2).

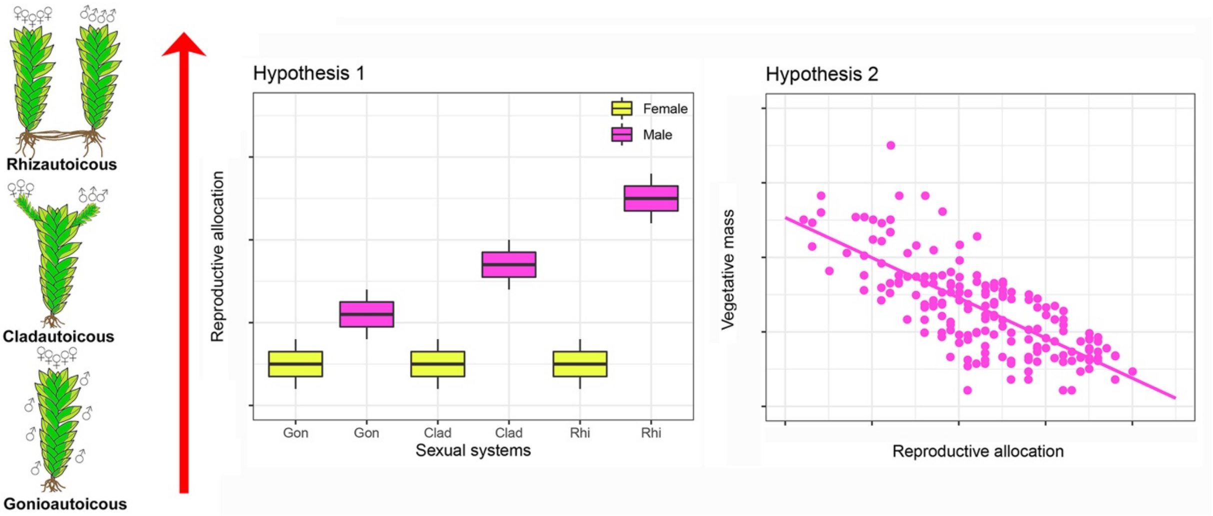

Fig. 2 Schematic diagram of the two hypotheses addressed in this study: (1) reproductive allocation differs between sexes and sexual systems and male reproductive allocation increases with increasing distance between male and female gametangia; and (2) allocation to reproductive structures is negatively related to vegetative growth. Abbreviations: Clad, cladautoicous; Gon, gonioautoicous; Rhi, rhizautoicous.

MATERIALS AND METHODS

Study area

We collected samples at Parque das Neblinas in the limits of Bertioga and Mogi das Cruzes municipally in the state of São Paulo (23°44′04.6″S, 46°10′53.0″W). With an area of 7000 ha of dense rainforest, the park is part of the largest continuum of Brazilian Atlantic Forest in the Serra do Mar State Park (Onofre *et al.*, 2010). The Bertioga region has a 'Tropical rainforest climate', according to the Köppen classification, with an average rainfall of ≥60 mm every month (Malhi and Wright, 2004). The annual average temperature is ~24 °C, and the average rainfall amounts to 3207 mm (Alvares *et al.*, 2013).

Studied species, reproductive structures and sample collection

We chose the genus *Fissidens* (Fissidentaceae, Bryophyta) because it presents a great diversity of sexual systems. Three species from the same subgenus (*Aloma*) were selected in order to reduce the potential effect of phylogenetic distance (Pursell, 2007; Bordin and Yano, 2013). The rhizautoicous *Fissidens scariosus* Mitt. (Figs 1 and 3A) is widely distributed in Brazil and occurs in the most diverse phytogeographical domains, such as the Amazon, Cerrado and Atlantic Forest, but predominantly in the last of these (Pursell, 2007; Bordin and Yano, 2013). We sampled *Fissidens pseudoplurisetus* Bordin, Pursell & O. Yano (Figs 1 and 3B), described as rhizautoicous or gonioautoicous by Bordin *et al.* (2011) but observed only as cladautoicous in our samples. In our collections, the rhizautoicous condition pointed out by Bordin *et al.* (2011) was not confirmed. *Fissidens pseudoplurisetus* has a very restricted distribution and occurs in mixed ombrophilous forests in South and Southeast Brazil. The gonioautoicous *Fissidens weirii* Mitt. (Figs 1 and 3C) is distributed in the Americas and Africa. In Brazil, it occurs in the Atlantic Forest of the South and Southeast regions (Pursell, 2007).

In bryophytes, sexual organs are formed on the gametophyte, and their position is used to define different mating systems (Haig, 2016; Fig. 3). Each antheridium produces numerous spermatozoids (male gametes), and each archegonium produces one egg cell (female gamete). Antheridia and archegonia are collectively termed gametangia. In mosses, the gametangia and the surrounding specialized photosynthetic leaves form reduced sexual branches called perichaetia in females and perigonia in males (collectively termed gametoecia; Fig. 3C). They are prezygotic reproductive structures. Here, we refer to male or female ramets (and male or female branchlets) when the gametophyte portion carries, respectively, male or female sexual structures (Fig. 3). The motile spermatozoid fertilizes the sessile egg cell in the perichaetium. The sporophyte develops on the maternal ramet after fertilization (postzygotic) and remains attached to it during its lifetime.

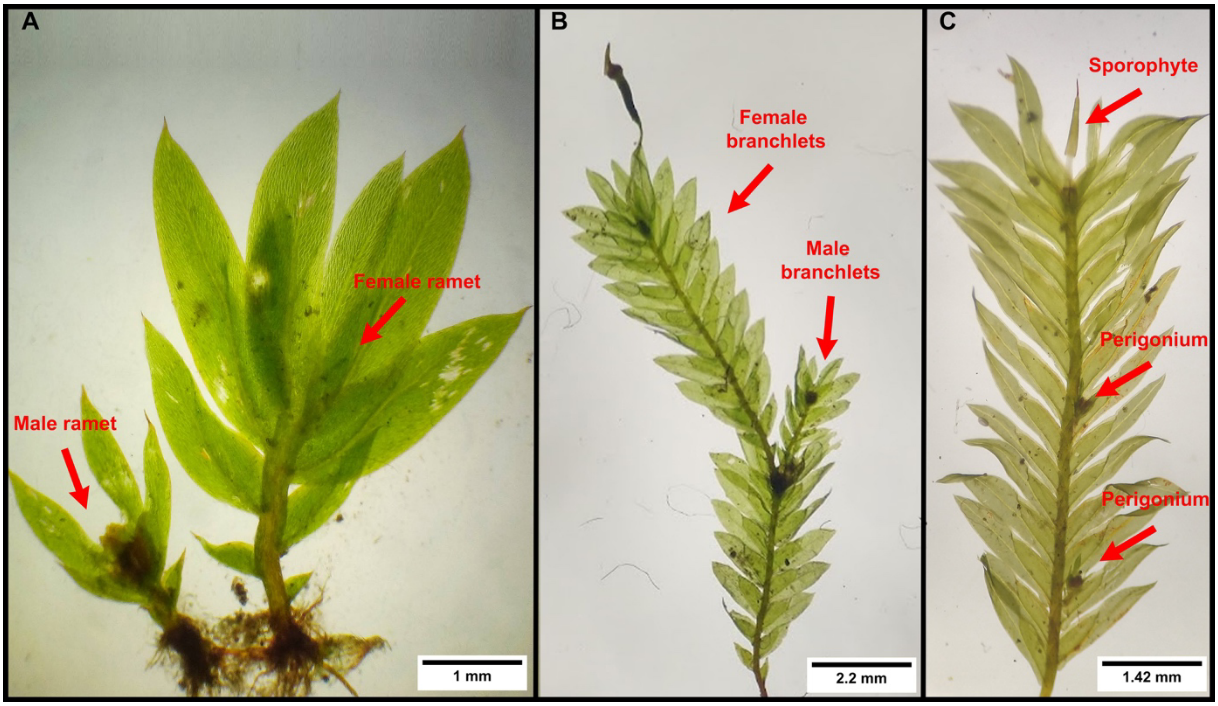

Fig. 3 Species studied, with their respective sexual systems. (A) *Fissidens scariosus* (rhizautoicous): on the left, male ramet attached by rhizoids to the female ramet on the right. (B) *Fissidens pseudoplurisetus* (cladautoicous): male and female branchlets on a common main ramet. (C) *Fissidens weirii* (gonioautoicous): ramet with perichaetia, with juvenile sporophyte in the apex and perigonia in the leaf axils.

Phenology of tropical bryophyte species is largely governed by precipitation, with high rates of sexual reproduction during the rainy period (Maciel-Silva and Válio, 2011; Glime and Bisang, 2017). Thus, we sampled all species during the rainy season in February 2021 from the same study area (7000 ha), which is covered by dense rainforest vegetation. For all three species, we collected colonies with ramets in the same phenophase (Greene, 1960). We therefore believe that our sampling design allows for interspecific comparison. We placed the samples in paper bags and noted the geographical location. Species identification took place in the laboratory, using specialized literature (Pursell, 2007; Bordin *et al.*, 2011; Bordin and Yano, 2013).

Reproductive allocation and association with vegetative mass

After identification, we cleaned and separated the ramets and classified them according to their sexual expression using a stereomicroscope and microscope. We selected 30 male ramets (with perigonia) and 30 non-sporophytic female ramets (with unfertilized perichaetia) of *F. scariosus* (rhizautoicous) and 30 ramets that bore both perigonia and perichaetia but no sporophytes of each of the other two species, *F. pseudoplurisetus* (cladautoicous) and *F. weirii* (gonioautoicous). We picked gametangia to represent the phenophase 'immature', implying gametangia of half to full length, green and with intact cap cells (Greene, 1960). We separated the perigonia and perichaetia from each ramet using fine-tipped forceps and a needle. In cladautoicous species, the perigonia and perichaetia sit on separate small branchlets of the main ramet (Fig. 3B). We quantified the vegetative mass of these branchlets, and not the vegetative mass of the main ramet, with respect to sex. For the vegetative parts of the ramet, we followed the methodology proposed by Bisang and Ehrlén (2002) and removed the basal brownish parts from the green photosynthetically active parts. We placed the vegetative parts of each ramet and the reproductive structures pooled separately according to each sex in small envelopes, wrapped them in aluminium foil and dried them for 72 h at 70 °C. The perichaetia and perigonia of each ramet were placed into a single small envelope, resulting in 30 envelopes of each sex per sexual system and a total of 180 envelopes. The vegetative part of each ramet was also placed into a separate envelope, resulting in 30, 60 and 60 envelopes of the gonioautoicous, cladautoicous and rhizautoicous species, respectively, and a total of 150 envelopes.

After drying, the content of each envelope with perigonia and perichaetia, the vegetative parts of the ramets of the gonioautoicous and rhizautoicous species, and the vegetative parts of the male and female branchlets of the cladautoicous species were weighed separately using an ultramicrobalance (SE2 ultramicrobalance; Sartorius, Goettingen, Germany; precision of 0.1 μg). For the gonioautoicous species, the perigonia found in the same ramet were weighed together.

Statistical analyses

Reproductive allocation (the proportion of resources allocated to reproduction; as a percentage) was calculated for each ramet separately based on the dry mass of its vegetative structures (VB; in milligrams) and reproductive structures (RB, in milligrams) as follows: RA = RB/(RB + VB) (McLetchie and Puterbaugh, 2000). We calculated the mean and s.d. of vegetative mass, reproductive mass and RA at ramet level for each species and sex. We compared the vegetative and reproductive mass among species and sexes by modelling each of them as the response variable of two separate general linear models (GLMs) with a Gaussian distribution and identity link function. We used species and sex as predictor variables except in the gonioautoicous species, in which it is not possible to model vegetative mass as a function of sex because both perigonia and perichaetia are produced on the same ramet; hence, in this case, species was the only predictor variable (Fig. 3). We applied *F*-tests to compare each model with a null model. We then applied *F*-tests to each full model separately to test whether the response variables varied between sexes. Error distributions were analysed, and neither of the two models showed underdispersion or overdispersion. We used Tukey's post hoc tests to check the differences between the three sexual systems.

We tested the first hypothesis using a GLM with a binomial distribution and logit link function. Initially, we created a full model, with RA as the response variable and with sex, sexual systems and their interactions as predictor variables. We compared the full model with a null model without a predictor, using a χ^2 test. The difference was significant, and thus we rejected the null model. We also removed the non-significant interactions from the model. A subsequent residual analysis of the error distribution revealed subdispersion, and thus we used a GLM with quasibinomial distribution to adjust the error distribution. We applied *F*-tests to check the model parameters and Tukey's post hoc comparisons between sexual systems.

We tested the second hypothesis with separate GLMs with a Gaussian distribution and identity link function to infer the effects of reproductive mass and sex on vegetative mass and the effects of RA and sex on vegetative mass. These effects were investigated in each sexual system separately. As explained above, we ran the models for the gonioautoicous species without sex, and thus without the interaction term as a predictor. In the models for the cladautoicous and rhizautoicous species, we removed non-significant interactions from the models. In the model testing the relationship between reproductive and vegetative mass in the rhizautoicous species, we encountered Simpson's paradox (Dunn and Smyth, 2018); that is, a statistical phenomenon in which a trend appears in multiple groups of data but disappears or reverses when these groups are combined. Thus, to solve this problem, models were fitted to each sex separately. For all models, we applied *F*-tests to compare the full and null models. Full models that were significantly different from the null models were submitted to residual analysis to evaluate the error distribution.

We used RStudio (RStudio Team, 2021) to perform the statistical analyses. The MULTICOMP package (Hothorn *et al.*, 2016) was used to make the pairwise comparisons, and the GGPLOT2 package was used to produce the graphics (Wickham *et al.*, 2016).

RESULTS

The vegetative mass differed among species with different sexual systems (d.f. = 4, F = 53.46, $P < 0.001$; Table 1). The mean vegetative mass of the gonioautoicous species was greater than that of the cladautoicous and rhizautoicous species in males and females (Fig. 4A). Mean vegetative mass between the cladautoicous and rhizautoicous species differed only in males, being greater in the rhizautoicous species. Intraspecifically, female and male vegetative mass in the cladautoicous species did not differ from each other, but females had a greater mean vegetative mass than males in the rhizautoicous species (Fig. 4A).

Table 1: Mean and standard deviation of vegetative and reproductive mass [mg] in male and female plants of three *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: *Fissidens weirii*, gonioautoicous; *F. pseudoplurisetus*, cladautoicous; *F. scariosus,* rhizautoicous; see Fig. 1 for sexual systems.

]

Sexual systems	Sex	Vegetative mass	Reproductive mass
Gonioautoicous	**Female**	0.102 ± 0.015^{a}	0.0081 ± 0.006^{a,b}
	Male		0.0050 ± 0.0012^{a}
Cladautoicous	**Female**	0.0273 ± 0.0358^{b,c,d}	0.0123 ± 0.0169^{b}
	Male	0.0175 ± 0.0111^{b}	0.0024 ±0.0014^{d}
Rhizautoicous	**Female**	0.0466 ± 0.0207^{c}	0.0090 ± 0.0038^{a,b}
	Male	0.0283 ± 0.0342^{d}	0.0087 ± 0.0060^{a,b}

Different superscript letters indicate significant differences at $p < 0.05$ based on separate generalized linear models for vegetative and reproductive mass.

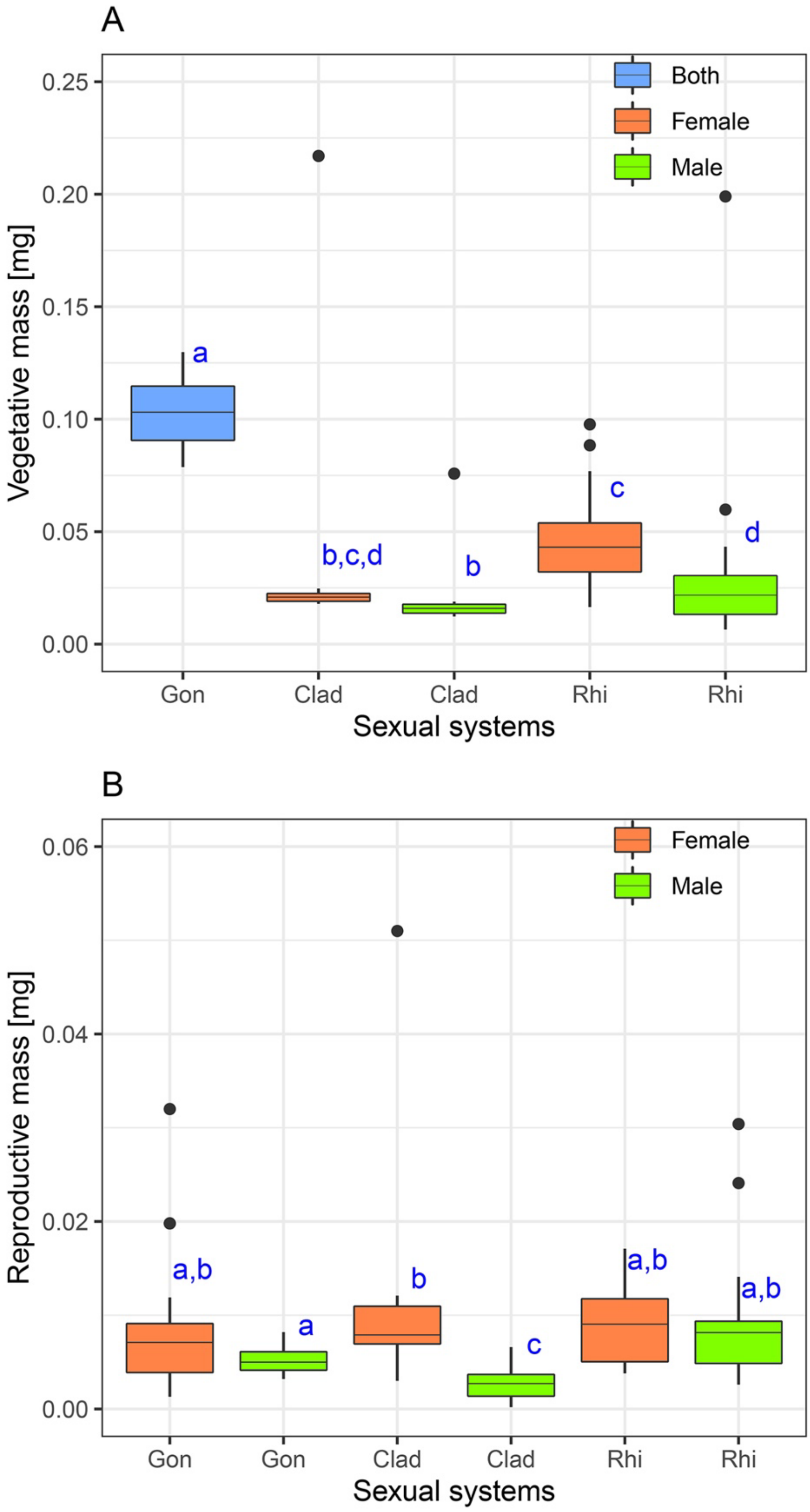

Fig. 4 Boxplot diagrams of reproductive and vegetative mass of *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: *Fissidens weirii*, gonioautoicous; *F. pseudoplurisetus*, cladautoicous; and *F. scariosus*, rhizoautoicus. (A) See Figure 3 for depiction of vegetative ramet or ramet parts. Abbreviations: Clad, total mass of male and female branchlets without the common ramet; Gon, ramet mass excluding perigonia and perichaetium; Rhiz, total mass of female and males ramets excluding sexual branches. (B) Reproductive mass of sexual branches (perigonia, perichaetia). Different letters

denote statistically significant differences based on Tukey's post hoc test. Black dots represent outliers.

Overall, reproductive mass also differed among sexual systems (d.f. = 5, $F = 5.59$, $P < 0.001$). The difference was attributable mainly to lower male reproductive mass in the cladautoicous species relative to the others, whereas female reproductive mass did not differ among the three species (Table 1; Fig. 4B). Intraspecifically, the mean reproductive mass did not differ between sexes in the gonioautoicous and rhizautoicous species, whereas it was greater in females than in males in the cladautoicous species (Fig. 4B; Table 1).

Reproductive allocation differed between sexes and among sexual systems (Table 2) and showed considerable variation within species, particularly in the rhizautoicous *F. scariosus* (Fig. 5). The mean RA of *F. scariosus* was higher than that of the gonioautoicous and cladautoicous species in both sexes (Fig. 5). Intraspecifically, mean RA did not differ between sexes in the gonioautoicous species, whereas it was higher in females than in males in the cladautoicous species, and higher in males than in females in the rhizautoicous species (Fig. 5).

Table 2. Effects of sex and sexual system on reproductive allocation in three *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo state, Brazil, based on a generalized linear model with a binomial distribution and logit link function

	Df	**Deviance**	**P**
Sex	1	0.292	< 0.01
Sexual systems	2	8.406	< 0.001
Observations			180
Null Deviance			20.034 (df = 179)
Residual Deviance			11.334 (df = 176)

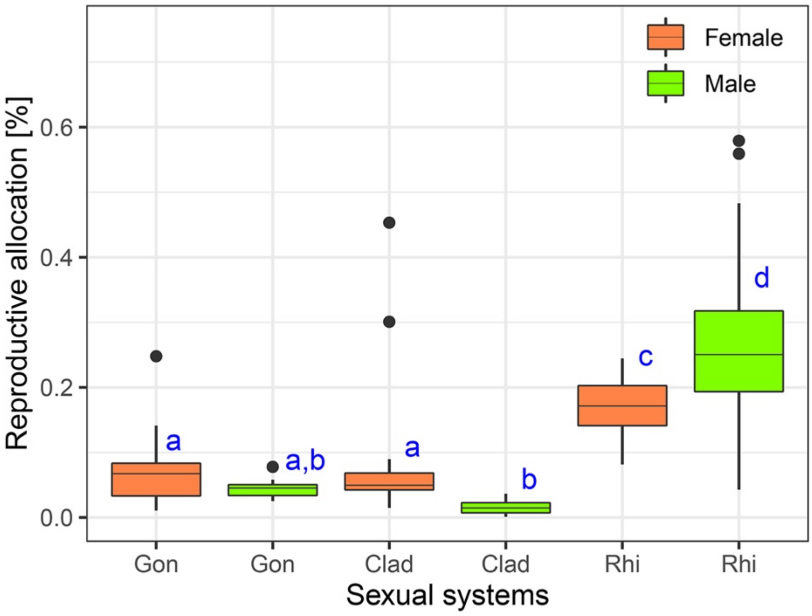

Fig. 5 Boxplot diagrams of reproductive allocation in males and females in *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo State, Brazil: *Fissidens weirii*, gonioautoicous (Gon); *F. pseudoplurisetus*, cladautoicous (Clad); and *F. scariosus*, rhizautoicous (Rhiz). Different letters denote statistically significant differences based on Tukey's post hoc test. Black dots represent outliers.

Vegetative mass was related to reproductive mass, and there was an effect of sex on reproductive mass (Table 3). Female reproductive mass increased with increasing vegetative mass in the rhizautoicous species, whereas we found a 'Simpson effect' in male ramets (Supplementary data, Supplementary material 1). When analysed in separate models for each sex, a positive association between vegetative and reproductive mass was confirmed for females (parameter estimate 3.62 ± 0.76, $P < 0.001$) but not for males (parameter estimate 0.55 ± 1.02, $P = 0.51$) (Table 3; Fig. 6C). No association between reproductive and vegetative mass was found in the gonioautoicous and cladautoicous species (Table 3; Fig. 6A, B). Vegetative mass decreased with increasing RA in female ramets of the rhizautoicous species and in male and female branchlets of the cladautoicous species (Table 4; Fig. 6E). In the gonioautoicous species, vegetative mass was unrelated to RA (Table 4; Figs 5 and 6A, D). The results of the comparison of the full and null GLMs are presented in the Supplementary data (Supplementary material 2).

Table 3. Effects of reproductive mass and sex on vegetative mass in three *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo state, Brazil, based on separate generalized linear models with a Gaussian distribution and identity link function. Sex was excluded as a predictor variable in the gonioautoicous species (see Table 1 for sexual systems)

Gonioautoicous					
			Df	Deviance	P
Reproductive mass			1	2.90^{-06}	> 0.05
	GLM – Summary				
	Estimate	Std. Error		t value	P
Reproductive mass	-0.048	0.44		-0.11	> 0.05
Observations					30
Akaike Inf. Crit.					-160.97
Null Deviance					0.007 (df = 29)
Residual Deviance					0.007 (df = 28)
Cladautoicous					
			Df	Deviance	P
Reproductive mass			1	0.0001	> 0.05
Sex			1	0.0013	> 0.05
	GLM – Summary				
	Estimate	Std. Error		t value	P
Female	-0.034	0.292		-0.118	> 0.05
Male	-0.01	0.007		-1.347	> 0.05
Observations					60
Akaike Inf. Crit.					-259.17
Null Deviance					0.042 (df = 59)
Residual Deviance					0.041 (df = 57)
Rhizautoicous - Female					
			Df	Deviance	P
Reproductive mass			1	-0.005	< 0.001
	GLM – Summary				
	Estimate	Std. Error		t value	P
Female	3.624	0.764		4.740^{***}	< 0.001
Observations					30
Akaike Inf. Crit.					-159.95
Null Deviance					0.012 (df = 29)
Rhizautoicous - Male					
			Df	Deviance	P
Reproductive mass			1	0.001	> 0.05
	GLM – Summary				
	Estimate	Std. Error		t value	P
Male	0.554	1.092		0.508	> 0.05
Observations					30
Akaike Inf. Crit.					-112.58
Null Deviance					0.034 (df = 29)
Residual Deviance					0.033 (df = 28)

Table 4: Effects of reproductive allocation and sex on vegetative mass in three *Fissidens* species with different sexual systems from Atlantic Forest in São Paulo state, Brazil, based on separate generalized linear models with Gaussian distribution and identity link function. Sex was excluded as a predictor variable in the gonioautoicous species. * Significant at $p < 0.05$; ** Significant at $p < 0.01$; *** Significant at $p < 0.001$.

	Gonioautoicous			
		Df	Deviance	P
Reproductive allocation		1	0.0008	> 0.05
	GLM - Summary			
	Estimate	Std. Error	t value	P
Reproductive allocation	-0.11	0.056	-1.96	> 0.05
Observations				30
Akaike Inf. Crit.				-160.97
Null Deviance				0.007 (df = 29)
Residual Deviance				0.006 (df = 28)
	Cladautoicous			
		Df	Deviance	P
Reproductive allocation		1	0.001	< 0.05
Sex		1	0.005	> 0.05
	GLM - Summary			
Sex	Estimate	Std. Error	t value	P
Female	-0.074	0.028	-2.617	< 0.05
Male	-0.022	0.008	-2.758	< 0.01
Observations				60
Akaike Inf. Crit.				-265.96
Null Deviance				0.042 (df = 59)
Residual Deviance				0.037 (df = 57)
	Rhizautoicous			
		Df	Deviance	P
Reproductive allocation		1	0.014	< 0.001
Sex		1	0.0001	> 0.05
	GLM - Summary			
Sex	Estimate	Std. Error	t value	P
Female	-0.145	0.037	-3.850	< 0.001
Male	-0.003	0.007	-0.394	> 0.05
Observations				60
Akaike Inf. Crit.				-265.26
Null Deviance				0.052 (df = 59)
Residual Deviance				0.037 (df = 56)

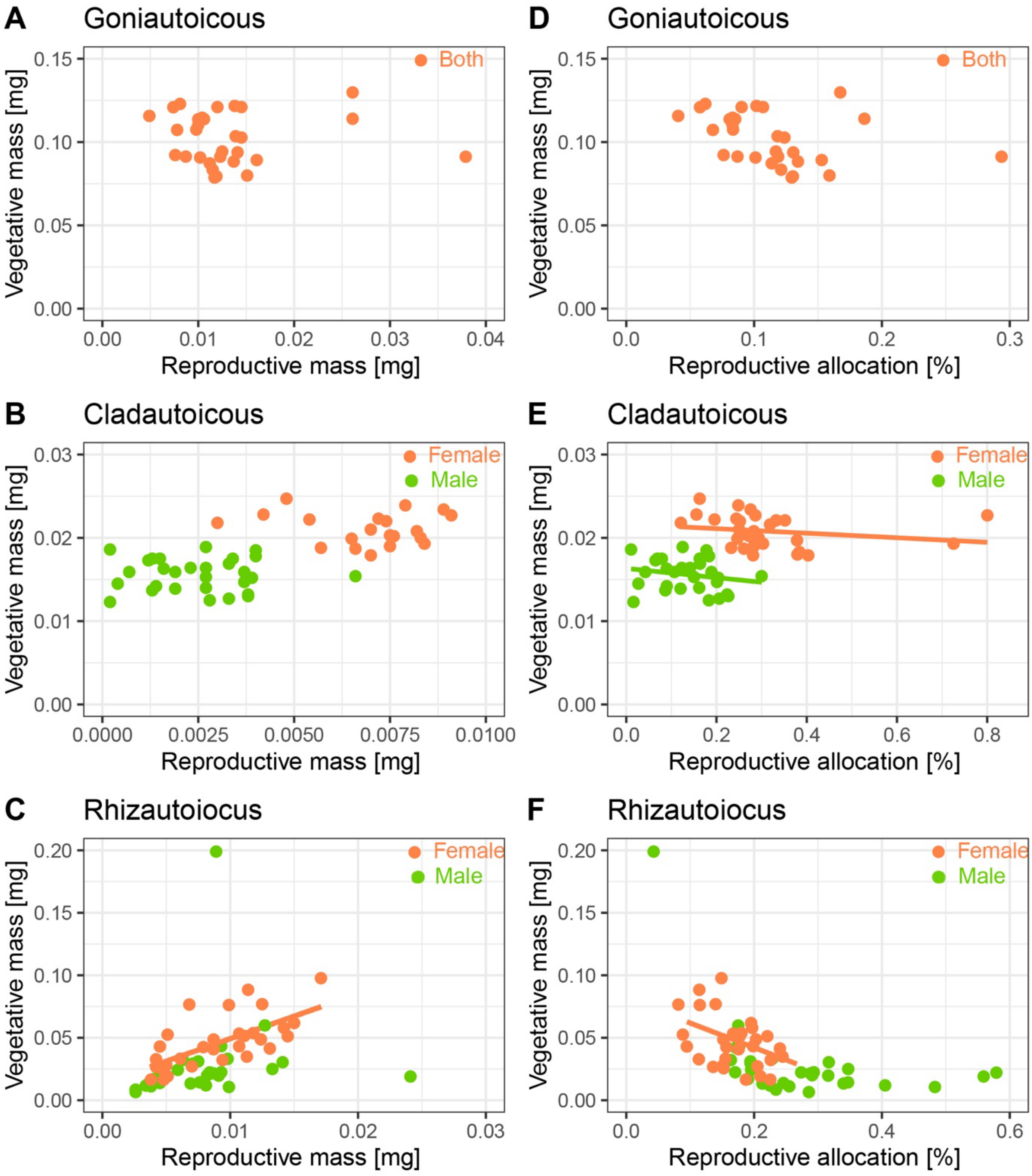

Fig. 6 Relationships between reproductive mass (A–C) or reproductive allocation (D–F) and vegetative mass. Male and female structures were pooled in the gonioautoicous sexual system and separated in the rhizautoicous and cladautoicous systems. Note the different scales between species on the *y*-axes.

DISCUSSION

This study is the first to quantify prezygotic RA in terms of relative resources devoted to reproduction in monoicous mosses with a sexual system other than rhizautoicous. We tested the predictions that RA to the male function increases with the distance between male and female sexual structures in monoicous mosses, as proposed by Stark and Brinda (2013), and that vegetative growth is negatively related to reproductive mass and RA. Neither of the two hypotheses could be supported fully. Instead, our results revealed a difference between species with 'truly' monoicous sexual systems and species that perform as functionally dioicous species. In gonioautoicous and cladautoicous systems, the sexual structures of both sexes are carried by a common vegetative ramet. In the rhizautoicous system, the male and female organs sit on individual ramets that are connected by rhizoids, which can eventually break apart. The male and female ramets still form a genet, but the distance is usually considerably longer than in the other systems (Fig. 1).

Reproductive allocation in the rhizautoicous sexual system

Reproductive allocation in the rhizautoicous system was higher in males than in females, contrary to the observations in the cladautoicous species. Previous studies that quantified prezygotic RA on a shoot basis in the rhizautoicous mosses *A. bifrons* (Stark and Brinda, 2013) and *F. flaccidus* (Santos *et al*., 2018) also found higher RA in males than in females. However, when estimated as the ratio between the number of male and female sexual branches (antheridia and archegonia) in earlier studies, RA was found to be higher in females than males in the rhizautoicous *Tortula muralis* Hedw., *Atrichum undulatum* (Hedw.) P. Beauv and *Weissia controversa* Hedw. (Anderson and Lemmon, 1972; Longton and Miles, 1982). We argue that estimates in terms of proportional mass devoted to reproduction provide a more accurate picture of resource distribution in a plant. This is basically because when we calculate the proportion of resources that a plant allocates to reproduction, we are measuring the real effort that the plant makes to reproduce (Karlsson and Méndez, 2005). In this way, we can exclude the non-photosynthetic parts, as suggested by Bisang and Ehrlén (2002), because they do not contribute to the production of resources allocated to reproduction.

The rhizautoicous sexual system can be considered a functionally dioicous system because the distance between male and female ramets can be in the same order of magnitude as in mixed populations of dioicous species (Stark and Delgadillo, 2001). Although genetically identical (because they emerge from a single spore or asexual propagule), the ramets can become physiologically independent if the rhizoid connection breaks up (Stark and Delgadillo, 2001; Stark and Brinda, 2013). Such separation might reduce competition for shared resources within an individual plant, but still offers benefits in terms of the maintenance of moisture within a patch of close neighbouring ramets (Rice *et al*., 2001: positive density dependence). This reduction of resource competition has been reported in monoecious angiosperms with sexual systems that promote some segregation of the sexes. For example, in gynodioecious species, in which plants bear either female or bisexual flowers (Bawa and Beach, 1981), the resource allocated to reproduction is higher in unisexual than in bisexual plants (Ashman, 1994; Vaughton and Ramsey, 2011). Our study adds valuable information on prezygotic reproductive allocation patterns in bryophytes, which is also scarce in dioicous species, in particular when it comes to estimates of RA based on relative mass. Prezygotic RA in terms of proportional mass allocated to sexual structures was higher in males than in females in the dioicous mosses *Weissia jamaicensis* (Mitt.) Grout. and *Syntrichia caninervis* Mitt. (Stark *et al*., 2000; Santos *et al*., 2022*a*). In the dioicous moss *Bryum argenteum* Hedw., individual perigonia weighed more than perichaetia, and RA was greater to the male than to the female function when calculated per unit area of culture media by Horsley *et al*. (2011). The latter method was also applied by Stark and Brinda (2013) for rhizautoicous *A. bifrons*. In *Drepanocladus trifarius* (F. Weber & D. Mohr) Broth., reproductive mass per sexual branch and per annual ramet and the RA in terms of proportional mass were higher in females than in males (Bisang *et al*., 2006). In contrast to the species mentioned earlier, *D. trifarius* is a pleurocarpous moss growing in wetlands. Prezygotic RA in *D. trifarius* did not result in decreased vegetative growth in males or females, indicating the lack of prezygotic reproductive costs (Bisang *et al*., 2006). No evidence of a negative relationship between RA in a given year and growth ability in the subsequent year was detected in *Polytrichum juniperinum* Hedw. either (Hedderson and Longton, 2008). Finally, in a study with the liverwort *Lophozia silvicola* H. Buch., female RA, including both pre- and postzygotic structures, and male RA were quantified by Laaka-Lindberg (2001).

Although the lack of a standardized methodology in the abovementioned examples makes data interpretation and comparisons difficult, they strongly suggest that reproductive allocation patterns depend on life-history traits, such as growth form, and environmental conditions, which could explain some of the differences observed among the species. Nevertheless, it is reasonable to think that in certain circumstances, an enhanced reproductive allocation to the male function in dioicous bryophytes increases the chance that female plants will be fertilized. Higher allocation to the male sex might eventually result in more male ramets, more male sexual structures and/or more sperm available for fertilization. Dioicous bryophytes commonly exhibit female-dominated ramet sex ratios (Bisang and Hedenäs, 2005). It is assumed that a single male plant can fertilize more than one female plant, but fertilization success in dioicous species has been shown to be limited by male mate availability and intersexual distances (Rydgren and Økland, 2001; Bisang *et al*., 2004). Although fertilization distances are an order of magnitude longer than commonly claimed (Bisang *et al*., 2004), investment in sperm without a guarantee that an archegonium will be fertilized might come at a cost (Haig, 2016). A premise is put forward by several authors that high allocation to reproductive structures might trade off with investment in other functions and eventually lead to higher mortality in the sex with higher RA (Stark *et al*., 2000; Rydgren *et al*., 2010; Haig, 2016). Indeed, the trade-off between investments in different functions might be one explanation for the low level of sexual expression of males in many populations of dioicous bryophyte species or even in the sexual dimorphism of the species, as in *Ceratodon purpureus* (Hedw.) Brid (Kollar *et al*., 2021).

Reproductive allocation in the gonioautoicous or cladautoicous species

The intraspecific pattern of RA differed between the three species (Figs 2 and 3). Although male RA was higher than female RA in the rhizautoicous species, we found the opposite in the cladautoicous species and no difference in the gonioautoicous species. Thus, a clear increasing trend in RA to the male function from synoicy to rhizautoicy, as predicted by Stark and Brinda (2013), was not evidenced (Fig. 1). Thus, we infer that RA differs between two main groups: (1) monoicous; and (2) dioicous and functionally dioicous (rhizautoicous) species. Further relationships between RA and sexual systems have been indicated in other studies. For example, in a comparative study with *Fissidens submarginatus* Bruch. (gonioautoicous) and *F. scariosus* (rhizautoicous), Santos *et al*. (2020) found: (1) higher sexual expression in the gonioautoicous species; (2) a greater number of gametangia produced per female archegonia than per male perigonia in both species; and (3) a significantly longer time for maturation of male gametangia in the rhizautoicous (4 months) than in the gonioautoicous species (3 months). Given that higher sexual expression is a striking feature of monoicous bryophytes (Glime and Bisang, 2017), the fact that sexual expression was higher in the gonioautoicous *F. submarginatus* and lower in the rhizautoicous *F. scariosus* indicates a trend towards dioicous functionality in the latter. In turn, although traits such as the timing and duration of maturation of sexual organs have not had their association with sexual systems and RA explored, evidence suggests that such relationships might exist. For example, in the rhizautoicous species *F. scariosus*, reproductive allocation was greater to the male function, and male sexual organs took longer to mature, indicating that the male sex might be the more expensive, requiring more resources and longer developmental time.

The gonioautoicous *F. submarginatus*, conversely, had a lower reproductive allocation and shorter developmental time. This protogynous development has been observed in monoicous species without a spatial separation between sexes, as, for example, in gonioautoicous species such as *Forsstroemia trichomitria* (Hedw.) Lindb. (Stark, 1985), *Trichostomum perligulatum* (Flowers) R.H. Zander (Stark and Castetter, 1995), *Syntrichia inermis* (Brid.) Bayrh. (Stark, 1997), and in the autoicous *Octoblepharum albidum* Hedw. (Egunyomi, 1978). In turn, protandry has been observed in other sexual systems, such as dioicous and rhizautoicous, such as in *Barbula lambarenensis* P. de la Varde (Fotoba, 1998), *L. silvicola* (Laaka-Lindberg, 2005) and *A. bifrons* (Stark and Brinda, 2013). The comparatively lower investment in the male function in gonioautoicous and cladouticous species can be explained by the short intersexual distances of another plant, which implies that there is no need for dedication of a large amount of resources to sperm production, contrary to what occurs in the rhizautoicous system.

Relationship between reproduction and vegetative growth

Estimates of reproductive and vegetative mass were positively associated only in female ramets of the rhizautoicous species, whereas no relationship between reproductive and vegetative mass was found in male plants or in other sexual systems. Again, a difference seems to be evident between functionally dioicous (rhizautoicous) and ‘truly’ monoicous species. A positive association between vegetative and reproductive mass has previously also been reported in female plants of the dioicous *Dicranum polysetum* Sw. (Ehrlén *et al*., 2000; Bisang and Ehrlén, 2002). This indicates that, in absolute values, a vegetatively strong female plant invests more in reproduction than a weaker plant, but only in the rhizautoicous species, and that the resources allocated to the formation of perichaetia do not trade off with vegetative growth. Rhizautoicous ramets supply resources to reproductive structures of only one sex, because they are likely to be physiologically independent, and in females the resources will then also be invested in the future development of sporophytes. In the dioicous moss *Ceratodon* *purpureus* (Hedw.), however, allocations to vegetative and reproductive gametophyte tissues were unrelated in females, while a negative association was evidenced in males (McDaniel, 2005).

Vegetative mass was negatively related to RA in female ramets of the rhizautoicous species and male and female branchlets of the cladautoicous species in the present study. This can be interpreted in different ways depending on the direction of the relationship. It might reflect the shortage of resources for vegetative growth resulting from RA in females (Convey and Smith, 1993; Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). Or, in contrast, RA decreases in vegetatively larger plants. However, this pattern is somewhat unexpected, because one would anticipate a perennial plant to ensure survival by investing in vegetative growth if it is small, and increasing the relative investment to reproduction if it is larger (Convey and Smith, 1993; Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). Interestingly, male RA in the rhizautoicous species was not related to vegetative mass. This could be explained by the phenology of this species, in that the production of male gametangia starts early and continues for relatively long time intervals, and resources can be supplied over a longer time span (Santos *et al.*, 2020). In contrast, Santos *et al.* (2018) reported a negative association between RA and vegetative mass in all sexual morphs (male, non-sporophytic and female sporophytic) of the rhizautoicous moss *F. flaccidus*. Thus, reproductive allocation patterns seem to vary among species. More species with different sexual systems and other life histories need to be investigated and more comprehensive predictions need to be tested before a more general pattern can emerge.

Conclusion

Our data suggest a possible trade-off between reproductive and vegetative allocation and that the allocation patterns vary among bryophyte species with different sexual systems. Our original hypotheses on RA were supported only in part. Rather than showing a gradient of increasing allocation to male reproduction in monoicous sexual systems with increasing levels of intersexual distance, the data revealed a distinction in different aspects of RA between 'truly' monoicous and monoicous but functionally dioicous sexual systems. To explore further the conditions in which males allocate more to reproduction than females in (functionally) dioicous bryophytes, as postulated by Stark and Brinda (2013), the life-history traits of the species and environmental factors need to be taken into account. For example, it is possible that trade-offs between reproduction and vegetative growth are associated with other reproductive traits in these sexual systems. We suggest that the hypotheses should be rephrased and tested further by comparing monoicous and dioicous species from different genera and environments. In addition, the postzygotic resource allocation in females in species with different sexual systems must be investigated to deepen our understanding of resource allocation patterns in bryophytes.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Supplementary 1 - "Simpson effect" in male ramets of rhizautoicous species.

Supplementary 2 - comparation of null and full models.

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Chapter 5

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Environmental gradient drives functional diversity variation: insights from *Fissidens* (Bryophyta) of Atlantic Forest

Wagner Luiz dos Santos[1], Kátia Cavalcanti Pôrto[2], Juçara Bordin[3], Fábio Pinheiro[1*], Mércia Patrícia Pereira Silva[2]

[1] Department of Plant Biology, University of Campinas, Rua Monteiro Lobato 255, Barão Geraldo, Campinas, São Paulo, Brazil

[2] Department of Botany, Federal University of Pernambuco, Avenida Moraes Rego, s/n, University City, Recife, Pernambuco, Brazil

[3] State University of Rio Grande do Sul, Machado de Assis, 1456, RS 95520-000, Brazil.

* For correspondence. E-mail biopin@unicamp.br

ABSTRACT

Understanding factors that influence biodiversity is crucial for ecological research, especially in bryology. Thus, investigating taxonomic, phylogenetic, and functional diversity in mosses like *Fissidens* (most diverse genus among mosses) is essential. This study focused on the impact of abiotic factors (altitude and precipitation) on *Fissidens'* functional diversity and composition in the diverse and threatened Brazilian Atlantic Forest. Fissidentaceae displays significant morphological and functional diversity, making it an ideal model for understanding biodiversity and ecosystem functioning. The research was conducted in the 'Serra do Mar' biogeographic region of Atlantic Forest, Southern Brazil, using 24 randomly selected plots. The functional strategies of each *Fissidens* species were described based on six traits, and climate data were obtained from CRU-TS 4.06 and WorldClim 2.1 datasets. We identified key traits characterizing the *Fissidens* community, including sexual segregation, limbidium presence, and distribution in the leaf and vaginant laminae. Our findings show that precipitation and altitude have a significant impact on the functional diversity of *Fissidens* in the Brazilian Atlantic Forest. Precipitation leads to greater functional richness, expanding the community's functional space. Altitude, on the other hand, increases functional dispersion, allowing for a wider range of viable morphological traits. While these traits relate to precipitation and altitude, their influence appears to be driven by phylogenetics, which should be considered in future studies. This study sheds light on the connections between *Fissidens'* functional diversity and environmental gradients in the Atlantic Forest. It highlights the significance of both environmental and functional diversity in bryophytes. Future research, including experimental approaches, should explore the ecological and evolutionary influences on functional traits in this diverse and ecologically important moss group. Such understanding is vital for conserving biodiversity in the highly threatened Atlantic Forest, especially amid climate change challenges.

Key words: Bryophyte ecology, community composition, environmental filters, functional traits.

INTRODUCTION

Elucidating how multiple factors regulate the spatial variation of biodiversity is a central topic in ecology and biogeography (Machac et al., 2011). Indeed, considering the research agenda for bryology (liverworts, mosses, and hornworts), the question “What are the main drivers of taxonomic, phylogenetic, and functional diversity in bryophytes?” has been recently pointed as the third of the 50 issues that are critical in advancing the discipline (Patiño et al., 2022). Several studies have addressed this question, however, the taxonomic facet of diversity has received way more attention (Amorim et al., 2017; Peñaloza-Bojacá et al., 2018; Batista et al., 2021; Araújo et al., 2022) compared to functional (Pardow et al., 2010; Ah-Peng et al., 2014; Henriques et al., 2017; Souza et al., 2020; Asplund et al., 2022), and phylogenetic (Heinrichs et al., 2009; Cox et al., 2010) diversity, which is even scarcer. Most of those authors agree with the idea that bryophyte diversity (regardless of the facet addressed) is based on environmental filtering. Environmental filters act on bryophyte assembly both in regional (e.g. disturbance, climate, or elevation; Pharo & Zartman, 2007; Santos & Costa, 2010; Amorim et al., 2017) and local scales (e.g. physical-chemical characteristics of the substrate, microhabitat or microclimate; Patiño & González-Mancebo, 2011; Silva et al., 2014; de Oliveira & ter Steege, 2015; Batista et al., 2021), which can be attributed to the poikilohydric ecophysiology of bryophytes: since most of the species are desiccation-tolerant (Proctor, 2000; Oliver, 2005; Proctor et al., 2007), thus their distribution is generally deterministic (niche based), driven by environmental filters (Mota De Oliveira et al., 2009).

Especially in the Neotropics, the diversity of bryophytes is dependent on habitat heterogeneity, mainly related to disturbance, vegetation type, climate, and/or elevation (Holz et al., 2002; Batista & Santos, 2016; Rodríguez-Quiel et al., 2022), suggesting that species coexist because they may specialize in different parts of the niche or share common morphological traits conferring abiotic/ disturbance tolerance (Mota De Oliveira et al., 2009). In this context, functional diversity is an interesting metric in characterizing communities, as well as phytogeographic domains and their respective vegetation types (Mason et al., 2005), since it allows for a comprehensive analysis of species composition and distribution in an ecosystem, providing a more complete understanding of its complexity and biological richness. Such morphological trait-based approaches to elucidate functional diversity become more relevant in highly diverse and threatened ecosystems, such as the Atlantic Forest. In the tropics, Atlantic Forest stands out as the most diverse and threatened Brazilian phytogeographic domain (INPE, 2001), being classified as a biodiversity hotspot (Sloan et al., 2014). Regarding bryophytes, the Atlantic Forest is the most species-rich phytogeographic domain in Brazil (Alvarenga & Pôrto, 2007; Silva & Pôrto, 2010; dos Santos et al., 2011), primarily due to its habitat heterogeneity, with wide altitudinal and latitudinal range (Perrigo et al., 2020).

The genus *Fissidens* belongs to the monotypic moss family Fissidentaceae and is one of the largest and most diverse in the world, with approximately 450 species currently recognized. These species are primarily found in tropical and subtropical regions (Pursell, 2007). In Brazil, Fissidentaceae stands out as the second richest family of mosses, occurring in all phytogeographic domains, but predominantly in the Atlantic Forest (Bordin & Yano, 2013). Currently, 55 species of *Fissidens* are reported for the Atlantic Forest, which corresponds to 7% of the total taxa of mosses occurring in the country (Flora e Funga do Brasil, 2023). *Fissidens* exhibits an unparalleled diversity of morphologies among bryophytes, encompassing a wide range of characteristics within this genus (Pursell, 2007; Bordin & Yano, 2013). For instance, all *Fissidens* species have a vaginant lamina, an “expanded laminae the base of the leaf which clasps the stem and the base of the leaf above it” (Magill, 1990). This trait, coupled with others, such as limbidium (differentiated leaf border), are highly variable and, according to Pursel's studies (Pursell & Vital 1986, Pursell, 1990, 2007), demonstrate a clear association with the environment, mainly altitude, although it has not yet been empirically tested. As a result, we can expect a wide range of morphological traits found in different species of *Fissidens*. Additionally, *Fissidens* exhibits a diversity of sexual systems (dos Santos et al., 2023; Santos et al., 2023), with different levels of gametangia segregation, however, the relation of the presence and frequency of the sexual systems of the genera and environmental factors is also still an open study field. Therefore, the diversity of morphological and reproductive traits makes the group *Fissidens* remarkable and capable of providing valuable insights into the relationship between plant functional traits and the environment (Pursell, 2007; Bordin & Yano, 2013).

Building on this knowledge, we used a regional scale to assess the role of abiotic (altitude and precipitation) factors on the functional diversity and composition of *Fissidens* assembly in the Atlantic Forest. We hypothesized that environmental filtering — via abiotic factors — drives functional diversity and composition patterns. Considering that previous studies have demonstrated that altitude and precipitation affect niche selection (Gabriel & Bates, 2005; Alvarenga & Pôrto 2007) and community assembly patterns in bryophytes, and that functional diversity decreases with increasing climatic/ abiotic stress (Gazol et al., 2017), we expect that lower temperatures with increasing elevation, combined with higher water availability, will act as an environmental filter and will increase the functional diversity of

Fissidens at a regional scale in the Atlantic Forest. Furthermore, we expect that different functional strategies will be selected along environmental gradients, and characteristics related to water conservation, such as the presence of papilla and limbidium through the leaf and bigger vaginant laminae will be more common at higher altitudes and in sites with lower levels of precipitation (Watson 1914). Yet, since sexual reproduction is dependent on water availability, we expect that the relative frequency of sex segregation will be lower in sites with low precipitation levels.

MATERIAL AND METHODS

Target taxon

Fissidentaceae is a widely distributed family of mosses throughout the world, with the genus *Fissidens* being the only genus within it (Pursell, 2007; Bordin & Yano, 2013). This family is present in various locations, except for Antarctica (Pursell, 2007; Bordin & Yano, 2013). Comprising small and delicate plants, these mosses are primarily found in humid habitats such as tropical and temperate forests (Pursell, 2007; Suzuki et al. 2018), but they also exhibit remarkable diversity in arid environments such as deserts and dry tropical forests (McCleary, 1959; Bastos et al., 1998). These mosses form dense and compact tufts on rocks, soil, or tree trunks, playing an essential role in the ecology of these environments.

Among bryophytes, the family Fissidentaceae stands out as the most diverse and variable in terms of morphological and functional characteristics among mosses (Pursell, 2007). The species belonging to Fissidentaceae display a wide variety of distinct attributes, including features such as leaf shape and structure, the presence of limbidium, papilla, and other relevant traits (Iwatsuki & Pursell, 1980; Bordin et al., 2011; Bordin & Yano, 2013; Guerra et al., 2021) (Fig. 1). In some cases, the variation in functional traits within this family in response to the environment is so significant that it can even be considered as distinctive taxonomic characteristics for the group, for instance in species *Fissidens wallisii* Müll. Hal. where specimens from regions more elevated in Atlantic Forest usually have teeth on the edge of the leaves that are clearly visible, larger ones, both at the apex of the leaves as in the vaginant laminae. The same occurs in *Fissidens weirii* Mitt. var. *weirii* where epixylic samples collected in high altitude, usually have oblong-lanceolate leaves, acute and gradually long acuminate apex and have bistratose limbidium, (Bordin & Yano, 2013). This remarkable diversity makes the study of the family Fissidentaceae fundamental for a broader understanding of biodiversity and the ecosystems where these mosses are present. Therefore, by investigating the morphological and functional characteristics of Fissidentaceae members, we can obtain valuable insights into their adaptations to the environment, their ecological interactions, and their importance for ecosystem functioning.

Study site and data sampling

This study was based on a dataset collected in the 'Serra do Mar' biogeographic region of Atlantic Forest, Southern Brazil (Table 1). The 'Serra do Mar' is an extensive mountain range that stretches over 1000 km along the south and southern coast of Brazil, spanning from the state of Espírito Santo to the northern part of Paraná (Almeida & Carneiro 1998), and holds the utmost significance for the country from both geographical and environmental perspectives. Its distinctive features and substantial contribution to biodiversity and geological processes render it truly remarkable within the coastal landscape (Almeida and Carneiro, 1998; Tabarelli et al., 2005; Carlucci et al., 2021). The 'Serra do Mar' harbors the largest refuge of the Atlantic Forest in Brazil, making it a region of most importance for the conservation of this ecosystem (Vieira & Gramani, 2015). A significant portion of this region is comprised of environmental preservation parks, which play a fundamental role in biodiversity protection and the maintenance of ecosystem services. A summary of the main environmental conditions of each plot is given in Table 1.

In the context of this study, sampling was conducted in 24 plots (100m² each) spanning a linear distance of 173.7 km and a minimum distance of 11.9 km between plots. To ensure representativeness and impartiality in the collections, a random sampling method was adopted (Santi et al., 2016). This approach allows for obtaining samples that are representative of the study area and minimizes possible influences or specific trends from certain regions within the study area. Therefore, the use of random collections contributes to obtaining more comprehensive and reliable data for analysis. The samples were carefully stored in paper bags, and properly labeled with the notation of the sampling plot and geographic coordinates. Additionally, each sample was accurately labeled with relevant information using appropriate literature. In the laboratory, the identification of the samples was carried out based on appropriate literature.

Table 1. Location and environmental data in the studied plots in 'Serra do Mar' biogeographic region, Southern Atlantic Forest.

Plot	Location	Mean Annual Precipitation (mm)	Elevation (m)
Pedro de Toledo 1	24°14'05.7"S 47°13'41.3"W	153.59	152.04
Pedro de Toledo 2	24°10'46.7"S 47°06'34.8"W	166.53	383.21
Pedro de Toledo 3	24°14'29.6"S 47°11'51.7"W	156.79	231.32
Pedro de Toledo 4	24°15'20.2"S 47°13'20.8"W	161.28	308.68
Peruíbe 1	24°24'04.3"S 47°08'48.8"W	170.85	62.84
Peruíbe 2	24°23'38.1"S 47°08'01.3"W	173.58	136.53
Peruíbe 3	24°23'31.8"S 47°07'09.6"W	172.02	88.08
Peruíbe 4	24°24'54.7"S 47°07'03.7"W	172.59	98.10
São Vicente 1	23°59'03.2"S 46°23'27.8"W	220.58	110.00
São Vicente 2	23°59'06.4"S 46°23'26.6"W	220.58	110.00
São Vicente 3	23°59'01.0"S 46°23'00.9"W	216.54	74.07
São Vicente 4	23°58'06.0"S 46°22'35.0"W	219.10	108.04
São Sebastião 1	23°45'59.2"S 45°36'23.4"W	202.12	188.68
São Sebastião 2	23°44'25.5"S 45°37'14.2"W	202.46	177.25
São Sebastião 3	23°44'28.5"S 45°37'17.6"W	203.58	149.00
São Sebastião 4	23°44'23.4"S 45°37'11.0"W	201.69	196.58
Parque das Neblinas 1	23°44'28.9"S 46°08'35.6"W	245.49	735.77
Parque das Neblinas 2	23°44'38.5"S 46°07'56.2"W	205.33	738.11
Parque das Neblinas 3	23°44'39.2"S 46°07'55.7"W	205.67	729.00
Parque das Neblinas 4	23°44'38.9"S 46°07'55.3"W	205.67	729.00
Bertioga 1	23°48'06.9"S 46°07'45.6"W	263.71	10.28
Bertioga 2	23°47'54.3"S 46°07'51.3"W	384.00	28.00
Bertioga 3	23°43'55.4"S 45°55'55.4"W	247.80	60.53
Bertioga 4	23°51'39.6"S 46°08'31.8"W	248.71	46.05

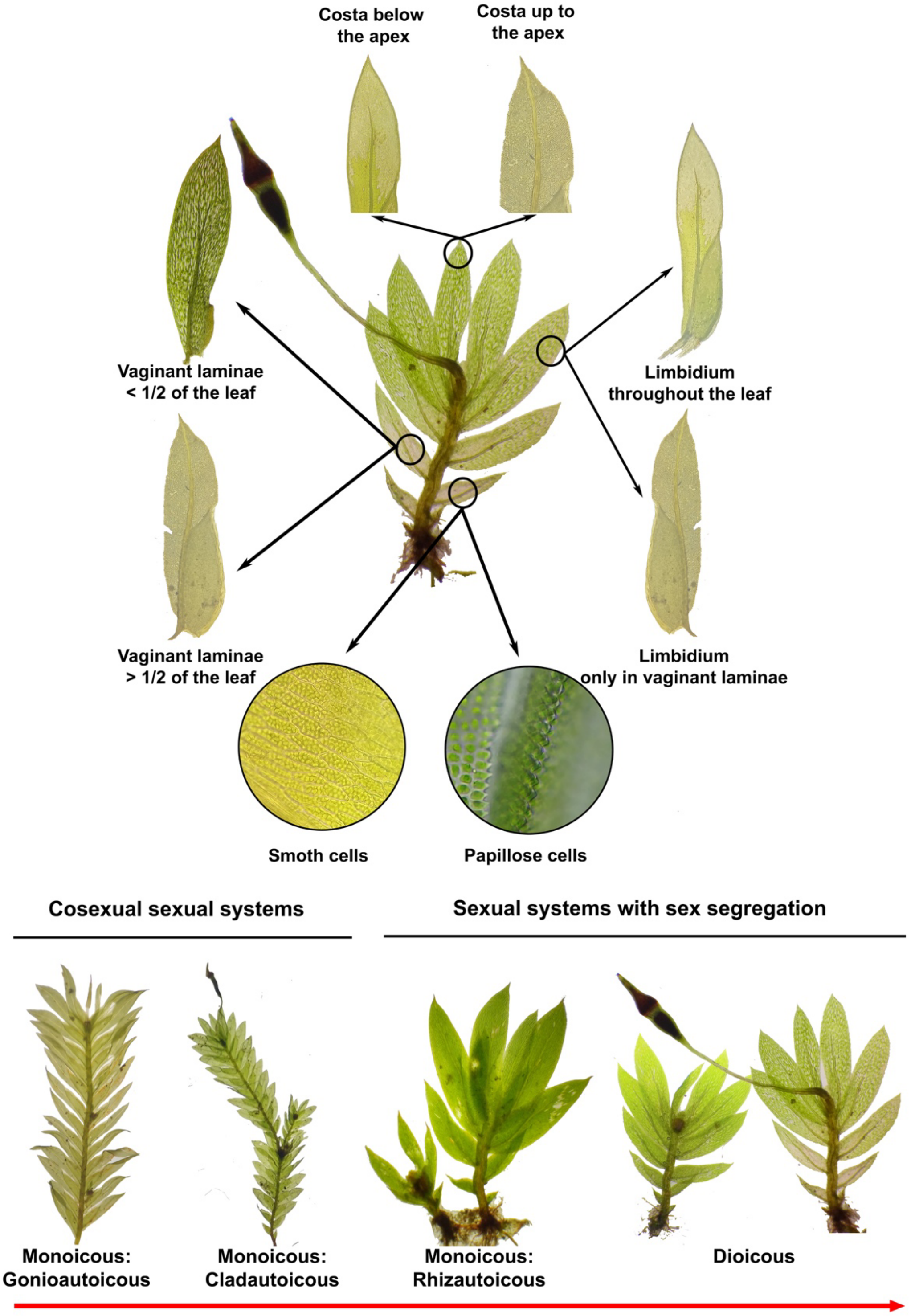

Fig. 1 Functional traits were used to measure functional diversity indices in the study, including characteristics such as the position of the costa relative to the leaf (from below to the apex and from costa up to the apex). Limbidium throuthg the leaf and limbidium only in the vaginant lamina. Smooth and papillose cells. Vaginant laminae> 1/2 of the leaf or being smaller than 1/2 of the leaf. In the lower part, the sexual systems of the studied species are represented, as well as whether the system is co-sexual (reproductive and female structures in the same shoot) or segregated (male and female reproductive structures in separate shoots). The red arrow indicates the direction in which the sexual systems increase the distance between the sexes.

Altitude and mean annual precipitation were obtained using climate dataset CRU-TS 4.06 (Harris et al., 2020) downscaled with WorldClim 2.1 (Fick & Hijmans 2017) with the resolution of 30s in the software QGis 3.32.0 (using 'Point Sampling Tools'). The functional strategy of each *Fissidens* species was described using six traits, following specialized literature (Table 2), computed as binary variables: 1 indicated presence and 0 absence. The costa plays a fundamental role in water absorption, being categorized as "ending at the leaf apex" or "ending at the leaf base." This classification indicates that species with costa ending at the apex have a greater water absorption capacity. The same criteria were applied to limbids, which were divided into "present throughout the leaf" or "present only in the vaginate blade." Additionally, the papilla, which plays a role in optimizing osmotic water uptake and regulation, was classified as "present" or "absent," and the size of the vaginate blade was categorized as "larger or smaller than half of the leaf." Regarding sexual systems, monoicous: rhizautoicous and dioicous species were considered as presenting sex segregation, since the male and female gametangia are spatially separated. On the other hand, monoicous gonioautoicous and cladautoicous species were considered cosexual, due to the greater proximity of male and female gametangia (Fig. 1).

Data analysis

The sampling sufficiency of each locality was calculated through sample coverage, based on the inventory completeness, using the iNEXT function in the 'iNEXT' package (Chao & Jost, 2012) in the R environment. We applied ACE estimator to evaluate the total species richness in each plot (Chao, 1984) and compared it with the observed species richness through a Chi-squared (*X2*) test.

Collinearity between environmental variables and between morphological traits was tested with the Spearman correlation (Zuur et al., 2010). For the environmental variables, as the correlation value found was low (rs = -0.14; p = 0.51), we assumed that our predictors were not collinear and both were included in the subsequent analysis (Neter et al., 1990) (Supplementary material). For the morphological traits, only limbidium throughout the leaf and limbidium only in vaginant laminae were significantly correlated (rs = 0.56; p = 0.0148), however we decided to include both traits in the analysis considering their ecological significance. One species with limbidium only in vaginant laminae and limbidium throughout the leaf may show a greater reduction in evapotranspiration than a species that has only one of these traits (Henriques et al. 2017).

Table 2. Traits used in the analyses of functional diversity and composition of *Fissidens*, with associated functions.

Traits	Function	Reference
Costa	Rapid water absorption and transport	(Proctor 1979; Frahm 1985)
Limbidium throughout the leaf	Reduction of evapotranspiration	(Henriques et al. 2017)
Limbidium only in vaginant laminae	Reduction of evapotranspiration	(Henriques et al. 2017)
Papilla	Optimization of osmotic water intake and regulation	(Proctor 1979)
Sex segregation	Different in the chances of female gamete fecundation	(Stark & Brinda 2013; dos Santos et al. 2020)
Size of vaginant laminae	Reduction of evapotranspiration	(Henriques et al. 2017)

Functional diversity along environmental gradients was evaluated per plot using four indices to describe the distance and the distribution of the species in the multidimensional niche space, performed through the function dbFD in the package 'FD' (Laliberté et al., 2014): FRic, FDiv, FEve and FDis. Functional richness (FRic) is measured as the number of unique trait value combinations in a community (in our study, the term community corresponds to a set of all species in each plot); Functional divergence (FDiv), which expresses the distance of the species frequencies from the center of the functional space (Villéger et al., 2008); Functional evenness (FEve), that describes the equity of distribution of traits in the functional space; Functional dispersion (FDis), the variance in a species' traits and where they are located in trait space , using both species relative abundances and the pairwise functional differences to summarize functional diversity (Schleuter et al., 2010). The relationship between the functional diversity indexes and abiotic variables was evaluated at the regional scale using generalized linear models GLM (Bates et al., 2009; Marschner et al., 2018). The functional indexes were the response variable and the models were fit using a Gaussian family error distribution.

We performed a non-metric multidimensional scaling (NMDS) with the morphological traits of the *Fissidens* species to identify main trait differences among the community. The NMDS was based on the Gower distance using the 'metaMDS' function in the 'vegan' package

(Oksanen et al., 2019). The same package was used to identify significant functional traits that differentiate the *Fissidens* species, performed with the 'envfit' function. Additionally, to graphically display the representativeness of each trait along the environmental we used a scatterplot matrix containing the scatter plots of the relative frequency of the traits and the environmental variables using Ggplot2 package (Kassambara, 2013). Additionally, we performed a GLM ('lme4' package), following the same methodology for the functional indexes, to investigate the relationship between the relative frequency of each trait with the environmental variables. All analyses were executed in R 4.3.1 environment (RStudio Team, 2021).

RESULTS

The database compiled 18 *Fissidens* species (Table 3). The most frequent species was *F. pseudoplurisetus* (occurences = 241; 18 plots), followed by *F. weirii* (occurrences = 218; 19 plots). In contrast, *F. goyazensis* and *F. guianensis* had only two occurrences, restricted to one plot each. The species number between plots varied from 1 to 10. Based on the effective species richness (q = 0), all plots presented a robust sample coverage, exceeding 90% (see Supplementary material). The observed species richness did not differ significantly from the ACE (X-squared = 168, df = 144, p-value = 0.0836) richness estimates.

Functional diversity varied significantly along the environmental gradients. The functional richness was positively related to the mean annual precipitation, while the functional dispersion was positively related to the altitudinal gradient (Table 4). The Functional divergence and Functional evenness indexes showed no relation with environmental gradients.

The NMDS showed one main trade-off among *Fissidens* species regarding the most representative traits. The trade-off was observed between the presence of sex segregation (r^2 = 0.8335, p = 0.001), costa up to the apex (r^2 = 0.7663, p = 0.001), limbidium throughout the vaginant laminae (r^2 = 0.7207, p = 0.001), limbidium throughout the leaf (r^2 = 0.6313, p = 0.001) and the absence of these traits (Figure 2). Only the traits papilla (r^2 = 0.2813, p = 0.098) and vaginant laminae > 1/2 of the leaf (r^2 = 0.1630, p = 0.275) showed no significant trade-offs.

Overall, *Fissidens* traits showed opposing trends along elevation and mean annual precipitation gradients, although not significant (Figure 3). Limbidium throughout the leaf showed a positive relation along the altitudinal gradient, while limbidium only vaginant

Table 3. Species of *Fissidens* collected in the Brazilian Atlantic Forest during this study, along with their respective functional traits.

Species	**Costa**	**Limbidium throughout the leaf**	**Limbidium only in vaginant laminae**	**Papilla**	**Size of vaginant laminae**	**Sexual segregation**
Fissidens anguste-limbatus Mitt.	1	1	1	0	1	1
Fissidens asplenioides Hedw.	0	0	0	0	1	1
Fissidens elegans Brid.	1	0	1	1	1	0
Fissidens flaccidus Mitt.	0	1	1	0	1	1
Fissidens goyazensis Broth.	1	1	1	1	1	1
Fissidens guianensis Mont.	0	0	1	1	1	0
Fissidens hornschuchii Mont.	0	0	1	1	0	1
Fissidens inaequalis Mitt.	0	0	0	0	0	0
Fissidens neglectus H.A. Crum	0	0	1	1	1	1
Fissidens oblongifolius Hook. f. & Wilson	0	0	0	0	1	1
Fissidens pellucidus Hornsch.	0	0	0	0	0	0
Fissidens psedoplurisetus Bordin, Pursell & O.Yano	1	0	0	1	1	0
Fissidens ramicola Broth.	1	0	1	1	1	1
Fissidens scariosus Mitt.	0	1	1	0	1	1
Fissidens serratus Müll. Hal.	1	0	0	0	1	0
Fissidens submarginatus Bruch	1	1	1	1	1	0
Fissidens weirii Mitt.	1	1	1	1	0	0
Fissidens zollingeri Mont.	1	1	1	0	1	1

laminae, papilla and vaginant laminae < ½ of the leaf displayed negative relation and costa, no trend. Along the mean annual precipitation gradient, all traits exhibited a positive relation, with exception of sex segregation, that expressed no trend. The GLM returned no relation between the relative frequency of the traits and the environmental variables (Supplementary material).

Table 4 Results of the generalized linear regression analysis regarding the effects of mean annual precipitation (MAP) and elevation on *Fissidens* functional diversity indexes. FRic = Functional richness; FDiv = Functional divergence; FEve = Functional evenness (FEve); FDis = Functional dispersion (FDis).

FRIc	Estimate	t	P
(Intercept)	0.001	4.55	< 0.001
MAP	< 0.001	2.11	< 0.05
Elevation	< 0.001	0.56	0.60
FDiv			
(Intercept)	< 0.001	11.49	< 0.001
MAP	< 0.001	0.81	0.50
Elevation	< 0.001	0.33	0.75
FEve			
(Intercept)	0.590	4.33	< 0.001
MAP	0.001	0.32	0.75
Elevation	0.001	1.47	0.15
FDis			
(Intercept)	< 0.001	2.30	< 0.05
MAP	< 0.001	0.28	0.78
Elevation	< 0.001	2.01	< 0.05

DISCUSSION

Functional traits of organisms are linked to their performance in the environment and to the overall structure, function, and diversity of ecosystems (Patiño et al., 2022). Therefore, understanding how the community composition of functional traits changes with the environment is key to understanding the role of climate in ecology (Wang et al., 2019). The functional diversity and functional composition of the *Fissidens* community in the Southern Atlantic Forest exhibited contrasting responses to environmental gradients. On one hand, functional diversity showed significant variations along these gradients, with positive associations with functional richness (in relation to the mean annual precipitation gradient) and functional dispersion (in relation to the altitudinal gradient). On the other hand, no relationship was observed between the relative frequency of *Fissidens* traits and environmental variables; however, general patterns of increased frequency for most traits with higher precipitation and decreased frequency with higher altitude were identifiable. These results highlight the importance of understanding the functional diversity and functional composition of bryophytes along environmental gradients, especially in highly diverse and endangered vegetation domains such as the Brazilian Atlantic Forest. This knowledge can help us to predict how bryophytes will respond to climate change and other environmental stressors, identify the bryophyte species that are most important for ecosystem function, and develop management strategies for conserving bryophyte diversity.

In accordance with the findings of Bordin & Yano (2013), who observed morphological variations in some species associated with higher altitudes, our study provides support for the assumption that more favorable environmental conditions for bryophytes contribute to increased functional diversity in *Fissidens* within the Southern Atlantic Forest. This is evident from the positive relationships we observed between functional richness and precipitation, as well as between functional dispersion and altitudinal gradients. Functional richness represents the amount of functional space filled by the community, indicating resource use, being considered a robust index underlying communities assembly rules (Wang et al., 2019). In our study, the increase in functional richness with precipitation reflects an increase in occupied functional space for *Fissidens* community, influenced by precipitation. In fact, in plots with high precipitation levels, there was a range of *Fissidens* traits. In the same community, there are *Fissidens* species with no papilla, limbidium through leaf, limbidium only in vaginant laminae and sexual system with sex segregation, such as *F. anguste-limbatus*, species with papilla, no limbidium and cosexual sexual system, such as *F. pseudoplurisetus*, and species hosting a myriad of trait combination between these. These trait variations expand the volume of the functional space, indicating a higher niche availability for *Fissidens* in the study area. Similar results were found by Henriques et al. (2017) on Azores archipelago, where the functional richness increased with elevation.

The wide functional space occupied by *Fissidens* community was also reflected in the functional dispersion. This index describes optimal plant strategies because it captures how abiotic and biotic factors can influence community structure (Daniel & Rooney, 2022). In our study, high elevation was associated with high functional dispersion, indicating an increase in viable morphological traits and strategies under environmentally favorable conditions provided by altitude and associated factors, such as low temperature. In other words, our results suggest that niche space increases with available resources on high elevation, which supports higher trait dispersion (Rabosky and Hurlbert, 2015). Cornwell and Ackerly (2009) along a precipitation gradient in California and Thakur and Chawla (2019b) in altitudinal transects in high altitude region of western Himalaya have shown the same directionality in functional diversity. For bryophytes, altitude is a known pivotal factor in the distribution and richness in tropical ecosystems (Dos Santos and Da Costa, 2010; Amorim et al., 2017; Thakur and Chawla, 2019a; Batista et al., 2021; Araújo et al., 2022). Here, we confirmed that altitude also drives the functional diversity of *Fissidens* in the southeastern Atlantic Forest.

The *Fissidens* functional diversity trends suggest that functionally more diverse species should co-exist (divergence in plant functioning) at higher elevations and precipitation levels. Considering that functionally diverse communities are more stable and functional diversity decreases along a climatic stress gradient (Cornwell and Ackerly, 2009; Gazol et al., 2017; Thakur and Chawla, 2019b), it has implications in biodiversity conservation and can be used to evaluate the impact of climate change on communities. In this context, *Fissidens communities* might be functionally harmed, in highly threatened environments such as the Atlantic Forest. The Atlantic Forest, renowned for its biodiversity richness, faces significant challenges due to climate change. With its intricate network of ecosystems and unique species, this region is highly susceptible to climatic impacts, including rising temperatures, shifts in rainfall patterns, and extreme weather events (Buytaert et al., 2011). The interconnection between plant functional diversity and ecosystem stability is pivotal for the resilience of the Atlantic Forest. Grasping the implications of functional diversity trends, as observed in the *Fissidens* study, provides valuable insights for conservation and environmental management strategies. In the face of escalating climatic impacts, adopting proactive approaches to preserve ecosystems as precious as the Atlantic Forest is imperative (Taylor, 2008). Exploratory research into plant functional diversity not only deepens our understanding of complex ecological processes but also empowers us to anticipate and mitigate adverse effects of climate change. By merging scientific knowledge and conservation efforts, we contribute to safeguarding the biological uniqueness of the Atlantic Forest and ensure a sustainable future for this region.

Regarding functional composition, the most representative traits of the community were sex segregation, costa up to the apex, and the presence of limbidium only in vaginant laminae and limbidium throughout the leaf. Among these traits, despite not being statistically significant to the environmental variables, all of them exhibited a positive relation with the precipitation, with exception of sex segregation, that expressed no trend. On the other hand, only limbidium throughout the leaf showed a positive relation along the altitudinal gradient. We found that the sexual system is not related to precipitation. Moisture is crucial for fertilization in mosses, particularly for dioecious species, since the swimming sperm is required to move between separate male and female gametophytes for fecundation to occur (Maciel-Silva & Pôrto 2014). Our results denote that the relative frequency of the sexual system in *Fissidens* of Southeastern Atlantic Forest is not related to moisture, which indicates that it is a feature with a strong phylogenetic signal. In fact, the sexual system is an efficient character to define Fissidentaceae subgenera and sections (Suzuki et al. 2018) and the phylogenetic signal was reported by Budke et al. (2022), using comparative phylogenies.

Most *Fissidens* sampled in our study are at one of the two ends of the spectrum for limbidium morphology, either with a limbidium present on the leaf and vaginant laminaeor with laminaethat is elimbate. Special border cells are rare among tracheophytes, suggesting that their presence indicates a function in bryophytes that is not useful in tracheophytes (Glime 2017). Functionally the limbidium may be involved in leaf structural support (Lowell, 1998), water storage (Daniels, 1998) and/or movement (Glime 2017). In *Fissidens*, limbidium absent is reconstructed as the ancestral condition for Fissidentaceae (Suzuki et al., 2018; Budke et al., 2022) and it has been reported that species with a limbidium present on all regions of the laminaeare associated with a wider niche breadth (Budke et al., 2022). One potential interpretation for our findings is that a more extensive limbidium enables these species to survive across a wider variety of habitats, provided by the altitudinal gradient, and thus to be more flexible in their niche choice (Budke et al., 2022). However, *Fissidens* morphological traits and their functional roles have not been explored experimentally and it is primordial further exploration in the context of taxonomy and functional ecology and evolution.

Conclusion

In summary, this study analyzed the influence of precipitation and altitude on the functional diversity of the *Fissidens* genus in the southeastern Atlantic Forest of Brazil. The results revealed that precipitation and altitude act in conjunction to define the functional diversity of these mosses. Furthermore, it was identified that sexual segregation stood out as the most explanatory trait of the community. Surprisingly, although precipitation showed more positive relationships than altitude with functional traits, none of these traits showed a significant relationship with the studied environmental gradients. This finding may indicate that these traits are primarily influenced by genetic factors and do not rely solely on the local environment (Henriques et al., 2017; Suzuki et al., 2018; Souza et al., 2020). Therefore, conducting future experimental studies is essential for a better understanding of the patterns of functional diversity and evolution of *Fissidens*. Further investigation of these mosses in different environmental contexts will help identify the key ecological and evolutionary forces shaping their functional characteristics. Consequently, based on the findings of this study, we emphasize the importance of considering both environmental and genetic factors when assessing the functional diversity of Fissidens. Only with an integrated approach, we can obtain a more comprehensive understanding of the ecology and evolution of these organisms crucial for the structure and maintenance of biodiversity in the Atlantic Forest.

CAPTIONS

Figures

Fig. 1 Functional traits were used to measure functional diversity indices in the study, including characteristics such as the position of the costa relative to the leaf (from below to the apex and from costa up to the apex). Limbidium throuthg the leaf and limbidium only in the vaginant lamina. Smooth and papillose cells. Vaginant laminae> 1/2 of the leaf or being smaller than 1/2 of the leaf. In the lower part, the sexual systems of the studied species are represented, as well as whether the system is co-sexual (reproductive and female structures in the same shoot) or segregated (male and female reproductive structures in separate shoots). The red arrow indicates the direction in which the sexual systems increase the distance between the sexes.

Fig. 2 Non-metric multidimensional scaling (NMDS) plot showing the distribution of *Fissidens* species and their traits in the 'Serra do Mar' Atlantic Forest, Southern Brazil. Stress = 0.0891, indicating a highly reliable representation of the data's dissimilarity.

Fig. 3 Relationship between the relative frequency values of *Fissidens* traits and the altitudinal gradient (A) and mean annual precipitation (MAP; B) in the study area.

TABLES

Table 1. Location and environmental data in the studied plots in ‘Serra do Mar’ biogeographic region, Southern Atlantic Forest.

Table 2. Traits used in the analyses of functional diversity and composition of *Fissidens*, with associated functions.

Table 3. Species of *Fissidens* collected in the Brazilian Atlantic Forest during this study, along with their respective functional traits.

Table 4. Results of the generalized linear regression analysis regarding the effects of mean annual precipitation (MAP) and elevation on *Fissidens* functional diversity indexes. FRic = Functional richness; FDiv = Functional divergence; FEve = Functional evenness (FEve); FDis = Functional dispersion (FDis).

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Chapter 6

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The impact of climate change on the fundamental niche and resilience of moss species with divergent sexual systems

Wagner Luiz dos Santos [1]; Paulo Weslem Portal Gomes[1,*]; Kátia Cavalcanti Pôrto[2]; Juçara Bordin[3]; Fabio Pinheiro[1]

[1] Department of Plant Biology, University of Campinas, Rua Monteiro Lobato., 250, Barão Geraldo, Campinas, SP, Brazil

[2] State University of Rio Grande do Sul, Machado de Assis, 1456, Porto Alegre, RS 95520-000, Brazil

[3] Department of Botany, Federal University of Pernambuco, Moraes Rego Av., S/N

Abstract

Plants showing different sexual systems may explore their habitats in different ways. For instance, dioicous species tend to occupy less seasonal, less stressful environments than monoicous species due to reproductive costs and reproductive assurance. Considering the modelled climate change scenarios, we expect different impacts on plants showing divergent sexual systems. Thus, we investigated the potential geographical distribution of the fundamental niche for four species of *Fissidens* (Bryophyta), assessing the potential impact of current environmental conditions and future climate change scenarios on their niche suitability, considering the influence of their different sexual systems. We are also exploring the importance of conservation units in the availability of the fundamental niche of species. Our hypothesis was that dioicous species would be more vulnerable to climate change than monoicous species. Furthermore, within the monoicous group, we assumed that increased sexual segregation would be associated with greater resistance to climate change. The results of this study partially supported our hypothesis, revealing that the loss of the fundamental niche was more pronounced in dioicous, and in monoicous species with cladautoicous systems, followed by gonioautoicous and rhizautoicous systems. This suggests that sexual segregation plays a crucial role in the resilience of these species to environmental changes. Additionally, we observed a strong association between the fundamental niche of *Fissidens* species and their sexual systems, with significant implications for their survival in the face of climate change. The absence of sexual reproduction in dioecious species emerged as a key factor in their increased vulnerability to climate change. This limits their ability to colonize new areas and reduces genetic diversity, making them more susceptible to extreme weather events and the adaptations required to survive in changing environments. In contrast, monoicous species, especially those with more segregated sexual systems, demonstrated greater resilience to climate change. This can be attributed to their higher dispersal capacity, enhanced genetic plasticity, and resource allocation favoring reproduction in more segregated sexual systems. Overall, this study underscores the importance of sexual reproduction and resource allocation in species' adaptation to climate change. It also highlights the close relationship between sexual systems and species resilience, emphasizing the need to incorporate this variable into conservation and species management studies, particularly in the context of climate change.

Keywords: Climate change; Species resilience; Sexual systems; Bryophytes

Introduction

Alarmingly high rates of environmental changes have been reported recently, and extensive changes in species distribution ranges are therefore predicted globally [1]. Changes in species distribution ranges triggered by environmental change may cause disruptions of ecological and evolutionary mutualistic interactions, which in turn may result in further changes in species distribution or even species loss [2–4]. For instance, when a plant species enters a new ecosystem and establishes itself, it has a high probability of disrupting the ecological balance [4,5]. This can happen through various mechanisms, including competition for resources, exclusion of key species, and initiation of unnecessary ecosystem functions [6]. Many of these factors can be caused by global warming, which has been investigated by several studies using Ecological Niche Models (ENMs). ENMs are empirical or mathematical approximations of the ecological fundamental niche of a species [7]. Based on the occurrence records of the target species associated with the set of climatic variables, the computational algorithms create a multidimensional environmental space that infers fundamental niche parameters and predicts the species distribution [8–10].

Studying plants using ENM offers distinct advantages, as acknowledged in previous research [11,12]. The inherent immobility of plants simplifies data collection, enhancing the precision of distribution information over extended timeframes [13]. Moreover, the considerable distribution range exhibited by many plant species permits a comprehensive understanding of their responses to environmental shifts across diverse habitats and climatic conditions [14]. Indeed, the geographic distribution of plant species with different sexual systems, such as dioecy and monoicy, is strongly influenced by environmental conditions, especially temperature and precipitation [15,16]. This raises the question of how sexual systems will respond to future climate change scenarios [17]. Most studies exploring this subject were performed with flowering plants, and results do not converge to general expectations. While some authors have shown a strong influence of future climate change scenarios in the plant’s geographic displacement according to their sexual system [18,19], other studies found no effect of the reproductive system on niche dynamics [20]. Such lack of agreement may be related to the limited number of studies on groups that may show a higher sensitivity to different climate conditions, such as bryophytes.

Within the domain of embryophytic land plants, bryophytes distinguish themselves by their absence of a lignified vascular system and subsequent poikilohydric physiological disposition [21]. Indeed, these distinctive characteristics confer upon bryophytes a notably heightened sensitivity to environmental variables, endowing them with a status as exemplary model organisms for elucidating ecological changes [21–23]. Another relevant characteristic of bryophytes is found in their sexual systems, which are intrinsically interconnected with the reproduction process, thus playing a crucial role in the conservation of bryophyte populations [24–26]. According to [25], dioicous sexual systems (plant with male and female gametangia in separated plants) can be classified as dioicous *stricto sensu* (when male and female gametangia is produced in different plants), and pseudoautoicous (a condition where a small male plant grows in female plants, being popularly called as dwarf male). Monoicous sexual systems (plants with male and female gametangia in the same plants) have subdivisions of sexual systems that form a gradient of the distance between male and female gametangia [25,27]. Being the closest to the farthest, they are classified into synoicous (male and female gametangia together and mixed); paroicous (male gametangia adjacent to female); gonioautoicous (terminal perichaetia and axillary perichaetia); cladautoicous (separated branch with male and female gametangia); and rhizautoicous (male ramet attached by female ramet by rhizoids) (Fig 1). The terms "dioicous" and "monoicous" are respectively analogous to "dioecious" and "monoecious" referred to angiosperms. This distinction arises from the fact that bryophytes express their sex during the haploid phase [24,28].

These classifications related to the position of male and female gametangia in the gametophyte are strongly associated with reproductive success (sporophyte formation) and reproductive dynamics of bryophytes species [29,30]. For example, dioicous bryophytes species have separate male and female gametangia and tend to have a lower reproductive success rate [28]. This lower fertilization rate in dioicous bryophytes is related to several factors such as reproductive cost, the difference in sexual expression rate, and sex distances [31–34]. On the other hand, the monoicous species have a great fecundation rate, since the male and female sex functions are near each other, and the sexual expression is higher in this sexual function [28]. However, the rhizautoiocous systems can be considerate functionally dioicous, since male and female ramets have it resources allocated in one function, beside present the same pattern of reproductive allocation of dioicous species [26,27,35,36,36].

The sexual systems within bryophytes play a vital role in colony maintenance and are closely linked to broader global implications, as evident from numerous studies establishing correlations between sexual systems and species biology. Among bryophyte families, the unigeneric Fissidentaceae (genus *Fissidens*) stands out for harboring a greater diversity of sexual systems [37]. In the Neotropical species of *Fissidens*, a discernible pattern characterizes the variability of sexual systems. Dioicous systems predominantly inhabit the southern regions of South America and colder, higher-altitude locations. Conversely, monoicous systems are distributed across South America, with a notable escalation in sexual system diversity closer to the Equator. This gradient of bryophyte sexual systems illustrates an increasing distance between sexes (from synoicous to dioicous) moving away from the Equator, while the reverse trend (from dioicous to synoicous) intensifies toward the equatorial regions [37,38].

Thus, in this study we aimed to investigate the possible impact of climate change on the fundamental niche and resilience of *Fissidens* species with different sexual systems, considering the current climate and in alternative future global warming scenarios. Additionally, our aim is to investigate how conservation units influence the formation of species' fundamental niches within the scenarios we are studying. The wide geographic distribution of *Fissidens* in Neotropics, coupled to its extensive diversity of species and sexual systems make the group an ideal model to investigate how climate change may have divergent impacts on plants showing different modes of reproduction. In addition, the genus was recently revised in the Neotropical region [37,39], conferring confidence in species recognition and delimitation. Moreover, compelling evolutionary evidence indicates that within the family, the dioicous sexual system is basal, whereas the monoicous system is considered a derivative form [40,41]. This evidence underscores a pivotal understanding of the sexual systems' evolutionary origins within the family. Our main question is whether species with different sexual systems exhibit divergent patterns of tolerance to projected climate change scenarios. Our hypothesis is that dioicous species are less tolerant to climate change than monoicous species. This is because dioicous species are found in colder environments and never reproduce sexually, which is a limiting factor for population maintenance. On the other hand, we expect that in monoicous species, sexual systems with the sexes closer to the farthest (gonioautoicous to rhizautoicous) would display higher tolerance to the projected climate change scenarios.

Materials and methods

Study area

The study area encompasses the entire South America, stretching from Ecuador to the subantarctic region [42]. This continent constitutes the southern portion of the Americas and is also considered a subcontinent within the broader American continent (Dietz and Holden, 1970). Covering an expansive 17.8 million square kilometers, it accounts for 12% of the Earth's land surface [43]. South America exhibits a remarkable climatic diversity, encompassing tropical, subtropical, and extratropical features [44]. Several biodiversity hotspots are situated within South America, including the Atlantic Forest, the Atacama Desert, Central Chile, the Brazilian Cerrado, the Chocó, the Darién, the Western Ecuador, and the Tropical Andes [45]. This wealth of biodiversity underscores the evident importance of studying this subcontinent.

Selection of species, occurrence records, and data cleaning

To conduct our study, we initially consulted the GBIF (GBIF.org (13 July 2023) GBIF Occurrence Download https://doi.org/10.15468/dl.8fh5s3) and SpeciesLink (https://specieslink.net/) databases to compile a comprehensive list of all *Fissidens* species found in South America. Subsequently, we categorized each species according to its sexual system. Among the various types of sexual systems identified within the *Fissidens* genus, we chose to focus on species exhibiting dioicous, rhizautoicous, cladautoicous, and goniautoicous sexual systems, as the others had limited collection records. From this initial selection, we chose four species from each of these four sexual systems. To make this choice, we considered criteria such as the fundamental niche of the species and the number of occurrences records available for each of them. However, due to the scarcity of records for most species, we opted to select those with the highest number of occurrence records in each category, namely: *Fissidens asplenioides* Hedw. (dioicous), *Fissidens elegans* Brid. (gonioautoicous), *Fissidens radicans* Mont. (cladautoicous), and *Fissidens zollingeri* Mont. (rhizautoicous) (Fig. 1).

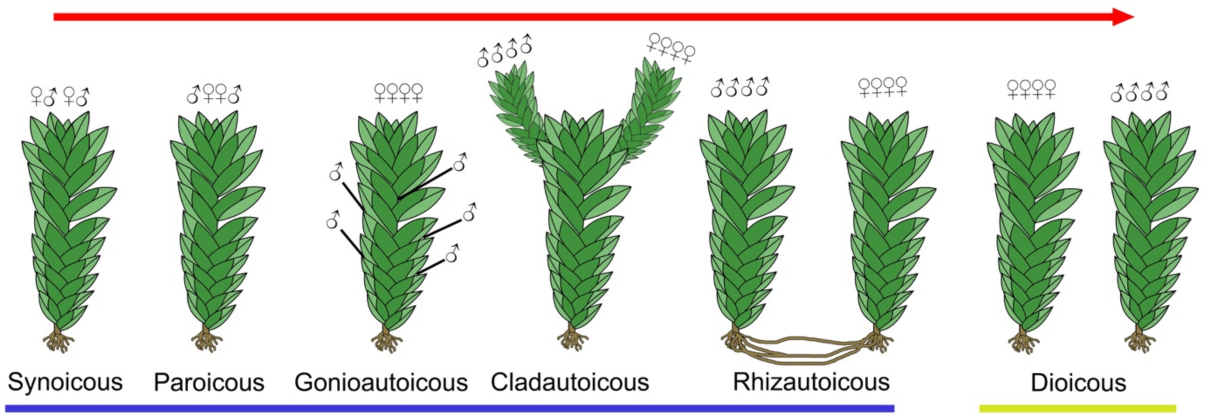

Figure 1. Illustrates the sexual system of the modeled species. The red line indicates the direction of systems that increase the distance between sexes. The blue line represents monoecious sexual systems, while the green line represents dioecious systems.

The correct scientific name, as well as any potential synonyms, present in the occurrence data were validated through consultation with the Flora do Brazil [38], the Taxonomic Name Resolution Service v5.0 (TNRS, https://tnrs.biendata.org/), and the World Flora Online (WFO, https://wfoplantlist.org/plant-list). We employed the R package CoordinateCleaner [46] to identify potentially problematic coordinates and utilized the spThin package[47] to reduce the sampling bias effects within a 5 km radius between each point of occurrence while retaining the maximum amount of useful information, resulting in a total of 460 occurrence records (Fig. 2). Please refer to the occurrence records in (Supplementary Table 1).

Current and future environmental data

We employed a set of 19 bioclimatic variables related to temperature and precipitation as predictors for assessing current and future conditions. These 19 bioclimatic variables were sourced from the WorldClim 2.1 dataset and cut out for South America region, characterized by a 5 arc-minute resolution, which is approximately equivalent to 10 kilometers. These data resources can be accessed at http://www.worldclim.org/ and are comprehensively documented in the study by [48].

In order to mitigate multicollinearity and reduce the dimensionality of predictor variables, a Principal Component Analysis (PCA) was conducted using current environmental variables as part of our methodological approach [49]. This analytical step aimed to prevent model overfitting, thereby ensuring the biological reliability of the resulting predictions [50]. The eigenvectors derived from the PCA were utilized to compute the scores for the first six principal components, which collectively accounted for 96.5% of the total variance [51] (Supplementary Table 2). Subsequently, these newly generated predictor variables, represented by the eigenvectors of the PCA, were employed in the development of Ecological Niche Models (ENMs) for all species. Furthermore, the same eigenvectors were employed to compute the scores of the principal components for future environmental scenarios.

We evaluated the effect of climate change on the distribution of *Fissidens* species using tree Shared Socioeconomic Pathways (SSPs) [52]. The first, SSP126 is the updated scenario of RPC2.6, which takes place in a sustainable world that follows a green path with low greenhouse gas emissions. The second, SSP245 is the updated RCP4.5 scenario that uses a moderate world at an intermediate level of greenhouse gas. Finally, SSP585, an updated scenario derived from RCP8.5, envisions a world characterized by rapid fossil fuel consumption [52], resulting in significantly elevated greenhouse gas emissions [53]. We used projections for 2090 (mean for the period from 2081 to 2100) using the General Circulation Model ISPL-CM6A-LR [52].

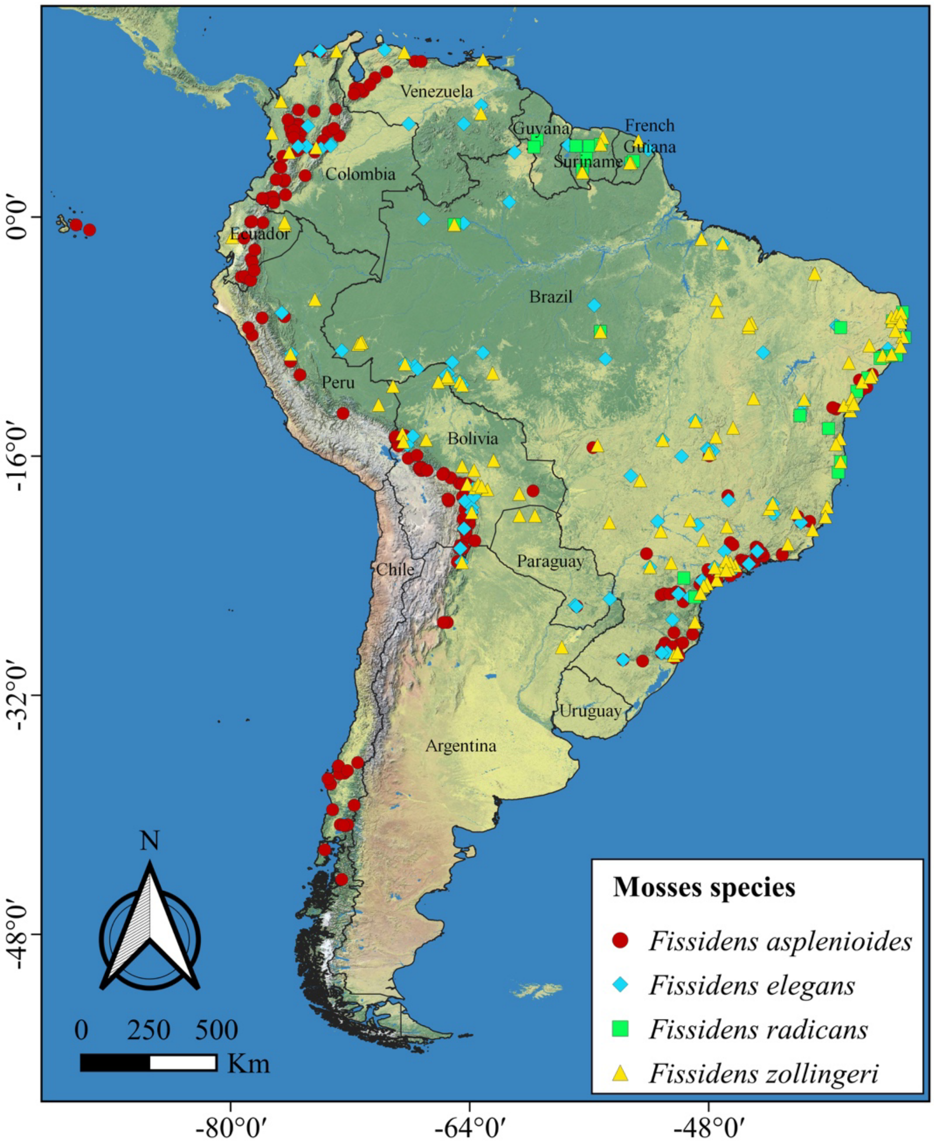

Figure 2. Spatial distribution of taxonomically validated records of *Fissidens* species.

Ecological niche models

We devised a methodology that incorporates three presence-absence modeling algorithms, namely Generalized Additive Models [54], Random Forest [55], and Support Vector Machine [56] (Supplementary Table 3). Numerous correlative methods have been proposed for the construction of ENMs, and their performance can vary depending on the modeling conditions and objectives [57]. Consequently, it is advisable to employ ensemble models that combine multiple algorithms to assess model performance [58]. In this study, we encountered a lack of georeferenced observations of absences. To address this limitation, we artificially generated absence points for each observed presence point of the species within the study area. The use of pseudo-absences serves as a surrogate for the true observation of absences [59].

To construct the models, we employed a methodology that encompasses the following procedures: a) Implemented a geographically structured pattern resembling a chessboard to mitigate potential spatial autocorrelations between the training and testing data, as suggested by [60]; b) Selected pseudo-absences based on the projections of a Bioclimatic model, and c) Established a threshold that optimizes the trade-off between sensitivity and specificity for model binarization, following the methodology proposed by [61]. Regarding the assessment of spatial autocorrelation in environmental variables, we adopted the following strategies: Utilized the training and testing subsets to calculate the Moran's I index, a metric ranging from -1.0 to 1.0, allowing discrimination between negative and positive spatial autocorrelation, as discussed by [56]. Additionally, we employed the Euclidean distance method to visualize distinct geographical areas, with the caveat that this approach assumes sphericity and is better suited for orthogonal covariates, i.e., those in which the covariance between variables approaches zero, as explained by [62].

To assess the performance of the model, we chose not to use common discrimination metrics such as Kappa, AUC and TSS, as these are influenced by the prevalence of species [63–65]. We used the Sørensen similarity index only with models ≥ 0.70 for two reasons. The first, since the lower the similarity value, the greater the number of false positives and false negatives, in relation to the number of true presences. Second, because similarity indices do not include true negatives, they are not influenced by a disproportionate number of true negatives. Thus, we propose to focus on an evaluation metric that maximizes true positives and minimizes both false positives and false negatives in relation to true positives [65].

The ultimate niche modeling for each species was established through an ensemble prediction approach. This ensemble prediction process involved the calculation of the arithmetic mean of suitability predictions generated by the top-performing algorithms for each species, specifically those models surpassing or equaling the average True Skill Statistic (TSS) threshold, as described in studies by [66] and [49]. All model training procedures were conducted utilizing the "ENMTML" package implemented in R software version 4.3.1 [67], following the methodology outlined by [68].

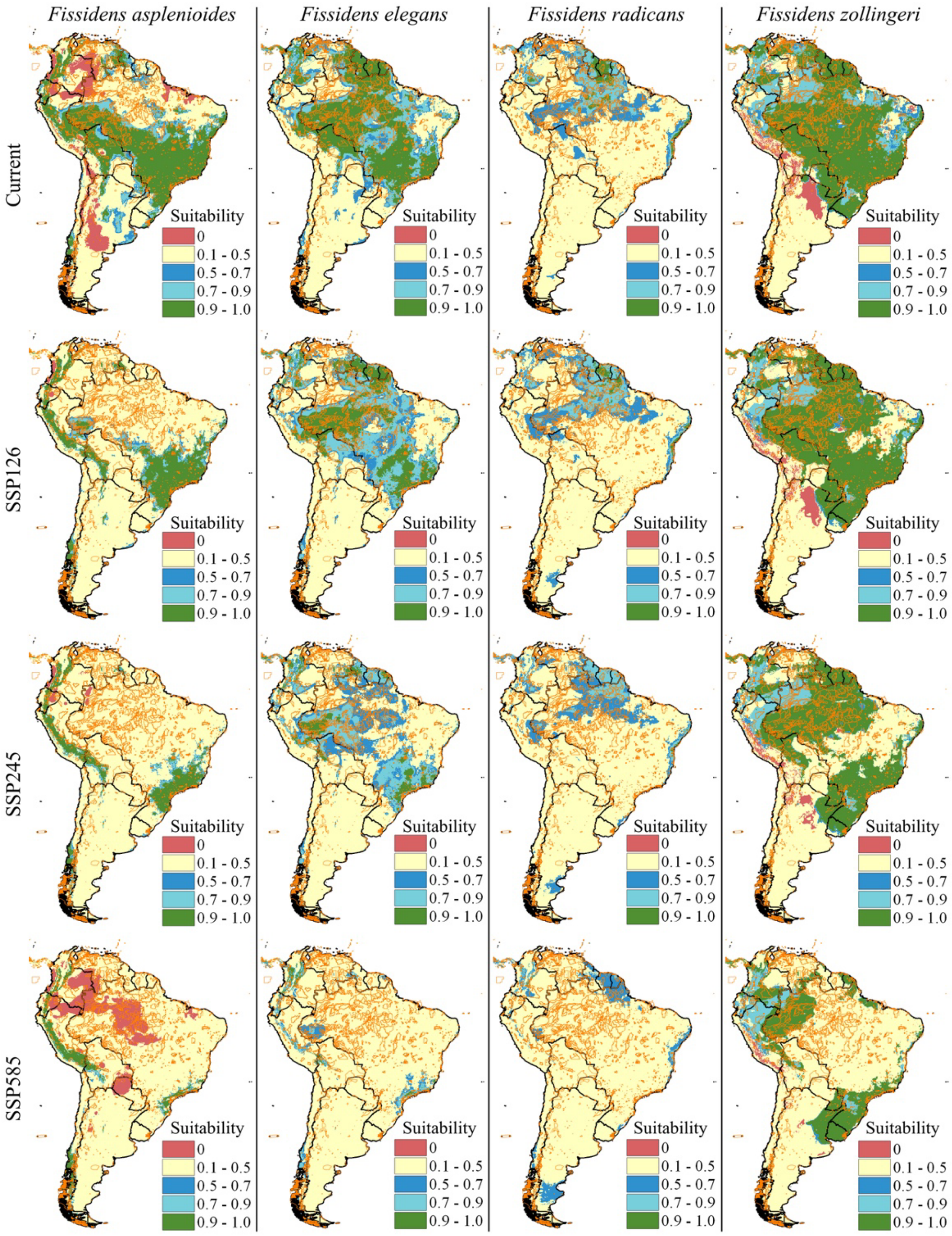

Figure 3. Map of South America showing the climatic suitability for *Fissidens* species in current conditions (top of the figure) and three future climate change scenarios (bottom of the figure). The highlighted orange lines represent the protected areas of South America. The suitability scale represents the value adopted for favorable and unfavorable habitats for the occurrence of species. SSPs are the Shared Socioeconomic Pathways, which are possible scenarios regarding the future emission of greenhouse gases. The SSP126 is an optimistic scenario that would occur in a sustainable world with low greenhouse gas emissions; SSP245 considers an intermediate level of greenhouse gas emissions; and SSP585 is the most pessimistic scenario and assumes that the use of fossil fuels will continue to increase. a – climate scenario of *Fissidens asplenioides* Hedw. (dioicous); b – *Fissidens elegans* Brid. (gonioautoicous), c – *Fissidens radicans* Mont. (cladautoicous), d – *Fissidens zollingeri* Mont. (rhizautoicous).

To partition species habitat based on climatic suitability areas, we considered a threshold cutoff of 0.5 that consistently separates favorable from unfavorable locations, regardless of species prevalence [69]. For unfavorable habitats, we considered two levels: unsuitable habitat (0), low suitable habitat (0.1–0.5). For favorable habitats, we considered three levels: moderate suitable habitat (0.5–0.7), high suitable habitat (0.7–0.9), and very high suitable habitat (0.9–1). Thus, our consensus maps were presented on a gradient spanning both habitats (favorable and unfavorable). To calculate fundamental niche areas in our models, we only considered the favorable habitat. Across all countries in South America, we computed the average potential habitat difference based on grid cells for current conditions and global warming scenarios. All models are available for download in our supplementary data. Therefore, those interested in the results can recalculate the data using the downloadable rasters in our supplementary material, as well as apply new analyses to the generated data.

Contrasting species coverage of ENMs

To contrast the possible potential habitat changes within South American Protected Areas to current conditions and global warming scenarios, we used the World Database on Protected Areas (WDPA), which is globally recognized as the most comprehensive database of terrestrial protected areas (UNEP-WCMC and IUCN, 2023). We overlay the South America shapefile over the Protected Areas shapefile to extract South American Conservation Units (CUs). Subsequently, we overlay the shapefile of Protected Areas on the raster files that represent the fundamental niche model for each species and for each scenario that was evaluated. Finally, we calculated the areas of climate adequacy within the CUs of each South American country using a threshold of 0.5. All of these analyses were conducted using the raster package [48] implemented in R software version 4.3.1 (R Core Team, 2023)[67].

Results

Model performance and current climate

The final models showed a satisfactory performance with Sørensen index values that ranged from 0.72 ± 0.009 to 0.94 ± 0.005 (Supplementary Table 4). Moran's I value ranged from 0.17 to 0.40 (Supplementary Table 5), indicating a low spatial autocorrelation of environmental variables. The Euclidean distance values ranged from 2.95 to 4.24.

All South American countries showed to be areas with potential occurrence for all species. The gonioautoicous (Fig. 3-B) and rhizautoicous (Fig. 3-D) species climate suitability areas occupy more than 50% of the extent of South America, with about 12.6 million km^2 (70%) and 11.4 million km^2 (64%) in extent, respectively. On the other hand, dioicous (Fig. 3-A) and cladautoicous (Fig. 3-C) species presented smaller potential occurrence in an extension of 7.4 million km^2 (41%) and 7.5 million km^2 (42%), respectively (Figure 3).

Climate change scenario simulation

The models showed that the species fundamental niche will be affected by future climatic conditions, even in the most optimistic scenario. Forecasts show that dioicous species will be one of the species most affected by climate change in South America. The model for this sexual system predicts that the species' fundamental niche will be reduced by more than 50% (3.75 million km^2) in the most optimistic scenario of change climate (SSP126). In a more pessimistic scenario (SSP585), dioicous species tends to lose about 84% (6.3 million km^2) of its current range, with total loss of suitability areas in French Guiana, Paraguay, Suriname and Uruguay (Fig. 3-A).

The predictions consider that until the period 2100 in a more pessimistic scenario (SSP585), the gonioautoicous species is a species qualified as Least Concern in the IUCN assessment categories. However, this category can change until the period of 2100 when we consider a pessimistic scenario (SSP585). Forecasts suggest the reduction of areas with potential distribution of gonioautoicous species in about 70% of its current area of extension, with total potential loss of distribution in Paraguay and Uruguay.

The cladautoicous and rhizautoicous species are the only species studied here that, even in the most pessimistic scenarios, continue to present areas with potential fundamental niche in all South American countries. The cladautoicous species had a potential distribution area of 6.5 million km^2 (87% of the current range) in the SSP245 scenario and 4.8 million km^2 (63% of the current range) in the SPP585 scenario. While the rhizautoicous species showed a greater loss of potential distribution, having a reduction in its climatic suitability area from 9.1 million km^2 (79.1% of the current range) in the SSP245 scenario to 4.9 million km^2 (43.1% of the current range) in the SSP585 scenario (Fig. 3-D).

Area of climatic suitability in protected areas

The potentially suitable areas for bryophyte occurrence in South American Conservation Units (CUs) varied among species, countries, and scenarios of climate change. Our analyses revealed that the areas with the highest potential distribution within South American CUs will be reduced in both optimistic and pessimistic scenarios of climate change. Currently, the suitability areas within the CUs represent approximately 11 million km² for dioicous species, 2.4 million km² for gonioautoicous species, 1.5 million km² for cladautoicous species, and 2.8 million km² for dioicous species. In the most optimistic climate change scenario (SSP126), the most affected species will be dioicous species, with a reduction to 610,000 km² of its suitable area (39.03% of the current extent), while rhiazautoicous species was the species with the most conserved potential distribution areas (2.7 million km², i.e., 97.24% of the current extent). In the most pessimistic climate change scenario (SSP585), the models predict a reduction of 87.6% and 86.6% in the potential distribution range for gonioautoicous (Fig. 3-A) and dioicous (Fig. 3-B) species, respectively.

Among individual South American countries, Brazil exhibited the highest average percentages of potential distribution areas among species for current climate conditions (63.5% to 76.5%), owing to its larger territorial extent compared to other South American countries. Dioicous species is likely to face significant conservation challenges by the end of this century since about 63.5% of its entire potential distribution area within CUs occurs in Brazil, and a reduction to 8.9% of the current potential distribution range is predicted in a more pessimistic scenario. These results may represent an irreversible loss for the maintenance and persistence of these species in the ecosystem, leading to the loss of thousands of years of evolutionary history.

Rhizautoicous displayed the highest percentages in the potential distribution range by countries for the current climate scenario, ranging from 0.01% in Uruguay to 67.5% in Brazil. Our assessment revealed that all species did not have potential distribution areas within CUs in at least one country, mainly Chile, Guyana, French Guiana, Uruguay, Paraguay, and Suriname. We observed that cladautoicous did not have potential distribution areas in CUs in Chile, Uruguay, and Paraguay from the current scenario up to the intermediate climate change scenario (SSP245); however, suitable areas shifted into CUs in Chile in the most pessimistic scenario (SSP585) (Fig. 3-C).

Discussion

The relationship between sexual systems and species resilience represents a relevant yet underexplored aspect in the scientific literature, underscoring the importance of considering this variable in conservation and species management studies in the context of climate change [70]. The aim of this study was to explore the current and future fundamental niche and resilience in the distribution of *Fissidens* species, while assessing the anticipated repercussions of climate change on their fundamental niche, taking into account their diverse sexual systems. Our hypothesis posited that dioicous species would experience more pronounced impacts from climate change compared to monoicous species. Furthermore, within the monoicous species group, we assumed that a higher degree of sexual segregation would be associated with greater resilience to climate change. Additionally, when analyzing the different monoicous sexual systems, we observed significant variation in resilience to climate change. Our hypothesis was partially corroborated, and our results indicated that fundamental niche loss was most pronounced in monoicous species with cladautoicous systems, followed by gonioautoicous and rhizautoicous systems. This suggests that sexual segregation plays a crucial role in the adaptive capacity of these species to environmental changes [34]. Moreover, we observed that the fundamental niche of *Fissidens* species is intrinsically associated to sexual systems, with significant implications for their survival in the face of climate change [37,39]. Our data not only inform us about how climate variables influence the geographic distribution of plant sexual systems, but also contain important information about the potential resilience of species with different reproductive strategies in the face of projected climate change scenarios.

Reproductive constraints and fundamental niche limitations in dioicous species

Our results indicate that the dioicous species was more vulnerable to climate change, regardless of the adopted projections, encompassing scenarios both more pessimistic and less pessimistic. Although several factors may contribute to this phenomenon, we believe that reproduction is one of the main factors leading to this outcome [32,71]. Therefore, it is plausible to infer that limited dispersal associated with the absence of sexual reproduction, as reported by [39] in dioicous species, hinders its ability to colonize new areas as comprehensively as species that engage in sexual reproduction [72–74]. Furthermore, the absence of sexual reproduction contributes to lower genetic diversity, which, in turn, negatively impacts the species' resilience in the face of climate change due to the lack of genetic variability [75]. Sexual reproduction is a fundamental strategy for introducing genetic variation into populations, allowing for greater adaptability to constantly evolving environmental pressures [76]. However, the absence of this reproductive mechanism in dioicous species results in lower genetic plasticity, making them more susceptible to extreme climatic events and adaptations required to survive in changing environments.

In this context, it is possible to draw an enlightening parallel with [77] research on bryophytes. Her findings clearly highlighted the evidence of dioicy related to NMDS, with *Metzgeria brasiliensis* Schiffn., a dioicous species, standing out as having the lowest equability compared to other species. Dioicy, a prominent feature in these organisms, tends to restrict sexual reproduction, favoring asexuality as a compensatory mechanism [25,71]. As suggested by [78], in this context, spores play a crucial role in long-distance dispersal, while asexually produced propagules have more limited dispersal over short distancesDioicous bryophyte species likely possess a limited dispersal capacity, reducing their ability to respond to geographic shifts caused by climate change. This could lead to genetic isolation and, ultimately, speciation. [79] study on Epidendrum fulgens Brongn., examining genetic differentiation and reproductive isolation in island organisms, presents a parallel. Their findings suggest gene flow restrictions due to seed dispersal, resulting in a pronounced plastid genetic structure between island and mainland orchid populations. Reduced nuclear genetic diversity and discrepancies in seed viability indicate post-zygotic barriers from low migration and a population bottleneck. This underscores the significance of reproduction and dispersal in species' adaptation to climate change, reinforcing the role of genetics in population differentiation. [19] study delves into ecological distinctions between sexes across multiple species, although the extent of sex-specific responses to climate change remains uncertain. Their extensive study on *Valeriana edulis* Nutt. spanning four decades and an 1800-meter elevation range revealed gender-specific reactions to changing climate conditions. The observed elevation increase correlated with amplified water availability, resulting in a rise in female frequency due to gender-specific adaptations in water usage efficiency and survival mechanisms. Recent aridification notably shifted male frequency upslope at an exceptional rate of 175 meters per decade, surpassing reported species' range shifts. This surge in male frequency alleviated pollen limitation and notably enhanced seedset. These findings, coupled with previous research on sex-specific arthropod communities, highlight the pivotal role of gender-based ecological differences in influencing biological responses to climate change. Furthermore, [18] work emphasizes the looming threat of heightened aridity, particularly due to climate change, on both plants and their associated biotic communities. Dioecious species, with spatial segregation of sexes and distinct physiological and morphological adaptations in separate microhabitats, face increased vulnerability. In species with sexual dimorphism, the potential suppression of trait overexpression by one gender under future climates is anticipated. Data suggests males exhibit lower sensitivity to increased aridity compared to co-occurring females, potentially leading to markedly skewed sex ratios across multiple populations. These imbalanced sex ratios are expected to cascade effects onto dependent community members reliant on specific genders for various ecological functions. This underlines the importance of sex-specific responses to climate change in shaping ecological dynamics within species and their associated communities.

Variability in fundamental niche in monoicous sexual systems under different climate predictions

When considering the monoicous species, a different pattern was observed. Fundamental niche increased from the gonioautoicous to the cladautoicous and rhizautoicous systems. In the rhizautoicous sexual system, the separate production of male and female reproductive structures is a characteristic that may contribute to its success in fundamental niche. [35] raise several advantageous aspects of this system, including the possibility of somatic mutations occurring in the regions where gametangia are produced. When these somatic mutations are differently incorporated into gamete production, male and female gametes become genetically distinct, thereby increasing genetic diversity [35]. This phenomenon confers greater tolerance to climate change, making the rhizautoicous sexual system more plastic and capable of coping with adverse conditions for the species that possess it. Additionally, reproductive allocation, i.e., the proportion of resources allocated to reproduction [80], plays a crucial role in this system. According to [27], there is a gradient of reproductive allocation in sexual systems that increases reproductive allocation in the male sexual function as the distance between the sexes increases (from monoicous to dioicous). Therefore, it is expected that in rhizautoicous and dioicous sexual systems, reproductive allocation in the male function is greater than in the female function. [34]confirmed that this pattern is observed in species studied so far in the rhizautoicous sexual system. Furthermore, [36] observed that this pattern is closely related to the population dynamics of the species *Fissidens flaccidus* Mitt., suggesting success in sporophyte production. These findings underscore the importance of reproductive structure and resource allocation in the success and adaptation of species with a rhizautoicous sexual system, that can be considered a functionally dioicous species [26,27].

Unlike dioicous and rhizautoicous sexual systems, gonioautoicous and cladautoicous systems are cosexual, meaning that both male and female reproductive structures are produced on the same ramets. The results have shown that gonioautoicous and cladautoicous systems exhibit lower fundamental niche among monoicous systems. Reduced reproductive allocation in cosexual monoicous sexual systems can lead to lower spore production, which, in turn, has the potential to decrease their fundamental niche. Furthermore, it is essential to highlight that protogyny (male reproductive organs of an organism mature before the female reproductive organs) is a common occurrence in the gonioautoicous system, as evidenced in various species such as *Forsstroemia trichomitria* (Hedw.) Lindb. By [29], *Trichostomum perligulatum* (Flowers) R.H. Zander by [81], *Syntrichia inermis* (Brid.) Bayrh. by [82], and the autoicous *Octoblepharum albidum* Hedw. by [83]. [30] in recent studies in *Fissidens* species observed that gonioautoicous species exhibited protogyny, while rhizautoicous species displayed protandry, with notable differences in reproductive parameters, such as sexual expression, being more pronounced in gonioautoicous species. Within the context of reproductive asynchrony, when the male reproductive organs mature earlier in relation to egg maturation, a temporal discrepancy arises in the release of antherozoids concerning the availability of eggs. As discussed by [84], this phenomenon leads to a substantial reduction in the likelihood of self-fertilization, concurrently favoring cross-fertilization, thereby promoting the expansion of genetic variability within the population. However, it is of paramount importance to emphasize that both the size and the quantity of produced spores play pivotal roles in the success of species fundamental niche [85]. Although specific data regarding the quantity of spores produced by each studied species are lacking, Caldeira et al. (2013) documented that in these same species, dimensions ranged from 10 to 14 µm for gonioautoicous species, from 8 to 11 µm for cladautoicous species, and from 10 to 12 µm for rhizautoicous species. Hence, it is reasonable to infer that as sexes become more separated within sexual systems, the availability of resources allocated to post-zygotic allocation tends to increase, thereby contributing to the success of fundamental niche in specific contexts.

The impact of climate change on bryophytes, their influence on ecosystems, and the crucial role of conservation parks in preserving these species

It is evident that global changes will have a significant impact on the distribution of the studied bryophyte species, regardless of whether they are located in protected areas or not [87]. However, it is crucial to note that protected areas can act as relatively more resilient refuges compared to unprotected areas, as illustrated in Figure 4. One of the factors that climate change, particularly temperature, has the most profound impact on is the physiology of bryophytes, as evidenced in the case *Bryum argenteum* Hedw. by [88], in *Anthelia juratzkana* and *Polytrichum* *sexangulare* Floercke by [89] and *Bryopteris filicina* (Sw.) Nees, *Leucoloma cruegerianum* (Müll. Hal.) A. Jaeger, and *Phyllogonium viscosum* (P. Beauv.) Mitt. By [90], to obtain additional information on a greater number of species, see in [91]. Therefore, the increase in temperature can result in a decrease in the photosynthetic development of these species, directly impacting their geographical distribution [91]. However, some results show the opposite of what was previously mentioned. For example, in a study conducted by [92], the authors analyzed bryophyte herbarium data in Switzerland, comparing records between 1880-1920 and 1980-2005. They observed a significant increase in the altitude of cryophilous species, indicating their expansion to higher elevations. At lower altitudes, there was evidence of a slow extinction process, possibly related to climate warming. These altitudinal changes reflect similar trends observed in vascular plants in Europe [92], highlighting the utility of herbaria in detecting shifts in species distribution. Therefore, it is evident that environmental changes can expand suitability areas where they did not previously exist and lead to extinction in areas where suitability once existed.

Despite limited research on the role of bryophytes in ecosystem processes, it is crucial to emphasize their significant influence on ecology, as highlighted by [70]. Specifically, studies conducted by [93] underscore the essential contribution of bryophytes to the colonization of epiphytic species in tropical rainforests. These plants serve as adherence substrates for the seeds of epiphytic species, enabling their germination and establishment on host trees. Furthermore, in this same humid ecosystems, bryophytes play a role in moisture maintenance due to their poikilohydry capacity. Conversely, in xeric environments, research by [94] and [95] indicates that bryophytes play a crucial role in the formation of soil crusts, where they establish beneficial associations with cyanobacteria. In this context, bryophytes benefit from the nitrogen released by cyanobacteria, while cyanobacteria benefit from the carbon released by bryophytes, resulting in a cycle of soil fertilization and moisture retention. However, it is imperative to recognize that ongoing climate change has the potential to destabilize these ecological interactions. The exclusion or decline of bryophytes in response to environmental changes can lead to significant ecological imbalances, affecting the availability of fundamental niche for other species and altering the dynamics of plant communities. Therefore, it is essential to maintain constant vigilance over these evolving dynamics as we continue to confront the challenges of environmental change.

Conclusion

Our study investigated the fundamental niche of *Fissidens* species and assessed the projected impact of climate change on their fundamental niche, considering different sexual systems. We found that dioicous species are more vulnerable to climate change than monoicous species. This is likely because dioicous species have a smaller gene pool since its produces spores less than monoicous and are less able to adapt to changing environmental conditions. Our findings also suggest that sexual reproduction and reproductive allocation play a crucial role in species adaptation to climate change. In addition, we observed that the niche fundamental of *Fissidens* species is closely linked to their sexual systems. This has important implications for their survival in the face of climate change. For example, dioicous species that are restricted to specific habitats are at greater risk of extinction if their habitats become unsuitable due to climate change. The relationship between sexual systems and species resilience is an understudied topic, but our findings highlight its importance for conservation and species management in the context of climate change. Climate change is also having a significant impact on the distribution and physiology of bryophytes, including *Fissidens* species. Bryophytes play a vital role in ecosystems, from facilitating the colonization of epiphytic species to maintaining moisture in humid environments and forming soil crusts in dry regions. However, climate change is disrupting these ecological interactions, affecting fundamental niches and altering plant community dynamics. As a result of climate change, the fundamental niche of protected areas is expected to shrink. Therefore, it is recommended to expand conservation units to preserve species. However, climate change is also likely to disrupt ecological interactions within protected areas, further exacerbating the threat to bryophytes. Close monitoring of the evolving dynamics of bryophyte communities is imperative as we confront the challenges of ongoing environmental changes.

Figure 1. Illustrates the sexual system of the modeled species. The red line indicates the direction of systems that increase the distance between sexes. The blue line represents monoecious sexual systems, while the green line represents dioecious systems.

Figure 2. Spatial distribution of taxonomically validated records of *Fissidens* species.

Figure 3. Map of South America showing the climatic suitability for *Fissidens* species in current conditions (top of the figure) and three future climate change scenarios (bottom of the figure). The highlighted orange lines represent the protected areas of South America. The suitability scale represents the value adopted for favorable and unfavorable habitats for the occurrence of species. SSPs are the Shared Socioeconomic Pathways, which are possible

scenarios regarding the future emission of greenhouse gases. The SSP126 is an optimistic scenario that would occur in a sustainable world with low greenhouse gas emissions; SSP245 considers an intermediate level of greenhouse gas emissions; and SSP585 is the most pessimistic scenario and assumes that the use of fossil fuels will continue to increase. a – climate scenario of *Fissidens asplenioides* Hedw. (dioicous); b – *Fissidens elegans* Brid. (gonioautoicous), c – *Fissidens radicans* Mont. (cladautoicous), d – *Fissidens zollingeri* Mont. (rhizautoicous).

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Chapter 7

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A comparative study between cultivated and field plants shows evidence for the shy male hypothesis in tropical genotypes of *Bryum argenteum*

Wagner Luiz dos Santos[a*]; *Kátia Cavalcanti Pôrto*[b]; *Joshua Greenwood*[c]; *Andie Davis*[c], *Fábio Pinheiro*[a], and *Lloyd R. Stark*[c]

[a] *Graduate Program in Ecology, Biology Institute, Department of Plant Biology, Universidade Estadual de Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil;* [b] *Biosciences Center, Department of Botany, Federal University of Pernambuco, Moraes Rego Av., s/n, Recife, PE 50670-901, Brazil;* [c] *School of Life Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, U.S.A.*

*Corresponding author: Wagner Luiz dos Santos, Department of Plant Biology, University of Campinas, Monteiro Lobato - 250, Barão Geraldo, Campinas, São Paulo, Brazil. wag.lsantos1@gmail.com, Phone: +55 35 991470469.

Wagner L. dos Santos: PhD candidate in Ecology Graduate Program at the University of Campinas. https://orcid.org/0000-0002-4434-2542
Fábio Pinheiro: Professor of Plant Biology at University of Campinas. https://orcid.org/0000-0003-3243-2652
Kátia C. Pôrto: Professor of Plant Biology at the University of Pernambuco. https://orcid.org/0000-0002-1861-0216
Andie Davis: undergraduate student of Biology at the University of Nevada Las Vegas.
Joshua Greenwood: Posdoc at University of Nevada Las Vegas
Llo Stark: Professor of Ecology at University of Nevada Las Vegas. https://www.researchgate.net/profile/Lloyd-Stark

ABSTRACT

Introduction

The female-biased sex ratio is recurrently observed in bryophyte populations, although the sex ratio of spores is usually balanced. The results of several studies have suggested explanations for this observation, one of which is the shy male hypothesis. The shy male hypothesis predicts that the sex ratio of populations is balanced in the field, but due to a low or lack of sex expression of male plants, the populations present a female bias when the sex ratio is based on observations of sex-expressing plants.

Methods

We tested the shy male hypothesis using samples of the moss *Bryum argenteum* collected from Brazilian seasonally dry tropical forests and the Brazilian Atlantic Forest. Field sex ratios were determined by classifying shoots as male, female, or non–sex-expressing. Non–sex-expressing shoots were further analysed for sex expression after culture regeneration.

Results and discussion

Our results showed that populations of *Bryum argenteum* in the field had a low rate of sex expression, and that most of the shoots expressed were female. However, when shoots without sex expression were cultured, a greater number of male shoots were observed compared with in the field plants. Because more males appeared in the shoots cultured from non–sex-expressing field plants, our data for tropical *B. argenteum* tend to support the shy male hypothesis. Some genotypes from the wet forest were synoicous (i.e. had male and female gametangia in the same gametoecia), suggesting polyploidy.

Keywords: Dioicy, gametangia production, labile sex, moss, sex ratio, sex expression

Introduction

One of the most intriguing questions regarding bryophyte reproduction is why dioicous populations mostly exhibit sex ratios with a bias towards female function (McLetchie and Puterbaugh 2000; Stark et al. 2001; Bisang and Hedenäs 2005; Stark et al. 2005, 2010; Bisang et al. 2020; Santos et al. 2022a). Therefore, understanding the origin of the sex bias in natural populations is a fundamental challenge for researchers investigating the reproduction and population dynamics of species with dioicous sexual systems (Söderström and Gunnarsson 2003). When sexual reproduction occurs in a population of bryophytes, the sex ratio plays an important role in sporophyte production (Hugonnot et al. 2014; Haig 2016). However, in several dioicous species of bryophytes, sporophytes rarely or never occur (Longton 1992). Thus, the sex ratios in populations of these dioicous species where sporophytes are unknown are frequently female-biased or completely female (Bisang and Hedenäs 2005).

Although the sex ratio of dioicous bryophyte populations is strongly biased towards females, this pattern is inconsistent with the sex ratio of the spores produced, which is expected to be balanced between male and female (Glime and Bisang 2017). This expected sex ratio (1:1) is due to the segregation of chromosomes during meiosis, when spores are formed. At meiosis, each sporogenous division (giving rise to spores) will produce a male spore and a female spore; at least, this is the pattern of the spore sex ratio in the studies reported so far (McLetchie 1992; Stark et al. 2010; Bisang et al. 2017).

Studies of the model moss *Ceratodon purpureus* (Hedw.) Brid. have provided experimental evidence indicating that the female bias in a bryophyte population may arise through processes occurring during the juvenile phases of gametophyte life history. During protonemal and young shoot development and growth, female plants became more common than male plants through sex-specific growth and/or survival differences that allow females to outcompete males for space (Eppley et al. 2018). There is still no consensus on the ultimate evolutionary causes of male rarity among dioicous species and populations. However, the authors of several studies have reported possible reasons for the formation of populations with female bias, for example, differences in branch growth rate, spore abortion, and stress tolerance (Table 1). These and other causes of male rarity are further discussed by Glime and Bisang (2017).

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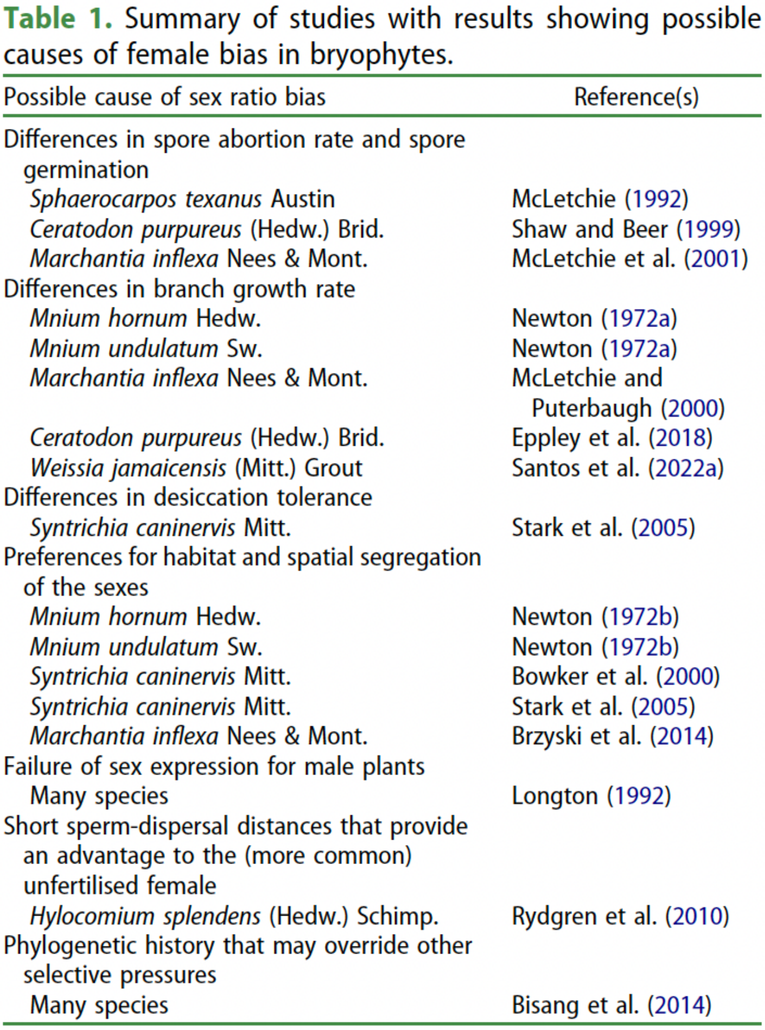


Table 1. Summary of studies with results showing possible causes of female bias in bryophytes.

Possible cause of sex ratio bias	Reference(s)
Differences in spore abortion rate and spore germination	
Sphaerocarpos texanus Austin	McLetchie (1992)
Ceratodon purpureus (Hedw.) Brid.	Shaw and Beer (1999)
Marchantia inflexa Nees & Mont.	McLetchie et al. (2001)
Differences in branch growth rate	
Mnium hornum Hedw.	Newton (1972a)
Mnium undulatum Sw.	Newton (1972a)
Marchantia inflexa Nees & Mont.	McLetchie and Puterbaugh (2000)
Ceratodon purpureus (Hedw.) Brid.	Eppley et al. (2018)
Weissia jamaicensis (Mitt.) Grout	Santos et al. (2022a)
Differences in desiccation tolerance	
Syntrichia caninervis Mitt.	Stark et al. (2005)
Preferences for habitat and spatial segregation of the sexes	
Mnium hornum Hedw.	Newton (1972b)
Mnium undulatum Sw.	Newton (1972b)
Syntrichia caninervis Mitt.	Bowker et al. (2000)
Syntrichia caninervis Mitt.	Stark et al. (2005)
Marchantia inflexa Nees & Mont.	Brzyski et al. (2014)
Failure of sex expression for male plants	
Many species	Longton (1992)
Short sperm-dispersal distances that provide an advantage to the (more common) unfertilised female	
Hylocomium splendens (Hedw.) Schimp.	Rydgren et al. (2010)
Phylogenetic history that may override other selective pressures	
Many species	Bisang et al. (2014)

The failure of sex expression in male plants relative to female plants of the same species or population is termed the *shy male hypothesis* and predicts a balanced genotypic sex ratio in the face of unbalanced phenotypic field sex ratios. However, this hypothesis was not confirmed when tested in several species (Hedenäs et al. 2010, Stark et al. 2010, Bisang and Hedenäs 2013, Ekwealor et al. 2022). The results of such studies implicate potential intersexual competition in mixed-sex populations prior to sex expression, consistent with the findings for *Ceratodon purpureus* (Eppley et al. 2018). The shy male hypothesis predicts that the sex ratio of dioicous populations is balanced, but due to the lack of male sex expression, the populations appear female-biased.

Stark et al. (2010), using the moss *Bryum argenteum* Hedw., tested the shy male hypothesis along with the hypothesis that spore sex ratios are unbiased. Their results did not support the shy male hypothesis, because the sex ratio of cultured plants presented the same sex ratio as that of the adult mixed-sex populations, a result that contrasted with the sex ratio of the spores produced, which was 1:1. The shy male hypothesis was also investigated by Bisang and Hedenäs (2013), using *Drepanocladus lycopodioides* (Brid.) Warnst. In that study, they investigated molecular markers in plants expressing and not expressing sex. Their results refuted the shy male hypothesis because the sex ratio was the same regardless of whether the quantification was performed using plants expressing sex or using all plants (i.e. those expressing and not expressing sex). In another study, the same authors found that the sex ratio of the spores of *D. lycopodioides*, like those of *B. argenteum* (Stark et al. 2010), was balanced (Bisang et al. 2017). Molecular analyses have been used to quantify the sex ratio in dioicous mosses, in *D. trifarius* (F.Weber & D.Mohr) Broth. (Hedenäs et al. 2010) and *Syntrichia caninervis* Mitt. (Ekwealor et al. 2022). In both species, the sex ratio was female-biased in both sex-expressing plants and non–sex-expressing plants, but in the latter species, the sex bias was significantly more female in plants expressing a sex.

There is a lack of support in the literature for the shy male hypothesis, and few studies have been carried out to explore it. Against this background, our objective in the present study was to test the shy male hypothesis by investigating, in plants collected in the field and plants cultured from the non–sex-expressing shoots, the sex ratio and sex expression in populations of *Bryum argenteum* in two tropical forests (dry and wet) with divergent characteristics. The forests where the plants were collected have very different climatic characteristics, mainly regarding precipitation, a factor that governs the reproduction of tropical bryophytes. Thus, the dry- and wet-forest *B. argenteum* genotypes studied are adapted to different climatic conditions.

For each forest type we used two conditions relating to sex expression of the shoots (expressing sex and not expressing sex), and two treatments (field-collected plants and plants cultured from non–sex-expressing shoots, hereafter referred to as field plants and cultured plants, respectively). To determine whether the shy male hypothesis is valid in tropical populations of *Bryum argenteum*, we addressed two questions. First, does sex expression differ between dry- and wet-forest plants in the different treatments? In the field plants, we expected sex expression to be higher in populations from dry forests than in those from wet forests, because a greater number of sporophytes were observed in dry-forest populations (in the wet forest, no sporophytes were observed). By contrast, we did not expect any difference in sex expression in the plants cultured from dry- and wet-forest *B. argenteum*, because the conditions of temperature, humidity and lighting were the same. Second, do sex ratio and sex bias differ between treatments and forest types? We expected the sex ratio to be female-biased in field plants from both forests but balanced in the cultured plants.

Material and methods

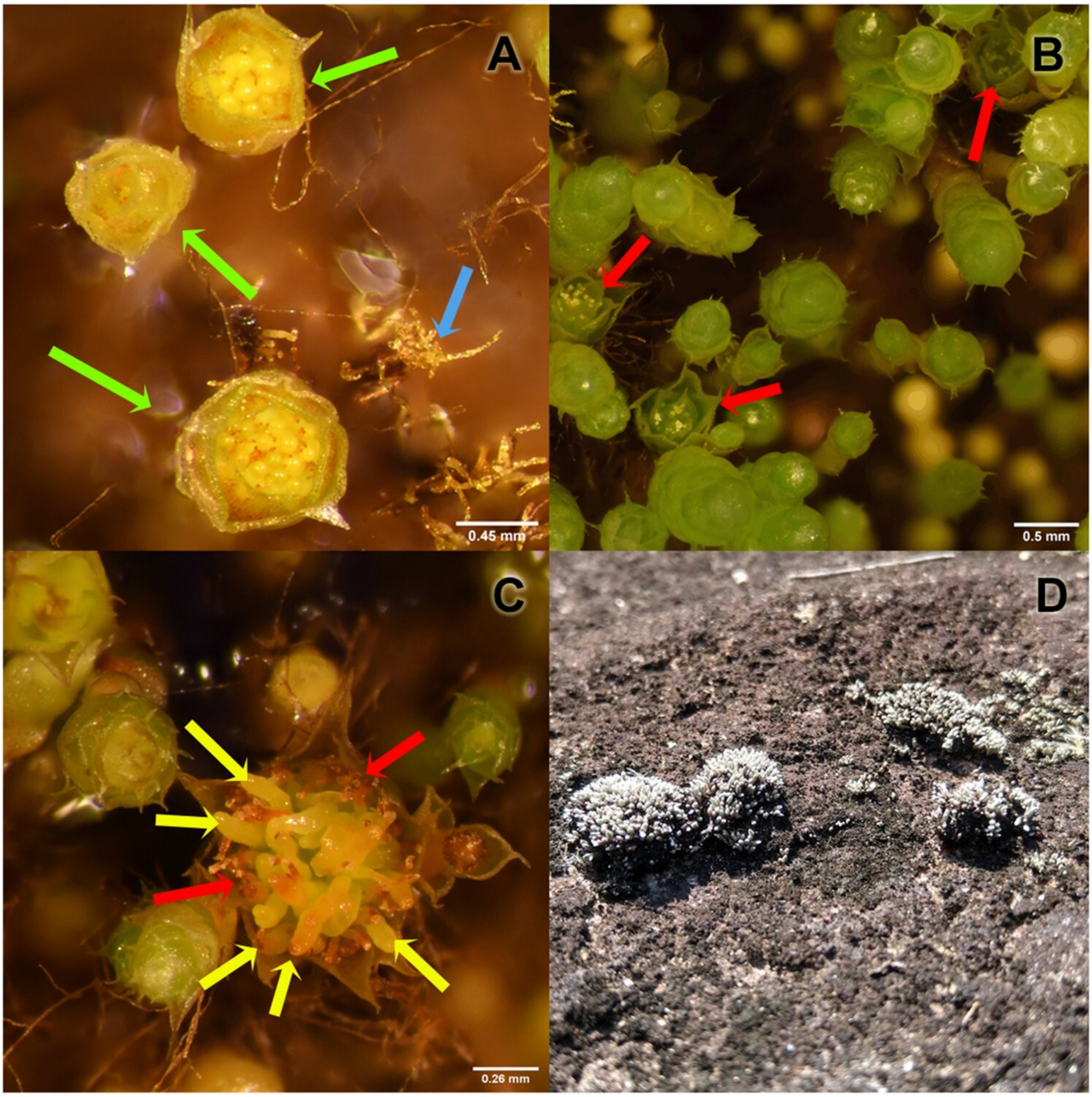
Species

Bryum argenteum, popularly known as silver moss or silver thread moss, is the most common species of moss in the Bryaceae family (Shaw and Albright 1990). The species is cosmopolitan, being found in a diversity of terrestrial ecosystems (Longton 1981; Pôrto et al. 2017; Gabriel et al. 2019; Yuqing et al. 2021). It is named for its silver coloration when dry, which is due to the hyaline appearance resulting from the absence of chloroplasts in the upper region of the leaves (Beever 1992).

Bryum argenteum has a dioicous sexual system: the male and female gametangia are produced on different plants (Moore et al. 2016). Perigonia (male inflorescences) are found at the tip of the male ramet, and perichaetia (female inflorescences) are mainly at the base of the female ramet (Horsley et al. 2011) (Figure 1A, B). Bulbils are also commonly found on shoots of *B. argenteum*, and asexual reproduction plays an important role in stabilising populations in the field (Glime and Bisang 2017).

The abundance of populations and easy culturing of *Bryum argenteum* have favoured its use as a model species in various studies aiming to, for example, elucidate aspects of reproductive biology (Moore et al. 2016; Pôrto et al. 2017; Castetter et al. 2019), desiccation tolerance (Cleavitt 2002), and high temperature tolerance (Stark et al. 2009).

Figure 1. (A–C) Cultured shoots of *Bryum argenteum*. (A) Male shoots with perigonia (green arrows) and protonema gemma (blue arrow). (B) Female shoots with perichaetia (red arrows). (C) Close-up image of synoicous inflorescence (archegonia at red arrows, antheridia at yellow arrows). (D) *Bryum argenteum* in its natural habitat in wet forest. Photographs: Wagner Luiz dos Santos.



Study area and sampling

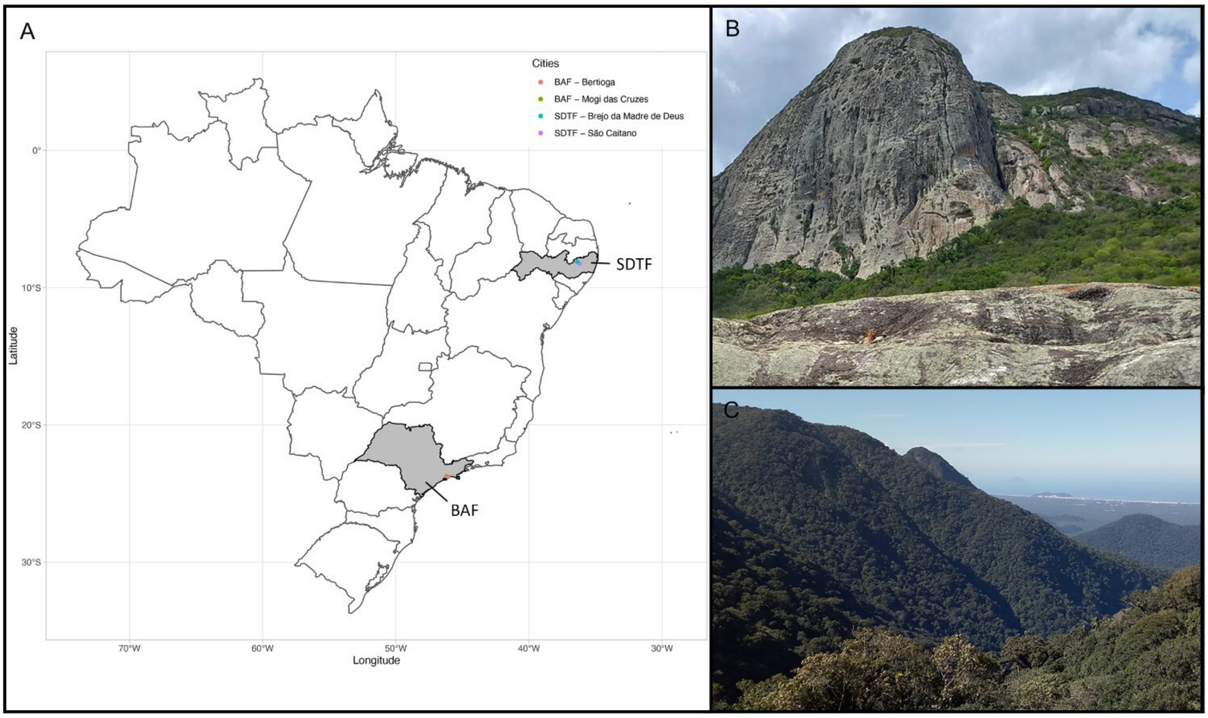
Samples of *Bryum argenteum* were collected from populations in two tropical phytogeographical domains with divergent characteristics: dry and wet tropical forests (Figure 2). The first phytogeographical domain was the Brazilian Seasonal Dry Forest (Caatinga), which represents an exclusively Brazilian ecosystem characterised by vegetation adapted to xeric conditions (Souza et al. 2015). In the Caatinga, the average annual precipitation is 300–1000 mm, being concentrated in the months of May to August, and the average temperature varies between 25 and 30°C (Silva et al. 2018; Queiroz et al. 2018). The populations studied were collected in the Reserva Particular do Patrimônio Natural (RPPN) Pedra do Cachorro, located in the municipality of São Caetano (8°14′12.8″S, 36°11′33.2″W), and in the RPPN Brejo da Made de Deus in the municipality of Brejo da Madre de Deus (8°08′40.2″S, 36°21′42.9″W). Both RPPNs are in the state of Pernambuco, Brazil. The second phytogeographical domain from which samples were collected, representing the wet forest, was the Brazilian Atlantic Forest (BAF). The BAF is considered the richest Brazilian phytogeographical domain in terms of number of bryophyte species (Costa and Peralta 2015). Characterised by dense forests with high humidity, the BAF is found along the entire coast from Northeast to South Brazil (Franke et al. 2005). The collections were made in Parque das Neblinas, located in the cities of Mogi das Cruzes and Bertioga, both in the state of São Paulo, Brazil (Onofre et al. 2010). In Parque das Neblinas, the average annual precipitation is 2400 mm, distributed fairly evenly throughout the year, and the temperature varies from 16 to 18°C (Onofre et al. 2010).

Expeditions were carried out between February and March 2022. When populations of *Bryum argenteum* were found, samples of approximately 80 cm^2 were collected with the aid of a pocketknife into Petri dishes. The different number of samples collected in the different forests was due to the rarity of *B. argenteum* in the wet forest. Samples were collected at a minimum distance of 1 m from each other, and each sample was considered one population. The samples were kept in paper bags and later identified in the laboratory and georeferenced using a GPS (model 64S, Garmin, Olathe, KS, USA). The presence of sporophytes and the type of habitat collected (rock or soil) were also recorded.

Sex ratios in field plants

For each field sample, the sex ratio was determined as follows. First, the sample was placed in the space between two 20 cm rulers positioned perpendicularly at the end of a tray, these acting as two axes (*x* and *y*) to select the shoots to be studied. Samples were placed on the tray such that they occupied a square or rectangular area. Because the samples varied in size, the size of the area occupied by the samples also varied. For each sample, we noted the total extent on the *x*- and *y*-axes.

Figure 2. (A) Map of Brazil showing sites where *Bryum argenteum* was collected in seasonally dry tropical forests (SDTFs) and the Brazilian Atlantic Forest (BAF). (B) View of the Reserva Particular do Patrimônio Natural Pedra do Cachorro. (C) View of Parque das Neblinas (BAF). Photographs: Wagner Luiz dos Santos.



Microsoft Excel was then used to generate 25 random numbers for each axis, in each case within the range of variation represented by the size of the sample. Thus, 25 individual shoots were randomly collected.

In the laboratory, each shoot was classified according to its sex: male (shoots with perigonia), female (shoots with perichaetia or sporophytes), or non–sex-expressing (shoots without sex expression). The sex ratio (ratio of male to female branches) was then quantified using the formula MM+F□□+□, where *M* = number of male shoots and *F* = number of female shoots. Sex bias was quantified by the chi-squared test (Bisang and Hedenäs 2005), and sex expression was determined as the proportion of shoots expressing a sex.

Sex ratio in cultured non–sex-expressing shoots

To quantify the sex ratio of non–sex-expressing shoots, 12 non–sex-expressing shoots were randomly selected for each sample, following a similar method to that described above (i.e. using rulers and randomly generated numbers). Each shoot tip, consisting of the shoot apex of about four to six leaves, was removed, and with the aid of needles and fine-tipped forceps, was cleaned in distilled water. Each tip was then washed for 5 s in a 2% conventional disinfectant solution (bleach).

For culture regeneration, the shoot tips were placed in separate wells in a 48-hole well plate containing sand previously sieved into a 345 μm^2 mesh and autoclaved at 121°C for 60 min. The contents of each well were hydrated with 3 drops of sterile distilled water. Subsequently, the well plates were placed in a Percival growth chamber (model E30B; Percival, Boone, IA, USA) under conditions of temperature, 22°C; relative humidity, 60–70%; and constant light, 100–410 $\mu mol\ m^{-2}\ s^{-1}$. The well plates were hydrated, alternating weekly between water and 30% Hoagland's solution for 3 months, and rotated weekly among growth chamber shelves to equalise photosynthetically active radiation among treatments. After 12 weeks, the cultured plant in each well was assessed for sex expression and classified into male, female, or non–sex-expressing. Sex ratio and sex expression were then quantified, and sex bias was determined.

Statistical analyses

To compare sex expression and sex ratio between samples from different forests (wet and dry) and from different treatments (field plants and cultured plants), we used the generalized linear model (GLM). First, to compare sex expression, we created a full model and a null model. In the full model, sex expression was used as a response variable and the following as predictor variables: treatments (i.e. field plants and cultured plants) and forest types (i.e. wet and dry) ($n = 4$). Both models used the binomial and logit functions. After creating the models, the null and full models were compared via the chi-squared test.

When the test showed a significant difference, the null model was excluded and the data were analysed using the full model. Further residual analysis of the error distribution revealed under-dispersion, and therefore we used a GLM with a quasibinomial distribution to fit the error distribution. After correcting the distribution of errors, the treatments were compared using Tukey's test.

Regarding sex ratio, the GLM model used did not present a good fit for the analysis. Therefore, we used the Kruskal–Wallis test to compare the sex ratio between forest types and between treatments, with the value of the sex ratio per sample being the continuous variable, and forest type and treatment being categorical variables. Dunn's test was used for multiple comparisons, and p values were adjusted with the Bonferroni correction. The Kruskal–Wallis test was also applied to compare the number of shoots that expressed male sex between treatments and between forest types. Dunn's test was used for multiple comparisons, and *p* values were adjusted with the Bonferroni correction.

All analyses were performed using the software RStudio (RStudio Team 2021). The treatments were compared using the MultComp package (Hothorn et al. 2016) and Dunn's test (Dinno and Dinno 2017). The graphics were generated using the ggplot packet (Wickham et al. 2016).

Results

Samples were collected from 52 populations: 9 in wet forest and 43 in dry forest (Table 2). Data on the presence of sporophytes and the type of habitat collected (rock or soil) are shown in Supplemental Table 1.

The results from the GLM model showed that sex expression varies between treatments and not between genotypes (i.e. those of dry- and wet-forest plants) ($df = 3$, Dev = 64.50, $p < 0.001$). The mean sex expression of field plants from dry and wet forest was 14.88% and 11.00%, respectively (Figure 3A). Cultured plants from dry and wet forests had sex expression of 92.60% and 91.40%, respectively (Figure 3B).

Regarding sex ratio, non-significant differences were found between treatments and between forest types ($\chi^2 = 22.87$, $df = 15$, $p = 0.09$) (see Figure 3B). Thus, both treatments and genotypes presented a population mostly biased towards females (see Table 2). However, the results indicate that the number of males expressing sex per population varies with treatment ($\chi^2 = 18.15$, $df = 6$, $p < 0.01$). Thus, the cultured plants, compared with the field plants, expressed a greater number of male plants (see Table 2).

In the wet-forest genotypes, seven individuals presented gametoecia with a synoicous sexual system (male and female gametangia mixed in the same gametoecium). Synoicous individuals (Figure 1C) were found in three populations, within which the other individuals that expressed sex formed only female structures. No synoicy was observed in plants from genotypes of the dry forest.

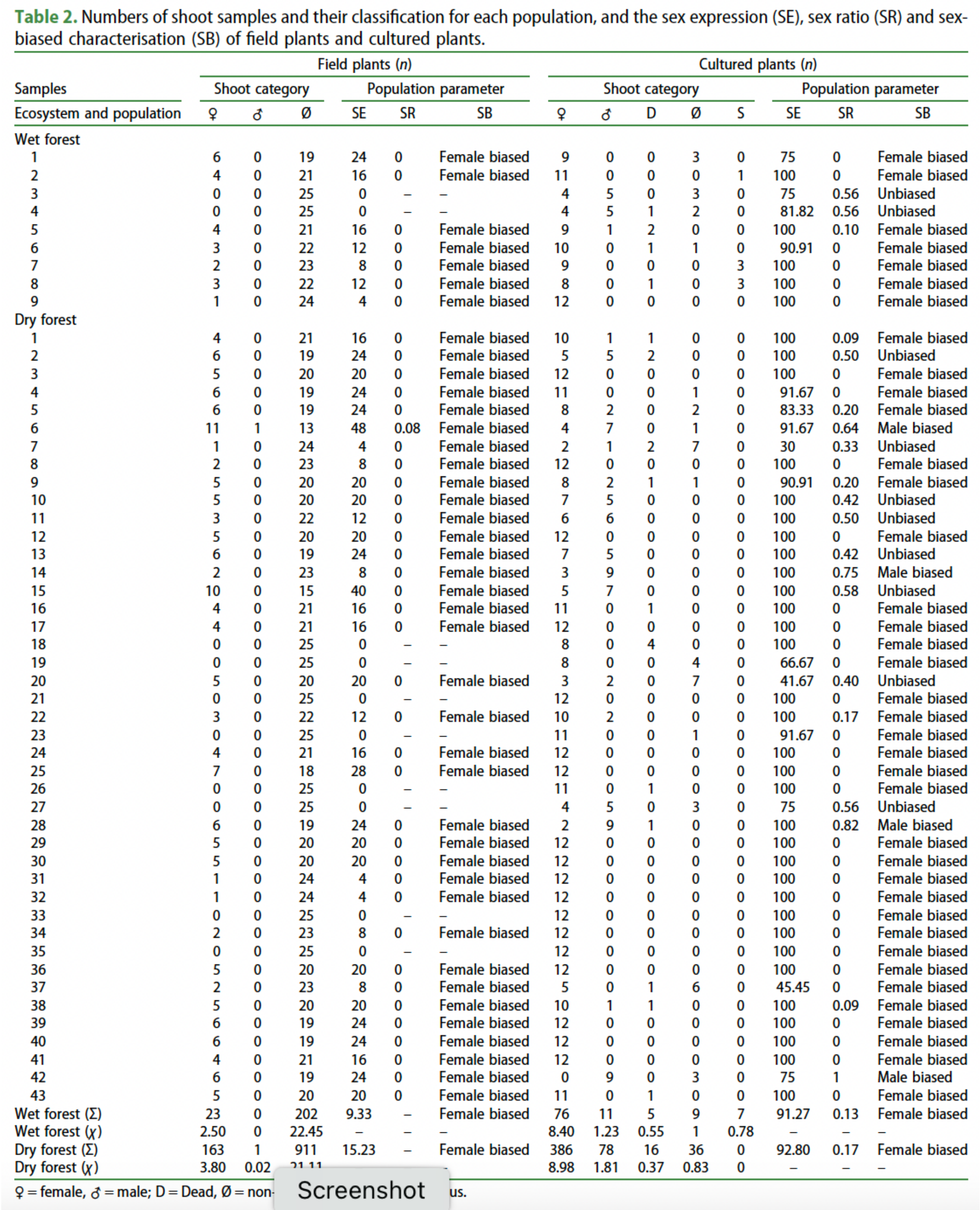
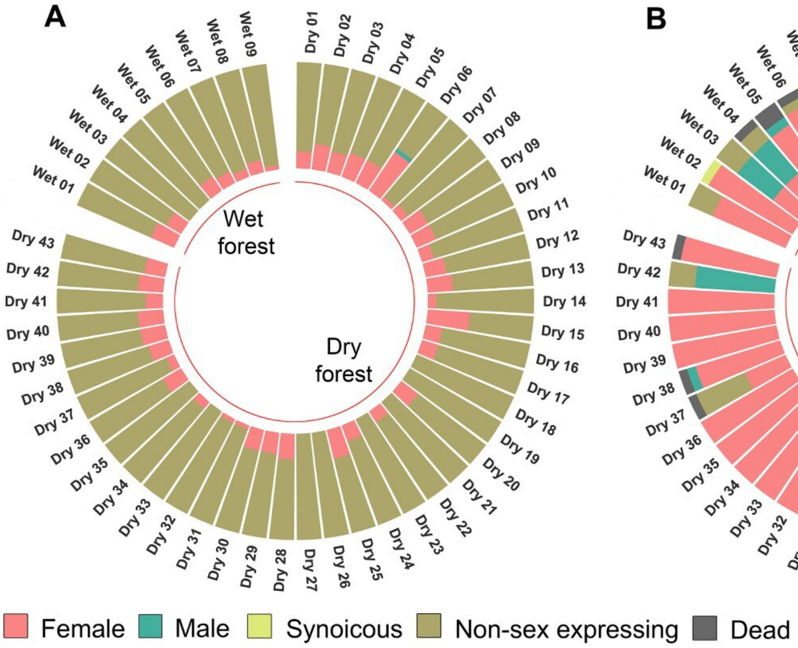


Table 2. Numbers of shoot samples and their classification for each population, and the sex expression (SE), sex ratio (SR) and sex-biased characterisation (SB) of field plants and cultured plants.

	Field plants (*n*)						Cultured plants (*n*)							
Samples	Shoot category			Population parameter			Shoot category					Population parameter		
Ecosystem and population	♀	♂	Ø	SE	SR	SB	♀	♂	D	Ø	S	SE	SR	SB
Wet forest														
1	6	0	19	24	0	Female biased	9	0	0	3	0	75	0	Female biased
2	4	0	21	16	0	Female biased	11	0	0	0	1	100	0	Female biased
3	0	0	25	0	–	–	4	5	0	3	0	75	0.56	Unbiased
4	0	0	25	0	–	–	4	5	1	2	0	81.82	0.56	Unbiased
5	4	0	21	16	0	Female biased	9	1	2	0	0	100	0.10	Female biased
6	3	0	22	12	0	Female biased	10	0	1	1	0	90.91	0	Female biased
7	2	0	23	8	0	Female biased	9	0	0	0	3	100	0	Female biased
8	3	0	22	12	0	Female biased	8	0	1	0	3	100	0	Female biased
9	1	0	24	4	0	Female biased	12	0	0	0	0	100	0	Female biased
Dry forest														
1	4	0	21	16	0	Female biased	10	1	1	0	0	100	0.09	Female biased
2	6	0	19	24	0	Female biased	5	5	2	0	0	100	0.50	Unbiased
3	5	0	20	20	0	Female biased	12	0	0	0	0	100	0	Female biased
4	6	0	19	24	0	Female biased	11	0	0	1	0	91.67	0	Female biased
5	6	0	19	24	0	Female biased	8	2	0	2	0	83.33	0.20	Female biased
6	11	1	13	48	0.08	Female biased	4	7	0	1	0	91.67	0.64	Male biased
7	1	0	24	4	0	Female biased	2	1	2	7	0	30	0.33	Unbiased
8	2	0	23	8	0	Female biased	12	0	0	0	0	100	0	Female biased
9	5	0	20	20	0	Female biased	8	2	1	1	0	90.91	0.20	Female biased
10	5	0	20	20	0	Female biased	7	5	0	0	0	100	0.42	Unbiased
11	3	0	22	12	0	Female biased	6	6	0	0	0	100	0.50	Unbiased
12	5	0	20	20	0	Female biased	12	0	0	0	0	100	0	Female biased
13	6	0	19	24	0	Female biased	7	5	0	0	0	100	0.42	Unbiased
14	2	0	23	8	0	Female biased	3	9	0	0	0	100	0.75	Male biased
15	10	0	15	40	0	Female biased	5	7	0	0	0	100	0.58	Unbiased
16	4	0	21	16	0	Female biased	11	0	1	0	0	100	0	Female biased
17	4	0	21	16	0	Female biased	12	0	0	0	0	100	0	Female biased
18	0	0	25	0	–	–	8	0	4	0	0	100	0	Female biased
19	0	0	25	0	–	–	8	0	0	4	0	66.67	0	Female biased
20	5	0	20	20	0	Female biased	3	2	0	7	0	41.67	0.40	Unbiased
21	0	0	25	0	–	–	12	0	0	0	0	100	0	Female biased
22	3	0	22	12	0	Female biased	10	2	0	0	0	100	0.17	Female biased
23	0	0	25	0	–	–	11	0	0	1	0	91.67	0	Female biased
24	4	0	21	16	0	Female biased	12	0	0	0	0	100	0	Female biased
25	7	0	18	28	0	Female biased	12	0	0	0	0	100	0	Female biased
26	0	0	25	0	–	–	11	0	1	0	0	100	0	Female biased
27	0	0	25	0	–	–	4	5	0	3	0	75	0.56	Unbiased
28	6	0	19	24	0	Female biased	2	9	1	0	0	100	0.82	Male biased
29	5	0	20	20	0	Female biased	12	0	0	0	0	100	0	Female biased
30	5	0	20	20	0	Female biased	12	0	0	0	0	100	0	Female biased
31	1	0	24	4	0	Female biased	12	0	0	0	0	100	0	Female biased
32	1	0	24	4	0	Female biased	12	0	0	0	0	100	0	Female biased
33	0	0	25	0	–	–	12	0	0	0	0	100	0	Female biased
34	2	0	23	8	0	Female biased	12	0	0	0	0	100	0	Female biased
35	0	0	25	0	–	–	12	0	0	0	0	100	0	Female biased
36	5	0	20	20	0	Female biased	12	0	0	0	0	100	0	Female biased
37	2	0	23	8	0	Female biased	5	0	1	6	0	45.45	0	Female biased
38	5	0	20	20	0	Female biased	10	1	1	0	0	100	0.09	Female biased
39	6	0	19	24	0	Female biased	12	0	0	0	0	100	0	Female biased
40	6	0	19	24	0	Female biased	12	0	0	0	0	100	0	Female biased
41	4	0	21	16	0	Female biased	12	0	0	0	0	100	0	Female biased
42	6	0	19	24	0	Female biased	0	9	0	3	0	75	1	Male biased
43	5	0	20	20	0	Female biased	11	0	1	0	0	100	0	Female biased
Wet forest (Σ)	23	0	202	9.33	–	Female biased	76	11	5	9	7	91.27	0.13	Female biased
Wet forest (χ)	2.50	0	22.45	–	–	–	8.40	1.23	0.55	1	0.78	–	–	–
Dry forest (Σ)	163	1	911	15.23	–	Female biased	386	78	16	36	0	92.80	0.17	Female biased
Dry forest (χ)	3.80	0.02	21.11			–	8.98	1.81	0.37	0.83	0	–	–	–

♀ = female, ♂ = male; D = Dead, Ø = non- us.

Figure 3. (A) Field sex expression in *Bryum argenteum* populations from the wet forests and dry forests of Brazil. (B) Sex expression in laboratory cultures from each population assessed: female (shoots with perichaetia), male (shoots with perigonia), synoicous (shoots with antheridia and archegonia in same gametoecia), non–sex-expressing (shoots without sex expression), and dead (shoots did not regenerate).



Discussion

In the present study, sex expression was found to differ in plants of *Bryum argenteum* grown under different conditions: those cultured in the laboratory under controlled conditions (in terms of temperature, luminosity, and available nutrients) had higher average sex expression than those growing in the field. This result is consistent with the findings of Castetter et al. (2019) for the same species; in their study, plants cultured in the laboratory had a higher rate of sex expression than those in an urban population. Together, these results indicate that sex expression in *B. argenteum* populations may be related to environmental conditions.

In the tropics, sex expression is strongly related to the phenology of the species. Several studies on phenology have shown that in the tropics the main factor stimulating sexual reproduction, which includes sex expression, is the rainy season (Fotoba 1998; Maciel-Silva and de Oliveira 2016; dos Santos et al. 2020). Our collections were made at the end of the rainy season in the wet forest and at the beginning of the rainy season in the dry forest. Therefore, although we expected plants from the wet forest to have a higher fertilisation rate than those from the dry forest, we found the opposite to be the case: populations of the Caatinga had a high fertilisation rate compared with wet-forest plants. This finding suggests that plants differ in their reproductive traits according to environmental and geographical distance (because the genotypes in the present study were geographically distant).

Despite the large number of populations with female bias in both forests studied, some populations of the dry-forest genotype had female plants with sporophytes whereas those of the wet-forest genotype did not. This result indicates that there are male plants expressing sex in dry-forest populations, and that this factor can be associated with environmental conditions. Differences in sex ratio related to environmental conditions have been rarely reported but exist in a few species. For example, female bias is more pronounced in populations of the moss *Pseudoscleropodium purum* (Hedw.) M.Fleisch. growing in warmer and wetter environments (Boquete et al. 2016, 2022). Additionally, populations of another moss, *Drepanocladus lycopodioides* (Brid.) Warnst., are female-biased in some regions of Sweden but to varying degrees depending on environmental conditions (Bisang et al. 2020). Finally, in an urban US *Bryum argenteum* population, colonies (transects) exposed to sunlight presented a female-biased sex ratio, whereas those in the shade presented a balanced sex ratio (Castetter et al. 2019). These results indicate that environmental variables can influence the sex ratio of populations. Therefore, the low rate of sex expression in field populations may be the result of stressful environmental factors that reduce the rate of male sex expression, which thus directly influences the sex ratio of populations.

Our results did not fully confirm the shy male hypothesis, because the sex ratio showed a female bias after the induction of sex expression in plants originally without sex expression. However, we observed that the number of non–sex-expressing shoots that later developed as males was significantly higher than in male plants that had already expressed their sex in the field. Some studies in which the shy male hypothesis was investigated yielded results refuting the hypothesis (Hedenäs et al. 2010; Stark et al. 2010; Bisang and Hedenäs 2013), whereas the results of others provided evidence supporting, but not confirming, it (Ekwealor et al. 2017, 2022). Of the former group of studies, that by Stark et al. (2010) found sex ratio with female bias in *Bryum argenteum* plants both expressing and not expressing sex. Studies using molecular markers found that in the mosses *Pseudocalliergon trifarium* (F.Weber & D.Mohr)

Loeske (Hedenäs et al. 2010) and *Drepanocladus lycopodioides* (Bisang and Hedenäs 2013), the sex ratio did not differ between sex-expressing and non–sex-expressing plants. By contrast, in *Syntrichia caninervis*, Ekwealor et al. (2017) found populations at high altitudes that were phenotypically strongly female-biased (17:1), while at low altitudes they did not express sex. In the same study, shoots without sex expression, investigated using molecular markers, a female-biased sex ratio (2:1) was found in high-altitude populations, while in low-altitude populations, only female plants were observed. The same pattern was observed in *S. caninervis* in a later study by Ekwealor et al. (2022); however, in that one, they found that, compared with female plants, male plants were found in more shaded places. Thus, our results are similar to the findings for *S. caninervis* and reinforce the idea that although the sex ratio is not balanced when shoots without sex expression are quantified, males fail in sex expression, that is, they are 'shy' males. Hence, it is evident that certain factors influence the sex ratio of populations from the moment of spore release until population stabilisation, because although the production of spores generates a balanced sex ratio, adult populations have a female bias. This is consistent with the results of Eppley et al. (2018) for *Ceratodon purpureus*.

Because some growth rates vary between the sexes in *Bryum argenteum* (Horsley et al. 2011), intersexual differences in competitive ability may underlie the greater number of female shoots. For example, McLetchie (1992) showed that the sex ratio of hepatic *Sphaerocarpus texanus* Austin is female-biased because the number of male spores aborted is recurrently higher in this species. In *Marchantia inflexa* Nees & Mont., McLetchie and Puterbaugh (2000) showed that female plants grow faster and stronger than males, showing that females are more competitive and that this consequently influences the sex ratio. In *B. argenteum*, Horsley et al. (2011) compared the development of male and female plants and reported that protonema growth does not differ between the sexes, but that intersexual differences exist in terms of number of ramets produced (greater in females), time for inflorescence production (greater in females), and number of inflorescences produced (greater in males), and that these variables also differ between genotypes from different states of the USA. We can observe that in addition to the possible greater competitiveness of females, geographical variation may influence reproductive traits and sex ratio, because plants of the same species, collected from geographically distant places and cultured under the same conditions, differ in their development. This may help explain our results, because the studied genotypes (wet and dry forest) are in geographically distant locations with very different climatic conditions affecting the biology of these populations.

Some individuals from the wet-forest populations of *Bryum argenteum* presented as synoicous, indicating instability regarding the sexual systems in these populations. This result may suggest possible polyploidy for the species, although this is rarely observed in bryophytes (Glime and Bisang 2017). According to Korpelainen (1998), environmental variables such as light, temperature and low moisture level favour male sex expression. Therefore, the very different environmental factors of wet and dry forests may influence sex expression in this species. However, male induction and female sex expression are governed by different factors in different bryophytes. For example, in some monoicous bryophyte species, temporal variation in sex expression is observed (Korpelainen 1998). In *B. argenteum*, Bhatla and Chopra (1981) found that phytohormones are related to the development of the gametangia; thus, the hormones auxin and gibberellin induce the formation of perichaetia in female plants, while a cytokine induces the formation of perigonia in male plants. However, in the monoicous species *Tetraphis pellucida* Hedw., population density induces female branches to produce perigonia (Kimmerer 1991), and in the rhizautoicous moss *Fissidens flaccidus* Mitt., high population density induces sex expression (Santos et al. 2022b). There remains no consensus regarding factors responsible for lability of sex expression in bryophyte shoots.

It is generally evident that the sex ratio is not a trivial topic, and that more studies are needed to understand the reasons why populations present sexual bias. We have shown that male plants fail in terms of sex expression in their natural environment, but that when cultured under controlled conditions have a high rate of sex expression. Additionally, our results show that the species studied presents a different pattern between tropical populations and populations in the northern hemisphere. However, to understand the reasons for female bias in *Bryum argenteum* populations, more studies are needed to investigate possible differences in development and stress tolerance in different stages of the life history of these plants in relation to sex, for example protonema and juvenile shoot growth dynamics (Eppley et al. 2018).

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental material

Supplemental material for this article can be accessed here: https://doi.org/10.1080/03736687.2023.2251776.

Supplemental Table 1. Summary of data for samples collected from 52 populations of *Bryum argenteum* in dry and wet forest in Brazil.

Additional information

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Notes on contributors

Wagner Luiz dos Santos

Wagner Luiz dos Santos is a Ph.D. candidate enrolled in the Ecology Graduate Programme at the University of Campinas, Brazil.

Kátia Cavalcanti Pôrto

Kátia Cavalcanti Pôrto is Professor of Plant Biology at the University of Pernambuco, Brazil.

Joshua Greenwood

Joshua Greenwood is a postdoctoral researcher at the University of Nevada, Las Vegas, USA.

Andie Davis

Andie Davis is an undergraduate student of biology at the University of Nevada, Las Vegas, USA.

Fábio Pinheiro

Fábio Pinheiro is Professor of Plant Biology at the University of Campinas.

Lloyd R. Stark

Lloyd R. Stark is Professor of Ecology at the University of Nevada, Las Vegas, USA (https://www.researchgate.net/profile/Lloyd-Stark).

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Chapter 8

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Phenological development and reproductive traits diverge in the silver moss *Bryum argenteum* genotypes of dry and wet tropical forests

Wagner Luiz dos Santos[a*]; Andrea Medina-Ramos[b]; Joshua Greenwood[b]; Kátia C. Pôrto[c]; Fábio Pinheiro[a]; Lloyd R. Stark[c]

[a]*Graduate Program in Ecology, Biology Institute, Department of Plant Biology, Universidade Estadual de Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil.*

[b] *School of Life Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, U.S.A.*

[c]*Biosciences Center, Department of Botany, Universidade Federal de Pernambuco, Moraes Rego Av., s/n, Recife, PE 50670-901, Brazil.*

[*]Correspondent author: wag.lsantos1@gmail.com

ABSTRACT

Sex-related disparities in development and reproduction are anticipated in plants. Nevertheless, limited knowledge exists regarding the influence of environmental factors on plant development and reproduction. We assessed phenological development in *Bryum argenteum* moss genotypes from two contrasting tropical ecosystems: the Brazilian Atlantic Forest (BAF) with low seasonality and high precipitation, and the Seasonal Dry Tropical Forest (SDTF) with high seasonality and low precipitation. Ten populations (5 from each ecosystem) were sampled, with a male and female shoot selected from each. These shoots were clonally propagated, and the process was repeated to eliminate environmental effects. Ultimately, 100 clones were established for analysis. Notably, male plants from the BAF displayed a reduced regeneration rate compared to other genotypes, illustrating a trade-off between sexual and asexual reproduction (bulbil production). Additionally, BAF plants exhibited delayed sexual expression initiation in contrast to SDTF genotypes. This study demonstrates the profound impact of environmental conditions on the life history traits of *B. argenteum*, with contrasting patterns of regeneration and reproduction in the same species from different ecosystems. Furthermore, our findings suggest that these genotypes adapt to their respective environments, displaying developmental traits that enhance their reproductive fitness in each ecosystem.

Key Words: Adaptation; asexual reproduction; bryophyte ecology; gametophyte development; sex-specific fitness; geographic distance effect; plant reproduction; protonema; regeneration; trade-off.

1 - INTRODUCTION

Reproduction is one of the most important traits in the life history of plants. Through reproduction, plants guarantee the maintenance of populations (Stearns, 1976, 1989; Oli & Coulson, 2016). Commonly, plants reproduce sexually when the male gamete fertilizes the female gamete, but asexual reproduction may also take place when clones are formed by vegetative propagules produced by the plant (Bengtsson & Ceplitis, 2000; Yang & Kim, 2016; Cronk, 2022). Furthermore, the regeneration process of vegetative propagules is critical in the life cycle of plants, as they are generally more robust and develop faster than the propagules provided by sexual reproduction as spores and seedlings (Harper & Ogden, 1970). Thus, vegetative propagules are expected to colonize a given area faster than a propagule arising from the sexual reproduction (Frey & Kürschner, 2011).

The production of vegetative propagules may differ between sexes in dioecious plant species (Douglas, 1981; Laaka-Lindberg, 2001; Van Drunen & Dorken, 2012). This difference is caused by the reproductive cost associated with each sex (Obeso, 2002). The reproductive cost arises due to limited resources available for other functions as a result of the substantial resource allocation in sexual reproduction (Kimmerer, 1991a,b; Fuselier & Mcletchie, 2002; Obeso, 2002). Thus, as the sexes have different patterns of reproductive allocation, it consequently generates different effects on the other reproductive traits of plants (Ashman, 1994; Bazzaz, Ackerly, & Reekie, 2005; Bisang, Ehrlén, & Hedenäs, 2006). Regarding the reproductive mode of plants, some studies have shown resource competition between sexual and asexual reproduction, and this competition between reproductive modes varies according to sex (Kimmerer, 1991b; Laaka-Lindberg, 2001; Fuselier & Mcletchie, 2002; Van Drunen & Dorken, 2012).

The differences between the sexes in the rate of reproduction, production of vegetative propagules, and phenological development can strongly affect the population's parameters, such as: sex expression, sex ratio, and population dynamics (Horsley, Stark, & McLetchie, 2011; Eppley *et al.*, 2018). For example, species expressing only one sex may have the advantage of allocating more resources to the production of vegetative propagules, increasing the chance of biasing the sex ratio towards that specific sex (McLetchie, 1992; McLetchie & Puterbaugh, 2000). Some studies have already shown that differences in development and reproduction can lead to changes in population structure, from sex distribution to genetic structure (Wang, Zhu, & Wang, 2012; Bona, Kulesza, & Jadwiszczak, 2019; Yin *et al.*, 2019). Furthermore, this difference between the sexes may vary on the micro and macro geographic scale, characterizing populations according to the environment (Bowker *et al.*, 2000; Cascante *et al.*, 2002; Castetter *et al.*, 2019; Yin *et al.*, 2019).

Geographic distance plays a significant role in influencing the phenology and reproductive biology of species, as it tends to increase the divergence of microhabitats (Leimu & Fischer, 2008; Gerst, Angert, & Venable, 2011; David, Seabloom, & May, 2016; Bisang, Ehrlén, & Hedenäs, 2020; Boquete *et al.*, 2023). When comparing two geographically distinct areas, factors such as latitude, altitude, climate, and environmental conditions can vary considerably (Maciel-Silva, Valio, & Rydin, 2012; Boquete *et al.*, 2023). These environmental variations can result in distinct phenological schedules, encompassing the stages of flowering, fruiting, and leaf fall (Satake, Nagahama, & Sasaki, 2022). Moreover, such environmental differences can also influence demographic aspects of populations, such as sex ratios, unveiling the remarkable phenological and biological diversity that unfolds across different regions (Satake *et al.*, 2022; Boquete *et al.*, 2023). Thus, geographic distance can influence plant phenology, leading to temporal variations in growth and reproductive phases across different areas. For example, in species where there are differences between sexes, such as in the case of the *Syntrichia caninervis* Mitt., male and female plants express their sexes under different environmental conditions (Bowker *et al.*, 2000). Therefore, understanding the relationship between the environment and the organism's biology is crucial to understanding how these organisms adapt to different conditions (Leimu & Fischer, 2008).

Compared to animals, plants are favorable organisms to use as models to understand the effect of contrasting environmental conditions on the organism's biology. Due to their sessile nature, plants provide greater ease of handling during experiments compared to animals (Szczepaniak & Biziuk, 2003). Among land plants, the bryophytes, a plant group whose monophyly is still a subject of debate (Harris *et al.*, 2020) that comprises the mosses, liverworts, and hornworts, have been considered more favorable than tracheophytes plants to be used as an ecological model. This is because bryophytes are morphologically simple compared to angiosperms, they are small in size, and exhibit a high rate of regeneration (McLetchie, 1992; Bisang & Ehrlén, 2002; Moore, Kollar, & McLetchie, 2016; Eppley *et al.*, 2018). Bryophytes exhibit reproductive structures in the haploid phase, occurring in both monoicous form (with male and female structures on the same plant) and dioicous form (with male and female structures on separate plants), similar to the nomenclature found in flowering plants: monoecious and dioecious respectively (Glime & Bisang, 2017). Therefore, studies that compare the development between different genotypes and sex are convenient when using bryophytes (Müller *et al.*, 2016). In addition, by presenting a simple morphological structure, the plants are more sensitive to environmental variables, and thus, organism responses are detected (Oliver, Velten, & Wood, 2000).

Species showing broad geographic ranges offer the opportunity to test the occurrence of heterogeneous plant reproductive responses to environmental variation (McLetchie & Puterbaugh, 2000; Pannell *et al.*, 2014; Bisang *et al.*, 2020). For instance, the silver moss, the *Bryum argenteum* Hedw. is a common species in the moss family Bryaceae (Shaw & Albright, 1990). It is cosmopolitan and is found on all terrestrial continents and in the most diverse ecosystems (Longton, 1981; Pôrto *et al.*, 2017; Gabriel *et al.*, 2019; Yuqing *et al.*, 2021). Furthermore, *B. argenteum* exhibits a dioicous sexual system, which facilitates the comparison of differences between the sexes. Therefore, in this study, we used *B. argenteum* to investigate phenotypic differences associated with sex and environmental factors. We conducted a study to investigate the phenological development, including shoot tip regeneration, as well as reproductive traits such as sex expression and gametangia maturation time in *B. argenteum* genotypes. These genotypes were collected from two tropical forests with distinct environmental characteristics. The first forest is the Brazilian Atlantic Forest (BAF), known for its low seasonality climate and high levels of rainfall. The second forest is the Seasonal Dry Tropical Forest (SDTF), characterized by a highly seasonal environment with low levels of rainfall (Alvares *et al.*, 2013). In this study, we investigated the following questions: [1] Does the rate of protonema growth and shoot production differ between the sexes and the different genotypes occurring in contrasting environments?; [2] Do study genotypes present differences in sex expression?; [3] Is there a trade-off between sexual reproduction (gametangia production) and asexual reproduction (bulbil production)?; [4] Does the development of gametangia differ between the sexes and genotypes studied?

2 - MATERIAL AND METHODS

Studied species

Bryum argenteum is characterized by the silver color when dry, caused by the absence of chloroplasts in the leaves upper region, making them hyaline (Beever, 1992). This species has sex-specific plants thus making it a dioicous system. Thus, male and female gametangia production in this species occurs in different plants (Liang, Sun, & Zhu, 2010). Perigonia (male gametangia) are found at the tip of the male ramet, and perichaetia (female gametangia) at the base of the female ramet, mostly (Liang *et al.*, 2010). Bulbil (asexual reproduction) is also commonly found in shoots. In Brazil, *B. argenteum* is found in all ecosystems, from the driest and hottest to the wettest and coldest (Costa & Peralta, 2015). *Bryum argenteum,* has a long history in terms of biology, related to the reproductive biology (Chopra & Rawat, 1977; Horsley *et al.*, 2011; Pôrto *et al.*, 2017), tolerance to desiccation (Proctor *et al.*, 2007; Stark *et al.*, 2016; Yuqing *et al.*, 2021), and high-temperature (Zhuo *et al.*, 2020). This is because the species is favorable to the stabilization and growth of colonies in the laboratory, favoring the performance of experiments.

Study site, sample, and growth

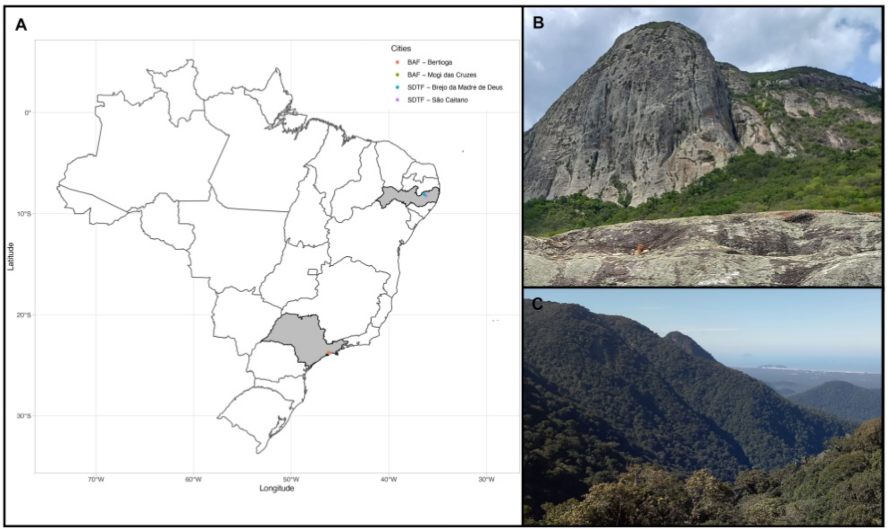

Fig 1 – A – Brazilian map with points collected in SDTF and BAF. B – View of Private Natural Heritage Reserve Pedra do Cachorro and C – View of Parque das Neblinas (BAF).

In this study, we collected populations of *B. argenteum* in two Neotropical biomes with divergent environmental characteristics. The first biome is a Seasonal Dry Tropical Forest (SDTF), also known as Caatinga (Fig. 1), which represents an exclusively Brazilian ecosystem characterized by vegetation adapted to xeric conditions (Souza, Artigas, & Lima, 2015). In the SDTF, the average annual precipitation is 300-1000 mm per year concentrated over a period of 3-5 months, and the average temperature varies between 25 and 30°C (de Queiroz *et al.*, 2018). Populations were sampled at Reserva Particular do Patrimônio Natural (RPPN) Pedra do Cachorro, located in the municipality of São Caitano (8°14’12.8” S 36°11’33.2” W), and at RPPN Brejo da Madre de Deus in the city of Brejo da Madre de Deus (8°08’40.2” S 36°21’42.9” W). Both RPPNs collected are in the state of Pernambuco – Brazil. The second biome collected, was the Brazilian Atlantic Forest (BAF). The BAF is considered the richest Brazilian biome in the number of species (Costa & Peralta, 2015). Characterized by dense forests with high humidity, the BAF is found along the entire coast from northeast to south of Brazil (Franke *et al.*, 2005). The collections were carried out in Parque das Neblinas, located in the cities of Mogi das Cruzes and Bertioga (23°44'13.0"S 46°10'37.7"W) (Fig. 1), both in the state of São Paulo – Brazil (Onofre, Engel, & Cassola, 2010). As for the precipitation in Parque das Neblinas, the average annual rainfall is 2400 mm all year, and the temperature varies from 16 to 18°C (Onofre et al., 2010).

Colonies of approximately 100 cm^2 were sampled from each population. The populations were collected with a minimum distance of 10 meters separation. This distance was established based on the observation that several sporophytic colonies containing male individuals did not influence the fertilization of female plants in colonies expressing sexual characteristics when they were located at distances less than 10 meters. Therefore, we assumed that this distance would ensure the presence of individuals with distinct genotypes. The samples were preserved in paper bags and kept at room temperature (appx 20°C). Species identity was confirmed following Canestraro and Peralta (2022). After specimens' confirmation, ramets that expressed sex (*i.e.,* ramets that had perigonia and perichaetium) were identified and separated. Thus, one male and female genotype was selected for each population and acuminated separately to be clonally propagated. Then, all the material used for the cultivation was prepared. The sand was collected in Las Vegas – NV, USA, near Red Rock Canyon National Conservation Area, sieved through a 350μm mesh, and autoclaved for 60 minutes at 121°C. As well as the sand, all materials used for the cultivation were autoclaved. After autoclaving, approximately 3 scoops of sand were placed in 20 Petri dishes of 35 mm diameter. Then they were hydrated with about 12 – 15 drops of water.

After the Petri dish preparation, the separated shoots were prepared for cultivation. First, with a stereomicroscope and fine-tipped tweezers, the ramet was analyzed and cleaned in water on a slide, removing all dirt and soil residue. In each new washing of the ramet, the tweezers were cleaned with 70% alcohol. Once cleaned, the tip was separated from the ramet and cleaned again in water. Subsequently, the tip was washed for 5 seconds in a 2% sodium hypochlorite solution (conventional bleach). After five seconds, the tip was placed in the center of the Petri dish previously prepared with sand and water. Each Petri dish was identified with adhesive tape at the bottom and a permanent marker at the top. Once the Petri dishes were all composed of the tips, they were placed in a germination chamber at a temperature of 20°C and constant light with an intensity of 100 to 410 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic active radiation. The Petri dishes were hydrated with water twice a week, and samples were changed in position in the growth chamber.

After two months of culture, when all dishes had developed ramets, each genotype was clonally propagated five times (In other words, each genotype was replicated five times). Thus, totaling 100 Petri dishes, 25 for each sex (male and female) of each genotype (SDTF and BAF). All tips placed to regenerate were washed for 5 seconds in a 2% sodium hypochlorite solution. All dishes were placed in a germination chamber at a temperature of 20°C and constant light with an intensity of 100 to 410 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic active radiation. Each Petri dish was hydrated twice a week, and once a week, each plate was photographed to follow the regeneration development. In the first month, the dishes were hydrated with distillate water. After the first month, hydration was alternated weekly between distilled water and 30% Hoagland solution.

Phenological observations

Protonema and shoot production

With photos taken weekly, the area occupied by the protonema and the number of ramets produced was quantified for 8 weeks. After 8 weeks, quantifying all ramets became problematic, as the larger shoots covered the smaller ones and thus made exact quantification impossible. Therefore, we quantified shoot production only until week eight. After week 8, only the shoots that expressed sex were quantified. For the growth of the protonema, in the first two weeks, pictures were taken under a stereomicroscope at 40x magnification. From the third week onwards, the photos were taken at 6.3x magnification, as the area produced by the protonema exceeded the visual limit of 40x magnification. The photos were captured with a Nikon DSLR camera, model D780, attached to a Leica trinocular stereomicroscope.

Furthermore, with the magnification of 0.63x, it was possible to take a picture of the entire area of the Petri dish. The ImageJ software (Rueden *et al.*, 2017) was used to quantify the area occupied by the protonema and the production of ramets. Photos of scales in mm were taken at each magnification photographed, which was later used for software calibration to calculate the area of protonema produced. Using the ImageJ software's Freehand selection tool, after the scale calibration, the area occupied by the protonema in the Petri dish was delimited, followed by the quantification of the protonema area. Finally, the day the protonema began to grow for each Petri dish was noted.

We used the ImageJ software particle analyzer tool to quantify the production of ramets. The photos were edited, and image parameters such as contrast, lighting, and shading were adjusted to leave the ramets with a more distinct color from the soil and protonema. These images were transformed into 8 bits and adjusted to black and white so that the ramets were highlighted, forming black points. With the black points (apex of each ramet), they were analyzed and quantified by the particle analysis tool. For each Petri dish, the day the shoots began to be produced was noted.

Growth rate of protonema and shoot

The rate of protonema growth and the shoot production were calculated for each dish, following the logistic curve equation $Nt = \frac{K}{1+\left(\frac{K-N0}{N0}\right)e^{-rt}}$ where: N(t) represents the quantity of shoots and the area of the protonema at time t, while N_0 represents the quantity of shoots and the area of the protonema at the initial time t_0. K is the carrying capacity, and r is the growth rate. The logistic curve equation was calculated using the Growthcurver package (Sprouffske & Wagner, 2016). From the various indices resulting from the logistic curve equation, we selected the following for analysis: the carrying capacity parameter (K) represents the maximum number of individuals of a species that the environment can support and sustain. It defines the upper limit that the population can reach before resource limitations or other factors impede further growth (Sprouffske & Wagner, 2016). The growth rate (r) refers to the rate at which a quantity or population increases over time (Sprouffske & Wagner, 2016). It quantifies the speed or intensity of growth, indicating how quickly the population expands or the quantity accumulates (Sprouffske & Wagner, 2016). Time med (T-med) represents the time at which the inflection point of the logistic curve occurs. This point is reached when the population load reaches half of the carrying capacity. It is a significant moment in the growth pattern, marking the transition from rapid growth to slower growth as the population approaches its maximum limit. These parameters, including the carrying capacity (K), growth rate (r), and T-med, were analyzed individually for each plate.

Sex expression

Weekly, sex expression was quantified in absolute terms (number of shoots that expressed sex per dish) and relative (proportion of shoots that expressed sex per dish). Relative sex expression was quantified up to the eighth week since, after that time, it was not possible to quantify all shoots without sex expression due to population density. At the end of the observation, we quantified the time for the beginning of protonema development, shoot production, and sex expression for each dish separately. The absolute sex expression was quantified until week 10.

Sexual reproduction versus *bulbil production*

Since the number of bulbils varied between genotypes, we investigated possible trade-offs between sexual and asexual reproduction. For this, we quantified the bulbils produced for each dish and the number of shoots that expressed the sex. Only the bulbils that were detached from the shoots were quantified. To quantify the bulbils, we hydrated the plates and then quantified them. This was done because, when hydrated, the bulbils float, facilitating their quantification. The bulbils were quantified in week 8, as week 8 was the week in which it was possible to quantify all the shoots (expressed and unexpressed), making it possible to investigate a potential trade-off between sexual reproduction and bulbil production.

Gametangia development

Regarding the development of gametangia, we classified and quantified the phenophases of gametoecia in three phases, namely: [1] – immature: when the gametangia are intact and green, that is, closed apex; [2] – mature: when the male gametangia are yellowish and/or not releasing sperm and the female gametangia with one or more open and receptive apex; [3] post-mature – when the gametangia are brownish and withered (**Fig. 2**). Weekly, for 10 weeks, the number of shoots that expressed sex and their respective phenophases were quantified, to monitor the development time of reproductive structures.

Statistical analysis

Protonema growth and shoot production

To compare the growth parameters (K, r, and T-med) of the protonema and shoot production, we used Generalized Linear Models (GLM). First, we created full models with the response variable being the growth indices (K, r, and T-med) and the type of shoot as the predictor variable for protonema growth rate and shoot production. Models were created separately for each response variable. Once the response variable has continuous data, we use the Gaussian distribution and the identity as link function. Each full model was compared via F-Test with a null model. The null models also presented the Family as Gaussian and the identity link function. The distribution of errors was checked, and the distribution was within the expected. Tukey's test was used with posthoc analysis with an alpha of 0.95.

Beginning of protonema, shoot, and sex expression

We used GLM to compare the beginning of protonema, shoot, and sex expression between the genotypes studied. For this, three models were created, with the genotype as a predictor variable and the beginning of protonema, the beginning of shoot production, and the beginning of sex expression as the response variable. Then, each model was compared via F-test to the equivalent null model (with the same response variable). The full and null models had the Poisson error family and the log as link function. Finally, the data dispersion was checked, and all models showed a dispersion not fitted to the model. Thus, the test family was changed to quasipoisson to adjust the data dispersion. Tukey's test was used with posthoc analysis with an alpha of 0.95.

Final absolute and relative sex expression rate

To compare absolute and relative sex expression at week 8 and absolute at week 10 between the sexes of BAF and SDTF, we used Kruskal-Wallis analysis, since the data did not show data normality, even after data transformation. Thus, three models were performed to compare sex expression with sex. Then, Dunn’s test was used for multiple comparisons, and p-values were adjusted with Bonferroni correction.

Sex expression versus *bulbil production*

We used the GLM (Generalized Linear Model) to investigate the potential relationship between sex expression and bulbil production. Two complete models were constructed for this purpose: [1] the response variable being bulbil production, with predictor variables being absolute sex expression at week 8 and sex; and [2] the response variable being bulbil production, with predictor variables being relative sex expression at week 8 and sex. Both models were tested using the Poisson family and the log link function. Each model was compared to a null model using a chi-square test. Comparing null and complete models is a common practice to assess the statistical significance of predictor variables. Since all complete models significantly differed from their respective null models, the null models were discarded, and the complete models were analyzed. The error distribution was examined by dividing the Deviance Residual by the degrees of freedom, and the resulting values were compared to the expected values according to the model. Both models exhibited overdispersion, indicating a greater variability in the data than expected. To address the overdispersion, the model's distribution was changed from Poisson to quasipoisson. Next, the relationship between absolute and relative sex expression and bulbil production was compared through GLM results and graphical analysis. When comparing the results of the model's correlation directionality and visually inspecting the plots, it was observed that the model with the response variable of relative sex expression exhibited Simpson's Paradox, a phenomenon observed in probability and statistics where a trend emerges within different data groups but disappears or even reverses when these groups are combined. This phenomenon was observed in the male sex in STDF (see Supplementary Table 1). Therefore, we separately analyzed the influence of sex and relative sex expression on bulbil production. Separate models were created for each sex, considering the different vegetations (SDTF and BAF). The Poisson distribution was used along with the Log link function. The same steps performed previously were applied to the separate groups. However, during the analysis of the models for data distribution, overdispersion was observed in all of them. To address this issue, an adjustment was made using the quasipoisson family and the log link function. When comparing the full model with the null model, a significant difference was found only in the case of female plants in the SDTF vegetation.

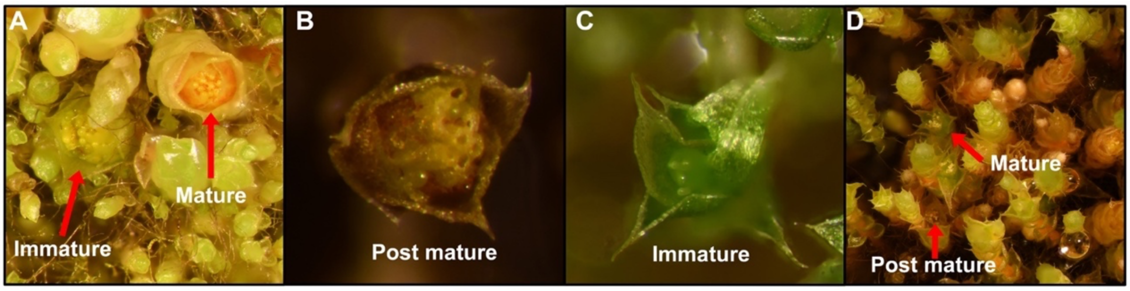

Fig 2 – Male and female gametangia with their respective phenophases analyzed in this study. A – male perigonia mature (yellowish antheridia) and immature (green antheridia); B – postmature perigonium (antheridia opened and brownish); C – immature perichaetium (archegonia green and closed); D – Perichaetia mature (open archegonia with colors yellowish and green) and post mature (open archegonia with brownish color).

Gametangia development

To analyze the development of gametangia, we chose to use the Kruskal-Wallis test due to the data being non-normally distributed. In this regard, we quantified the initial occurrence week and the peak week (representing the time when the highest quantity of the respective phenophase occurred) for both the immature and mature phases within each group. To handle missing data, represented by 0 values, we calculated the mean of the initial occurrence week and the peak week for each variable and group individually. This approach was adopted to avoid distortions in the temporal analysis since assigning a value of 0 would erroneously imply that the phenophase occurred instantaneously. By replacing the missing values with the mean, we could more accurately represent the occurrence of the phenophase and its peak week without compromising data integrity. After performing the Kruskal-Wallis analysis, we conducted post-hoc comparisons between the investigated groups using the Dunn's test. To control the Type I error, the Dunn's test was adjusted with the Bonferroni correction method, which accounts for multiple simultaneous comparisons. However, due to the limited number of observations in the post-maturation phase, it was not possible to include it in the statistical analyses conducted. Therefore, our analysis focused on the earlier stages of phenological development.

All analyzes were performed using the Rstudio software (RStudio Team, 2021). The Growthcurver package (Sprouffske & Wagner, 2016) was used to calculate population growth (protonema and shoots). The Dunn's test package (Dinno & Dinno, 2017) was used to perform Posthoc Kruskal-Wallis analyses. The Circular analyses were performed using the Circular package (Agostinelli & Lund, 2022). The graphics were made by the packages GGPLOT2 (Wickham, Chang, & Wickham, 2016) and PLOTRIX (Lemon *et al.*, 2009).

3 - RESULTS

Protonema and shoot growth

Of all the models analyzed for comparison of growth rates, except the model that tests the difference between the growth index (r) to shoot production, were not significant (**Table 1**). On the other hand, all other models showed significance in the variation of indices about the genotype. The K index for protonema growth, male genotypes of BAF was the only category that differed from the others, with a lower average (**Table 1**). The protonema growth rate (r) was lower in male SDTF genotypes but did not differ in the female genotypes of the SDTF, which in turn did not differ among the other genotypes (**Fig. 3**). The female and male SDTF and female BAF genotypes showed no differences from T-med. Still, the female SDTF and male BAF genotypes had no significant differences (**Table 1**).

Regarding the K index for shoot production, the male genotypes from the BAF were significantly lower than the other genotypes, while the other shoots did not differ from each other (**Table 1**). For the T-med index, the male genotypes from the BAF was significantly lower compared to the male genotypes from the SDTF and the female genotypes from the BAF, but did not differ from the female genotypes from the SDTF (**Table 1**). The male and female genotypes from the SDTF and the female from the BAF did not differ from each other (**Table 1**). No differences were observed among the genotypes related to r growth (**Table 1; Fig. 3**).

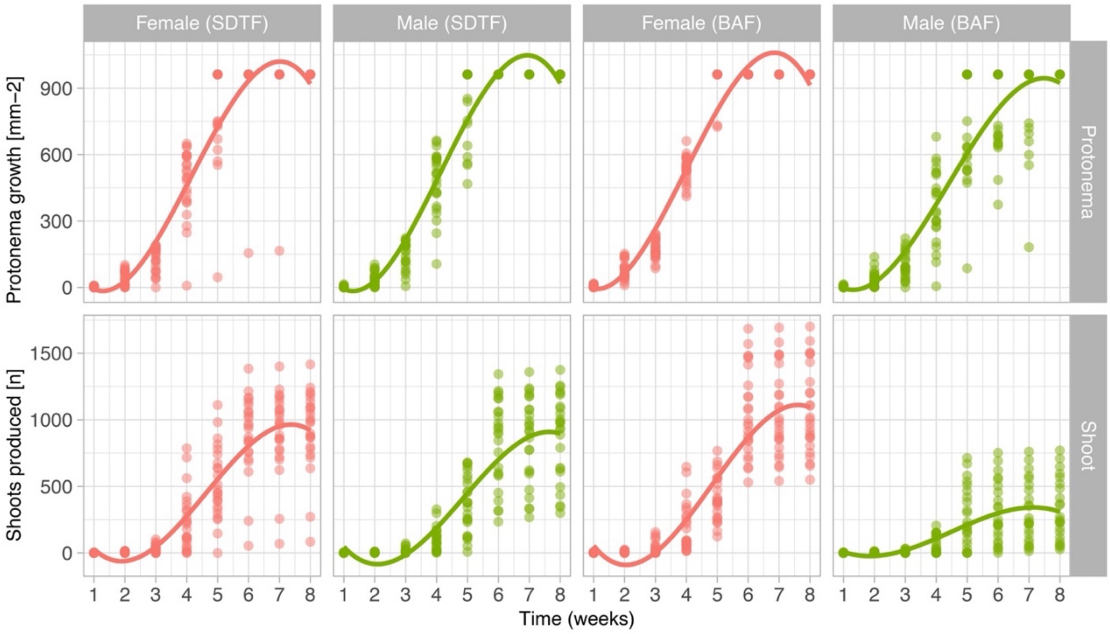

Fig 3 – Scatterplot with two lines: the first line displays the growth of protonema area [mm-^{2}] over time [weeks], while the second line represents shoot production [n] over time [weeks]. The data points on the plot represent colonies cultivated in the laboratory, while the drawn line depicts the logistic growth curve.

Beginning of protonema, shoot, and sex expression

The beginning of protonema production did not differ between the genotypes studied (GLM, df = -3, Dev = -1.73, P = 0.17) (**Fig. 4**). On the other hand, the production of shoots showed a significant difference (GLM, df = -3, Dev = -14.80, P < **0.001**). In this sense, the male genotype from the BAF showed a significantly longer time for the start of shoot production when compared to the other genotypes that did not differ from each other (**Fig 4**). The beginning of sex expression differed between genotypes (GLM, df = -3, Dev = -14.96, P < 0.001), male and female genotypes from the SDTF had a shorter time for sex expression and did not differ from each other. The female BAF and SDTF genotypes did not differ in time for the sex expression. However, the female BAF genotypes had a significantly higher time to begin the sex expression when compared to the male SDTF genotypes. The male genotypes from the SDTF presented the longest time for sex expression, and this genotype differs from all the other genotypes studied (**Fig. 4**).

Table 1 – Mean with standard deviation of population growth parameters with GLM results. K (carrying capacity); r (growth rate) T-med (the time at the inflection point of the logistic curve occurs at half the capacity load). The subscription represents the difference between the groups. Values in bold mean that the *P* value is significant.

Life phase	**Growth parameters**	**SDTF**		**BAF**		**GLM**		
		Female	Male	Female	Male	Deviance	F	P
Protonema	K	975.67 ± 6.32^{a}	977.79 ± 9.24^{a}	973.99 ± 4.59^{a}	776.69 ± 341.74^{b}	-695951.00	8.39	**< 0.001**
	R	1.98 ± 0.39^{a,b}	2.02 ± 0.41^{b}	2.04 ± 0.35^{b}	1.62 ± 0.80^{a}	-2.71	3.43	**< 0.05**
	T-mid	4.06 ± 0.35^{a,b}	4.02 ± 0.38^{a}	3.85 ± 0.13^{a}	4.56 ± 0.93^{b}	-6.68	8.07	**< 0.001**
Shoots	K	938.19 ± 285.13^{a}	876.46 ± 324.27^{a}	1087.61 ± 344.67^{a}	311.46 ± 227.85^{b}	-8147469.00	30.12	**< 0.001**
	R	3.29 ± 2.09^{a}	3.33 ± 1.50^{a}	3.01 ± 1.27^{a}	3.41 ± 1.37^{a}	-2.19	0.29	0.08
	T-mid	4.79 ± 0.59^{a,b}	5.01 ± 0.39^{b}	5.06 ± 0.20^{b}	4.50 ± 1.13^{a}	-4.62	3.52	**< 0.05**

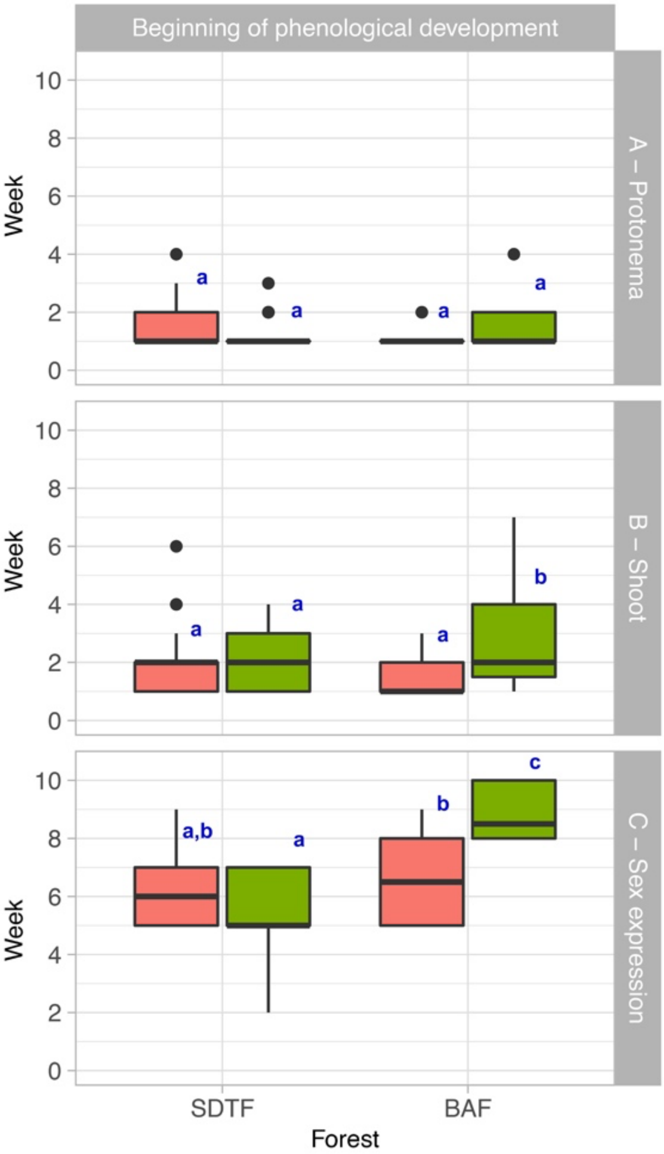

Fig 4 –Boxplot displays the onset of development (time taken to initiate development of these traits) of protonema, shoots, and sex expression for male and female colonies of BAF and SDTF varieties. The legend below the plot indicates the significance parameter between the groups, based on the posthoc test result of the generalized linear model (GLM).

Final absolute and relative sex expression rate

Regarding the absolute sex expression rate at week 8, the analyzes showed significant differences between the sexes ($X^2 = 49.27$, df = 3, $P < 0.001$). Absolute sex expression at week 8 was higher in SDTF males, while female plants (SDTF and BAF) did not differ. The male plants from the BAF had the lowest average, differing from all genotypes of SDTF and BAF females (**Fig. 5**). The analysis of relative sex expression (week 8) also showed significant differences ($X^2 = 47.38$, df = 3, $P < 0.001$), with male plants from the SDTF having the highest average compared to the other categories. The female plants (BAF and SDTF) did not differ.

BAF male plants differed from all sexes (**Fig. 5**). Finally, absolute sex expression at week 10 showed a significant difference ($X^2 = 52.61$, df = 3, $P < 0.001$). The difference followed the same pattern observed in comparing the sexes about relative sex expression (**Fig. 5**).

Fig 5 – Boxplot displays: A) absolute sex expression at week 8; B) relative sex expression at week 8; C) absolute sex expression at week 10, for male and female cultures of BAF and SDTF varieties. The legend below the plot represents the significance parameters generated by the posthoc test of the generalized linear model (GLM).

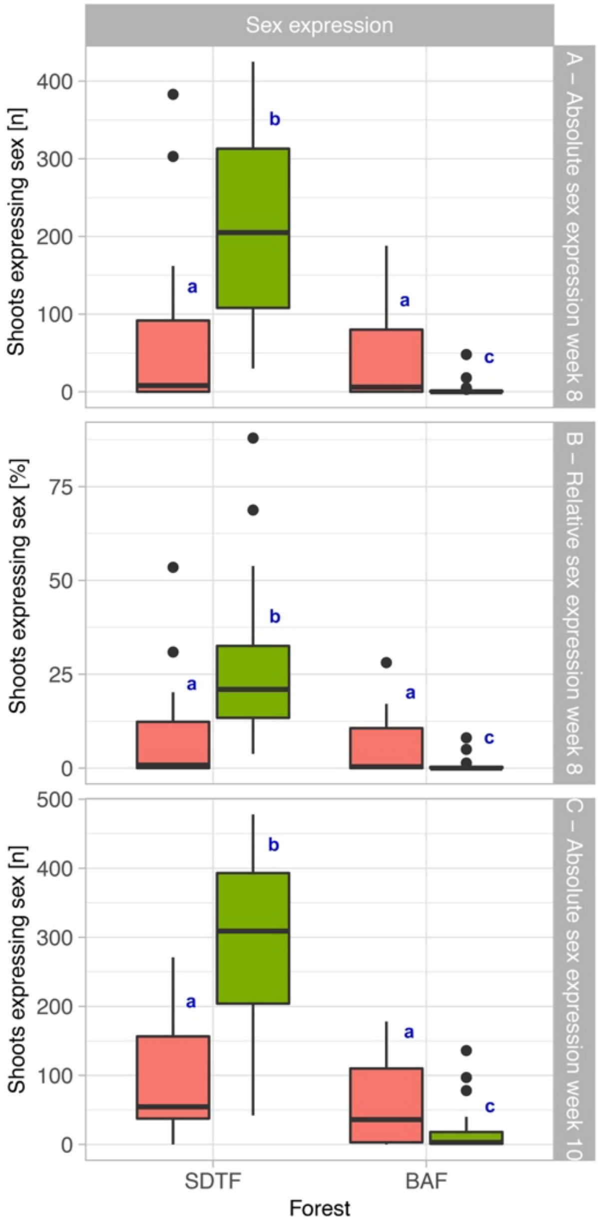

Table 2 – GLM results for the effect of absolute sexual expression and sex on bulbil production. Bold values indicate statistical significance ($P < 0.01$).

Response variable - Bubil production			
	Df	***Deviance***	***P***
Absolute sex expression	1	1035.37	**< 0.001**
Sex	3	295.53	0.08
	Estimate	***t value***	***P***
Female (BAF)	-0.520	-2.168	**0.01**
Male (BAF)	-0.209	-0.943	0.30
Female (SDTF)	-0.003	-2.528	**0.01**
Male (SDTF)	-0.684	-1.912	> 0.05

Sex expression* versus *bulbil production

The GLM results revealed that absolute sex expression had a significant impact on bulbil production, while sex did not show a statistically significant influence (Table 2). Therefore, the model analysis indicated that female plants from BAF and SDTF exhibit a negative trade-off between bulbil production and absolute sex expression, whereas males do not show this relationship (Figure 6). Regarding the GLMs investigating the relationship between relative sex expression, sex, and bulbil production, only female plants from SDTF showed a significant negative association (Estimate = -0.07; t-value = -2.70; P = 0.01). The other groups, SDTF males (Estimate = 0.001; t-value = 0.09; P = 0.93), BAF females (Estimate = -0.01; t-value = -0.372; P = 0.71), and BAF males (Estimate = -0.001; t-value = -0.05; P = 0.95), did not exhibit an association between bulbil production and relative sex expression (Figure 7).

Gametangia development

The initial stage of gametangia development (immature phenophase) showed noticeable variations among the examined genotypes ($X^2 = 28.67$, df = 3, $P < 0.001$). The results revealed that the mean duration of the immature phenophase stage did not display significant differences between sexes in SDTF. However, in BAF, notable distinctions were evident with male plants in BAF showing a significantly higher mean. Consequently, the means for both sexes in BAF were higher than those in SDTF. Regarding the peak of the immature phenophase, no significant differences were found among the different compared groups ($X^2 = 5.63$, df = 3, P = 0.10). In regard to the mature phenophase, the Kruskal-Wallis test revealed a significant difference in the results ($X^2 = 8.85$, df = 3, $P < 0.01$). However, the difference between groups was observed only when comparing the mean of male plants from SDTF with female plants from SDTF and male plants from BAF. It was found that, in relation to female plants from SDTF, male plants showed a higher mean, while in relation to male plants from BAF, male plants from SDTF exhibited a lower mean. No significant differences were found among the other groups. The Kruskal-Wallis test also indicated a significant difference in the results for the peak of the mature phenophase ($X^2 = 22.78$, df = 3, $P < 0.001$). In terms of these differences, male plants from SDTF were significantly different from the other male and female groups from BAF and female SDTF (Fig. 8).

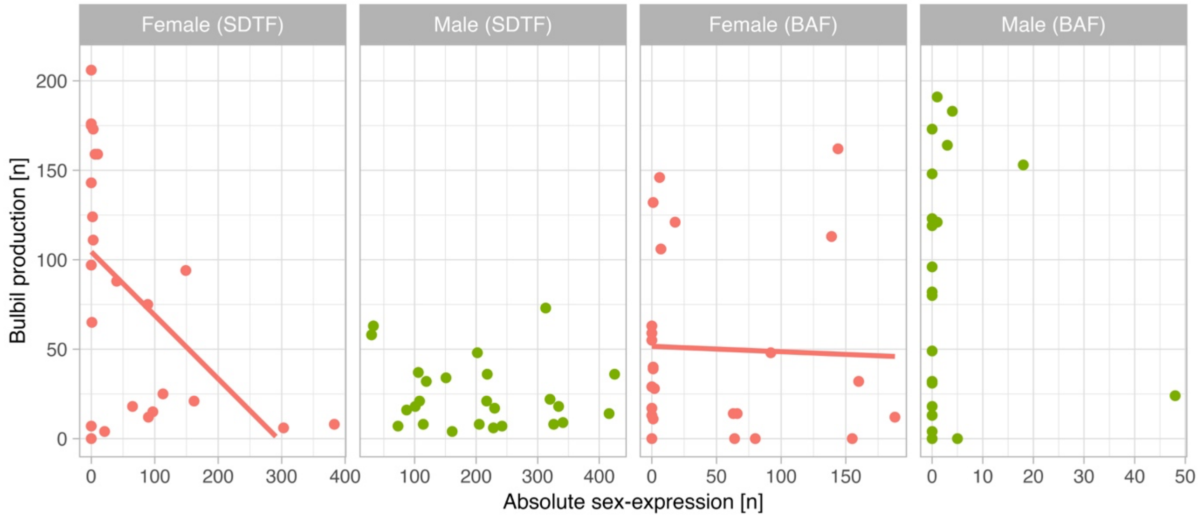

Fig 6 – Scatterplot illustrates the relationship between the variables: bulbil production on the y-axis and absolute sex expression on the x-axis for each sex and forest environment. The data points represent colonies cultivated in the laboratory, while the drawn line depicts the data trend. It is important to note that the absence of a line does not indicate any statistical significance between the variables.

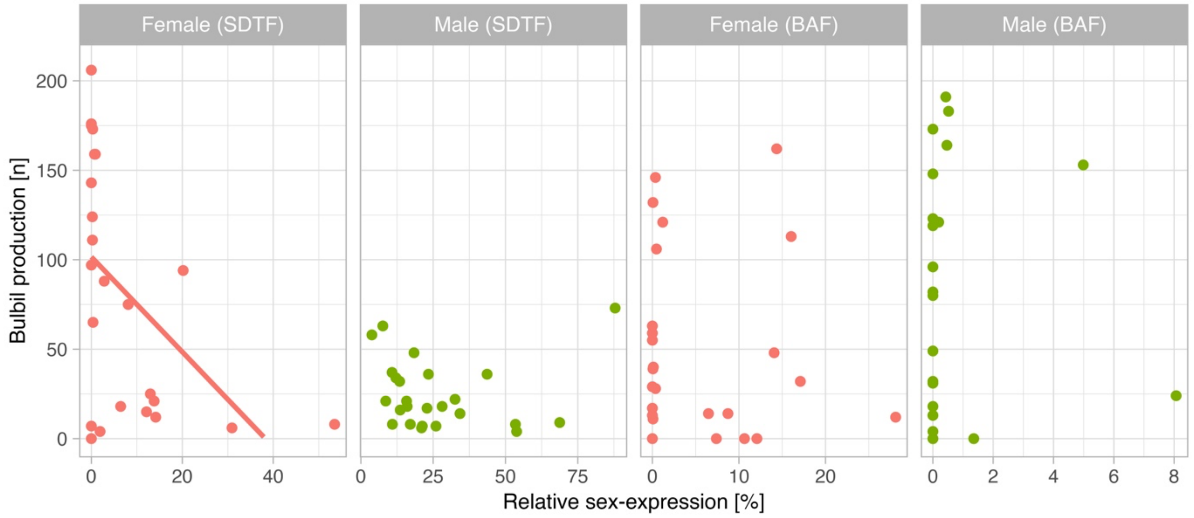

Fig 7 – Scatterplot illustrates the relationship between the variables: bulbil production on the y-axis and relative sex expression on the x-axis for each sex and forest environment. The data

points represent colonies cultivated in the laboratory, while the drawn line depicts the data trend. It is important to note that the absence of a line does not indicate any statistical significance between the variables.

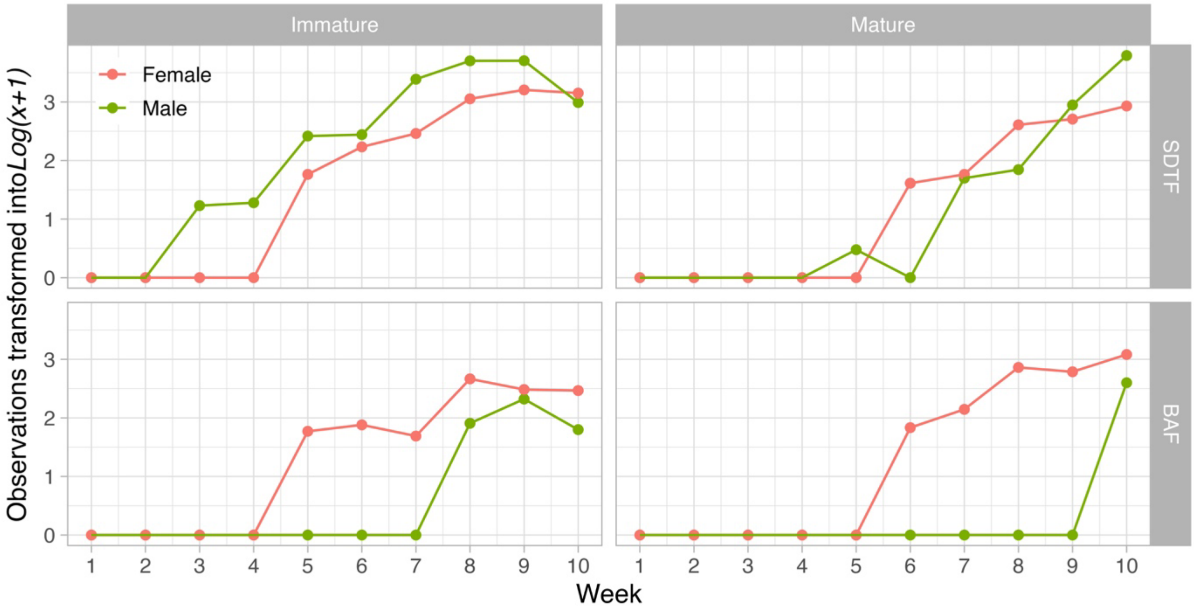

Fig 8 – Point plot representing the immature and mature phenophases for male and female cultures of both studied genotypes (SDTF and BAF). The Y-axis represents the total observations made each week, as depicted on the X-axis. The data has been transformed using a log(x+1) for better observation.

4 - DISCUSSION

Reproductive traits and phenological development differ between sex and genotype in many aspects of *B. argenteum,* as observed in some genotypes of *B. argenteum* (Horsley *et al.*, 2011). However, the differences are more explicit in male plants of SDTF genotypes. Regarding phenology development, our main findings showed that the production of male protonema in the SDTF genotype is slower than others genotypes and sex, and the carrying capacity of shoot production is small in male plants of the SDTF genotype. In addition, except for male plants of BAF genotypes, all other genotypes show the trade-off between sex expression and bulbil production. The plants of genotype BAF spend more time on the maturation of their gametangia. Our results show different patterns between samples from divergent environments, suggesting populations may be adapted to specific abiotic conditions. For example, the high rate of male sex expression in SDTF plants indicates a possible higher reproductive fitness for SDTF plants than BAF plants. In addition, some results show potential evidence of local speciation related to humid and dry environments, where populations show different strategies associated with reproductive fitness.

The genotypes show differences in growth parameters and suggest competitiveness between the sexes

Growth parameters of *B. argenteum* varied among the different sexes and genotypes (Fig. 3). However, male plants from the BAF tended to present low protonema growth and shoot production. These results demonstrated that *B. argenteum* plants do not exhibit a consistent pattern of regeneration, and regeneration varies between sexes, as different effects are observed for the same species. For instance, Horsley et al. (2011) found differences in the rate of protonema growth and shoot production in some populations of *B. argenteum* collected in distinct environments, while other genotypes did not differ in such traits. In the dioicous moss *Pleurozium schreberi* (Willd. ex Brid.) Mitt. Longton and Greene (1979) reported in populations of this species that there are no differences in several reproductive traits, among them in the vegetative growth rate, which is like our findings for the SDTF. Other results in moss species show sex-specific differences, such as in *Polytrichum commune* Hedw., the production of male plants was higher compared to female plants (Wyatt & Darda, 1997). On the other hand, results found demonstrate a greater advantage for female plants, as in *Pogonatum dentatum* (Menzies ex Brid.) Brid., (Hassel, Pedersen, & Söderström, 2005), and *Syntrichia caninervis* Mitt. female plants regenerate faster than male (Stark *et al.*, 2005; Stark & McLetchie, 2006). Finally, the dioicous moss *Weissia jamaicensis* (Mitt.) Grout, female plants produce more shoots (Santos, Pôrto, & Pinheiro, 2022).

This faster development in female plants is strongly related to the reproductive cost of the plants (Obeso, 2002; Karlsson & Méndez, 2005). Considering the pattern of reproductive allocation in bryophytes (greater allocation in male functions in rhizautoicous and dioicous systems at the prezygotic level), this generates a limitation of resources in male plants to other vital functions of plants (McLetchie and Puterbaugh, 2000; Stark et al., 2000; Hedderson and Longton, 2008; Horsley et al., 2011; Stark and Brinda, 2013; Santos et al., 2018, 2022; 2023). In *B. argenteum,* the prezygotic reproductive allocation is significantly higher in males compared to females reported by Horsley et al. (2011). This favorable reproductive allocation to male function entails the reproductive cost. Thus, a good development for female plants is expected at the prezygotic level. Still, our findings indicate that populations of *B. argenteum* behave differently according to the environment where they are found. Indeed, several growth parameters differ between genotypes of SDTF and BAF (Fig. 4). For example, the male plants from the SDTF presented a faster development than the female genotypes. In contrast, the male plants from the BAF had the opposite observed.

The inflection point (T-med) represents the fastest-growing moment of the reproductive traits measured in this study. Our results suggest possible advantages for the female plants of the SDTF. Regarding protonema growth, the BAF genotype indicated that the T-med of female plants (3.85) is lower than that of males (4.56). This means that a greater area of protonema had been produced at the given time for each sex. Accordingly, this finding reinforces the possible greater competitiveness for females in protonema growth. On the other hand, the opposite was observed for the production of the shoot in the BAF genotype, where the T-med was higher for females. However, in any case, it can still be considered that females are more competitive since the carrying capacity (K) of shoots was significantly higher in female plants. Thus, if a female and male plant grow together, the female plant will initially have a larger protonema area, limiting the site for the male development plants. Thus, consequently generating a female-biased population, according to data tracing the female-biased sex ratio in *Ceratodon purpureus* (Hedw.) Brid. to early gametophytic development (Eppley *et al.*, 2018). Few studies have investigated the competitive ability between sexes in bryophytes. Of these studies, all presented populations with female bias, as McLetchie (1992) reports for the species *Sphaerocarpos texanus* Austin and Shaw et al., (1999) for the moss *C. purpureus* report that male spores undergo more abortions compared to female spores. In *Marchantia inflexa* Nees & Mont. (McLetchie & Puterbaugh, 2000) and in *Weissia jamaicensis* (Mitt.) Grout (Santos *et al.*, 2022), female plants produce more branches than male ones. In general, the variation in life history characteristics found in *B. argenteum* is associated with different sexes and environments. Thus, suggesting that the location where these plants are found is related to the reproductive strategies. Therefore, with our findings, we reinforce that the traits of the life history of *B. argenteum* vary about the sexes and genotypes since the genotype of the BAF and SDTF present very different characteristics from each other. Furthermore, the growth pattern of the SDTF genotype indicates a possible cause for female bias in *B. argenteum* populations (Glime and Bisang 2017).

Sex expression is more evident in SDTF plants and differs from what is observed in the field

Absolute and relative sex expression was higher in male plants from the SDTF compared to the other sexes and genotypes (Fig. 5). This result is intriguing since, in field conditions, male sex expression is rare (Pôrto *et al.*, 2017; Castetter *et al.*, 2019). Therefore, we expected the lowest rate of sex expression for male plants, as observed in the male genotype from the BAF. One of the possible causes that we believe may affect sex expression is the reproductive cost that each sex incurs. The reproductive cost has already been tested for sex expression by Stark et al. (2000). The authors used *S. caninervis,* which had previously been reported to have a high sex ratio with female bias by Bowker et al. (2000). They tested the hypothesis that the sex that is more expensive is found to express sex less in the population. The authors corroborated the hypothesis since male plants' reproductive allocation was greater. The same pattern was found in the moss *W. jamaicensis*, reports Santos et al. (2022). On the other hand, Bisang et al. (2006) investigated the same hypothesis in the moss *Pseudocalliergon trifarium* (F. Weber & D. Mohr) Loeske. They found the opposite, showing that the hypothesis was invalid for the studied species. Consequently, our results show that the relationship between reproductive cost and sex ratio can vary in the same species, so the environment can influence these reproductive traits. Furthermore, these results show that the pattern is not universal and may differ between species.

Another important factor regarding sex expression is that there is a trade-off between sex expression and bulbil production. The type of reproduction plays different roles in the colonization of the species (Vigalondo *et al.*, 2019). For example, sexual reproduction (spore) ensures long-distance dispersal, while asexual reproduction favors local colonization (Glime and Bisang 2017). Newton and Mishler (1994) state that vegetative propagules have a greater ability to colonize previously occupied areas compared to spores, but they can also achieve greater success in uncolonized locations. Therefore, we can expect that populations of *B. argenteum* in different ecosystems show different patterns in the dispersal and colonization of their reproductive propagules, which can influence populations at genetic levels to population dynamics. The relationship between the type of reproduction with the genetic structure has been demonstrated in some species of bryophytes. For instance, Wang et al. (2012) used microsatellite molecular markers to understand the effect of sexual and asexual reproduction in two moss species, namely *Hypnum plumaeforme* Wilson (predominantly asexual reproduction) and *Pogonatum inflexum* (Lindb.) Sande Lac. (predominant sexual reproduction) that was artificially isolated in Thousand-Island Lake (Province of Zhejiang, China). They observed that for the species *H. plumaeforme,* the genetic diversity of island populations was significantly greater than that of riverine populations. On the other hand, in *P. inflexum*, genetic diversity was not significantly different between the island and riverine populations. These results show that reproduction by sexual reproduction substantially influences the structure and genetic diversity of species. Therefore, it is logical to expect that the studied populations may present different degrees of genetic diversity caused by the plant reproduction type since the SDTF genotype had more sexual reproduction than the BAF one.

The difference in the development of gametangia may indicate speciation in the studied genotypes

The corresponding male and female phenophases (same phenophase) vary with sex in each genotype (SDTF and BAF). Thus, the onset of gametangia development (immature gametangia) was faster in males compared to females in the SDTF genotypes, while in the BAF genotypes, the opposite was observed. Regarding the mature phenophase, both genotypes showed protogyny, and the BAF genotype matured one week later than the SDTF genotype. This result suggests a possible adaptation in the genotypes since SDTF plants need to reproduce in 4-5 months, while BAF plants have resources (precipitation) to produce throughout the year. Regarding the production and development of gametangia in bryophytes, tropical species have a shorter development time than species from temperate regions (Glime, 2017). In the UK, for example, *B. argenteum* develops its male gametangia (from sex expression to maturation) over 6-8 months compared to 2-3 months for females (Miles, Odu, & Longton, 1989). In Brazil, although no phenological studies have been carried out with *B. argenteum*, climate patterns suggest that reproduction is faster compared to the findings of (Miles *et al.*, 1989) since the limiting factor for the reproduction of tropical species is the rain which in several Brazilian ecosystems are strongly seasonal (Maciel-Silva & De Oliveira, 2016). Of the species studied phenologically from the tropics, all developed over shorter periods (Miles *et al.*, 1989; Fotoba, 1998; Oliveira & Porto, 2001; Pôrto & de Oliveira, 2002; MacIel-Silva & Marques Válio, 2011; dos Santos, Maciel-Silva, & Pôrto, 2020). On the other hand, species from temperate and polar regions present the development of gametangia for a long time. For example, in some species of *Ptychomitrium* in Japan, Deguchi and Takeda (1986) showed that antheridia typically required 9 months, whereas archegonia only needed 1 month to develop. We can interpret that species from the tropical region are adapted to develop more quickly, and consequently, this pattern can vary between the genotypes found in different tropical ecosystems, which can be affected by geographic distance.

5 - CONCLUSION

This study supports the contention that a species differs in its life traits according to the ecosystems for which it is specialized in different habitats. In general, the SDTF genotypes showed faster development and greater sex expression, while the BAF genotypes showed the opposite. This faster development of SDTF plants can be suggest as a possible speciation for different habitats, as plants from this ecosystem need to complete their reproductive cycle in a few months, due to the strong seasonality of the ecosystem. Furthermore, the mode of reproduction varies about genotypes, which is strongly associated with the trade-off between sexual and asexual reproduction, and which can genetically structure populations in different ecosystems. Therefore, we conclude this study by showing that genotypes are strongly associated with the ecosystems where they are specialized. Future studies to investigate the tolerance of abiotic factors such as desiccation and temperature, and genetic structure in the different genotypes studied would help to better understand the relationship between the biology of the species and the ecosystem in which it is found. However, it is necessary to broaden the inclusion of species from different ecosystems, as this will contribute to a more comprehensive understanding of this process.

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AUTHOR CONTRIBUTIONS

WLS collected the material. WLS, KCP, and FP sorted the collected material. WLS experimented with the help of JG and AMR. WLS, LRS, and JG conceived the ideas. Finally, WLS analyzed the data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Chapter 9

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Unveiling the impact of shoot phenophase and life phase on desiccation tolerance and in regeneration in the silver moss *Bryum argenteum*

Wagner Luiz dos Santos[1*], Andrea Medina-Ramos[2], Joshua Greenwood[2], Kátia Cavalcanti Pôrto[3], Fábio Pinheiro[1], Lloyd R. Stark[2]

[1]Graduate Program in Ecology, Biology Institute, Department of Plant Biology, Universidade Estadual de Campinas, Monteiro Lobato 255, Campinas, SP 13083-862, Brazil.

[2]School of Life Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, U.S.A.

[3]Biosciences Center, Department of Botany, Universidade Federal de Pernambuco, Moraes Rego Av., s/n, Recife, PE 50670- 901, Brazil.

Running title: Investigating desiccation tolerance associated with reproduction.

*Correspondent author: wag.lsantos1@gmail.com

ABSTRACT

Background and Aims**:** Desiccation tolerance (DT) is crucial for survival in arid environments, where organisms develop strategies in reproduction, maintenance, and defense to cope with water scarcity. Therefore, investigating the relationship between reproduction and DT is essential to understand the ecology and adaptive strategies of species. This study explores the connection between the development of male and female gametangia in the moss *Bryum argenteum* and the decrease in DT during the progression of phenological phases in gametangia and protonema.

Methods: Samples collected from a dry tropical forest in Brazil were cultivated, cloned, and subjected to desiccation. Subsequently, the physiological parameters of shoots and protonemata were analysed. Shoot and protonema regeneration were monitored for 28 days after the physiological analyses. Both phases were subjected to control and desiccation treatments.

Key Results**:** Significant effects of desiccation and sex on the physiological parameters and regeneration capacity of shoots and protonemata were found. Male shoots generally exhibited lower values in Fm, Fv/Fm, and ϕPSII, while females demonstrated better recovery after desiccation. Protonemata also showed variations in Fm and Fv/Fm over time and sex, with no significant differences in ϕPSII between them. Desiccated male shoots had higher mortality rates and produced fewer new shoots. Regarding the females, the regeneration patterns varied between the desiccation-exposed groups and the control, with decreased shoot production, and some protonemata growing into filaments without forming shoots.

Conclusion: These findings improve our understanding of bryophytes ecological responses to desiccation stress and provide insights into their adaptive strategies in challenging environments, such as the possible rarity of males in dioicous mosses populations.

Key words: Adaptive strategies; bryophytes; ecological responses; phenophase advancement; physiological parameters; regeneration patterns; sex-specific difference.

INTRODUCTION

Desiccation tolerance (DT) is an essential characteristic for the survival of many organisms, particularly in arid environments subject to periodic droughts (Oliver 2005; Alpert 2006; Ryabova *et al.* 2020; Chovsepian *et al.* 2022). This ability enables these organisms to survive in a highly dehydrated state, even with humidity levels below 50%, and subsequently revive or recover upon rehydration (Alpert and Oliver 2002). This evolutionary adaptation plays a crucial role in enabling these organisms to withstand extreme and challenging conditions, ensuring their persistence and success in hostile environments (Bewley 1979; Oliver *et al.* 2000; Gaff *et al.* 2013).

In plants, desiccation occurs differently among plant groups (Oliver 2005; Alpert 2006; Farrant *et al.* 2009; Gaff *et al.* 2013). Angiosperms possess characteristics that favour a lower occurrence of desiccation, such as a well-developed cuticle, stomata that control the opening and closing to prevent excessive water loss, specialized transport cells ensuring efficient distribution of nutrients and water, and lignified cells providing greater resistance and protection against dehydration (Gaff *et al.* 2013). Although bryophytes, characterized by the absence of lignified cells and their poikilohydric nature, which means they are unable to actively regulate water loss to the environment, become highly vulnerable to desiccation (Oliver 2005; Gao *et al.* 2017; Stark *et al.* 2022; Morales-Sánchez *et al.* 2022). Recent findings demonstrate advanced vascular functions in *Polytrichum commune* Hedw., displaying strong functional parallels with higher vascular plants (Brodribb *et al.* 2020). These parallels include resilient vascular conduits transporting water under tension and leaves regulating transpiration, allowing photosynthetic gas exchange without vascular cavitation. This advancement contrasts with their leaves' highly inefficient water use, underlining the significance of stomatal evolution for photosynthesis above ground. However, adaptive strategies to cope with low moisture environments are observed in most bryophytes, such as the ability to enter a state of dormancy or reversible dehydration, where their metabolic activities are suspended until they are rehydrated (Oliver 2005; Gao *et al.* 2017; Stark *et al.* 2022). These strategies help bryophytes survive in harsh environments and endure unfavourable conditions, showing higher desiccation tolerance compared to angiosperms (Stark *et al.* 2017). However, specific differences between the sexes can directly influence DT (Stark *et al.* 2005; Silva-e-Costa *et al.* 2022).

Sexual differences in bryophytes are commonly observed across various aspects of life, including reproductive allocation (Bisang *et al.* 2006; Stark and Brinda 2013; Santos *et al.*

2023), phenology (Stark 1985; dos Santos *et al.* 2020), sexual expression (Castetter *et al.* 2019; Bisang *et al.* 2020), reproductive cost (Bisang *et al.* 2006), and tolerance to abiotic stresses such as desiccation and thermotolerance (Stark *et al.* 2005; Silva-e-Costa *et al.* 2022). This sexual differentiation is broadly explained by life history theory and is predominantly associated with reproduction (Obeso 2002). Many of these differences are related to reproductive cost, referring to the effect of resource allocation to reproductive structures (Obeso 2002). This implies that the sex investing more resources in reproduction will have fewer resources available for maintaining other aspects of its life history, such as maintenance and defence (Bazzaz *et al.* 2005). For instance, in dioicous bryophyte species, it's common to observe a higher resource allocation to male function compared to female function (Glime and Bisang 2017). Consequently, it's typical for male individuals in these species to exhibit reduced performance in other life history aspects when compared to female plants (Glime 2017). For instance, studies have reported that reproductive allocation in the moss *Syntrichia caninervis* Mitt. is several times higher in the male function than in the female function (Stark *et al.* 2000). This disparity in the allocation of reproductive resources has indirectly resulted in an extreme difference in desiccation tolerance between the sexes in *S. caninervis* (Stark *et al.* 2005) which may influence the forms of desiccation response.

The plants exhibit two forms of response to desiccation, which are constitutive and inducible DT. Constitutive tolerance refers to the inherent ability of certain plants to survive in dry conditions due to their specific structural and physiological characteristics (Charron and Quatrano 2009). On the other hand, inducible tolerance is an acquired response by plants when facing drought conditions, activating protective and adaptive mechanisms (Charron and Quatrano 2009). Both forms of DT are adaptive strategies that enable plants to survive in water-deficient environments. However, constitutive tolerance is innate, while inducible tolerance is acquired in response to water stress (Charron and Quatrano 2009; Stark *et al.* 2022). These two response forms are strategies observed in various life stages of plants, given that organisms display desiccation tolerance at different stages of their life. For instance, bryophytes are known for exhibiting DT in all life stages (Oliver *et al.* 2000; Oliver 2005; Stark *et al.* 2022). From the protonema stage, where spores or vegetative propagules germinate and form protonemata (Pressel and Duckett 2010; Greenwood *et al.* 2019), to the initial development of the gametophyte, and through later stages such as the mature gametophyte (Greenwood *et al.* 2019), spores (Proctor *et al.* 2007), gametes (Shortlidge *et al.* 2012; Stark *et al.* 2016), vegetative propagules (Greenwood *et al.* 2019), and sporophytes (Stark and Brinda 2015).

Despite the investigation of various aspects of bryophyte biology related to DT, there is a scarcity of studies on how the development of reproductive structures, namely different phenological stages, relates to DT (Morales-Sánchez *et al.* 2022). It's crucial to focus research efforts on this aspect, particularly because tropical arid environments are especially susceptible to the effects of global warming (Huang *et al.* 2017; Li *et al.* 2018), and the impacts of drought (DT) on species viability are still poorly understood, it may be of special interest to understand how phenological changes may influence the survival capacity of these plants under extremely dry conditions. This understanding could provide valuable insights for the conservation and management of these sensitive ecosystems (Kramer *et al.* 2000).

Although other species, such as *Syntrichia caninervis* Mitt., and others Pottiaceae species have often been used as models for desiccation tolerance (DT) studies, in recent years, the moss *B. argenteum* has emerged as a widely employed model species. This moss is utilized to investigate the effects of desiccation across various phases of the plant life cycle, employing diverse experimental and molecular approaches. The choice of *B. argenteum* as a model species is due to its widespread distribution on Earth, easy identification, and its ability to be easily cultivated and used in experiments (Stark *et al.* 2010; Horsley *et al.* 2011; Zhuo *et al.* 2020). This species has proven to be essential for understanding the underlying mechanisms of DT and has made significant contributions to advancing knowledge in this research area. Therefore, we used *B. argenteum* to investigate the relationship between (male and female) gametangium development on DT in shoots, as well as DT in the protonema. Based on this, we investigated two hypotheses, namely:

Hypothesis 1 - As the gametophyte phenophase advances, the recovery of the photosynthetic system in the aerial part is less pronounced after desiccation, with a more marked recovery in females compared to males.

Hypothesis 2 - Protonemata from male plants exhibit lower DT compared to female plants.

MATERIAL AND METHODS

Study area

The samples used in this study were collected at the Reserva Particular do Patrimônio Natural Pedra do Cachorro (RPPN-PC) located in the city of São Caetano, in the state of Pernambuco, Brazil (8° 14' 12.019" S, 36° 11' 33.104" W). The RPPN-PC encompasses a diverse vegetation consisting of Tropical Dry Forest, commonly known as Caatinga, which is an exclusively Brazilian biome, renowned for its flora adapted to the water scarcity and extreme climatic variations of the region (Soares *et al.* 2016; da Silva *et al.* 2018). The average annual precipitation in RPPN-PC is approximately 439.6 mm, with the rainy season occurring mainly from May to July (Soares *et al.* 2016; de Queiroz *et al.* 2018). The region has an average temperature of 26°C during the warmest months and an average temperature of 19°C during the colder months (Soares *et al.* 2016). The unique environmental conditions at RPPN-PC offer a distinct habitat for local fauna and flora, thus rendering it an area of great importance for research and biodiversity conservation.

Studied species and sampling

Bryum argenteum Hedw., known as silver moss or silver thread moss, is a widespread species of moss belonging to the Bryaceae (Shaw 1990; Canestraro and Peralta 2022). It is found in various terrestrial ecosystems, and its name is derived from the silver coloration it exhibits when dry, which is caused by the absence of chloroplasts in the upper region of its leaves (Horsley *et al.* 2011). The species exhibits a dioicous sexual system, with male and female gametangia being produced on separate plants (Horsley *et al.* 2011; Canestraro and Peralta 2022). Male gametangia are located at the apex of male ramets, while female inflorescences are primarily situated at the base of female ramets (Stark *et al.* 2010). Bulbils, a form of asexual reproduction, are also commonly observed on shoots of *B. argenteum*, playing a significant role in population stability in natural habitats (Canestraro and Peralta 2022). Due to its abundant populations and ease of cultivation, *B. argenteum* has served as a model species in various studies focusing on reproductive biology, DT, and high-temperature tolerance (Cleavitt 2002; Moore *et al.* 2016; Pôrto *et al.* 2017; Castetter *et al.* 2019).

Field work was conducted within the RPPN-PC to survey and collect georeferenced populations of *B. argenteum*. Collected samples were carefully stored in paper bags, and pertinent information regarding substrate type and the presence or absence of sporophytes was documented. For this study, our sampling strategy focused on selecting populations with sporophytes, as this indicated the presence of male shoots within the colony. Furthermore, to ensure the inclusion of distinct populations, we deliberately chose samples that were spatially distant from one another (approx. 300m). Consequently, five distinct populations were selected, and from each population, one male and one female shoot were chosen for further investigation.

Culture technique

Protonemata and shoots production

Shoots were cultivated following the protocol described by Horsley *et al.* (2011). The soil was collected near Red Rock Canyon National Park, Located in the state of Nevada, United States, it's 24 kilometres west of Las Vegas. Soil was sieved through a 0.35 mm mesh and autoclaved at 121°C for 60 minutes. Two spoonfuls of this soil were then placed in sterile 3.5 cm diameter Petri dishes and hydrated with 5-8 drops of water to encourage shoot growth. The shoots underwent a cleaning process, with their tips separated, washed with water and after in a 2% bleach solution for 5 seconds, and returned to the Petri dishes for regeneration. Weekly hydration involved around 5 drops of water for the initial month, followed by alternating hydration with a 30% Hoagland solution (Hoagland and Arnon 1950) and water. Cultivation extended for approximately three months until colonies displayed necessary phenophases for experimentation (Fig 1 -A, B). During this time, water was added to reach the soil level, ensuring the regenerating shoots were not submerged. The same process was carried out for protonema production, although a larger volume of water was added. The Petri dishes were placed in a Percival growth chamber (Percival model E30B, Boone, IA, USA) with controlled conditions: 22°C temperature, 60-70% relative humidity, and constant light levels ranging from 100 to 410 µmol m -2 s –$^{1.}$

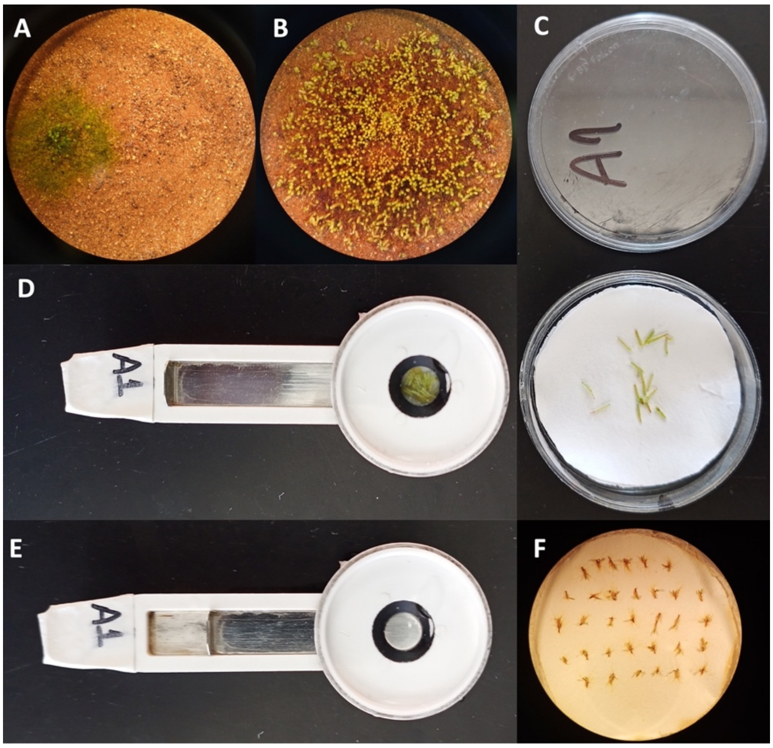

Figure 1: A) Two-week-old regenerating tip showing a green mass consisting of protonema and 8 shoots; B) Colony of shoots at approximately 35 days; C) Shoots placed for drying using the fast dry method; D) Clip holding shoots on the paper; E) Clip containing the shoots, closed

for 30 minutes to adapt to darkness before conducting photosynthetic parameters; F) Shoots arranged for regeneration.

Experimental design

Colony numbers

In each of the five studied populations, one male and one female shoot were selected and cloned for five successive generations to minimize the potential influence of environmental factors on their biology. In the fifth generation of cloning, we selected five male and five female shoots from each population to create new clones. In this way, 50 cultures were established, with each population represented by five male and five female clones. The same number of Petri dishes used to produce shoots were also made to produce protonema. Each genotype had an additional clone (shoots and protonemata) serving as the control group in the experiment. Totalling 110 cultures (55 for shoots and 55 for protonema).

Studied phenophases, shoots and protonema selection

The shoots were classified into three different phenophases, as described below:

1. Non-sex expressing: Signifying absence of reproductive structures, devoid of male or female gametangia, displaying no reproductive progression.
2. Immature: Characterized by green, closed gametangia, indicating their presence without readiness for reproduction.
3. Mature: Marked by yellow, closed male gametangia or releasing sperm (antherozoids) and open female gametangia, receptive to fertilization, the phase conducive to reproduction.

We selected 30 to 40 shoots from each phenological phase (non-sex expressing, immature, and mature) on each plate. Each set of shoots on a dish represented a sampling unit, which was subsequently used for comparison. The selection of shoots was performed using tweezers, which were cleaned with 70% alcohol after each selection. Subsequently, the upper part of the shoots (tips) was carefully trimmed to 2 mm, while the basal part was discarded. For protonema selection, we collected the mass of protonema and transferred it to a 1 ml microtube. The microtube was then vortexed for approximately 5 seconds, allowing the sand residues to separate from the protonemata.

Subsequently, selected shoots and protonemata were positioned on a paper tissue inside a Petri dish filled with distilled water. This setup aimed to provide an ideal environment for their sustenance during the experiment. Using a clip ensured stability and accurate positioning, eliminating unwanted interferences. The paper tissue served as a substrate, retaining water, offering hydration to the shoots, and facilitating gas exchange. This controlled setting allowed precise observation and monitoring of shoot behavior throughout the experiment.

Desiccation tolerance

For the DT experiment, shoots and protonema were categorized into three groups based on treatments (dried and control), sexes (male and female), and phenophases (non-sex expressing, immature, and mature). The dried group underwent desiccation and subsequent rehydration, aimed at assessing photosynthetic system regeneration (Fig. 2 - C). Conversely, the control group remained consistently hydrated throughout the experiment without undergoing desiccation (Fig. 2 - D).

Subsequently, the shoots had their photosystem measured using a fluorometer (Hansatech Model FMS 2), and the following parameters were measured: [1] Fm - the maximum chlorophyll fluorescence, representing the maximum amount of light absorbed by the shoots' photosystems (Maxwell and Johnson 2000), [2] Fv/Fm - a measure of photosynthetic efficiency under ideal conditions, representing the ratio between the maximum variation in chlorophyll fluorescence (Fv) and the maximum chlorophyll fluorescence (Fm) (Ruban 2016), and [3] ϕPSII - plays a fundamental role in generating the energy required for ATP and NADPH synthesis (Maxwell and Johnson 2000; Ruban 2016).

The measurements of the photosynthetic parameter were performed at five distinct time points. The first time point corresponded to the control time, where measurements were taken before the shoots underwent desiccation. After the control measurement, the shoots were subjected to a rapid drying process using two layers of filter paper at a temperature of 20°C and a relative air humidity of 0% for a period of 24 hours (Fig. 1 -C)(Stark *et al.* 2022). After 24 hours, the shoots were placed in the clips and then hydrated (Fig. 1 -D). After 30 minutes, the measurements of the photosynthetic parameters were taken, corresponding to time 0.5. Subsequently, the same measurements were repeated after 6, 12, and 24 hours. All shoots and protonema were kept in the dark for 30 minutes prior to the measurements (Fig. 1 - E).

Protonema and shoot regeneration

Following time 24 measurements, all shoots and protonemata underwent a regeneration process. Shoots from each clip were transferred to 35 mm Petri dishes, ensuring all shoots from a clip were in a single dish. Dishes, layered with paper towel and filter paper, were hydrated, arranging shoots appropriately. Regeneration occurred under prior growth conditions, monitored for 28 days (Fig. 1 - F). Protonemata regeneration followed a similar process, grown in distilled water without filter paper.

After observations, the following parameters were measured for each plate: rate of dead shoots, number of shoots solely with protonemata, number of shoots generating new shoots, total shoots produced, number of shoots expressing sex, and those exhibiting apical growth. As for protonema parameters, they included: count of dead protonemata, quantification of those producing new filaments, and those generating shoots with or without sexual expression. These measurements provided insights into the regeneration capacity of both shoots and protonemata. Tracking these parameters over time enabled a comprehensive understanding of developmental patterns and reproductive behaviors following the regeneration process.

Statistical analyses

Desiccation tolerance

To investigate intra- and intersexual variations across various phenophases, we employed Generalized Linear Models (GLMs). Each photosynthetic parameter (FV, FV/FM, and ϕPSII) was analysed concerning the three phenophases (Non-sex expressing, immature, and mature), resulting in 9 models to assess DT. In the analysis of protonema, due to the absence of distinct phenophases, we developed only 3 corresponding models, one for each photosynthetic parameter. This approach facilitated a detailed analysis of shoots' and protonemas' response to desiccation across different developmental stages, considering their interaction with the examined photosynthetic parameters.

Essentially, three main models were created, and replicated for each phenophase:

[1] Fm as the response variable, with time and sex as predictor variables, using a Poisson family and a log link function.

[2] Fv/Fm as the response variable, with time and sex as predictor variables, using a binomial family and a logit link function.

[3] ϕPSII as the response variable, with time and sex as predictor variables, using a binomial family and a logit link function.

Each model was compared to a null model, and all complete models showed significant differences compared to the null model, leading to the rejection of the null models. Subsequently, the data dispersion was examined, and in cases of overdispersion or underdispersion, the error distribution was adjusted from Poisson to quasipoisson and from binomial to quasibinomial, respectively. To finish, we compared with pairs of male and female groups at control time, 0.5 and 24 using Sidak Adjustment.

Shoots regeneration

The comparison between sexes, phenophase, and treatment in different shoot regeneration parameters was based on Generalized Linear Models (GLMs). One model was used for each parameter, resulting in a total of 6 models. The response variables for the models were: [1] mortality rate; [2] shoots with only protonema; [3] shoots with new shoots; [4] produced shoots; [5] shoots expressing sex; [6] shoots with growth apex. The predictor variables included: [1] phenophase; [2] sex; [3] treatment. Each model had its distribution and test family correctly adjusted.

After analyzing each GLM model, we conducted pairwise comparisons using the Sidak Adjustment. The aim was to contrast the means between sexes at each time point (Control, 0.5, 6, 12, and 24). We chose the Sidak Adjustment method due to its ability to control errors associated with multiple comparisons, providing a robust analysis of mean differences between sexes over time while reducing the likelihood of Type I errors. This strategy ensures a more reliable and accurate interpretation of the observed discrepancies.

Protonemata regeneration

We conducted comparisons between the dry and control treatments, as well as between male and female sexes, in the following parameters: [1] number of dead protonemata, [2] number of new protonemal filaments, [3] number of shoots produced, and [4] number of shoots expressing sex. We employed the Kruskal-Walli's test and to assess significant post-hoc differences, we used the Dunn's test.

All analyses were performed using RStudio (RStudio Team 2021). The emmeans(Lenth *et al.* 2019), multcomp (Hothorn *et al.* 2016), and multcompView packages (Graves *et al.* 2015) were employed to conduct post-hoc analyses on the GLMs, computing adjusted means and applying corrected multiple comparisons to identify significant differences among groups. Post-hoc analyses for the Dunn test were carried out using the Dunn package (Dinno and Dinno 2017), while the graphical representations were generated using the ggplot2 package (Wickham *et al.* 2016).

RESULTS

DT of shoots Fm, Fv/Fm, and ϕPSII

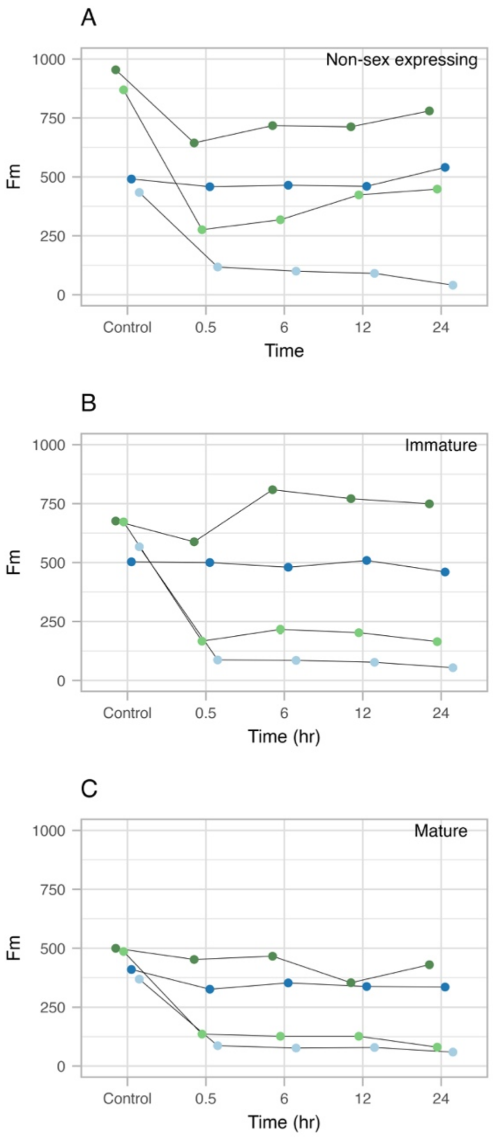

Figure 2: This scatterplot displays the variation of maximum fluorescence (Fm) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in Fm. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

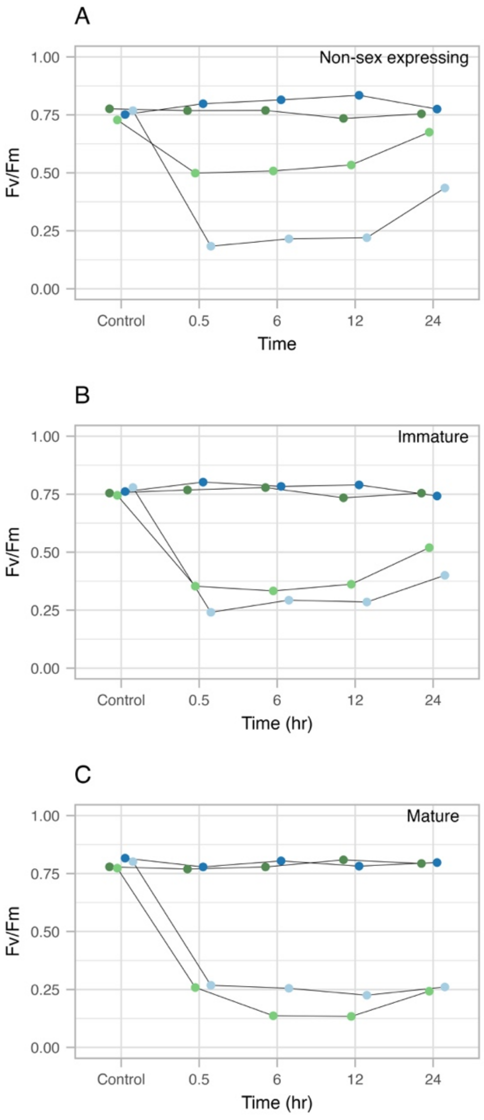

Figure 3: This scatterplot displays the variation of maximum fluorescence (Fm) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in Fm. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

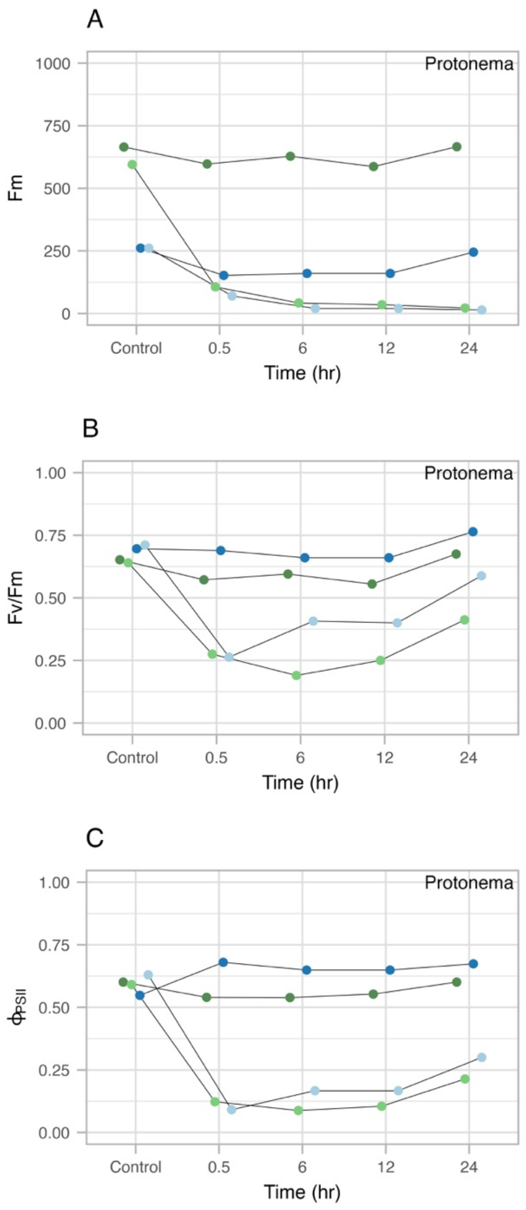

Figure 4: This scatterplot displays the variation of the effective quantum yield of photosystem II (ϕPSII) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in ϕPSII. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

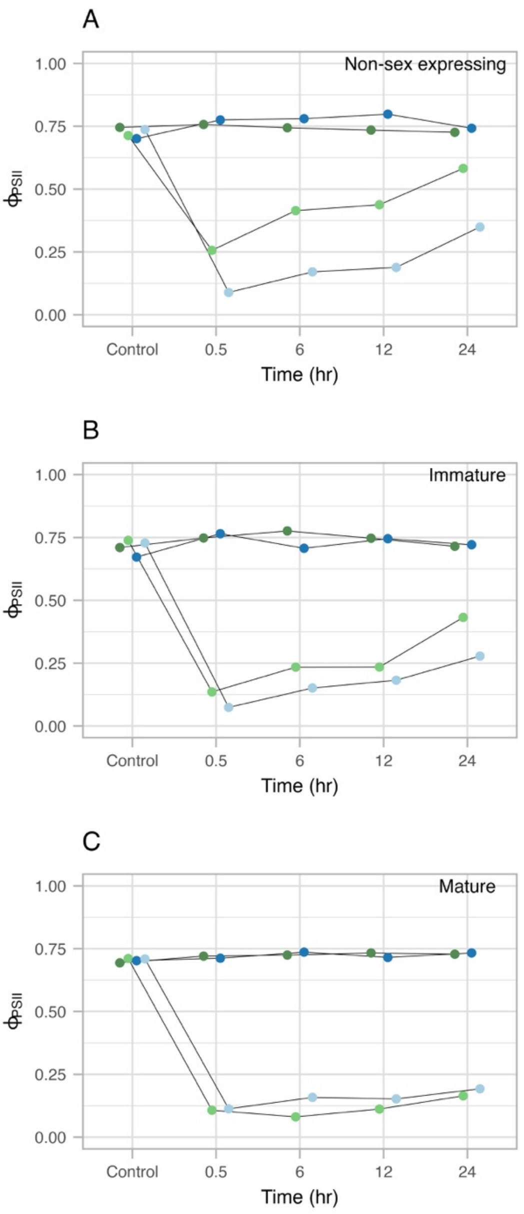

Figure 5: This image displays three scatterplots A, B, and C, each representing the respective photosynthetic parameters Fm, Fv/Fm, ϕPSII during protonema regeneration. The X-axis in all graphs represents time, with categories (Control, 0.5, 6, 12, and 24) indicated. On the Y-axis, we have the corresponding variable for each photosynthetic parameter (Fm, Fv/Fm, ϕPSII). The points at each time point represent the average of the variable for different groups. Dark and light blue points correspond to males in control and desiccated groups, respectively. Similarly, dark and light green points represent females in control and desiccated groups, respectively.

In shoots not expressing sexual, significant effects were observed due to time ($\chi^2 = 12173$; df = 2; $p < 0.001$) and sex ($\chi^2 = 11316$; df = 1; $p < 0.001$) on the Fm variable. Initially, males exhibited lower mean values than females at the control time for parameter Fm (estimate = -439.6; $P < 0.001$), a trend that persisted post-desiccation at time 0.5 (estimate = 171.6; $P < 0.05$). By time 24, female shoots displayed more recovery compared to males (estimate = 371.9; $P < 0.001$), who exhibited no recovery since desiccation (Figure 2 - A). Within the immature phenophase, the GLM revealed significant effects of both time ($\chi^2 = 14649.5$; df = 2; $p < 0.001$) and sex ($\chi^2 = 3126.8$, df = 1, $p = 0.001$) on Fm. Initially, males demonstrated lower mean values than females at the control time for Fm (estimate = 167.70; $P < 0.05$). Post-desiccation at time 0.5, no sex differences were observed (estimate = 126.20; $P = 0.25$). By time 24, male shoots displayed no recovery, while females exhibited higher recovery mean (estimate = 188.3; $P < 0.05$) (Figure 2 - B). In mature shoots, significant effects of both time ($\chi^2 = 12959.0$; df = 2, $p < 0.001$) and sex ($\chi^2 = 888.2$, df = 1, $p < 0.01$) on the Fm variable were noted. Initially, males had lower mean values in parameter Fm at the control time (estimate = 112.8; $P < 0.01$). Post-desiccation, no sex difference was observed at time 0.5 (estimate = 62.4; $P = 0.61$) or at time 24 (estimate = 33.80; $P = 0.99$) (Figure 2 - C).

In non-sex expressing phases, both time ($\chi^2 = 12.7226$, df = 2, $p < 0.001$) and sex ($\chi^2 = 4.0333$, df = 1, $p < 0.001$) significantly impacted the Fm/Fv variables. Initially, male and female shoots displayed similar Fv/Fm means during the control period (estimate = -0.02; $P = 0.99$). However, after desiccation at time 0.5, male shoots exhibited significantly lower means compared to females (estimate = 0.25; $P < 0.001$). By time 24, females fully recovered, while males maintained a similar mean as at time 0.5 (estimate = 0.20; $P < 0.001$) (Fig. 3 - A). Within the immature phase, both time ($\chi^2 = 17.6111$, df = 2, $p < 0.001$) and sex ($\chi^2 = 0.5039$, df = 1, $p = 0.05$) significantly influenced the Fm/Fv variables. Initially, no differences were observed between sexes in Fv/Fm during the control (estimate = -0.02; $P = 1.0$). After desiccation, both sexes exhibited lower means without significant differences between them (estimate = 0.08; $P = 0.62$). By time 24, both sexes displayed slight regeneration, higher than at time 0.5 but lower than the control (estimate = 0.09; $P = 0.35$) (Fig. 3 - B). The analysis showed significant influence of time ($\chi^2 = 31.4583$, df = 2, $p < 0.001$) on Fm/Fv variables in the immature phase, while sex did not ($\chi^2 = 0.0703$, df = 1, $p = 0.5$). Initially, during the control, the Fv/Fm mean was notably higher in female shoots (estimate = -0.2; $P = 1.0$). After desiccation, the mean decreased similarly for both male and female shoots, showing no difference in the mean (estimate = -0.01; $P = 1.0$), remaining consistent across subsequent time points (time 0 and time 3) (estimate = -0.01; $P = 1$) (Fig. 3 - C).

The analysis revealed a statistically significant effect of time on the variable ϕPSII in the Non-sex expressing phase ($\chi^2 = 31.4583$, df = 2, $p < 0.001$), while the impact of sex, though present, was less pronounced ($\chi^2 = 0.0703$, df = 1, $p < 0.05$). There were no differences between male and female sexes in ϕPSII during the control time (estimate = -0.01; P = 1.0). However, at 0.5 hours (estimate = 0.14; $P < 0.05$) and 24 hours (estimate = -12; $P < 0.01$), the female mean was significantly higher than the male (Fig. 4 - A). In the immature phase, only time had a significant impact on ϕPSII ($\chi^2 = 28.5951$, df = 2, $p < 0.001$), while sex did not have a notable influence ($\chi^2 = 0.5687$, df = 1, p = 0.07). Therefore, the sex means showed no significant differences across corresponding time periods (Fig. 4 - B). Regarding the mature phase, time was statistically relevant ($\chi^2 = 35.583$, df = 2, $p < 0.001$), but sex did not exhibit statistical relevance ($\chi^2 = 0.019$, df = 1, p = 0.7). In summary, no differences were identified between sexes across any of the evaluated time intervals (Figure 4 - C)

DT of protonema Fm, Fv/Fm, and ϕPSII

The GLM analysis revealed a significant impact of both time ($\chi^2 = 27771.2$, df = 2, $p < 0.001$) and sex ($\chi^2 = 2277.8$, df = 1, $p < 0.001$) on the Fm variable. Initially, female averages surpassed male averages (estimate = 283.90; $p < 0.001$). However, at time intervals 0.5 (estimate = 26.50; p = 0.99) and 24 (estimate = 18.2, p = 1.0), there was no difference between the averages. Regarding the Fv/Fm variable, the model unveiled significant effects of both time ($\chi^2 = 18.0567$, df = 2, $p < 0.001$) and sex ($\chi^2 = 2.1412$, df = 1, $p < 0.001$). The male and female averages did not differ at the control time (estimate = -0.07, p = 0.10). However, at time 0.5 (estimate = -0.28, $p < 0.001$) and at time 24 (estimate = -0.28, $p < 0.001$), the male average exceeded the female average. Finally, the GLM results demonstrated that ϕPSII was significantly influenced by time ($\chi^2 = 28.3340$, df = 2, $p < 0.001$) but not by sex ($\chi^2 = 0.0061$, df = 1, p = 0.70). Thus, the sex averages did not vary among themselves at the control time (estimate = 0.04; p = 0.81), at 0.5 (estimate = 0.01; p = 1), or at 24 (estimate = 0.04; p = 0.08).

Shoots regeneration

The analysis revealed that Treatment ($\chi^2 = 10.2812$, df = 1, p = 2.529e-09) and Sex ($\chi^2 = 10.3114$, df = 1, $p < 0.001$) significantly impacted shoot mortality, while Phenophase ($\chi^2 = 0.7352$, df = 2, p = 0.30) did not show statistical relevance. There was consistently high mortality in male shoots across various phenophases, except in desiccated male shoots in the non-sex expressing phase (Table 1). For shoots generating only protonema, Phenophase ($\chi^2 = 182.09$, df = 2, $p < 0.001$), Treatment ($\chi^2 = 173.32$, df = 1, $p < 0.001$), and Sex ($\chi^2 = 525.82$, df

Table 1: Shoot category characteristics. Values are represented as mean ± standard deviation. Subscript letters indicate significantly different parameters. Please note that letters should be compared within each column separately. GLM results are detailed in the text.

	Shoot category	**Motality rate**	**Shoots with only protonema**	**Shoots with new shoots**	**Shoots produced**	**Shoots expressing sex**	**Shoots with grow apex**
Non-sex expressing	*Female control*	$0 \pm 0^{a,b}$	$0 \pm 0^{a,b}$	$34.6 \pm 3.05^{a,b}$	184.8 ± 67.07^{f}	$3.6 \pm 4.34^{a,b}$	13.2 ± 4.87^{a}
	Male control	$0 \pm 0^{a,b}$	$1 \pm 2.24^{a,b,c}$	$30.4 \pm 2.3^{a,b,c}$	$118.6 \pm 40.65^{d,e,f}$	28.2 ± 6.87^{c}	21 ± 8.28^{b}
	Female dried	0.42 ± 1.64^{a}	0.71 ± 1.73^{a}	$29.46 \pm 7.76^{a,b}$	97.04 ± 37.26^{e}	1 ± 2.21^{a}	2.75 ± 3.43^{c}
	Male dried	8.26 ± 5.63^{c}	$16.3 \pm 6.63^{d,e}$	2.61 ± 2.23^{d}	11.96 ± 11.58^{a}	0 ± 0^{a}	0.04 ± 0.21^{d}
Immature	*Female control*	$0 \pm 0^{a,b}$	$1.4 \pm 3.13^{a,b,c}$	$29.8 \pm 3.56^{a,b,c}$	$65.6 \pm 22.14^{a,b,c,d,e}$	2.4 ± 3.78^{a}	$4.8 \pm 3.11^{c,e}$
	Male control	$0.2 \pm 0.45^{a,b}$	$13.2 \pm 8.76^{b,c,d,e}$	$28.8 \pm 9.65^{a,b,c}$	$80.4 \pm 23.78^{c,d,e}$	1.8 ± 2.49^{a}	0 ± 0^{d}
	Female dried	0.91 ± 1.95^{a}	$9.74 \pm 8.5^{b,c}$	$21.09 \pm 10.43^{c,e}$	$63.43 \pm 32.56^{c,d}$	$1.52 \pm 3.57^{a,b}$	3.96 ± 3.39^{c}
	Male dried	$7.87 \pm 7.06^{b,c}$	18.52 ± 9.06^{e}	3.39 ± 5.95^{d}	$14.7 \pm 24.57^{a,b}$	0.3 ± 1.26^{a}	0 ± 0^{d}
Mature	*Female control*	$0 \pm 0^{a,b}$	$0.2 \pm 0.45^{a,b}$	$19.4 \pm 2.88^{a,c,e}$	$70 \pm 20.94^{b,c,d,e}$	$7 \pm 7.87^{b,d}$	$8.6 \pm 4.62^{a,e}$
	Male control	$0.6 \pm 0.89^{a,b}$	$1.8 \pm 1.48^{a,b,c}$	$34.6 \pm 1.82^{a,c,e}$	182.4 ± 86.97^{f}	12 ± 10.37^{d}	0 ± 0^{d}
	Female dried	$4.63 \pm 5.48^{a,b,c}$	2.46 ± 4.37^{a}	17.46 ± 9.06^{e}	49.42 ± 30.48^{c}	0.08 ± 0.41^{a}	$2.54 \pm 2.45^{c,d}$
	Male dried	$9.75 \pm 12.21^{b,c}$	$11.21 \pm 7.35^{c,d}$	1.46 ± 4.94^{d}	51.42 ± 38.3^{c}	0.04 ± 0.2^{a}	0 ± 0^{d}

Table 2: Results derived from the Kruskal-Wallis test, analyzing the impact of treatment on different parameters among females and males in control and dried conditions. The table showcases mean values accompanied by their standard deviations (± SD). Subscript letters denote significance observed in pairwise comparisons.

Parameters	**Treatment**				**Kruskal-Wallis**		
	Female		**Male**				
	Control	**Dried**	**Control**	**Dried**	X^2	**df**	**P**
Dead protonema (mean ± SD)	0 ± 0 ^{a}	0.28 ± 0.45 ^{a}	0 ± 0 ^{a}	0.24 ± 0.43 ^{a}	3.37	3	> 0.05
New protonemas filaments (mean ± SD)	0 ± 0 ^{a}	0.24 ± 0.44 ^{a}	0 ± 0 ^{a}	0.28 ± 0.45 ^{a}	3.38	3	> 0.05
Shoots produced (mean ± SD)	33.40 ± 15.82 ^{a,b}	13.28 ± 22.85 ^{a,c}	40.00 ± 12.28 ^{b}	12.40 ± 26.97 ^{c}	14.33	3	< 0.01
Shoots expressing sex (mean ± SD)	0.42 ± 0.84 ^{a}	0 ± 0 ^{a}	5.60 ± 4.27^{b}	0 ± 0^{a}	39.36	3	< 0.001

= 1, $p < 0.001$) had significant impacts. Male shoots exhibited a higher mean in producing only protonema (Table 1). Regarding shoots generating other shoots, Phenophase ($\chi^2 = 82.25$, df = 2, $p < 0.01$), Treatment ($\chi^2 = 384.20$, df = 1, $p < 0.001$), and Sex ($\chi^2 = 737.83$, df = 1, $p < 0.001$) had highly significant influences. Generally, the control treatment shoots showed a considerably higher mean (Table 1). Considering the total shoots produced, Phenophase ($\chi^2 = 371.87$, df = 2, p $p < 0.01$), Sex ($\chi^2 = 741.87$, df = 1, $p < 0.001$), and Treatment ($\chi^2 = 1621.74$, df = 1, $p < 0.001$) had highly significant impacts. Dried male shoots exhibited a lower mean, while control treatments registered a higher mean. For shoots expressing sex, Phenophase ($\chi^2 = 63.67$, df = 2, $p = 0.08$) and Sex ($\chi^2 = 26.66$, df = 1, $p = 0.15$) did not show statistical significance, but Treatment ($\chi^2 = 638.60$, df = 1, $p < 0.001$) was highly significant. Regarding shoots growing at the apex, Phenophase ($\chi^2 = 68.793$, df = 2, $p < 0.001$), Sex ($\chi^2 = 134.068$, df = 1, $p < 0.001$), and Treatment ($\chi^2 = 281.848$, df = 1, $p = 1.804e-15$) had a significant impact. However, this pattern was less common in male shoots, especially the dried ones. (Table 1).

Protonema regeneration

In general, there were no differences observed between control and desiccated treatments within the same gender, except for the parameters 'Shoots Produced' and 'Shoots Expressing Sex,' where the desiccated shoots exhibited a lower mean in both shoot production and expression of sex (Table 2). Among desiccated female protonemata, 7 died, yielding no shoots or new protonema filaments. Surviving desiccated female protonemata produced 332 shoots (an average of ~13 per dish), notably lower than the control's 167 shoots (~33.4 per dish). Desiccated male protonemata produced an average of 12 shoots per dish, while the male controls had 205 shoots (~46 per dish). Additionally, 7 female and 6 male protonemata produced new protonema filaments but no shoots. Interestingly, only 2 shoots from the female control group exhibited sexual expression, whereas none from desiccated females did. In contrast, 28 shoots from male controls and desiccated females expressed sex, differing from shoots produced from desiccated females.

DISCUSSION

In this study, we explore the relationship between the phenological stage of gametangia and desiccation resistance in *B. argenteum*. Our hypotheses suggest that as the phenological stage progresses, desiccation resistance influences the recovery of the photosynthetic system in shoots, with a more pronounced recovery in females compared to males. Additionally, we hypothesize that protonemata from male plants exhibit lower desiccation resistance compared to female plants. Our findings indicate that as phenology advances, the regeneration of photosynthesis in shoots decreases, implying a reproductive cost that varies between sexes, thus supporting our initial hypothesis. Concerning protonema, while some parameters were regenerated, others were not, and in certain cases, male protonemata showed greater success in regeneration, partially supporting our second hypotheses. These results enhance our understanding of how reproductive costs manifest in response to water deficit conditions in a species inhabiting a hostile environment with seasonal cycles of natural drought. Moreover, they highlight intrinsic factors related to gender in protonema desiccation tolerance.

Early phenophases and female shoots show greater desiccation recovery

We observed that in several measured parameters (Fm, Fv/Fm and ϕPSII), female shoots had higher averages than male shoots when subjected to desiccation, even when sexual structures had not been formed (non-sex expressing phenophase). This observation suggests that female shoots have a greater capacity to cope with and recover from the adverse effects of desiccation. Differences between sexes are expected due to the reproductive cost in different sexual functions (Obeso 2002). However, few studies have reported sex-based differences in the recovery of the photosynthetic system, and the majority of those that did focused on liverworts. Investigating the liverwort *Marchantia inflexa* Nees & Mont., Marks *et al.* (2016) examined the extent to which DT varies among populations and sexes of a species expected to exhibit a moderate DT phenotype. The results of this study confirmed the hypothesis that *M. inflexa* can tolerate moderate desiccation. Additionally, it was observed that females showed greater DT than males. This sex difference suggests the existence of DT variation within the species. However, when the populations were cultivated in a common garden, no significant differences in DT were observed. This indicates that DT variation may be influenced by the environment in which the shoots grow. In our experiment, both male and female genotypes were cloned to eliminate the effect of the environment, and even so, the patterns observed were different between the sexes. Comparing our findings with those reported by Marks *et al.* (2016), the difference in DT observed in *B. argenteum* suggests the presence of an intrinsic factor that influences this trait. Given that both male and female shoots, which do not express sexual characteristics, exhibited distinct patterns even without the influence of reproductive cost.

In Silva-e-Costa *et al.* (2022) they investigated the variation in DT in the liverwort *Plagiochila porelloides* (Torrey ex Nees) Lindenb. by considering colonies and sexes within the same riparian zone. Through shoot collection, controlled desiccation, and rehydration, the authors evaluated water status, photosynthesis, net carbon, and efficiency of photosystem II (PSII). The authors mentioned significant differences in DT between colonies and sexes (with female shoots showing better recovery of the photosynthetic system), especially in a more exposed colony. They also observed PSII recovery within 72 hours and positive carbon gain within 30 days after rehydration. These results highlight the adaptive plasticity of the species in response to desiccation and its ability to recover from environmental stresses. These studies are important for understanding the ecology and physiology of shoots in riparian environments, where water availability can vary considerably. Furthermore, the authors emphasize the importance of considering not only differences between colonies but also differences between sexes when studying DT in shoots species. In these two studies reporting DT in liverworts Marks *et al.* (2016) and Silva-e-Costa *et al.* (2022), noted that the difference between sexes is smaller in males compared to females due to a greater allocation of resources for reproduction in male shoots before zygote formation, resulting in costs in the recovery of the photosynthetic system..

It is reasonable to hypothesize that more advanced reproductive phenophases allocate a greater quantity of resources to reproductive structures compared to more recent phenophase, as predicted by life history theory (Oli and Coulson 2016). Consequently, it is expected that gametophytes have fewer resources as the phenophase advances, which could provide a plausible explanation for the obtained results. In all measured parameters, the recovery was higher in younger phenophase compared to older ones (from non-sex expressing to immature to mature). Indeed, there are examples in the literature showing that the younger life phase (protonema) is less tolerant to desiccation (Glime 2017). For example, Hájek and Vicherová (2014) reported that DT in *Sphagnum* mosses is inducible through hardening treatments, being more pronounced in certain species and in response to seasonal conditions, resulting in a trade-off between water retention capacity and the ability to develop physiological desiccation tolerance. Additionally, protonema stages are found to be less tolerant to desiccation than in shoots. On the other hand, in *Physcomitrella patens* (Hedw.) Bruch & Schimp., shoots can tolerate desiccation, but protonema stages do not, as reported by Gao *et al.* (2017). In contrast, Greenwood *et al.* (2019) reported that protonema stages of *B. argenteum*, when subjected to desiccation, exhibit decreased photosynthetic parameters but recover slowly compared to shoots. Furthermore, Coe *et al.* (2021) demonstrated that protonema and gemmae are more susceptible to desiccation damage compared to adult shoots of *S. caninervis*. Our findings are consistent with previous studies, highlighting the greater sensitivity to desiccation and slower regeneration observed in the protonema stage. One possible explanation for this increased sensitivity could be attributed to the simpler morphology of the protonema compared to the shoots.

Shoot and protonema regeneration explain patterns in adult populations of B. argenteum

The difference in growth phenophases revealed that the regeneration rate varies with sex and phenophase. Several measured parameters indicated that more advanced phenophases have a negative effect on regeneration. For example, the mortality rate is higher in the mature phenophase and lower in the non-sexually expressing phenophase, with the male sex consistently showing the highest mortality rate across all phenophases (Table 1). Our explanation for these findings is related to reproductive allocation in different sexual functions and phenophases, as observed in the dioicous moss *Weissia jamicenseis* (Mitt.) Grout. In this moss, Santos et al. (2022) observed a reproductive allocation (reproductive mass) gradient among shoots, increasing from non-sexually expressing female shoots to male shoots and then to female sporophytic shoots. On the other hand, the production of new shoots was inversely proportional to reproductive allocation in the shoots, indicating a trade-off between reproductive investment and the formation of new shoots, as observed in our findings. Regarding regeneration associated with desiccation tolerance (DT), Newton (1972) provided empirical evidence of the vulnerability of regenerating male leaves of *Mnium* to desiccation stress compared to female leaves. Furthermore, it has been hypothesized that sex-specific differential desiccation stress contributes to the increased mortality observed in male individuals expressing maleness in *Polytrichum commune* Hedw. In a 1-year study, all 50 plants that recently expressed maleness died, while non-expressing stems had a survival rate of only 34%. However, female stems were not evaluated, limiting the extent of sex-specific comparisons. Mortality in *P. commune* peaked during summer drought in the northern forest (Watson 1975). The production of a relatively costly antheridial cup may compromise the shoots ability to withstand severe desiccation stress. Additionally, male rarity (relative to females) has been indirectly linked to sex-specific water stress in the liverwort *Riccia frostii* Austin (Pettet 1967) and the moss *Syrrhopodon texanus* Sull. (Reese 1984), as well as in *S. caninervis* (Stark *et al.* 2005).

Based on the previously discussed studies, it becomes evident that male plants are more sensitive to desiccation compared to female plants. This pattern can be a limiting factor for adult population structures, especially sex ratio. In literature, is evident that the sex ratio in dioicous species tends to be biased towards the female sex (Bisang and Hedenäs 2005), with few species exhibiting populations biased towards the male sex (Shaw *et al.* 1992; Bisang and Hedenäs 2005; Laaka-Lindberg 2005; Alvarenga *et al.* 2013), since there are several reasons that bias populations. For instance, in the context of the joint growth of male and female plants, the female plant initially presents a larger protonema area, which restricts the space available for the development of male plants. This results in a population skewed towards the female sex, a trend supported by the female-biased sex ratio in *Ceratodon purpureus* (Hedw.) Brid. during the early stages of gametophytic development (Eppley *et al.* 2018). On the contrary, in *Syntrichia caninervis* Mitt., Ekwealor et al., (2017) found that populations at higher altitudes showed a significantly skewed female phenotype (17:1), while at lower altitudes, they lacked evident sexual dimorphism. Conversely, among shoots without sexual differentiation, employing molecular markers, a female sex ratio (2:1) was recorded within high-altitude populations, whereas at lower altitudes, only female plants were visible. A similar trend was evident in *S. caninervis* according to Ekwealor et al. (2022); nevertheless, in this scenario, the researchers note the identification of male plants in shadier locales compared to their female counterparts. One of the factors discussed in the literature is that abiotic factors such as desiccation and temperature can affect the sex ratio due to differences in abiotic stresses between the sexes (Glime 2017). *B. argenteum* is a species that exhibits a strong female sexual bias in adult populations, with evidence of greater sensitivity to desiccation stress in male plants (Gao *et al.* 2017; Greenwood *et al.* 2019). Therefore, it is expected that these differences are found, especially considering that reproductive allocation in the sexual function of *B. argenteum* is strongly accentuated in the male sexual function compared to the female function without sporophyte (Horsley *et al.* 2011). In a natural environment, it is expected that this species predominantly consists of female plants, as desiccation events are more frequent, resulting in higher mortality in male plants, as observed in *S. caninervis* (Stark *et al.* 2005). Additionally, other limiting factors for population structure are found in the environment, such as shading, solar radiation, and humidity, playing a crucial role in regulating the distribution and abundance of plants and directly influencing the species' adaptive response. One of our results that may favour a bias towards the female sex is that in female plants, when they are dried, their apex continues to grow, while in males it does not. Thus, it becomes evident that in dry cycles within populations, female plants tend to continue apex growth, while male plants stop apex growth and initiate the production of new protonemata and new shoots.

In this study, we explored the relationship between the phenological stage of gametangia and desiccation resistance in *B. argenteum*. Our hypotheses suggest that as the phenological stage progresses, desiccation resistance influences the recovery of the photosynthetic system in shoots, with a more pronounced recovery in females compared to males. Additionally, we hypothesized that protonemata from male plants exhibit lower desiccation resistance compared to female plants. Our results indicate that as phenology advances, the regeneration of photosynthesis in shoots decreases, implying a reproductive cost that varies between sexes, thus supporting our initial hypothesis. Within the desiccation context, several measured parameters (Fm, Fv/Fm, and ϕPSII) showed higher averages in female shoots compared to male shoots when subjected to desiccation, even in non-sex expressing phenophases. This observation suggests that female shoots possess a greater capacity to handle and recover from the adverse effects of desiccation. While some protonema parameters were regenerated, others were not, and in certain cases, male protonemata exhibited greater success in regeneration, partially supporting our second hypothesis. These findings expand our understanding of how reproductive costs manifest in response to water deficit conditions in a species inhabiting a hostile environment with seasonal cycles of natural drought. Furthermore, we suggest intrinsic factors related to sexes in protonema desiccation tolerance, as in this phase, the influence of sexual structures to incur reproductive costs is absent, yet differences were still observed.

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AUTHOR CONTRIBUTIONS

Material was collected by WLS, then sorted by WLS, KCP, and FP. Experimentation involved WLS with JG and AMR's assistance. Conceptual ideas were conceived by WLS, LRS, and JG. WLS performed data analysis and manuscript writing. All authors provided critical input to drafts and approved the final publication.

CONFLICT OF INTEREST

No conflict of interest is declared.

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LEGENDS

Figures

Figure 2: This scatterplot displays the variation of maximum fluorescence (Fm) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in Fm. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

Figure 3: This scatterplot displays the variation of maximum fluorescence (Fm) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in Fm. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

Figure 4: Figure 2: This scatterplot displays the variation of maximum quantum efficiency of photosystem II (Fv/Fm) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in Fv/Fm. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

Figure 5: This scatterplot displays the variation of the effective quantum yield of photosystem II (ϕPSII) over time for different phenophases (Non-sex expressing, immature, and mature). The X-axis depicts time (Control, 0.5, 6, 12, and 24), while the Y-axis reflects the variation in ϕPSII. The points on the graph represent the average of the variable for different groups. The dark and light blue points indicate males in the control and desiccated groups, respectively. Similarly, the dark and light green points represent females in the control and desiccated groups, respectively.

Figure 6: This image displays three scatterplots A, B, and C, each representing the respective photosynthetic parameters Fm, Fv/Fm, ϕPSII during protonema regeneration. The X-axis in all graphs represents time, with categories (Control, 0.5, 6, 12, and 24) indicated. On the Y-axis, we have the corresponding variable for each photosynthetic parameter (Fm, Fv/Fm, ϕPSII). The points at each time point represent the average of the variable for different groups. Dark and light blue points correspond to males in control and desiccated groups, respectively. Similarly, dark and light green points represent females in control and desiccated groups, respectively.

Tables

Table 1: Shoot category characteristics. Values are represented as mean ± standard deviation. Subscript letters indicate significantly different parameters. Please note that letters should be compared within each column separately. GLM results are detailed in the text.

Table 2: Results derived from the Kruskal-Wallis test, analyzing the impact of treatment on different parameters among females and males in control and dried conditions. The table showcases mean values accompanied by their standard deviations (± SD). Subscript letters denote significance observed in pairwise comparisons.

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GENERAL CONCLUSION

The analysis of the different parts of this thesis reveals a comprehensive and detailed approach to the reproductive biology, distribution, and ecophysiology of tropical mosses, with specific investigations into aspects such as sexual expression, reproductive allocation, and costs, as well as detailed examinations of the distribution patterns of moss species, particularly focusing on the relationship between sexual systems and functional traits within *Fissidens* species. Additionally, thorough investigations into the ecophysiology of *Bryum argenteum* across different forest ecosystems are presented, highlighting phenological variations and genotypic differences in response to environmental conditions.

In the first part, the importance of investigating various aspects of reproductive biology, such as sexual expression, reproductive allocation, costs, and trade-offs, is emphasized to better understand the reproduction of tropical acrocarpous mosses. The studies highlight differences in reproductive strategies among different species, as well as the need for standardization in methodologies and terminology to advance research in this area.

The second part of the thesis explores the relationship between the sexual systems of Fissidens species and their functional traits, as well as the distribution of these species in different environments. The results indicate significant variations in functional traits among sexual systems, suggesting specific adaptations that may influence the survival and distribution of Fissidens species, especially in response to global climate change.

The third part addresses ecophysiological studies of Bryum argenteum in different forest ecosystems. These studies highlight genotypic differences between populations from different environments, as well as distinct phenological patterns between male and female genotypes. Additionally, the research underscores the importance of considering phenophases in understanding the desiccation tolerance of these species.

The thesis provides a comprehensive insight into various aspects of tropical moss biology, highlighting the importance of interdisciplinary research to understand the adaptations of these plants in different environments. The findings contribute not only to the basic knowledge of reproductive biology, distribution, and ecology of mosses but also have practical implications for the conservation and management of these species in the face of environmental changes, especially in the context of global climate change. Standardization of methodologies and terminology emerges as a critical need to advance the field and enable meaningful comparisons between studies.

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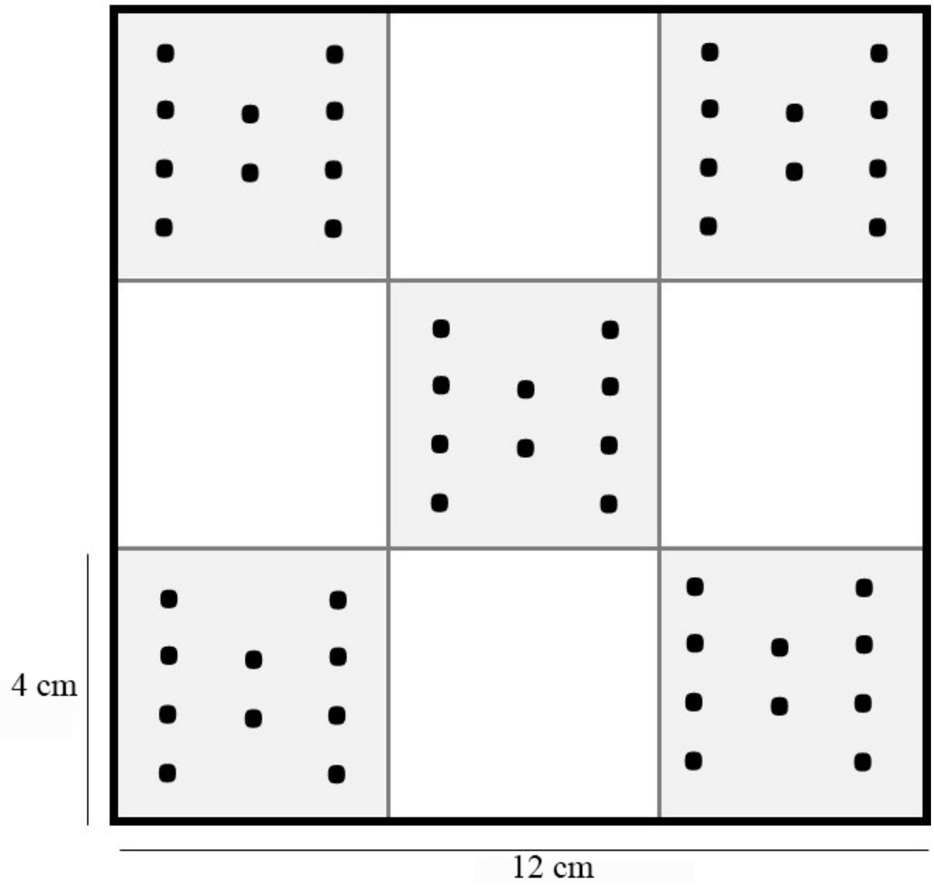
APPENDICES

Chapter 1

Santos *et al*.—American Journal of Botany 2022—Appendix S1

Appendix S1 -



Appendix S1 – Scheme of the five 4 × 4 cm quadrants (highlighted in gray) selected in each germination box for collection of ramets of *Weissia jamaicensis*. Black dots indicate the points where ramets were intentionally collected.

Chapter 2

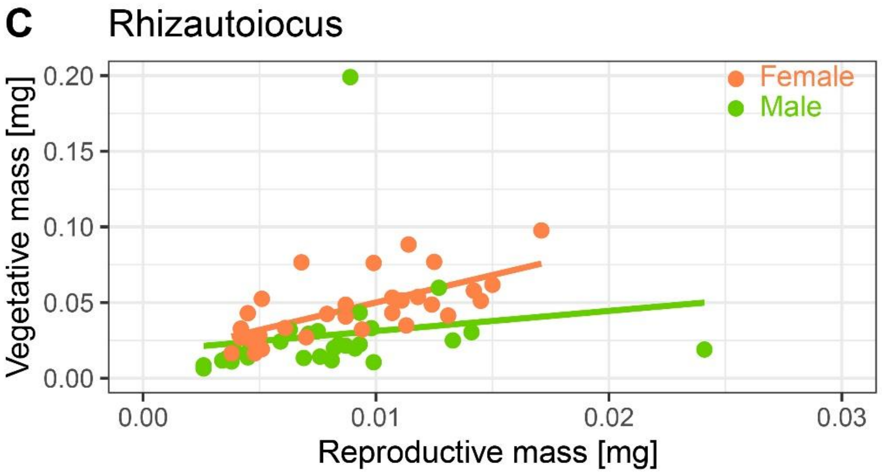
Chi-square of sex ratio

Metapopulation	X^2	Df	P
1	868.43	1	$< 2.2^{e-16}$
2	530	1	$< 2.2^{e-16}$
3	744.24	1	$< 2.2^{e-16}$
4	127	1	$< 2.2^{e-16}$
5	-	-	-
6	729.67	1	$< 2.2^{e-16}$
7	812.13	1	$< 2.2^{e-16}$
8	250	1	$< 2.2^{e-16}$
9	216	1	$< 2.2^{e-16}$
10	350.94	1	$< 2.2^{e-16}$

Chapter 3

Supplementary 1 - “Simpson effect” in male ramets of rhizautoicous species

Rhizautoicous			
	Df		Deviance
Reproductive mass	1		0.003*
Sex	1		0.005*
GLM - Summary			
	Estimate	Std. Error	t value
Female	1.463	0.729	2.00*
Male	-0.017	0.007	-2.50*
Observations			60
Akaike Inf. Crit.			-257.5
Null Deviance			0.052 (df = 59)
Residual Deviance			0.041 (df = 56)



Supplementary 2

Null *vers.* Full models Hypothesis 1						
	Resid. Df	Resid. Dev	Df	Deviance	Pr(>Chi)	
1	174	7,8963				
2	179	20,034	-5	-12,138	0,03295	*

Supplementary 2 - comparation of null and full models.

Chapter 5

species	longitude	latitude		species	longitude	latitude
Fissidens_asplenioides	-46,5	-21,916667		Fissidens_elegans	-65,38333	-10,8
Fissidens_asplenioides	-50,933889	-28,512222		Fissidens_elegans	-65,43333	-10,48333
Fissidens_asplenioides	-65,5	-27,13333		Fissidens_elegans	-68,08333	6,23333
Fissidens_asplenioides	-46,561389	-21,787778		Fissidens_elegans	-65,58333	-10,58333
Fissidens_asplenioides	-74,36666	4,35		Fissidens_elegans	-67,66666	-9,96666
Fissidens_asplenioides	-74,0244	11,1228		Fissidens_elegans	-68,33333	-9,83333
Fissidens_asplenioides	-74,035278	11,073611		Fissidens_elegans	-67,5	-10,16666
Fissidens_asplenioides	-73,4972	5,6875		Fissidens_elegans	-64,66666	-11,33333
Fissidens_asplenioides	-72,7275	5,44806		Fissidens_elegans	-64,5	-11,25
Fissidens_asplenioides	-72,38333	-37,21666		Fissidens_elegans	-46,942222	-22,372222
Fissidens_asplenioides	-71,13	8,4		Fissidens_elegans	-64,66666	-11,01666
Fissidens_asplenioides	-71,66666	8,61666		Fissidens_elegans	-41,633333	-12,433333
Fissidens_asplenioides	-72,98333	7,2		Fissidens_elegans	-44,358611	-9,074444
Fissidens_asplenioides	-71,5	-36,51666		Fissidens_elegans	-64,416667	-0,416667
Fissidens_asplenioides	-78,56666	-7,9		Fissidens_elegans	-67,089167	-0,130278
Fissidens_asplenioides	-72,48333	-13,15		Fissidens_elegans	-74,91666	4,71666
Fissidens_asplenioides	-77,9	-6,75		Fissidens_elegans	-36,011667	-8,827778
Fissidens_asplenioides	-78,81666	-7,41666		Fissidens_elegans	-46,533333	-23,462778
Fissidens_asplenioides	-55,75	-15,43333		Fissidens_elegans	-63,23333	7,46666
Fissidens_asplenioides	-44,446667	-22,468889		Fissidens_elegans	-76,58333	-6,41666
Fissidens_asplenioides	-78,66666	-0,33333		Fissidens_elegans	-64,4	6,23333
Fissidens_asplenioides	-70,33333	9,31666		Fissidens_elegans	-63,11666	-9,08333
Fissidens_asplenioides	-71,73333	-39,36666		Fissidens_elegans	-45,31	-23,221667
Fissidens_asplenioides	-76,71666	3,36666		Fissidens_elegans	-54,916667	-9,5
Fissidens_asplenioides	-72,31666	-40,75		Fissidens_elegans	-50,426944	-26,960833
Fissidens_asplenioides	-72,66666	-40,66666		Fissidens_elegans	-55,25	-7,66666
Fissidens_asplenioides	-90,35	-0,53333		Fissidens_elegans	-55,66666	-5,91666
Fissidens_asplenioides	-46,86	-23,489722		Fissidens_elegans	-48,765556	-20,613056
Fissidens_asplenioides	-89,45	-0,86666		Fissidens_elegans	-69,7	11,18333
Fissidens_asplenioides	-47,925556	-24,387778		Fissidens_elegans	-41,833333	-20,45
Fissidens_asplenioides	-47,459722	-24,281389		Fissidens_elegans	-48,9	-13,616667
Fissidens_asplenioides	-44,959722	-23,079722		Fissidens_elegans	-74,83333	6,08333
Fissidens_asplenioides	-44,8	-22,716667		Fissidens_elegans	-51,418889	-20,367222
Fissidens_asplenioides	-44,6	-22,683333		Fissidens_elegans	-43,669722	-19,88
Fissidens_asplenioides	-51,18333	-25,3		Fissidens_elegans	-65,16666	-9,71666
Fissidens_asplenioides	-52,425833	-29,7175		Fissidens_elegans	-51,083056	-14,920278
Fissidens_asplenioides	-45,839722	-22,956667		Fissidens_elegans	-43,714444	-19,168889
Fissidens_asplenioides	-45,886944	-23,179444		Fissidens_elegans	-50,033333	-25,216667

Fissidens_asplenioides	-44,323333	-22,683611		Fissidens_elegans	-49,273056	-25,427778
Fissidens_asplenioides	-73,7	-42,35		Fissidens_elegans	-54,61666	-25,56666
Fissidens_asplenioides	-64,2	-21,46666		Fissidens_elegans	-56,95	-25,98333
Fissidens_asplenioides	-78,633	-2,8		Fissidens_elegans	-75,487747	4,710416
Fissidens_asplenioides	-79,11666	-1,41666		Fissidens_elegans	-44,75	-22,36666
Fissidens_asplenioides	-67,66666	10,41666		Fissidens_elegans	-48,45	-24,33333
Fissidens_asplenioides	-67,24722	10,4125		Fissidens_elegans	-46,28333	-23,45
Fissidens_asplenioides	-51,133333	-28,883333		Fissidens_elegans	-53,267	3,6
Fissidens_asplenioides	-75,08333	4,7		Fissidens_elegans	-56,88333	4,75
Fissidens_asplenioides	-77,85	-0,3833		Fissidens_elegans	-55,23333	4,86666
Fissidens_asplenioides	-71,35	8,56666		Fissidens_elegans	-52,05	4,5
Fissidens_asplenioides	-52,1675	-22,5325		Fissidens_elegans	-53,2	3,583
Fissidens_asplenioides	-45,555278	-23,026389		Fissidens_elegans	-64,55	-23,1
Fissidens_asplenioides	-76	-9,66666		Fissidens_elegans	-56,85	-26,06666
Fissidens_asplenioides	-45,31	-23,221667		Fissidens_elegans	-73,281389	4,849444
Fissidens_asplenioides	-76,4	-6,66666		Fissidens_elegans	-73,316667	4,766667
Fissidens_asplenioides	-69,56666	9,71666		Fissidens_elegans	-72,561667	-8,936389
Fissidens_asplenioides	-41,243889	-20,368333		Fissidens_elegans	-67,81166	-14,66
Fissidens_asplenioides	-68,11666	-16,15		Fissidens_elegans	-56,45666	3,14
Fissidens_asplenioides	-50,977778	-25,213056		Fissidens_elegans	-56,45083	3,00083
Fissidens_asplenioides	-65,27	-17,4594		Fissidens_elegans	-64,31888	-19,00527
Fissidens_asplenioides	-50,604444	-25,23		Fissidens_elegans	-64,62944	-22,18305
Fissidens_asplenioides	-50,326111	-27,816111		Fissidens_elegans	-36,366667	-9,266667
Fissidens_asplenioides	-44,675	-22,501667		Fissidens_elegans	-47,066667	-1,766667
Fissidens_asplenioides	-67,23333	-16,75		Fissidens_elegans	-63,84666	-19,45138
Fissidens_asplenioides	-56,83333	-26,05		Fissidens_elegans	-63,86111	-19,75833
Fissidens_asplenioides	-73,5872	4,73611		Fissidens_elegans	-48,105	-15,505833
Fissidens_asplenioides	-71,75	8,2333		Fissidens_elegans	-64,38055	-20,855
Fissidens_asplenioides	-75,36666	-10,56666		Fissidens_elegans	-47,727333	-15,663167
Fissidens_asplenioides	-48,053056	-23,591667		Fissidens_elegans	-35,156667	-7,0425
Fissidens_asplenioides	-44,8333	-22,0833		Fissidens_elegans	-47,612222	-23,445556
Fissidens_asplenioides	-78,583	-0,3		Fissidens_elegans	-68,166667	-15,25
Fissidens_asplenioides	-70,68333	8,85		Fissidens_elegans	-48,046944	-15,581111
Fissidens_asplenioides	-75,416667	6,166667		Fissidens_elegans	-63,718333	-18,702222
Fissidens_asplenioides	-75,5031	6,22444		Fissidens_elegans	-63,773889	-18,195833
Fissidens_asplenioides	-74,416667	7,083333		Fissidens_elegans	-46,235	-23,499444
Fissidens_asplenioides	-75,25	5,916667		Fissidens_elegans	-41,890556	-13,2875
Fissidens_asplenioides	-75,3	5,66666		Fissidens_elegans	-41,852778	-13,086944
Fissidens_asplenioides	-76,08333	6,41666		Fissidens_elegans	-50,806944	-29,144444
Fissidens_asplenioides	-72,16666	-40,66666		Fissidens_elegans	-53,738	-29,629611
Fissidens_asplenioides	-75,91666	5,58333		Fissidens_elegans	-51,154639	-29,172833
Fissidens_asplenioides	-48,593056	-24,585556		Fissidens_elegans	-39,46025	-7,273075

Fissidens_asplenioides	-75,5	6,06666		Fissidens_elegans	-39,328611	-7,364722
Fissidens_asplenioides	-67,21666	-16,96666		Fissidens_elegans	-51,928	-23,430611
Fissidens_asplenioides	-67,28333	-16,95		Fissidens_radicans	-38,081389	-11,660278
Fissidens_asplenioides	-76,0203	5,965		Fissidens_radicans	-65,033333	-0,5
Fissidens_asplenioides	-72,71666	-37,26666		Fissidens_radicans	-35,691667	-6,963333
Fissidens_asplenioides	-50,161944	-25,095		Fissidens_radicans	-34,881111	-8,053889
Fissidens_asplenioides	-73,095	5,9275		Fissidens_radicans	-39,98333	-14,14972
Fissidens_asplenioides	-77,1881	1,1775		Fissidens_radicans	-55,25	-7,666667
Fissidens_asplenioides	-37,42213	-11,39628		Fissidens_radicans	-35,142056	-6,741861
Fissidens_asplenioides	-69	-14,75		Fissidens_radicans	-53,2	3,617
Fissidens_asplenioides	-76,16666	6,5		Fissidens_radicans	-59,4833	5,16667
Fissidens_asplenioides	-75,46666	7,16666		Fissidens_radicans	-59,7	4,68333
Fissidens_asplenioides	-75,505947	4,710384		Fissidens_radicans	-56,88333	4,75
Fissidens_asplenioides	-67,53333	-15,95		Fissidens_radicans	-56	4,75
Fissidens_asplenioides	-75,78333	5,78333		Fissidens_radicans	-55,23333	4,86666
Fissidens_asplenioides	-77,65	1,23333		Fissidens_radicans	-39,172222	-16,379722
Fissidens_asplenioides	-75,375	5,041667		Fissidens_radicans	-53,03583	3,75194
Fissidens_asplenioides	-76,63333	3,31666		Fissidens_radicans	-56,1975	3,91888
Fissidens_asplenioides	-77,291667	1,258333		Fissidens_radicans	-56,46083	3,15138
Fissidens_asplenioides	-77,36666	1,3		Fissidens_radicans	-35,943333	-9,306667
Fissidens_asplenioides	-75,01666	2,76666		Fissidens_radicans	-36,35	-9,366667
Fissidens_asplenioides	-75,78333	5,31666		Fissidens_radicans	-36,516667	-9,466667
Fissidens_asplenioides	-75,53333	4,45		Fissidens_radicans	-35,023611	-6,374444
Fissidens_asplenioides	-76,4261	2,38083		Fissidens_radicans	-35,430278	-9,259167
Fissidens_asplenioides	-77,11666	1,16666		Fissidens_radicans	-37,342222	-10,754444
Fissidens_asplenioides	-67,38333	-16,8		Fissidens_radicans	-48,919306	-25,437778
Fissidens_asplenioides	-67,31666	-16,78333		Fissidens_radicans	-49,666528	-24,164028
Fissidens_asplenioides	-76,3833	2,4917		Fissidens_radicans	-39,325278	-17,090556
Fissidens_asplenioides	-76,9547	2,50222		Fissidens_radicans	-41,890556	-13,2875
Fissidens_asplenioides	-77,18333	1,35		Fissidens_radicans	-39,169722	-7,406075
Fissidens_asplenioides	-44,75	-22,36666		Fissidens_zollingeri	-60,67777	-19,98194
Fissidens_asplenioides	-48,45	-24,33333		Fissidens_zollingeri	-45,141111	-7,084444
Fissidens_asplenioides	-75,4975	4,61333		Fissidens_zollingeri	-45,333333	-7,383333
Fissidens_asplenioides	-74,3	4,53333		Fissidens_zollingeri	-68,38333	11,03333
Fissidens_asplenioides	-64,033333	-18,483333		Fissidens_zollingeri	-46,369444	-14,093056
Fissidens_asplenioides	-64,08527	-18,51805		Fissidens_zollingeri	-79,9	-1,333
Fissidens_asplenioides	-46,38333	-21,93333		Fissidens_zollingeri	-47,92944	-15,77972
Fissidens_asplenioides	-76,33333	1,48333		Fissidens_zollingeri	-45	-12,13333
Fissidens_asplenioides	-77,88333	1,25		Fissidens_zollingeri	-47,52444	-14,70583
Fissidens_asplenioides	-77,11666	0,96666		Fissidens_zollingeri	-40,072222	-19,391111
Fissidens_asplenioides	-79,3	-4		Fissidens_zollingeri	-48,365833	-21,603333
Fissidens_asplenioides	-76,51388	4,06527		Fissidens_zollingeri	-51,206667	-21,028333

Fissidens_asplenioides	-65,73333	-17,15		Fissidens_zollingeri	-47,416389	-6,329444
Fissidens_asplenioides	-64,83333	-23,06666		Fissidens_zollingeri	-47,491667	-5,526389
Fissidens_asplenioides	-65,76666	-27,13333		Fissidens_zollingeri	-63,11666	10,55
Fissidens_asplenioides	-72,433333	-40,716667		Fissidens_zollingeri	-42,139167	-19,789722
Fissidens_asplenioides	-64,16722	-17,975		Fissidens_zollingeri	-46,221389	-23,315556
Fissidens_asplenioides	-72,56666	-44,33333		Fissidens_zollingeri	-54,646389	-20,442778
Fissidens_asplenioides	-73,8367	5,10917		Fissidens_zollingeri	-74,36666	-5,51666
Fissidens_asplenioides	-64,03333	-18,56666		Fissidens_zollingeri	-76	-9,16666
Fissidens_asplenioides	-78,55	-3,03333		Fissidens_zollingeri	-47,459722	-24,281389
Fissidens_asplenioides	-78,95	-4,1		Fissidens_zollingeri	-46,783333	-20,716667
Fissidens_asplenioides	-78,983333	-4,05		Fissidens_zollingeri	-65,38333	-10,8
Fissidens_asplenioides	-64,23333	-17,83333		Fissidens_zollingeri	-65,51666	-10,66666
Fissidens_asplenioides	-39,466667	-12,85		Fissidens_zollingeri	-68,33333	-9,83333
Fissidens_asplenioides	-73,31666	-37,95		Fissidens_zollingeri	-64,66666	-11,01666
Fissidens_asplenioides	-73,48333	-37,6		Fissidens_zollingeri	-64,5	-11,25
Fissidens_asplenioides	-72,78333	-36,73333		Fissidens_zollingeri	-46,5525	-23,123611
Fissidens_asplenioides	-63,7	-18,18333		Fissidens_zollingeri	-41,616667	-12,2
Fissidens_asplenioides	-64,01666	-18,66666		Fissidens_zollingeri	-38,616667	-9,75
Fissidens_asplenioides	-78,38333	-2,2		Fissidens_zollingeri	-63,25	6,93333
Fissidens_asplenioides	-64,68333	-17,83333		Fissidens_zollingeri	-48,119444	-24,532778
Fissidens_asplenioides	-72,18333	-37,05		Fissidens_zollingeri	-65,033333	-0,5
Fissidens_asplenioides	-39,7	-12,75		Fissidens_zollingeri	-46,636111	-23,5475
Fissidens_asplenioides	-63,80027	-18,10888		Fissidens_zollingeri	-35,018333	-8,002222
Fissidens_asplenioides	-64,01666	-20,53333		Fissidens_zollingeri	-35,691667	-6,963333
Fissidens_asplenioides	-64,31666	-19,56666		Fissidens_zollingeri	-40,190833	-20,057222
Fissidens_asplenioides	-64,31888	-19,00527		Fissidens_zollingeri	-42,704444	-21,887778
Fissidens_asplenioides	-64,48527	-18,72861		Fissidens_zollingeri	-55,25	-7,666667
Fissidens_asplenioides	-65,38555	-18,93527		Fissidens_zollingeri	-48,9175	-27,098056
Fissidens_asplenioides	-68,370278	-14,620833		Fissidens_zollingeri	-50,518611	-23,150833
Fissidens_asplenioides	-68,44833	-15,03		Fissidens_zollingeri	-51,938611	-23,425278
Fissidens_asplenioides	-64,54944	-22,24916		Fissidens_zollingeri	-47,2225	-23,656389
Fissidens_asplenioides	-64,62944	-22,18305		Fissidens_zollingeri	-35,158611	-8,663611
Fissidens_asplenioides	-64,63055	-21,97916		Fissidens_zollingeri	-43,95	-19,53333
Fissidens_asplenioides	-64,52555	-22,03444		Fissidens_zollingeri	-50,333333	-29,333333
Fissidens_asplenioides	-63,91805	-18,06166		Fissidens_zollingeri	-50,08333	-29,13333
Fissidens_asplenioides	-64,07111	-19,30083		Fissidens_zollingeri	-48,333333	-24,7
Fissidens_asplenioides	-64,43111	-22,15861		Fissidens_zollingeri	-48,881944	-13,653611
Fissidens_asplenioides	-64,70694	-22,175		Fissidens_zollingeri	-52,7	5,11666
Fissidens_asplenioides	-73,183	-39,667		Fissidens_zollingeri	-46,838889	-23,005833
Fissidens_asplenioides	-43,071944	-22,553889		Fissidens_zollingeri	-76,383333	-0,5333333
Fissidens_asplenioides	-43,071944	-22,620556		Fissidens_zollingeri	-46,84	-23,639722
Fissidens_asplenioides	-68,83888	-15,20833		Fissidens_zollingeri	-62,466389	-10,438889

Fissidens_asplenioides	-63,67222	-18,28361		Fissidens_zollingeri	-51,083056	-14,920278
Fissidens_asplenioides	-64,24027	-21,01472		Fissidens_zollingeri	-43,714444	-19,168889
Fissidens_asplenioides	-64,2725	-21,0925		Fissidens_zollingeri	-35,142056	-6,741861
Fissidens_asplenioides	-79,0775	-3,97138		Fissidens_zollingeri	-41,05	-20,93333
Fissidens_asplenioides	-78,66166	-4,24361		Fissidens_zollingeri	-76,08333	4,33333
Fissidens_asplenioides	-78,42888	-3,57916		Fissidens_zollingeri	-76,63333	7,75
Fissidens_asplenioides	-64,03333	-20,3225		Fissidens_zollingeri	-38,510833	-12,971111
Fissidens_asplenioides	-51,16666	-29,16666		Fissidens_zollingeri	-38,68	-12,6275
Fissidens_asplenioides	-68,76777	-15,31944		Fissidens_zollingeri	-39,21666	-14,83333
Fissidens_asplenioides	-65,415	-19,0075		Fissidens_zollingeri	-72,916667	11,1
Fissidens_asplenioides	-65,46611	-18,87972		Fissidens_zollingeri	-75,349998	10,55
Fissidens_asplenioides	-59,75972	-18,33055		Fissidens_zollingeri	-38,955833	-12,618333
Fissidens_asplenioides	-68,58777	-14,77333		Fissidens_zollingeri	-53,2	3,617
Fissidens_asplenioides	-63,86111	-19,75833		Fissidens_zollingeri	-77,25	5,61666
Fissidens_asplenioides	-49,075	-27,920833		Fissidens_zollingeri	-38,419167	-12,135556
Fissidens_asplenioides	-64,34861	-20,14444		Fissidens_zollingeri	-57,833	-28,783
Fissidens_asplenioides	-64,44361	-20,21444		Fissidens_zollingeri	-71,473056	-8,5233333
Fissidens_asplenioides	-64,23916	-19,95916		Fissidens_zollingeri	-71,209722	-8,3533333
Fissidens_asplenioides	-64,38055	-20,855		Fissidens_zollingeri	-71,308333	-8,4108333
Fissidens_asplenioides	-63,65833	-21,66611		Fissidens_zollingeri	-62,81527	-18,23888
Fissidens_asplenioides	-65,812778	-17,234722		Fissidens_zollingeri	-55,08333	5,33333
Fissidens_asplenioides	-63,9	-18,733333		Fissidens_zollingeri	-55,23333	4,86666
Fissidens_asplenioides	-63,923611	-18,665833		Fissidens_zollingeri	-53,283	3,617
Fissidens_asplenioides	-68,9775	-14,69		Fissidens_zollingeri	-74,285	4,65556
Fissidens_asplenioides	-68,000278	-14,772222		Fissidens_zollingeri	-39,172222	-16,379722
Fissidens_asplenioides	-46,16	-23,757222		Fissidens_zollingeri	-64,51666	-23,1
Fissidens_asplenioides	-64,255833	-18,366111		Fissidens_zollingeri	-55,4459	-15,2738
Fissidens_asplenioides	-37,342222	-10,754444		Fissidens_zollingeri	-59,63333	-19,98333
Fissidens_asplenioides	-64,506389	-17,876945		Fissidens_zollingeri	-64,16666	-17,88333
Fissidens_asplenioides	-66,861389	-16,953611		Fissidens_zollingeri	-63,63333	-18,11666
Fissidens_asplenioides	-37,906111	-10,906111		Fissidens_zollingeri	-63,43333	-17,9
Fissidens_asplenioides	-48,918278	-25,436194		Fissidens_zollingeri	-60,68333	-18,51666
Fissidens_asplenioides	-49,70375	-25,741056		Fissidens_zollingeri	-38,283333	-12,516667
Fissidens_asplenioides	-46,7175	-18,652778		Fissidens_zollingeri	-63,2	-18,03333
Fissidens_asplenioides	-47,966111	-15,983917		Fissidens_zollingeri	-62,4	-16,28333
Fissidens_asplenioides	-44,531194	-22,430333		Fissidens_zollingeri	-64,50722	-16,66555
Fissidens_asplenioides	-42,04199	-20,04352		Fissidens_zollingeri	-69,133333	-11,316667
Fissidens_asplenioides	-36,435254	-9,236536		Fissidens_zollingeri	-70,1	-12,566667
Fissidens_asplenioides	-49,957639	-25,189167		Fissidens_zollingeri	-56,47194	3,09472
Fissidens_asplenioides	-50,324111	-29,505333		Fissidens_zollingeri	-56,45083	3,00083
Fissidens_asplenioides	-50,93725	-28,866333		Fissidens_zollingeri	-35,778444	-6,6901389
Fissidens_asplenioides	-46,562222	-24,012778		Fissidens_zollingeri	-40,908889	-3,783333

Fissidens_asplenioides	-50,161278	-29,065278		Fissidens_zollingeri	-35,422222	-6,5376389
Fissidens_asplenioides	-53,738	-29,629611		Fissidens_zollingeri	-68,51083	-14,50333
Fissidens_asplenioides	-49,975217	-28,598203		Fissidens_zollingeri	-68,448333	-15,03
Fissidens_asplenioides	-49,739444	-28,5		Fissidens_zollingeri	-36,366667	-9,266667
Fissidens_asplenioides	-50,006528	-29,404361		Fissidens_zollingeri	-47,066667	-1,766667
Fissidens_asplenioides	-50,914722	-28,664722		Fissidens_zollingeri	-35,755556	-9,184722
Fissidens_asplenioides	-50,45	-28,756944		Fissidens_zollingeri	-66,10194	-11,02805
Fissidens_asplenioides	-41,837222	-20,41		Fissidens_zollingeri	-63,86111	-19,75833
Fissidens_asplenioides	-44,728056	-22,139111		Fissidens_zollingeri	-48,024167	-15,830278
Fissidens_asplenioides	-37,05966	-10,52876		Fissidens_zollingeri	-76,3975	-0,3447222
Fissidens_asplenioides	-37,69718	-11,53017		Fissidens_zollingeri	-49,246389	-20,288333
Fissidens_elegans	-57,4	4,81666		Fissidens_zollingeri	-35,115833	-6,409167
Fissidens_elegans	-56,16666	4,68333		Fissidens_zollingeri	-66,916667	-14,9
Fissidens_elegans	-46,636111	-23,5475		Fissidens_zollingeri	-35,156667	-7,0425
Fissidens_elegans	-49,810278	-16,020278		Fissidens_zollingeri	-47,612222	-23,445556
Fissidens_elegans	-74,0225	11,122778		Fissidens_zollingeri	-35,509444	-7,616111
Fissidens_elegans	-73,9619	4,56556		Fissidens_zollingeri	-52,592814	-17,623581
Fissidens_elegans	-75,93333	-9,15		Fissidens_zollingeri	-63,715278	-16,93
Fissidens_elegans	-46,673611	-18,945833		Fissidens_zollingeri	-45,316667	-7,183333
Fissidens_elegans	-53,215278	-17,314722		Fissidens_zollingeri	-48,55278	-25,1925
Fissidens_elegans	-61,35	1		Fissidens_zollingeri	-37,245972	-8,585639
Fissidens_elegans	-61	4,33333		Fissidens_zollingeri	-48,490178	-1,455731

species	longitude	latitude
Fissidens_zollingeri	-39,483333	-15,2
Fissidens_zollingeri	-37,737003	-11,034709
Fissidens_zollingeri	-37,05966	-10,52876
Fissidens_zollingeri	-37,17584	-10,69972

Table S2. Coefficients of the principal components selected from the principal component analysis performed with data from niche modeling for climate change scenarios.

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Annual Mean Temperature	0.268	0.254	-0.094	0.065	0.077	-0.004
Mean Diurnal Range	-0.193	0.236	-0.083	-0.459	-0.133	-0.504
Isothermality	0.239	-0.008	0.325	-0.061	0.106	-0.557
Temperature Seasonality	-0.244	0.046	-0.401	-0.001	-0.174	0.130
Max Temperature of Warmest Month	0.150	0.374	-0.318	0.004	-0.109	-0.046
Min Temperature of Coldest Month	0.300	0.114	0.056	0.179	0.091	0.017
Temperature Annual Range	-0.251	0.147	-0.311	-0.214	-0.194	-0.056
Mean Temperature of Wettest Quarter	0.211	0.303	-0.192	-0.062	0.161	-0.014
Mean Temperature of Driest Quarter	0.273	0.176	0.000	0.166	-0.062	-0.048
Mean Temperature of Warmest Quarter	0.200	0.317	-0.302	0.093	-0.013	0.052
Mean Temperature of Coldest Quarter	0.291	0.183	0.056	0.059	0.108	-0.048
Annual Precipitation	0.274	-0.200	-0.077	-0.193	-0.156	0.084
Precipitation of Wettest Month	0.277	-0.075	0.078	-0.282	-0.277	0.255
Precipitation of Driest Month	0.154	-0.347	-0.305	-0.027	0.094	-0.330
Precipitation Seasonality	-0.043	0.301	0.396	-0.374	-0.158	-0.038
Precipitation of Wettest Quarter	0.278	-0.086	0.072	-0.281	-0.270	0.243
Precipitation of Driest Quarter	0.164	-0.346	-0.301	-0.029	0.066	-0.297
Precipitation of Warmest Quarter	0.175	-0.161	-0.163	-0.548	0.447	0.236
Precipitation of Coldest Quarter	0.207	-0.193	-0.039	0.148	-0.651	-0.140
Variance explained for each PC (%)	52.59	20.46	11.09	5.51	4.16	2.73
Cumulative variance explained (%)	52.59	73.06	84.15	89.66	93.82	96.55

Table S3. Algorithms used to build species distribution models.

Algorithm	Acronym	Entry data	Model Overview	Reference
Generalized additive model	GAM	Presence and pseudo-absence	A non-parametric extension of the Generalized Linear Model (GLM), in which a smoothing function is used to adjust non-linear relationships. GAM is the only species distribution model in which each explanatory variable has one additive effect, resulting in the sum of functions from each variable.	Austin (2002)
Random Forest	RDF	Presence and pseudo-absence	A Machine Learning Method that clusters a high number of generated regression trees from randomly selected data subsamples. The present study used RDF to set 500 regression trees.	Breiman (2001); Liaw & Wiener (2002)
Support Vector Machine	SVM	Presence and pseudo-absence	A Machine Learning Method based on maximum-margin classifiers that incorporates non-linear limits. The predictor variables (i.e., support vectors) are projected in a multidimensional space applying a kernel density estimation, and then an ideal hyperplane is adjusted using an optimization function between training samples and the hyperplane itself. The present study adjusted models with the “C-svc” classifier and used a kernel function as the Gaussian radial basis.	Tax & Duin (2004); Karatzoglou et al. (2004)

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Table S4. TSS and AUC values for *Fissidens* species.

Species	**Algorithm**	**Sorensen**	**Sorensen***
Fissidens asplenioides Hedw.	Generalized Additive Models	0.94806	0.00551
	Random Forest	0.90307	0.05890
	Support Vector Machine	0.91696	0.04527
	Average of the best models	0.94806	0.00551
Fissidens elegans Brid.	Generalized Additive Models	0.84703	0.03751
	Random Forest	0.80884	0.03157
	Support Vector Machine	0.88336	0.04567
	Average of the best models	0.88336	0.04567
Fissidens radicans Mont.	Generalized Additive Models	0.72077	0.00918
	Random Forest	0.81318	0.06216
	Support Vector Machine	0.89192	0.03162
	Average of the best models	0.85736	0.05541
Fissidens zollingeri Mont.D.	Generalized Additive Models	0.81750	0.02999
	Random Forest	0.86007	0.06492
	Support Vector Machine	0.81750	0.02999
	Average of the best models	0.86007	0.06492

*Standard deviation

Scenarios	**Grid Number**	**Cell Size**	**Moran's I index Grid**	**Mess value Grid**	**SD Grid**
Fissidens asplenioides Hedw.	25	6.925	0.176	4.243	0.137
Fissidens elegans Brid.	24	6.643	0.400	3.817	0.167
Fissidens radicans Mont.	20	5.517	0.346	2.956	0.202
Fissidens zollingeri Mont.D.	22	6.080	0.330	3.653	0.046

Table S5. Moran’s, I index and Multivariate Environmental Similarity Surface

Chapter 7

Supplemental Table 1. Summary of data for samples collected from 52 populations of *Bryum argenteum* in dry and wet forest in Brazil.

Population	Latitude	Longitude	Substrate	Forest	Sporophyte
1	–8.23965900	–36.18717000	Rock	Dry forest	No
2	–8.23968300	–36.18718600	Rock	Dry forest	No
3	–8.23959700	–36.18712200	Rock	Dry forest	Yes
4	–8.23962100	–36.18714900	Rock	Dry forest	No
5	–8.24006800	–36.18745900	Rock	Dry forest	No
6	–8.24011100	–36.18764300	Rock	Dry forest	No
7	–8.24104600	–36.18721700	Rock	Dry forest	Yes
8	–8.24102300	–36.18717800	Rock	Dry forest	Yes
9	–8.24132500	–36.18716900	Rock	Dry forest	No
10	–8.24135800	–36.18719300	Rock	Dry forest	Yes
11	–8.24173600	–36.18720100	Rock	Dry forest	Yes
12	–8.24183100	–36.18733500	Rock	Dry forest	No
13	–8.24189100	–36.18757300	Rock	Dry forest	No
14	–8.24174700	–36.18691300	Rock	Dry forest	Yes
15	–8.24129200	–36.18680800	Rock	Dry forest	Yes
16	–8.24668800	–36.19091500	Rock	Dry forest	No
17	–8.24683900	–36.19149200	Rock	Dry forest	Yes
18	–8.24685500	–36.19151100	Rock	Dry forest	No
19	–8.24689600	–36.19149700	Rock	Dry forest	No
20	–8.24696700	–36.19152000	Rock	Dry forest	No
21	–8.23211900	–36.16291600	Rock	Dry forest	Yes
22	–8.23216500	–36.16296200	Rock	Dry forest	No
23	–8.23215900	–36.16294400	Soil	Dry forest	No
24	–8.23215300	–36.16300600	Rock	Dry forest	No
25	–8.23313600	–36.17131700	Rock	Dry forest	No
26	–8.23312800	–36.17128400	Rock	Dry forest	Yes
27	–8.23311500	–36.17125700	Rock	Dry forest	No
28	–8.23310200	–36.17118700	Rock	Dry forest	No
29	–8.23308800	–36.17114400	Rock	Dry forest	No
30	–8.23310200	–36.17115600	Rock	Dry forest	Yes
31	–8.24150100	–36.18586100	Rock	Dry forest	No
32	–8.24130100	–36.18647400	Rock	Dry forest	No
33	–8.23946000	–36.18677700	Rock	Dry forest	No
34	–8.23972900	–36.18721900	Soil	Dry forest	Yes
35	–8.24172100	–36.18722500	Rock	Dry forest	No
36	–8.24179900	–36.18729200	Rock	Dry forest	Yes
37	–8.24179300	–36.18729300	Rock	Dry forest	Yes
38	–8.24178100	–36.18728400	Rock	Dry forest	No
39	–8.24168400	–36.18722800	Rock	Dry forest	No

40	–8.17218300	–35.46161300	Rock	Dry forest	No
41	–8.17225900	–35.46167300	Rock	Dry forest	Yes
42	–8.17219100	–35.46182000	Rock	Dry forest	No
43	–8.17220000	–35.46180800	Rock	Dry forest	Yes
1	–23.73445600	–46.18153900	Rock	Wet forest	No
2	–23.73574900	–46.18460500	Rock	Wet forest	No
3	–23.73509700	–46.18471000	Rock	Wet forest	No
4	–23.73553800	–46.18481700	Rock	Wet forest	No
5	–23.73549300	–46.18478900	Rock	Wet forest	No
6	–23.73554300	–46.18480100	Rock	Wet forest	No
7	–23.73582400	–46.18324300	Rock	Wet forest	No
8	–23.73544000	–46.18300100	Rock	Wet forest	No
9	–23.73553600	–46.18324300	Rock	Wet forest	No

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Assinatura : ______________________________
Nome do(a) orientador(a): **Fábio Pinheiro**
RG n.° 28312820

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Caixa Postal 6109. 13083-970, Campinas, SP, Brasil
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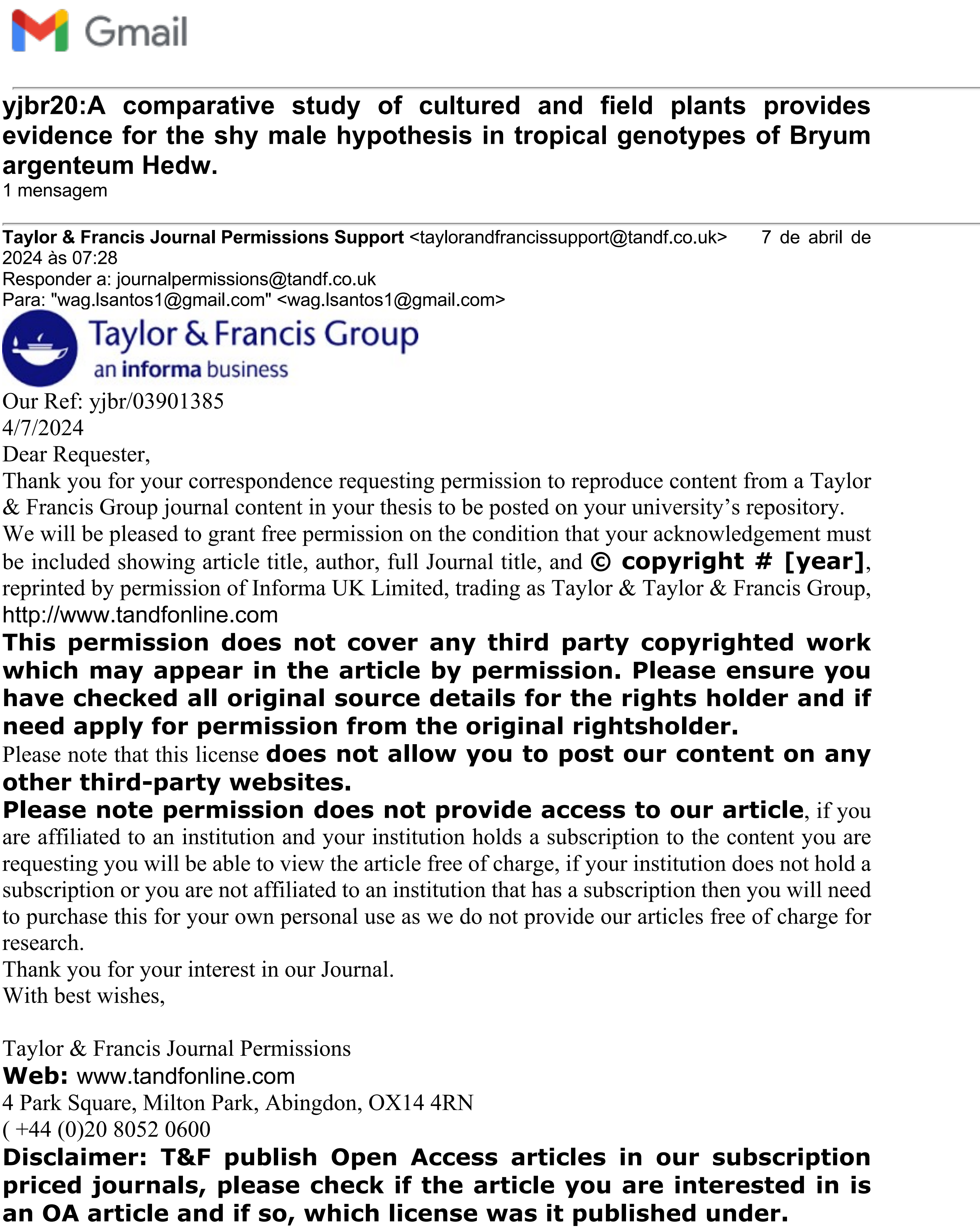

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Author name: Wagner Luiz dos Santosa*https://orcid.org/0000-0002-4434-2542, Kátia Cavalcanti Pôrtobhttps://orcid.org/0000-0002-1861-0216, Joshua Greenwoodc, Andie Davisc, Fábio Pinheiroahttps://orcid.org/0000-0003-3243-2652 & Lloyd R. Starkca Graduate Programme in Ecology, Biology Institute, Department of Plant Biology, Universidade Estadual de Campinas, Monteiro Lobato 255, 13083-862 Campinas – SP, Brazilb Biosciences Center, Department of Botany, Federal University of Pernambuco, Avenida Moraes Rego, s/n, 50670901 Recife – PE, Brazilc School of Life Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, USA Wagner Luiz dos Santos is a Ph.D. candidate enrolled in the Ecology Graduate Programme at the University of Campinas, Brazil.
Kátia Cavalcanti Pôrto is Professor of Plant Biology at the University of Pernambuco, Brazil.
Joshua Greenwood is a postdoctoral researcher at the University of Nevada, Las Vegas, USA.
Andie Davis is an undergraduate student of biology at the University of Nevada, Las Vegas, USA.
Fábio Pinheiro is Professor of Plant Biology at the University of Campinas.
Lloyd R. Stark is Professor of Ecology at the University of Nevada, Las Vegas, USA (https://www.researchgate.net/profile/Lloyd-Stark).
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Name: Wagner Luiz dos Santos
Street address: Alaor Ventura, 47.
Town: Itaú de Minas
Email: wag.lsantos1@gmail.com
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