UNIVERSIDADE FEDERAL DE MINAS GERAIS

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

Orvalina Augusta Teixeira

DE BAIXO PARA CIMA:

minhocas como carreadoras de propágulos de briófitas

Belo Horizonte - MG

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Orvalina Augusta Teixeira

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Orientador: Profa. Dra. Adaíses Simone Maciel Silva

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A amizade é uma palavra sagrada, é uma coisa santa e só pode existir entre pessoas de bem, só se mantém quando há estima mútua; conserva-se não tanto pelos benefícios quanto por uma vida de bondade (Étienne de La Boétie, 1577)

RESUMO

Briófitas também se beneficiam da zoocoria para sua dispersão, tal como as traqueófitas. Pode-se citar, por exemplo, moscas, lesmas, formigas e vertebrados, como aves e mamíferos. O campo das interações entre animais e briófitas é composto majoritariamente por estudos de campo, com algumas pesquisas experimentais. Portanto, possui um grande potencial para testar hipóteses envolvendo esses organismos. As briófitas exercem importantes serviços ecológicos como estabilização do solo, reabilitação de solo erodido e facilitar o estabelecimento de plantas vasculares. Realizando tantos papéis no ecossistema, é preciso conhecer as diversas formas como as briófitas se reproduzem e se dispersam. Dado sua importância, este estudo avaliou a capacidade de dispersão de briófitas por minhocas através de um experimento controlado. Foram utilizadas três espécies de briófitas, *Chryso-hypnum diminutivum* (Hampe) W.R. Buck, *Hyophila involuta* (Hook) A. Jaeger, *Fossombronia porphyrorhiza* (Nees) Prosk., para serem ingeridas por minhocas vermelhas californianas, *Eisenia andrei* Bouché. Aplicou-se dois tratamentos: "pó de briófitas", que consistiu em fragmentos de plantas obtidos através de atrito contra uma peneira, e "bloco de briófitas", uma parcela de 16cm^2 de colônia coletada. Os anelídeos entraram em contato com as plantas por um período de 7 dias e foram afastados para jejum. Após 2 dias em isolamento, as fezes foram coletadas e colocadas para germinar em recipientes hermeticamente fechados com vermiculita. Cada tratamento com 6 repetições, incluindo os grupos controles. Observou-se o crescimento de briófitas em 47,22% das unidades amostrais com inoculação de plantas por fezes de anelídeo, corroborando a hipótese inicial. A espécie que se saiu melhor foi *H. involuta*, seguida por *F. porphyrorhiza* e então *C. diminutivum.* Supõe-se que a espécie *H. involuta* obteve maior dispersão de unidades devido à produção de gemas, que foram ingeridas e dispersadas em grande quantidade. Enquanto *F. porphyrorhiza* conseguiu se dispersar, provavelmente, pela produção de esporos grandes, característicos do gênero. O tratamento pó de briófitas demonstrou mais emergências de plantas inoculadas com fezes do que o bloco de briófitas. A eficiência do pó de briófitas pode ser explicada pelos fragmentos mais fáceis de serem ingeridos pelos animais. Este é o primeiro experimento controlado que corrobora com a hipótese de que as minhocas conseguem dispersar briófitas. Este estudo também abre um leque de possibilidadespara se pensar como as minhocas interagem e impactam as colônias de briófitas terrícolas. Por exemplo, construir estruturas robustas de metapopulações ou como o trato digestivo de minhocas pode influenciar na quebra de dormência de gemas e esporos.

Palavras-chave: Annelida. Banco de diásporos. Gema. Dispersão. Zoocoria.

ABSTRACT

Bryophytes also benefit from zoocoria for their dispersal, as do tracheophytes. One can cite, for example, flies, slugs, ants, and vertebrates such as birds and mammals. The field of animal-bryophyte interactions is mostly composed of field studies, with a few experimental researches. Therefore, it has great potential for testing hypotheses involving these organisms. Bryophytes perform important ecological services such as soil stabilization, rehabilitation of eroded soil, and facilitating the establishment of vascular plants. By performing so many roles in the ecosystem, it is necessary to know the various ways in which bryophytes reproduce and disperse. Given their important role, this study evaluated the dispersal ability of bryophytes by earthworms through a controlled experiment. Three species of bryophytes were used, *Chryso- hypnum diminutivum* (Hampe) W.R. Buck, *Hyophila involuta* (Hook) A. Jaeger, *Fossombronia porphyrorhiza* (Nees) Prosk., to be ingested by red worms, *Eisenia andrei* Bouché. There were two treatments: "bryophyte dust", which consisted of plant fragments obtained by grinding against a sieve, and "bryophyte square", a 16cm² plot of the collected colony. The annelids came in contact with the plants for a period of seven days and were removed for fasting. After 2 days in isolation, the feces were collected and placed to germinate in airtight containers with vermiculite. Each treatment with 6 repetitions, including the control groups. Bryophyte growth wasobserved in 47.22% of the sample units with inoculation of plants by annelid feces, corroborating the initial hypothesis. The species that did best was *H. involuta*, followed by *F. porphyrorhiza* and then *C. diminutivum*. It is assumed that the species

H. involuta obtained greater dispersal of units due to the production of gemmae, which were ingested and dispersed in large numbers. While *F. porphyrorhiza* was able to disperse, probably, by the production of large spores, characteristic of the genus. The bryophyte dust treatment showed more plant emergings inoculated with feces than the bryophyte square. The efficiency of the bryophyte dust can be explained by the fragments being easier for the animals to ingest. This is the first controlled experiment that supports the hypothesis that earthworms can disperse bryophytes. This study also opens up a range of possibilities for thinking about how earthworms interact with and impact terrestrial bryophyte colonies. For example, building robust metapopulation structures or how the digestive tract of earthworms can influence the breakdown of gemmae and spore dormancy.

Keywords: Annelida. Diaspore bank. Dispersal. Gemmae. Zoochory.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

°C	degrees Celsius
cm^2	square centimetres
F	F-value
g	grams
h	hours
max.	maximum
min.	minimum
mL	millilitres
mm	millimetres
P	P-value
μm	micrometres
μmol	micromol

SUMÁRIO

APRESENTAÇÃO

Não é difícil constatar que para organismos terrestres sésseis, a dispersão é de suma importância para estabelecimento de metapopulações e aumento da variabilidade genética (OUBORG et al., 1999; SAASTAMOINEN et al., 2018). Há muitas formas que uma planta pode se dispersar pelo seu ambiente, uma delas é a anemocoria, dispersão pelo vento, presente em uma multitude de clados vegetais e principal forma de dispersão de briófitas (OUDTSHOORN; ROOYEN, 1999, VANDERPOORTEN et al., 2019). As briófitas são plantas terrestres poiquilohidricas que diferem de outras embriófitas por ter o gametófito (haploide) como fase de vida dominante e o esporófito (diploide) sendo uma etapa mais curta de seu ciclo de vida (GRADSTEIN et al., 2001; PROCTOR et al., 2007). Este grupo é composto por três filos: Bryophyta (musgos), Marchantiophyta (hepáticas) e Anthocerotophyta (antóceros) (GLIME, 2017). Essas plantas possuem ampla importância ambiental, tais como estabilização do solo para evitar erosão, reabilitação de solo erodido e melhorar o estabelecimento de plantas vasculares (LABERGE, et al., 2015; MEDEIROS et al., 2023). Quando encontradas no solo ou em rocha, juntamente com cianobactérias, líquenes, fungos e bactérias, são conhecidas como as crostas biológicas do solo e guardam um valor ecológico valioso de tal forma a serem reconhecidas como engenheiras de ecossistemas (BELNAP & LANGE, 2001; OLIVEIRA; MACIEL-SILVA, 2022). Sabemos que a dispersão afeta as (meta)populações de briófitas de várias maneiras. Como exemplo, muitos propágulos encontrados no ambiente não são oriundos da comunidade mais próxima e sim podendo ter origem em comunidades distantes. Além disso, características temporais, espaciais e estruturais do ambiente possuem maior influência na dispersão do que a distância (BARBÉ, et al., 2016).

Outro termo importante ao falar sobre dispersão de plantas é o “banco de diásporos”. Trata-se de uma reserva de propágulos vegetais assexuados ou sexuados que aguardam no solo até o momento oportuno para germinar (DURING, 2001). Briófitas que produzem muitos propágulos atuam como fontes para os bancos de diásporos e são muito importantes para a conservação de suas metacomunidades (LÖBEL; SNÄLL; RYDIN, 2006). Esses diásporos também estão sujeitos à influência de animais.

A relação de dispersão entre espécies vegetais e animais data do Paleozoico (TIFFNEY, 2004) e evoluiu a ponto de apresentar uma especificidade surpreendente entre dispersor e planta, apesar de ser mais raro do que se imagina (HERRERA, 1985). A dispersão de diásporos por animais, tanto vertebrados quanto invertebrados, zoocoria, adiciona um nível de complexidade capaz até de mudar a estrutura genética espacial de populações vegetais (VAN DER PIJL, 1982; GELMI- CANDUSSO; HEYMANN; HEER, 2017). Essa dispersão pode se dar em dois espaços distintos: fora do corpo do bicho, ao que chamamos de epizoocoria, ou dentro dele, podendo ser defecado ou regurgitado, denominando-se endozoocoria (VAN DER PIJL, 1982).

Apesar de existirem menos estudos sobre briófitas em comparação com espermatófitas, não podemos afirmar que essas pequenas plantas estejam isentas de serem transportadas e dispersadas por animais. No caso dos vertebrados, as aves desempenham um papel fundamental nesse processo, tanto por epizoocoria quanto por endozoocoria, podendo formar colônias de briófitas em locais distantes de sua origem (CHMIELEWSKI; EPPLEY, 2019). Além disso, cervos e javalis carregam fragmentos de plantas na superfície do corpo, e raposas-voadoras apresentam propágulos viáveis em suas fezes (HEINKEN et al., 2001; PARSONS et al., 2007). No entanto, os estudos com vertebrados são escassos e, na maioria das vezes, limitam-se a observações.

No que diz respeito aos invertebrados, há um número significativo de registros na literatura. Muitos desses registros envolvem anelídeos, aracnídeos e insetos, como besouros, moscas, formigas e gorgulhos, em contato com propágulos de briófitas. No entanto, alguns desses artigos não fornecem evidências sólidas de dispersão, representando apenas uma interação superficial. Um exemplo disso é o comportamento das formigas, que cortam as cápsulas de briófitas em épocas de escassez de sementes, mas as briófitas não conseguem se dispersar efetivamente por meio desse comportamento (LORIA; HERRNSTADT, 1980). Outro exemplo é o trabalho de Revill et al. (1967), que originalmente buscava registrar a dispersão de algas e protozoários por típulas e mosquitos, mas acabou identificando protonemas nos resultados. É importante ressaltar, no entanto, o caso da família Splachnaceae, também conhecida como "dung mosses" (musgos de esterco, em tradução livre). Essa família recebeu atenção considerável com vários estudos, tantoobservacionais quanto experimentais, dedicados a compreender a relação de entomofilia entre esses musgos e as moscas (MARINO; RAGUSO; GOFFINET,2009).

As minhocas, por sua vez, possuem um arcabouço teórico um pouco mais abrangente quando se trata de dispersão de sementes. Sabe-se que minhocas são capazes de ingerir sementes de até 3mm e elas podem dispersar verticalmente estas sementes e revirar o solo, trazendo para a superfície sementes que estavam inertes no subsolo. Além disso, os buracos escavados por estes organismo contribuem para a germinação e o recrutamento de plântulas. Sendo assim, minhocas possuem amplo impacto no banco de sementes do solo. Contudo, a maioria dos experimentos são feitos com apenas a espécie anécica, *Lumbricus terrestris* L. (DECAËNS et al., 2003; FOREY et al., 2011; MCTAVISH; MURPHY, 2021; MILCU; SCHUMACHER; SCHEU, 2006; REGNIER et al., 2008; SHUMWAY; KOIDE, 1994). De forma simplificada, as minhocas se dividem em três grupos ecológicos: Anécicas, que cavam túneis verticais no solo e alimentam-se de liteira em sua superfície; Epigeicas, vivem na liteira da superfície e se alimentam dela; Endogeicas, vivem em túneis verticais e horizontais nos horizontes superficiais do solo e se alimentam de matéria orgânica presente no mesmo (CARDOSO, 2016; CHATELAIN; MATHIEU, 2017).

Trabalhos que envolvem dispersão de esporos por minhocas limitam-se a: dois sobre briófitas, um sobre fungos e um com pteridófitas (DURING et al., 1987; GANGE, 1993; HAMILTON; Lloyd, 1991; VAN TOOREN; DURING, 1988). Os artigos que abordam a dispersão de minhocas por briófitas não envolvem experimento controlado, porém uma observações de campo. A publicação During et al. (1987) objetivava coletar informações sobre o banco de diásporos em Barcelona, sendo a dispersão por minhocas um comentário extra. Já Van Tooren; During (1988)é mais focado em dispersão por minhocas, porém de diásporos de diversas plantas, entre elas, briófitas. Este último trabalho abre brechas para questionamento da metodologia e não avalia bem a interação entre minhocas e briófitas.

Tendo em vista o arcabouço teórico disponível, esta dissertação busca testarpor meio de um experimento controlado em laboratório a capacidade de minhocas em dispersar briófitas. Este trabalho possui dois tratamentos: 1) Pó de briófitas, no qual fragmentos da planta (e seus diásporos) são misturados ao solo e apresentado aos anelídeos; 2) Bloco de briófitas, neste, uma parcela da colônia de plantas é colocada em cima do solo e apresentado aos anelídeos. Assim, é possível ter uma ideia de qual condição é a mais ideal para dispersão. As três espécies de briófitas escolhidas são terrículas, sendo duas do filo Bryophyta e uma do filo Hepatophyta. As plantas foram selecionadas com base no tamanho da colônia e habitat. Além disso, cada uma é capaz de produzir diásporos especializados e não especializados(fragmentação do gametófito). A minhoca escolhida para o experimento, *Eisenia andrei* Bouché (1972), é uma espécie epigeica e exótica à fauna brasileira. Por ser amplamente comercializada, é possível usar centenas de espécimes para os fins do experimento sem causar dano ambiental por coleta excessiva. Este estudo apresenta perspectivas sobre as interações animal-planta fora do escopo de vertebrados e traqueófitas, além de ver esse tema sob o prisma de um ambiente tropical.

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1. Manuscrito

Go down to go up:
earthworms as vectors of bryophyte propagula

Manuscrito submetido na revista Journal of Bryology

Go down to go up: earthworms as vectors of bryophyte propagula

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Abstract

Similar to tracheophytes, bryophytes also benefit from zoochory as a mechanism for their dispersion, even though it is not species-specific or well explored. In order to assess the temporal dispersion of bryophytes facilitated by earthworms, we subjected three distinct bryophyte species (*Chryso-hypnum diminutivum*, *Fossombronia porphyrorhiza*, *Hyophila involuta*) to two distinct treatments: “bryophyte dust” and “bryophyte squares”. Our observations revealed bryophyte growth in 47.22% of the sampled units, providing compelling support for our initial hypothesis. *Hyophila involuta* emerged as the top-performing species, with bryophyte dust exhibiting the highest efficacy. This is the first controlled experiment to support the hypothesis of bryophyte dispersion by earthworms. The observed efficacy of bryophyte dust in the results may be attributed to the ease with which minute plant fragments can be ingested by the worms. This suggests a potential ecological role for earthworms in the dispersal of bryophytes in natural settings.

Keywords: dispersal, gemmae, herbivory, liverworts, mosses, zoocory.

Introduction

Animals exert significant influence on plant communities, ranging from shaping community composition to promoting seed germination through herbivore digestive processes, particularly in spermatophytes (Howe and Smallwood 1982; Archer and Pyke 1991). It is noteworthy that bryophytes, despite lacking seeds, canalso derive advantages from zoochory for their dispersion, as comprehensively documented in Glime (2017) and references therein.

Bryophytes, classified as gametophyte-dominant plants, play a pivotal role invarious ecological processes, including but not limited to soil stabilization to mitigate erosion, rehabilitation of eroded soils, and enhancement of the establishment of vascular plants (Belnap and Lange 2001; Laberge et al. 2015; Medeiros et al. 2023). Additionally, they provide essential habitat and sustenance for a diverse microfauna community, including tardigrades, rotifers, and nematodes (Young et al. 2018).Terrestrial bryophytes, in conjunction with cyanobacteria, lichen, fungi, and bacteria, collectively form biological soil crusts, holding substantial ecological significance and garnering recognition as ecosystem engineers (Oliveira and Maciel-Silva 2022).

Considering the ecological role played by bryophytes, it is imperative to delveinto the mechanisms underpinning the sustainability of their populations. Bryophytesemploy sexual reproduction via spore formation, a strategy that facilitates extensive dispersal over considerable distances (Longton 2006). Furthermore, asexual reproduction mechanisms such as gemmae production, tuber formation, and the development of caducous structures serve as vital strategies enabling these organisms to increase and perpetuate their colonies (Frey and Kürschner 2011).

The main mode of spore and propagula dispersal is wind mediated (Vanderpoorten et al. 2019). Nevertheless, noteworthy instances of bryophyte dispersal via animal vectors have been documented, including scenarios involving significant journeys on avian carriers or slower transport facilitated within the digestive tracts of slugs, as elucidated (Chmielewski and Eppley 2019; Boch et al. 2013). Despite the wealth of observational evidence, our understanding of bryophytedispersal mechanisms via animal vectors remains predominantly speculative, with limited empirical investigations, apart from notable studies involving flies (Marino et al. 2009).

As we can see, bryophytes are able to reproduce in many ways, however, inreference to dispersion, it is a cloudy field, particularly tropical regions. In order to lighten this scenario, we intend to answer the following question: are earthworms able to disperse bryophytes? To test the hypothesis that annelids have a role in bryophyte dispersion, we assess the interaction between earthworm *Eisenia andrei*Bouché and liverwort *Fossombronia* *porphyrorhiza* (Nees) Prosk., and mosses *Chryso-hypnum diminutivum* (Hampe) W.R. Buck and *Hyophila involuta* (Hook) A. Jaeger. We hope our data contribute to possible implications to tropical species in this group of plants.

Observations indicate bryophytes exhibit diverse reproductive strategies. Yet, themechanisms underlying their dispersal, especially in tropical regions, remain ambiguously understood. To elucidate this aspect, this study poses the question: canearthworms facilitate the dispersion of bryophytes? We evaluate this hypothesis by examining the interaction between the annelid, *Eisenia andrei* Bouché, and select bryophyte species: the liverwort *Fossombronia porphyrorhiza* (Nees) Prosk., and the mosses *Chryso-hypnum diminutivum* (Hampe) W.R. Buck and *Hyophila involuta* (Hook) A. Jaeger. Our findings aim to provide insights that could have significant implications for the dispersion strategies of tropical bryophyte species.

Materials and Methods

Study site and bryophyte sampling

In the present study, we investigated three bryophyte species: the liverwort *Fossombronia porphyrorhiza*, and the moss species *Chryso-hypnum diminutivum* and *Hyophila involuta* (see Figure 1). We collected 36 samples (16 cm²) for each bryophyte species. Sampling was conducted utilizing spatulas to ensure precision, after which sampleswere stored in paper bags to maintain their integrity. The collection was carried out in June/2022 at the Universidade Federal de Minas Gerais campus, located in Belo Horizonte, Brazil (19°51’57” S 43°57’58” W, 749 m a.s.l).

Additionally, we collected one more 16 cm² sample of each species for identificationpurposes. To identify these specimens at the species level, we used stereo and optical microscopes in combination with specialized literature (Allen 2002; Gradstein and Costa 2003; Oliveira-da-Silva and Ilkiu-Borges 2018).

Experimental design

We applied two experimental treatments: (1) 'Bryophyte Square' which encompassed a 16 cm² soil patch overlaid with intact bryophytes, and (2) 'BryophyteDust', signifying a 16 cm² patch of fragmented bryophytes obtained post-sieve processing to yield fragments of approximately 0.5 mm in size (see Figure 2). To eliminate potential confounders arising from the intrusion of exogenous bryophyte propagula, we instituted three distinct control conditions: a) 'Worm Control': soil samples ingested solely by earthworms, devoid of any bryophytes; b) 'Soil Control': the unadulterated substrate employed throughout the experiment to study animal- plant interaction. c) 'Vermiculite Control': the specific substrate utilized for the cultivation of earthworm excreta.

For each of the aforementioned treatments and controls, six replicates were used. Bryophytes were carefully positioned in 250 mL plastic containers, each containing 40 g of potting soil, and were sealed with lids. These pots were housed ina growth chamber, subjected to a consistent 12-hour light photoperiod (radiation of 34–78 μmol.m^{-2}.s^{-1}) and an average temperature of 20.8°C (min. 15.6°C, max.
26.3°C). Furthermore, the positions of the containers were systematically randomized on a weekly basis to mitigate any positional effects.

Earthworms

Earthworms used in this experiment belong to the species *Eisenia andrei* and were acquiredfrom a professional breeder. The animals selected (adults with developed clitellus, as possible) went through a fasting period of 48 hours. After fasting, they were submitted to a triple bath with distilled water and eight worms were placed in each pot of *bryophyte square*,*bryophyte dust* and control treatments. After seven days, earthworms were removed from the pots containing bryophytes and controls and went through another triple bath with distilled water and once more a fast for 48 hours. In this last fasting, feces were collected and transferred to 5g vermiculite filled 20 mL pots with leads. It is in this last stage we add vermiculite and soil controls.

The earthworms employed for this study, identified as *Eisenia andrei*, were acquiredfrom a reputable breeding source. Selected specimens, preferably mature adults with a prominent clitellum, were subjected to a preparatory fasting duration of 48 hours. Following this deprivation period, each specimen was consecutively rinsed thrice using distilled water.

Thereafter, a cohort of eight such pre-treated earthworms was allocated to each designated treatment pot, encompassing the “bryophyte square”, “bryophyte dust”, and the control setups. After a temporal span of seven days, the earthworms were meticulously extracted from these pots and once more subjected to the triple distilled water rinse, followedby an additional 48-hour fasting phase.

During this concluding fasting window, earthworm excreta was systematically harvested and introduced into 20 mL containers pre-filled with 5g of vermiculite. These containers were subsequently sealed. It was at this terminal stage that the vermiculite andsoil controls were incorporated into the experimental design.

Data analysis

We performed an Analysis of Variance (ANOVA) to evaluate the differences betweentreatments and species. A post hoc Tukey test was further employed to discern significant interspecies differences. Prior to executing the ANOVA and Tukey tests, the area (mm^2) of bryophytes within the sampling units, as quantified via the ImageJsoftware (Rasband, 2012), underwent a logarithmic transformation. Specifically, theANOVA assessed the impact of treatments and bryophyte species on the transformed area values. All statistical analyses were executed using R Statistical Software (version 4.2.2; R Core Team, 2022).

Results

Bryophyte growth was evident in 47.22% of the sampling units inoculated with earthworm droppings. Our data support the hypothesis that, under controlled conditions, earthworms play a role in bryophyte dispersal (Figure 3). Notably, nobryophyte growth was detected in any of the control units.

In terms of bryophyte dispersal efficiency, the 'bryophyte dust' treatmentoutperformed significantly ($F = 14.13$; $P < 0.05$), registering a success rate of 66.67%. This contrasts with the 27.78% efficacy observed with the 'bryophyte square' treatment. Furthermore, the 'bryophyte dust' treatment revealed an enhanced plant cover per unit area (Figure 4).

Regarding species-specific performance (F = 5.24; $P < 0.05$), the moss *H. involuta* emerged as the most efficient, demonstrating a success rate of 66.67% in terms of successful inoculation within sample units. This was followed by the liverwort *F. porphyrorhiza*, which exhibited a 50% success rate. Conversely, the moss *C. diminutivum* demonstrated a reduced efficiency with a success rate of only25%. When evaluating vegetation cover, *H. involuta* consistently recorded superior results (Figure 5).

A notable abundance of gemmae was documented within the *H. involuta* sampling units. Contrastingly, within units inoculated with *C. diminutivum*—where growth was evident in 25% of the samples—the emergent bryophyte growth predominantly did not correspond to the initially collected species. Notably, speciesfrom the *Fissidens* genus were detected in 3 out of the 12 samples, with a singular sample manifesting the presence of *C. diminutivum* (Figure 3B). This phenomenonelucidates the larger bryophyte coverage observed in *C. diminutivum*-inoculated samples compared to those of *F. porphyrorhiza*. Specifically, *Fissidens* occupied a considerable expanse of the container, overshadowing *F. porphyrorhiza*, even though the latter species manifested in a higher frequency across the sampling units(Figure 5).

In addition to bryophytes, vascular plants (mostly ferns) that propagate via spores were identified in 16.67% of the sampling units. Specifically, these were distributed as follows: two within the *F. porphyrorhiza*, six within the *C. diminutivum*sampling units, and one within the soil control units.

Discussion

This study is the first controlled experiment to support the hypothesis that earthworms can both ingest and subsequently disperse bryophytes. We assessed the temporal dispersion viability of propagula within the annelid digestive tract, for a duration extending to two days following initial contact. Furthermore, this study highlighted the efficacy of earthworms in facilitating the propagation of spore-bearing plants, as illustrated by the emergence of fern gametophytes across several samples.

The superior efficacy of the “bryophyte dust” over the “bryophyte square” maybe attributed to the ease with which earthworms ingest smaller plant fragments, gemmae, or spores prevalent in the former. In contrast, the bryophyte square presents plants entirely atop the soil. Nonetheless, one might contend that naturally occurring bryophyte colonies resemble the bryophyte square more than the bryophyte dust. It's pertinent to note that bryophytes typically produce sexual propagula (i.e., spores), often adapted for anemochory. These spores can settle proximally or distally from the colony onto unoccupied soil (During, 1979).

Fragmentation of bryophytes is also a naturally observed phenomenon (McDaniel & Miller, 2000; Frey & Kürschner, 2011), playing a pivotal role in forming new communities (Robinson & Miller, 2013). Natural vectors for this fragmentation include strong winds (Miller & Ambrose, 1976), vertebrate activities (Heinken et al., 2001; Chmielewski & Eppley, 2019), ingestion by vertebrates (Parsons et al., 2007),and even human-induced actions (Studlar, 1983; Amélio et al., 2021). We must alsorecognize caducous organs: specialized asexual reproductive structures where a segment of the gametophyte (such as leaves, leaf apices, shoots, branches, or bulbils) intentionally detaches, essentially serving as a form of "planned fragmentation." Additionally, gemmae, vegetative propagula comprising a few cells,can give rise to protonema upon germination (Laaka-Lindberg et al., 2003; Frey & Kürschner, 2011).

Soils harboring a substantial quantity of viable, yet dormant, spores and asexual propagula are termed as diaspore banks (During, 2001). In fact, our data suggest the earthworms interacted not only with bryophytes but also with the adjacent soil and its diaspore reserves. Analogous to its namesake, this "bank" serves as a vital reservoir for bryophyte diversity (Jonsson and Esseen 1998; Maciel-silva et al 2012; Bisang and Bergamini 2021). These repositories facilitate rapid colonization in perturbed ecosystems (Jonsson, 1993) and act as genetic safeguard for local populations (Hock et al., 2008).

It's imperative to note the potential synergy between diaspore banks and annelids. As posited by During et al. (1987), annelids, with their soil-turning activities,could inadvertently expose or bury bryophyte propagula. This cyclical action fosters conditions conducive for propagula to be ingested and subsequently dispersed—an interaction analogously observed with seeds (Grant, 1983). Adding to this, earthworms' innate affinity for organically rich soils and their quest for mating partners (Caro et al., 2013) could further bolster bryophyte dispersion, synergizing with soil turnover activities.

The three species employed in this study have interesting features that can beexplored by earthwormns and other invertebrates upon the diaspore banks. *Hyophilainvoluta* is a dioicous species, noted for its gemmae (>100 µm; Zander and Eckel 1993; Sharma and Chopra 1986). These gemmae not only demonstrate an ability forprolific regeneration into extensive protonemata but also have the potential to further produce more gemmae (unpublished data). The prevalence of these gemmae in our sample units underscores their significance for dispersal mechanisms. Consistent with findings from Maciel-Silva et al. (2012), dioicous species exhibit a pronounced presence in diaspore banks compared to extant vegetation. This observation accentuates the critical role of vegetative propagula in tropical ecosystems.

The monoicous liverwort *Fossombronia porphyrorhiza* showcased robust performance in our study, with fast-development and a profusion of sporophytes replete with spores. The genus *Fossombronia* is characterized by its large (> 35 µm)and thick-walled spores capable of withstanding desiccation (Gradstein and Costa 2003). These attributes are conducive to prolonged storage within diaspore banks, especially amidst environmental perturbations.

Lastly, *C. diminutivum* is identified as an autoicous moss (Gradstein et al. 2001). Monoicous mosses generally allocate substantial energy towards sexual reproduction, culminating in a prolific spore production that increase the diaspore bank density (Maciel-Silva et al. 2012). However, spores of *C. diminutivum* are small(>20 µm) and very green, indicating a likely short-term longevity into the soil (Maciel-Silva et al. 2012; Maciel-Silva et al. 2014).

During our study, we fortuitously observed the emergence of plants belongingto the *Fissidens* moss genus in both *H. involuta* and *C. diminutivum* inoculated samples. This observation implies a probable abundance of *Fissidens* diaspores in the soil's diaspore bank. It is conceivable that the subdued growth of *C. diminutivum*,in contrast to *H. involuta* and *F. porphyrorhiza*, provided a favorable environment for *Fissidens* establishment.

Invertebrates, such as ants (Rudolphi 2009), weevils (Gressitt & Samuelson 1968), harvestmen (Machado & Vital 2001), and slugs (Kimmerer & Young 1995; Boch et al 2013)have been documented to act as bryophyte dispersers. Particularly, flies have been extensively studied in this context due to their well-documented interaction with dung- mosses (Pyysalo et al. 1978; Cameron and Wyatt 1986; Goffinet et al. 2004; Marino et al. 2009; Jofre et al. 2011; Wyatt et al. 2022).

The potential of earthworms in this role remains a vast, uncharted territory. While Tooren & During (1988) and During et al. (1987) pioneered studies on zoochory involving worms and spore-bearing plants over three decades ago, the potential capacities of these coelomate worms have since remained underexplored. Notably, there has been a lack of renewed interest in this topic, especially concerning tropical ecosystems. These foundationalstudies did not exclusively focus on bryophytes, but rather considered the general viability ofplant diaspores within the earthworm digestive system. Subsequent research has shed light on annelids as dispersal agents for seeds (Forey et al. 2011) and spores from fungi (Reddelland Spain 1991, Gange 1993, Rantalainen et al. 2004) and ferns (Hamilton and Lloyd 1991).

Earthworms have the potential to bolster the emergence of new colonies in habitat patches, thereby strengthening the metapopulation structure of bryophytes (Söderström andDuring 2005). Moreover, the journey through an earthworm's digestive tract might facilitate the breaking of dormancy in diaspores. This is supported by our observation of prolific germination of gemmae following *H. involuta* inoculation. Nevertheless, this hypothesis warrants meticulous examination over time, mirroring other studies (Eisenhauer et al. 2009, Asshoff et al. 2010, Forey et al. 2011).

These perspectives highlight numerous avenues for future research endeavors.

Notably, several ecological and physiological facets pertaining to the symbiotic interactionsbetween bryophytes and their animal dispersers remain unexplored, particularly within tropical ecosystems.

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Figures

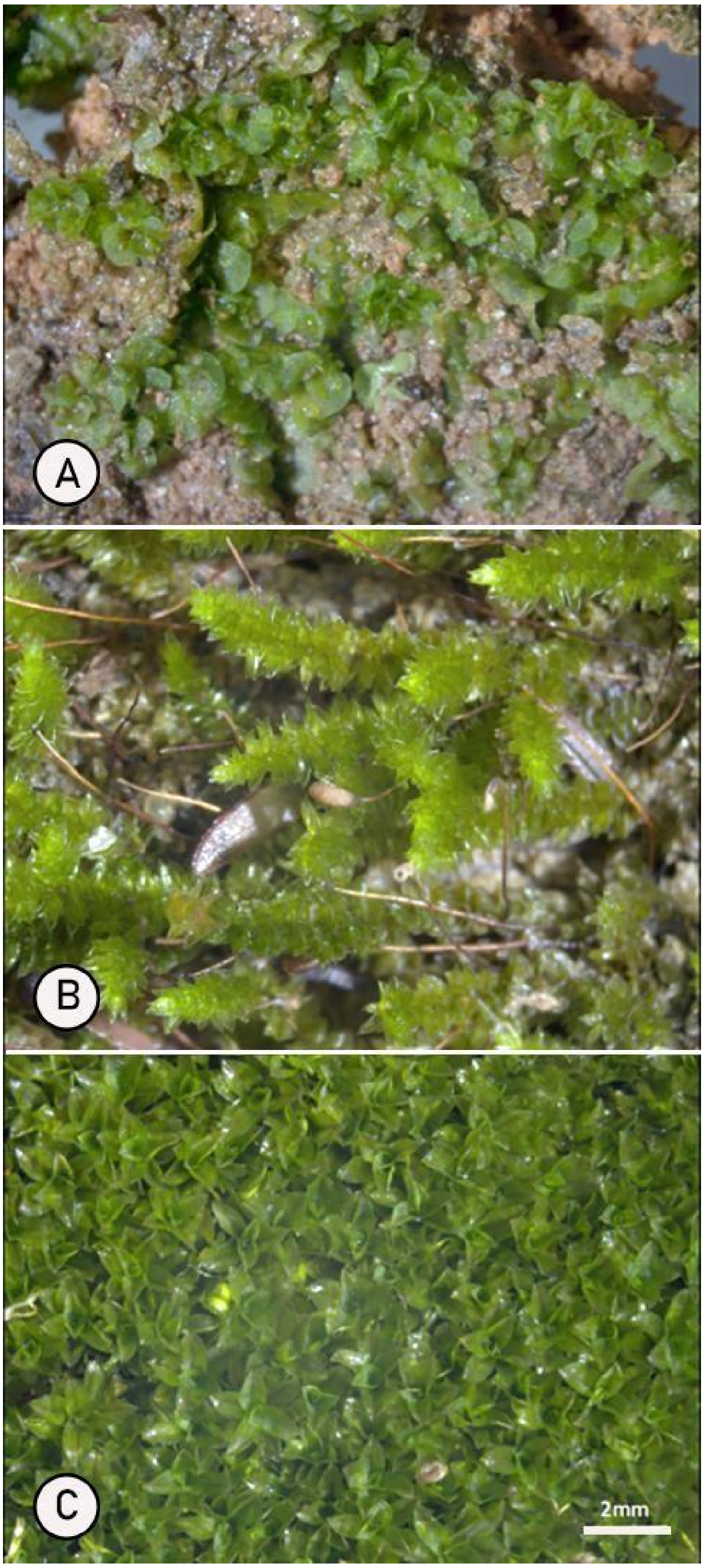

Figure 1. Bryophyte species selected for experimental assessment of earthworm feeding preferences and consequent temporal dispersal. A) *Fossombronia porphyrorhiza*; B) *Chryso-hypnum diminutivum*; C) *Hyophila involuta*.

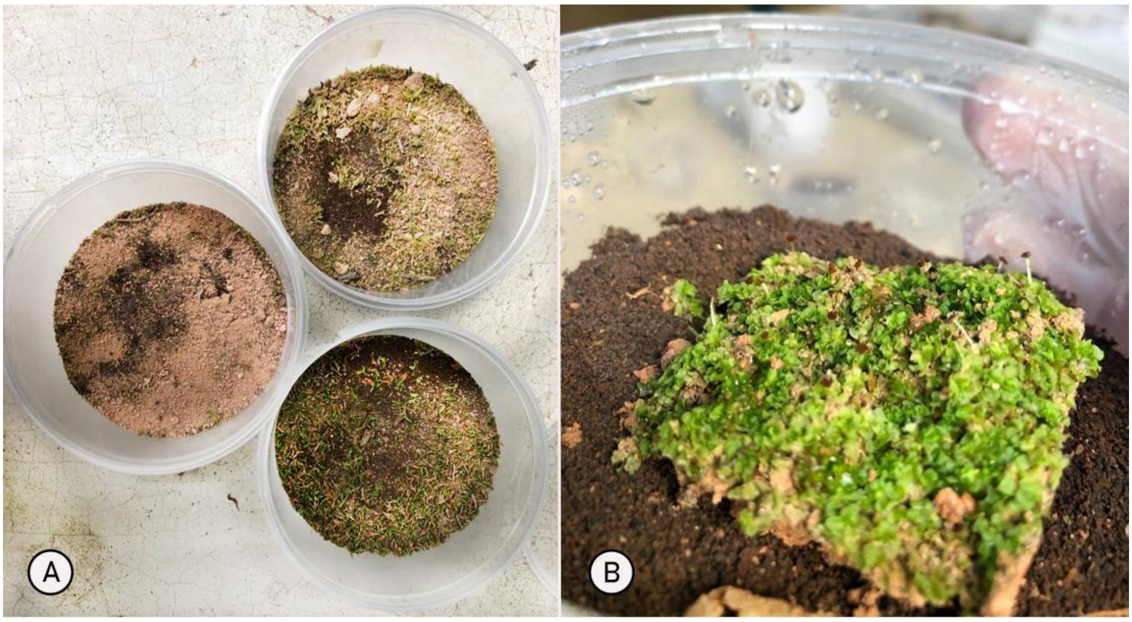

Figure 2. Bryophyte treatments employed in the experimental evaluation of earthwormfeeding preferences. A) Bryophyte dust; B) Bryophyte square

Figure 3. Sample units illustrating bryophyte colonization via earthworm fecal deposits on vermiculite and Worm Control. A) Worm Control. Arrow points to non-enoculated fecal matter. B, C) Samples inoculated by *F. porphyrorhiza.* Arrow in C indicates a small *F. porphyrorhiza* gametophyte with leaves; D, E, F) *C. diminutivum* inoculated samples. Arrow in D points to *C. diminutivum* alongside with *Fissidens sp.* and fern gametophyte. In E and F,*Fissidens sp.* emerged where *C. diminutivum* was supposed to be;G, H, I) Samples inoculated by *H. involuta.* Arrow points to *H. involuta* alongside *Fissidens sp.* In I, arrow indicates gemmae not yet formed into gamethophyte.

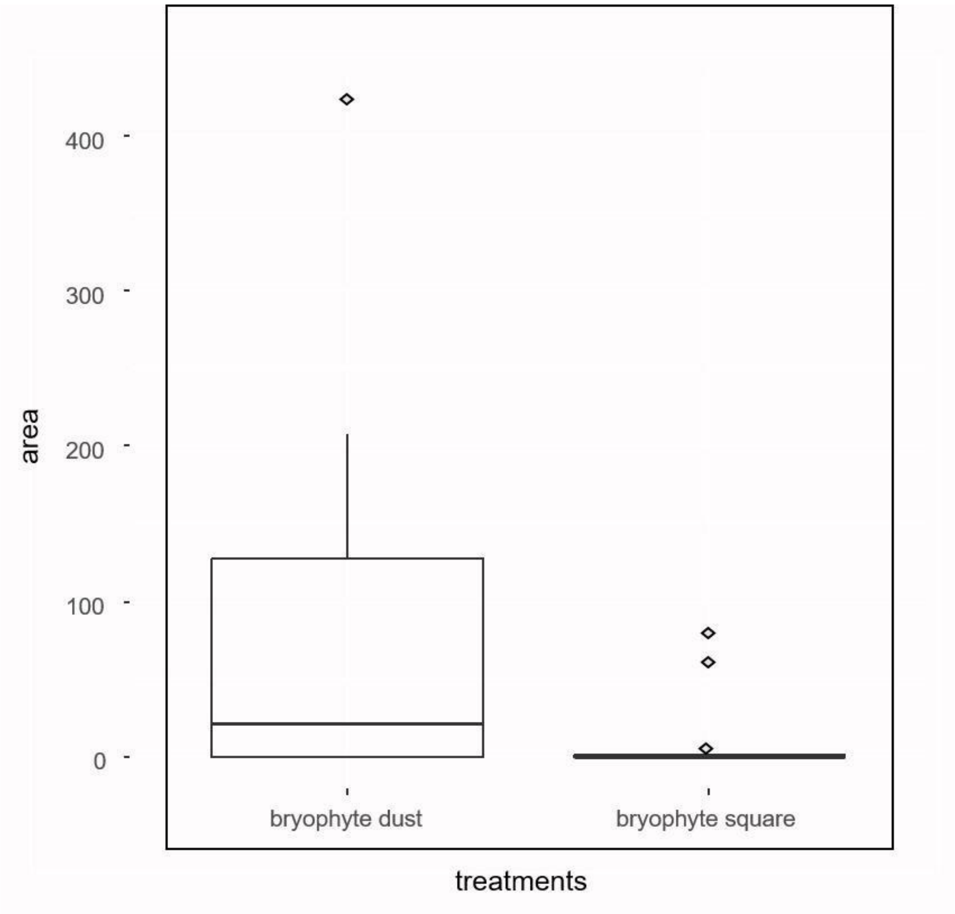

Figure 4. Bryophyte cover area (mm^2) per treatment. Box plot shows the 25th-75th percentile of data, horizontal line in the middle of the box indicates the median, n = 18 samples per treatment. Diamonds indicate outliers.

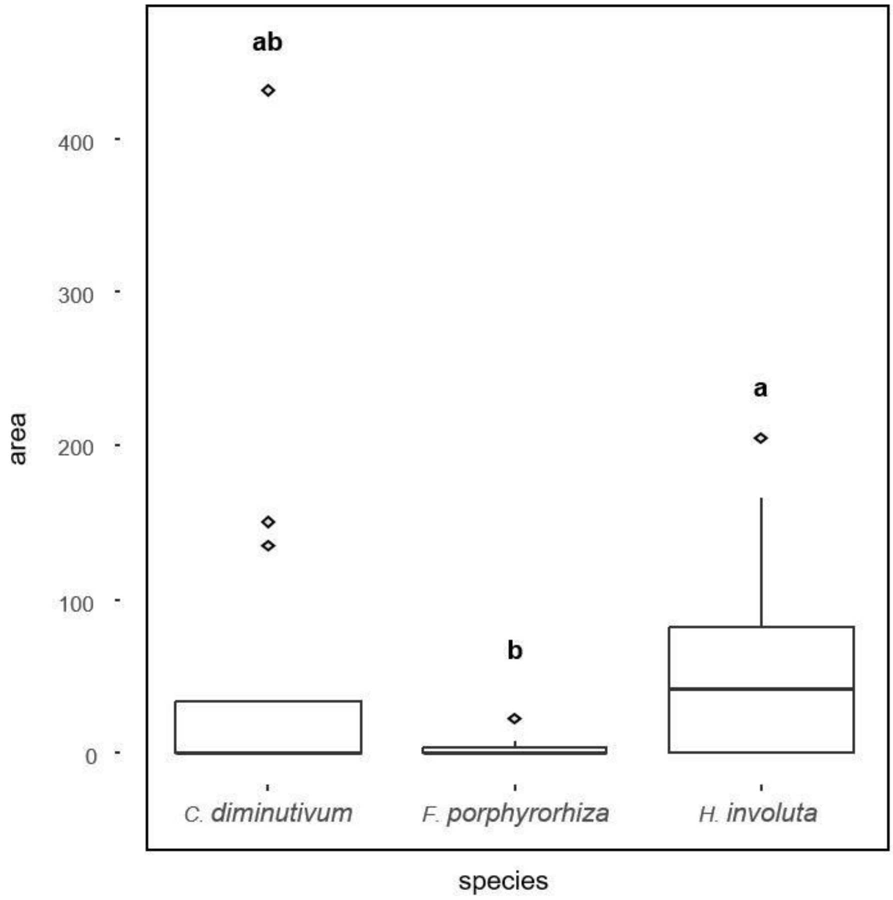

Figure 5. Bryophyte cover area (mm^2) per species. Box plot shows the 25th-75th percentile of data, horizontal line in the middle of the box indicates the median, n = 12 samples per species and diamonds indicates outliers. Different letters point to significant differences according to Tukey test.

CONCLUSÕES

Esse estudo contribui para a ampliação do conhecimento acerca da dispersão de briófitas por invertebrados. Com ele, saímos do escopo da observação para analisar através de dois tratamentos a melhor forma que as minhocas podem entrar em contato com briófitas no habitat natural. Além disso, esse é um dos poucos estudos a incluir a brioflora da região tropical sob a análise de zoocoria. Quais mais outros invertebrados ou vertebrados da fauna sul-americana entram em contato com as briófitas?

Podemos também acrescentar, com os dados e observações obtidos a partir deste trabalho, a forma mais provável que as minhocas entram em contato com as briófitas: através dos bancos de diásporos do solo, visto que o tratamento com fragmentação de plantas obteve maior sucesso que o tratamento de bloco. Os bancos de diásporos contém uma verdadeira reserva de esporos, fragmentos e outros propágulos de briófitas (DURING, 2001) de tamanho adequado a serem ingeridas pelos pequenos anelídeos Portanto, ao contrário das lesmas e caracóis que predam ativamente as briófitas, ou as moscas que pousam em seus esporofitos e colhem os esporos sem querer (MARINO et al., 2009; BOCH, 2013), as minhocas entrarão em contato com a reserva de diásporos do solo, adicionando mais um utensílio ao nosso conhecimento de bancos de diásporos.

Para perspectivas futuras dentro do tema de dispersão entre plantas com gametófito dominante, podemos pensar em quão longe elas podem ser carreadas por animais sem pernas. Ou passando pelo escopo da fisiologia, investigar se a passagem pelo trato digestivo de invertebrados altera a taxa de germinação de esporos ou gemas. Outra perspectiva interessante são estudos de campo que registrem interações entre anelídeos selvagens de regiões tropicais e a brioflora local, que também seria um ótimo argumento para a preservação não só de minhocas, que são muito caçadas para a pesca (DRUMOND et al., 2015), mas também de briófitas que são coletadas irrestritamente em várias épocas do ano a ponto de comprometer suas populações (AMÉLIO et al. 2021).

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