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WAGNER LUIZ DOS SANTOS

DIMORFISMO SEXUAL E INVESTIMENTO REPRODUTIVO EM *Fissidens* Hedw. (FISSIDENTACEAE, BRYOPHYTA)

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Dissertação apresentada ao Programa de Pós Graduação em Biologia Vegetal da Universidade Federal de Pernambuco como requisito para obtenção do título de mestre em Biologia Vegetal

Área de concentração: Ecologia

Orientadora: Dr.ª Kátia Cavalcanti Pôrto

Coorientadora: Dr.ª Lisi D. Pereira Alvarenga

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BANCA EXAMINADORA

Profa. Dra. Kátia Cavalcanti Pôrto (Orientadora)
Universidade Federal de Pernambuco

Profa. Dra. Adaíses Simone Maciel da Silva (Examinadora Externa)

Universidade Federal de Minas Gerai

Dr. Oswaldo Cruz Neto (Examinador Interno)

Universidade Federal de Pernambuco

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RESUMO

A teoria de história de vida prediz que todo o recurso produzido por um organismo é investido na reprodução, crescimento e defesa. Dentre estes traços do histórico de vida, a reprodução é considerada o processo mais dispendioso. Por isso, demandas conflitantes são comumente observados entre o investimento reprodutivo (proporção de recurso alocado na reprodução) e o crescimento vegetativo, o que de certa forma acarreta diferenças morfológicas entre os sexos, e, consequentemente, causam dimorfismo sexual. Esse estudo foi direcionado a elucidar o investimento e *a* demanda conflitantereprodutiva em espécies de distribuição tropical do gênero *Fissidens* (Fissidentaceae, Bryophyta). Os objetivos do trabalho foram: (1) investigar se o dimorfismo sexual no musgo rizautoico (ramos masculinos presos aos femininos pelos rizoides) *Fissidens flaccidus* Mitt. se relaciona ao investimento reprodutivo, e (2) se a compartimentalização das funções sexuais induz ao maior investimento reprodutivo na função masculina no sistema rizautoico comparado ao sistema gonioautoico (perigônios axilares e periquécios terminais na mesma rameta), presente na espécie congênere *F. submarginatus* Bruch. O dimorfismo sexual foi confirmado em *F. flaccidus*, com base em análises morfométricas (tamanho e largura das rametas, e quantidade de filídios por rameta). As rametas masculinas apresentaram menor tamanho em todos os parâmetros morfométricos, e maior investimento reprodutivo comparativamente as rametas femininas esporofíticas e não esporofíticas. Análises de correlações evidenciaram que quanto mais recurso é investido na reprodução, menor é o desenvolvimento vegetativo. Quanto à comparação do investimento reprodutivo nos dois sistemas sexuais, a espécie rizautoica apresentou maior alocação de recurso para a formação de perigônios e esporófitos. Por sua vez, não houve diferença significativa quanto ao recurso investido na formação do periquécio. Nós concluímos que os sistemas sexuais se relacionam com o investimento reprodutivo.

Palavras-chave: Dimorfismo sexual. Musgos. Reprodução sexual. Esforço reprodutivo. Custo reprodutivo. Rizautoico.

ABSTRACT

The life-history theory predicts that all resources produced by an organism are invested in reproduction, growth and defense. Among life-history traits, reproduction is considered the more expensive process. Because of that, trade-offs are commonly observed between reproductive investment (proportion of resources allocated to reproduction) and vegetative growth, which in some cases entail morphological differences between the sexes, and consequently, sexual dimorphism. This study was directed to elucidate the reproductive investment and trade-offs in species with tropical distribution of the genus *Fissidens* (Fissidentaceae, Bryophyta). The goals of the study were: (1) to investigate if sexual dimorphism in the rhizautoicous moss (male shoots attached to female shoots by rhizoids) *Fissidens flaccidus* Mitt. is related to reproductive investment, and (2) if the compartmentalization of sexual functions entails highest reproductive investment to the male function when comparing the rhizautoicous *F. flaccidus* with a gonioautoicous species (axillary perigonia and terminal perichaetia in the same ramet) of the same genus, *F. submarginatus* Bruch. Sexual dimorphism was confirmed in *F. flaccidus* based on morphometric analyses (ramet size and width, and amount of leaves per ramet). The male ramets showed smaller sizes in all morphometric parameters, and conversely higher reproductive investment compared to sporophytic and non-sporophytic female ramets. Correlation analyses showed that the more resource is invested in reproduction, the lower was the vegetative development. As for the comparison of the reproductive investment in the two sexual systems, the rhizautoicous species presented higher allocation of resources to the formation of perigonia and sporophytes. On the other hand, there was no significant difference as to the invested function in the formation of the perichaetia. We conclude that systems are related to reproductive investment.

Keywords: Sexual dimorphism. Mosses. Sexual reproduction. Reproductive effort. Reproductive cost. Rhizautoicous.

SUMÁRIO

1 INTRODUÇÃO

Desde que foi publicado o livro *The different forms of flowers on plants of the same species* (DARWIN, 1877), o dimorfismo sexual tem recebido grande atenção nos estudos que envolvem a reprodução das plantas (ATLAN et al., 1992, GALLOWAY; MAUREEN; STANTON, 1996, DELPH, 1999). Levando-se em consideração que toda a funcionalidade de um organismo advém de recurso próprio, torna-se evidente que toda a energia produzida é particionada entre as funções vitais, tais como crescimento, defesa e reprodução (STEARN, 1989, 2000; DELPH, 1999). Desse modo, em muitos casos, o dimorfismo sexual se apresenta relacionado à reprodução do organismo, uma vez que as demandas conflitantes são comumente observados entre a reprodução e o desenvolvimento vegetativo (CHARLESWORTH; CHARLESWORTH, 1991; OBESO, 2002).

Comparando-se os dados de diversos estudos que elucidaram a biologia reprodutiva das briófitas, observa-se que as demandas conflitantes são comuns entre as funções reprodutivas e as vegetativas (LAAKA-LINDBERG, 2001, HORSLEY; STARK; McLETCHIE, 2011), acarretando, muitas vezes, o dimorfismo sexual nas espécies (McDANIEL, 2005; SANTOS; ALVARENGA; PÔRTO, 2018). Os sistemas reprodutivos (monoico e dioico) nas briófitas são subdivididos em diversos tipos conforme a distribuição das estruturas reprodutivas nos gametófitos (WYATT, 1987; GOFFINET; SHAW, 2009; MACIEL-SILVA; PÔRTO, 2014). Algumas espécies dioicas e monoicas que possuem segregação das funções sexuais apresentam diferenças entre os sexos no que diz respeito à história de vida, as quais são, muitas vezes, relacionadas ao investimento alocado na reprodução (STARK; BRINDA, 2013; BORDIN; YANO, 2014; SANTOS; ALVARENGA; PÔRTO, 2018).

O investimento reprodutivo é definido como a proporção de recurso alocado na reprodução (KARLSSON; MÉNDEZ, 2005). Os estudos quais retratam as briófitas têm sido realizados com espécies dioicas e monoicas rizautoicas (BISANG; EHRLÉN; HEDENÄS, 2006; STARK; BRINDA, 2013). Dentre as espécies monoicas, o sistema sexual rizautoico tem recebido pouca atenção quanto a sua biologia reprodutiva. Embora esse sistema seja geneticamente monoico, funcionalmente, ele se comporta como dioico, visto que há a compartimentalização das funções sexuais.

Diante desse contexto, o presente estudo objetivou investigar os parâmetros reprodutivos de espécies do gênero *Fissidens* Hedw. (Fissidentaceae, Bryophyta). Os resultados desse estudo são apresentados em dois capítulos. O primeiro aborda a relação do dimorfismo sexual ao investimento reprodutivo na espécie rizautoica *Fissidens flaccidus* Mitt.. No segundo capítulo investigou-se o fato de a compartimentalização das funções sexuais induzir ao maior investimento reprodutivo no sistema rizautoico de *F. flaccidus*, se comparado ao sistema gonioautoico (periquécio terminal e perigônios axilares), de *F. submarginatus*.

2 REVISÃO DE LITERATURA

2.1 Reprodução nas briófitas

Segundo a teoria da história de vida, toda a funcionalidade de um organismo é dependente do recurso por ele produzido. Oli (2006) define a teoria da história de vida como cronogramas de eventos no ciclo de vida de um indivíduo. Assim, segundo essa teoria, toda a energia proveniente do organismo está direcionada aos traços da história de vida, tais como reprodução, crescimento e defesa (STEARNS, 1992, 2000; DELPH, 1999). Richards (1997) defende que, entre os traços da história de vida, a reprodução é o evento mais importante, uma vez que garante a manutenção e a diversidade das populações.

O sistema sexual das briófitas é dividido em dioico e monoico, os quais são subdivididos em vários sistemas sexuais (WYATT, 1985; MACIEL-SILVA; PÔRTO, 2014; HAIG, 2016). Para que ocorra a reprodução sexuada, nas briófitas, a presença de água é indispensável, visto que o gameta masculino (anterozoide) produzido no anterídio necessita nadar até o gameta feminino (oosfera) produzido no arquegônio. Após a fecundação, há a formação do esporófito que é dependente nutricionalmente do gametófito, o esporófito é formado e produz esporos. Em muitos casos, essa é a fase mais custosa da reprodução (GOFFINET; SHAW, 2009).

2.2 Investimento reprodutivo nas briófitas

Durante longo tempo, um dos grandes problemas ao se discutir investimento reprodutivo era a ambiguidade referente às terminologias adotadas (KARLSSON; MÉNDEZ, 2005). Por exemplo, o termo esforço reprodutivo foi utilizado por WILLIAMS (1966) como parâmetro demográfico; já por LOVETT, (1989) e STEARNS, (1992), como custo reprodutivo e por FISHER (1930), STEARNS (1976) E ANTONOVICS (1980), como a proporção de recurso destinado à reprodução. Diante dessa imprecisão terminológica, KARLSSON; MÉNDEZ (2005) realizaram uma meta-análise, a qual pode ser encontrada no capítulo do livro *Reproductive Allocation in Plants*, cujo objetivo era uniformizar estas terminologias. Em nosso estudo, adotamos o investimento reprodutivo como a proporção de recurso alocado para a reprodução, e o custo reprodutivo como o efeito do investimento reprodutivo nos demais traços da história de vida (BISANG; EHRLÉN; HEDENÄS, 2006; STARK; BRINDA, 2013).

Nas plantas, o investimento reprodutivo tem sido relatado em dois momentos do ciclo de vida (GLIME; BISANG, 2017b). Tem-se o investimento reprodutivo pré-zigótico, que é a proporção de recurso destinada à formação dos perigônios e periquécios (LAAKA-LINDBERG, 2001; STARK; BRINDA, 2013), e o investimento reprodutivo pós-zigótico, que é destinado à proporção de recurso alocado na formação do esporófito e que recai sobre as plantas femininas (EHRLÉN; BISANG, 2000; RYDGREN; ØKLAND, 2002, 2010).

Nas últimas décadas, as briófitas de regiões temperadas têm sido utilizadas como modelo para os estudos envolvendo a alocação de recursos para a reprodução (LAAKA-LINDBERG, 2001; EHRLÉN; BISANG, 2000; RYDGREN; ØKLAND, 2002, 2010, STARK; BRINDA, 2013). Alguns fatores tornam as briófitas excelentes plantas para investigação sobre o investimento reprodutivo. O primeiro deles é devido ao seu curto ciclo de vida se comparado às espermatófitas, facilitando o acompanhamento de futuras reproduções (BISANG; EHRLÉN; HEDENÄS 2006; RYDGREN; ØKLAND 2010). Segundo, as briófitas apresentam uma grande diversidade de sistemas sexuais e de estratégias reprodutivas (WYATT 1985; MACIEL-SILVA; PÔRTO 2014). Por fim, o pequeno tamanho das briófitas favorece a determinação da biomassa das suas estruturas reprodutivas e vegetativas (McLETCHIE; PUTERBAUGH, 2000). Apesar disso, Proctor (1977) comenta que mensurar o investimento reprodutivo pós-zigótico nas briófitas, é complicado, visto que a ocorrência de fotossíntese na fase inicial de desenvolvimento do esporófito pode prejudicar a comparação entre as espécies.

Um dos primeiros estudos a quantificar o investimento reprodutivo em briófitas foi realizado por Convey; Smith (1993), que quantificaram o investimento reprodutivo em musgos da Antártica em dois níveis: (1) na proporção da biomassa alocada na formação do esporófito; e (2) no número de esporos produzidos por cápsulas, relacionando-os com as estratégias de vida propostas por During (1979). Esses autores observaram que ambas as mensurações em espécies anuais e de vida curta, eram maiores do que nas espécies perenes. Por outro lado, objetivando investigar o investimento reprodutivo de gemas, McLetchie; Puterbaugh (2000) e Fuselier; McLetchie (2002) observaram que na hepática *Marchantia inflexa* Nees & Mont., os gametófitos masculinos investiram mais em produção de gema se comparados aos gametófitos femininos.

Após os trabalhos de Convey; Smith (1993) e de McLetchie; Puterbaugh (2000) perceberam-se a necessidade de se aprofundar o conhecimento a respeito das funções sexuais masculinas e femininas. Portanto, com a finalidade de elucidar o investimento reprodutivo masculino e feminino, Laaka-Lindberg (2001) estudou a hepática *Lophozia silvicola* H. Buch*.* e constatou que os gametófitos femininos investiram mais em reprodução sexuada que os masculinos, confirmando a demanda conflitante entre a reprodução sexuada com a reprodução vegetativa (produção de gemas). Stark; McLetchie; Mishler (2001) relataram, para o musgo *Syntrichia caninervis* Mitt., resultados diferentes dos obtidos por Laaka-Lindberg (2001), tendo em vista que não observaram diferença no investimento reprodutivo pré-zigótico entre os sexos.

A fim de compreender o investimento em níveis pré e pós-zigótico em gametófitos femininos, Bisang; Ehrlén (2002) observaram o mais alto investimento reprodutivo na formação dos esporófitos para o musgo *Dicranum polysetum* Brid. Posteriormente, McDaniel (2005) observou no musgo *Ceratodon purpureus* (Hedw.)

Brid*.,* menor investimento reprodutivo na formação das estruturas pré-zigóticas femininas e relacionou este investimento ao dimorfismo sexual observado na espécie.

Atendo-se a uma abordagem ecológica, Bisang; Ehrlén; Hedenäs (2006) questionaram a predição proposta por Stark; Mishler; McLetchie (2000) que sugere que o sexo que necessita de maior investimento reprodutivo se apresenta em menor número na população. Para tanto, estudaram o musgo *Pseudocalliergon trifarium* (F. Weber & D. Mohr) Loeske. reportando o maior investimento reprodutivo na formação de periquécios. Esses autores concluíram que o investimento reprodutivo nem sempre influencia a razão sexual, uma vez que as populações estudadas se apresentaram tendenciosamente femininas. Em oposição aos resultados constatados no musgo *Pseudocalliergon trifarium,* Horsley; Stark; McLetchie et al. (2010) observaram em nível pré-zigótico de *Bryum argenteum* Hedw., que os gametófitos masculinos investiram significativamente mais em reprodução, quando comparados aos femininos. Além disso, observaram que a razão sexual era equilibrada (1:1), sugerindo que o investimento reprodutivo não é determinante na razão sexual, mas sim fatores relacionados à expressão sexual masculina, o que levou esses autores a sugerirem a hipótese do macho tímido (“s*hy male”)* (STARK; McLETCHIE; EPPLEY, 2010).

O investimento reprodutivo em espécies rizautoicas têm despertado atenção, uma vez que elas apresentam compartimentalização das funções sexuais. Stark; Brinda (2013) foram os precursores do estudo de investimento reprodutivo em espécie rizautoica, utilizando, como modelo, o musgo *Aloina bifrons* (De Not.) Delgad. relataram o investimento reprodutivo pré-zigótico para ambas as funções sexuais e observaram que o mais alto investimento reprodutivo foi direcionado a função sexual masculina. Resultado similar foi recentemente observado por Santos; Alvarenga; Pôrto (2018), para a espécie *Fissidens flaccidus* Mitt.

2.3 Custo reprodutivo em briófitas

Uma vez que a reprodução causa limitação de recurso para os demais traços da história de vida, há a ocorrência do custo reprodutivo (WILLIAMS, 1996; DELPH, 1999; OBESO, 2002; STARK; BRINDA; McLETCHIE, 2009a). Embora alguns autores considerem que o custo reprodutivo seja quando a reprodução influencia negativamente a futura reprodução (JÖNSSON, 2000; BISANG; EHRLÉN, 2002), outros acreditam que podem ser relacionados a outros traços da história de vida (DELPH, 1999; RYDGREN; ØKLAND, 2002). Obeso (2002), em uma extensa revisão bibliográfica sobre o assunto, relata que o custo reprodutivo pode influenciar tanto a futura reprodução, quanto o crescimento e a sobrevivência da espécie.

O custo reprodutivo tem sido reportado desde os tempos de Darwin (1877). Em seu livro "*The different forms of flowers on plants of the same species*", Darwin retratava a limitação de recurso como Lei da compensação. Ele alegava que, quando um recurso não era alocado para uma função, naturalmente era realocado para outra. Como exemplo, explanou que em flores de angiospermas monoicas com pistilos muito desenvolvidos, apresentavam estames menores. Atualmente, essa condição é referida como demanda conflitante*offs* (CHARLESWORTH; MORGAN, 1991). Stark; Mishler; McLetchie (2000) foram pioneiros ao testar a hipótese do custo reprodutivo em briófitas, atribuindo que o sexo mais custoso é encontrado em menor proporção nas populações. Para esse estudo, utilizaram, como modelo, o musgo *Syntrichia caninervis* Mitt. e confirmaram o custo reprodutivo em relação ao maior investimento pré-zigótico masculino e a formação de esporófitos. Ehrlén; Bisang (2000) investigaram o custo da formação do esporófito no musgo *Dicranum polysetum* Brid. Esses autores verificaram que, quando os periquécios eram removidos, os gametófitos apresentavam maior crescimento apical devido ao fato de que o recurso que seria investido na formação do esporófito era alocado no crescimento. Resultado similar foi observado no musgo *Entodon cladorrhizans* (Hedw.) Müll. Hal., que apresentou crescimento vegetativo reduzido no momento da maturação do esporófito (STARK and STEPHENSON 1983).

Com o objetivo de analisar a dinâmica populacional do musgo *Hylocomium splendens* (Hedw.) Schimp., Rydgren; Økland (2002) compararam o crescimento em populações esporofíticas e não esporofíticas durante cinco anos. Como conclusão, perceberam que a formação do esporófito reduz a taxa de crescimento populacional a longo período, embora, em curto período, com a formação dos esporófitos, a formação de novos ramos é reduzida.

2.4 Dimorfismo sexual nas briófitas

As espécies que investem muito recurso na reprodução para formação de um determinado sexo apresentam menor vigor nas demais funções vegetativas (STEARNS, 1992; DELPH, 1999; LAAKA-LINDBERG, 2001). Conforme relatado na seção anterior, é notório que o custo reprodutivo afeta a morfologia dos indivíduos. Especificamente nas briófitas, o dimorfismo sexual tem ocorrido em diferentes estágios ontogenéticos, *e.g*., fisiológico e morfológico (UNE, 1984; McLETCHIE, 2001; STARK et al., 2004, GLIME AND BISANG, 2017a).

2.4.1 Dimorfismo sexual em traços fisiológicos

Um dos primeiros relatos sobre dimorfismo sexual em traço fisiológico foi feito por McLETCHIE (2001) para a hepática *Sphaerocarpos texanus* Austin*.* O autor observou uma maior resistência na germinação dos esporos femininos quando comparado aos masculinos. Sendo assim, concluiu que a mortalidade dos esporos masculinos implicou diretamente na razão sexual da população, uma vez que o maior número de esporos viáveis a germinação eram femininos.

Em *Syntrichia caninervis,* tem-se observado o dimorfismo sexual em traços fisiológicos quanto à termotolerância, estresse hídrico e regeneração de partes vegetativas (STARK; McLETCHIE; MISHLER, 2005; STARK; McLETCHIE, 2006). Estes trabalhos relatam que as plantas femininas de *S. caninervis* toleram mais o estresse hídrico, apresentam maior taxa de crescimento do protonema e se regeneram mais rápido do que as plantas masculinas.

Recentemente, Marks; Burton, McLetchie (2016) exploraram a tolerância à dessecação nos sexos masculinos e femininos em populações de *Marchantia inflexa* constatando que essa espécie é capaz de tolerar moderadas desidratações, e que, independente da população, os gametófitos femininos exibem maior tolerância à dessecação se comparados aos masculinos.

2.4.2 Dimorfismo sexual em traços morfológicos dos gametófitos

Lewin (1988) afirma que “o mundo é cheio de grandes fêmeas” (“*the world is full of great females*”), em relação aos animais. Embora sejam poucas as espécies de briófitas que apresentam dimorfismo sexual, o padrão de maior tamanho nos gametófitos femininos tem sido observado (SÖDERSTRÖM; GUNNARSSON, 2003). A necessidade de os organismos femininos serem maiores que os masculinos, relaciona-se à reprodução, visto que as fêmeas necessitam produzir e nutrir os seus descendentes (LEWIN, 1988; DELPH, 1999; OBESO, 2002).

Quanto às diferenças de tamanho dos gametófitos das briófitas, observam-se plantas femininas maiores do que as masculinas, plantas masculinas maiores do que as femininas e plantas com dimorfismo sexual pouco pronunciado (Tabela 1). Dentre as plantas terrestres, um extremo dimorfismo sexual é encontrado exclusivamente nas briófitas, os “machos anões” (*dwarf male)* (GLIME; BISANG, 2017), que ocorrem em espécies dioicas de musgos, com sistema sexual pseudautoico, onde esporos masculinos germinam sobre as plantas femininas, formando gametófitos diminutos. (ROSENGREN et al., 2014; ROSENGREN; CRONBERG, 2014). Estudos sugerem que a redução do tamanho destes gametófitos é regulada por hormônios voláteis (GLIME; BISANG, 2017 b).

Além dos machos anões, gametófitos masculinos pequenos podem ser observadas ocorrendo naturalmente em espécies dioicas de musgos, ou seja, sem estarem epifitando uma planta feminina (GLIME; BISANG 2017b). A maioria das espécies com dimorfismo sexual apresenta indivíduos masculinos menores do que os femininos e, no geral, o menor tamanho dos gametófitos ou rametas masculinas relaciona-se à reprodução (HORSLEY; STARK; McLETCHIE, 2011).

No que concerne ao menor tamanho dos gametófitos masculinos, a demanda conflitante, entre o investimento reprodutivo e o desenvolvimento vegetativo, foi observado no acarretamento do dimorfismo sexual nas espécies (Tabela 1). Em contrapartida, nas espécies em que os gametófitos femininos são menores que os masculinos, os estudos relacionam o dimorfismo sexual com a formação do esporófito (Tabela 1).

2.5 Fissidentaceae

A família objeto desta dissertação, Fissidentaceae, apresenta musgos acrocárpicos, amplamente distribuídos em todos os continentes excetuando na

Antártica, tendo a maior diversidade nas regiões tropicais. É unigenérica, sendo *Fissidens* Hedw. representado pelos subgêneros *Aloma* Kinfb., *Fissidens* Hedw., *Octodiceras* (Brind.) Broth. e *Pachyfissidens* (Müll. Hall.) Kindb. (PURSELL; BRUGGEMAN-NANNENGA, 2004). As espécies de Fissidentaceae são facilmente distinguidas das demais espécies de musgos pelo peristômio com dentes divididos até ao meio, disposição dística dos filídios e pela lâmina vaginante (PURSELL 2007; BORDIN; YANO 2014). São conhecidas aproximadamente 400 espécies de *Fissidens* em todo o mundo, destas, 93 são ocorrentes no Neotrópico (PURSELL 2007) e 72 são citadas para o Brasil (BORDIN, 2015).

Quanto ao sistema sexual, as espécies neotropicais de *Fissidens* podem ser classificadas em: monoicas, com sistema rizautoico (rametas perigoniais ligadas a rametas periqueciais pelos rizoides), goniautoico (androécios axilares e ginoécios terminais), cladautoico (com androécio em um ramo separado), sinoico (anterídios e arquegônios misturados na mesma inflorescência), polioico (com várias formas de gametângios na mesma planta), e dioicas (inflorescência masculinas e femininas em plantas diferentes) *sensu stricto* e pseudautoica (plantas masculinas anãs epifitando planta feminina) (PURSELL 2007). O dimorfismo sexual é assinalado na família, onde os ramos esporofíticos são menores que os ramos não expressos, sendo esta uma característica estável e a ponto de ser utilizada na distinção taxonômica de espécies. No Brasil, apresentam dimorfismo as seguintes *Fissidens curvatus* Hornsch., *F. scalaris* Mitt. e *F. taylorii* Müll. Hal.

Sendo assim, Fissidentaceae é uma família que oferece a possibilidade de testar relação entre o investimento reprodutivo e o dimorfismo sexual, em espécie de condição sexual rizautoica comparativamente a outros sistemas sexuais. Para isso foram selecionados neste estudo representantes de duas espécies com sistemas reprodutivos distintos: rizautoico (*Fissidens flaccidus*) e goniautoico (*F. submarginatus*).

Tabela 1. Traços de história de vida para várias espécies de briófitas dioicas e rizautoicas com dimorfismo sexual.

Espécie	**Sistema sexuais**	**Tamanho do gametófito**	**Limiar para a primeira reprodução**	**Frequência de expressão sexual**	**Investimento reprodutivo pré-zigótico**	**Investimento reprodutivo pós-zigótico**	***Demanda conflitante***	**Razão sexual**	**Referências**
Aloina bifrons (De Not.) Delgad.	Rizautoico	F > M	—	F < M	F < M	—	Tipo 1	F < M	Stark e Brinda (2013)
Anastrophyllum hellerianum (Nees ex Lindenb.) R.M.Schust.	Dioico	F > M	—	—	—	—	Tipo 1	—	Pohjamo e Laaka-Lindberg (2004)
Bryum agenteum Hedw.	Dioico	F < M	—	—	F < M	—	Tipo 1	F > M	Horsley; Stark; McLetchie (2011)
Ceratodon purpureos (Hedw.) Brid.	Dioico	F > M	—	F > M	F < M	—	Tipo 1	F > M	Shaw e Beer (1999); Shaw e Gaughan (1993); McDaniel (2005)

Dicranum polysetum Sw.	Dioico	F > M	—	—	F < E	F < E	Tipo 1	—	Bisang e Ehrlén (2002)
Fissidens flaccidus Mitt.	Rizautoico	F > M	—	F > M	F < M	F < E <M	Tipo 1	F > M	Santos et al. (2018)
Hylocomium splendens (Hedw.) Schimp.	Dioico	F < M	—	—	—	—	Tipo 1	F > M	Rydgren e Økland, (2002) Cronberg et al. (2006)
Lophozia silvícola H. Buch.	Dioico	F < M	—	—	F > M*	—	Tipo 1; Tipo 3	—	Laaka-Lindberg (2001)
Marchantia inflexa Nees & Mont.	Dioico	F > M	—	F > M	F < M^{G}	—	Tipo 4	F > M	McLetchie e Puterbaugh (2000)
Pseudocalliergon trifarium (F. Weber & D. Mohr) Loeske.	Dioico	F = M*	—	—	F > M	—	Tipo 1	F < M	Bisang et al. (2006)
Sphaerocarpus	Dioco	F > M	—	—	—	—	Tipo 2	F > M	McLetchie,

texanus Austin									2001.
Syntrichia carninervis Mitt.	Dioico	F = M*	> 2 mg	—	F = M	—	Não observado	F > M	Stark et al. (2001)

F = feminino não esporofítico, E= feminino esporofítico, M = masculino expresso e NE = indivíduo ou *ramet* não expressando sexo.

[G] Investimento reprodutivo calculado com base na produção de gemas.

* Dimorfismo sexual pouco proeminente.

Demanda conflitante: Tipo 1 (Reprodução sexual *vs.* crescimento vegetativo); Tipo 2 (Reprodução assexual *vs.* crescimento vegetativo); Tipo 3 (Reprodução sexual *vs.* reprodução assexual); Não observado (Quando não ocorreu *Demanda conflitante*).

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3 SEGREGATION OF SEXUAL FUNCTIONS PROMOTE THE HIGHEST REPRODUCTIVE INVESTMENT IN *Fissidens* (FISSIDENTACEAE, BRYOPHYTA)

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Segregation of sexual functions promote the highest reproductive investment in *Fissidens* (Fissidentaceae, Bryophyta)

Wagner L. dos Santos[1,2]

Lisi D. P. Alvarenga[1]

Kátia C. Pôrto[1]

[1] Federal University of Pernambuco, Department of Botany, Moraes Rego Av., s/n, University City 50670-901 - Recife, Pernambuco, Brazil

[2]Corresponding author (e-mail: wag.lsantos1@gmail.com)

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ABSTRACT

Premise of the study

Life history theory predicts that reproduction is expensive. Male and female organisms usually have different patterns of reproductive investment. In this study, we proposed the hypothesis that separation of sex functions in a rhizautoicous moss species (male ramets attached to female ramets by rhizoids) entails a higher reproductive investment in the male function in relation to the female function in the prezygotic phase, than that observed in a gonioautoicous (terminal perichaetia and axillary perigonia) taxonomically related moss.

Methods

The studied species were *Fissidens flaccidus* Mitt. and *F. submarginatus* Bruch., rhizautoicous and gonioautoicous, respectively. We selected 100 ramets of *F. flaccidus* of each of the following categories: male, sporophytic female, non-sporophytic female. In the case of *F. submarginatus*, 100 ramets each of sporophytic and non-sporophytic categories were selected. The ramets were cleaned, dried for 72 h at 70 °C, and weighed individually for quantification of the total and reproductive biomass in order to calculate the reproductive investment of each ramet individually.

Key results

In the rhizautoicous moss, male reproductive investment was significantly higher than prezygotic female investment, while in the gonioautoicous species no significant differences were found. GLM models showed that reproductive investment compromises the vegetative development in both species, indicating the existence of reproductive cost.

Conclusions

Our findings confirmed our hypothesis, showing that resource limitation of vegetative development is related to the reproductive investment of the ramet. So that, as the distance between the sexual structures increases, so does the reproductive investment in male function.

Key words

Gonioautoicy; life history; reproductive cost; reproductive effort; resource allocation; resource limitation; rhizautoicy; sexual systems.

INTRODUCTION

According to the theory of life history in plants, all resources produced by an individual are allocated to traits, such as reproduction, defense, and growth (Delph, 1999). Among these traits, reproduction is crucial because it ensures the maintenance of populations in ecosystems and is considered the most important event in the life cycle of plants (Richards, 1986). For a plant to reproduce, it is essential that part of the energy and nutrients captured by it be directed to the formation of sexual structures, whose proportional values correspond to the definition of reproductive investment (Karlsson and Méndez, 2005). Reproductive investment involves two moments of the life cycle, namely, the prezygotic phase corresponding to the formation of male and female gametangia, and the postzygotic phase, exclusive of females, corresponding to the formation of offspring (Jesson and Garnock-Jones, 2012).

In dioecious species, males and females present different patterns of reproductive investment (Atlan et al., 1992; Suzuki, 2005; Bisang et al., 2006). In angiosperms, the prezygotic reproductive investment is always highest in the male (Goldman and Willson, 1986; Cruden, 2000). However, plants with hermaphrodite flowers present a smaller reproductive investment in male function when compared to dioecious species (Cruden, 2000). In turn, the production of fruits and seeds is always more expensive than prezygotic sex functions (Lloyd and Webb, 1977; Richards, 1986). As for the bryophytes, few studies have investigated the relative prezygotic reproductive investment of the two sex functions, but the highest values have been reported more frequently for the male than the female in dioicous and rhizautoicous species (Stark et al., 2000; Horsley et al., 2011; Stark and Brinda, 2013; Santos et al., 2018), while the opposite was reported by Bisang et al. (2006). In turn, postzygotic investment, i.e. the formation of sporophytes, is always more expensive.

In case of resource limitation, reproduction can compete with other vital functions of the plant, and such an effect is called reproductive cost (Obeso, 2002). Measuring the reproductive cost in hermaphrodite angiosperms is difficult because the invested resource in reproduction is directed for two sexual functions (Delph, 1999). In bryophytes, both prezygotic and postzygotic reproductive investment have been reported to cause deficits in vegetative functions such as defense and growth (Laaka-Lindberg, 2001; Bisang and Ehrlén, 2002; Santos et al., 2018). In these plants, the reproductive cost of sporophyte production is quite often documented (Rydgren and Økland, 2003; Stark et al., 2000), while a few studies suggest the possibility of reproductive cost to be also entailed in gametangia production (Laaka-Lindberg, 2001; Santos et al., 2018).

Reproductive investment and cost have been more researched in dioicous (Stark et al., 2000; Rydgren and Økland, 2003; Bisang et al., 2006; Brzyski et al., 2014; Horsley et al., 2011) than monoicous bryophyte species (Stark and Brinda, 2013; Santos et al., 2018), because the separation of sexual functions calls attention to comparative analyses between sexes.

Several properties make bryophytes an excellent model system to evaluate the relationship between reproductive investment and reproductive cost in different sexual systems. First, bryophytes present a rapid life cycle compared to angiosperms, making it easier to follow up reproductive events (Ehrlén et al., 2000). Second, the small size of these plants facilitates the quantification of reproductive investment, which is defined as the proportion of biomass directed to reproductive structures in relation to vegetative parts. Finally, bryophytes present a large diversity of sexual systems with a gradual distancing of sex functions, which makes it possible to carry out comparative studies on the variety of reproductive investments across gradients of segregation of sex functions (Maciel-Silva and Pôrto, 2014).

Sexual systems influence the pattern of reproductive investment in bryophytes. Stark and Brinda (2013) call attention to the possible gradient of probability of cross-fertilization among the sexual systems of bryophytes, where the lowest chance is predicted in the synoecious system (sex organs in the same inflorescence), increasing in other sexual systems as the physical distance between male and female reproductive organs increases, to a maximum in the dioicous system. They predicted that this gradient may reflect the male reproductive investment, which is expected to be higher in cases of greater sex segregation (Table 1). This hypothesis, although widely discussed in flowering plants, has not yet been tested in other studies with bryophytes, other than by Stark and Brinda (2013), who proposed it.

Therefore, we aimed to understand the relationship between reproductive investment and competition for resources implied in the separation of sex functions in bryophytes. As a model system, two tropical species of the genus *Fissidens* were chosen. We studied *Fissidens flaccidus* Mitt. and *F. submarginatus* Bruch., which are rhizautoicous (reproductive organs produced in distinct plants connected by rhizoids) and gonioautoicous (reproductive organs produced in the same plant) species, respectively. In this study, we tested the following hypothesis: (1) reproductive investment in male function in relation to female function is highest in the rhizautoicous species when compared to the gonioautoicous; and (2) resource limitation in the systems studied is indicated by the fact that reproductive investment implies less resources left for vegetative growth in both sexual systems studied.

MATERIALS AND METHODS

Studied species, site and sampling

Fissidens flaccidus is an acrocarpous moss with a monoicous sexual system of the rhizautoicous type, found in the tropics (Pursell, 2007; Bordin and Yano, 2013). In Brazil, it is found in all phytogeographical domains (Amazon, Caatinga, Cerrado, Atlantic rainforest, Pampa and Pantanal), growing on artificial substrates, rocks and, more frequently soil, at altitudes from 40–1900 meters (Pursell, 2007; Bordin and Yano, 2013; Bordin, 2015). The population analyzed in the present study was found on soil, in the campus of the Federal University of Pernambuco, Recife, PE (-8°.05'05"18 S, -34°.94'86"32 W).

Fissidens submarginatus is an acrocarpous moss with a monoicous sexual system of the gonioautoicous type, and distributed in the Americas and Africa (Pursell, 2007; Bordin and Yano, 2013). As *F. flaccidus*, it is found in all Brazilian phytogeographical domains and, according to Bordin and Yano (2013), it grows on soil, rock, and tree bases. The population selected for the study was found on soil, in Dois Irmãos State Park, Recife, PE (8°7'30"S and 34°52'30"W).

The climate in Recife is hot and wet, according of Köppen's classification, with a mean temperature of 23 °C (Alvares et al., 2013). The rainy season occurs in the autumn-winter (March-August), and the rainiest months are June and July (Coutinho et al., 1998).

For each population, 40 1-cm² samples were randomly collected. One hundred ramets of each of the following categories were selected in *F. flaccidus*: expressing male (ramets with perigonia), non-sporophytic female (ramets with perichaetia), and sporophytic female (ramets with sporophyte with intact operculum). In the case of *F. submarginatus*, 100 ramets of the following categories were selected: non-sporophytic ramets, (ramets with perigonia and perichaetia) and sporophytic ramets (ramets with perigonia and sporophytes).

Biomass and reproductive investment

Antheridia and archegonia in the ramets were counted under microscope (40x magnification). The simple and distinct structure of ramets of *Fissidens* species made it possible to count gametangia in the present study through non-destructive observation. Reproductive structures (perigonia, perichaetia and sporophytes) were detached from the vegetative parts and then oven-dried at 70 °C for 72 h (Bisang and Ehrlén, 2002). After, the structures were deposited in a styrofoam box with silica gel until weighing on a semi-microanalytical scale Mettler Toledo scale (maximum value 320g, resolution of 0.01mg/0.1mg).

Because perigonia and perichaetia were too light to be weighed individually, the biomass of each perigonium and archegonium was indirectly estimated. To this end, all perichaetia of the 100 non-sporophytic ramets of *F. submarginatus* were weighed altogether, and the value obtained was divided by the total number of archegonia of the set. The individual biomass of each perichaetia was indirectly estimated by multiplying this constant value by the number of archegonia of each perichaetia. The same procedure was used to determine the biomass of perigonia and the perichaetia of the other ramets of *F. submarginatus* and *F. flaccidus*. Sporophytes were weighed individually.

We calculated the sexual reproductive investment for each ramet of *F. flaccidus* and *F. submarginatus* individually, according to the formula: RI = RB/ (RB + VB), where RI is the reproductive investment (proportion of biomass invested in reproduction), RB is the biomass of reproductive structures and VB is the biomass of the vegetative portion of the ramet (McLetchie and Puterbaugh, 2000).

Statistical analysis

Data normality was tested with the Shapiro-Wilk test. As the data did not present a normal distribution even after transformation, non-parametric tests were applied to compare the reproductive investments. The Kruskal-Wallis test was applied to compare the perigonial reproductive investment of male ramets of *F. flaccidus* with non-sporophytic and sporophytic ramets of *F. submarginatus*. In turn, the Mann-Whitney test was used to compare the perichaetial reproductive investment of non-sporophytic female ramets of *F. flaccidus* and non-sporophytic ramets of *F. submarginatus*. The same test was applied to compare the biomass invested in sporophytes in sporophytic ramets of *F. flaccidus* and *F. submarginatus*. Total reproductive investment of each type of ramet was compared with the Kruskal-Wallis test. Prezygotic male and female reproductive investment in the rhizautoicous species was compared using the Mann-Whitney test, whereas Kruskal-Wallis test was used for the gonioautoicous species.

Resource limitation in growth

We investigated the existence of reproductive cost using generalized linear models (GLM) as an indication of resource limitation in the studied species. First, two models were created. In both models, the vegetative biomass and type of ramet were the predictor variable; in model 1, reproductive biomass was the response variables, and in model 2, reproductive investment (proportion of resource allocated in reproduction) was the response variable.

The Akaike's Information Criterion adjusted for small samples ($AIC_{c)}$ was calculated for the models. The AIC_c compares models in order to indicate which is more representative of the realization of statistical analyses (Rieman et al., 2006). The AIC_c results interpretation is based on weight valor of test, between 1 and 0, and the higher the value of the weight, the more representative the model (Rieman et al., 2006). Then, interactions in the models for each species were tested, removing the interaction term when non-significant. The models were compared through a variance test (ANOVA) to confirm if there was any significant explanation. The summary of the model was analyzed to identify which of the predictor variables and interactions had greater explanation power over the response variable. The GLMs were analyzed separately for each species. All of the statistical analyses were performed with R Studio Software (RStudio Team, 2015), and the MiMuin (Barton, 2009) package was used for the AIC_c analysis.

RESULTS

Reproductive investment

Perigonial investment differed significantly between sporophytic and non-sporophytic ramets of the gonioautoicous species and male ramets of the rhizautoicous species (Kruskal-Wallis chi-squared = 224.18, df = 2, $P < 0.001$) (Table 2).

As for perichaetial investment, there was no significant difference between species when comparing non-sporophytic female ramets of the rhizautoicous species and non-sporophyte ramets of the gonioautoicous species, (Mann-Whitney U test = 4522.5, P = 0.412). On the other hand, investment in sporophytes was significantly higher in the rhizautoicous species (Mann-Whitney U test = 2303, P < 0.001) (Table 2).

Regarding total reproductive investment, non-sporophytic ramets of the gonioautoicous species did not differ from the non-sporophytic female and male ramets of the rhizautoicous species, while the other ramets were significantly different from each other (Kruskal-Wallis chi-squared = 220.49, df = 4, P <0.001) (Tab. 2). Within the rhizautoicous species, male ramets presented higher reproductive investment than non-sporophytic female ramets (Mann-Whitney U test = 90, P < 0.001). On the other hand, investments in perichaetia and perigonia in the gonioautoicous species, were not statistically different (Mann-Whitney U test = 4814.5, P = 0.975).

Resource limitation

The AIC_c results indicated irrelevance for model 1 for both species (Table 3), thus we only used model 2 for statistical analyses. The GLM results showed that reproductive investment entails resource limitation for vegetative growth (Table 4). Vegetative growth was negatively associated with reproductive investment in all categories of ramets of the rhizautoicous species and in sporophytic ramets of the gonioautoicous species (Table 5).

DISCUSSION

The results of this study suggest that the rhizautoicous sexual system presents the feature of highest prezygotic reproductive investment directed to the male function, while no differences between male and female function were seen when it comes to prezygotic reproductive investment in the gonioautoicous sexual system. This confirms the prediction proposed by Stark and Brinda (2013). The advantage of lack of competition for resources in the rhizautoicous species is supported by the assumption of a limited pool of resources, and this in turn was confirmed in our study by the existence of cost of sex expression. We found that the highest reproductive investment implies resource limitation; thus, the sexual system that presented the highest reproductive investment consequently displayed the highest resource limitation for vegetative growth.

Our findings reinforce the hypothesis that compartmentalization of sexual functions leads to an increase in reproductive investment in the male function. This pattern, also observed in dioecious angiosperms, is explained by the fact that greater male investment aims to increase fertilization rates, as stamens of dioecious species have been found to produce a greater number of pollen grains than monoecious species (Armstrong and Irvine, 1989). This pattern in bryophytes may be explained by the segregation of sexual functions observed in the rhizautoicous and dioicous systems. In these systems, the number of ramets expressing male reproductive structures (perigonia) is found in a lower proportion than those that produce female structures. There are several theories and predictions that try to explain this phenomenon (Anderson and Lemm, 1972; Bisang and Hedenäs, 2005; Stark et al., 2010). The reproductive investment in sexual systems with compartmentalization of sexual functions, with male ramets producing more gametangia and consequently more sperm, must increase fertilization rates.

The compartmentalization of sexual functions to increase male reproductive investment compared to the female, also increases reproductive investment in each sexual function. Atlan et al. (1992) reported that unisexual individuals of gynodioicous angiosperms *Thymus vuigaris* L. invest more in reproduction compered to bisexuals, suggesting that the absence of resource competition provides, in the former, a higher resource allocation in seeds in the case of unisexual individuals. In bryophytes, Maciel-Silva et al. (2012) reported a higher gametangia production in tropical dioicous species, what we believe to be a possible indication that compartmentalization of sexual functions as separate shoots, increases the performance of the sexual function of individual shoots. Although biomass is used to quantify reproductive investment, the number of gametangia may be indicative of reproductive investment in species. In our findings, a higher reproductive investment in male and sporophytic shoots was observed in the rhizautoicous species than in the gonioautoicous. In turn, there was evidence of resource competition in the latter species, since non-sporophytic ramets presented higher perigonial investment than those that formed sporophytes. Thus, we can infer the resources that would be directed to the production of antheridia, thus limiting the male sexual function, in sporophytic ramets was reallocated to sporophyte development.

Reproductive investment is related to the life history of the organism. Our results showed that the reproductive investment of a rhizautoicous moss was larger than that of a gonioautoicous moss. In the classification of life strategies proposed by During (1979) for bryophytes, and the relation of reproductive investment with life strategies has been already reported in bryophytes (Convey and Smith, 1993; Rydgren and Økland, 2002; Rydgren and Økland, 2003 Stark and Brind, 2013; Horsley et al., 2011; Pereira et al., 2016).

Assuming that the resources available for vital functions of the plant are not unlimited, trade-offs among the life history traits are expected. Limited resource in the sexual systems studied was confirmed by the fact that reproductive investment was linked to a smaller vegetative growth of ramets. Darwin (1877) argued that when a resource is not invested in one function, it is randomly relocated to another, a process alleged as a compensation law. This process is now called a trade-off, and in the case of reproductive functions, reproductive cost (Charlesworth and Charlesworth, 1981). In the case of *F. flaccidus*, reproductive cost was shown to be related to sexual dimorphism (Santos et al., 2018), where male ramets, which invest proportionally more resources than females in production of gametangia, are typically of small size, with gametophytes being described as gemmiform in the literature. Although it is common for studies to report resource limitation at the individual level, some studies related it to several phases of the population dynamics of bryophytes. For instance, in *Anastrophyllum hellerianum* (Nees ex Lindenb.), R.M. Schust., Pohjamo and Laaka-Lindberg (2003) showed that sporophyte-forming branches had a higher mortality rate than non-sporophytic ones. On the other hand, Alvarenga et al. (2016) observed that the gametophytes of the dioicous epiphytllous moss *Crossomitrium patrisiae* (Brid.) Müll Hal. that reproduced sexually (sporophyte formation) and asexually (propagule production) had the benefit of living for longer times but presented a reduction in growth rate.

CONCLUSIONS

In conclusion, our results supported the prediction of Stark and Brinda (2013) that sexual systems with compartmentalization of sexual functions tend to invest more in male than in female reproduction. We also conclude that segregation of sexual functions in the rhizautoicous species induced a higher reproductive investment in relation to the gonioautoicous system, and that there is a limited pool resources, demonstrated by a tradeoff between reproduction and vegetative growth.

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Author contributions

Wagner Santos, Lisi Alvarenga, and Kátia Pôrto elaborate together the main ideas and the writing of the manuscript. Kátia Pôrto and Lisi Alvarenga were involved in planning and supervising the work. Wagner Santos processed the experimental data and performed the analyses. All the authors worked together in the discussion and in the comments of the results

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Tables

Table 1

Sexual system	Description	Reproductive investment
Dioicy	**Male and female in different plants**	**LARGER**
Rhizautoicy	**Separate male and female shoots connected by rhizoids**	↑
Cladautoicy	**Perigonia and perichaetia on separate branches but both branches connected above ground**	
Autoicy	**Separate male and female inflorescences along same branch**	
Gonioautoicy	**Terminals perichaetia and axillary perigonia**	
Paroicy	**Antheridia adjacent to archegonia, very close together**	
Synoicy	**Antheridia and archegonia in same inflorescence mixed**	**SMALLER**

Table 1. Table reporting the different sexual systems. Arrow indicates the direction in which the male reproductive investment increases. Adapted from Stark and Brinda (2013).

Table 2

	Reproductive investment %			
	Perichaetia $^{n.s.}$	Perigonia ***	Sporophyte ***	Total***
	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD	$\bar{X}$ SD
Fissidens flaccidus				
(Rhizautoicous)				
Sex-expressing male	-	35.15 ± 4.70^{a}	-	35.15 ± 4.70^{a}
Non-sporophytic female	12.61 ± 6.78^{a}	-	-	12.61 ± 6.78^{b}
Sporophytic female	-	-	25.4 ± 7.85^{a}	25.4 ± 7.85^{c,d}
Fissidens submarginatus				
(Goniautoicous)				
Non-sporophytic	12.70 ± 8.61^{a}	12.20 ± 7.54^{b}	-	24.90 ± 14.82^{d,e}
Sporophytic	-	4.73 ± 3.00^{c}	17.64 ± 6.10^{b}	22.38 ± 7.13^{e}

Table 2. Mean of reproductive investment (%) of rhizautoicous and gonioautoicous species. The letters are significance parameters for comparison of reproductive investment between the sexual systems. The letters correspond to the comparison of the same column. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001; $^{n.s.}$ is a non-significant value for Kruskal-Wallis and Mann-Whitney tests. For rhizautoicous species “Sporophytic female” ramets possess a sporophyte, “Non-sporophytic female” ramets possess perichaetia, and “Sex-expressing male” ramets possess perigonia. For gonioautoicous, “Non-sporophytic” ramets possess perichaetia and perigonia, “Sporophytic” ramets have a sporophyte and perigonia.

Table 3

Fissidens flaccidus – rhizautoicous

Model	Intercept	df	LogL	AIC_C	Δ AIC_C	Weight
Model-1	0.9969	7	66.548	-118.7	297.55	0
Model-2	2.2960	7	215.322	-416.3	0.00	**1**

Fissidens submarginatus – gonioautoicous

Model	Intercept	df	LogL	AIC_C	Δ AIC_C	Weight
Model-1	1.078	5	60.716	-111.1	112.24	0
Model-2	2.264	5	116.834	-223.4	0.00	**1**

Table 3. Akaike's Information Criterion adjusted for small sample AIC_c results. Model 1: (predictor variable: vegetative biomass and ramet type; answer variable: reproductive biomass); Model 2: (predictor variable: vegetative biomass and ramet type; answer variable: reproductive investment). Log-likelihood (LogL), Akaike's Information Criterion with the small-sample bias adjustment (AICc), Δ AICc, and Akaike weights (w).

Table 4

	Df	Deviance	F	P- value
Fissidens flaccidus				
(Rhizautoicous)				
Vegetative biomass	1	11.149	784.089	**< 0.001**
Ramets	2	3.595	126.443	**< 0.001**
Vegetative biomass *vs* Ramets	2	0.638	4.180	**< 0.001**
Fissidens submarginatus				
(Gonioautoicous)				
Vegetative biomass	1	7598.5	77.776	**< 0.001**
Ramets	1	84.7	0.866	**< 0.001**
Vegetative biomass *vs* Ramets	1	1.4	0.014	0.668

Table 4. GLM ANOVA results of Model 1. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001; $^{n.s.}$ is a non-significant value.

Table 5

Table

Source	Estimate	Error	P-value
Fissidens flaccidus **(Rhizautoicous)**			
Main effects			
Non-sporophytic female	2.295	0.090	**< 0.001**
Sporophytic female	-0.454	0.109	**< 0.001**
Expressing male	-0.482	0.136	**< 0.001**
Interaction effects			
Vegetative biomass *vs* Non-sporophytic female	-0.660	0.048	**< 0.001**
Vegetative biomass *vs* Sporophytic female	0.398	0.059	**< 0.001**
Vegetative biomass *vs* Expressing male	0.354	0.130	**< 0.05**
Fissidens submarginatus **(Gonioautoicous)**			
Main effects			
Sporophytic ramet	2.263	0.188	**< 0.001**
Non-sporophytic ramet	-0.102	0.196	0.602
Interaction effects			
Vegetative biomass *vs* Sporophytic ramet	-0.518	0.106	**< 0.001**
Vegetative biomass *vs* Non-sporophytic ramet	-0.048	0.113	0.668

5. GLM summary.*p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001; $^{n.s.}$ is a non-significant value.

4 CONCLUSÕES

Diante dos trabalhos resultantes dessa dissertação que analisou o investimento reprodutivo de duas espécies de musgo do gênero *Fissidens*, podem ser destacadas as seguintes conclusões:

✓ O investimento reprodutivo está associado ao menor crescimento vegetativo masculino e causa dimorfismo sexual no musgo rizoautoico *Fissidens flaccidus.*

✓ Em diferentes tipos de sistemas monoicos o investimento reprodutivo nas funções sexuais se apresenta com padrões diversos.

✓ A segregação espacial dos sexos acarreta maior investimento pré-zigótico da função masculina.

✓ A compartimentalização das funções sexuais no sistema rizautoico determina o maior investimento reprodutivo devido à ausência de competição de recurso entre as funções sexuais.

✓ No sistema goniautoico o investimento esporofítico gera limitação de recurso para a formação dos perigônios.

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ANEXO A – ARTIGO PUBLICADO NO PERIÓDICO *CRYPTOGAMIE, BRYOLOGIE*

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Sexual dimorphism, vegetative growth and reproductive investment in the rhizautoicous moss

Fissidens flaccidus (Fissidentaceae, Bryopsida)

Wagner Luiz dos SANTOS[*], *Lisi dámaris Pereira ALVARENGA*

& Kátia Cavalcanti PÔRTO

Botany departament, Federal University of Pernambuco,

Av. Prof. Moraes Rego s/n Bioscience Center, 50670-901 Recife, PE, Brasil

Abstract – Reproduction is one of the most important and expensive processes in the life history of plants. The cost commonly incurred by reproductive investment in plants with separate sexes may result in sexual dimorphism. Among bryophytes, sexual dimorphism has been observed in some sex-expressing dioicous species and in some sex-expressing monoicous species with a rhizautoicous sexual system. In the present study, we have investigated sexual dimorphism in morphological features and relative reproductive investments in sporophytic female, non-sporophytic female, sex-expressing male and non-expressing ramets in a population of the rhizautoicous *Fissidens flaccidus.* Morphometric analyses confirmed sexual dimorphism; gemmiform male ramets were smaller than the other ramet morphs, and exhibited greater sexual investment. Sexual reproductive investment was approximately 35 % in males, 13% in non-sporophytic females and 25% in sporophytic females. Our results indicate trade-offs between reproductive investment and vegetative growth for both sexes in this species.

Sexual system / reproductive allocation / reproductive cost / rhizautoicy / trade-offs / mosses / bryophytes

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

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INTRODUCTION

Male and female individuals in many dioicous species may differ in morphology, physiology and life history traits, such as sexual maturation and mortality (Dawson & Geber, 1999; Holzapfel & Bradshaw, 2002). Although more frequently seen among unisexual species, dissimilarity between the sexes has been also observed in some cosexual species of bryophytes and angiosperms (Delph, 1999; Stark & Brinda, 2013). Among bryophytes, this is the case with some rhizautoicous species, where small male shoots are connected to female shoots through rhizoids (Wyatt, 1985; Maciel-Silva & Pôrto, 2014).

Sexual dimorphism in bryophytes is generally more subtle than in seed plants. Not only is sexual dimorphism expressed exclusively in the gametophytic phase (Glime & Bisang, 2017a), but sexual expression is also relatively rare (= gametangia) in dioicous bryophytes, which makes sex-specific morphological differences difficult to detect, with the exception of nanandrous (dwarf males) species (Hedenäs & Bisang, 2011; Pichonet & Gradstein, 2012). Sexual dimorphism

can be expressed in morphological, physiological and life history traits and at various ontogenetic stages. For example, male spores have been reported to be smaller in a few species (anisospory) (Une, 1984; Hedderson & Zander, 2007; Glime & Bisang, 2017b), and may have lower germination rates (McLetchie, 1992), while male gametophytes have shown lower water stress tolerance (Marks *et al.*, 2016), or their gametangia have been observed to mature earlier (Milne, 2001; Stark & Brinda, 2013). Conversely, sex-expressing females have distinctly greater biomass than males in some species (Shaw & Gaughan, 1993; McLetchie & Puterbaugh, 2000; Pohjamo & Laaka-Lindberg, 2004; McDaniel, 2005; Horsley *et al.*, 2011), and smaller in other species (Laaka-Lindberg, 2001; Rydgren & Okland, 2003; Holá *et al.*, 2014), while in some species, morphological sexual dimorphism is minimal (Stark *et al.*, 2001).

Life history theory predicts that the resources produced by a plant are directed to three essentials functions: growth, defense and reproduction (Delph, 1999). The proportion of resources allocated to reproduction is defined as reproductive investment or reproductive effort (Hirshfield & Tinkle, 1975; Karlsson & Méndez, 2005). In many cases, reproductive investment entails a cost because the reproductive function competes with others functions. If such a cost differs between the sexes, sexual dimorphism may result (Laaka-Lindberg, 2001; Obeso, 2002; Karlsson & Méndez, 2005).

Reproductive cost has received little attention in monoicous bryophytes with functionally separate sexes, such as rhizautoicous species. On the other hand, there are indications of reproductive costs in dioicous species in terms of reduced growth rates and clonal propagation as a result of prezygotic (Laaka-Lindberg, 2001; Pereira *et al.*, 2016) and postzygotic investment, i.e. sporophyte formation (Ehrlén *et al.*, 2000; Stark *et al.*, 2000; Bisang & Ehrlén, 2002; Rydgren & Økland, 2002, 2003; Stark *et al.*, 2009). Prezygotic investment has been reported to be higher in males than in females (McLetchie & Puterbaugh, 2000; Horsley *et al.*, 2011; Stark & Brinda, 2013), lower in males than in females (Laaka-Lindberg, 2001; Pohjamo & Laaka-Lindberg, 2004; Bisang *et al.*, 2006), and equal between the sexes (Stark *et al.*, 2001). The formation of sporophytes usually consumes more resources than the development of gametangia (Laaka-Lindberg, 2001; Holá *et al.*, 2014).

In this study, we aimed to investigate the relationship between reproductive investment and sexual dimorphism in sex-expressing individuals of a tropical bryophyte species, the rhizautoicous *Fissidens flaccidus* Mitt. This species has been described as monomorphic, with equal-sized female and male stems (Pursell, 2007). However, preliminary statistical analyses indicated clear morphological differences between the sexes. Here, we quantify sexual dimorphism in *F. flaccidus* and test if this is associated with reproductive investment. We hypothesize that higher reproductive investment is associated with reduced vegetative growth; i.e. the sexual morph with higher allocation to sexual function has smaller sizes.

MATERIALS AND METHODS

Species studied, study site and sampling

Fissidens flaccidus is a monoicous acrocarpous moss with a rhizautoicous sexual system, distributed in the Neotropical region, Africa, New Guinea and Australia (Pursell, 2007; Bordin & Yano, 2013). In Brazil, the species is found in all phytogeographical domains (Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa and Wetland), where it grows on artificial substrates, rocks and, more frequently, soils, at 40 to 1900 m a.s.l. (Pursell, 2007; Bordin & Yano, 2013; Bordin, 2015).

Forty 1-cm^2 samples were randomly collected from a large terricolous population (approximately 2 m^2) of *Fissidens flaccidus* at the end of the rainy season on the campus of the Federal University of Pernambuco, in the city of Recife (-8°.05’05’’18 S, -34°.94’86’’32 W). The weather in Recife is tropical, hot and humid according to Köppen’s classification, with an average monthly temperature of 23°C (Alvares *et al.*, 2013). The rainy season occurs in the autumn-winter period (March-August), with June-July being the rainiest months (Coutinho *et al.*, 1998). The studied population of *Fissidens flaccidus* began to stabilize early in the rainy season and withered during the dry season. Gemmae and spores were observed in the studied population.

All ramets of each 1-cm^2 sample were examined (10 ramets in average). After screening, we picked 100 ramets of the each one of the following sexual morphs: sex-expressing male (producing perigonia), non-sporophytic female (producing archegonia), sporophytic female (sporophyte in the phenophase “late operculum intact”) (Greene, 1960) and non-expressing (*i.e.* unknown sex) ramets. Thus, a total of 400 ramets were selected from the sample set of 40 cm^2. The sex of the ramets was identified under a dissection microscope based on the presence of sexual structures. Ramets were washed to remove residual substrate (soil) and had their rhizoids cut off and gemmae eliminated under a dissection microscope.

Morphometric analysis

The individual ramets were placed on a slide with distilled water. The slides with non-sporophytic females, sporophytic females and non-expressing ramets were photographed using a Leica EZ4 stereomicroscope (3x magnification), while sex-expressing male ramets had to be photographed with a Leica DM500 microscope (10x magnification) because of their smaller size.

All images were used for making morphometric measurements. The following parameters were measured on each individually photographed ramet using ImageJ software (Abràmoff *et al.*, 2012): length of the ramet, width of the middle region of the ramet, length and width of leaves in the middle region of the ramet (one randomly chosen leaf per ramet) (Fig. 1) and total number of leaves.

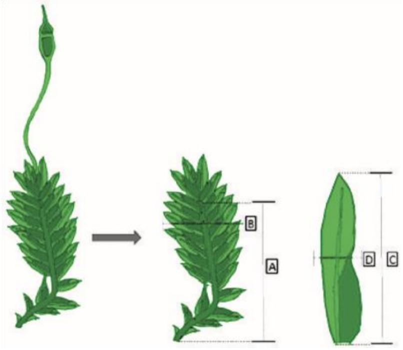

Fig. 1. Schematic drawing of sporophytic female ramets showing the traits measured for morphometric analysis of *Fissidens flaccidus.* **A.** Ramet length, **B.** Ramet width, **C.** Leaf length, **D.** Leaf width.

Biomass and reproductive investment

After morphometric measurements, female and male ramets were analyzed microscopically (40x magnification) for quantification of the number of antheridia per perigonium and archegonia per perichaetia. The flat and simple structure of ramets made it possible to count gametangia through non-destructive observation. Next, we excised the reproductive structures (perigonia, perichaetia and sporophytes) and stored them separately from the vegetative parts.

Vegetative and reproductive structures were oven-dried at 70°C for 72 h until a constant weight (Bisang & Ehrlén, 2002), and deposited in a styrofoam box with silica gel until weighing in a semi-microanalytical Metler Toledo scale (maximum value 320g, resolution of 0.01 mg/0.1 mg).

Sex-expressing male ramets were too light to be weighed individually and so biomass was estimated indirectly. For this, the number of leaves was the morphometric variable chosen as indicative of weight increment because this variable had the strongest correlation with weight in the other ramet categories (nonsporophytic female, sporophytic female and non-expressing ramets), which were weighed on individual basis. Thus, to estimate the weight of individual male ramets, the weight of 100 male ramets was determined, including leaves and stems. This value was then divided by the sum of the number of leaves of the entire set of male ramets. Finally, the resulting value was used as a constant to estimate the biomass of each individual ramet by multiplying it by the number of leaves. The same indirect estimation of biomass was used for gametoecia, because of their small size. The perichaetia of the 100 non-sporophytic females were weighed all together and this value was divided by the total number of archegonia in the whole set; individual biomass of each perichaetium was then obtained by multiplying this constant value by the number of archegonia of each perichaetium. The same was done in the case of sex-expressing males and sporophytic females. Sporophytes were weighted separately.

Sexual reproductive investment was calculated for each individual ramet according to the formula: $Ri = Rb/(Rb + Vb)$, where Rb is the reproductive biomass, Vb is the vegetative biomass and Ri the proportion of resources invested in reproduction (McLetchie & Puterbaugh, 2000).

Statistical analysis

Sexual dimorphism. Measures of centrality and dispersion (mean, standard deviation and Pearson coefficient of variation) were used to describe morphometric variables and biomass.

In order to investigate the presence of dimorphism, the normality of data was first checked by the Shapiro-Wilk test. Since several traits were not normally distributed, we used the Kruskal-Wallis test to compare morphometric data and biomass between morphs. The Dunn's test was used for multiple comparisons and p-values were adjusted with Bonferroni correction.

Sexual dimorphism *versus* reproductive investment. In order to investigate how reproductive investment relates to dimorphism, we used a Principal Component Analysis (PCA). Since many morphometric variables were internally correlated, ramet length was used to represent the morphometric variables for comparisons of ramet vegetative biomass and reproductive investment. The scale of variation of the data was standardized (Ranging). The Spearman's correlation index was used to investigate the statistical significance of the relationship between these variables. P-values were adjusted with Bonferroni correction. All analyses were performed using Rstudio Team (2015) version 1.0.143 and the figures were prepared with the R package GGPLOT (Wickham, 2009).

RESULTS

Sexual dimorphism

Sex-expressing male ramets were significantly smaller in all morphometric aspects (Table 1; Fig. 2; ramet length H2 = 170.85, $p < 0.0001$; ramet width H2 = 165.93, $p < 0.0001$; leaf length H2 = 178.91, $p < 0.0001$; leaf width H2 = 182.23 , $p < 0.0001$; number of leaves H2 = 143.58, $p < 0.0001$) and had lower vegetative biomass (H2 = 226.73, $p < 0.0001$) (Table 1) than the other morphs. Non-sporophytic and sporophytic female ramets had more leaves than non-expressing ramets, and sporophytic females also had longer leaves (Tab. 1) than non-expressing ramets. The total biomass of sporophytic and non-sporophytic females was similar and significantly greater than the biomass of sex-expressing males (H2 = 182.23 , $p < 0.0001$).

Fig. 2. Principal Component Analysis (PCA) of ramet length, reproductive investment, and vegetative biomass of three different sexual morphs of *Fissidens flaccidus*. Legend: R.L, Ramet length (Axis 1 = 0.56; Axis 2 = –0.65); V.B, Vegetative biomass (Axis 1 = 0.61; Axis 2 = –0.07); R.I, Reproductive investment (Axis 1 = –0.55; Axis 2 = –0.75). Variation explained by the first two axes: 95.6 %.

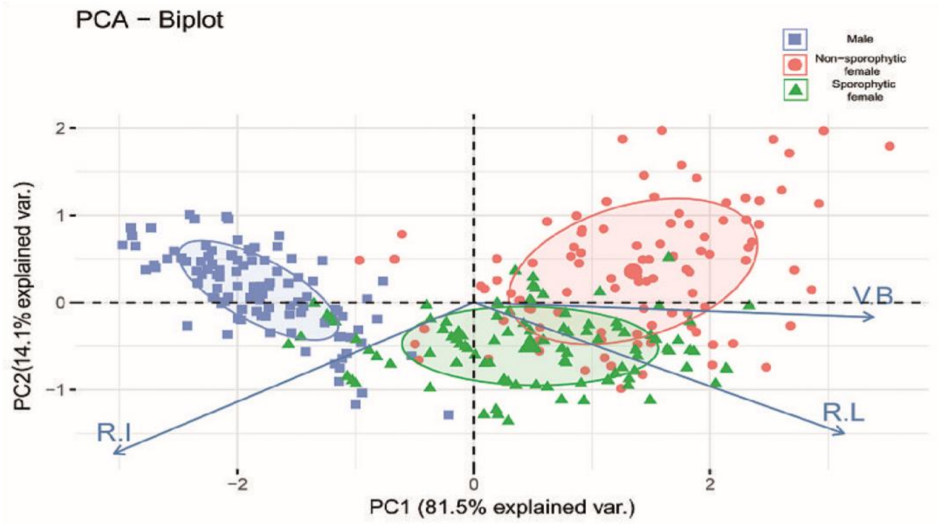

Table 1. Mean ($\bar{X}$) standard deviation (SD) and Pearson's coefficient of variation (CV) of morphometric data, vegetative biomass, reproductive biomass, total biomass and reproductive investment. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001; n.s. non-significant values of Kruskal-Wallis test. "Non-expressing" ramets are of unknown sex, "Sporophytic female" ramets possess a sporophyte, "Non-sporophytic female" ramets possess perichaetia, and "Sex-expressing male" ramets possess perigonia

	Sex-expressing male		*Non-sporophytic female*		*Sporophytic female*		*Non-expressing*	
	$\bar{X}$ *SD*	*CV*	$\bar{X}$ *SD*	*CV*	$\bar{X}$ *SD*	*CV*	$\bar{X}$ *SD*	*CV*
Morphometric measures (mm)								
Ramet length***	1.55 ± 0.80^{a}	0.51	3.99 ± 1.66^{b}	0.41	3.75 ± 1.27^{b}	0.33	3.77 ± 1.40^{b}	0.34
Ramet width***	1.50 ± 0.45^{a}	0.3	2.57 ± 0.41^{b}	0.15	1.99 ± 0.48^{c}	0.24	2.22 ± 0.66^{b}	0.29
Number of leaves ***	10.11 ± 2.75^{a}	0.27	16.70 ± 3.49^{b}	0.20	14.36 ± 4.55^{b}	0.31	17.15 ± 4.97^{c}	0.28
Leaf length***	1.11 ± 0.25^{a}	0.22	1.84 ± 0.27^{b}	0.14	1.55 ± 0.28^{c}	0.18	1.48 ± 0.39^{c}	0.26
Leaf width***	0.20 ± 0.05^{a}	0.25	0.36 ± 0.06^{b}	0.16	0.28 ± 0.04^{c}	0.14	0.31 ± 0.07^{b}	0.22
Biomass (µg)								
Vegetative biomass***	6.20 ± 1.70^{a}	0.27	80.30 ± 43.84^{b}	0.54	66.36 ± 46.87^{b}	0.70	78.5 ± 55.96^{b}	0.71
Reproductive biomass***	7.80 ± 3.40^{a}	0.43	11.69 ± 6.30^{a}	0.53	29.90 ± 16.84^{b}	0.56	–	–
Total biomass***	14.20 ± 4.05^{a}	0.28	91.00 ± 44.46^{b}	0.48	96.25 ± 57.00^{b,c}	0.59	78.5 ± 55.96^{c}	0.71
Reproductive investment (%)***	35.15 ± 4.70^{a}	0.13	12.61 ± 6.78^{b}	0.53	25.4 ± 7.85^{c}	0.30	–	–

Table 2. Spearman's Correlation coefficient and associated p-values between reproductive and vegetative traits of three sexual morphs of *Fissidens flaccidus*. n.s. indicates non-significant correlation

sex-expressing male	*R_2*	p- *value*
Reproductive investment vs. Ramet length	– 0.25	= 1.0n.s.
Reproductive investment vs. Vegetative biomass	– 0.53	< 0.001
Non-sporophytic female	*R_2*	p- *value*
Reproductive investment vs. Ramet length	– 0.19	= 1.0n.s.
Reproductive investment vs. Vegetative biomass	– 0.62	< 0.001
sporophytic female	*R_2*	p- *value*
Reproductive investment vs. Ramet length	– 0.19	1.0n.s.
Reproductive investment vs. Vegetative biomass	– 0.72	< 0.001

Sexual dimorphism *versus* reproductive investment

Reproductive investment was significantly different between males, nonsporophytic females and sporophytic females (H2 = 339.89, p < 0.0001) (Table 1). Although sporophyte biomass was significantly higher than that of perigonia and perichaetia (H2 = 310.66, p < 0.0001), the reproductive investment, i.e. the average proportional allocation to sex structures was higher in males (35.15%) than sporophytic females (25.40%) and non-sporophytic females (12.61%). The first two PCA–axes explained 95.6% of the variance (Axis 1, 81.5%; Axis 2, 14.1%; Fig. 2). The first PCA-axis was strongly positively correlated with vegetative biomass (0.61) and ramet length (0.56). The second axis was strongly and negatively correlated with reproductive investment (-0.75). The Spearman's correlation test showed that reproductive investment was negatively correlated with vegetative growth in all ramet categories (Table 2).

DISCUSSION

The objective of this study was to quantify sexual dimorphism in *Fissidens flaccidus* and test whether this is associated with reproductive investment. Sexual dimorphism was confirmed for length and width of ramets, as well as for number, length and width of leaves and total biomass. Interestingly, the reproductive biomass did not differ between the sexes as long as no sporophytes were formed; in other words, perichaetia mass did not differ from perigonia mass at the ramet level. This finding implies that although absolute reproductive biomass is similar in both sexes, the reproductive investment is higher in males because they produce similar biomass despite being much smaller. Their limited size may be a result of earlier sexual expression, with resources being channelled to reproduction to the detriment of vegetative growth.

Many theories have sought to explain the possible causes of sexual dimorphism, and all involve reproduction as the determining factor (Price, 1984). Darwin (1877) and Lewin (1988) suggested that female organisms needed to reach greater sizes to initiate sexual expression because their function of producing and maturing offspring, which often involves a large number of descendants, is very costly. In this case, females must be larger at onset of sex expression to cope with the high future reproductive cost. In turn, Delph (1999) and Obeso (2002) suggest that reproductive cost leads to sexual dimorphism by causing the most expensive sex to grow less and become relatively smaller. In the present study, male ramets of

F. flaccidus invested proportionally more in reproduction than non-sporophytic and sporophytic females. To cope with the metabolic cost of forming perigonia, it has been suggested that maturation of gametes takes longer in male bryophytes (Lackner, 1939; Stark & Brinda, 2013), which we presume may have happened in *F. flaccidus*. The early onset of sex investment may have competed with vegetative development, leading males to become gemmiform.

Sexual dimorphism is common among some rhizautoicous species of *Fissidens*, including three species that occur in Brazil, namely, *Fissidens curvatus* Hornsch., *F. scalaris* Mitt., and *F. taylorii* Müll. Hal. In these species, male ramets are gemmiform and distinctly smaller than sporophytic females, which in turn are smaller than non-expressing ramets (Pursell, 2007; Bordin & Yano, 2013). In comparison with these species, sexual dimorphism in *F. flaccidus* is less pronounced.

The morphometric and biomass similarity between female and nonexpressing ramets may indicate that the latter are less likely to be males, because males are consistently smaller in the population. A higher proportion of females among sex-expressing ramets seems to be common in rhizautoicous mosses such as *Tortula muralis* Hedw. and *Atrichum undulatum* (Hedw.) P. Beauv., as reported by Longton & Miles (1982), and *Weissia controversa* Nees & Hornsch. (Anderson & Lemmon, 1972). An exception is *Aloina bifrons* (Stark & Brinda, 2013). The sampling in the present work was not designed for determining the sex ratio of *F. flaccidus* and, thus, this characteristic still needs to be investigated.

A higher proportional investment to formation of gametangia in males than in non-sporophytic females also has been reported by Stark & Brinda (2013) in the rhizautoicous *Aloina bifrons* (De Notaris) Delgadillo, although sporophytic ramets were not observed and reproductive investment was estimated at the population level only. Among the reproductive systems of bryophytes, the pseudautoicous system follows a similar trend of high sexual investment in the male function. Male plants of pseudautoicous species that grow on female ramets are dwarf and apparently have the main function of producing antheridia (Pichonet & Gradstein, 2012; Glime & Bisang, 2017b). While the mechanisms ruling the facultative reduction of males has yet to be investigated, higher reproductive investment of male ramets has been previously reported (Pursell, 2007; Stark & Brinda, 2013), and was suggested to imply higher reproductive success (Stark & Delgadillo, 2001).

The association between high reproductive investment, i.e. proportional allocation of resources to sex organs (Bisang *et al.*, 2006) and lower vegetative performance seems straightforward. Plants that invest proportionally more and earlier in reproduction may have little energy left over for growth (Laaka-Lindberg, 2001; Horsley *et al.*, 2011). Smaller size may result in relatively lower vigour and defense in the face of inclement weather or other harsh environmental conditions. For example, male populations of the dioicous liverwort *Marchantia inflexa* Nees & Mont. that reproduce better asexually have lower success under stressful conditions (McLetchie & Puterbaugh, 2000; Marks *et al.*, 2016). Larger females of the moss *Entodon cladorrhizans* (Hedw.) Müll. Hal. were observed to produce larger sporophytes, but the development of sporophytes resulted in decreased vegetative growth (Stark & Stephenson, 1983).

In the present study, reproductive investment was higher in sex-expressing males, followed by sporophytic females, and non-sporophytic females, and the accumulation of vegetative biomass decreased in this same order. This finding may indicate that these functions, growth and reproduction, compete for resources. Competition for resources, or trade-offs, among tropical mosses has been poorly investigated until present, but a trade-off between sexual (archegonia and antheridia) and asexual (gemmae) structures has been reported in species of *Calymperes* (Pereira *et. al.,* 2016). In order to confirm the presence of trade-offs in *F. flaccidus*, however, temporal studies, and preferably the inclusion of different genets and environmental gradients, are necessary (Horsley *et al.*, 2011; Brzyski *et al.*, 2014).

In the present study, we have confirmed that sex-expressing females and male ramets of *F. flaccidus* are dimorphic in many morphometric parameters, and although absolute prezygotically invested biomass does not differ among the sexes, the reproductive investment, i.e. the proportional biomass invested in sexual structures, was greater in male ramets, and this likely led them to grow less, thus indicating a trade-off between reproduction and vegetative growth.

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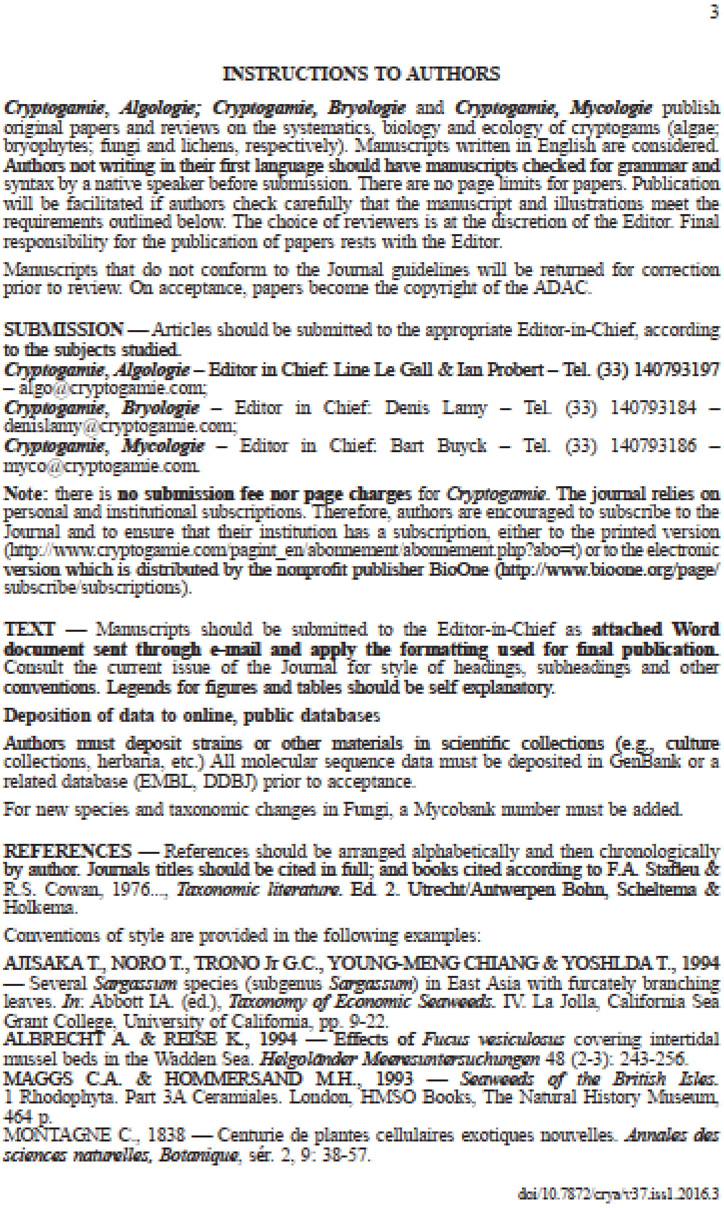
ANEXO B – NORMAS DO PERIÓDICO *CRYPTOGAMIE BRYOLOGIE*

ttp://www.cryptogamie.com/pagint_en/instructions/instructions_authors.pdf



INSTRUCTIONS TO AUTHORS

Cryptogamie, Algologie; Cryptogamie, Bryologie and ***Cryptogamie, Mycologie*** publish original papers and reviews on the systematics, biology and ecology of cryptogams (algae; bryophytes; fungi and lichens, respectively). Manuscripts written in English are considered. Authors not writing in their first language should have manuscripts checked for grammar and syntax by a native speaker before submission. There are no page limits for papers. Publication will be facilitated if authors check carefully that the manuscript and illustrations meet the requirements outlined below. The choice of reviewers is at the discretion of the Editor. Final responsibility for the publication of papers rests with the Editor.

Manuscripts that do not conform to the Journal guidelines will be returned for correction prior to review. On acceptance, papers become the copyright of the ADAC.

SUBMISSION — Articles should be submitted to the appropriate Editor-in-Chief, according to the subjects studied.
Cryptogamie, Algologie – Editor in Chief: Line Le Gall & Ian Probert – Tel. (33) 140793197 – algo@cryptogamie.com;
Cryptogamie, Bryologie – Editor in Chief: Denis Lamy – Tel. (33) 140793184 – denislamy@cryptogamie.com;
Cryptogamie, Mycologie – Editor in Chief: Bart Buyck – Tel. (33) 140793186 – myco@cryptogamie.com.

Note: there is **no submission fee nor page charges** for *Cryptogamie*. The journal relies on personal and institutional subscriptions. Therefore, authors are encouraged to subscribe to the Journal and to ensure that their institution has a subscription, either to the printed version (http://www.cryptogamie.com/pagint_en/abonnement/abonnement.php?abo=t) or to the electronic version which is distributed by the nonprofit publisher BioOne (http://www.bioone.org/page/subscribe/subscriptions).

TEXT — Manuscripts should be submitted to the Editor-in-Chief as **attached Word document sent through e-mail and apply the formatting used for final publication.** Consult the current issue of the Journal for style of headings, subheadings and other conventions. Legends for figures and tables should be self explanatory.

Deposition of data to online, public databases

Authors must deposit strains or other materials in scientific collections (e.g., culture collections, herbaria, etc.) All molecular sequence data must be deposited in GenBank or a related database (EMBL, DDBJ) prior to acceptance.

For new species and taxonomic changes in Fungi, a Mycobank number must be added.

REFERENCES — References should be arranged alphabetically and then chronologically by author. Journals titles should be cited in full; and books cited according to F.A. Stafleu & R.S. Cowan, 1976..., *Taxonomic literature*. Ed. 2. Utrecht/Antwerpen Bohn, Scheltema & Holkema.

Conventions of style are provided in the following examples:

AJISAKA T., NORO T., TRONO Jr G.C., YOUNG-MENG CHIANG & YOSHLDA T., 1994 — Several *Sargassum* species (subgenus *Sargassum*) in East Asia with furcately branching leaves. *In*: Abbott IA. (ed.), *Taxonomy of Economic Seaweeds*. IV. La Jolla, California Sea Grant College, University of California, pp. 9-22.
ALBRECHT A. & REISE K., 1994 — Effects of *Fucus vesiculosus* covering intertidal mussel beds in the Wadden Sea. *Helgoländer Meeresuntersuchungen* 48 (2-3): 243-256.
MAGGS C.A. & HOMMERSAND M.H., 1993 — *Seaweeds of the British Isles*. 1 Rhodophyta. Part 3A Ceramiales. London, HMSO Books, The Natural History Museum, 464 p.
MONTAGNE C., 1838 — Centurie de plantes cellulaires exotiques nouvelles. *Annales des sciences naturelles, Botanique*, sér. 2, 9: 38-57.

doi/10.7872/crya/v37.iss1.2016.3

UNIVERSIDAD AUTONOMA DE MADRID
Facultad de Ciencias

ANEXO C – ACEITE CRYPTOGAMIE BRYOLOGIE

CRYPTOGAMIE, BRYOLOGIE

December 18th, 2017

Mazimpaka, Vicente

Edificio de Biología (Botánica)

Facultad de Ciencias, UAM.

Campus de Cantoblanco

E-28049 Madrid (Spain).

e-mail: vicente.mazimpaka@uam.es

Dr. Katia Pôrto
Department of Botany
Federal University of Pernambuco
Brasil

Dear Dr Pôrto,

I am pleased to inform you that your paper entitled– “***Sexual dimorphism, vegetative growth and reproductive investment in the rhizautoicous moss Fissidens flaccidus (Fissidentaceae, Bryopsida)”,*** submitted by *Wagner Luiz dos SANTOS, Lisi Dámaris Pereira ALVARENGA* and yourself, has been accepted for publication in *Cryptogamie, Bryologie*.
It will appear in the 2018 spring issue of the journal.
For any question regarding its status in the future, please contact the Editor-in-Chief (D. Lamy).

Yours sincerely,

Dr. Vicente Mazimpaka
Guest Editor
Cryptogamie, Bryologie.

ANEXO D – NORMAS DO PERIÓDICO *AMERICAN JOURNAL OF BOTANY*

http://www.amjbot.org/site/misc/ifora.xhtml

Review Procedure and Policy

All manuscripts are evaluated by members of the Editorial board to determine whether the paper should go forward for peer review. Papers sent out for review will, typically, be sent to two or three independent referees. Authors may suggest possible referees, or indicate referees to be excluded. In both cases, justification should be provided. Papers are evaluated for innovations in, significant contributions to, and noteworthy advances in the theoretical or conceptual bases of the subdisciplines of plant biology, and/or novel insights of general relevance to fundamental questions of biology (see http://www.botany.org/ajb/AJB_Reviewer_Instructions.pdf for review criteria). Phylogenetic analyses will generally only be considered if the phylogeny is used to test explicit evolutionary and/or ecological hypotheses or morphological associations—essentially a significant discussion of the impact and/or use of the phylogeny.

Final acceptance of a manuscript is contingent upon compliance with Journal requirements as outlined below. Articles that have undergone complete peer review and copyediting, as well as full review by the authors, will be posted online as soon as possible.

Publication Policies

The Journal editors expect authors to follow the ethical standards required of scholarly research and the ethics guidelines of the Botanical Society of America (BSA) (www.botany.org/governance/ethics.php).

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