Sexual dimorphism, vegetative growth and reproductive investment in the rhizautoicous moss *Fissidens flaccidus* (Fissidentaceae, Bryopsida)

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**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**

**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

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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; Hederson & 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 dioicus 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
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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-cm² samples were randomly collected from a large terricolous population (approximately 2 m²) 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-cm² sample were examined (10 ramets in average). After screening, we picked 100 ramets of 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 cm². 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.](image-url)
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 (perigonium, 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 & Ehrén, 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 (non-sporophytic 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: \( R_i = \frac{R_b}{(R_b + V_b)} \), where \( R_b \) is the reproductive biomass, \( V_b \) is the vegetative biomass and \( R_i \) 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 GGPPLOT (Wickham, 2009).

RESULTS

Sexual dimorphism

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

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.

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

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>p-value</th>
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<tr>
<td>Sex-expressing male</td>
<td></td>
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<tr>
<td>Reproductive investment vs. Ramet length</td>
<td>– 0.25</td>
<td>= 1.0 n.s.</td>
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<tr>
<td>Reproductive investment vs. Vegetative biomass</td>
<td>– 0.53</td>
<td>&lt; 0.001</td>
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<td>Non-sporophytic female</td>
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<tr>
<td>Reproductive investment vs. Ramet length</td>
<td>– 0.19</td>
<td>= 1.0 n.s.</td>
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<tr>
<td>Reproductive investment vs. Vegetative biomass</td>
<td>– 0.62</td>
<td>&lt; 0.001</td>
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<td>Sporophytic female</td>
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<tr>
<td>Reproductive investment vs. Ramet length</td>
<td>– 0.19</td>
<td>1.0 n.s.</td>
</tr>
<tr>
<td>Reproductive investment vs. Vegetative biomass</td>
<td>– 0.72</td>
<td>&lt; 0.001</td>
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</table>

**Sexual dimorphism versus reproductive investment**

Reproductive investment was significantly different between males, non-sporophytic 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 non-expressing 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 in flexa* 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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**REFERENCES**


DARWIN C., 1877 — The different forms of flowers on plant of the same species. London, John Murray.

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