

UNIVERSIDADE ESTADUAL DE CAMPINAS

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**Estratégias reprodutivas de briófitas em dois habitats  
distintos da Floresta Atlântica do Parque Estadual da Serra  
do Mar**

Este exemplar corresponde à redação final  
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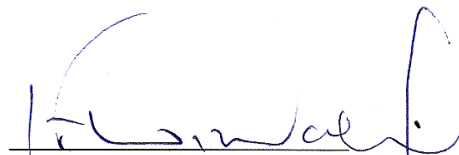
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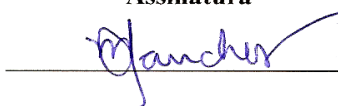
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**A todas as formas de vida, conhecidas ou não,**

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## RESUMO

O objetivo da tese foi aumentar o conhecimento e o entendimento sobre estratégias reprodutivas de briófitas presentes em floresta tropical úmida, especificamente em dois habitats distintos da Floresta Ombrófila densa (Floresta Montana e de Restinga) do Parque Estadual da Serra do Mar, São Paulo. A seguinte hipótese foi testada: o sistema reprodutivo (e.g. monóico *versus* dióico) das espécies e o habitat que ocupam influenciam as estratégias reprodutivas e conseqüentes características de história de vida em briófitas de dois habitats distintos de Floresta Atlântica. Três principais perguntas foram acessadas: (1) o desenvolvimento de estruturas reprodutivas (de gametângios a esporófitos) varia entre populações de briófitas presentes em habitats distintos de floresta tropical? (2) O desempenho reprodutivo (via reprodução sexuada) de briófitas presentes em floresta tropical é influenciado pelo sistema reprodutivo das espécies ou pelas características do habitat que ocupam? (3) Características do habitat e sistemas reprodutivos das espécies são importantes para explicar os padrões de bancos de diásporos de briófitas observados em dois habitats de floresta tropical?

O desenvolvimento de estruturas reprodutivas seguiu os índices de pluviosidade nos dois habitats de floresta (apenas com pequenos ajustes), com elevada fertilização durante os períodos mais chuvosos e dispersão de esporos nos períodos mais secos. Tanto o sistema reprodutivo quanto o habitat foram importantes fatores que explicam o desempenho reprodutivo de briófitas. Nem todas as espécies dióicas falharam em produzir estruturas sexuadas sob números similares aos das espécies monóicas, porém as fases de fertilização e produção dos esporófitos foram reduzidas nas espécies dióicas. A raridade de colônias masculinas, assim como as

altas razões sexuais de gametângios ( $>1 \text{ ♀:♂}$ ) entre plantas da Floresta de Restinga comparadas àquelas da Floresta Montana, auxiliam a entender como as plantas de Restinga compensaram o baixo desempenho de alguns estádios do ciclo reprodutivo investindo em mais estruturas reprodutivas nas fases iniciais do ciclo e aumentando as probabilidades de fertilização. Características das espécies, sobretudo filo e sistema reprodutivo, foram importantes na formação de bancos de diásporos em distintos substratos (solo < casca de árvore < tronco morto em decomposição). Os dois habitats de floresta, embora com pequenas diferenças, apresentam reservas compostas principalmente por diásporos de musgos monóicos, que investem largamente em reprodução sexuada, e de musgos dióicos com eficientes métodos de propagação assexuada. Quando comparadas as proporções de espécies dióicas vs. monóicas presentes no banco de diásporos dos dois habitats de floresta estudados, com levantamentos das espécies de briófitas em vegetação da mesma floresta, a contribuição de espécies dióicas foi superior na vegetação, enquanto que espécies monóicas foram mais bem representadas no banco de diásporos. Briófitas presentes na Floresta Atlântica apresentaram diferentes estratégias reprodutivas, moldadas pelo sistema reprodutivo das espécies e pelo habitat que ocuparam. Mais especificamente, outros fatores como filo e formas de crescimento das espécies, e características microambientais também influenciaram as estratégias de vida adotadas pelos grupos de briófitas. A presente tese é pioneira na investigação dos temas abordados aqui do ponto de vista das briófitas, sobretudo em florestas tropicais. Os dados referentes à fenologia reprodutiva, desempenho da reprodução sexuada e formação de bancos de diásporos de briófitas auxiliarão a compreender como e porque essas plantas têm papel destacado em florestas tropicais úmidas.

## SUMMARY

The main goal of this thesis was to increase the knowledge and understanding on reproductive strategies of bryophytes in tropical rain forests, specifically in two habitats of the Ombrophilous Dense Forest (montane and sea level sites) of the Serra do Mar State Park, São Paulo, Brazil. The following hypothesis was tested: breeding system and habitat drive the reproductive strategies and life-history traits of bryophytes in two habitats of a Brazilian Atlantic Forest. Three questions were assessed: (1) Do the development of sexual reproductive structures (gametangia to sporophytes) vary among populations in two distinct habitats of tropical rain forest? (2) Is the sexual reproductive performance of bryophytes in tropical rain forest influenced by breeding system or habitat characteristics? (3) Are habitat and breeding system important to explain the patterns of bryophyte diaspore banks in tropical rain forests?

Development of sexual reproductive structures followed the pluviosity in the two Forest sites, with slight adjustments. There was high fertilization during the wettest periods and the spore dispersal was common in the driest months. Both breeding system and habitat were important factors explaining the reproductive performance of bryophytes. Some dioicous species do not fail to produce sexual reproductive structures, presenting similar values to monoicous ones. However, dioicous species fail mostly in relation to fertilization and sporophyte production. The rarity of male colonies and female biased sexual ratios of gametangia among plants at sea level compared to montane, help us to understand how plants in the sea level site compensate the low performance of some sexual phases by investing more in structures at initial cycle, and increasing the fertilization chances. Species traits, such

as phylum and breeding system of bryophytes were relevant for diaspore bank formation from different substrates (soil < tree bark < decaying wood). The two forest sites, although with little differences, had reserves mostly composed by monoicous mosses that invest in sexual reproduction, and dioicous mosses with efficient asexual propagation. When we compared the diaspore bank and vegetation composition from previous bryophyte surveys in the same forest, different breeding system roles were found. The dioicous bryophytes were over-represented in the vegetation and monoicous ones well represented in the diaspore bank. Bryophytes in the Atlantic Forest had different reproductive strategies, driven by the breeding system and habitat of the species. More specifically, other factors such as phylum and growth form, and microhabitat characteristics also influenced the life strategies of the bryophyte groups. This thesis is pioneer investigating the subjects here studied for bryophytes, mostly in tropical forests. The data on reproductive phenology, sexual performance and diaspore bank formation of bryophytes will help us to understand how and why bryophytes have an important role in tropical rain forests.



## INTRODUÇÃO GERAL

As briófitas compõem um grupo não monofilético de pequenas plantas que compartilham inúmeras características como dominância da geração haplóide, poiquilohidria e reprodução por esporos e diferentes diásporos assexuados (Longton & Schuster, 1983; Shaw, 2000; Glime, 2007). Esse grupo é composto pelos filos Bryophyta (musgos), Marchantiophyta (hepáticas) e Anthoceroophyta (antóceros) com aproximadamente 15.000 espécies (Gradstein *et al.*, 2001; Rydin, 2009), que compõem uma parte proeminente da vegetação em habitats como florestas temperadas, florestas tropicais de terras baixas a montanhas, desertos, pântanos e brejos, campos polares, campos alpinos e tundras (Glime, 2007). Nesses ambientes, briófitas apresentam um importante papel no armazenamento de água, estoque de carbono, retenção de nutrientes e manutenção de interações com pequenos animais e outras plantas (Proctor, 2000; Frahm, 2003; Kürshner, 2004).

Colônias de briófitas tendem a ser distribuídas como pequenas manchas separadas umas das outras no ambiente, comportando-se como sub-populações de uma mesma metapopulação (Söderström & Herben, 1997), nas quais a expressão das características genéticas dentre os indivíduos (diferentes rametas, mas geneticamente similares) é fortemente determinada pelo(s) diásporo(s) que originou cada população. Assim, é possível encontrar populações exclusivamente femininas ou masculinas de espécies dióicas, cujo grau de isolamento das demais populações influencia as chances de fertilização dos indivíduos femininos e conseqüente formação de esporófitos (Longton, 1992; Longton & Schuster, 1983; Oliveira & Pôrto, 2002). Dificuldade na produção de esporófitos em briófitas dióicas é uma das causas para explicar a raridade de muitas espécies dióicas em campo (Longton, 1992; Laaka-Lindberg, *et al.*; 2000; Longton, 2006), associadas ao fato de que muitas espécies não contam com eficazes meios de propagação assexuada. Por outro lado, a raridade de espécies

monóicas está relacionada aos problemas conseqüentes da autofertilização obrigatória (Longton, 1992; Longton, 2006).

Características da história de vida em briófitas, tais como fenologia reprodutiva, produção, germinação e estabelecimento de diásporos, entre outras, quando combinadas com o estudo do sistema sexual das espécies (monóicas *versus* dióicas) auxiliam no entendimento das estratégias de reprodução desses grupos de plantas (Söderström & Gunnarsson, 2003). Uma vez que são plantas pequenas, com populações bem delimitadas, de ciclo de vida curto em relação à maioria das traqueófitas, poiquilohídricas (uma vez secas, param seu crescimento e mantém o mesmo estágio fenológico, que é facilmente acessado via reidratação), e com diversidade de sistemas reprodutivos, briófitas são um bom modelo para investigação de parâmetros envolvidos na reprodução de plantas.

#### *Reprodução sexuada, sistemas reprodutivos e estratégia de vida*

Entre briófitas de ecossistemas temperados é comum a combinação da condição dióica com gametófitos perenes apresentando propágulos assexuados e baixa produção de esporófitos, ou táxons dióicos colonizadores com freqüente reprodução assexuada e sexuada (Longton & Schuster, 1983; Longton, 1998). Já em táxons monóicos, são comuns espécies colonizadoras, anuais e fugitivas, sendo o esforço reprodutivo, nos dois últimos casos, transferido principalmente à reprodução sexuada por meio de esporos (During, 1979). Desta forma, parece que espécies monóicas tendem a investir mais em reprodução sexuada comparativamente às espécies dióicas. Exceção à regra são as hepáticas, nas quais a reprodução assexuada é bastante representativa, mesmo entre as espécies monóicas (Longton & Schuster, 1983; Longton, 1998). Outra demanda conflitante parece existir entre longevidade do gametófito e número de esporos produzidos. Por exemplo, musgos perenes produzem

esporos pequenos e em alto número por cápsula, enquanto que musgos anuais têm esporos grandes e em menor número (Longton & Schuster, 1983).

Os mecanismos de liberação dos esporos em briófitas tropicais são, em geral, promovidos durante condições quentes e secas (Oliveira & Pôrto, 1998, 2001; Pôrto & Oliveira, 2002) para ascensão dos esporos na coluna de ar, embora em florestas tropicais úmidas alguns táxons liberem seus esporos sob condições de alta umidade (Rydin 2009). A fenologia da reprodução sexuada de briófitas em florestas tropicais tende a seguir os índices de pluviosidade locais, com alta frequência de gametângios fertilizados durante a estação chuvosa e dispersão dos esporos na estação seca (Ugunyomi, 1979; Oliveira & Pôrto, 1998, 2001; Pôrto & Oliveira, 2002). Dessa forma, esporos menores (8-12 $\mu$ m) tendem a ser levados a longas distâncias, enquanto que esporos maiores (>25 $\mu$ m) e propágulos vegetativos tendem a ser dispersos próximos às populações originais, constituindo muitas vezes bancos de diásporos temporários ou permanentes (Longton & Schuster, 1983; Schuster, 1983; Shaw, 2000).

#### *Bancos de diásporos*

Bancos de diásporos de briófitas são reservas de esporos e principalmente propágulos assexuados especializados como gemas, “tubers” e bulbilhos, ou não especializados ao exemplo de fragmentos do caulídio e filídio (During & ter Horst, 1983; During *et al.*, 1987). Diferentemente de pteridófitas e de plantas com semente, os bancos de briófitas não são constituídos em sua maioria por diásporos de origem sexuada (esporos), mas sim pelos de origem assexuada (During, 2001). Os bancos de diásporos de briófitas em ambiente temperado seguem os seguintes padrões de ocorrência: (1) espécies presentes apenas acima do solo, com raros fragmentos no solo (principalmente musgos pleurocárpicos e hepáticas); (2) espécies em clareiras temporárias acima do solo, mas presentes abaixo do solo em grandes números, como esporos; (3) espécies formadoras de “tubers”, comuns abaixo do solo e raras na vegetação; (4)

espécies que também possuem “tubers”, abundantes tanto acima do solo como no banco (During, 2001). A proporção de briófitas perenes nos bancos de diásporos (baixa em sua maioria) está em concordância com a estratégia de vida apresentada por essas espécies. Por outro lado, para espécies de curta duração de vida (e.g. anuais e fugitivas), modelos sugerem que bancos de diásporos são importantes principalmente em substratos efêmeros (Sundberg & Rydin, 2000).

No entanto, em ambientes tropicais pouco é sabido sobre bancos de diásporos de briófitas, havendo apenas trabalhos que comparam superficialmente as espécies emergidas do banco com aquelas presentes na vegetação (Bisang *et al.*, 2003; During, 2007; Maciel-Silva & Lins-Silva, 2007), sem investigação das características dessas espécies e prováveis estratégias que permitam a formação dessas reservas de diásporos. Da mesma forma, são escassas informações que associem a reprodução de briófitas às suas estratégias de vida em diferentes habitats tropicais.

### *Objetivos*

O objetivo geral da tese foi levantar dados que contribuirão para um melhor conhecimento das estratégias reprodutivas de briófitas presentes em floresta tropical úmida, especificamente de dois habitats distintos da Floresta Ombrófila densa (Floresta Montana e de Restinga) do Parque Estadual da Serra do Mar, São Paulo. Com base na informação já disponível para espécies de ambientes temperados e nos esforços desenvolvidos principalmente no campo da biologia reprodutiva de briófitas tropicais no Brasil (Oliveira & Pôrto, 1998, 2001, 2002; Pôrto & Oliveira, 2002), foi elaborada a seguinte hipótese: O sistema reprodutivo das espécies, assim como o fator habitat, influencia as estratégias reprodutivas e conseqüentes características bionômicas em briófitas de Floresta Atlântica.

Mais especificamente, as seguintes questões foram avaliadas:

- O desenvolvimento de estruturas reprodutivas (gametângios e esporófitos) varia entre populações de briófitas presentes em habitats distintos de floresta tropical? (**Capítulo I**)
- O desempenho reprodutivo via reprodução sexuada (e.g. produção de estruturas sexuais como ramos sexuais, gametângios e esporófitos) de briófitas presentes em floresta tropical é influenciado pelo sistema reprodutivo das espécies e/ou pelas características do habitat que ocupam? (**Capítulo II**)
- Características do habitat e sistemas reprodutivos das espécies são importantes para explicar os padrões de bancos de diásporos de briófitas observados em dois habitats de floresta tropical? (**Capítulo III**)

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# Capítulo I



**Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep**

(Manuscrito formatado segundo as normas do periódico *The Bryologist*)

## **Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep**

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### **Abstract**

Events of the sexual reproductive cycle of 11 bryophyte species (mosses and liverworts with monoicous and dioicous breeding systems) were described for two different sites (montane and sea level) of a Brazilian tropical rain forest, during a period of 15 months. The sex expression of many species was continuous over the whole period, irrespective of the seasons and the forest sites. The sexual systems of dioicous species did not change, but synoicous species had strategies to increase the chances of out-crossing, bearing unisexual branches in addition to bisexual ones. The pattern of gametangia and sporophyte development was different among the species, but the same between the sites. There was a seasonal effect on the maturation of gametangia and sporophytes. Male gametangia were mostly mature from the end of the dry season. Fertilization occurred during the wettest months and sporophytes developed during the dry season, spreading spores mostly towards the end of the dry season. Female gametangia were receptive over the whole period, with many mature gametangia before the start of the rainy season. Male gametangia, in contrast to female, took longer to develop and aborted at high numbers. Bryophytes in tropical rain forests are favored by the wet weather and mild temperatures, and similar reproductive phenological patterns were found in different habitats with some minor adjustments.

**Key-words:** Brazilian Atlantic Forest, breeding system, liverworts, mosses, phenophases, sexual reproduction, sexual system.

## Introduction

The relationship between time and events of growth and reproduction in the life cycle of a species is described by phenology (Glime 2007; Stark 2002). The seasonality of these events and the recognition of different stages or phenophases characterize these types of studies (Rathcke & Lacey 1985; Stark 2002). To understand reproductive phenology is crucial to resolve and interpret aspects of reproductive ecology and life strategy, such as the breeding system and fertilization biology, as well as traits related to the production of spores and propagules, and the longevity of individuals in a species (During 1979; Stark 2002). In addition, to verify the influence of biotic (e.g. herbivory, parasitism and competition) and abiotic (e.g. light, photoperiod, moisture, temperature, nutrients and pH) factors on growth and reproduction one must clarify which environmental factors determine the events of the life cycle in a species (Laaka-Lindberg 2005; Stark 2002).

Many studies on reproductive phenology of bryophytes have been carried out from the late middle of the XIX century (see references in Stark 2002), mostly conducted with mosses from temperate to polar areas (Ayukawa *et al.* 2002; Laaka-Lindberg 2005; Lloret Maya 1987; Milne 2001; Stark 1983). Some studies from tropical ecosystems have generally reported the adaptation of the reproductive phenophases of tropical bryophytes to the seasonal alternation of the wet and dry seasons (Egunyomi 1979; Makinde & Odu 1994; Oliveira & Pôrto 2001, 2002; Salazar Allen 1992). However, the influence of different sites from the same ecosystem, breeding system and phylum on the reproductive behaviour of tropical bryophytes is still unknown.

Our general aim was to describe the events of the sexual reproductive cycle of 11 bryophyte species (mosses and liverworts with monoicous and dioicous breeding systems) from two different sites (montane and sea level) of a Brazilian tropical rain forest, during a period

of 15 months. We addressed the following questions: (1) is sex expression continuous during a whole year for all species at both sites? (2) Are the sexual systems invariable among the species and sites? (3) Is there any difference in the gametangial and sporophytic development between the two sites and the species?

## Material and Methods

### Study sites

The study sites were situated in the “Núcleo Picinguaba” (23° 34' S - 45° 02' W and 23° 17' S - 45° 11' W) and in the “Núcleo Santa Virgínia” (23° 17' - 23° 24' S and 45° 03' - 45° 11' W) of the *Serra do Mar State Park* – São Paulo State, Brazil (Veloso *et al.* 1991). This park is covered by a tropical rain forest, the Atlantic Ombrophilous Dense Forest (Veloso *et al.* 1991), which is characterized by temperatures of about 25°C and a high precipitation evenly distributed over the year. The study sites were established within the project “floristic composition, structure and functioning of the *Serra do Mar State Park* forest” (for details see Alves *et al.* 2010), and were chosen to represent altitudinal extremes of the Atlantic Ombrophilous Dense Forest. The low altitude forest is known as “Restinga forest”, a close to sea and seasonally flooded forest, and referred to as a “sea level” site (*Núcleo Picinguaba*), whereas the high-altitude site (*c.* 1,000 m a.s.l.) is denominated “montane forest” (*Núcleo Santa Virgínia*). These two sites are *ca.* 35 km apart and differ in soil nutrients and total biomass (Alves *et al.* 2010).

We characterized the climate of both sites by carrying out monthly or bimonthly measurements of minimum and maximum temperatures in the understory of the forests. We used two max-min thermometers at each site, positioned on tree trunks *ca.* 150 cm above the ground. These measurements were made from September 2007 to December 2008. For

precipitation we used data from two meteorological stations (INPE) near the study sites (distance *ca.* 100 m from the sea level site and *ca.* 10 km from the montane site; Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos – CPTEC/INPE; Project “Estudos da Previsibilidade de Eventos Meteorológicos Extremos na Serra do Mar”).

### Sampling and study species

At each site (sea level and montane), one area (10,000 m<sup>2</sup>) was established and sub-divided in 100 plots of 100 m<sup>2</sup> each. We collected 244 and 167 bryophyte samples from the sea level and montane sites, respectively, from bark and decaying wood in the majority of the 100 m<sup>2</sup> plots. These samples were preliminarily identified at the species or genera level. From this information, we selected species for study according to the occurrence, breeding system (monoicous and dioicous), sexual system (sub-division into the breeding system), and phylum (Bryophyta – mosses; Marchantiophyta – liverworts). Mosses were separated according to growth form as pleurocarpous, cladocarpous and acrocarpous (La Farge-England 1996). We selected 11 species (seven genera of mosses and liverworts), according to the variation of these traits (Table 1). When one species did not appear or was not so common in the two sites, two closely related species with the same breeding system and growth form were chosen. Colonies of the same species were selected at least five meters apart. We selected four to six colonies of each species and site, and samples were collected bimonthly during 15 months, from October 2007 to December 2008. From each colony, squares of at least 4 cm<sup>2</sup> for liverworts and 9 cm<sup>2</sup> for mosses were collected, put in paper bags and taken to the laboratory. The colony size varied from approximately 100 cm<sup>2</sup> (for liverworts) to 1,000 cm<sup>2</sup> (for mosses). The samples were collected from different parts of the colony, at each time, to minimize disturbance (Laaka-Lindberg 2005).

In October 2007 we randomly picked out ten shoots per colony and six shoots in the other months. Samples of *Bazzania* spp. were analyzed as single units, since the “shoots” were not so noticeable. We carefully cleaned these shoots, removing fragments of leaf litter. Individual shoots in each sample were classified under a dissecting microscope as: sex non-expressing or sex-expressing. Shoots bearing sex-expressing male, female and bisexual branches were recorded. To distinguish the proportion of sex-expressing shoots in each species between the two sites, we used a  $\chi^2$  test to compare the total and month mean frequencies of shoots in the montane and sea level.

The developmental stages of the gametangia and the sporophytes were classified on the basis of the stages described by Greene (1960) and Laaka-Lindberg (2005), and here adapted to fit the traits of moss and liverwort species (Table 2). We selected perichaetia and perigonia from the current growth intervals avoiding older tissues. Archegonia and antheridia in each sexual branch were classified and counted under a light microscopy. The development of gametangia and sporophytes was demonstrated by the maturity index (Longton & Greene 1967):  $M_i = \sum(n_x I) / \sum n$ , where  $n_x$  is the number of gametangia or sporophyte representing each developmental stage,  $I$  is the rank of the index value of the developmental stage (for gametangia: 1 – 4; for sporophyte: 1 – 7; Table 2), and  $\sum n$  is the total number of scored gametangia or sporophyte pooled per sexual branch. Maturity indices of gametangia and sporophytes were calculated separately for each sexual branch. We calculated the mean of  $M_i$ s for each shoot, and the mean values of  $M_i$ s in each colony were used for the statistical analyses.

Mean values of maturity indices for gametangia and sporophytes were compared using repeated measures Anovas. We performed the analyses considering the between-subjects effect (site effect), and the within-subjects effect (time effect) and interactions (site x time).

Analyses were conducted separately with the mean  $M_i$ s of male and female gametangia and sporophytes. In this model, the effect of species was not assessed due to insufficient degrees of freedom, since many species (mainly dioicous) did not express sex and consequently lacked any gametangia or sporophytes. We conducted the same analyses using only the monoicous species *Pyrrhobryum spiniforme* and *Neckeropsis* spp. Linear model assumptions, such as the residuals normality and variances homogeneity were checked. We used the software Statistica 8.0, StatSoft, Inc. to perform the analyses.

## Results

### Temperature and precipitation measurements

The temperature in the montane site was lower than at sea level, varying from 4°C in the winter to 27°C in the summer in the montane, and from 12°C in the winter to 32°C in the summer at the sea level site (Figure 1A and 1B). The summer was rainy and the winter dry. The monthly mean precipitation was higher than 100 mm in both sites, except for the driest months from May to July in the montane and from May to September at sea level, with precipitation about or lower than 60 mm (Figure 1A and 1B).

### Sex expression of shoots

Sex-expressing shoots were found in all species in both sites during the whole study period, except for *Leucobryum crispum* in the montane (Figure 2N). The sex-expression was high in many species (above 40% of the shoots were sex-expressing), including monoicous mosses and dioicous liverworts (Figures 2A – 2J). The dioicous mosses *Leucobryum clavatum* and *Leucoloma serrulatum* had the lowest values of sex-expressing shoots during all months (Figures 2K – 2M). No seasonal increasing of the sex expression was observed among the different species. The total and month mean frequencies of sex-expressing shoots in each



species varied significantly between the two sites (total:  $\chi^2 = 88.89$ ,  $P < 0.001$ ; month mean:  $\chi^2 = 18.72$ ,  $P = 0.004$ ; Table 3), where more sex-expressing shoots were found at sea level.

The sexual system of some species was not strictly fixed, varying from synoicous to autoicous in the moss *P. spiniforme* (Figures 3A – 3B), and from dioicous to synoicous (low proportions) in the liverwort *P. martiana* (Figures 3H). Sexual branches in *P. spiniforme* were not strictly bisexual, but the plants also expressed female branches at sea level, and female and male in the montane site. The male sexual branches were generally found in the axils of female sexual branches (gonioautoicous) or bisexual branches, and in the bisexual branches the male gametangia were located around the female gametangia, but rarely a complete mixture was found. *Neckeropsis disticha* and *N. undulata* were mostly autoicous and autoicous/synoicous, respectively (Figures 3C – 3D). Apart from the liverwort *P. martiana*, other dioicous species did not present bisexuality events.

#### Development of gametangia and sporophytes

Mature gametangia were produced during the whole year in both sites of forest, but mature male gametangia were more conspicuous than female gametangia and mostly present in the wettest months (Figure 4). The maturity indices of gametangia, as well as indices of sporophytes did not differ significantly between the sites, but they differed over time. No interaction for site  $\times$  time was observed (Table 4). In a general way, the maturity indices of male gametangia were high over all months, decreasing to values about 2 (mature stage) at the end of the dry season (August 2008) in the montane and at the start of the rainy season (August 2008 to October 2008) at sea level. Mature and mostly recently dehisced female gametangia (dehisced stage 1) were found at all times in both sites. The phase of spore dispersal was more conspicuous in Aug-08 for the both sites (Figures 1C – 1D). The maturity

indices of gametangia were in general inversely related to those of sporophytes, with male gametangia becoming mature mainly around the end of the dry season, and sporophytes spreading spores before the start of the rainy season.

Each species had different patterns of gametangial and sporophytic development, and the same species had slightly different responses in the two sites (Figures 1E – 1L). No significant differences of gametangial and sporophytic maturity indices were found between the two sites, and the monoicous species *Neckeropsis* spp. and *P. spiniforme* differed significantly only in male gametangia maturity indices (Table 4; Figures 1E– 1H). There was a significant effect of time for all maturity indices of gametangia and sporophytes, and some interactions of time  $\times$  species and site were also significant for male and female gametangia (Table 4). Among the species with large sex expression, only *Phyllogonium viride* at sea level had no sporophyte.

The frequencies of abortive gametangia were recorded for the species with high numbers of gametangia. Frequencies of abortive gametangia were low (about 10%) in all species, except for *P. viride* at sea level with values reaching 100% of abortive gametangia in different months (Figure 5F). Abortive male gametangia were more frequent than abortive female gametangia for both sites and all species (Figures 5A – 5E).

## Discussion

The sex expression of many species was continuous during the period from October of 2007 to December of 2008, irrespective of the seasons in the forest sites. The sexual systems of dioicous species generally did not change, but the synoicous species did not have fixed sexual systems, presenting both bisexual and unisexual branches simultaneously. The development pattern of gametangia and sporophytes was different among the species, but with

no difference between the sites. There was a seasonal effect on the maturation of gametangia and sporophytes, and male gametangia were mostly mature from the end of the dry season, whilst sporophytes spread spores during the dry season. Female gametangia were receptive to fertilization (dehisced stage 1; Table 2) over the whole period.

#### Constant sex expression over time

The pattern of constant sex expression over time is characterized as opportunistic, since the species are favored by the mild temperatures and the high water availability in the tropical rain forests producing new sex organs constantly. This opportunistic pattern is commonly observed in some mosses of tropical environments lacking different climatic seasonality (Makinde & Odu 1994; Moyá 1992; Oliveira & Pôrto 1998; Pôrto & Oliveira 2002). In contrast, the pattern in temperate habitats is generally annual, characterized by a growth season when the new reproductive structures are produced, after months of snow cover and mean temperatures below 0°C (Ayukawa *et al.* 2002; Laaka-Lindberg 2005; Une 2000).

Since bryophytes are poikilohydric plants and need water to continue growing (Proctor & Tuba 2002), these plants are able to maintain growth and reproduction during a whole year in humid habitats such as tropical rain forests. Both sea level and montane sites are extremely humid with a short dry period from May to August, where the precipitation is reduced but never absent. The main distinctions between these two sites of Atlantic forest are due to the elevation above the sea, with periodical flooding at the sea level site (from December to March), higher temperatures at sea level than in the montane, and the constant presence of fog in the montane site. Consequently, the same or related species have different reproductive behavior in these two sites, where the frequencies of sex-expressing shoots at sea level outnumber the frequencies in the montane. The functional mechanism behind these patterns is

not completely understood, but plants at the montane site compensate the low values of sex-expressing individuals and production of sexual branches (compared to plants at sea level) by unbiased sex ratios of gametangia and high values of fertilization, which result in numbers of sporophytes similar to those found in the sea level site (Maciel-Silva, Válio & Rydin in prep.). *Phyllogonium viride*, an exception, has a higher number of sex-expressing individuals in the montane than in the sea level site. In addition, this moss species has high percentages of male abortive gametangia at sea level, explaining the absence of sporophytes in that forest site. In this case, high temperatures at sea level possibly can damage male gametangia development compared to that of the female (Stark 2002). Still, species from the genus *Phyllogonium* are commonly associated to shaded understory into the forest due to their physiological responses to low light (Léon-Vargas *et al.* 2006; Romero *et al.* 2006), explaining at least in part the reproductive constraint of this species at sea level, a lighter habitat than the montane site (Maciel-Silva, Válio & Rydin in prep.). However, the mechanisms behind the constraints on gametangia development of bryophytes in the field are still completely unknown.

#### Infrequent sex-expression among some dioicous species

In contrast to the monoicous mosses, the dioicous *Leucobryum* and *Leucoloma* spp. have a low or no frequency of sex-expressing shoots over the whole period in both sites. Similar findings were observed in other dioicous acrocarpous mosses, such as *Bryum apiculatum* in a Brazilian Atlantic Forest with about 87% of sex nonexpressing gametophytes and only 13% female (Oliveira & Pôrto 2002), and *Didymodon nevadensis* in a North-American desert with no male and 54% of female expressing shoots (Zander *et al.* 1995). The pattern of infrequent sexual expression, in addition to the spatial segregation and skewed distribution of the sexes are the main causes to explain the failure of the dioicous species to

produce sporophytes (Bisang & Hedenäs 2005; Longton 2006; Longton & Schuster 1983; Stark 2002). It seems that the fertilization likelihoods in dioicous acrocarpous mosses are low, due to a single unisexual branch being produced at the shoot apex per growing season (La Farge-England 1996). In contrast, in pleurocarpous mosses the chances of fertilization are increased with many sexual branches along the main stem or side-branches. In agreement with this idea, the dioicous pleurocarpous moss *P. viride* produces continually sex-expressing individuals, and the dioicous liverworts, which also have many active sexual branches along the stem, present a similar strategy with many sex-expressing shoots. The high sexuality of liverworts seems to be associated with reproductive trade-offs, since the total number of spores produced per capsule (Glime 2007; Schuster 1983) and plants generated from each spore (Nehira 1983) are generally lower than in mosses.

Sexual systems are not always fixed

The monoicous species *P. spiniforme* and *N. disticha* described in the literature as synoicous (Gradstein *et al.* 2001; Merced-Alejandro & Sastre-de-Jesús 2009) have strategies to reduce self-fertilization and increase the chances of out-crossing. The presence of unisexual branches together with the bisexual branches can guarantee high fertilization rates by intragametophytic selfing at the level of the same perichaetium or different perichaetia, intergametophytic selfing (gametophytes from spores originated by the same sporophyte), or even out-crossing (Crawford *et al.* 2009). Although the self-fertilization in monoicous bryophytes is the general rule (Eppley *et al.* 2007), the high probability of new colonization events by spores from different populations must be important to increase the genetic variability inside the patch and explain the advantages to escape from the purely synoicous condition of sexual branches. In addition, animal vectors (e.g. springtails and mites, see

Cronberg *et al.* 2006) transporting sperm among different colonies should be much more efficient than the water vector, since water-mediated fertilization in terrestrial bryophytes is restricted to only a few centimeters (maximum distance about 200 cm by splashing, see Glime 2007).

#### Seasonality of the gametangia and sporophytic development

Both male and female gametangia are produced over the whole period, but the highest production of immature male gametangia occurs at the end of the dry season in the montane (June-August) and the start of the rainy season at sea level (August-October), and consequently there are many mature male gametangia during the rainy season. Fertilization occurs mainly during the wettest period in the montane (December) and sea level (October to February) sites. Sporophytes reach the dehisced stage mostly in the dry season, when spores are spread. Spores are favored by the low temperatures and dry air at that time (August). After that, the spores must to reach safe places and establish as new plants during the rainy season.

The gametangia and sporophyte phenophases of tropical bryophytes are adapted to the seasonal alternation of the wet and dry seasons, with adjustments related to the habitat type. Similar findings were observed for the moss *Octoblepharum albidum* in savanna (Egunyomi 1979) and tropical rain forest (Pôrto & Oliveira 2002; Salazar Allen 1992), the mosses *Archidium ohioense*, *Bryum coronatum*, *Fissidens minutifolius* and *Trachycarpidium tisserantii* dominant in the African savanna (Makinde & Odu 1994), and *Sematophyllum subpinnatum* in tropical rain forest (Oliveira & Pôrto 2001). In our study area, the wet season is warm whilst the dry is cool, different from the tropical rain forest of the northeast of Brazil, where the dry season is hot and the wet season has mild temperatures. Adjustments are obvious, but the pattern of gametangia and sporophyte development is the same, with

gametangia maturing mostly at the end of the dry season or start of the rainy season, high fertilization in the wettest period, and spore dispersal occurring mainly during the dry season.

Little regulation is observed between the sea level and montane sites of our study, this involving mostly a reduced development of gametangia in the coolest months at the montane site. But, this observation is far from the complete resting phase of gametangia and sporophytes observed in bryophytes of cold-climate sites or xeric habitats, where these structures take more than one year to complete their development (Milne 2001; Laaka-Lindberg 2005; Stark 1997; Une 2000).

Contrary to male gametangia, with the predominating mature phenophase, the female gametangia are mostly found dehisced, indicating faster development of female gametangia compared to male. A longer time to maturation for the male is commonly observed, and male gametangia are generally initiated before female (Milne 2001; Stark 1983, 1997; Stark & Castetter 1995). Since male gametangia produce thousands of antherozoids immersed in a lipid medium, whilst each female gametangium contains a single egg (Paolillo 1979), it seems that the production of male gametangia is more expensive than the female gametangia production. Because of this, male gametangia development takes longer time than female gametangia, and is also more susceptible to abortion.

In summary, bryophytes in the Brazilian Atlantic forests are favored by the wet weather and mild temperatures. Related species, although with some small adjustments, have similar reproductive phenological patterns in different sites. Species with a mixture of sexual branches have strategies to increase the chances of out-crossing, bearing unisexual branches together with bisexual ones. Male gametangia, as opposed to female, need more time to develop and abort at high numbers, but both male and female gametangia complete their

development in less than a one-year period. Fertilization time is extremely dependent on the wettest period and spore dispersal depends on the driest months.

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**Figure 1.** Maximum and minimum monthly temperatures (°C) and precipitation (mm month<sup>-1</sup>) from montane (A) and sea level (B) sites (September 2007 to December 2008). Precipitation data are from meteorological stations; note that data were not available for all months at both sites. Development of male and female gametangia, and sporophytes for all species in the montane (C) and sea level (D) sites, and for each species at both sites (E – L) of an Atlantic forest, Brazil, from October 2007 to December 2008. Data from *Bazzania* spp., *Leucobryum* spp. and *Leucoloma* spp. are not shown in detail, since there was no record of gametangia and sporophytes for some months. For more details about the maturity index calculations see Tabela 2.

**Figure 2.** Percentage of sex-expressing and nonexpressing shoots of monoicous (A–D) and dioicous species (E–N) in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008. Values above the columns are the total shoots analyzed.

**Figure 3.** Percentage of shoots bearing of female, male and bisexual branches of monoicous (A–D) and dioicous species (E–L) in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008. Values above the columns are the total shoots analyzed.

**Figure 4.** Percentage of mature gametangia per sexual branch of bryophytes in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008.

**Figure 5.** Percentage of abortive gametangia per sexual branch of mosses in the sea level (A, C and E) and montane (B, D and F) sites of an Atlantic forest, Brazil, from October 2007 to December 2008.

**Table 1.** Species of mosses and liverworts from two sites of an Atlantic forest, Brazil.

Species	Site		Growth form	Breeding system (sexual system)
	Montane	Sea level		
<b>Mosses</b>				
<i>Leucobryum</i>	–	<i>clavatum</i>	Acrocarpous	Dioicous (Pseudautoicous)
<i>Leucobryum</i>	<i>crispum</i>	–	Acrocarpous	Dioicous (Pseudautoicous)
<i>Leucoloma</i>	<i>serrulatum</i>	<i>serrulatum</i>	Acrocarpous	Dioicous
<i>Pyrrhobryum</i>	<i>spiniforme</i>	<i>spiniforme</i>	Cladocarpous	Monoicous (Synoicous)
<i>Neckeropsis</i>	–	<i>disticha</i>	Pleurocarpous	Monoicous (Synoicous)
<i>Neckeropsis</i>	<i>undulata</i>	–	Pleurocarpous	Monoicous (Autoicous/Synoicous)
<i>Phyllogonium</i>	<i>viride</i>	<i>viride</i>	Pleurocarpous	Dioicous
<b>Liverworts</b>				
<i>Plagiochila</i>	–	<i>disticha</i>	Leafy	Dioicous
<i>Plagiochila</i>	<i>martiana</i>	–	Leafy	Dioicous
<i>Bazzania</i>	–	<i>phyllobola</i>	Leafy	Dioicous
<i>Bazzania</i>	<i>heterostipa</i>	–	Leafy	Dioicous

**Table 2.** Stages of development and index values (*I*) of gametangia and sporophytes of mosses and liverworts adapted from Greene (1960) and Laaka-Lindberg (2005).

<b>Stages</b>		<b>Index</b>	<b>Event marking beginning of phase</b>
		<b>value (<i>I</i>)</b>	
<b>Gametangia</b>			
Immature		1	Gametangia reaching half length of mature gametangia
Mature		2	Gametangia reaching length of dehisced gametangia and cap cells are fragile or freshly ruptured
Dehisced 1		3	Gametangium hyaline or green in appearance; Antheridia are completely empty and archegonia have cap cells lacking
Dehisced 2		4	Gametangia with brownish coloration with ruptured apices
Abortive		A	Gametangia with brownish coloration or shriveled appearance with unruptured apices
<b>Sporophytes</b>			
<i>Mosses</i>		<i>Liverworts</i>	
Swollen venter	Swollen venter	1	Venter of archegonium is swollen
Early calyptra in perichaetium	Differentiating embryo	2	Calyptra becomes recognizable as a distinct structures, but remains immersed in perichaetial bracts for mosses; embryo begins to differentiate
Early calyptra intact	Immature capsule	3	Calyptra becomes fully exerted from bracts; Capsule remains inside calyptra and perianth
Late calyptra intact	Mature capsule inside calyptra and perianth	4	Capsule reaches full width and volume
Operculum intact	Mature capsule outside perianth	5	Capsule color darkens to olive-green; seta elongates
Operculum fallen	Capsule dehisced	6	Operculum falls (or valves open) and capsule retains > 1/2 spores
Capsule empty	Capsule empty	7	Capsule retains < 1/2 spores
Aborted	Aborted	A	Apex of sporophyte (in Index 1, 2 or 3) turns hyaline, brown, or shrivels.

**Table 3.** Total and monthly mean frequencies of sex-expressing shoots of mosses and liverworts in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008 (total number of shoots analyzed in brackets).

Species	Sea level		Montane	
	% sex-expressing shoots			
	total	month mean ± sd	total	month mean ± sd
<b>Mosses</b>				
<i>Pyrrhobryum spiniforme</i>	84.3 (300)	31.4 ± 4.8	51.3 (302)	19.4 ± 5.34
<i>Leucobryum</i> spp.	12.0 (299)	4.5 ± 2.9	0 (294)	0
<i>Leucoloma serrulatum</i>	19 (300)	7.1 ± 2.6	10.8 (286)	3.8 ± 3.3
<i>Neckeropsis</i> spp.	68.7 (304)	26.1 ± 10	57.2 (255)	18.2 ± 7.7
<i>Phyllogonium viride</i>	38.5 (262)	12.6 ± 3.8	57.6 (257)	18.5 ± 5.5
<b>Liverworts</b>				
<i>Plagiochila</i> spp.	78.5 (205)	20.1 ± 9.1	44.4 (223)	12.4 ± 5.3
<i>Bazzania</i> spp.	54.4 (44)	3 ± 1.2	27.4 (39)	2.9 ± 3.4
<b>Total mean</b>	50.8 ± 28.5	15.0 ± 11.1	35.5 ± 23.2	10.7 ± 8.3



**Table 4.** Summary of the repeated measures Anovas ( $\alpha = 0.05$ ) for the maturity index of male and female gametangia and sporophytes of bryophytes from two sites of an Atlantic forest, Brazil.

<b>All species</b>	♂ gametangia		♀ gametangia		Sporophytes	
<i>Between-subjects effects</i>	F	P	F	P	F	P
Site	0.01	0.930	0.42	0.538	0.12	0.759
<i>Within-subjects effects</i>	F	P	F	P	F	P
Time	2.75	<b>0.034</b>	3.11	<b>0.008</b>	3.61	<b>0.019</b>
Site x time	0.77	0.617	0.10	0.445	0.50	0.816
<b>Only monoicous species</b>	♂ gametangia		♀ gametangia		Sporophytes	
<i>Between-subjects effects</i>	F	P	F	P	F	P
Site	3.87	0.089	1.67	0.232	0.50	0.529
Species	83.61	<b>&lt;0.001</b>	1.96	0.198	3.73	0.149
Site x species	0.58	0.471	0.96	0.355	0.19	0.689
<i>Within-subjects effects</i>	F	P	F	P	F	P
Time	6.04	<b>&lt;0.001</b>	4.39	<b>&lt;0.001</b>	2.4196	<b>0.055</b>
Time x site	1.25	0.293	1.21	0.310	0.13	0.995
Time x species	4.28	<b>&lt;0.001</b>	3.20	<b>0.006</b>	0.58	0.764
Time x site x species	1.49	0.193	2.54	<b>0.024</b>	0.32	0.934

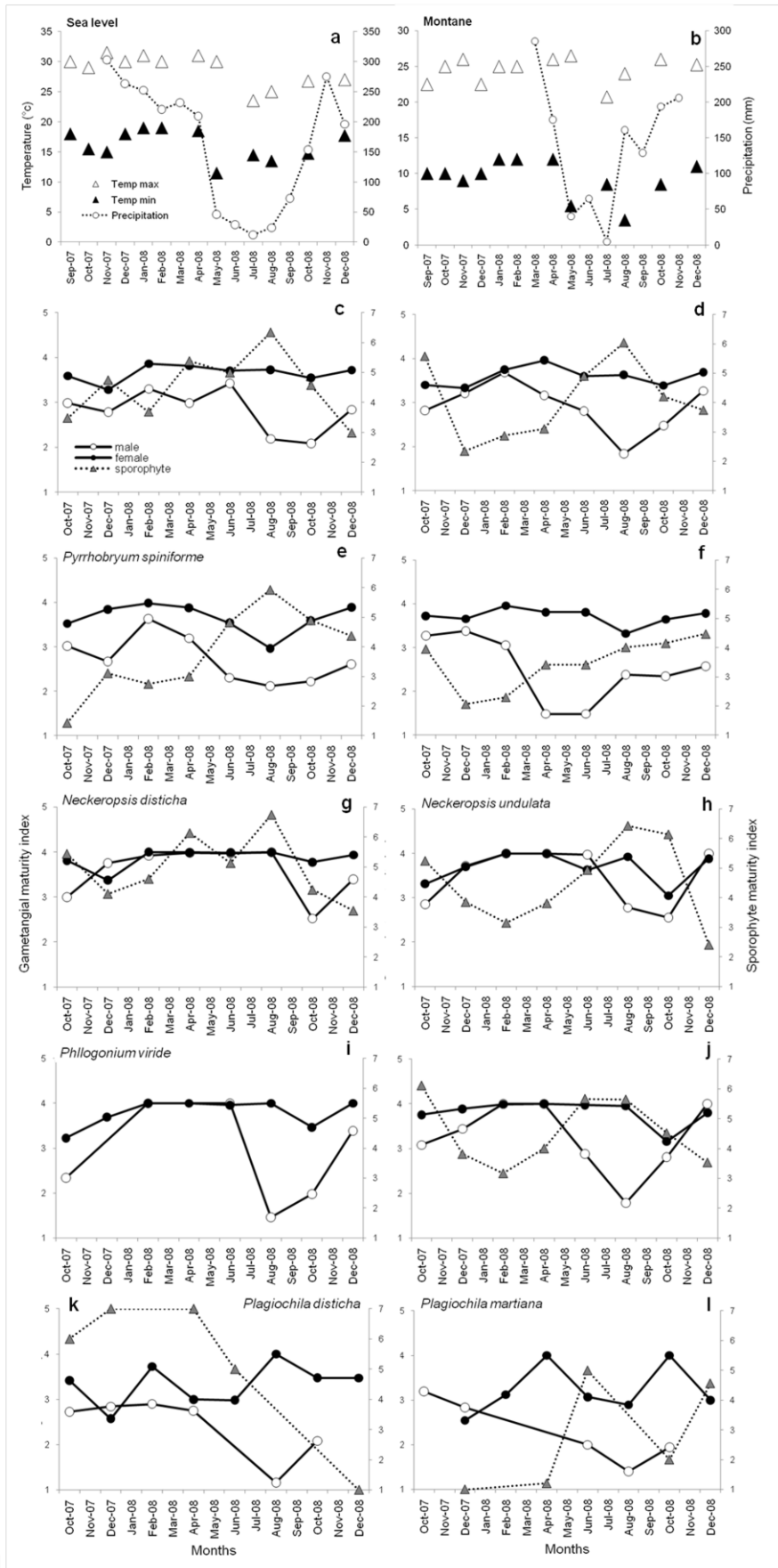


FIGURE 1

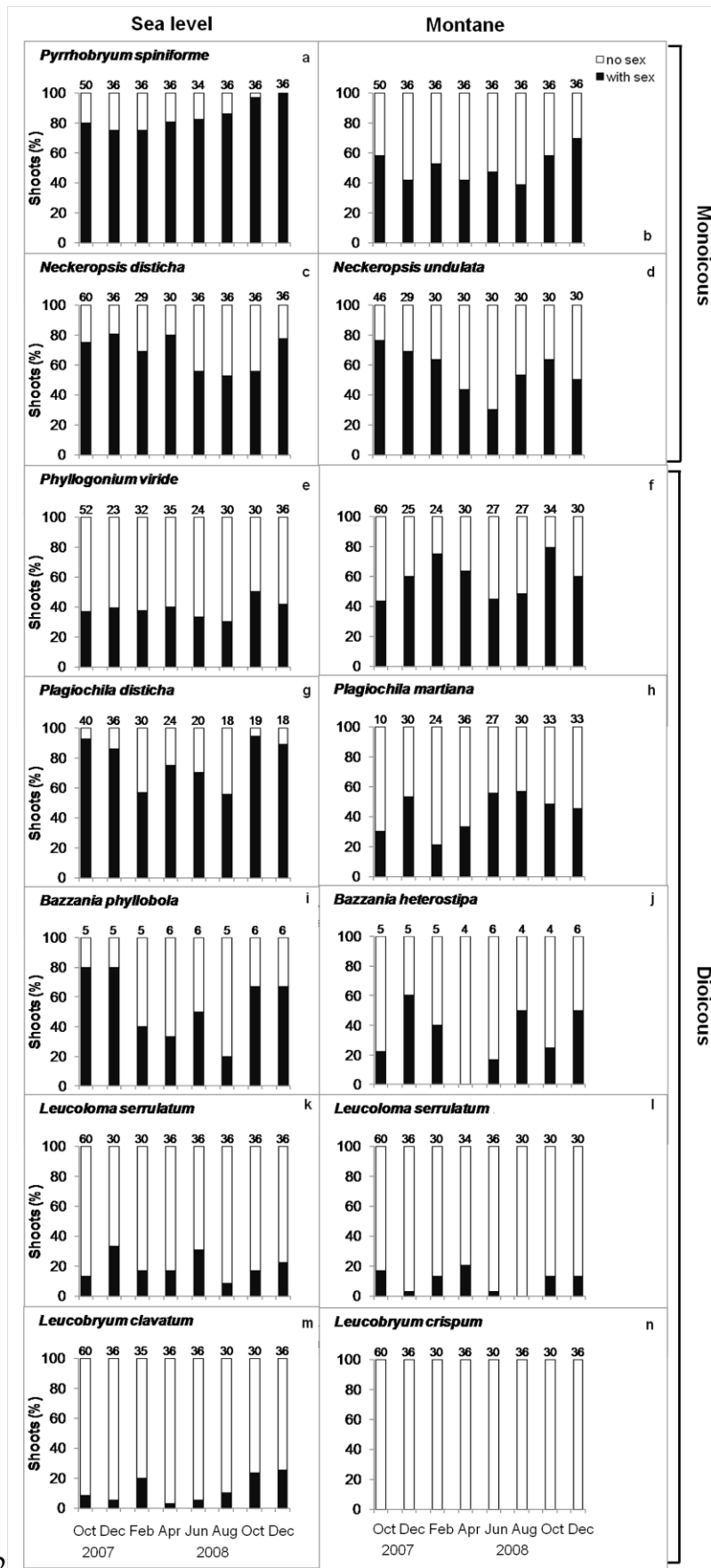


FIGURE 2

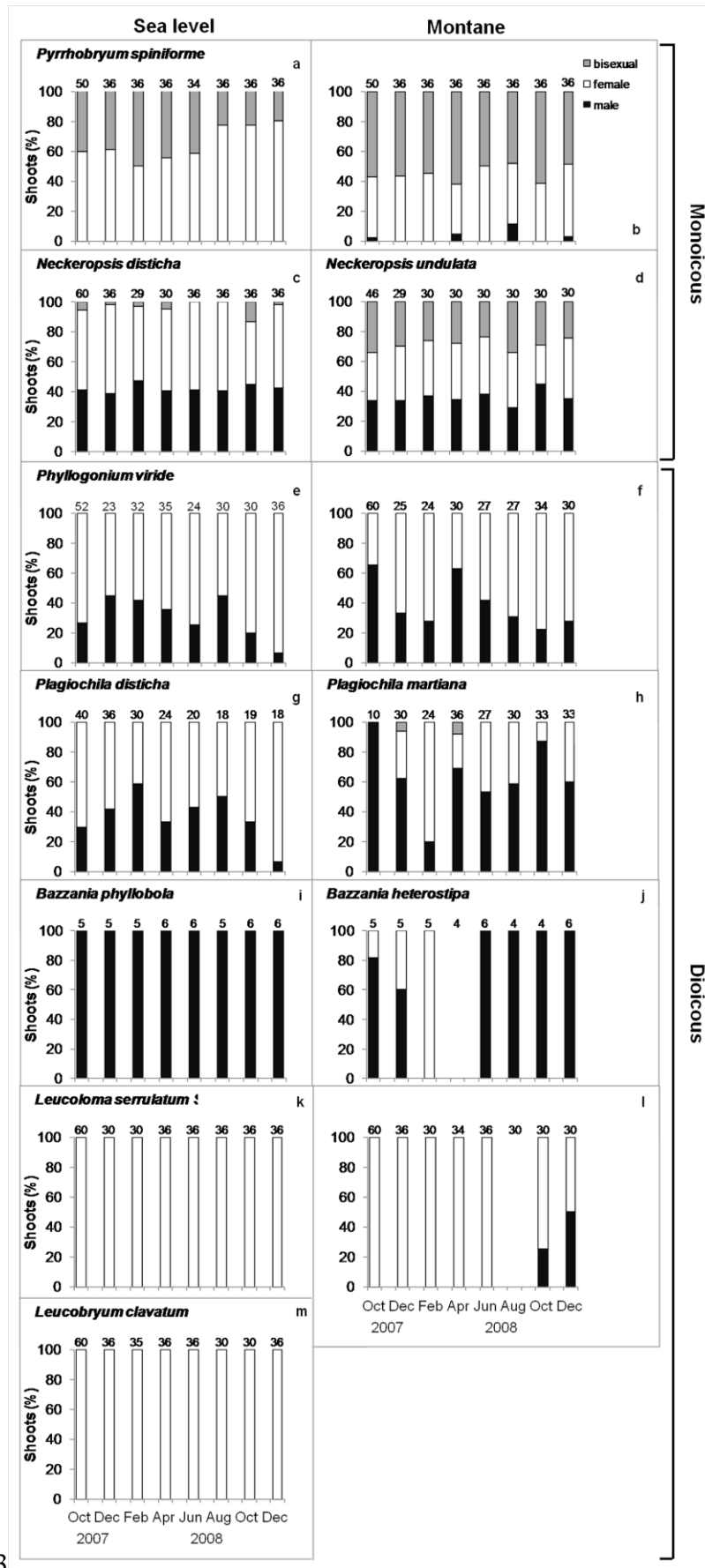


FIGURE 3

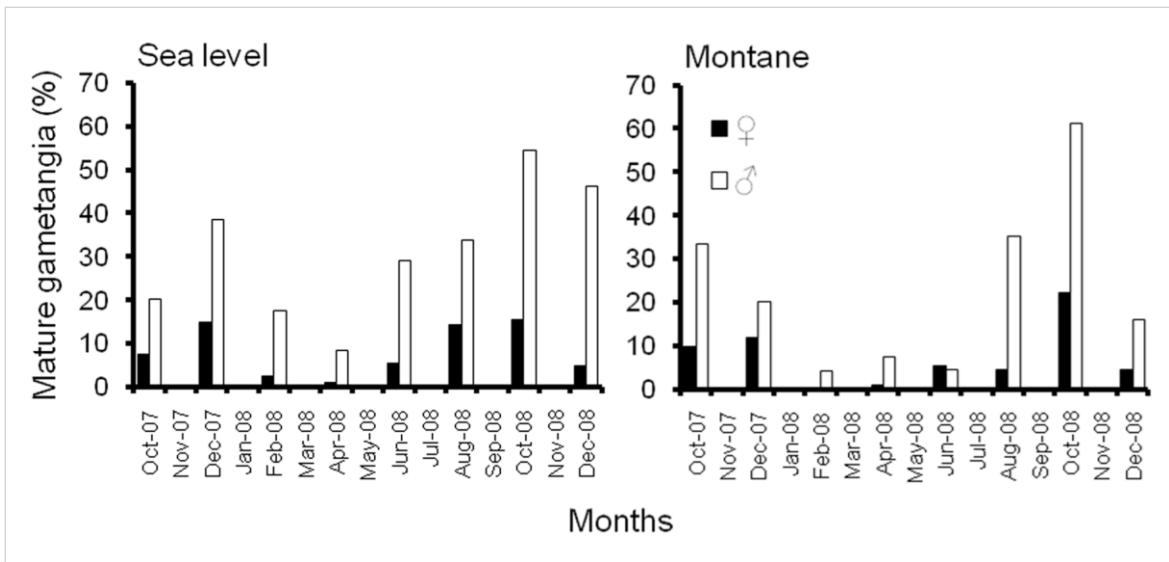


FIGURE 4

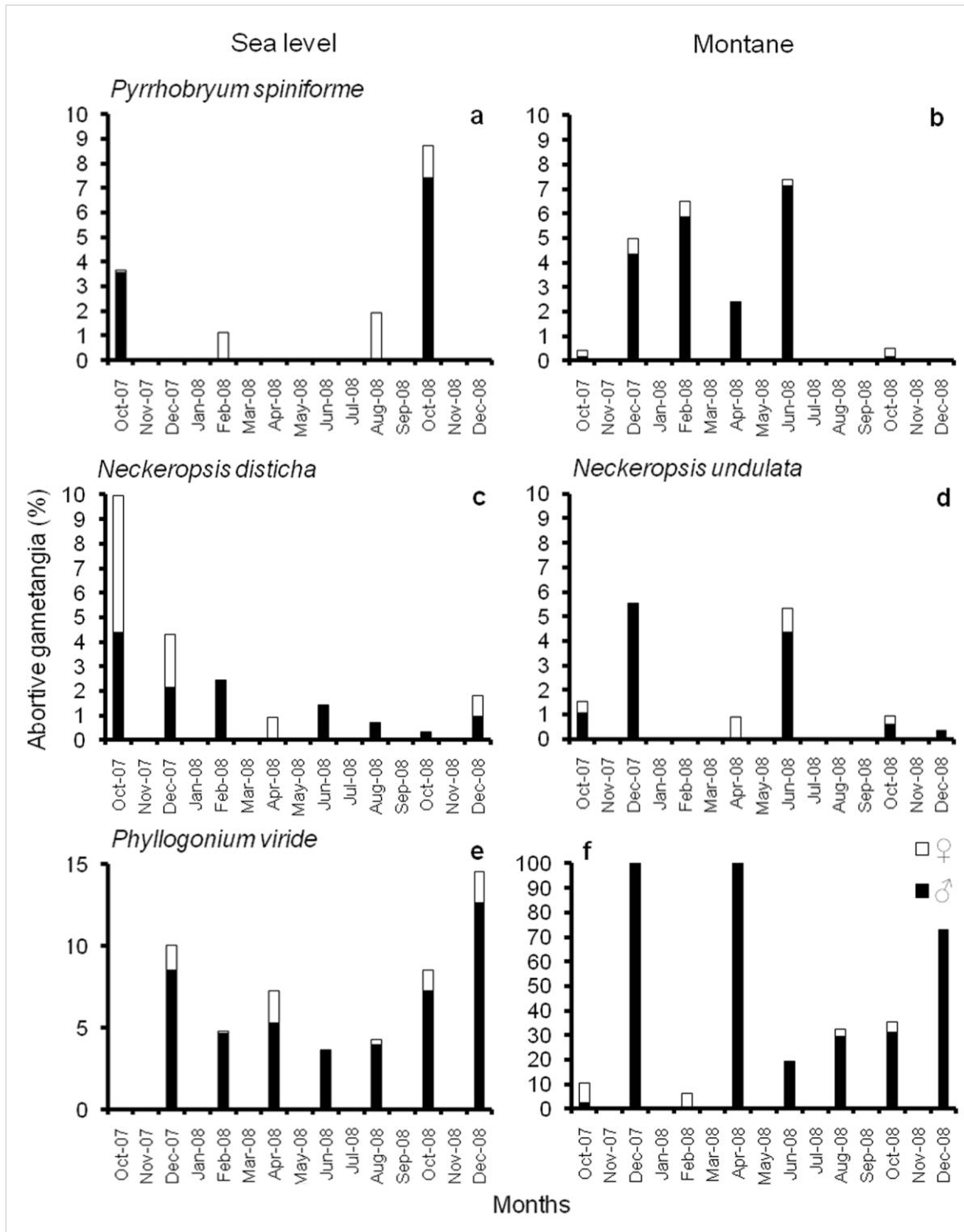
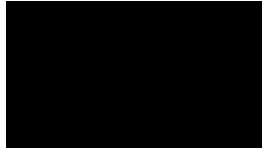


FIGURE 5

## Capítulo II



**Altitude affects the reproductive performance in monoicous and dioicous bryophytes:  
examples from a Brazilian Atlantic Rainforest**

(Manuscrito formatado segundo as normas do periódico *Annals of Botany*)



**Altitude affects the reproductive performance in monoicous and dioicous bryophytes:  
examples from a Brazilian Atlantic Rainforest**

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**Abstract**

**Background and Aims** Bryophytes are good models to understand plant reproductive strategies, since these plants have short life cycle and trade-offs linked to breeding systems. Our aim was to test if differences in sexual reproductive performance of bryophytes in tropical rain forests are driven by the breeding system of the species (monoicous and dioicous), or mainly affected by the habitat.

**Methods** The reproductive performance (sexual branches, gametangia, fertilization and sporophyte production) of eleven species was analysed from populations in the sea level and montane sites of a Brazilian Atlantic Rainforest during fifteen months.

**Key Results** Monoicous species had the highest reproductive performance, particularly for male gametangia, fertilized gametangia and sporophyte production. Species in the sea level site produce more sexual branches and female gametangia than species in the montane site, but the fertilization is frequent at montane, and sporophytes occur similarly in the two sites. Fertilization occurs mostly in the rainiest periods (October to December).

**Conclusion** Not only the breeding system influences the reproductive performance of bryophytes, but the habitat is an important factor determining life-history differentiation. Since the establishment likelihoods of sea level plants seem low due to periodical disturbances, these plants compensate with high production in reproductive structures at the initial phases of the reproductive cycle.

**Key-words:** Atlantic Forest, fertilization rate, gametangia, liverworts, mosses, sexual reproduction, sporophytes.

## **Introduction**

The regenerative phase (Grime 2001) is crucial in the life cycle of the plants, and since young individuals must find new safe-sites to establish and survive (Harper *et al.* 1965), the reproductive phase is strongly driven by the selective forces. Different reproductive strategies have been developed among plants by means of both asexual and sexual types of reproduction, in accord to their life-histories and the environment where they live (Bengtsson & Ceplitis 2000, Obeso 2002). In a general view, the reproductive strategies based on long life-span are related to low growth-rate and low reproductive effort, whilst fast growth rate and high reproductive effort are linked to short life-span (Grime 2001, García *et al.* 2008). Other traits can be related direct or indirectly to the longevity of plants, such as breeding systems (unisexual or bisexual), seasonality of reproduction, and formation of diaspore banks (During 1979, Grime 2001), where the probability and frequency of the reproductive events generally decrease with the increasing life-span.

Plant life-history traits change along different environmental gradients, sometimes within short distances, involving growth and reproductive parameters (Hassel *et al.* 2005, von Arx *et al.* 2006, Milla *et al.* 2009, Hautier *et al.* 2009). Different strategies permit plants to explore a

range of distinct habitats. Along altitudinal gradients it is common to find many species of high-altitude with a conservative approach, investing more in growth than in reproduction, and low-altitude investing more in reproduction (von Arx *et al.* 2006, Hautier *et al.* 2009). These findings are generally related to the restricted conditions in the high mountains, with low temperatures, a period of snow cover and low productivity (Körner 2007), but we do not know if they apply to altitudinal gradients of tropical rain forests.

Tropical rain forests are extremely threatened environments, despite of their high richness and diversity of species (Gómez-Pompa *et al.* 1972, Murray-Smith *et al.* 2009). For instance, the Atlantic forest in Brazil has nowadays less than 7% of its initial cover (Tabarelli *et al.* 2005), and is restricted to small forest fragments and a few large nature reserves, where altitudinal gradients of forest from the sea-level to the mountain tops are very scarce (e.g. Atlantic Forest covering the “Serra do Mar” in the southeastern Brazil). The Brazilian Atlantic forest retains a high number of plant species, with a total of about 15,800 (7,155 endemic species), of which 1,230 are bryophytes (Stehmann *et al.* 2009).

Bryophytes are a large and important component in tropical rain forests, covering substrates such as tree bark, leaves and decaying wood, and contributing to the high species richness and diversity (Whitmore *et al.* 1985, Frahm & Gradstein 1991), beyond functioning of these ecosystems (nutrient and water cycling, and habitat for micro fauna – Nadkarni 1984, Veneklaas 1990, Schofield 1985, Turetsky 2003). Since these plants (liverworts, mosses and hornworts) have in common a dominant haploid generation, reduced size, similar types of breeding system, and in general a shorter cycle life than the seed plants cycle (Glime 2007), they are excellent models to study factors affecting sexual reproduction. Specifically, it is quite interesting to use bryophytes to understand life-history strategies involving the

reproductive performance of liverworts and mosses, monoicous and dioicous species in different habitats of the same ecosystem.

Our aim was to test if differences in sexual reproductive performance of bryophytes in tropical rain forests are driven by the breeding system of the species (monoicous and dioicous), or mainly affected by the habitat.

Since monoicous species tend to produce sporophytes more frequently than dioicous ones (Gemmell 1950, Longton & Schuster 1983, Longton 1992), we expected that monoicous species have higher gametangia production, fertilization rates and sporophyte production than dioicous species. We also expected to find differences in these features in similar species between different sites, since the reproduction can be differently triggered by abiotic factors (e.g. light, temperature, humidity; Chopra & Bhatla 1983, Kumra & Chopra 1983, Longton 1990). We studied the sexual branches and gametangia production, fertilization rate, and sporophyte production of bryophytes in two contrasting sites of Atlantic tropical rain forests (montane and sea level) in Brazil. We specifically investigated the following questions:

- (1) Do monoicous and dioicous species differ in their sexual reproductive output?
- (2) If so, at what stages of the reproduction do the differences occur (production of male and female structures, fertilization rate, sporophyte production).
- (3) Do the same, or closely related, species differ in sexual performance between habitats?
- (4) Is there a seasonal variation in reproduction?
- (5) Do abiotic factors such as pH and moisture of substrates influence the reproduction?

## **Material and Methods**

### *Study sites*

We selected two different altitudinal sites of an Atlantic Ombrophilous Dense Forest (Veloso *et al.* 1991; Alves *et al.* 2010), located in the “Serra do Mar” State Park, Brasil. This park is covered by a tropical rain forest characterized by temperatures around 25°C and high precipitation evenly distributed over the year, with a short dry season during only one to three months. The low altitude forest is locally known as “Restinga forest”, a close to sea and seasonally flooded forest, referred in our study to as “sea level site” (*Núcleo Picinguaba*, 23° 34' S - 45° 02' W and 23° 17' S - 45° 11' W), and the high-altitude forest (*ca.*1,000 m a.s.l.) is referred to “montane site” (*Núcleo Santa Virgínia*, 23° 17' - 23° 24' S and 45° 03' - 45° 11' W).

Monthly or bimonthly measurements of minimum and maximum temperatures were taken in the understory of the two sites, using two max-min thermometers at each site. These measurements were taken from September 2007 to December 2009. We used precipitation data from two meteorological stations (INPE) next to the study sites (*ca.* 100 m from the sea level site and *ca.* 10 km from the montane site; Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos – CPTEC/INPE; Projeto “Estudos da Previsibilidade de Eventos Meteorológicos Extremos na Serra do Mar”).

The temperature in the montane site varies from 4°C in the winter to 25°C in the summer, whereas in the sea level site varies from 12°C in the winter to 32°C in the summer (Figure 1a and 1b). The annual mean precipitation in the montane is > 2,000 mm and in the driest months (June to August) the month precipitation is about or lower than 60 mm (Setzer 1966; Figure 1a), and the VPD (vapour pressure deficit) of the forest is 0.6 kPa (Rosado *et al.* 2009). In the sea level site the annual mean precipitation is > 2,200 mm and in the driest months about or lower than 80 mm (Setzer 1966; Figure 1b), and the forest VPD is 0.2 kPa (Rosado *et al.* 2009). The air relative humidity is similar in these two sites, but the montane site is more shaded at understory level than the sea level, and the montane site has lower

photosynthetically active radiation and red:far red ratio (Maciel-Silva, Válio & Rydin, in prep).

### *Sampling and study species*

For each forest site (sea level and montane) we established one area (10,000 m<sup>2</sup>) subdivided in 100 plots of 100 m<sup>2</sup> each. We initially collected 244 and 167 bryophyte samples in the sea level and montane sites, respectively, from bark and decaying wood in the majority of the 100 m<sup>2</sup> plots. This was used as basis to select study species according to the occurrence, breeding system, and phylum (Bryophyta – mosses; Marchantiophyta – liverworts) (Table 1). Mosses were separated according to growth form as pleurocarpous (one or more spore capsules along the main stem or on side-branches) and acrocarpous (a single capsule is produced at the shoot apex) (La Farge-England 1996). Here we considered the cladocarpous moss *Pyrrhobryum spiniforme* as pleurocarpous, since there are one or more capsules along the main stem. To cover the variation in these attributes we selected 11 species from seven genera of mosses and liverworts (Table 1). When one species did not appear or was not so abundant at both sites, two congeneric species were chosen, with the same breeding system and growth form. For simplicity, we refer to species, or species pair as 'genus' in the following.

We selected five to six colonies of each species and site, and samples were bimonthly collected during 15 months (from October 2007 to December 2008). The colony size varied from approximately 100 cm<sup>2</sup> (for liverworts) to 1,000 cm<sup>2</sup> (for mosses). Colonies of the same species were selected at least five meters apart. From each colony, squares being at least 4 cm<sup>2</sup> for liverworts and 9 cm<sup>2</sup> for mosses were collected (put in paper bags and carried to laboratory). Samples contained both plants and substrate. At each time, the samples were taken from different parts of the colony to minimize disturbance (Laaka-Lindberg 2005).

We randomly picked out ten shoots per colony in October 2007, and six shoots in the other months. We carefully cleaned these shoots, removing fragments of other bryophyte species and leaf litter. The shoots were air dried in laboratory (temperature around 20-27°C, air relative humidity of 60%), and maintained in paper bags up to the weighing process (we did not dry these plants in an oven to avoid damaging the gametangia). The number of sexual branches and sporophytes per mass was assessed from December 2007 to December 2008, whilst the number of sexual branches and sporophytes per shoot, together with gametangia production and fertilization rate were assessed also from the first sampling (Oct 2007). All variables were assessed on the same shoots and in the statistical analyses we used the mean values for the shoots in a colony.

#### *Production of sexual branches*

We weighed the shoots collectively from each colony using an analytical balance with accuracy of 10 µg (Ohaus Model AP250D-0). With dissecting microscopy we counted their sexual branches (♂ - perigonia, ♀ - perichaetia, and bisexual branches in some species). The production of sexual branches was expressed as number per mass. We also assessed the mean number of sexual branches per shoot for each colony.

#### *Production of gametangia*

From each shoot we assessed the number of gametangia (i.e., sex organs: ♂ - antheridia, ♀ - archegonia) per sexual branch on the actively developing gametophyte part, using dissecting and light microscopes. We also calculated the sex ratio of each species at both sites from the mean number of female and male gametangia produced per sexual branch.

#### *Fertilization rate and production of sporophytes*

We counted the total number of fertilized archegonia (with swollen venter) in each sexual branch. We calculated the fertilization rate in two ways: 1) The number of fertilized

archegonia divided by the total number of archegonia produced in each sexual branch; 2) The inverse number of fertilized archegonia (1/fertilized archegonia), since one perichaetium can support several archegonia but normally only one archegonium becomes sporophyte. To assess the sporophyte production we counted the number of sporophytes per mass unit and also per shoot.

#### *pH and moisture of substrates*

Samples of substrates (bark and decaying wood) of each species at both forests were collected (December 2008), put in plastic bags, sealed and brought to laboratory as fast as possible. A part of each sample was separated to measure the pH. We used around 1 g suspended in 5 mL of deionised water over night at 27°C (Watson *et al.* 1988, Farmer *et al.* 1990). The other part was immediately weighed (accuracy of 1 mg). These samples were dried for three days at 70°C, and again weighed. The substrate moisture was expressed in percent as (fresh mass – dry mass) x 100/fresh mass. Since the preliminary analyses showed that gametangia production was the response most influenced by habitat, we related the gametangia production in both sites to these measurements of pH and moisture for December 2008.

#### *Statistical analysis*

Since we collected samples from the same colonies during 15 months, a model of repeated measures was proper. To investigate the effect of habitat, genus and time on the reproductive performance of the bryophytes, we performed repeated-measures ANOVAs. For the variables “production of sexual branches” and “sporophyte production”, the data were log transformed ( $\log x + 1$ ) to achieve normality of residuals and homogeneity of variances. We performed the analyses considering the between-subjects effect (site and genera effects), the within-subjects effect (time effect) and interactions. To detect differences in the “production



of male and female gametangia”, and “fertilized female gametangia”, the analyses were performed considering the between-subjects effect (site effect), the within-subjects effect (time effect) and interactions. However, the degrees of freedom did not allow us to test the effect of genus for the variables “male and female gametangia”, and “fertilized female gametangia”. The two variables “fertilization rate” were not statistically analyzed, since few data were recorded (mainly only in the monoicous species). In addition, we also performed simple linear regressions to detect possible effects of pH and moisture on the female and male gametangia, fertilization and sporophyte production of monoicous and dioicous species in the two sites.

## **Results**

An overview of the results is shown in Table 2, with ANOVAs summaries in Table 3. Most aspects of reproduction varied between the sites or among genera, and also showed clear phenological patterns (for detailed time variation see Figure 2 and SM 1). There were, however, no instances with site x genus interactions.

### *Production of sexual branches*

The production of sexual branches per shoot did not differ statistically between the two sites (Table 3), despite of a production slightly higher at sea level than in the montane site, except for *Phyllogonium* and *Neckeropsis*, which had more sexual branches in the montane. Genera differed in their production of sexual branches per shoot (Table 3), and the monoicous *Neckeropsis* and *Pyrrhobryum*, and the dioicous *Phyllogonium* had the highest numbers, whilst the dioicous *Leucoloma* and *Leucobryum* had the lowest production of sexual branches. *Leucobryum* in the montane site did not express sex. Active sexual branches were found

during all months for many genera, with a significant increasing in the months of October and December (SM 1a).

When the production of sexual branches was evaluated per mass, the genus had significantly more sexual branches at sea level than in the montane site, except for *Phyllogonium* (Table 2 and 3). Genera differed in their production of sexual branches per mass (Table 2 and 3), where the monoicous *Neckeropsis* and dioicous *Phyllogonium*, *Plagiochila* and *Bazzania* had the highest numbers of sexual branches, differing over the time (Figure 2a).

#### *Gametangium production*

Many genera at sea level produced more gametangia per sexual branch than in the montane site (Table 2). The production of female gametangia differed significantly between the two sites, but no significant difference in male gametangia production was observed (Table 3). Different from the other genera, *Phyllogonium* had more female gametangia in the montane site (Table 2).

Female gametangia production was generally overrepresented, compared to the male one (Table 2). The gametangial F:M sexual ratio was higher at sea level than in the montane site for the two monoicous genera *Pyrrhobryum* and *Neckeropsis*. Among the dioicous genera, *Phyllogonium* had a higher sex ratio in the montane site. *Bazzania*, *Leucobryum* and *Leucoloma* produced only one sex at sea level, and *Leucobryum* had neither male nor female gametangia at the montane site (Table 2). The male and female gametangia production did not vary significantly over time (Table 3; Figure 2b and 2c).

#### *Fertilization rate and sporophyte production*

There was no overall effect of site on fertilized gametangia (Table 3), but several genera had a higher fertilization rate at the montane site (Table 2). The fertilization rates were frequent for the monoicous genera *Pyrrhobryum* and *Neckeropsis* in the two forest sites, whilst

the dioicous *Phyllogonium* and *Plagiochila* had conspicuous fertilization rates only in the montane site. Fertilization rates were rarely recorded for the dioicous *Bazzania* and *Leucoloma*, and no rate observed for *Leucobryum* in both sites. Gametangia fertilization was observed over different months for monoicous and dioicous genera in the two sites, but it significantly differed over time (Table 3), occurring mainly in October and December (Figure 2d). High fertilization rates were observed in the dry season (June to August), mainly for the monoicous *Pyrrhobryum* and *Neckeropsis* in the montane site (SM 1c).

The number of sporophytes per shoot and per mass did not differ significantly between the two sites (Table 3). With exception of *Neckeropsis*, where the number of sporophytes per mass was higher at sea level, all genera had similar production of sporophytes in the two sites (Table 2). Genera differed significantly in the number of sporophytes per shoot and mass (Table 3), and the monoicous *Neckeropsis* and *Pyrrhobryum* had the highest numbers of sporophytes both per shoot and mass (SM 1b; Table 2). The dioicous *Phyllogonium* and *Plagiochila* had sporophytes mostly found in the months April, June and December. *Bazzania*, *Leucobryum* and *Leucoloma* were found bearing rare or none sporophyte (Figure 2e).

#### *Effect of pH and moisture*

The pH and moisture had no significant effects on the fertilization and sporophyte production. Significant effects of pH and moisture were only detected on the gametangia production, and were rather weak ( $R^2 \leq 0.3$ ), and mainly observed in monoicous species at sea level. The pH had a significant negative effect on the number of male and female gametangia of monoicous species in the sea level forest (male:  $y = 34.81 - 4.57x$ ,  $R^2 = 0.06$ ,  $P = 0.05$ ; female:  $y = 41.58 - 4.98x$ ,  $R^2 = 0.06$ ,  $P = 0.01$ ). The gametangial production of both sexes occurred from pH 4.4 to 5.6, decreasing with increasing pH. For dioicous species in the sea level site, female gametangia increased with increasing pH ( $y = -24.47 + 7.95x$ ,  $R^2 = 0.30$ ,  $P <$

0.001; male gametangia data were too few for a regression). Moisture had positive effect on the number of male and female gametangia of monoicous species at sea level (male:  $y = 5.59 + 0.13x$ ,  $R^2 = 0.07$ ,  $P = 0.029$ ; female:  $y = 5.15 + 0.23x$ ,  $R^2 = 0.23$ ,  $P < 0.001$ ). Similarly, male gametangia of monoicous species in the montane site increased significantly with increasing moisture ( $y = -9.66 + 0.36x$ ,  $R^2 = 0.27$ ,  $P < 0.001$ ).

## Discussion

Regarding to the initial questions, our main findings were: (1) The monoicous species had a generally higher reproductive output, and some dioicous species failed in the production of male gametangia, fertilization or sporophyte production. (2) Within some genera, production of sexual branches and female gametangia differed between the two sites, and in many traits several genera performed better at sea level compared to the montane site. (3) A noticeable seasonal variation was recorded, and fertilized gametangia occurred mostly in the rainiest periods (October to December). (4) pH and moisture could to some degree explain the gametangia production, but did not directly affect fertilization or sporophyte production.

### *Influence of the breeding system on the reproductive performance*

The sex-expression of bryophytes in tropical rain forests seems to be influenced by the breeding system of the species, where some dioicous ones fail to express one or both sexes and consequently fail to produce many sporophytes. In fact, many authors relate the failure of dioicous bryophytes to produce sporophytes due to the spatial separation of sexes and/or absence of at least one sex (generally the male one) (Gemmell 1950, Longton & Schuster 1983, Bowker *et al.* 2000, Oliveira & Pôrto 2002, Bisang & Hedenäs 2005). Some dioicous species in our study had high numbers of sexual branches and gametangia, but still failed to produce high number of sporophytes (e.g. *Bazania* and *Leucoloma* in the montane site). This

observation must be related to the distance between populations of the two sexes and absence of male plants (mostly at sea level).

Compared with seed plants, the breeding systems in bryophytes assume different roles. The dioicium among bryophytes is considered ancestral to the monoicium, whereas the dioecism in seedplants is derived (Bawa & Beach 1981, Longton & Schuster 1983) (note that the suffix “-oicium” is used instead of “-oecism” to distinguish between gametophytic and sporophytic breeding systems, Allen & Magill 1987, Mishler 1988). In seed plants, the evolution of the pollination mechanisms selects some strategies that favor large genetic recombination (Bawa 1980, Bawa & Beach 1981), but in bryophytes the water dependence of the sperm to perform fertilization establishes trade-offs. The relationship of fertilization likelihood (and offspring output) *versus* genetic recombination is generally opposite for monoicous and dioicous bryophytes. Monoicous species have frequently more sporophytes compared to dioicous ones, but inbreeding events are more common among monoicous species (Longton & Schuster 1983, Longton 1992, Longton 1997, Eppley *et al.* 2007). Since the breeding likelihoods can be low for dioicous bryophytes, these plants should compensate via an increased life-span compared to monoicous ones (During 1979, Longton 1997).

The breeding system is not the only factor acting on the sexual reproduction of the bryophyte species studied here. The growth form seems to be important to characterize different strategies. For instance, except for the acrocarpous mosses *Leucobryum* and *Leucoloma*, other dioicous species have a significant production of sexual branches and gametangia. The pleurocarpous moss *Phyllogonium* and the liverworts *Plagiochila* and *Bazzania* can have more than one reproductive branch per shoot, in contrast to acrocarpous species. This strategy is based on increased breeding likelihoods by means of investment in more sexual branches. In addition, the monoicous mosses *Pyrrhobyum* and *Neckeropsis* are

also able to produce many sexual branches per shoot. Alternatively, compared to the species with high sexual reproductive output, the dioicous mosses *Leucobryum* and *Leucoloma* have efficient means of asexual reproduction by regeneration of detached leaves (Maciel-Silva unpubl. data). That trait must be important for propagation of these species, since the sexual reproduction is rare in the two forest sites investigated.

#### *The role of the habitat on reproductive performance*

Sexual reproductive performance of bryophytes varies in relation to the forest site. Species at sea level, compared to the montane site, have the highest production of sexual branches per mass and also of female gametangia per sexual branch. The reproductive strategy of bryophytes at sea level is less conservative (more reproduction) than the strategy of bryophytes in the montane site, where the reproductive output is reduced. In addition, since the gametangial sex-ratios are generally female-biased at sea level, and unbiased in montane, the fertilization events are more frequent in the montane site. Similarly, distinct strategies in the same or closely-related species inhabiting at different altitudes have been recorded. These studies give evidence to the important role of trade-offs between reproductive investments and vegetative growth, as for plants at highest altitudes with low reproductive effort (Hassel *et al.* 2005, von Arx *et al.* 2006, Hedderson & Longton 2008, Hautier *et al.* 2009). Since the abiotic conditions on the mountains tend to be harder than at lowlands, mostly due to low temperatures (Körner 2007), plants with conservative strategies must be favored in these habitats.

In our forest sites the climate at montane cannot be considered harmful for bryophytes, but rather has temperature and water availability that would favor the growth and reproduction of bryophytes (Oliveira & Pôrto 1998, 2001). Contrary to the montane site, the temperatures are elevated at sea level, reaching above of 30°C in the summer. During the winter, important

period for induction of gametangia and mostly male gametangia of the species at both sites (Maciel-Silva & Válio in prep.), the temperatures are around 20°C in montane and 25°C at sea level. The induction and development of sexual structures in bryophytes are susceptible to variation in abiotic factors (Bowker *et al.* 2000, Glime 2007). Since high temperatures can promote the reproductive growth of structures for one or both sexes (Chopra & Bhatla 1981, Chopra & Rahbar 1982), we could expect that the highest reproductive output, mostly for female gametangia, of bryophytes at sea level can be related to the temperatures in that site. But, the elevated temperatures that can promote the female sex at sea level perhaps do not act similarly on the male structures.

In addition to the temperature, another important factor for bryophyte reproduction must be the photoperiod. In fact, the sexual organs induction was concentrated mostly at the long-day months (warm and wet season) compared to the other months (cool and dry season), in both forest sites. Similarly, other studies have recorded an increased flower production in different plants at high temperatures and long days (Talora & Morellato 2000, Marques *et al.* 2004). These factors are correlated in tropical forests at higher latitudes, and together to precipitation they trigger reproductive events among tropical plants (Wright 1996, Marques *et al.* 2004).

When we analyze the reproductive output (e.g. sexual branches) of the species per mass unit or shoot, similar findings for two sites appear, except for *Neckeropsis*. The number of sexual branches per shoot of *Neckeropsis* at sea level tends to be lower than in the montane site. However, an opposite pattern is observed when the reproductive output is measured per mass. Different from the other species, the *Neckeropsis* plants differ considerably in size. Plants at montane are larger than those ones at sea level. These two plants do not differ in stem long, but they differ in branch long, where *N. undulata* (montane) has branches up to 5

cm and *N. disticha* (sea level) has branches up to 3 cm (Buck 1998). Because of this, *Neckeropsis* plants in the sea level site have low reproductive output at shoot-level, but elevated output at mass-level (fixed carbon). Since in bryophytes, as well as in other clonal organisms, the role of the individual (e.g. shoot) is less relevant than the role of the clone (Crowley & McLetchie 2002, Winkler *et al.* 2009), populations producing more reproductive structures per mass can be favored at unstable habitats. The sea level site, for instance, is characterized by seasonal flooding (Alves *et al.* 2010) and is an unstable habitat for many species and mainly for plants that lose many diaspores and juveniles per year.

Although the fertilization occurs in different months, there is a strong influence of the rainiest periods on fertilization among bryophytes in both sites. Other studies in tropical rain forests also corroborate the importance of the rainiest period for fertilization in bryophytes (Oliveira & Pôrto 1998, 2001, 2002). Invertebrate-mediated fertilization in bryophytes is possible without a water film (Cronberg *et al.* 2006). Such interactions between bryophytes and invertebrates may be important in tropical rain forests, but unassisted fertilization is also favoured in these generally wet habitats.

Despite of the weak effect of pH and moisture on the gametangia production, these two abiotic factors can clarify some constraints on the gametangia production of bryophytes. The moistest substrates support high numbers of gametangia compared to the driest substrates, evidencing the importance of the water availability for the success of the gametangial formation (Egunyomi 1979). Since microhabitats with high water-holding capacities can support colonies producing many gametangia, we expect that epiphytic colonies, different from epixilic ones in a same species, have generally low sexual reproductive output. Additionally, the spatial separation of sexes in the dioicous species must be still more important than the effect of moisture on fertilization and sporophyte production (Stark *et al.*



2005), explaining the low reproductive output (e.g. sporophytes) in species with conspicuous production of reproductive organs (e.g. sexual branches and gametangia). The pH effect on gametangia production, observed only for plants at sea level, is different for monoicous and dioicous, and monoicous species are favoured by the lowest pH values. Since the three bark pH at sea level has low values compared to the montane site (sea level = mean  $3.83 \pm \text{sd } 0.61$ ; montane = mean  $4.43 \pm \text{sd } 0.16$ ), plants should take advantages from this trait at sea level.

Whereas plants in the montane site have a reproductive strategy based on quality, plants at sea level invest quantitatively in the initial phases of the sexual reproductive cycle, bearing many sexual branches and female gametangia. This pattern is observed for monoicous and dioicous species inhabiting both forest sites, although it is more clear for monoicous ones (Figure 3). Summarizing, both monoicous and dioicous at sea level tend to produce more female gametangia than male ones. Consequently, the fertilization rates at sea level are low compared to those in the montane site. Monoicous species have similar sporophyte production in both sites, whilst the dioicous (mainly at sea level) have the lowest sporophyte production. The female-biased gametangial sex ratios and the probable spatial separation of sexes among dioicous species at sea level must reduce the fertilization rates and sporophyte development. Alternatively, in the montane site, the unbiased sex ratios results in conspicuous fertilization rates and sporophyte production. Finally, the monoicous species in both sites have the highest reproductive output (sporophyte production) compared to the dioicous ones.

The reproductive performance of plants at sea level must be associated to periodical disturbances in that habitat (e.g. flooding over summer). Since plants at habitats under disturbance dynamics can have adapted life history traits (Milla *et al.* 2009), we expect that bryophytes in the sea level site, compared to plants at the montane site, have low likelihoods of germination and establishment. Conversely, plants produce many sexual structures (sexual

branches and female gametangia), which increase the fertilization chances and keep the production of sexual diaspores to colonize safe sites.

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**Table 1.** Phylum, growth form and breeding system of the bryophyte species studied in two sites of a Brazilian Atlantic Rainforest.

Genus	Species		Phylum	Growth form	Breeding system
	<i>Montane</i>	<i>Sea level</i>			
<i>Bazzania</i>	<i>heterostipa</i>	<i>phyllobola</i>	Liverwort	-	Dioicous
<i>Plagiochila</i>	<i>martiana</i>	<i>disticha</i>	Liverwort	-	Dioicous
<i>Leucobryum</i>	<i>crispum</i>	<i>clavatum</i>	Moss	Acrocarpous	Dioicous
<i>Leucoloma</i>	<i>serrulatum</i>		Moss	Acrocarpous	Dioicous
<i>Phyllogonium</i>	<i>viride</i>		Moss	Pleurocarpous	Dioicous
<i>Neckeropsis</i>	<i>undulata</i>	<i>disticha</i>	Moss	Pleurocarpous	Monoicous
<i>Pyrrhobryum</i>	<i>spiniforme</i>		Moss	Pleurocarpous	Monoicous

**Table 2.** Reproductive parameters (production of sexual branches per mass, gametangia per sexual branch, gametangial sex-ratio and fertilized gametangia per sexual branch, and sporophytes per mass) measured in bryophytes in two sites of a Brazilian Atlantic Rainforest (M= montane site, SL = sea level site; BS = breeding system; Di = dioicous, Mo = monoicous). Data are means  $\pm$  standard deviation (for more explanation see the section “sampling and study species”).

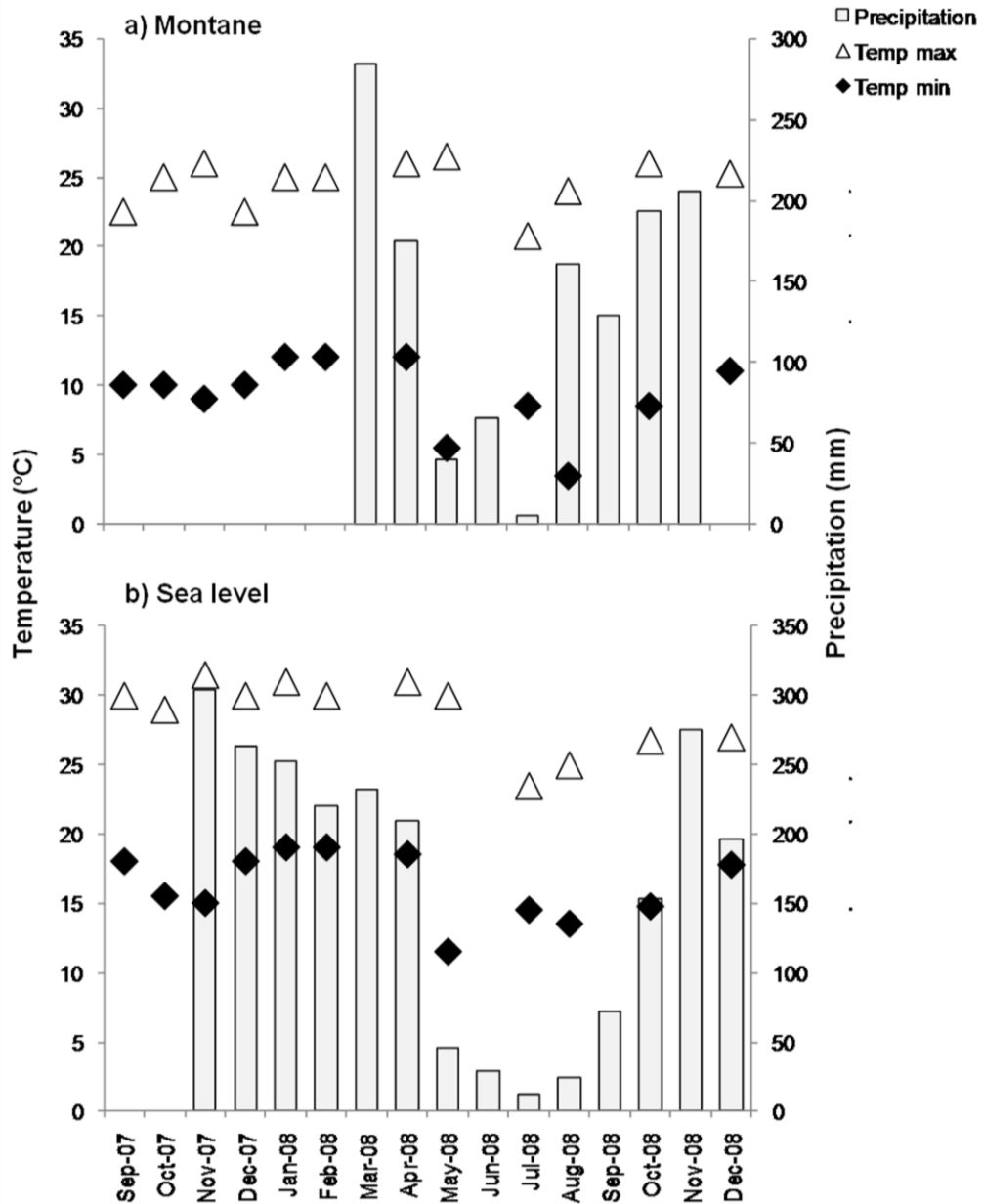
Genus	BS	Site	Sexual branch production (n° cg <sup>-1</sup> )	Female gametangia per sexual branch	Male gametangia per sexual branch	Gametangial sex-ratio F:M	Fertilized gametangia per sexual branch	Sporophyte production (n° cg <sup>-1</sup> )
<i>Bazzania</i>	Di	M	1.92 $\pm$ 1.95	8.4 $\pm$ 0.1	4.6 $\pm$ 0.5	1.8	0.05 $\pm$ 0.07	0
		SL	6.52 $\pm$ 9.30	0	4.2 $\pm$ 0.7	Only ♂	-	0
<i>Plagiochila</i>	Di	M	4.78 $\pm$ 2.58	11.8 $\pm$ 3	12.5 $\pm$ 2.7	0.9	0.59 $\pm$ 0.80	0.21 $\pm$ 0.24
		SL	7.46 $\pm$ 3.50	15.5 $\pm$ 4.9	14.9 $\pm$ 6.8	1.0	0.003 $\pm$ 0.01	0.17 $\pm$ 0.26
<i>Leucobryum</i>	Di	M	0	0	0	No sex	-	0
		SL	0.55 $\pm$ 0.61	7.7 $\pm$ 1.86	0	Only ♀	-	0
<i>Leucoloma</i>	Di	M	0.78 $\pm$ 1.12	18.6 $\pm$ 6.8	7.6 $\pm$ 1.9	2.4	0	0
		SL	1.17 $\pm$ 0.76	23.4 $\pm$ 5.4	0	Only ♀	0.08 $\pm$ 0.23	0
<i>Phyllogonium</i>	Di	M	4.19 $\pm$ 2.66	14.4 $\pm$ 2.8	11.3 $\pm$ 2.1	1.3	0.07 $\pm$ 0.07	0.27 $\pm$ 0.16
		SL	1.68 $\pm$ 0.90	7.8 $\pm$ 1.7	8.9 $\pm$ 3.7	0.8	0	0
<i>Neckeropsis</i>	Mo	M	0.41 $\pm$ 0.15	8.3 $\pm$ 1.2	6.8 $\pm$ 0.8	1.2	0.15 $\pm$ 0.15	0.41 $\pm$ 0.15
		SL	7.74 $\pm$ 3.60	11.5 $\pm$ 1.4	7.6 $\pm$ 0.8	1.5	0.10 $\pm$ 0.10	1.42 $\pm$ 0.47
<i>Pyrrhobryum</i>	Mo	M	1.90 $\pm$ 0.97	13.1 $\pm$ 3.3	11.5 $\pm$ 5.4	1.1	0.18 $\pm$ 0.10	0.49 $\pm$ 0.06
		SL	3.54 $\pm$ 1.58	17.4 $\pm$ 2.6	9.7 $\pm$ 3.3	1.8	0.05 $\pm$ 0.06	0.38 $\pm$ 0.08

**Table 3.** Summary of the repeated measures Anovas for sexual features of bryophyte genera in two sites of a Brazilian Atlantic Rainforest. The response variables “sexual branches” and “sporophytes” were transformed as  $\log x + 1$  prior to analysis.

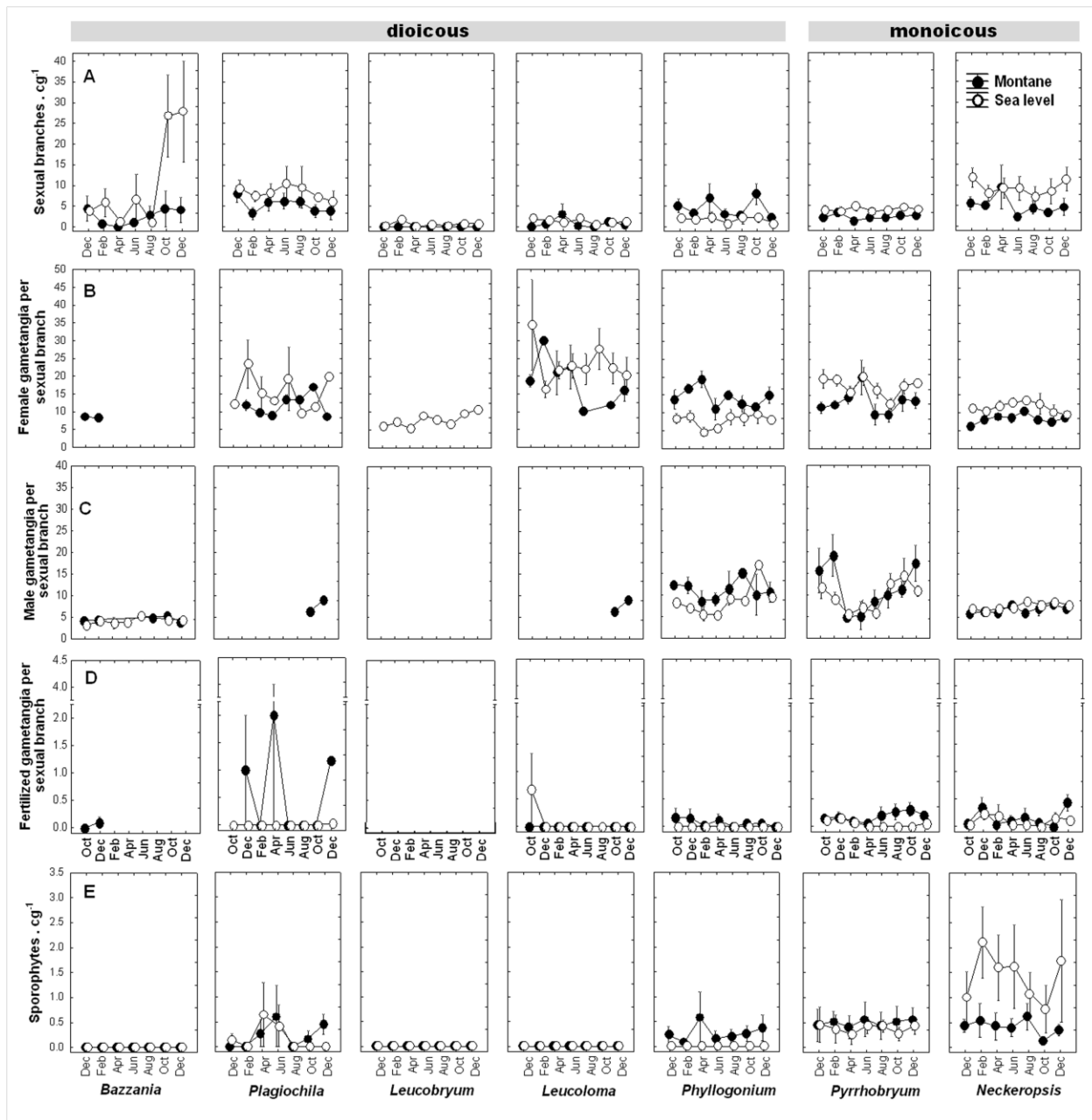
Source	Repeated measures anova			
	d.f.	F	MS	P
<b><i>Sexual branches per shoot</i></b>				
<i>Between-subjects effects</i>				
Site	1	1.595	0.742	0.214
Genus	6	8.496	3.956	<b>&lt;0.001</b>
Site x Genus	6	1.419	0.661	0.230
Error	41		0.465	
<i>Within-subjects effects</i>				
Time	7	3.692	0.208	<b>&lt;0.001</b>
Time x Site	7	1.000	0.053	0.431
Time x Genus	42	2.438	0.137	<b>&lt;0.001</b>
Time x Site x Genus	42	1.086	0.061	0.339
Error	287		0.056	
<b><i>Sexual branches per mass</i></b>				
<i>Between-subjects effects</i>				
Site	1	7.657	2.174	<b>0.008</b>
Genus	6	14.107	4.006	<b>&lt;0.001</b>
Site x Genus	6	1.643	4.665	0.159
Error	42		0.284	
<i>Within-subjects effects</i>				
Time	6	1.994	0.159	0.067
Time x Site	6	0.990	0.079	0.432
Time x Genus	36	2.212	0.177	<b>&lt;0.001</b>
Time x Site x Genus	36	0.864	0.691	0.692
Error	252		0.080	
<b><i>Male gametangia per sexual branch</i></b>				
<i>Between-subjects effects</i>				
Site	1	0.271	15.002	0.618
Error	7		32.116	
<i>Within-subjects effects</i>				
Time	7	0.387	21.234	0.905
Time x Site	7	0.664	8.747	0.701
Error	28		7.439	
<b><i>Female gametangia per sexual branch</i></b>				
<i>Between-subjects effects</i>				
Site	1	7.441	63.940	<b>0.029</b>
Error	7		235.75	
<i>Within-subjects effects</i>				
Time	7	1.039	4.310	0.416
Time x Site	7	0.835	7.370	0.563
Error	28		11.110	

**Table 3.** continued.

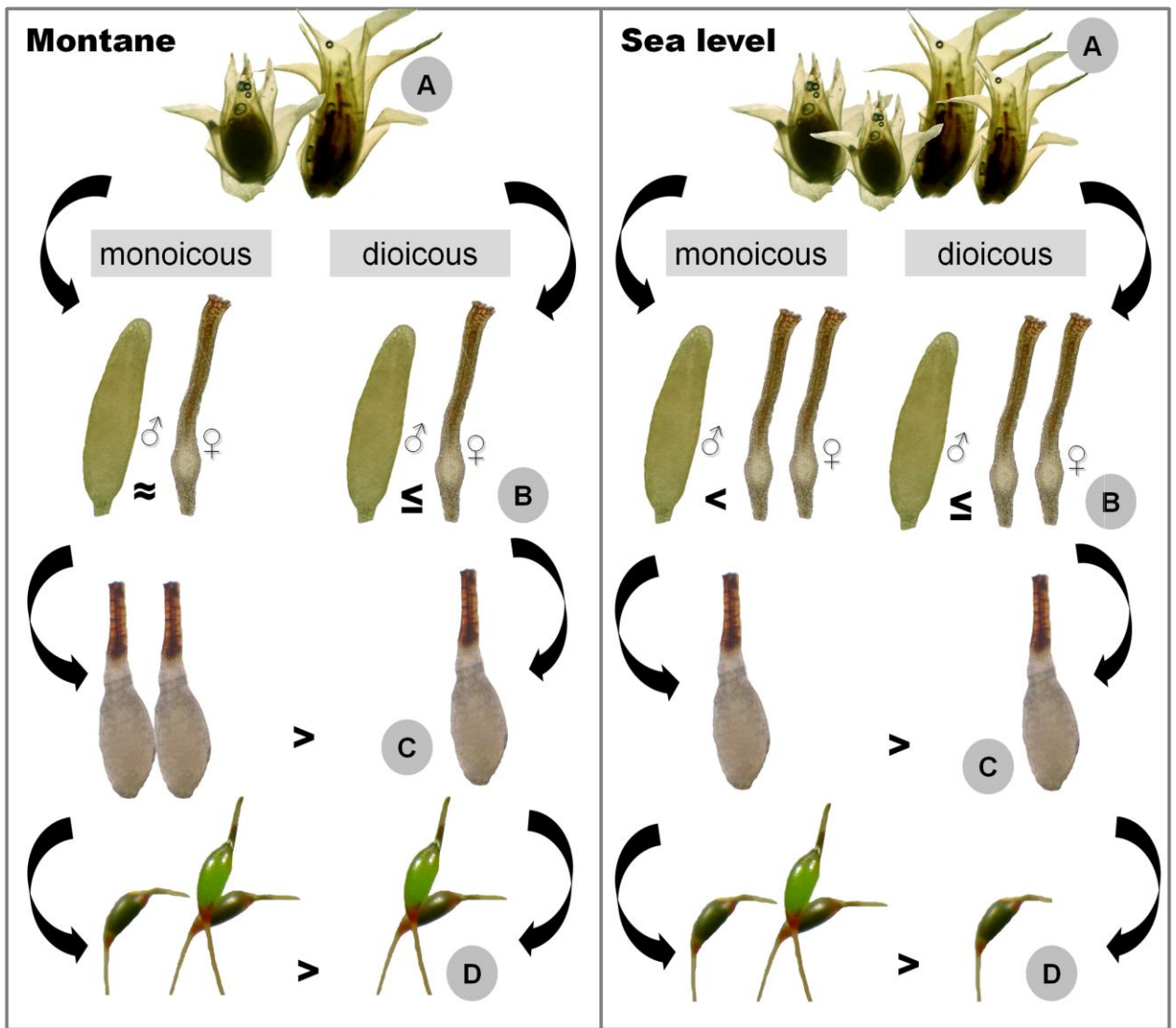
<b><i>Fertilized gametangia per sexual branch</i></b>				
<i>Between-subjects effects</i>				
Site	1	0.467	0.145	0.532
Error	4		0.019	
<i>Within-subjects effects</i>				
Time	7	2.854	0.012	<b>0.022</b>
Time x Site	7	8.747	0.009	0.348
Error	28		0.011	
<b><i>Sporophyte per shoot</i></b>				
<i>Between-subjects effects</i>				
Site	1	0.235	0.021	0.630
Genus	6	4.760	0.417	<b>0.001</b>
Site x Genus	6	0.224	0.019	0.966
Error	41		0.087	
<i>Within-subjects effects</i>				
Time	7	0.573	0.005	0.770
Time x Site	7	0.254	0.002	0.971
Time x Genus	42	0.593	0.005	0.981
Time x Site x Genus	42	0.471	0.004	0.998
Error	287		0.009	
<b><i>Sporophyte per mass</i></b>				
<i>Between-subjects effects</i>				
Site	1	0.103	0.006	0.749
Genus	6	6.999	0.407	<b>&lt;0.001</b>
Site x Genus	6	1.365	0.079	0.251
Error	42		0.058	
<i>Within-subjects effects</i>				
Time	6	0.778	0.009	0.587
Time x Site	6	0.823	0.010	0.553
Time x Genus	36	0.564	0.007	0.980
Time x Site x Genus	36	0.595	0.007	0.968
Error	252		0.012	



**Figure 1.** Maximum and minimum monthly temperatures (°C) and precipitation (mm month<sup>-1</sup>) from montane (a) and sea level (b) sites (September 2007 to December 2008). Precipitation data are from meteorological stations; note that data were not available for all months at both sites. (source: “Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos – CPTEC/INPE” for precipitation data).

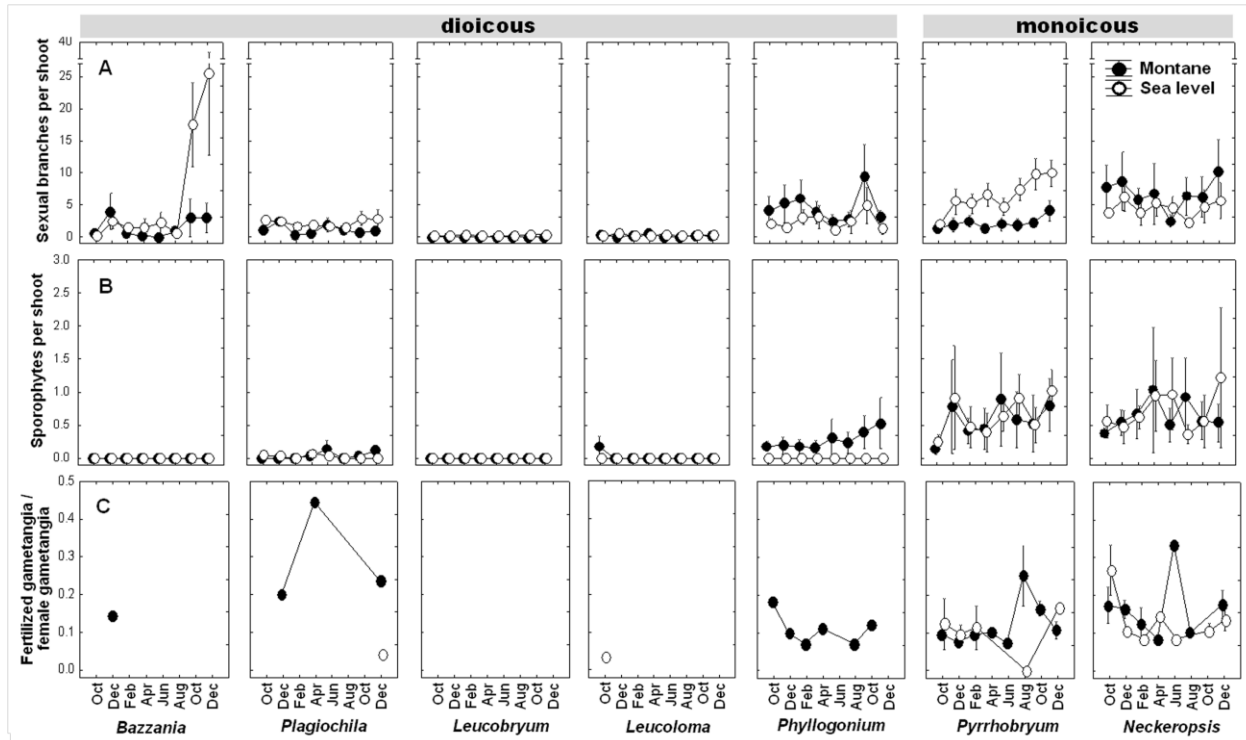


**Figure 2.** Number of sexual branches per mass (A), number of female (B) and male (C) gametangia per sexual branch, number of fertilized female gametangia per sexual branch (D), and number of sporophytes per mass (E) of dioicous and monoicous bryophyte species in two sites of a Brazilian Atlantic Rainforest. Note that data were not available for all species at both sites. Vertical bars denote standard errors.



**Figure 3.** Summary of the sexual reproductive performance for monoicous and dioicous bryophytes in montane and sea level sites of a tropical rain forest, Brazil. A. Sexual branches; B. Male and female gametangia; C. Fertilized gametangia; D. Sporophytes. Different numbers of structures denote differences between the sites, and symbols represent comparisons between the breeding systems in the same site.

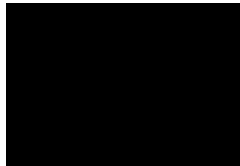
## Supplementary material



**SM 1.** Number of sexual branches (A) and sporophytes per shoot (B), and fertilized rate as “fertilized gametangia per total female gametangia in each sexual branch” (C) of dioicous and monoicous bryophyte species in two sites of a Brazilian Atlantic Rainforest. Note that data were not available for all species at both sites. Fertilized rate data as “inverse number of fertilized archegonia” are omitted since it is very similar to the “fertilized gametangia/female gametangia” shown here. Vertical bars denote standard errors.



## Capítulo III



**Diaspore bank of bryophytes in tropical rain forests: the importance of breeding system,  
phylum and micro-habitat**

(Manuscrito formatado segundo as normas do periódico *Journal of Ecology*)

## **Diaspore bank of bryophytes in tropical rain forests: the importance of breeding system, phylum and micro-habitat**

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### **Summary**

**1.** Diaspore banks are key subjects for the maintenance and resilience of plant communities. Although seed banks have received large attention in different ecosystems, diaspore banks of bryophytes remain poorly known, especially from tropical ecosystems. For instance, the dynamics of bryophyte dispersal and establishment remain unknown in tropical rain forests, despite the fact that these forests harbour high species richness. This is the first study with focus on the role of the transient diaspore banks of bryophytes in tropical rain forests.

**2.** Our general aim was to test if micro-habitat characteristics (substrate type) and species traits (breeding system and phylum) are important to explain the pattern of bryophyte diaspore banks in tropical rain forests. We assessed, through samples cultivated in the laboratory, the number of species and shoots emerging from samples of bark, decaying wood and soil from two sites of the Atlantic rain forest (montane and sea level) in Brazil, comparing the contribution of species differing in phylum (mosses and liverworts) and breeding system (monoicous and dioicous).

3. More species emerged from bark (68) and decaying wood (55) than from soil (22). Similar numbers of species were found in the montane and sea level sites. Mosses were more numerous in species and shoot number, and monoicous species dominated over dioicous species in both sites, although proportionally more dioicous ones emerged at the montane site. The substrate pH had only weak effects on the emergence of species in the diaspore banks. Species commonly producing sporophytes and gemmae in the field had a high contribution to the diaspore banks.

4. These superficial diaspore banks represented rather well the extant vegetation, but had an overrepresentation of short-lived species. We propose a conceptual model for the dynamics of these diaspore banks driven by species traits and micro-habitat characteristics.

5. *Synthesis.* We suggest that short-term diaspore banks of bryophytes in tropical rain forests can contribute to fast (re)establishment of many species after disturbances and during succession, and are particularly important for dioicous mosses investing in asexual reproduction and monoicous mosses investing in sexual reproduction.

**Key-words:** Asexual diaspores; Brazilian Atlantic Forest; breeding system; establishment; liverworts; mosses; pH; spores.

## Introduction

Diaspore banks are important for maintenance of genetic variability and resilience of plant communities, mainly in ecosystems having an unfavourable dry or cold season (Thompson 2000; Walck *et al.* 2005; Hock *et al.* 2008). Seed banks have been widely studied in different ecosystems (Thompson & Grime 1979; Hyatt & Casper 2000; Sletvold & Rydgren 2007; Caballero *et al.* 2008; Aguiar & Tabarelli 2010), mainly to determine which characteristics of the habitat or species that affect the seed bank composition. For instance,

lack of seed germination can be due to absence of suitable environmental conditions or seed dormancy triggered by abiotic (e.g. cold, photoperiod) and intrinsic factors (hormone balance, coat thickness). The longevity of the seed bank depends on features both of seeds and habitats (Thompson 2000; Walck *et al.* 2005). In a broad way, seed banks are divided into persistent banks with seeds surviving for at least one year, and transient banks with seeds surviving at most one year (Thompson & Grime 1979). The contribution of small seeds to a soil bank is generally higher compared to large ones. They are often produced in large numbers, have high likelihood to become buried, suffer less from predation, and they survive longer time in the soil than large seeds (Thompson 2000).

Compared to seed banks, diaspore banks of bryophytes remain little understood, with some surveys from temperate and boreal areas (During & ter Horst 1983; Jonsson 1993; Bisang 1995; Sundberg & Rydin 2000) and there are few studies from tropical ecosystems (During 1997; Bisang, Piippo & Hedenäs 2003; During 2007; Maciel-Silva & Lins-Silva 2007). Diaspore banks of bryophytes differ in two main points from the seed banks. First, in addition to spores they may contain a large diversity of asexual diaspores, such as gemmae, caducous leaves and buds, and fragments from any part of the bryophyte capable to regenerate. Second, they have a high number of large spores and asexual diaspores, since small spores tend to be long-range dispersed and seem to be more short-lived than large ones (Jonsson 1993; During 2001; Glime 2007; Löbel & Rydin 2010).

Since there is a considerable variation in life histories among bryophytes characterized by different reproductive trade-offs (During 1979), diaspore banks offer interesting models to investigate the influence of breeding systems (monoicous, dioicous and polyoicous), diaspore types (spores, gemmae, fragments), phylum (mosses, liverworts and hornworts) and habitat features on the dispersal and establishment processes.

Contrasting the scarce knowledge of the role and functioning of bryophyte diaspore banks in tropical rain forests, bryophytes are a large and important component in these forests, contributing to the high species richness and diversity (Whitmore, Peralta & Brown 1985; Frahm & Gradstein 1991), and functioning of these ecosystems (nutrient and water cycling, and habitat for micro fauna – Nadkarni 1984; Veneklaas 1990; Schofield 1985; Turetsky 2003). Tropical rain forests are extremely threatened, especially in the countries under fast economical development. For example, the Atlantic forest in Brazil has nowadays less than 7% of its initial cover (Tabarelli *et al.* 2005), and is restricted to small forest fragments and a few large nature reserves. Despite the intense deforestation, the Brazilian Atlantic forest retains a high number of plant species, with a total of 15,782 (7,155 endemic species), of which 1,230 are bryophytes (Stehmann *et al.* 2009).

Our general aim was to test if micro-habitat characteristics, such as substrate type, and species traits (breeding system and phylum) are important to explain the pattern of bryophyte diaspore banks in tropical rain forests. Specifically, we compared the emergence (species occurrence and shoot abundance) of mosses and liverworts, differing in breeding system (monoicous, dioicous and polyicous), from the main substrates (decaying wood, bark, soil) in two sites to cover the range of environmental variation of the Brazilian Atlantic Ombrophilous Dense Forest (montane and sea level forests, see Alves *et al.* 2010), over two seasons (dry and rainy). Our hypotheses were:

- (1) The diaspore bank should be dominated by monoicous species since they produce more frequently sporophytes than dioicous ones (During 1979; Longton 1992).
- (2) There should be more mosses than liverworts in the diaspore bank since the richness of mosses in the Brazilian Atlantic forests is generally higher than that of the liverworts (Costa 2009). However we expected more liverworts at sea level than in the montane site because

their richness tend to be higher in lowland tropical forests (Cornelissen & Gradstein 1990; Costa 1999; Visnadi 2005).

(3) The number of species in the diaspore bank should be lower in soil than in decaying wood and bark, reflecting the richness typically found in these substrates in tropical rain forests (Visnadi 2005; Santos & Costa 2008; Alvarenga & Lisboa 2009). In addition, we expected that the substrate pH should have different effects on the establishment of mosses and liverworts with different breeding systems (Löbel & Rydin 2009; 2010).

(4) We expected more species in the diaspore bank in samples collected in the dry season when more species have a peak in spore dispersal (Pôrto & Oliveira 2002; Maciel-Silva unpubl. data).

## **Material and Methods**

### *Study sites*

The field sites selected for this study were established within a larger project dealing with floristic composition, structure and functioning of the Serra do Mar State Park (Alves *et al.* 2010). This park is covered by a tropical rain forest, referred to as Atlantic Ombrophilous Dense Forest (Veloso, Rangel Filho & Lima 1991), which is characterized by temperatures of about 25°C and a high precipitation that is rather evenly distributed over the year. The study sites were situated in the “Núcleo Picinguaba” (23° 34' S - 45° 02' W and 23° 17' S - 45° 11' W) and in the “Núcleo Santa Virgínia” (23° 17' - 23° 24' S and 45° 03' - 45° 11' W) of the Serra do Mar State Park – São Paulo State, Brazil (Veloso *et al.* 1991). The “Núcleo Santa Virgínia” has approximately 5,000 ha of forest, from 850 to 1,100 m a.s.l., and the “Núcleo Picinguaba” has approximately 47,500 ha of forest, from sea level to 1,340 m a.s.l.

The study sites were chosen to represent the extremes of an altitudinal and floristic variation of the Atlantic Ombrophilous Dense Forest (see Alves *et al.* 2010). The low altitude

forest is locally known as “Restinga forest”, a close to sea and seasonally flooded forest, and in our study is referred to as “sea level” site (N. Picinguaba), whilst the high-altitude site (about 1,000 m a.s.l.) is called “montane forest” (N. Sta Virginia). The soil is rich sand/clay in the montane and nutrient-poor sandy at the sea level site, and the total biomass is higher in the montane than at sea level (Alves *et al.* 2010). The two sites are about 35 km apart.

We did monthly or bimonthly measurements of minimum and maximum temperatures, relative air humidity, PAR (photosynthetically active radiation from 400 to 700 nm) and red:far-red ratio (650 : 730 nm) in the understory of the two sites. We used two max-min thermometers at each site, and a digital thermohygrometer. We used a quantum and a spectro-radiometer (a quantum sensor *LI-190 SA* coupled to a Li-1000 data logger, Li-Cor for PAR measurements; and Skye quantum sensor for R: FR ratio measurements). We recorded maximum and minimum humidities during days of field work. Measurements of light were done from 11:00h to 14:00h during cloud-free days, in different places of the forests, with paired measures of tree-covered and open conditions (e.g. sunfleck). These measurements were done from August 2007 to May 2009. We also used precipitation data from two meteorological stations (INPE) near the study sites (distance c. 100 m from the sea level site and c. 10 km from the montane site; Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos – CPTEC/INPE; Projeto “Estudos da Previsibilidade de Eventos Meteorológicos Extremos na Serra do Mar”).

The temperature in the montane site is lower than at sea level, and varies from 4°C in the winter to 27°C in the summer (Table 1; Fig. 1a), annual mean precipitation is > 2,000 mm and in the driest months, June to August, total precipitation is about or lower than 60 mm (Setzer 1966; Fig. 1b), and the VPD (vapour pressure deficit) of the forest is 0.6 kPa (Rosado, Oliveira & Aïdar 2009). In the sea level site the temperatures varies from 12°C in the winter to



32°C in the summer (Table 1; Fig. 1a). Annual mean precipitation is > 2,200 mm and in the driest months about or lower than 80 mm (Setzer 1966; Fig. 1b), and the forest VPD is 0.2 kPa (Rosado *et al.* 2009). The air relative humidity is similar in these two sites (Table 1). Finally, the montane site is more shaded at understory level than the site at sea level, in general with less photosynthetically active radiation and lower red:far red ratio (Table 1).

#### *Chemical characteristics of substrates*

Samples of soil, decaying wood and bark from all patches, where diaspore bank samples were collected, were dried at 40°C for 72h. Bark and decaying wood were ground, and stored. We measured pH in all samples using 1 g suspended in 5 mL of deionised water over night at 27°C (Watson, Hawksworth & Rose 1988; Farmer, Bates & Bell 1990). Combined samples of each substrate type per site and season (n = 12) were chemically analyzed by the Laboratory of Soil Fertility (CENA - USP); available nutrients for soils, and total concentrations for bark and decaying wood (Silva 1999; van Raij *et al.* 2001). Macro (N, P, K, Ca, Mg and S) and micro nutrients (Fe, Cu, Zn and Mn) were measured.

In general, substrates from the sea level had lower values of all nutrients than substrates from the montane site (Table 2). Bark generally contained more nutrients than decaying wood. In bark and decaying wood higher differences between the sites were observed for N, K, S, Ca, Cu and Mn values during both seasons. Calcium and manganese were higher in bark than in decaying wood in the montane site, but at sea level these nutrients were higher in decaying wood than in bark. Potassium had low values recorded from bark at sea level compared to bark from montane site (Table 2).

pH values differed significantly among substrates (Wald = 56.54;  $P < 0.001$ ) and between sites (Wald = 9.82;  $P = 0.002$ ), and the interaction between substrate and site was significant (Wald = 23.44;  $P < 0.001$ ; for statistics details see the section Data analysis). The

pH values were high in bark, followed by decaying wood and soil (Table 2). In general, pH values of decaying wood and soil were similar between the two sites, but bark pH values at sea level were extremely low contributing to a difference of pH between the sea level and montane sites. These pH differences probably were associated with different levels of Ca, Mn and K recorded in bark and decaying wood from the two sites.

#### *Diaspore bank sampling*

One area (10,000 m<sup>2</sup>) was established in each site of the forest (sea level and montane) and sub-divided into 100 plots of 100 m<sup>2</sup> each. We collected samples of soil, decaying wood, and tree bark. Leaves are also important for bryophytes in tropical rain forests (Gradstein, Churchill & Salazar Allen 2001), but were excluded because of their short lifespan (Bentley 1987; Coley, Kusar & Machado 1993). We selected randomly 31 (montane) and 32 (sea level) plots and collected a sample of 150 cm<sup>2</sup> of soil, decaying wood and bark in each plot, using knife and shovel. The soil layer corresponded to the first 3 cm below the litter layer (which is usually about 5 cm thick). Bark samples were taken from the outer layer from the tree base to 150 cm height. Decaying wood was collected from the outer 2 cm on fallen wood with stages of decay from 3 to 5 (Pyle & Brown 1999). After each sample the shovel and knife were carefully cleaned. Samples were collected where there were no visible bryophyte plants (adults or juveniles gametophytes). Each sample was separately put in a black plastic bag and immediately brought to the laboratory. We collected both in the dry (August 2007) and rainy (January 2008) seasons.

#### *Growth conditions*

The samples were homogenized and put in 150 cm<sup>2</sup> containers on top of a fine vermiculite layer to keep the samples moist, with a substrate thickness of about 1 cm. The containers were covered with transparent plastic bags and kept humid using distilled water

during the experiment (Bisang 1995; Ranal 2003). Initially, we conducted experiments in growth chambers with temperatures from 20 to 25 °C, photoperiod of 12h and PAR of approximately 20  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (fluorescent lamps). These conditions were intended to simulate field conditions in the understory. After six months, when plants were established, we transferred the containers to a growth room with artificial and natural light, with temperatures from 18 to 27 °C, photoperiod of 12 to 14h (fluorescent lamps were responsible for 12h light, but in the summer natural light prolonged this to about 14h), and PAR varying from 40 to 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The containers were repositioned every seventh days to remove the effects of spatial variation in light intensity.

### *Species identification*

We monitored shoot emergences of morphospecies from each sample monthly, under a dissecting microscope. After one year, when many plants were sexually mature, we identified them to species or family level. Nomenclature follows Goffinet, William & Shaw (2008) for mosses and Crandall-Stotler, Stotler & Long (2008) for liverworts. The number and occurrence of species were assessed from the dry and rainy season separately, and we also counted, as far as possible, the number of shoots per species.

Bryophyte species were grouped by phylum (mosses – Bryophyta and liverworts – Marchantiophyta) and breeding system (monoicous – two sexes together in the same gametophore; dioicous – sexes in different gametophores; and polyoicous – the same species with two different breeding systems, following Allen & Magill (1987)). Data on breeding system were available in Gradstein *et al.* (2001) and references therein. We used information from the literature (Gradstein *et al.* 2001 and references therein) and observations in the field and in the laboratory to characterize the species in relation to their frequency of sporophytes

and gemmae or gemmae-like propagules in two classes: “present to common” or “lacking to rare” following Jonsson (1993).

#### *Occurrence in extant vegetation*

We collected 244 and 167 bryophytes samples in the sea level and montane sites, respectively, from bark and decaying wood in the same plots as used for diaspore bank sampling. These samples were preliminarily identified, but many could only be identified to the family level. In addition, we used information from Visnadi (2005, 2009) and preliminary data from a floristic survey (N.D. Santos pers. comm.). Since no complete and quantitative bryophyte survey exists from these sites, we assigned a rough occurrence ranking of the species in a scale from 1 (least frequent) to 10 (most frequent) for a comparison of their occurrence in the diaspore bank.

#### *Data analysis*

We used generalized linear models (Glim) to analyze the effect of micro-habitat characteristics on the occurrence of species in the diaspore banks. Since the response variable “number of species” are count data and did not fulfil the requirements for general linear models (homocedasticity of variances and normality of residuals, checked by histograms of residuals, scatterplots of residuals versus predicted values, beyond proper tests as Levene and Kolmogorov-Smirnov), we used Glim with Poisson distribution and log link function (Quinn & Keough 2002) to test the effects of site, substrate, season, and pH. The models were evaluated by analysis of deviance and codeviance (effect of pH), using the Wald-test.

To analyze the effect of micro-habitat characteristics on the abundance of species in the diaspore banks, we used general linear models (Glm). The variable “shoot number” was  $\log(x + 1)$  transformed prior to use Analyses of variance (factorial Anovas). The variables “site” and “substrate” were treated as fixed factors, and “species” as a random factor.

First, we analyzed all micro-habitat effects (site, substrate and season) on the mean number of species, and on each category (number of monoicous mosses, monoicous liverworts, dioicous mosses and dioicous liverworts). Since “season” did not affect the general results (also checked by graphics with error bars), we removed it from further analyses and graphics.

To test the effect of pH we used the same model as above, but adding the covariate pH. We used mean number of species and shoots in each category of phylum and breeding system. We also analyzed separately the effects of pH on the number of species and shoots using linear regressions. All analyses were conducted using the software Statistica version 8.0 (StatSoft, Inc.).

## **Results**

### *Total number of species*

We found in total 85 species (or rather taxa; not all could be identified to species level) of bryophytes (58 at the montane site and 53 at sea level) from the three different substrates. Of these, 45 were mosses and 13 liverworts in the montane site and 31 mosses and 22 liverworts at sea level. In both sites considerably more species were found in bark (Fig. 2a; 46 in the montane and 42 at sea level) and decaying wood (42 and 34) than in soil (18 and 19). The patterns were similar for the two seasons. Monoicous species were more numerous than dioicous (Fig. 2b). The two sites had similar number of monoicous (montane: 30, sea level: 30), dioicous (25 and 19) and polyoicous species (3 and 4).

The most abundant species were *Isopterygium subbrevisetum*, *Syrrhopodon incompletus*, *Octoblepharum albidum*, *Trichosteleum papillosum* (mosses), *Riccardia digitiloba* and *Telaranea nematodes* (liverworts) at sea level; and *I. subbrevisetum*,

*Syrrhopodon prolifer*, *Trichosteleum pusillum*, *Campylopus julicaulis*, *Syrrhopodon gaudichaudii* (mosses) and *Riccardia digitiloba* (liverwort) in the montane site (Table SM1). Of these species *Syrrhopodon* spp. and *Campylopus julicaulis* are dioicous whilst the others are monoicous.

#### *Number of species per patch*

The number of species per patch differed significantly among the three substrates at both sites, in general with higher number in decaying wood, followed by bark and lower values in soil samples (Wald = 195.176,  $P < 0.001$ ; Fig. 3). The number of species per patch did not differ between the sites (Wald = 1.306,  $P = 0.253$ ), but the interaction between substrate and site was significant (Wald = 9.117,  $P = 0.010$ ), mainly because of a higher species number in decaying wood in the montane site (Fig. 3). There was no effect of the collection season on the number of species (Wald = 2.252,  $P = 0.133$ ).

The substrate type had significant effects on mosses and liverworts with different breeding systems (Table 3). There were more species of mosses than liverworts per patch in both sites, with the highest number in decaying wood, followed by bark and soil (Figs. 4a – 4d). Proportionally, mosses had considerable numbers of species in the montane site (Figs. 4a and 4b), whilst liverworts had noticeable record at sea level (Figs. 4 c and 4d). In general, there were more monoicous followed by dioicous species in both sites, with a higher percentage of monoicous species among the mosses (Fig. 4a). The high number of monoicous and dioicous mosses in decaying wood in the montane site contributed to the significant interactions “site x substrate” (Figs. 4a and 4b, Table 3). The forest site had significant effect only on dioicous mosses (Table 3).

### *Number of shoots*

The number of shoots grew slowly during the first five months of incubation and final data were collected after seven months (Fig. 5a – 5d). During the five first months of incubation, shoot numbers varied around 10 and 50 per patch at the montane and sea level site, respectively. After seven months these numbers increased to around 150 shoots per patch in both sites, possibly because of the change in light conditions (see “Growth conditions” section). After seven months, it was not possible to count shoots due to shoot and species intermixing. In the sea level site, species with the highest number of shoots were the mosses *Isopterygium* spp., *Pterogonidium pulchellum*, *Octoblepharum albidum*, *Syrrhopodon* spp., *Trichosteleum papillosum*, Pilotrichaceae spp. (mainly *Callicostella pallida/rufescens*), and the liverworts Lejeuneaceae spp. (mainly *Cheilolejeunea* spp. and *Ceratolejeunea* spp.), *Riccardia digitiloba*, *Telaranea nematodes* and *Arachniopsis monodactyla*. Abundant in the montane site were the mosses *Isopterygium* spp., *Syrrhopodon* spp., *Trichosteleum pusillum*, Pilotrichaceae spp. (mainly *Thamniopsis incurva*), *Pyrrhobryum spiniforme*, and the liverworts *Riccardia digitiloba*, Lejeuneaceae spp. (mainly *Metalejeunea cuculata*), *Telaranea nematodes* and *Lophocolea* spp. All these species are monoicous, with exception of *Syrrhopodon* spp. (dioicous) and *Ceratolejeunea* spp. (polyoicous).

The number of shoots in each species per patch mimicked the pattern recorded for the number of species per patch, i.e. decaying wood had highest values, followed by bark and soil (Fig. 5a – 5d; Wald test = 8.53, P = 0.001). The substrate had significant effect only on monoicous mosses (Wald test = 5.12, P = 0.017), but no effect was recorded on dioicous mosses and liverworts. There were more shoots of mosses than liverworts at the final record (after seven months), mostly among dioicous mosses at sea level (Fig. 5c). No significant

effect of site or site-interactions was observed on shoots of mosses and liverworts. In general monoicous were followed by dioicous and polyoicous species (data not shown).

#### *Effect of substrate pH*

Main significant effects of pH were detected on species number of monoicous mosses (Wald test = 4.55, P = 0.030), dioicous mosses (Wald test = 7.18, P = 0.007), monoicous liverworts (Wald test = 24.87, P = <0.001) and dioicous liverworts (Wald test = 5.50, P = 0.019). However, the explanatory power of pH was low. For instance, we only detected significant, but extremely weak, negative effects of pH on the number of monoicous liverworts in decaying wood from the sea level and montane sites ( $y = 3.48 - 0.48x$ ,  $R^2 = 0.09$  and  $y = 2.81 - 0.47x$ ,  $R^2 = 0.09$ , respectively), and a weak positive effect on the number of monoicous mosses in decaying wood from montane site ( $y = -0.44 + 0.90x$ ,  $R^2 = 0.10$ ). The analyses of shoot number indicated no effect of pH, neither direct or by interactions.

#### *Relationship between diaspore bank and vegetation*

Both in the vegetation and in the diaspore bank mosses were more numerous than liverworts, and monoicous species were more numerous than dioicous species (Table 4,  $\chi^2$ -tests showed no differences in these relationships between vegetation and diaspore bank). Monoicous species with sporophytes, as well as dioicous species with gemmae were well represented in the two sites, both in the diaspore bank and the vegetation (Fig. 6). Gemmae-producing monoicous liverworts were more frequent than dioicous liverworts in the diaspore bank and in the vegetation. Dioicous liverworts and mosses with gemmae were mainly frequent in the diaspore bank.

In general, many species that were well represented in the diaspore bank were also found in the vegetation (Table SM1). A few species were well represented in the vegetation but not in the diaspore bank, and these were mainly dioicous species, including both mosses



(*Meteoridium remotifolium* at both sites) and liverworts (*Ceratolejeunea cornuta*, *Plagiochila disticha*, *Bazzania phyllobola* at sea level). The monoicous liverwort *Riccardia digitiloba*, which is commonly found with gemmae and sporophytes, was abundant in the diaspore bank, but not in the vegetation. *Sphagnum* cf. *recurvum*, from the montane site, was the only species in the diaspore bank that was not observed in the vegetation.

## **Discussion**

It is clear from our results that there is a bryophyte diaspore deposit in substrates such as decaying wood, bark and soil in tropical rain forests. However, these diaspore banks found on the surface represent the extant vegetation rather well, somewhat contrasting with buried bryophyte diaspore banks that have low proportion of species commonly found in the vegetation (During & ter Horst 1983; During *et al.* 1987; Bisang 1995; Ross-Davis & Frego 2004; Clarke & Dorji 2008).

We corroborated at least partially, the hypotheses 1 to 3. Mosses were more numerous than liverworts, and monoicous species more numerous than dioicous ones. Dioicous species were proportionally more common at the montane site, and liverworts at sea level. The substrates bark and decaying wood supported the highest numbers of species. However, the effects of substrate pH were very weak on the different groups, and seasonal differences were not important.

### *Effects of habitat and season*

The pattern of diaspore bank among different substrates corresponds with surveys of bryophytes in tropical rain forests, with high richness of epiphytic, saxicolous, epixylic, epiphyllous but few terricolous species (Visnadi 2005; Santos & Costa 2008; Alvarenga & Lisboa 2009). The scarcity of bryophytes on the forest floor is due to a thick layer of leaf litter

covering the soil (Frahm & Gradstein 1991). This is different from bryophytes in boreal forests, which abundantly cover soil, logs, stumps and rocks on the forest floor, whilst epiphytes are largely confined to deciduous trees with higher pH than the conifers. A reason for this is probably an interaction between low pH and low water potentials (Wiklund & Rydin 2004; Löbel & Rydin 2009, 2010) inhibiting the establishment of many bryophytes. In tropical rain forests the high humidity and constant rains can maintain bark moisture, facilitating plant establishment and growth even at low pH.

There were only small differences between montane and sea level sites in the diaspore bank, such as a higher number of species in decaying wood at the montane site. Since the bryophyte species richness generally increases from lowland to montane tropical rain forests (Frahm & Gradstein 1991) and more species are present in bark and decaying wood in these forests, a “site  $\times$  substrate” interaction effect was expected. It was also expected from the higher values of nutrients from bark and decaying wood in the montane compared to the sea level site. However, it appears that the microclimatic differences between the sites are not large enough to affect the diaspore bank. Even though there is no marked dry season there is a peak in spore dispersal mainly from April to October (Maciel-Silva unpubl. data). However, this did not cause a seasonal pattern in the diaspore bank.

#### *The role of breeding system and phylum*

Species traits, such as breeding system and phylum are important to determine the diaspore bank patterns of bryophytes in tropical rain forests, with a high contribution of mosses and monoicous species. These findings differ from the dominance of dioicous species in the vegetation from bryophyte surveys in the same forest (Visnadi 2005). Data reveal a proportion of approximately 64% and 36% of dioicous vs. monoicous species, respectively

(from 353 species, Visnadi 2005) in the vegetation against 45% and 55% of dioicous vs. monoicous ones at diaspore bank (from the present study).

Monoicous species seem favoured by their frequent sporophyte production (Longton 1992). In addition, capsules contain high number of spores (from thousands to millions, depending on species – Frahm 2008). Although the relationship between frequent sporulation and monoicism in mosses was recently questioned in an analysis accounting for phylogeny by Crawford, Jesson & Garnock-Jones (2009), the correlations remained significant when the species were analysed as independent data points. Interestingly, we recorded a higher representation of monoicous liverworts with gemmae, compared to dioicous species with gemmae, in the diaspore bank. It is surprising that many monoicous liverworts invest largely in asexual reproduction even when they are able to produce many sporophytes and spores. Possibly this is a strategy to compensate for the high sensitivity of their spores to severe conditions during germination and establishment. For example, Löbel & Rydin (2010) found spores of liverworts to be more sensitive to high and low pH than spores and gemmae of mosses. Moreover, the higher contribution in the bank than in the vegetation of dioicous species with gemmae suggests an important role of asexual diaspores for the formation of bryophyte banks in these tropical forest types.

Frequency of both monoicous and dioicous mosses commonly producing sporophytes and gemmae, respectively, was high in the diaspore bank, explaining in part the predominance of mosses over liverworts in the bank. That is, mosses with success in the diaspore banks seem to invest strongly in spores or specialized asexual structures. In addition, differences such as high germination and protonemal growth rate, as well as tolerance to desiccation in mosses, and fast transition from protonema to shoot stage in liverworts (Maciel-Silva, Simabukuro & Pôrto 2006; Löbel & Rydin 2010) may determine the patterns observed here. Beyond this,

many shoots can develop clonally from the large filamentous protonema of mosses, whilst the globose protonema of liverworts develops into a single shoot (Nehira 1983).

#### *Weak influence of pH*

pH values recorded for the main substrates are related to degree of decay and acidic organic matter present, decreasing from bark to decaying wood and soil. The extremely low pH values recorded in the sea level site (pH 3.7 – 3.8) are exceptional for pH of bark in tropical rain forests (pH 4 - 5, ter Steege & Cornelissen 1989) and have a direct relationship with nutrients in the substrates, especially the low amounts of calcium, manganese and potassium. However, despite the marked differences of pH between bark from sea level and montane sites, neither species number nor shoot production was strongly affected by pH. Only two weak effects were noted: monoicous liverworts and mosses responded negatively and positively, respectively, to increased pH. This general lack of pH effects is surprising, since in boreal forests bryophyte establishment and growth are strongly hampered by low bark pH (about 4.7 to 7.6, Löbel & Rydin 2010). The high humidity in tropical rain forests possibly counteracts the negative effect of pH.

#### *The dynamics of the bryophyte diaspore banks in tropical rain forests*

Diaspore banks are not exclusive for bryophytes and other plants, but are also found in algae, fungi, bacteria and micro-crustaceans (Hoffmann & Santelices 1991; Kjølner & Bruns 2003; Decaestecker, Lefever & Meester 2004). Many of them are formed not only by “spores” but by intermediate forms between the main diaspore and the adult organism, analogous to seedling and sapling banks in angiosperms (Aguiar & Tabarelli 2010). These forms remain in a dormant stage waiting for an environmental signal, such as increasing light, water, nutrients, or changes in temperature (Hoffmann & Santelices 1991). Based on our results we propose a

conceptual model for the discussion of the dynamics of the bryophyte diaspore banks in tropical rain forests (Fig. 7).

Diaspores (spores and asexual diaspores) reach the substrates after short or long-range dispersal by wind, rain or animals (Frahm 2008; Rudolphi 2009). Microscopic forms (post-germination stages such as protonema and gametophytic buds or small shoots) may develop and remain inactive due to lack of suitable conditions (see references in Schofield 1981; Thomas, Proctor & Maltby 1994; Proctor *et al.* 2007). Spores and asexual diaspores contribute differently to the banks, with a high contribution of spores and asexual diaspores from near sources, followed by spores, and with least contribution by asexual diaspores from distant sources (Miles & Longton 1990; Pohjamo *et al.* 2006). In the case of long-range dispersal of asexual diaspores, animal vectors can be important (Parssons *et al.* 2007; Rudolphi 2009). Mosses and liverworts distinctly contribute to the banks, with more monoicous mosses and fewer dioicous liverworts. Decaying wood and bark contain many species and high numbers of diaspores, whilst soil supports few diaspores that are also rapidly buried under a thick leaf litter.

Diaspores can be activated under suitable conditions, with higher contribution of mosses than liverworts due to the clonal growth of mosses during the protonemal phase. So, mosses and liverworts have different roles after germination of diaspores, since from one spore of moss many shoots can be developed in the protonema. Losses are caused by decay, predation, parasitism and burial. In the latter case, persistent diaspores can emerge after soil disturbance (e.g. treefall).

We do not know the real longevity of the diaspores present in this bank, but morphological traits such as green color of spores (high metabolism associated to low longevity – Lloyd & Klekowski 1970) in many species from tropical forests, and low survival

of fragments over long time (due to fast chlorosis) suggest that these diaspore banks are transient rather than persistent. Longevity of bryophyte diaspores is poorly known. For example, essays in laboratory record a few months of longevity under dry conditions. The viability of small spores and asexual specialized diaspores decreases from around 80 to 100% to lower than 30 and 70%, respectively, after 50 days (Löbel & Rydin 2010). Spores of epiphytic and epixylic species have their viability reduced from around 90% to near zero after 48 – 240 days and 25 - 30 days, respectively (Egunyomi 1978; Wiklund & Rydin 2004; Maciel-Silva, Pôrto & Simabukuro 2009). In contrast, spores of *Sphagnum* could retain high viability (around 70%) after three years of burial in peat (Sundberg & Rydin 2000).

We propose that short-term diaspore banks of bryophytes in tropical rain forests can contribute to fast (re)establishment of many species after disturbances and during the species succession. Pioneer species investing highly in asexual reproduction (e.g. dioicous mosses) during the first months, or sexual reproduction (e.g. monoicous mosses) in the first year after establishment are especially important. These surface banks should have an important role after small-scale disturbances (e.g. animal activities, treefall gaps), but since they are probably transient rather than persistent, they can be strongly affected by environmental changes such as desiccation and high irradiance.

Many questions remain to be answered regarding the functioning and sensitivity of diaspore banks in tropical rain forests. We know very little about types and proportions of diaspores and microscopic forms, their longevity in the field, influence of intrinsic (size, storage of diaspores) and extrinsic factors (desiccation, irradiance, parasitism, and predation). The next step is also a quantitative exploration of the relationships between species composition of the diaspore bank and the vegetation.

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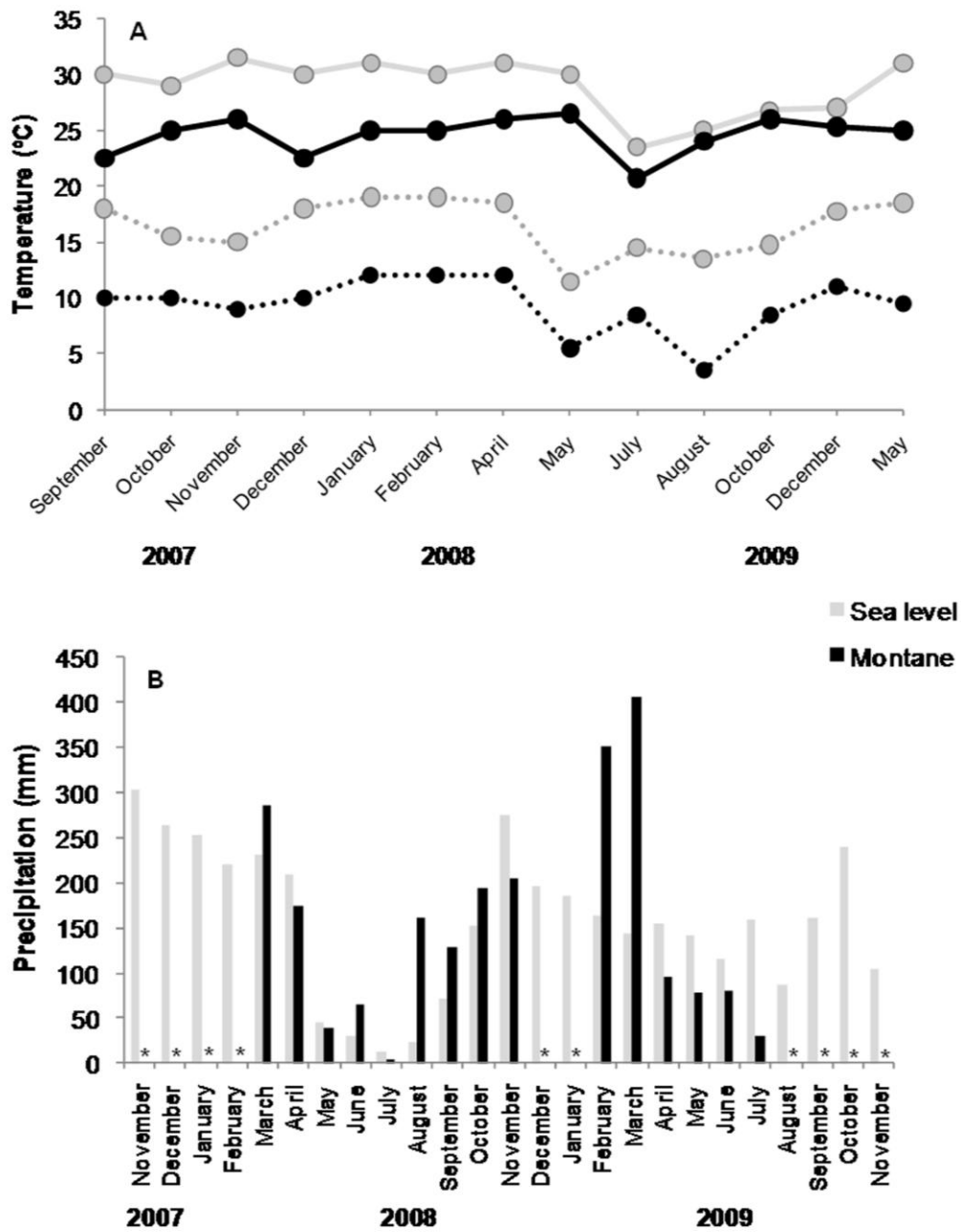
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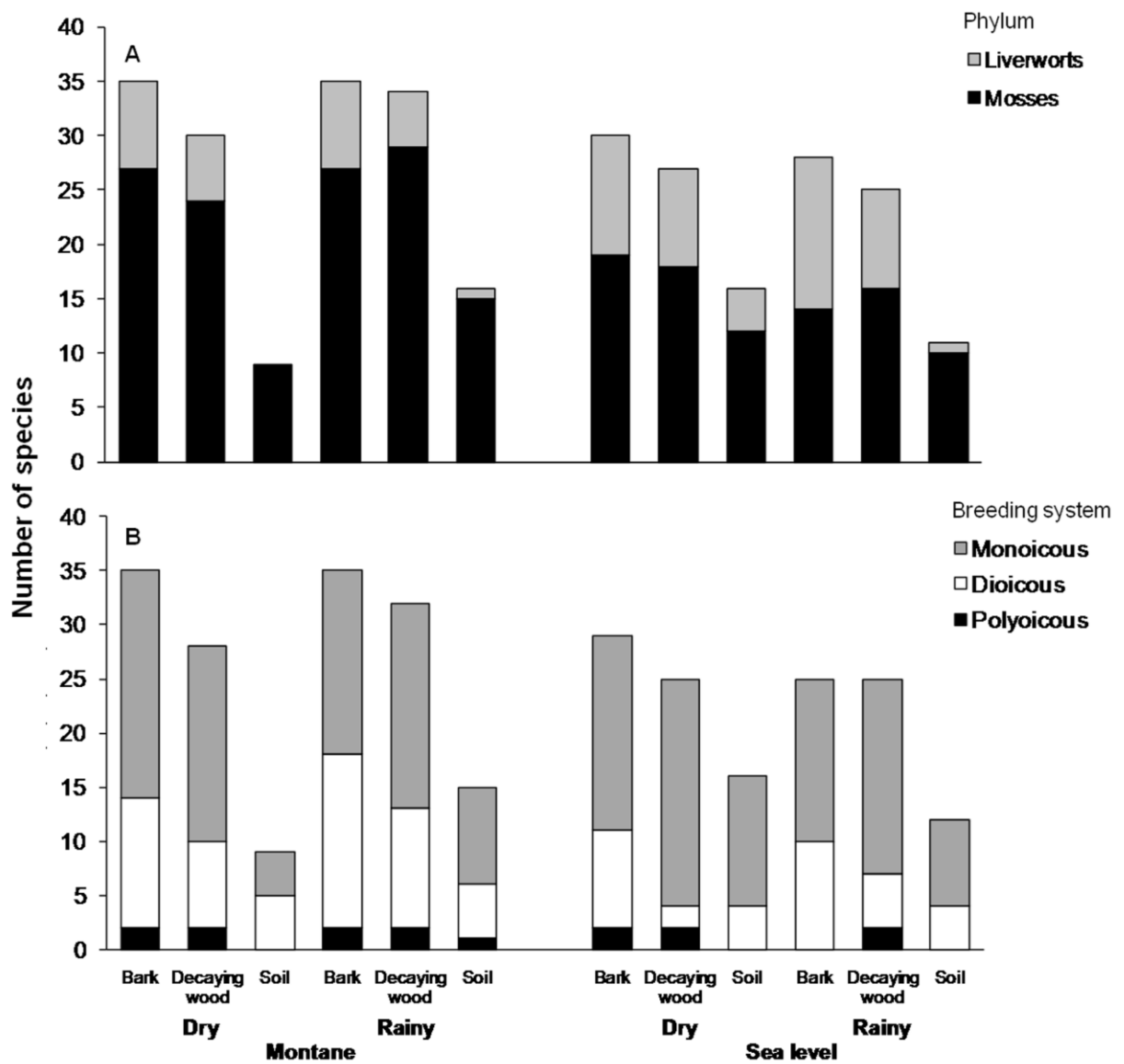
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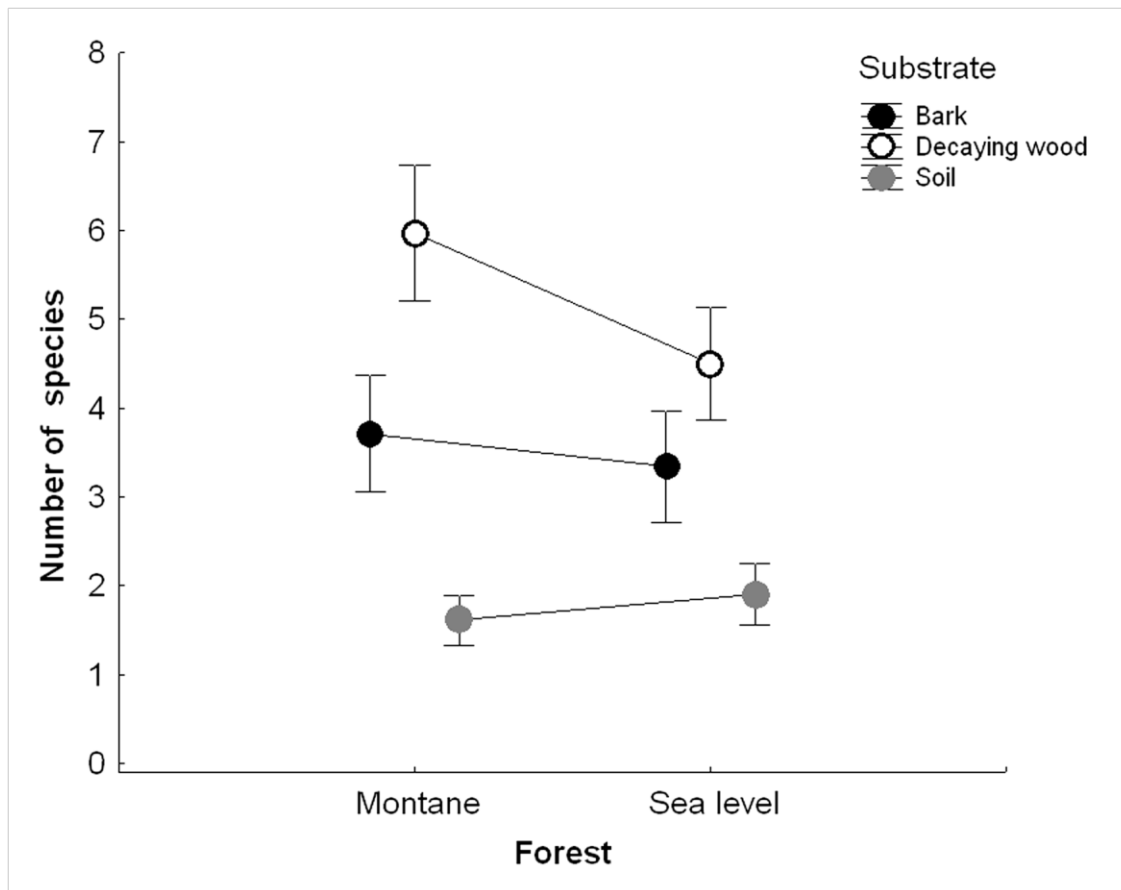


**Fig. 1** (A) Maximum (continuous lines) and minimum (dotted lines) temperatures (°C) from measurements in the sea level (grey) and montane sites (black). (B) Precipitation (mm) from meteorological stations near the sea level and montane sites; \* indicates that data were not available for all months at the montane site. (Source: “Plataforma de coleta de dados, Centro de Previsão de Tempo e Estudos Climáticos – CPTEC/INPE” for precipitation data)

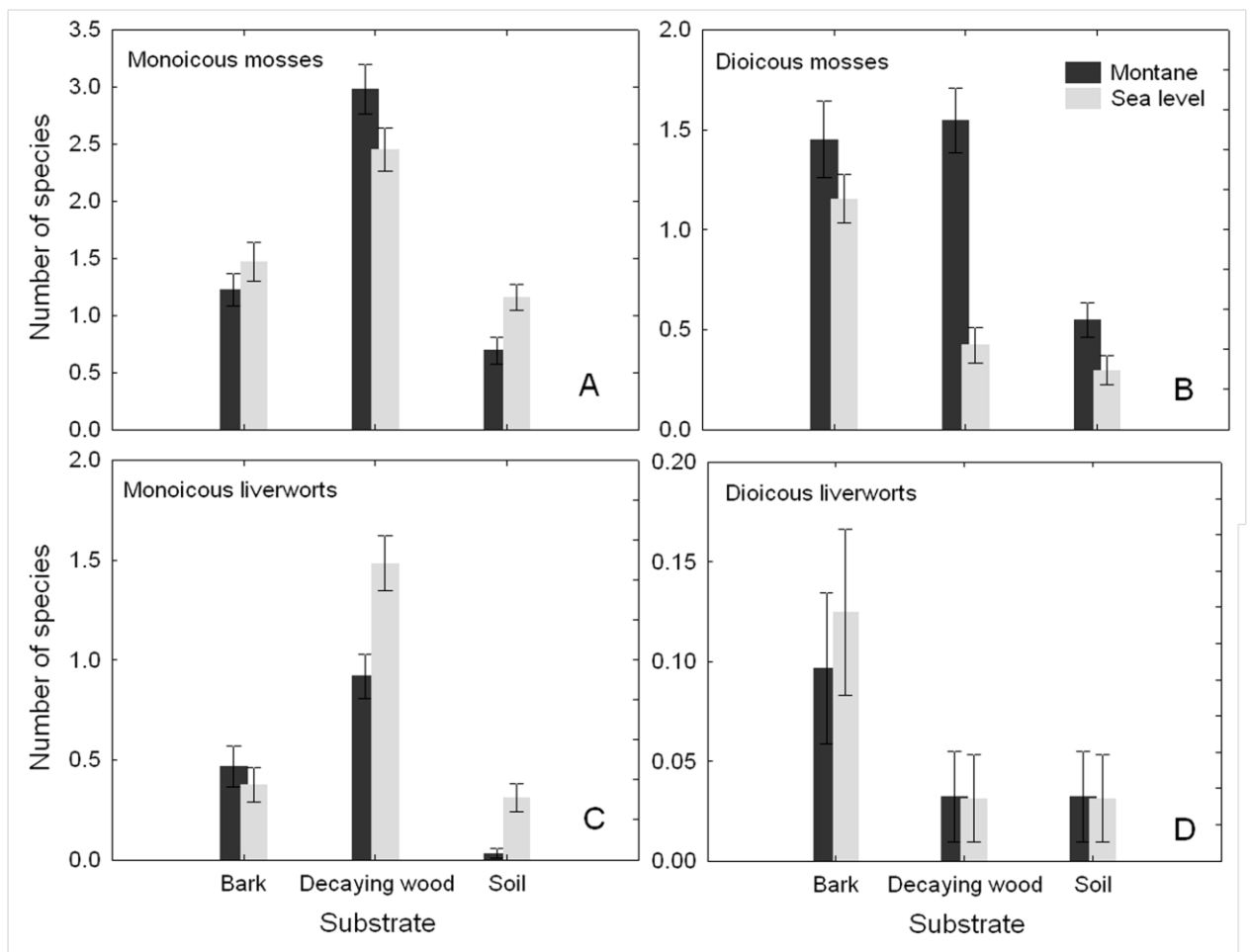




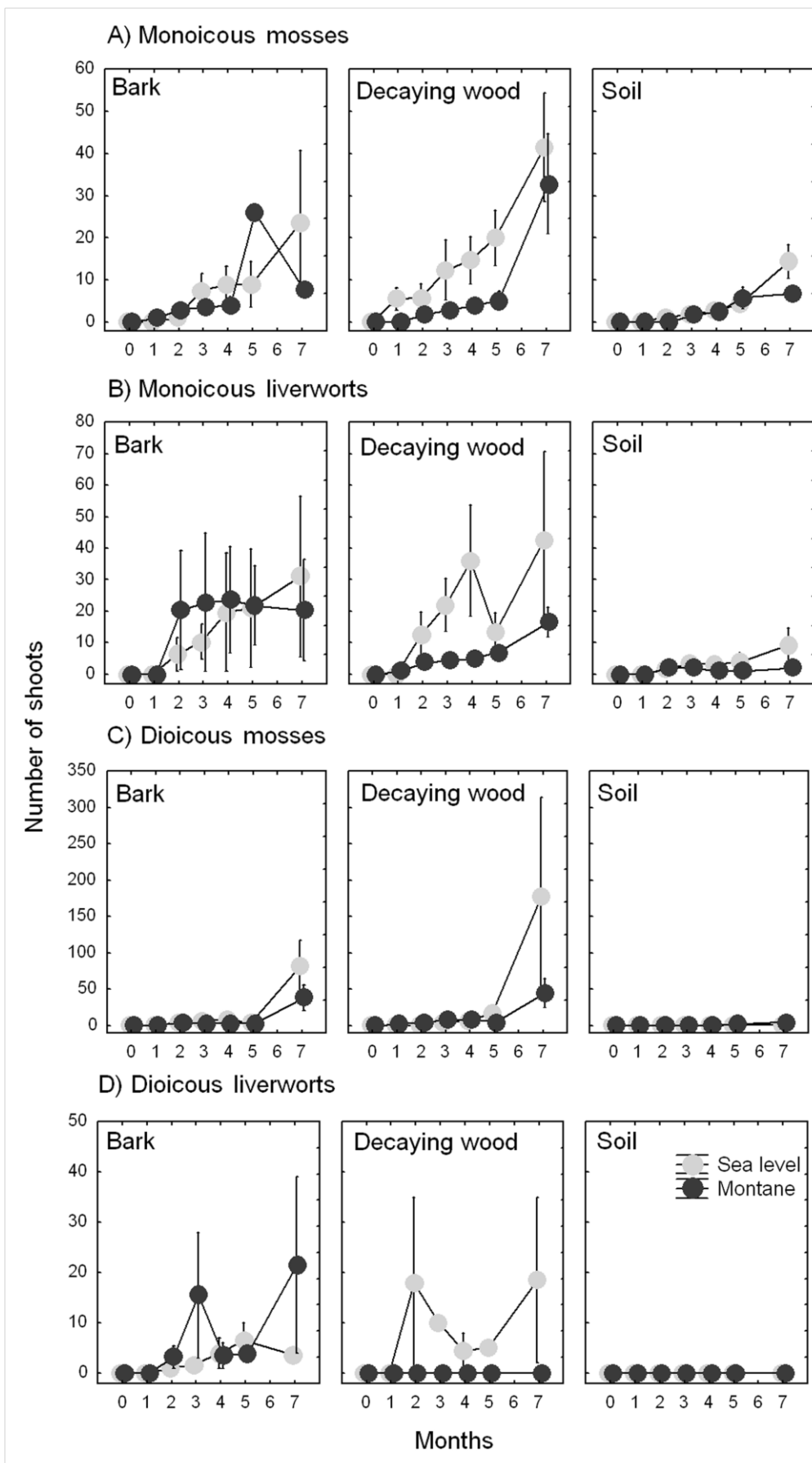
**Fig. 2.** Total number of moss and liverwort species (A), and monoicous, dioicous and polyoicous species (B) emerged from bark, decaying wood and soil collected in two different sites of a Brazilian Atlantic Forest, in the dry and rainy seasons.



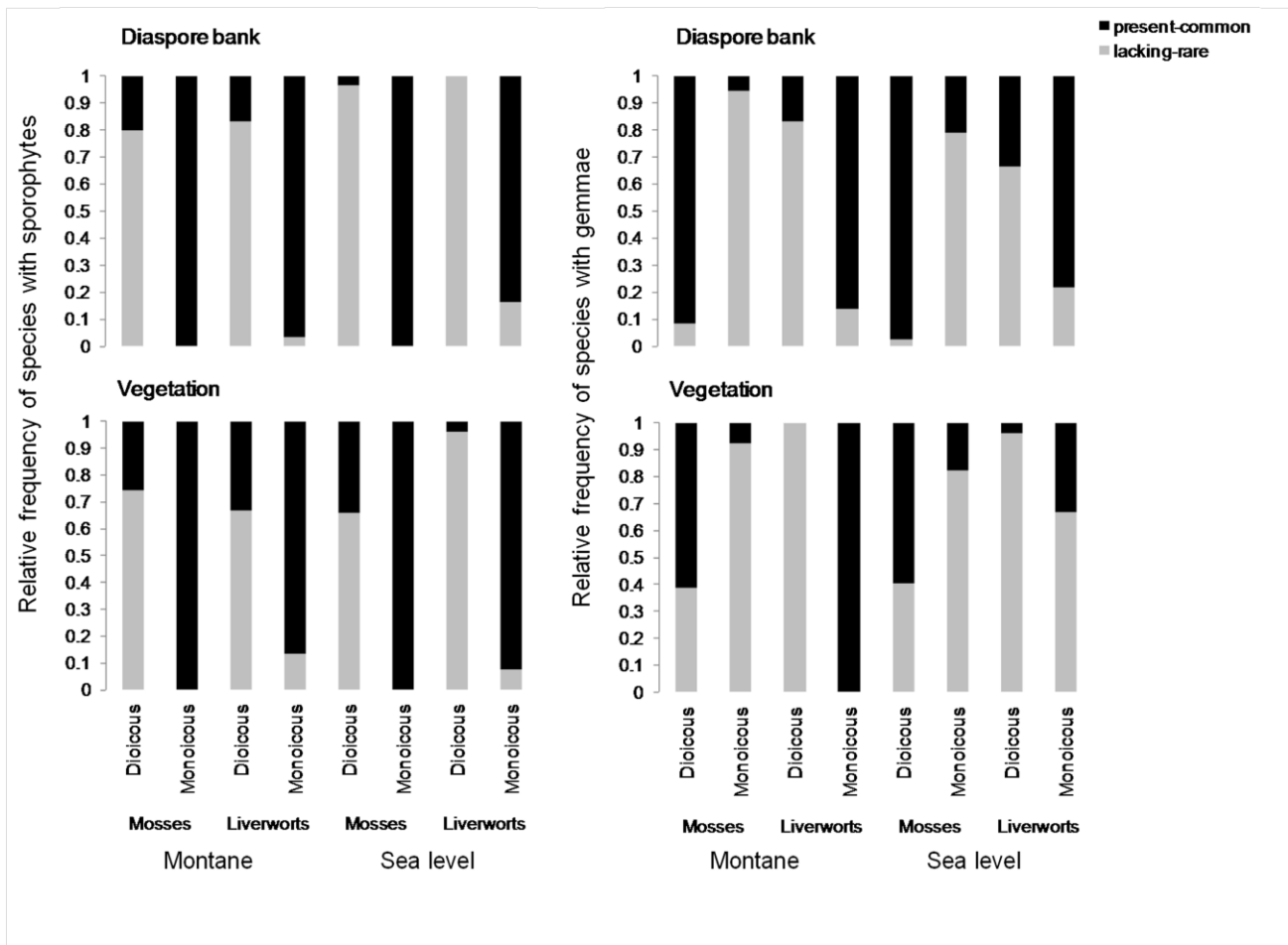
**Fig. 3.** Interaction diagram of the number of bryophyte species emerged per sample (150 cm<sup>2</sup>) from bark, decaying wood and soil collected in two different sites of a Brazilian Atlantic Forest. Data are mean number of species and error bars show  $\pm 1$  SE.



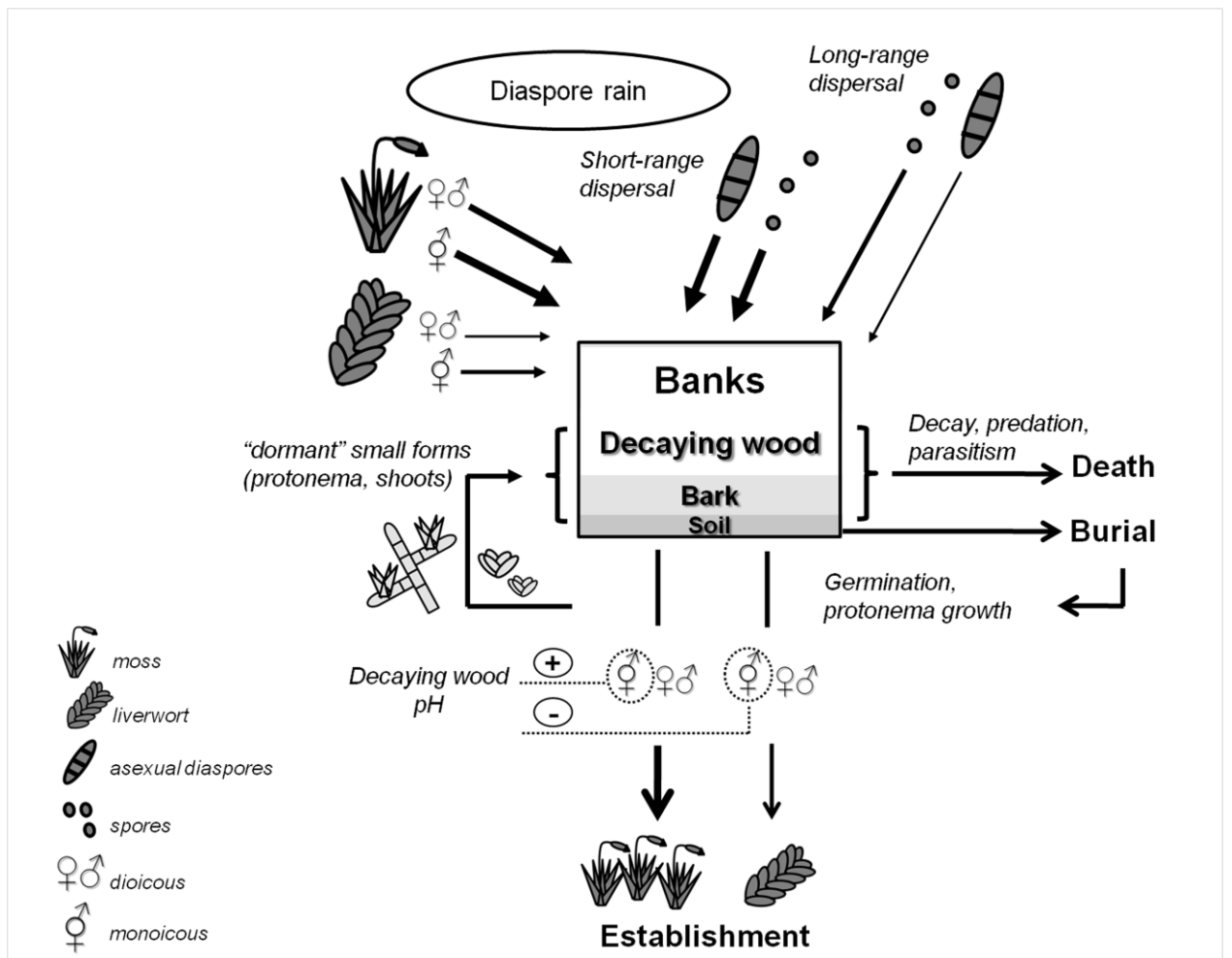
**Fig. 4.** Number of monoicous moss and liverwort species (A and C), and dioicous moss and liverwort species (B and D) emerged per sample (150 cm<sup>2</sup>) from bark, decaying wood and soil collected in two different sites of a Brazilian Atlantic Forest. Data are mean number of species and error bars show  $\pm 1$ SE.



**Fig. 5.** Number of shoots of liverwort and moss with different breeding systems (A and C – monoicous; B and D - dioicous) emerged per sample (150 cm<sup>2</sup>) from bark, decaying wood and soil collected in two different sites of a Brazilian Atlantic Forest. Data are mean number of shoots in each species per sample and error bars show  $\pm 1$  SE.



**Fig. 6.** Relative frequency of monoicous and dioicous mosses and liverworts species with present-common or lacking-rare sporophytes and gemmae from diaspore banks and vegetation in two different sites of a Brazilian Atlantic Forest.



**Fig. 7.** Proposed conceptual model of the dynamics of bryophyte diaspore banks in tropical rain forests. Arrow thickness indicates diaspore number in the diaspore rain, and number of established shoots in mosses and liverworts. Dashed lines denotes weak pH effect.

**Table 1.** Microclimatic parameters measured in the montane and sea level sites, from August of 2007 to May of 2009. Values are medians with upper and lower quartiles for light measurement, and mean  $\pm$  standard deviation for humidity and temperature.

<b>Parameters</b>	<b>Montane</b>	<b>Sea level</b>
PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) – sunfleck	140 (39 – 448, n = 12)	712 (198 – 1035, n = 8)
Red:Far red ratio – sunfleck	1.0 (0.69 – 1.11, n = 12)	1.2 (0.80 – 1.40, n = 6)
PAR ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) – tree covered	2.3 (1.6 – 5, n = 11)	11 (1.7 – 37, n = 10)
Red:Far red ratio – tree covered	0.15 (0.09 – 0.19, n = 11)	0.19 (0.11 – 0.28, n = 10)
Relative air humidity (%)	80.80 $\pm$ 19.09 (n = 9)	84.01 $\pm$ 8.17 (n = 9)
Maximum temperature ( $^{\circ}\text{C}$ )	24.6 $\pm$ 1.4 (n = 10)	29.2 $\pm$ 2.2 (n = 10)
Minimum temperature ( $^{\circ}\text{C}$ )	9.5 $\pm$ 2.5 (n = 10)	16.7 $\pm$ 2.0 (n = 10)



**Table 2.** Total macro and micronutrients (decaying wood and bark), available macro and micronutrients (soil) and pH of substrates from montane and sea level sites, collected in dry and rainy seasons. Mean  $\pm$  SD; n = 31 and 32 for montane and sea level, respectively.

<b>Sample</b>	<b>pH</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>S</b>	<b>Ca</b>	<b>Mg</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Zn</b>
	Mean $\pm$ SD	----- (g kg <sup>-1</sup> ) -----					----- (mg kg <sup>-1</sup> ) -----				
<i>Montane – dry</i>											
Decaying wood	3.82 $\pm$ 0.51	9.6	0.20	0.57	1.11	3.31	0.83	5.67	433.5	193.9	17.4
Bark	4.43 $\pm$ 0.16	13.0	0.27	2.05	2.66	4.54	0.96	11.13	358.1	237.3	21.9
<i>Sea level – dry</i>											
Decaying wood	3.98 $\pm$ 0.54	6.4	0.16	0.54	0.66	4.40	1.05	3.18	381.6	347.9	16.9
Bark	3.83 $\pm$ 0.61	10.1	0.26	0.59	1.28	2.44	0.97	8.95	300.6	57.3	11.1
<i>Montane – rainy</i>											
Decaying wood	4.08 $\pm$ 0.59	9.9	0.20	0.57	1.05	3.36	1.05	5.32	320.7	222.6	20.1
Bark	4.61 $\pm$ 1.21	11.4	0.28	2.13	2.39	4.94	1.28	14.28	93.9	227.9	21.8
<i>Sea level – rainy</i>											
Decaying wood	3.95 $\pm$ 0.79	7.4	0.16	0.51	0.78	4.24	1.00	3.95	249.0	204.0	16.4
Bark	3.74 $\pm$ 0.49	9.1	0.19	0.71	1.42	2.67	1.09	7.44	275.0	54.4	9.9
	<b>pH</b>	<b>N</b>	<b>P</b>	<b>K</b>	<b>S-SO<sub>4</sub></b>	<b>Ca</b>	<b>Mg</b>	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>Zn</b>
<b>Soil</b>	Mean $\pm$ SD	g kg <sup>-1</sup>	mg kg <sup>-1</sup>	mmolc kg <sup>-1</sup>	mg kg <sup>-1</sup>	mmolc kg <sup>-1</sup>	mmolc kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>
<i>Montane – dry</i>	3.46 $\pm$ 0.17	5.65	40.30	3.18	22.74	16.73	12.86	0.56	354.68	11.98	2.92
<i>Sea level – dry</i>	3.56 $\pm$ 0.28	2.38	25.64	1.56	19.85	7.47	8.54	0.15	192.08	5.70	1.93
<i>Montane – rainy</i>	3.53 $\pm$ 0.24	6.87	60.51	4.64	38.31	28.36	19.07	0.57	319.47	19.06	3.80
<i>Sea level – rainy</i>	3.43 $\pm$ 0.18	3.35	25.28	1.71	19.41	7.31	7.27	0.14	268.55	2.92	2.23

**Table 3.** Glim analyses of the effect of site, season and substrate on the number of mosses and liverworts with two breeding system per patch, using Poisson distribution with a log link function. P values <0.05 are in bold. Values of scaled deviance / d.f. up to 1.5 indicate a good model for the analysis (Quinn & Keough 2002).

Source	<i>Monoicous mosses</i>			<i>Dioicous mosses</i>			<i>Monoicous liverworts</i>			<i>Dioicous liverworts</i>		
	Wald test	P	d.f.	Wald test	P	d.f.	Wald test	P	d.f.	Wald test	P	d.f.
Site (S)	2.206	0.137	1	24.284	<b>&lt;0.001</b>	1	0.647	0.421	1	0.274	0.600	1
Season (Ss)	0.054	0.816	1	3.785	0.051	1	0.016	0.898	1	0.401	0.526	1
Substrate (Sb)	100.51	<b>&lt;0.001</b>	2	47.592	<b>&lt;0.001</b>	2	30.453	<b>&lt;0.001</b>	2	16.161	<b>&lt;0.001</b>	2
S x Ss	0.785	0.375	1	0.064	0.801	1	0.019	0.888	1	1.495	0.221	1
S x Sb	8.711	<b>0.013</b>	2	14.450	<b>0.001</b>	2	4.157	0.125	2	1.046	0.592	2
Ss x Sb	1.352	0.508	2	3.146	0.121	2	0.860	0.650	2	1.046	0.592	2
S x Ss x Sb	0.193	0.908	2	2.334	0.321	2	0.259	0.878	2	3.897	0.142	2
<b>Goodness of the model</b>	Statistics			Statistics			Statistics			Statistics		
Scaled deviance	378.00			366			378			366		
df	366			366			366			366		
Scaled deviance / df	1.03			1			1.03			1		

**Table 4.** Number of moss and liverwort species, and monoicous and dioicous species found in the diaspore bank and vegetation in two different sites of a Brazilian Atlantic Forest.

Forest	Source	Phylum		Breeding system	
		<i>Mosses</i>	<i>Liverworts</i>	<i>Monoicous</i>	<i>Dioicous</i>
Montane	Vegetation	30	11	21	18
Montane	Diaspore bank	45	13	30	25
Sea level	Vegetation	27	15	23	17
Sea level	Diaspore bank	31	22	30	19

**Table SM1.** Occurrence rank of bryophyte taxa from the diaspore banks (in bark, decaying wood - DW and soil) and vegetation (V; rank from 1 to 10, where 1 means least frequent species) in two sites of a Brazilian Atlantic Forest. Data from both seasons are combined. Species are classified according to phylum (Ph; M = mosses, L = liverworts) and Breeding system (BS; M = monoicous species, D = dioicous species, P = polyoicous, and M/D = both monoicous and dioicous species present).

Species	Montane							Species	Sea level						
	BS	Ph	Bark	DW	Soil	Total	V*		BS	Ph	Bark	DW	Soil	Total	V*
Number of patches	-	-	62	62	62	<b>186</b>	-	Number of patches	-	-	64	64	64	<b>192</b>	-
<i>Acroporium exiguum</i>	M	M	1	1	0	<b>2</b>	-	<i>Acroporium exiguum</i>	M	M	2	1	0	<b>3</b>	3
<i>Actinodontium sprucei</i>	D	M	17	10	1	<b>28</b>	-	<i>Actinodontium sprucei</i>	D	M	1	1	0	<b>2</b>	-
<i>Aerobryopsis</i> cf. <i>capensis</i>	D	M	3	0	0	<b>3</b>	-	<i>Arachniopsis monodactyla</i>	M	L	1	8	0	<b>9</b>	3
<i>Bazzania heterostipa</i>	D	L	4	0	0	<b>4</b>	3	<i>Bazzania heterostipa</i>	D	L	2	0	0	<b>2</b>	5
<i>Bryopteris fillicina</i>	P	L	0	0	0	<b>0</b>	4	<i>Bazzania phyllobola</i>	D	L	1	0	0	<b>1</b>	9
<i>Bryum</i> cf. <i>limbatum</i>	D	M	3	1	2	<b>6</b>	2	<i>Bryopteris diffusa</i>	P	L	0	0	0	<b>0</b>	5
<i>Callicostella depressa</i>	M	M	6	16	0	<b>22</b>	3	<i>Callicostella depressa</i>	M	M	0	3	1	<b>4</b>	-
<i>Callicostella pallida</i>	M	M	1	3	0	<b>4</b>	-	<i>Callicostella pallida</i>	M	M	4	4	3	<b>11</b>	-
<i>Callicostella rufescens</i>	M	M	0	1	0	<b>1</b>	-	<i>Callicostella rufescens</i>	M	M	2	14	1	<b>17</b>	3
<i>Calypogeia peruviana</i>	M	L	0	1	0	<b>1</b>	3	Calymperaceae sp.1	D	M	1	0	0	<b>1</b>	-
<i>Campylopus dichrostis</i>	D	M	5	5	0	<b>10</b>	3	<i>Calypogeia peruviana</i>	M	L	1	4	1	<b>6</b>	-
<i>Campylopus filifolius</i>	D	M	6	11	0	<b>17</b>	3	<i>Campylopus julicaulis</i>	D	M	0	0	1	<b>1</b>	3
<i>Campylopus julicaulis</i>	D	M	4	20	24	<b>48</b>	5	<i>Ceratolejeunea cornuta</i>	P	L	1	2	0	<b>3</b>	10
<i>Cyclodictyon varians</i>	M	M	2	3	0	<b>5</b>	3	<i>Ceratolejeunea cubensis</i>	P	L	6	2	0	<b>8</b>	4
<i>Fissidens angustifolius</i>	M	M	3	3	4	<b>10</b>	2	<i>Cheilolejeunea adnata</i>	M	L	2	0	0	<b>2</b>	-
<i>Fissidens</i> cf. <i>submarginatum</i>	M	M	0	0	1	<b>1</b>	-	<i>Cheilolejeunea</i> cf. <i>acutangula</i>	M	L	1	0	0	<b>1</b>	2
<i>Haplolejeunea cucullata</i>	M	L	2	0	0	<b>2</b>	-	<i>Cheilolejeunea discoidea</i>	M	L	4	9	0	<b>13</b>	-
<i>Hymenodon aeruginosus</i>	D	M	1	0	2	<b>3</b>	-	<i>Cheilolejeunea</i> sp.1	M	L	1	0	0	<b>1</b>	-
<i>Hypopterygium tamarisci</i>	M	M	4	1	0	<b>5</b>	3	<i>Fissidens angustifolius</i>	M	M	0	0	2	<b>2</b>	-
<i>Isopterygium subbrevisetum</i>	M	M	28	50	19	<b>97</b>	5	<i>Groutiella</i> sp.1	D	M	0	1	0	<b>1</b>	-
<i>Isopterygium tenerum</i>	M	M	1	6	11	<b>18</b>	5	<i>Haplolejeunea cucullata</i>	M	L	0	1	0	<b>1</b>	-

**Table SM1.** Continued.

<i>Kurzia capillaris</i>	D	L	1	0	0	<b>1</b>	-	<i>Helicodontium capillare</i>	M	M	1	0	0	<b>1</b>	-
Lejeuneaceae sp.1	M/D	L	1	1	0	<b>2</b>	-	<i>Isopterygium subbrevisetum</i>	M	M	34	49	39	<b>122</b>	5
<i>Lepidopilum</i> cf. <i>scabrisetum</i>	P	M	4	1	0	<b>5</b>	-	<i>Isopterygium tenerum</i>	M	M	2	3	6	<b>11</b>	5
<i>Lepidopilum ovalifolium</i>	D	M	6	3	0	<b>9</b>	-	<i>Lejeunea flava</i>	M	L	0	0	0	<b>0</b>	5
<i>Lepidopilum</i> sp.1	M/D	M	0	0	1	<b>1</b>	-	<i>Lejeunea laetevirens</i>	M	L	0	0	0	<b>0</b>	3
<i>Leucobryum</i> cf. <i>martianum</i>	D	M	0	1	0	<b>1</b>	-	<i>Lejeunea</i> sp.1	M/D	L	2	1	0	<b>3</b>	-
<i>Leucobryum crispum</i>	D	M	3	1	1	<b>5</b>	3	Lejeuneaceae sp.2	M/D	L	1	0	0	<b>1</b>	-
<i>Leucobryum</i> sp.1	D	M	2	1	0	<b>3</b>	-	<i>Leucobryum clavatum</i>	D	M	0	0	0	<b>0</b>	4
<i>Leucoloma serrulatum</i>	D	M	2	3	0	<b>5</b>	3	<i>Leucobryum crispum</i>	D	M	0	1	0	<b>1</b>	1
<i>Leucomium strumosum</i>	M	M	0	2	0	<b>2</b>	-	<i>Leucoloma serrulatum</i>	D	M	1	0	0	<b>1</b>	3
<i>Lophocolea martiana</i>	M	L	1	7	0	<b>8</b>	10	<i>Leucophanes molleri</i>	D	M	2	0	1	<b>3</b>	5
<i>Lophocolea perissodonta</i>	M	L	1	13	1	<b>15</b>	2	<i>Lophocolea martiana</i>	M	L	1	3	2	<b>6</b>	5
<i>Lophocolea</i> sp.1	M	L	0	2	0	<b>2</b>	-	<i>Metalejeunea cucullata</i>	M	L	3	3	0	<b>6</b>	-
<i>Lopidium concinnum</i>	P	M	0	0	0	<b>0</b>	4	<i>Meteoridium remotifolium</i>	D	M	0	0	0	<b>0</b>	8
<i>Macrodictyum proliferum</i>	D	M	1	0	0	<b>1</b>	-	<i>Mnioloma</i> cf. <i>parallelogramma</i>	D	L	1	0	0	<b>1</b>	-
<i>Metalejeunea cucullata</i>	M	L	10	0	0	<b>10</b>	-	<i>Neckeropsis</i> spp.	M	M	1	0	0	<b>1</b>	4
<i>Meteoridium remotifolium</i>	D	M	1	1	0	<b>2</b>	10	<i>Neurolejeunea breutelii</i>	D	L	0	1	0	<b>1</b>	-
<i>Meteorium</i> cf. <i>nigrescens</i>	D	M	0	1	0	<b>1</b>	5	<i>Octoblepharum albidum</i>	M	M	29	16	12	<b>57</b>	6
<i>Metzgeria</i> spp.	M/D	L	0	0	0	<b>0</b>	4	<i>Odontoschisma falcifolium</i>	D	L	2	0	0	<b>2</b>	-
<i>Neckeropsis</i> spp.	M	M	0	0	0	<b>0</b>	4	<i>Phyllogonium viride</i>	D	M	0	0	0	<b>0</b>	3
Pallaviciniaceae spp.	D	L	0	0	0	<b>0</b>	1	<i>Plagiochila disticha</i>	D	L	2	0	0	<b>2</b>	7
<i>Phyllogonium viride</i>	D	M	5	0	0	<b>5</b>	3	<i>Plagiochila</i> sp.1	D	L	1	0	0	<b>1</b>	-
<i>Plagiochila gymnocalycina</i>	D	L	1	0	0	<b>1</b>	5	<i>Pterogonidium pulchellum</i>	M	M	4	7	5	<b>16</b>	4
<i>Porella</i> spp	D	L	0	0	0	<b>0</b>	3	<i>Pyrrhobryum spiniforme</i>	M	M	0	0	0	<b>0</b>	6
<i>Porotrichum</i> spp.	D	M	0	0	0	<b>0</b>	3	<i>Radula</i> spp.	D	L	0	0	0	<b>0</b>	1
<i>Pyrrhobryum spiniforme</i>	M	M	4	18	1	<b>23</b>	10	<i>Riccardia digitiloba</i>	M	L	2	45	2	<b>49</b>	1
<i>Radula</i> cf. <i>elliottii</i>	D	L	1	0	0	<b>1</b>	-	<i>Schlotheimia</i> cf. <i>jamesonii</i>	D	M	1	0	0	<b>1</b>	-
<i>Riccardia digitiloba</i>	M	L	9	33	0	<b>42</b>	3	Sematophyllaceae sp.1	M	M	0	1	0	<b>1</b>	-

**Table SM1.** Continued.

<i>Sematophyllaceae</i> sp.1	M	M	0	2	0	<b>2</b>	-	<i>Sematophyllum adnatum</i>	M	M	0	1	0	<b>1</b>	1
<i>Sematophyllum galipense</i>	M	M	2	0	0	<b>2</b>	4	<i>Sematophyllum galipense</i>	M	M	2	4	2	<b>8</b>	3
<i>Sematophyllum</i> sp.1	M	M	0	1	0	<b>1</b>	-	<i>Sematophyllum subpinnatum</i>	M	M	6	5	0	<b>11</b>	5
<i>Sematophyllum</i> sp.2	M	M	1	0	0	<b>1</b>	-	<i>Sematophyllum subsimplex</i>	M	M	0	12	0	<b>12</b>	5
<i>Sematophyllum subpinnatum</i>	M	M	2	2	0	<b>4</b>	5	<i>Syrrhopodon gaudichaudii</i>	D	M	17	3	2	<b>22</b>	4
<i>Sematophyllum subsimplex</i>	M	M	1	1	1	<b>3</b>	5	<i>Syrrhopodon incompletus</i>	D	M	38	16	11	<b>65</b>	5
<i>Sphagnum</i> cf. <i>recurvum</i>	D	M	0	0	1	<b>1</b>	-	<i>Syrrhopodon ligulatus</i>	D	M	1	1	0	<b>2</b>	1
<i>Syrrhopodon gaudichaudii</i>	D	M	22	18	2	<b>42</b>	8	<i>Syrrhopodon prolifer</i>	D	M	13	6	3	<b>22</b>	5
<i>Syrrhopodon incompletus</i>	D	M	1	0	0	<b>1</b>	2	<i>Telaranea diacantha</i>	M	L	0	0	0	<b>0</b>	5
<i>Syrrhopodon prolifer</i>	D	M	25	29	2	<b>56</b>	9	<i>Telaranea nematodes</i>	M	L	9	22	15	<b>46</b>	3
<i>Telaranea nematodes</i>	M	L	5	3	0	<b>8</b>	4	<i>Thamniopsis incurva</i>	M	M	1	0	0	<b>1</b>	4
<i>Thamniopsis incurva</i>	M	M	6	25	3	<b>34</b>	4	<i>Trichosteleum papillosum</i>	M	M	6	35	4	<b>45</b>	3
<i>Thamniopsis langsdorffii</i>	M	M	3	11	0	<b>14</b>	3	<i>Vesicularia vesicularis</i>	M	M	0	2	0	<b>2</b>	5
<i>Thuidium tamariscinum</i>	D	M	0	1	0	<b>1</b>	3	<i>Zelometeorium patulum</i>	D	M	0	0	0	<b>0</b>	5
<i>Trachyxiphium guadalupense</i>	M	M	6	16	0	<b>22</b>	2								
<i>Trichosteleum pusillum</i>	M	M	11	41	3	<b>55</b>	3								

\* - indicates insufficient information to rank the species.

## DISCUSSÃO FINAL

Diferentes organismos, tais como plantas, fungos e animais podem ser todos agrupados de acordo com o tipo de estratégia que possuem em relação a sua história de vida, captura e utilização dos recursos, resposta ao clima e biologia regenerativa (Grime, 2001). Especificamente em plantas, estratégias (ou ainda tipos funcionais) são o agrupamento de características genéticas similares ou análogas que ocorrem amplamente entre espécies ou populações, resultando em similaridades ecológicas (Grime, 2001). O tema tem sido amplamente explorado desde 1979, mas foi nos últimos anos que a discussão sob o foco de características, tipos e diversidade funcionais desde a escala de populações a ecossistemas (Reich *et al.*, 2003; Violle *et al.*, 2007; Powes & Tiffin, 2010) vem se expandindo. Do ponto de vista de ecossistemas tropicais ainda há muito a se pesquisar e entender, sobretudo devido à alta diversidade de espécies e conseqüentemente diferentes estratégias usadas por essas plantas (De Deyn *et al.*, 2008; Powes & Tiffin, 2010). Por exemplo, grupos de plantas como as briófitas *sensu lato*, importantes para a composição e o funcionamento de ecossistemas ao exemplo das florestas tropicais (Nadkarni, 1984; Schofield, 1985; Whitmore *et al.*, 1985; Frahm & Gradstein 1991; Veneklaas, 1990; Turetsky 2003), são extremamente desconhecidas do ponto de vista de sua história de vida e estratégias nesses ambientes.

A presente tese contribui para o conhecimento e a melhor compreensão dos padrões e processos reprodutivos de diferentes briófitas, entre musgos e hepáticas, presentes em dois habitats (nível do mar e montano) de Floresta Atlântica do PE Serra do Mar, São Paulo. Os resultados e conclusões obtidos provavelmente podem ser generalizados para espécies ocorrendo em outras localidades de floresta tropical, embora com adaptações ao clima e histórico de uso das florestas. A seguir, são respondidas as perguntas iniciais e abordadas as conclusões chave da tese. (1) A fenologia reprodutiva de briófitas sofre leves ajustes de acordo

com o habitat que ocupam, mas o desenvolvimento de estruturas reprodutivas segue os índices de pluviosidade de maneira similar nos dois habitats de floresta. O padrão de elevada fertilização durante os períodos mais chuvosos e dispersão de esporos nos períodos mais secos, já verificado entre briófitas em savanas (Egunyomi 1979) e Floresta Atlântica Nordestina (Oliveira & Pôrto 1998, 2001, 2002; Pôrto & Oliveira 2002), se repete para briófitas em Floresta Atlântica do sudeste do país. Isso significa que, mesmo quando presentes em um ambiente bastante úmido como a Floresta Ombrófila Densa, essas plantas são dependentes de períodos com extrema disponibilidade hídrica para que haja alta frequência de fertilização dos gametângios, desenvolvimento dos esporófitos nos meses subsequentes e pico de dispersão dos esporos coincidindo com a redução das chuvas. (2) Tanto o sistema reprodutivo quanto o habitat são importantes fatores que explicam o desempenho reprodutivo de briófitas. Nem todas as espécies dióicas falham em produzir estruturas sexuadas sob números similares aos das espécies monóicas, porém as fases de fertilização e produção dos esporófitos são reduzidas nas espécies dióicas. Esse fenômeno entre as espécies dióicas pode ser explicado pela segregação espacial dos sexos (separação espacial das colônias femininas e masculinas; Longton & Schuster, 1983; Bowker *et al.*, 2000; Oliveira & Pôrto, 2002) atrelada à forte dependência de água para a fertilização, uma vez que, em geral, os anterozóides não atingem distâncias de deslocamento superiores a 200 cm (Glime 2007). Em adição, a raridade de colônias masculinas, assim como as altas razões sexuais de gametângios ( $>1 \text{ ♀}:\text{♂}$ ) entre plantas da Floresta de Restinga comparadas àquelas da Floresta Montana, auxiliam a entender como as plantas de Restinga compensam o baixo desempenho de alguns estádios do ciclo reprodutivo investindo em mais estruturas reprodutivas nas fases iniciais do ciclo e aumentando as probabilidades de fertilização. (3) Características das espécies, sobretudo filo e sistema reprodutivo, são importantes na formação de bancos de diásporos de briófitas em



florestas tropicais a partir de distintos substratos (solo < casca de árvore < tronco morto em decomposição). Os dois habitats de floresta, embora com pequenas diferenças, tem reservas compostas principalmente por diásporos de musgos monóicos, que investem largamente em reprodução sexuada, e de musgos dióicos com eficientes métodos de propagação assexuada. Quando comparadas as proporções de espécies dióicas vs. monóicas presentes no banco de diásporos dos dois habitats de floresta estudados com levantamentos das espécies de briófitas em vegetação da mesma floresta (Visnadi, 2005), a contribuição de espécies dióicas é superior na vegetação, enquanto que espécies monóicas são mais bem representadas no banco de diásporos.

Briófitas presentes na Floresta Atlântica apresentam diferentes estratégias reprodutivas, moldadas pelo sistema reprodutivo das espécies e pelo habitat que ocupam. Mais especificamente, outros fatores como filo e formas de crescimento das espécies, e características microambientais também influenciam as estratégias de vida adotadas pelos grupos de briófitas. A presente tese é pioneira na investigação dos temas abordados aqui do ponto de vista das briófitas, sobretudo em florestas tropicais. Os dados referentes à fenologia reprodutiva, desempenho da reprodução sexuada e formação de bancos de diásporos de briófitas auxiliarão a compreender como e porque essas plantas têm papel destacado em florestas tropicais úmidas. Esforços futuros para entender (1) como os bancos de diásporos são formados (tipos, longevidade, germinabilidade e velocidade de estabelecimento de diferentes tipos de diásporos), (2) como gradientes de recursos (ex. água, luz e temperatura) influenciam a reprodução (sexuada e assexuada), (3) como os mecanismos de dispersão são estabelecidos (via plasticidade fisiológica ou seleção de habitat), e (4) como diferentes tipos funcionais são representados em uma escala mais ampla de ecossistemas (habitats xéricos vs. úmidos), serão importantes não só do ponto de vista da briologia, mas também ampliarão a compreensão dos

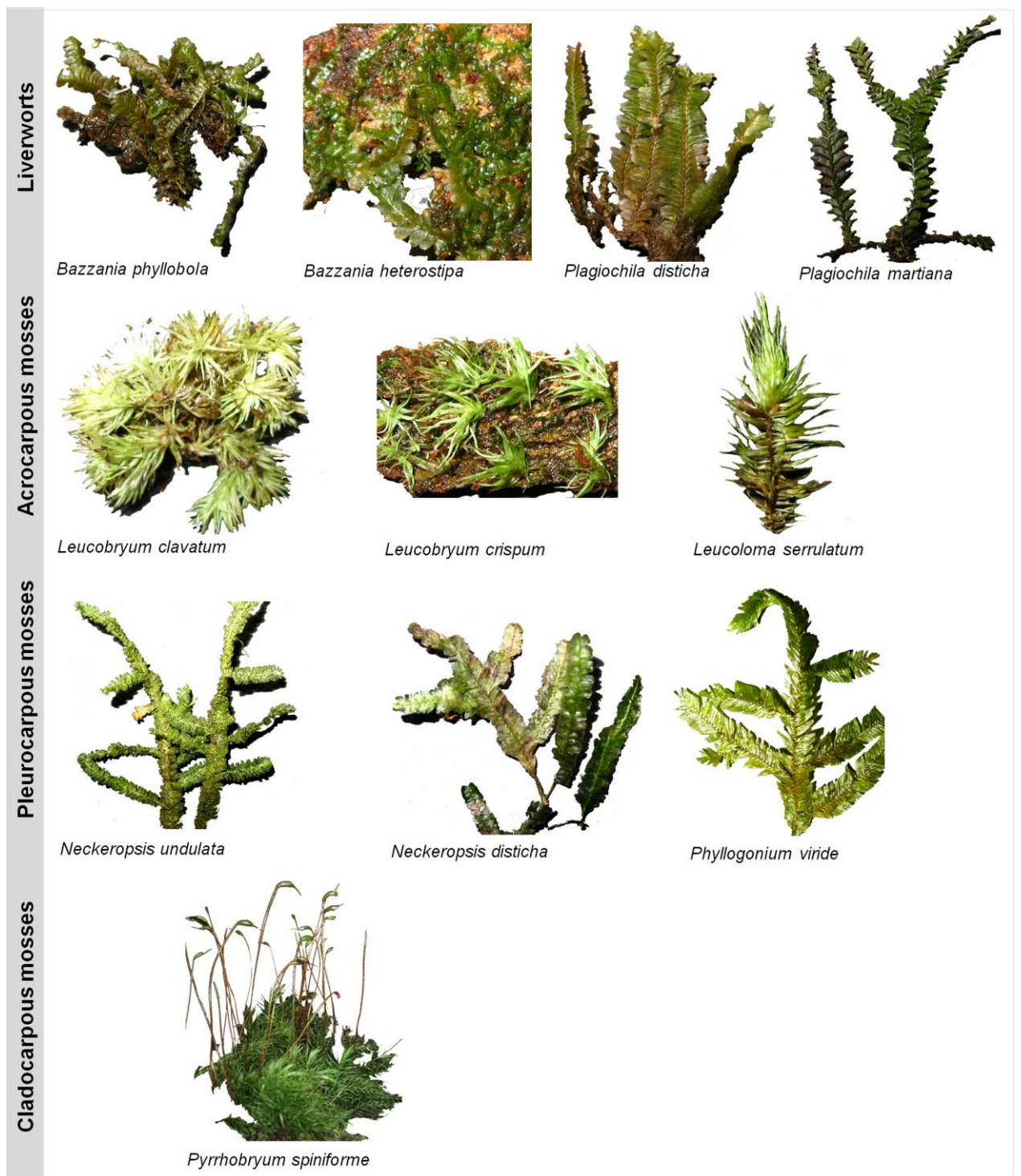
processos ecológicos de comunidades vegetais, cujo foco quase sempre está apenas nas traqueófitas.

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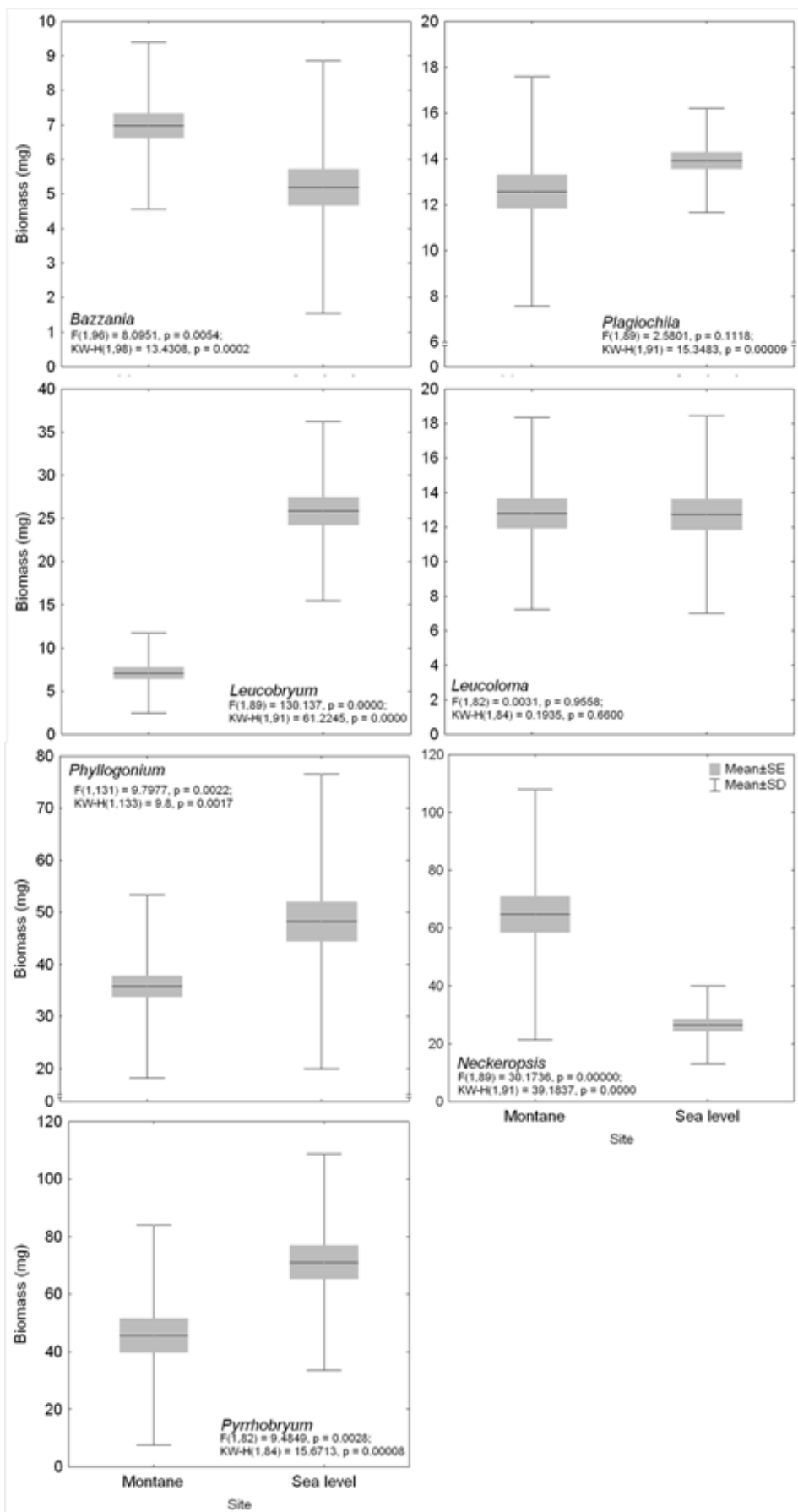
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## **ANEXOS**



**Figura 1.** Espécies de briófitas (hepáticas; musgos acrocárpicos, pleurocárpicos e cladocárpicos) estudadas nos Capítulos I e II da presente tese e coletadas a partir de duas localidades (Floresta Montana e Foresta de Restinga) de Floresta Altântica do Parque Estadual da Serra do Mar – SP.



**Figura 2.** Biomassa (mg) média de sete gêneros de briófitas (musgos e hepáticas) presentes em duas localidades (Montane = Floresta Montana; Sea Level = Floresta de Restinga) de Floresta Altântica do Parque Estadual da Serra do Mar – SP. Os valores de biomassa representam a média da massa conjunta de seis indivíduos por colônia (4 a 6 colônias por espécie em cada localidade de estudo; para mais detalhes ver a seção “Materials and Methods” do Capítulo II). Testes t-Student comparam valores entre as duas localidades de floresta (valores significativos de  $P > 0.05$  estão evidenciados em negrito).