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# 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 sexual conditions) were described for two different sites (montane and sea level) of a Brazilian tropical rain forest, during a 15 month-period. The sex expression of many species was continuous over the whole period, irrespective of the seasons and the forest sites. The specific sexual conditions 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 by the end of the dry season. Fertilization occurred during the wettest months and sporophytes developed during the dry season, dispersal of 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 in high numbers. Bryophytes in tropical rain forests are favored by wet weather and mild temperatures. Similar reproductive phenological patterns were found in different habitats with minor variations.

**KEYWORDS.** Brazilian Atlantic Forest, liverworts, mosses, phenophases, monoicous, dioicous, sexual reproduction.



The relationship between time and events of growth and reproduction in the life cycle of a species is described by phenology (Glime 2007). 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 sexual condition 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). In addition, to verify the influence of biotic and abiotic 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

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2002), mostly conducted with mosses from temperate to polar areas (e.g., 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 in the same ecosystem, sexual condition and phylum on the reproductive behavior 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 sexual conditions) 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 specific sexual conditions 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 located in the “Núcleo Picinguaba” (23° 31′–23° 34′ S and 45° 02′–45° 05′ 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). 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 seasonally flooded forest adjacent to the sea, and referred to as the “sea level” site (Núcleo Picinguaba), whereas the high-altitude site (c. 1,000 m a.s.l.) is denoted “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).

The climate at both sites was characterized by monthly or bimonthly records of minimum and

maximum T° in the understory of the forests. At each site, two max-min thermometers were placed on tree trunks ca. 150 cm above the ground. Data were recorded from September 2007 to December 2008. Precipitation data was obtained 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), an area 10,000 m<sup>2</sup> was established and divided into 100 plots of 100 m<sup>2</sup> each. We collected 244 and 167 bryophyte samples mostly from bark and decaying wood from the sea level and montane sites, respectively. These samples were identified to species or genus. The species for the phenological study were selected from these samples according to occurrence degree, sexual condition (monoicous and dioicous), specific sexual condition (sub-division into the sexual condition), and phylum (Bryophyta – mosses; Marchantiophyta – liverworts) (Table 1). 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. When one species did not appear or was not so common in the two sites, two closely related species with the same sexual condition 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. Since the bryophyte colonies in our forests were frequently small (see below the size), we decided to carry out collects bimonthly to reduce disturbance on the colony development. In addition, tropical bryophytes seem to produce frequently new reproductive organs, permitting us to observe all gametangia and sporophytes developmental phases along different months. 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).

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

Species	Site		Growth form	Sexual condition (specific sexual condition)
	Montane	Sea level		
<b>Mosses</b>				
<i>Leucobryum</i>	<i>crispum</i>	<i>clavatum</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>undulata</i>	<i>disticha</i>	Pleurocarpous	Monoicous (Autoicous/ synoicous; Synoicous)
<i>Phyllogonium</i>	<i>viride</i>	<i>viride</i>	Pleurocarpous	Dioicous
<b>Liverworts</b>				
<i>Plagiochila</i>	<i>martiana</i>	<i>disticha</i>	Leafy	Dioicous
<i>Bazzania</i>	<i>heterostipa</i>	<i>phyllobola</i>	Leafy	Dioicous

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 noticeable as isolated ramets. 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, sex-expressing male, sex-expressing female and sex-expressing bisexual. 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 sites.

Developmental stages of gametangia and sporophytes were classified following Greene (1960) and Laaka-Lindberg (2005), and adapted to fit the traits of moss and liverwort species (**Supplementary on-line Table S1**). We selected only newly produced perichaetia and perigonia for this study. Archegonia and antheridia on each sexual branch were classified and counted under a light microscope. Developmental stages of gametangia and sporophytes were assessed by their maturity index (Longton & Greene 1967):  $M_i = \sum(n_x I) / \sum n$ , where  $n_x$  is the number of gametangia or sporophytes representing each developmental stage,  $I$  is the rank of the index value of the developmental stage (for gametangia: 1–4; for sporophyte: 1–7;

**Supplementary on-line Table S1**), and  $\sum n$  is the total number of scored gametangia or sporophytes pooled per sexual branch. Maturity indices of gametangia and sporophytes were calculated separately for each sexual branch. 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  $\times$  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) lacked any gametangia or sporophytes in some months. 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.*

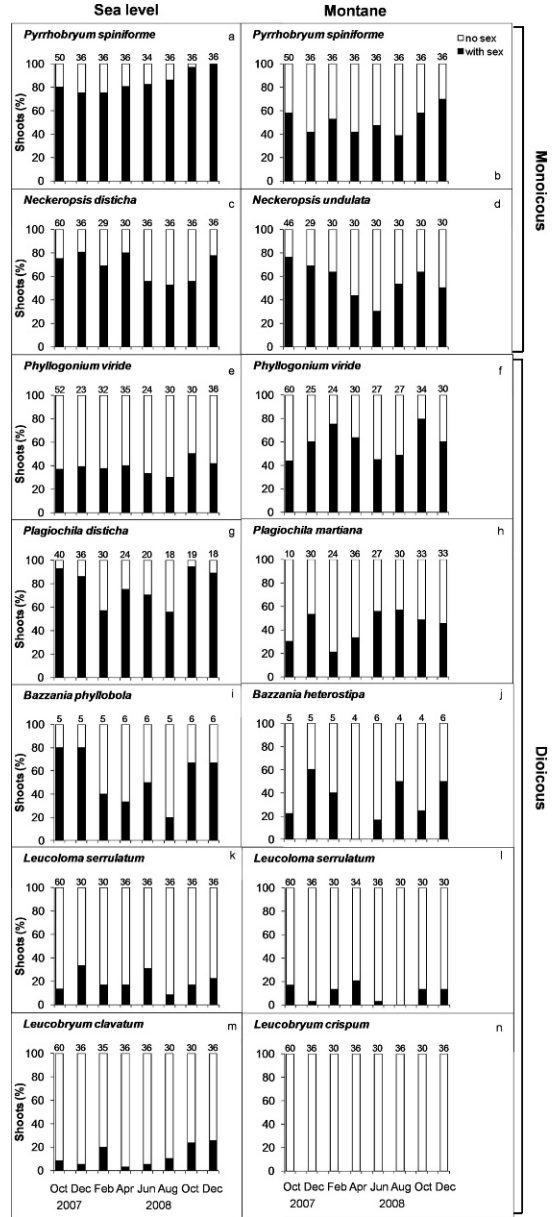
The temperature in the montane site was lower than at sea level, varying from 4°C in the winter to 27°C in the summer in the montane, and from 12°C in the winter to 32°C in the summer at the sea level site (**Supplemental on-line Fig. S1**). The summer was

rainy and the winter dry. The mean monthly 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 (Supplemental on-line Fig. S1).

**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 (Fig. 1N). The sex-expression was high in many species (above 40% of the shoots were sex-expressing), including monoicous mosses and dioicous liverworts (Figs. 1A–J). The dioicous mosses *Leucobryum clavatum* and *Leucoloma serrulatum* had the lowest values of sex-expressing shoots during all months (Figs. 1K–M). 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:  $\chi^2 = 18.72$ ,  $P = 0.004$ ; Supplementary on-line Table S2), where more sex-expressing shoots were found at sea level.

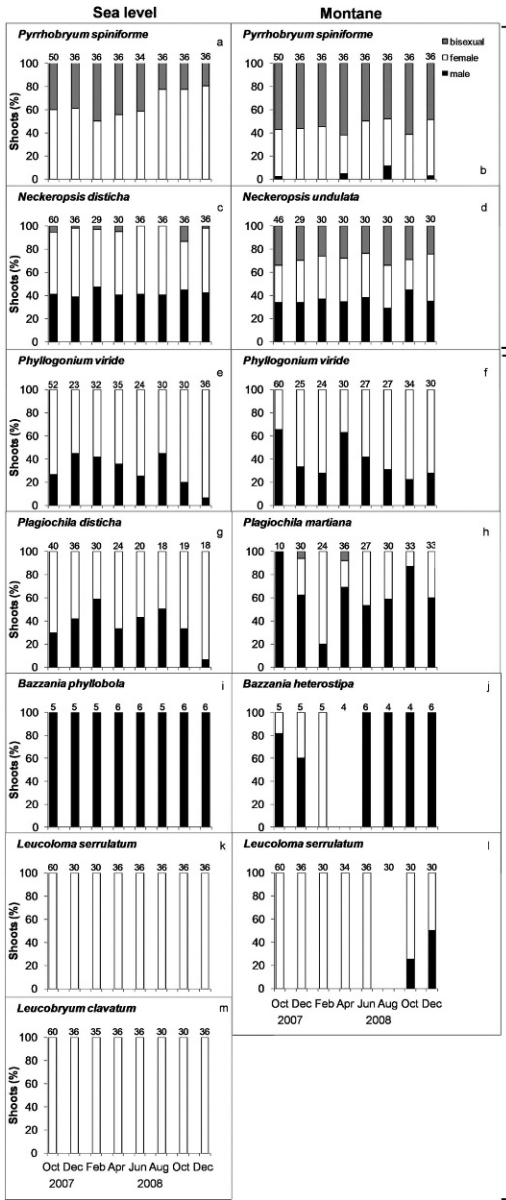
The specific sexual condition of some species was not strictly fixed, varying from synoicous to autoicous in the moss *Pyrrhobryum spiniforme* (Figs. 2A–B), and from dioicous to synoicous (low proportions) in the liverwort *P. martiana* (Fig. 2H). Sexual branches in *P. spiniforme* were not strictly bisexual, but the plants also expressed female branches at sea level, and female and male branches in the montane site. The male sexual branches were generally found in the axils of female sexual branches (gonioautoicous), 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 (Figs. 2C, D). Except for the liverwort *P. martiana*, other dioicous species did not express bisexuality (Figs. 2E–M).

**Development of gametangia and sporophyte.** Mature gametangia were produced during the whole year in both sites of forest, but mature male gametangia were mostly present in the wettest months (Fig. 3). Maturity indices of gametangia, as well as indices of sporophytes did not differ



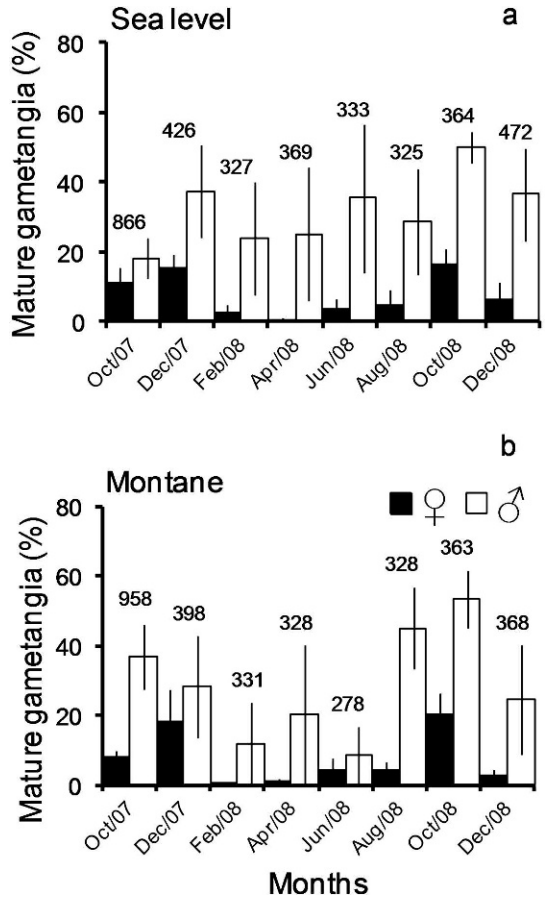
**Figure 1.** Percentage of sex-expressing and nonsex-expressing shoots of monoicous (A–D) and dioicous species (E–N) in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008. Values above the columns are the total shoots analyzed.

significantly between the sites, but they differed over time. No significant interaction for site  $\times$  time was observed (Table 2). 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



**Figure 2.** Percentage of shoots bearing female, male and bisexual branches of monoicous (A–D) and dioicous species (E–M) in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008. Values above the columns are the total shoots analyzed. Data from *Leucobryum crispum* are not shown, since there was no record of sex organs in this species.

montane and at the start of the rainy season (August 2008 to October 2008) at sea level. Mature and mostly recently dehiscid female gametangia were found at all times in both sites. The phase of spore dispersal was more conspicuous in Aug-08 in both sites (Figs. 4A, B). The maturity indices of



**Figure 3.** Percentage of mature gametangia per sexual branch of bryophytes in two sites of an Atlantic forest, Brazil, from October 2007 to December 2008. **A.** At sea level. **B.** At a montane site. Data are means from colonies of seven species in each site  $\pm$  standard errors (values above the columns are the total sexual branches analyzed per month).

gametangia were in general inversely related to those of sporophytes, with male gametangia maturing mainly by the end of the dry season, and sporophytes dehiscid before the start of the rainy season.

Each species had different patterns of gametangial and sporophytic development, and the same or related species had slightly different responses in the two sites (Figs. 4C–J). No significant differences of gametangial and sporophytic maturity indices were found between the two sites, and the monoicous species *Pyrrhobryum spiniforme* and *Neckeropsis* spp. differed significantly only in male gametangia maturity indices (Table 2; Figs. 4C–F). There was a significant effect of time for all maturity

**Table 2.** 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.

All species	♂ gametangia		♀ gametangia		Sporophytes	
	F	P	F	P	F	P
<i>Between-subjects effects</i>						
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 × time	0.77	0.617	0.10	0.445	0.50	0.816
Only monoicous species	♂ gametangia		♀ gametangia		Sporophytes	
	F	P	F	P	F	P
<i>Between-subjects effects</i>						
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 × 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 × site	1.25	0.293	1.21	0.310	0.13	0.995
Time × species	4.28	<b>&lt;0.001</b>	3.20	<b>0.006</b>	0.58	0.764
Time × site × species	1.49	0.193	2.54	<b>0.024</b>	0.32	0.934

indices of gametangia and sporophytes, and some interactions of time  $\times$  species and site were also significant for male and female gametangia (Table 2). 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 *Phyllogonium viride* at sea level with values reaching 100% of abortive gametangia in different months (Fig. 5E). Abortive male gametangia were more frequent than abortive female gametangia at both sites and all species (Figs. 5A–F).

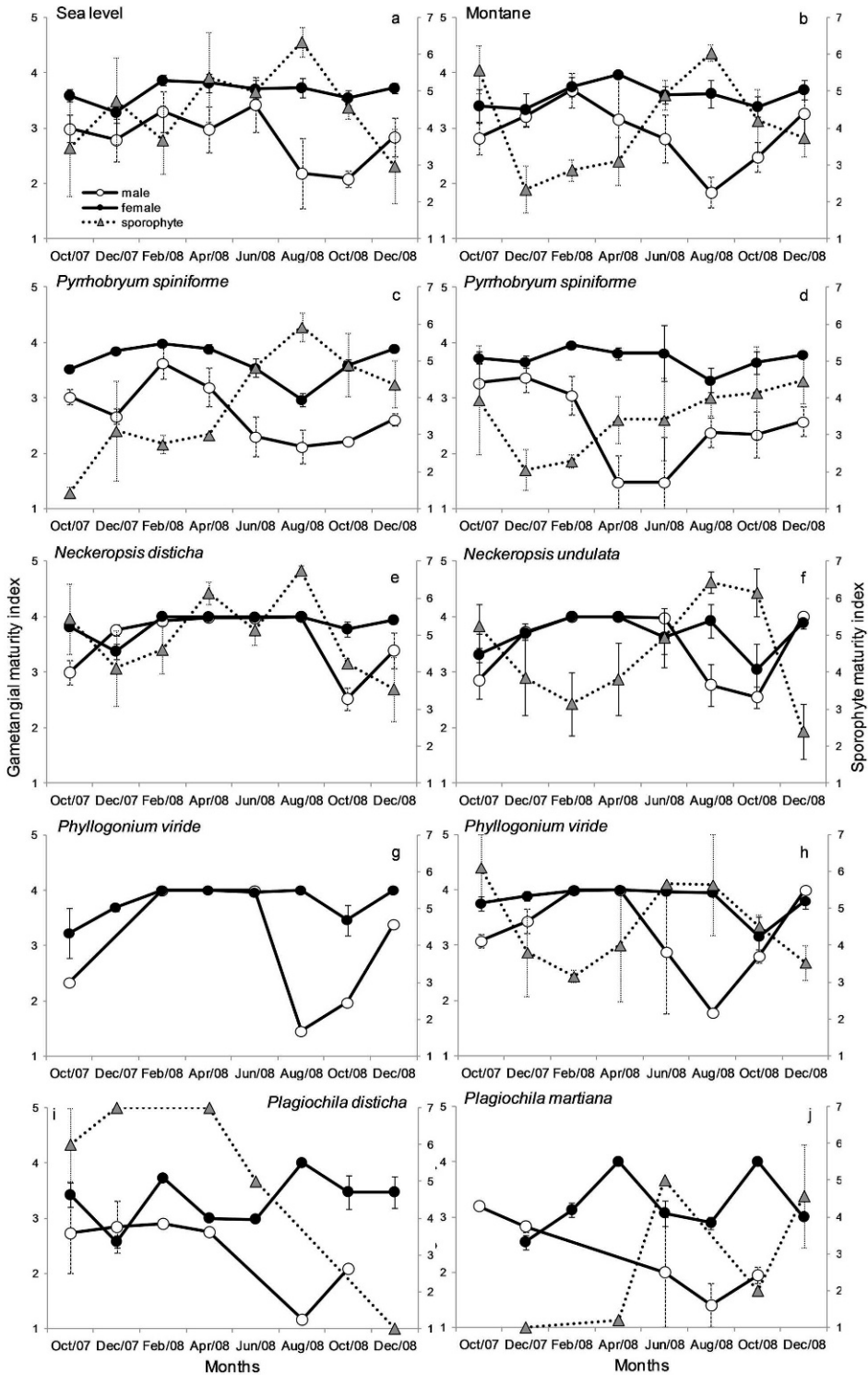
## DISCUSSION

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 specific sexual conditions of dioicous species generally did not change, but synoicous species did not seem to have a fixed specific sexual condition, producing both bisexual and unisexual branches simultaneously. Developmental 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 at the end of the dry season, whilst sporophytes were dehiscent during the dry season. Female gametangia were receptive to fertilization over the whole period.

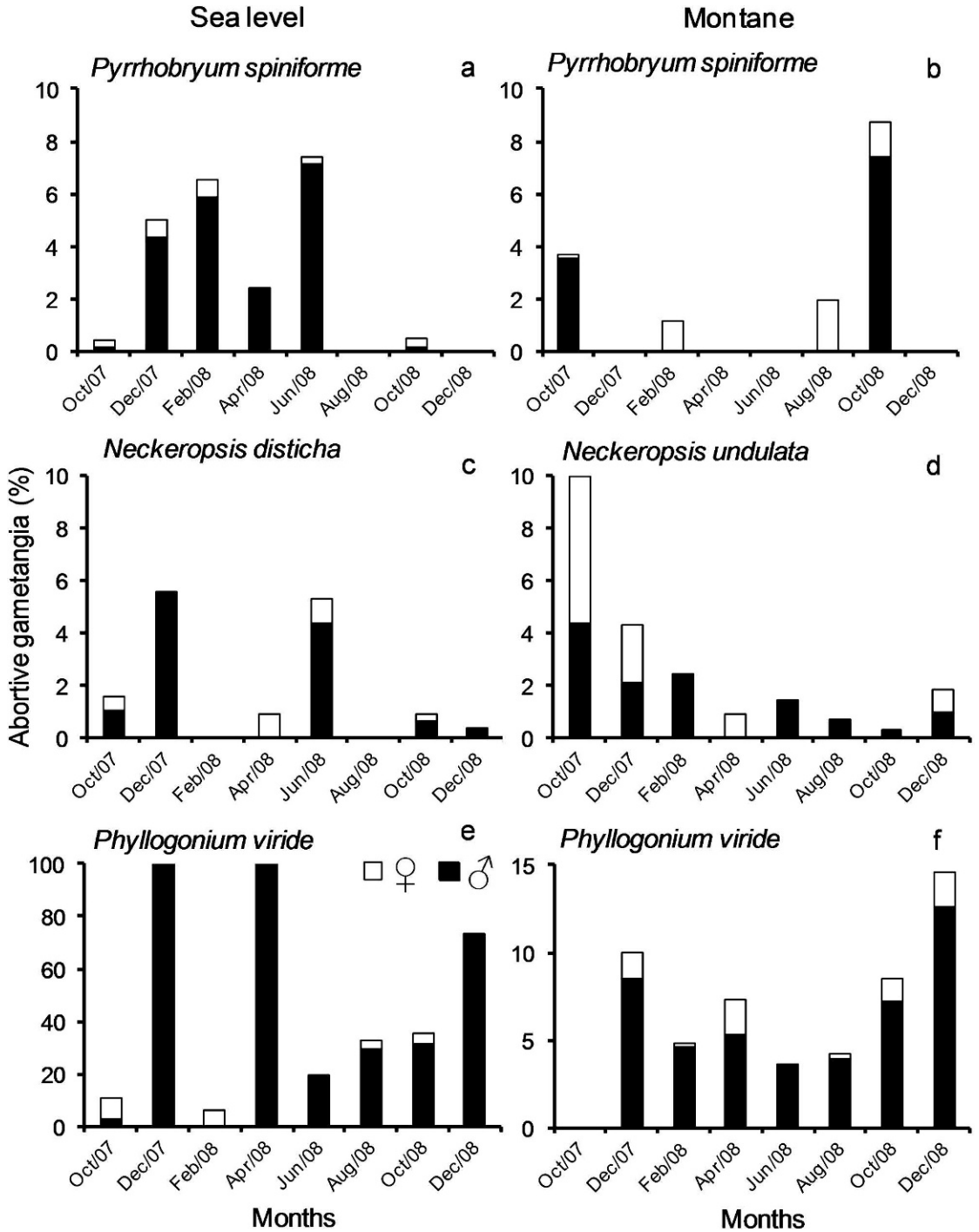
**Constant sex expression over time.** The pattern of constant sex expression over time is characterized as opportunistic. In tropical rain forests, mild temperatures and relatively high water availability favor the continuous production of sex organs. This opportunistic pattern is commonly observed 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 where new reproductive structures are produced, after months of snow cover and mean temperatures at or 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 differences between these two sites of Atlantic forests are due to



**Figure 4.** Development of male and female gametangia, and sporophytes for all species in the sea level (A) and montane (B) sites, and for each species at both sites (C–J) 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. Data are means  $\pm$  standard errors.





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

the elevation above sea level, the periodical flooding (from December to March) and higher temperatures at sea level, and the constant presence of fog in the montane site. 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 site. Beyond abiotic factors, other species specific factors are

relevant to explain the differences among the reproductive characters found in the two sites. The functional mechanism behind these patterns is not completely understood, but plants in 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 a number of sporophytes similar to those found at sea level (Maciel-Silva, Válio & Rydin in prep.). *Phyllogonium viride*, an exception, has a higher number of sex-expressing individuals at the montane than at sea level. In addition, this moss species has high percentages of male abortive gametangia at sea level, explaining the absence of sporophytes in this forest site. In this case, high temperatures at sea level possibly can damage male gametangia development compared to that of the female (Stark 2002), since male gametangia are mainly matured during the rainy season (higher temperatures). However, the mechanisms behind the constraints on gametangia development in bryophytes under natural conditions are still unknown.

**Infrequent sex-expression among some dioicous species.** In contrast to the monoicous mosses, the dioicous *Leucobryum* spp. 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 gametophytes with non expressing sex condition and only 13% female (Oliveira & Pôrto 2002), and also *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 likelihood of fertilization in dioicous acrocarpous mosses is low, due to the production of a single unisexual branch at the shoot apex every 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 on lateral branches. Thus, 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, have 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.

**Specific sexual conditions 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 a patch and may explain, at least in part, 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 may 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). 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 discharged. Spores are favored by the low temperatures and dry air at that time (August).

Gametangia and sporophyte phenophases of tropical bryophytes are adapted to the seasonal alternation of 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 forests (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 warm and the wet season has mild temperatures. There are minor variations, 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 ones. 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. Thus, male gametangia development takes longer time than female gametangia, and it is also more susceptible to abortion.

Bryophytes in the Brazilian Atlantic forests are favored by the wet weather and mild temperatures. Related species 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 rates, but both male and female gametangia complete their development in less than a one-year period. Fertilization time is dependent on the wettest period and spore dispersal occurs on the driest months.

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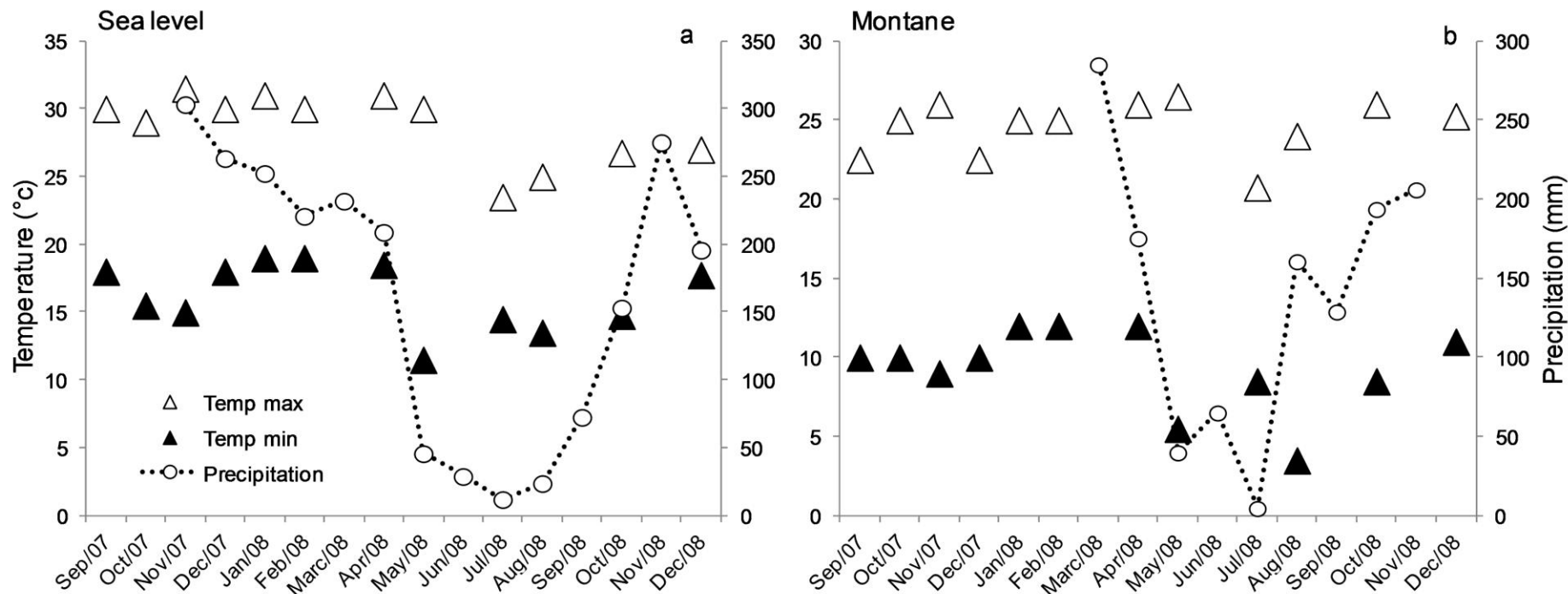
**Supplemental on-line documents:**

**Figure S1.** Maximum and minimum monthly temperatures ( $^{\circ}\text{C}$ ) and precipitation ( $\text{mm month}^{-1}$ ) from montane and sea level.

**Table S1.** Stages of development and index values (*I*) of gametangia and sporophytes of mosses and liverworts.

**Table S2.** Total and monthly mean frequencies of sex-expressing shoots of mosses and liverworts in two sites of an Atlantic forest.

**Supplementary on-line Figure for Maciel-Silva, A.S. & I.F. Marques Válio. (2011). Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep. The Bryologist 114(4): 708–719.**



**Figure S1.** Maximum and minimum monthly temperatures ( $^{\circ}\text{C}$ ) and precipitation ( $\text{mm month}^{-1}$ ) from sea level (A) and montane (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.

**Supplementary on-line Table for** Adaisés S. Maciel-Silva<sup>1</sup> and Ivany Ferraz Marques Válio

(2011). Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep. *The Bryologist* 114(4): 708–719.

**Table S1.** Stages of development and index values (*I*) of gametangia and sporophytes of mosses and liverworts adapted from Greene (1960) and Laaka-Lindberg (2005).

Stages		Index	Event marking beginning of phase
		value ( <i>I</i> )	
<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 structure, 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.

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**Table S2.** 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