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PALYNOLOGY AND ASPECTS OF REPRODUCTIVE BIOLOGY OF *PLAGIOCHILA* (DUMORT.) DUMORT. (PLAGIOCHILACEAE)

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, da Universidade Federal de Juiz de Fora, como parte dos requisitos necessários à obtenção do Título de Doutor em Ecologia Aplicada ao Manejo e Conservação de Recursos Naturais.

Orientadora: Profa. Dra. Andrea Pereira Luizi Ponzo

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Para minha família.

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“Quando vejo os teus céus, obras dos teus dedos, a lua e as estrelas que preparastes;

Quem é o homem mortal para que te lembres dele? E o filho do homem, para que o visites?”

Salmos 8: 3 e 4

ABSTRACT

Plagiochila (Dumort.) Dumort is a group of great importance among bryophytes due to its taxonomic and ecologic implications and it is the richest and more complex genus in Plagiochilaceae, possessing proximally 700 accepted names. Despite the various attempts made to solve taxonomic issues, there are few studies on *Plagiochila* dealing with palynological and reproductive investigations. Thus, the goals of this dissertation were to develop a palynological study of *Plagiochila* (Dumort.) Dumort. (Chapter one) and to investigate the strategies regarding the reproductive biology (Chapter two) and desiccation tolerance (Chapter three) of one species belonging to this genus. To develop the palynological study, spores of seventeen species of *Plagiochila* were analyzed following standard palynological techniques and observed through light and electron microscopy; morphological and ultrastructural data were obtained and interpreted considering their relevance to taxonomy. *Plagiochila porelloides* was the species selected as model to perform the reproductive biology and water stress tolerance studies. For the first, we assessed the sex ratio and fertilization success to test for sex ratio variation among populations and for possible relationships between the sex ratio and environmental factors. For the latter, field collected healthy plants, among male and female individuals from three different population, were subjected to a desiccation tolerance assay at 40% of RH for a period of 22 h, pursuing fordesiccation tolerance ability and any sex differences in tolerance. Our results demonstrate that the spores were found to be apolar, spheroidal, released in monads, and varying in size from 13µm to 58µm (small to large size). The sporoderm comprises three layers and, based on spore surface ornamentation (variable granules in shape and morphology), species were assembled into four different spore types. Hierarchical cluster analysis using both taxonomic and palynologic information revealed five different groups of species. Regarding the sex ratio, the

expressed sex ratios in *P. porelloides* populations were heterogeneous, having a male-biased ratio (0.375 ♀:1 ♂), and it was associated with fertilization; but no significant relationship was found in relation to any environmental aspects. Concerning to water stress, *P. porelloides* shoots survived the low RH of 40%, demonstrating that the species is DT; females were found to be more DT than males. Our finds bring important information about spore morphology and reproductive ecology for *Plagiochila*, revealing the importance of spore description in further phylogenetic studies, and the relevance of sex ratio and sex differences in water stress tolerance to reproductive biology and population dynamics.

Key Words: Bryophytes; Desiccation Tolerance; Ecology; Liverwort; Male Bias; Palynology; Reproductive Ecology; Sex Ratio; Spore; Sporoderm.

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Chapter one

SUMMARY

INTRODUCTION

Plagiochilaceae Müll. Frib. & Herzog (Lophocoleineae Schljakov) is a leafy liverwort family, characterized as a group of cryptogamic and avascular land plants, that has a life cycle with alternation of heteromorphic phases: a diploid, perennial and photosynthetic gametophyte, and a diploid, ephemeral and non-photosynthetic sporophyte. Together with thalloid liverworts, mosses, and hornworts, they constitute a group known as "bryophytes", which shares ancestry with green algae, supported by morphological and molecular analyses (CRUM, 2001; MISHLER et al., 1994).

According to GOFFINET & SHAW (2009), bryophytes include three monophyletic divisions: Anthocerotophyta (hornworts), Bryophyta (mosses), and Marchantiophyta (leafy and thalloid liverworts). However, recent phylogenetic analyses have risen the discussion about 'bryophytes' monophyly, suggesting that liverworts and mosses form a monophyletic clade (Setaphyta), which branched first among land plants, and hornworts are reported as an independent lineage, closely related to tracheophytes (COX, 2018; WICKETT et al., 2014; PUTTICK et al., 2018). Bryophytes are the second largest group amongst embryophytes, with approximately 18,000 species worldwide, showing great diversity in the Neotropics (GOFFINET & SHAW, 2009).

The family Plagiochilaceae includes plants with a robust stem, which may be ascending or pending, and often presents a rhizome-like creeping base. Leaves are alternate or opposite (rarely), always succubous, with a reflex dorsal margin, which may be ciliated or entire; the underleaves are generally absent, but when present, they are rudimentary. Laminal cells are tremendously variable. Branches in this family may be terminal or intercalary, rarely central intercalary; this character state is a very important

taxonomic feature (GRADSTEIN et al., 2001; GRADSTEIN & COSTA, 2003; HEINRICHS, 2002).

Plagiochilaceae constitutes a large group at the tropical region due to their richness (GRADSTEIN & REINER-DREHWALD, 1995) but it is also diverse in subtropical and temperate regions (JAMY et al., 2016). There are ten genera worldwide distributed (CRANDALL-STOTLER et al., 2009; SÖDERSTRÖM et al., 2013; SÖDERSTRÖM et al., 2016), and *Plagiochila* (Dumort.) Dumort. is the richest genus, including more than 95% of the species of the family (GRADSTEIN et al., 2001; GRADSTEIN &COSTA, 2003; HEINRICHS, 2002; JAMY et al., 2016; SÖDERSTRÖM et al., 2016).

Plagiochila is a nearly cosmopolitan genus, most common and diverse in oceanic regions or montane rain forests (SCHUSTER, 1980). GRADSTEIN et al. (2001) reported occurrence of *Plagiochila* species on several types of substrata: bark, rotten WOODs, moist rocks, soil, and occasionally on living leaves. In the Neotropics, one of its centers of diversity (SCHUSTER, 1980), plants can be found from the lowlands to over 4,000 meters (HEINRICHS & GRADSTEIN, 2000).

Plagiochila is a taxonomically complex group, with more than 2,300 published names (INOUE, 1989), or even proximally 3,000 names (ELPT database). SO and GROLLE (2000) reported c.a 450 species worldwide distributed. GRADSTEIN (2015a, 2015b) reviewed and contributed to synonymization and lectotypification of several names, and more recently, numbers reach 700 currently accepted species (SÖDERSTRÖM et al., 2016). SCHUSTER (1980) quote that “the extreme polymorphism of the majority of the taxa is responsible for the chaos” of its taxonomy.

Historically, numerous attempts to classify this genus into infrageneric groups have been made; the first ones relied on some important gametophyte characters. The

very first attempt was a proposal made by LINDENBERG (1839) which was based on leaves and perianth shape, and frequency of branching. Later, SPRUCE (1885) presented a new subdivision based on perianth position and branching type: *Ramiflorae* and *Cauliflorae*. SCHIFFNER (1900), STEPHANI (1902), DUGAS (1929), and CARL (1931) have also attempted to subclassify *Plagiochila* considering leaf and cell shape, perianth morphology, and branching patterns.

Later, SCHUSTER (1959, 1960), INOUE and SCHUSTER (1971), and more recently, SO (2001), and HÄSSEL (2004, 2006) have also tried to classify the genus based on gametophyte morphology. Molecular phylogenies studies on *Plagiochila* and Plagiochilaceae (GROTH et al., 2003; HEINRICHS, 2002; JAMY et al., 2016; PATZAK et al., 2016; SÖDERSTRÖM et al., 2016) have reviewed the morphological classification, attempting to improve the current taxonomic arrangement.

The great amount of systematics and phylogenetics studies on *Plagiochila* (e.g. AMORIM et al., 2011; GRADSTEIN, 2015; HEINRICHS et al., 1998; HEINRICHS et al., 2000, 2002, 2004, 2006; 2016; HEINRICHS & GRADSTEIN, 1999; INOUE & SCHUSTER, 1971; INOUE, 1981; 1989; MULLER et al., 1999; SO & GROLLE, 2000; PATZAK et al., 2016; among others) contrasts the scarce information about spore morphology (ERDTMAN, 1965; VOJTKO, 1993) and reproductive strategies (BERRIE, 1974; LONGTON & SCHUSTER, 1983; MACIEL-SILVA & MARQUES-VÁLIO, 2011; PIIPO, 1992; SCHUSTER, 1980).

Reproductive strategies and the spore morphology were crucial to enhance plants land life transition. The evolution of reproductive and ecological strategies, especially related to the ability to respond to water scarcity enables plants to develop features to survive life under scarce water accessibility, e.g. desiccation tolerance (LEVITT, 1980;

ALPERT, 2005). Concerning to spore, the development of a durable and protective spore wall is considered to be essential for the success of the life on land (ARTEAGA-VAZQUEZ, 2016; BROWN & LEMMON, 1988; RENZAGLIA *et al.*, 2000; WELLMAN, 2004; WALLACEet al., 2011;).

Desiccation tolerance is considered a very ancient characteristics of dry land life (PROCTOR et al., 2007), once desiccation tolerant species is widepread in living organisms, and the genes associated with this strategy appears to be homologous (ALPERT, 2000; ALPERT, 2005 and references within). Many authors have studied the desiccation tolerance strategy and various definitions have been formulated. It can be defined as the ability to dry to equilibrium with air, which may be moderately to extremely dry, and then return to normal metabolic activity after rewetting (ALPERT, 2005; OLIVER, 2009; VANDERPOORTEN & GOFFINET, 2009). For an ecological definition, desiccation tolerance is the ability to withstand under intermittent water availability (PROCTOR, 2009). Among plants, the ability to tolerate drought is known in several groups, but it is only in bryophytes that this strategy is found in both vegetative and reproductive tissue (ALPERT, 2000; GLIME, 2017; OLIVER et al., 2000; PROCTOR et al., 2007; STARK, 2017).

Desiccation tolerance is very common amongst bryophytes but not a mandatory feature (PROCTOR, 1990; PROCTOR & PENCE, 2002; PROCTOR et al., 2007; PROCTOR, 2009; STARK 2017; WOOD, 2007;), and varies greatly between species (PROCTOR & TUBA, 2002). There are various degrees of tolerance that have been seen in mosses and liverworts (PROCTOR & TUBA, 2002). These degrees fluctuate between true desiccation tolerance - where plants can tolerate an absolute water content < - 100 MPa (approximately < 10%), returning to normal metabolism and growth if rewetted; and

dehydration tolerance - plants that can tolerate drought down to – 10 MPa, surviving and recovering cellular water loss (MARKS et al., 2016; OLIVER, 2009; OLIVER et al., 2010). Therefore, a certain degree of drought tolerance is crucial, especially in habitats where the bryophytes are not kept constantly moist (VANDERPOORTEN & GOFFINET, 2009).

Sex differences in water stress are found in seed plants but the sex that is more tolerant varies among the different species (FREEMAN & MC ARTHUR, 1982; JUVANY & MUNNÉ-BOSCH, 2015; SINCLAIR et al., 2012). Among a few bryophytes, greater water stress tolerance (DT or DhT) were reported as being higher in females than in males (MARKS et al., 2016; NEWTON, 1972; STIEHA et al., 2014). These studies suggested that this difference in water stress tolerances might give female a survival advantage contributing to a female bias sex ratio, that is common in bryophytes (BISANG & HEDENAS, 2005; GLIME & BISANG, 2017; HAIG, 2016). Because females are the sex that bears the sporophyte after fertilization (HAIG, 2016), this DhT advantage may enhance spore production.

The spores are the first stage of gametophyte in liverworts life cycle and consist of a single cell produced by meiosis, they are involved by a special cell wall, the sporoderm (BROWN & LEMMON, 1988). For liverworts, two layers are observed on sporoderm: intine, the inner layer, constituted by polysaccharides, and related to spore germination; and exine, a more external stratum, composed by sporopollenin (BLACKMORE& BARNES, 1987; BROWN & LEMMON, 1988; NEIDHART, 1979; MOGENSEN, 1983; OLESEN & MOGENSEN, 1978; RENZAGLIA et al., 2000; WALLACEet al., 2011). Sporopollenin is a highly resistant polymer that provides resistance and protection, either against desiccation, or against the destructive action of

pathogens, ensuring spore content protection, greater longevity, and resistance (BROWN AND LEMMON, 1988; ITO et al., 2007; RENZAGLIA et al., 2000; WALLACEet al., 2011).

Many studies on spores refer to different aspects of various groups (BLACKMORE& BARNES, 1987; BROWN & LEMMON, 1980, 1984a, 1984b, 1988, 1991; CALDEIRA et al., 2006, 2009, 2013; ESTÉBANEZ et al., 1997; HECKMAN, 1970; LUIZI-PONZO & BARTH, 1998; 1999; LUIZI-PONZO & MELHEM, 2006; SILVA-E-COSTA et al., 2017; ROCHA et al., 2008; RODRIGUES and LUIZI-PONZO, 2015; SAVAROĞLU, 2015; SAVAROĞLU et al., 2017; YANO & LUIZI-PONZO, 2006; 2011). However, on the genus *Plagiochila*, little information is known about spore morphology.

In fact, it is worthy to note that sporophytes are infrequent in *Plagiochila* (HEINRICHS 2002). Dioicy is reported for this genus (SCHUSTER, 1980; GRADSTEIN *et al.*, 2001; HEINRICHS, 2002), which may be a reason for low sporophyte production due to unbalanced sex ratios and spatial segregation of the sexes, very common in species with unisexual individuals (LAAKA-LINDBERG et al., 2000; LONGTON & SCHUSTER, 1983; MACIEL-SILVA & PÔRTO, 2014; REESE, 1984;), especially when sperm distance dispersal is limited (KNIGHT *et al*., 2005; LONGTON, 1976; LONGTON & SCHUSTER, 1983; RYDREN et al., 2006; VAN DER VELDE et al., 2001).

An unbalanced population sex ratio can negatively impact sexual reproduction and, consequently, population maintenance and genetic variation (VANDEPITTE et al., 2010). In populations with absence or low levels of sexual reproduction, population persistence occurs mainly by asexual reproduction via specialized propagules and plant

fragmentation (see reviews by FREY & KURSCHNER, 2011; LAAKA-LINDBERG, 2000).

Population sex ratios vary and can be characteristically biased against one sex or the other across major plant taxa. Among seed plants, the sex ratio is generally male biased (ABE et al., 2002; ANDERSON & LEVINE, 1982; BARRET et al., 2010; GARCIA & ANTOR, 1995; GLODLEY, 1964; NICROTA, 1998; LENZA & OLIVEIRA, 2006; OPLER & BAWA, 1978; SINCLAIR et al., 2012). In bryophytes and herbaceous seed plants, on the other hand, sex ratios are mostly female biased (see e.g. BISANG & HEDENAS, 2005; BOWKER et al., 2000; FIELD et al., 2012; GLIME & BISANG, 2017; HAIG, 2016; LLOYD, 1974; RYDGREN et al., 2010; PUCHOLT et al., 2017). As mentioned above, unequal sex ratio may be due to the reproductive condition and spatial segregation of the sexes, but also can be associated with sexual differences in stress tolerance and physiological requirements.

Investigating and exploring the ecological features and physiological mechanisms in bryophytes, a group that has close relations with the first extant land plants, brings benefits to plant systematics, phylogeny, and evolutionary studies. As stated by PROCTOR et al. (2007), "Bryophytes are no less highly evolved and sophisticated than vascular plants, just smaller, with all the biological differences their size entails."

Thus, the objectives of this dissertation were to develop a palynological study of *Plagiochila* (Dumort.) Dumort. species and to investigate the reproductive strategies (including reproductive biology and desiccation tolerance) of one species belonging to this genus. Along these lines, this dissertation is divided into three chapters, that have specific aims:

Chapter One: To perform a palynological evaluation of species of *Plagiochila*, we analyzed spore size, sporoderm ornamentation, and sporoderm ultrastructure of seventeen species;

Chapter Two: To investigate the reproductive biology, a sex ratio study was developed, using the species *Plagiochila porelloides* (Torr. ex Nees) Lindenb. as study organism. We tested for variation in the expressed sex ratios among population and its correlation with environmental factors;

Chapter Three: To evaluate desiccation tolerance and sex differences in water stress tolerance, we developed an experimental assay, submitting healthy individuals of *Plagiochila porelloides* (Torr. ex Nees) Lindenb. to a desiccation stress, evaluating sexual differences in damage and recovery.

Chapter One

Spores of *Plagiochila* (Dumort.) Dumort.: the taxonomic relevance of morphology and ultrastructure

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Spores of *Plagiochila* (Dumort.) Dumort.: the taxonomic relevance of morphology and ultrastructure.

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ABSTRACT

Plagiochilaceae is a family of leafy liverworts that are distributed worldwide. It is of great importance due to its taxonomic and ecologic implications among bryophytes. Most species of the family belong to the genus *Plagiochila*, but there is no consensus regarding its infrageneric circumscription. There have been few palynological studies involving Plagiochilaceae and *Plagiochila*. Here, we describe the spore morphology of seventeen species of *Plagiochila* and discuss the taxonomic value of palynological characters for these taxa. The spores were processed by standard palynological techniques and analyzed using light and electron microscopy. The spores were found to be apolar, spheroidal, released monads that vary in size from 13µm to 58µm (small to large size). The sporoderm comprises an intine (stratified), a nexine, and a sexine. The spore surface is ornamented with granules that vary in shape and morphology, thus allowing the studied species to be grouped into four spore types: regular and delicate granulate, irregular and coarse granulate, long granules with flattened apices, and long and straight granules. Hierarchical cluster analysis revealed five different groups of species, evidencing the importance of spore information for taxonomic and phylogenetic studies.

Key words: bryophytes, liverworts, morphology, palynology, SEM, taxonomy, TEM, ultrastructure.

INTRODUCTION

Plagiochilaceae Müll. Frib. & Herzog (Lophocoleineae Schljakov), a leafy liverwort family, include robust, ascending or pending plants (GRADSTEIN & COSTA, 2003; HEINRICHS, 2002). The leaves are alternate or opposite, succubus, with a dorsal margin reflex, which may be ciliated or entire, and the underleaves are generally absent (GRADSTEIN et al., 2001; GRADSTEIN & COSTA, 2003). Plagiochilaceae are an important group of bryophytes at the tropical region due to their richness (GRADSTEIN & REINER-DREHWALD, 1995) but are also diverse in subtropical and temperate regions (JAMY et al., 2016). There are ten genera worldwide distributed (CRANDALL-STOTLER et al. 2009; SÖDERSTRÖM et al., 2013; SÖDERSTRÖM et al., 2016), and *Plagiochila* (Dumort.) Dumort. is far the richest one, including ca 96 % of the species of the family (GRADSTEIN et al., 2001; GRADSTEIN & COSTA, 2003; HEINRICHS, 2002; JAMY et al., 2016, SÖDERSTRÖM et al., 2016).

Plagiochila (Dumort.) Dumort. is a taxonomically complex group, with more than 2,300 published names (Inoue 1989), or even proximally 3,000 names (ELPT database). SO & GROLLE (2000) reported ca 450 species worldwide distributed, GRADSTEIN (2015a, 2015b) reviewed and contributed to synonymization and lectotypification of several names, and more recently numbers reach 700 currently accepted species (SÖDERSTRÖM et al., 2016).

Historically, numerous attempts to classify this genus into infrageneric groups have been made; the first ones relied on some important gametophyte characters as leaf shape, leaf cell pattern, and branching type (CARL, 1931; DUGAS, 1929; LINDENBERG, 1839; SCHIFFNER, 1900; STEPHANI, 1902; SPRUCE, 1885). Later,

studies of SCHUSTER (1959; 1960), INOUE & SCHUSTER (1971), and more recently SO (2001), and HÄSSEL (2004; 2006), have also tried to classify it based on gametophyte morphology. Molecular phylogenies studies on *Plagiochila* and Plagiochilaceae (GROTH et al., 2003; HEINRICHS, 2002; JAMY et al., 2016; PATZAK et al., 2016; SÖDERSTRÖM et al., 2016) have reviewed the morphological classification, attempting to improve the current taxonomic arrangement.

In spite of different studies that have been conducted dealing with *Plagiochila*, information about spores are scarce (ERDTMAN, 1965; VŐJTKO, 1993) or reduced to brief commentaries on taxonomic descriptions (GROLLE & HEINRICHS, 1999; HEINRICHS & GRADSTEIN, 1999; HEINRICHS et al., 2000, 2001; HEINRICHS, 2002; HÄSSEL, 2004; MÜLLER et al., 1999; among others). ERDTMAN (1965) and VŐJTKO (1993) presented a general spore surface description, and size evaluating, with a small sample size. Spore description in taxonomic studies are limited to a superficial and sometimes inaccurate comments on ornamentation and size measurements.

During the plants land life transition, several characters were crucial to enhance life, but the development of a durable and protective spore wall was essential (ARTEAGA-VAZQUEZ, 2016; BROWN & LEMMON, 1988; RENZAGLIA, et al. 2000; WELLMAN, 2004; WALLACE, et al. 2011;). The spore, a single cell produced by meiosis, is the first stage of gametophyte in liverworts life cycle (BROWN & LEMMON, 1988). For liverworts, two layers are observed on sporoderm, the special spore cell wall: intine, the inner, constituted by polysaccharides, and related to spore germination; and exine, a more external stratum, formed by sporopollenin, a highly resistant polymer that provides resistance, and protection (BLACKMORE & BARNES, 1987; BROWN & LEMMON, 1988; ITO et al., 2007; MOGENSEN, 1983;

NEIDHART, 1979; OLESEN & MOGENSEN, 1978; RENZAGLIA et al., 2000; WALLACE et al., 2011).

Many studies on spores refer to different aspects of various groups (BLACKMORE & BARNES, 1987; BROWN & LEMMON, 1980; 1984a, 1984b, 1988, 1991; ESTÉBANEZ, et al., 1997; HECKMAN, 1970; LUIZI-PONZO & BARTH, 1998, 1999; LUIZI-PONZO & MELHEM , 2006; CALDEIRA et al., 2006, 2009, 2013; ROCHA et al., 2008; RODRIGUES & LUIZI-PONZO, 2015; SAVAROGLU, 2015; SAVAROGLU et al. , 2017; SILVA-E-COSTA et al., 2017; YANO & LUIZI-PONZO, 2006, 2011). However, on the genus *Plagiochila*, little information is known about spore morphology. In fact, is worthy to note that sporophytes are infrequent in *Plagiochila* species (HEINRICHS, 2002).

The present study aimed to perform a palynological evaluation of species of *Plagiochila*, in order to (1) analyze spore size variation intra and interspecifically, (2) describe spore ornamentation and (3) sporoderm structure, and (4) consider if infrageneric circumscription is supported by spore characterization.

MATERIALS AND METHODS

Sample selection and studied material

The research was developed using herborized botanical materials loaned or donated by the following herbaria: Botanical Garden of Rio de Janeiro Herbarium (RB), Brazilian National Museum Herbarium (R), Herbarium Anchieta (PACA), Santa Cecília University Herbarium (HUSC), University of Kentucky Herbarium (KY), and Professor Leopoldo Krieger Herbarium (CESJ). Acronyms follow Thiers (2018).

As previously mentioned, the rarity of sexual reproduction occurrence in *Plagiochila* is remarkable and, consequently, the difficulty to find sporophytes on these plants is a fact (GRADSTEIN & COSTA, 2003; HEINRICHS, 2002; SCHUSTER, 1980). To cope with this intrinsic feature of those species, proximally 1000 specimens of various species of *Plagiochila* were examined, from the different herbaria mentioned above, seeking for specimens with sporophyte. All species that had sporophyte and enough material available for the development of the study were included in our analyzes, a total of thirty-four specimens and seventeen species studied. Here we present the analyzed species, preceded by the name of the respective section: sect. *Arrectae* Carl - *Plagiochila bifaria* (Sw.) Lindenb; sect. *Fuscolutea* Carl – *P. fuscolutea* Taylor; sect. *Glaucescentes* Carl - *P. buchtiniana* Steph.; sect. *Hylacoetes* Carl - *P. macrostachya* Lindenb.; sect. *Plagiochila – P. asplenioides* (L.) Dumort. and *P. porelloides* (Tor ex. Ness) Lindenb.; sect. *Rutilantes* Carl - *P. gymnocalycina* (Lehm. & Lindenb.) Lindenb., *P. heteromalla* Lehm. & Lindenb., *P. rutilans* Lindenb., and *P. trichostoma* Gottsche.; and sect. Vagae Lindenb. - *P. corrugata* (Nees) Nees & Mont, *P. crispabilis* Lindenb., *P. disticha* (Lehm. & Lindenb.) Lindenb., *P. laetevirens* Lindenb., *P. patula* (Sw.) Lindenb., *P. raddiana* Lindenb., and *P. simplex* (Sw.) Lindenb. Species circumscription follows GRADSTEIN (2015b) and the names are in accordance to Tropicos database (2018).

Light Microscope Observation

For observation under light microscopy (LM), the spores were submitted to the preparation according to the method of WODEHOUSE (1935), for observation of cellular content; and acetolysis method proposed by ERDTMAN (1960). Both techniques were performed following modifications recommended by LUIZI-PONZO

& MELHEM (2006). Spores were described based on the terminology proposed by PUNT et al. (2007) and the definitions of size classes follow ERDTMAN (1952).

Scanning Electron Microscopy Observation

For observation under scanning electronic microscopy (SEM), the capsules were fixed in 2.5% glutaraldehyde for 24 hours and then washed in 0.05 M fosfate buffer solution. Post-fixation was performed with 2% osmium tetroxide (OsO4) in buffer solution for a period of two hours. Then, the capsules were dehydrated in ethanol series and taken to Critical Point dryer (SILVEIRA, 2007). The capsules were opened under stereoscopic microscopy and the spores were dispersed upon stubs with double-sided carbon tape covered with 20nm gold layer, led to SEM and observed. Non-fixed spores were also observed. These analyses were generated at the Laboratory of Electron Microscopy of Federal University of Juiz de Fora, Microscopy Center of Federal University de Minas Gerais and the Microscopy and Microanalysis Center of Federal University of Viçosa.

Transmission Electron Microscopy Observation

For observation under transmission electron microscopy (TEM), mature capsules were separated and fixed 2.5% glutaraldehyde for 24 hours and then washed in 0.05 M fosfate buffer solution, and post fixed in 2% osmium tetroxide, buffer solution. After, the capsules were dehydrated under ethanolic increasing sequence, embedded in Spurr resin, and heated at 70ºC for 48 hours. The material was cut in ultrathin sections (65-70nm) and was stained with uranyl acetate and lead citrate (REYNOLDS, 1963). Finally, the material was led to TEM and observed. These analyses were generated at Microscopy Center of Federal University de Minas Gerais.

Statistical Analyses

In order to access the spore size, the acetolyzed material were measure under light microscopy. To all species studied, and always, if possible, more than one specimen was examined, named Reference (RS) and Comparison Specimens (CS), and they were selected relying on the availability of palynological material. For the Reference Specimen, fifty spores were measured, randomly chosen in three microscope slides, for estimation of larger diameter. When available, a Comparison Specimen was analysed by measuring thirty spores, randomly chosen in three microscope slides. The resulting data were analysed under descriptive statistics, calculating arithmetic mean (X), size range (X_{min}–X_{max}), standard deviation (S), standard error (S_x), variation coefficient (VC% - obtained by the formula $(S/X)* 100$) (SOKAL & ROHLF, 1995), confidence level at 95% (CL95%), and confidence interval (CI 95% - $X \pm CL(95\%)$) (SOKAL & ROHLF, 1995) using Microsoft Excel (2016). For sporoderm strata thickness, ten non-acetolyzed spores were measured, randomly chosen in three microscope slides, for the Reference Specimen, and only the arithmetic mean is presented.

The measurements obtained were submitted to Shapiro-Wilk normality test and showed that our data do not present a normal distribution (p value < 0.05). Consequently, a Kruskall-Wallis test was performed, followed by the Dunnett's test (which are more appropriate tests for non-parametric data), to check for intra and interspecific differences. Graphic evaluation presenting median values and data distribution was provided. The statistical analyses and graphic construction were performed in the software R 3.5.1 (R CORE TEAM, 2018) and JMP® 12 (SAS Institute, Cary, North Carolina, USA).

Cluster analysis

In order to evaluate the degree of association between the species studied, a cluster analysis was performed, using the unweighted pair-group average (UPGMA) algorithm, and Jaccard similarity index, and presenting the cophenetic correlation coefficient, using the software Past 3.21 (HAMMER et al., 2001).

Palynological, gametophytic and one ecological data (Table 3) were plotted on a qualitative binary matrix (Table 4). The palynological information are: spore size (< 26 μm or ≥ 26 μm), spore ornamentation (granules or long granules), and sporoderm thickness (two classes of sporoderm thickness were established using the formula $\mathbf{h} = \frac{\mathbf{A}}{\mathbf{k}}$; where **h** is the class amplitude, **A** is the spore size amplitude, and **k** is the number of classes (CORREA, 2007). The gametophytic information are: Branching type (*Frullania*-type or *Plagiochila*-type), Androecia shape (single or fan-shaped), Androecia position (terminal or intercalary), Perianth base (naked or covered by bracts), and Asexual reproduction (absent or present). The ecological variable is related to substratum: the species are reported as exclusive, when occurring in only one type of substratum; and generalist, when occurring in more two or more types of substratum. The species *Plagiochila bifaria* was not included into this test due to lack of some of the information used here.

Specimen Investigated

Plagiochila asplenioides (L.) Dumort.

GERMANY: Niedersachsen: Hannover, 11/V/1902, *Hanner 5020* (MN*).

Plagiochila bifaria (Sw.) Lindenb.

BRAZIL: Rio de Janeiro: Itatiaia, Agulhas Negras, 16/I/1925, M.C. *Vaughan Bandeira* s/n (RB*).

Plagiochila buchtiniana Steph.

BOLIVIA: La Paz: Nor Yungas, Parque Nacional Cotapata, 08/X/1997, *J. Heinrichs* et al. *4128* (RB*).

Plagiochila corrugata (Nees) Nees & Mont.

BRAZIL: Minas Gerais: Juiz de Fora, 24/VIII/2011, *L.A. Paiva & E.T. Amorim 320* (CESJ*). **Rio Grande do Sul**: Gramado, 28/XII/ 1945, *A. Sehnem 4742* (PACA). **Santa Catarina:** Santa Cruz, 26/XII/1946, *A. Sehnem 2407* (PACA); São Leopoldo, 30/VII/1941, *A. Sehnem 1103* (PACA).

Plagiochila crispabilis Lindenb.

BRAZIL: Rio de Janeiro: Itatiaia, XII/1924, *P. Occhioni s/n* (RB). **São Paulo**: São Luiz do Pacaitinga, 29/X/2009, *D.P. Costa et al. 5100* (RB*); Eldorado, 26/XI/1974, *D. M. Vital 4946* (RB).

Plagiochila disticha (Lehm. & Lindenb.) Lindenb.

BRAZIL: Acre: Tarauacá, Vale do Alto Juruá, 22/XI/1995, *D.P. COSTA et al. 2769* (RB); 25/XI/1995, *D.P.Costa et al. 2895* (RB*); 18/XI/1995, *D.P. Costa et al. 2685* (RB); *D.P. Costa et al. 2615*(RB); 19/XI/1995, *D.P. Costa et al. 2623* (RB).

Plagiochila fuscolutea Taylor

BOLIVIA: La Paz: Nor Yungas, 05/XI/1997, *H. Anton and J. Heinrichs s/n* (RB*); *J. Henrichs 3915* (RB).

Plagiochila gymnocalycina (Lehm. & Lindenb.) Lindenb.

BRAZIL: Rio de Janeiro: Santa Maria Madalena, 15/V/2007, *N.D. Santos et al. 840* (RB*); Itatiaia, 10/V/2000, *S.R. Gradstein and D.P.Costa 3876* (RB); Serra da Bocaina, 15/V/2000, *S.R. Gradstein and D.P.Costa 3890* (RB).

Plagiochila heteromalla Lehm. & Lindenb.

BRAZIL: Rio de Janeiro: Rio de Janeiro, 19/IV/2006, *N.D. Santos et al. 475* (RB*).

Plagiochila laetevirens Lindenb.

BRAZIL: Rio de Janeiro: Itatiaia, 18/X/1926, *M. Bandeira s/n* (RB).

Plagiochila macrostachya Lindenb.

BRAZIL: Minas Gerais: Serra do Papagaio, 01/XI/2016, *E.T. Amorim 210* (CESJ);

Plagiochila patula (Sw.) Lindenb.

BRAZIL: Acre: Tarauacá, Vale do Alto Juruá, 25/XI/1995, D.P. *Costa et al. 2885* (RB); **São Paulo:** Ubatuba, 28/X/2009, *D.P. Costa et al. 5054* (RB*).

Plagiochila porelloides (Torr. ex. Ness) Lindenb.

UNITED STATES OF AMERICA: Kentucky: Clayhole, 11/IV/2018, *D. N. McLetchie s/n* (UKY).

Plagiochila raddiana Lindenb.

BRAZIL: Acre: Tarauacá, 19/XI/1995, *D.P. Costa et al. 2659* (RB); 17/XI/1995, *D.P. Costa et al. 2596* (RB); Alto Juruá, 01/XII/2000, *D.P. Costa et al. 5067* (RB). **Rio de Janeiro**: Nova Iguaçu, 01/VIII/1957, *E.C. Rente 426* (R*).

Plagiochila rutilans Lindenb.

BRAZIL: **Rio de Janeiro**: Teresópolis, 24/III/1926, *M.C. Vaughan Bandeira s/n* (RB*); Petrópolis, 16/III/1968, *D. Sucre 2453 & P.I.S. Braga 295* (RB).

Plagiochila simplex (Sw.) Lindenb.

BOLIVIA: Cochamamba: Chapare, 08/XII/2007, *D.P. Costa et al. 4863* (RB).

BRAZIL: **Rio de Janeiro**: Itatiaia, 11/V/1902, *P. Dusén 450* (R*)

Plagiochila trichostoma Gottsche

COSTA RICA: San Jose, 20/IX/1999, *J. Heinrichs et al. 4323* (RB*).

RESULTS

The spores of *Plagiochila* genus are isomorphic, monads, apolar to weakly heteropolar, small to large sized, varying form 13.00 µm to 57.80 µm (Table 1, Figs. 1 and 2), subcircular amb; inaperturate. Spore surface ornamentation is formed by granules, but the feature of these processes is variable between the seventeen species studied (Figs. 1 and 2). Based on this variation, four palynological types could be identified under LM and SEM observations: (1) Regular and Delicate Granulate (RD), (2) Irregular and Coarse Granulate (IC), (3) Long Granules with Flattened Apices (LF), and (4) Long and Straight Granules (LS).

Ornamentation pattern I- RD - consists of granules homogeneously distributed on spore surface and presenting a regular shape (Fig. 1A - C). This pattern is represented by *Plagiochila asplenioides* (Fig. 1A), *P. disticha* and *P. patula* (Figs. 1B, C). The spore size in these three species varies from small to medium (Table 1), with a thin sporoderm (Table 2). In observations under LM, this sculpture seems as a blur in optical cut due to its small and fine magnification (Figs. 1A, B), but under SEM, the granules can be easily identified (Fig. 1C).

Ornamentation pattern II – IC - comprises irregular shaped granules, disorderly distributed on spore surface (Figs. 1 D - H), and it is represented by *P. gymnocalycina* (Fig. 1D)*, Plagiochila heteromala, P. laetevirens* (Fig. 1E), *P. porelloides* (Fig. 1F, G) and *P. raddiana* (Fig. H). The spore size varies from small to medium (Table 1) and presents a variable sporoderm thickness (Table 2). *P. gymnocalycina* shows a more elaborated version of this pattern, with overlapped and united granule (Fig. 1D), leading the aspect of gemmae.

Ornamentation pattern III – LF – includes species whose spores present sporoderm surface ornate by elongate granules, irregularly distributed, and having a flattened apex (Figs. 2 A- F). The species that belong to this pattern are *Plagiochila crispabilis* (Figs. 2A, C) and *P. simplex* (Figs. 2B, D, E, F). This kind of granules can barely be seen under LM (Fig. 2A), however, under SEM analyses, it is clear the presence of a flattened apex region (Figs. 2 C-F). Besides, a region with smaller granules restricted in a particular area (Fig. 2D) suggests that this region might was the local of contact during tetrad stage.

Ornamentation pattern IV – LS – this ornamentation pattern consists of spore surface ornate by elongate granules that have a smooth and straight shape, resembling bacula (Fig. 2G-I). The species that belongs to this pattern are: *Plagiochila bifaria*, *P. buchtiniana*, *P. corrugata* (Fig. 2H), *P. fuscolutea*, *P. macrostachya*, *P. rutilans*, and *P. trichostoma* (Figs. 2G, I). We also observed in some species, a region with smaller granules suggesting that the area is the spore proximal pole. In this group, may be highlighted that, in *P. corrugata*, we observed endosporic and intracapsular germination (Fig. 2H).

For all the species studied (Tab. 2), sporoderm thickness varied from 0.9 µm to 1.8 µm. When observed under TEM, the sporoderm presented one (or two) electrontranslucent layer(s) visible, both corresponding to intine (Figs. lI, 2F – inner intine and outer intine); and two electron dense layer compounded by lamellae deposition are also visible (Figs. lI, 2F), corresponding to an exine divided in nexine and sexine. The sexine lamellae have a perpendicular to inclined arrangement (Fig. 1I, 2F). On the spores of *P. disticha*, a stratified intine was observed (Fig. 1I): an inner layer, in contact with cell content, is granular and presents a mix of electrontranslucent

and eletron dense components, while the outer layer, in contact with nexine, has visibly great amount of eletrontranslucent elements.

Descriptive statistical analyses showed that the species analyzed here present statistically different spore size (Fig. 3), and in those species that a comparison specimen was available (see Table 1), the intraspecific spore size variation was also significant (Fig. 4); except in *P. simplex* (Fig. 4I), in which the specimens analyzed did not present any spore size statistical significance.

The size variability of the spores is confirmed by the variation coefficient (Table 1). Lowest values around 8% were observed in *P. buchtiniana* and among RS and CS1 in *P. cripabilis*. Values around 10% were observed in a great number of species, and values bigger than 12% of variation coefficient were detected in at least one specimen analyzed of *P. crispabilis*, *P. fuscolutea*, *P gymnocalycina*, and *P. raddiana*; besides *P. asplenioides*, *P. patula*, and *P. porelloides*. Considering the confidence interval (CI), in almost all species that a comparison specimen could be analyzed, the mean value found at the comparison specimens did not fit the CI established for the reference specimen (Table 1).

Cluster analysis revealed five groups above 0.5 of similarity relation (Fig. 5 - G1 to G5) and presenting a cophenetic correlation coefficient of 0.8138. G1 group assembles *P. disticha*, *P. laetevirens*, *P. patula*, and *P. raddiana*; G2 group reunites *P. asplenioides*, *P. buchtiniana*, *P. corrugata*, and *P. porelloides*. G3 is the lager one and is represented by five species: *P. fuscolutea*, *P. gymnocalycina*, *P. rutilans*, *P. trichostoma*, and *P. simplex*; G4 is formed by *P. crispabilis* and *P. macrostachya*, and G5 is represented by only one species, *P. heteromalla*. Observing the binary matrix (Table 5), it is possible to unravel the shared characteristics between species of the same

group. In G1, species share type of ornamentation, which is rounded granules, the sporoderm thickness, *Frullania*-type as the type of branching, generalists regarding substratum occupation, single androecia, perianth covered by bracts, and presence of any type of asexual reproduction. In G2, species also shares single androecia and perianth covered by bracts, but do not present am asexual reproduction structure, and are exclusive in regard to substratum occupation. Besides, the terminal androecia position is also a joining variable, and the branching type is variable. In G3, species are generalist and present *Plagiochila*-type as branching type and single androecia; while in G4, species are generalist, fan-shaped androecia, and perianth covered by bracts. In this last group, species shared some spore characteristics as ornamentation and sporoderm thickness. *P. heteromalla* were placed on its own separate group, named G5.

DISCUSSION

Plagiochila (Dumort.) Dumort. is an important genus, worldwide distributed, and represents one of the most speciose among liverworts; palynological information can lead to a better understanding on its taxonomy and ecology of these species. Here, we presented that spores in this genus are variable, especially concerning to spore size and ornamentation of sporoderm, that allow separating the studied species into four spore types.

Spore Size in Plagiochila *(Dumort.) Dumort*.

Spore size average values in *Plagiochila* varied from 13.80 µm to 57.05 µm, being classified from small to large (ERDTMAN, 1952). Among liverworts, spore size is a variable characteristic. For example, in *Chonecolea doelligeri* (Ness) Grolle (Chonecoleaceae), spores presented 16.00 µm at polar view (YANO & LUIZI-PONZO, 2006). In other species belonging to Frullaniaceae Lorch (ZHAO et al., 2011),

Dumortieraceae D.G. Long (YANO & LUIZI-PONZO, 2011), Porellaceae Cavers (ZHAO et al., 2011), and Ricciaceae Rchb. (STEIKAMP & DOYLE, 1979; ZHAO et al., 2011), spore size varied drastically from 31 µm (medium size) to 127 µm (giant size).

ERDTMAN (1965) described the spores of *P. asplenioides* with 15 µm of average size, and VOJTKO (1993) described the spores of *P. porelloides* with 17.80 µm, related to our finds. Some taxonomic studies brought notes about spores in this genus, especially due to size and ornamentation surface. HEINRICHS & GRADSTEIN (2000) described *P. disticha* with size amplitude of 16 µm to 28 µm (- 47 µm), and *P. raddiana* with 18 µm to 25 µm (- 45 µm); HEINRICHS et al. (2000) noted *P. buchtiniana* spores with amplitude of 33 µm to 54 µm; HEINRICHS et al. (2001) observed that spores in *P. rutilans* had amplitude of 23 µm to 28 µm; HEINRICHS et al. (2004a) noted *P. corrugata* spores with 18 µm to 52 µm. We presented in this study a slighter variation of amplitude to the spores of these species.

Lophocoleineae Schljakov, which includes Plagiochilaceae, presents exosporic spore germination (CRANDALL-STOTLER et al., 2009), but some studies (HEINRICHS et al., 2000; HEINRICHS et al., 2002, 2004a) report *Plagiochila* spores as released with 1-8 cells. We also observed endosporic germination, and the releasing of spores with 1-5 cells (and even intracapsular germination) in *P. corrugata*.

We observed a variation coefficient of spore size ranging from 8% to 12 %. A variation coefficient around 10% is commonly found in palynological treatments of bryophytes, as observed in the works of LUIZI-PONZO & BARTH (1998, 1999), LUIZI-PONZO & MELHEM (2006), ROCHA et al. (2008), CALDEIRA et al. (2009; 2013), YANO & LUIZI-PONZO (2011), and RODRIGUES & LUIZI-PONZO (2015).

For *Plagiochila*, HEINRICHS (2002) stated that the average size of the spores in *Plagiochila* may vary considerably, and HEINRICHS & GRADSTEIN (1999) observed a variation of ca 50% on spore size in *Plagiochila longiramea* Steph.

Sporoderm structure and surface ornamentation

Two layers of sporoderm were observed, intine and exine, as expected for bryophytes spores (CLARKE, 1979; MOGENSEN, 1983; NEIDHART, 1979). ERDTMAN (1965) pointed that spore of *P. asplenioides* had a very thin exine. The sporoderm of the species studied is quite thin, with no more than 2 µm of thickness and even thinner than 1 µm for some species.

Analyses made under TEM revealed that exine is divided into nexine, an inner layer, and sexine, an outer layer. Similar configurations to this pattern of exine were observed in mosses and liverwort spores (BROWN et al., 2015; BROWN & LEMMON, 1988; CALDEIRA et al., 2013; ESTÉBANEZ et al., 1997; HECKMAN, 1970; ROCHA et al., 2008; RODRIGUES & LUIZI-PONZO, 2015; SILVA-E-COSTA, 2015; STEIKAMP & DOYLE, 1979; YANO & LUIZI-PONZO, 2006, 2011).

Lamellar deposition of exine elements in liverworts has been early reported by HECKMAN (1970) and BROWN & LEMMON (1988). In our study, we observed a perpendicular sexine elements in two species, P. *disticha* and *P. simplex*. Similar sexine patterns were observed in two other liverworts, *Lophocolea heterophylla* (Schrad.) Dumort. and *Chiloscyphus polyanthos* (L.) Corda, in the work of Heckman 1970. The author described these elements as “lamellar slips”, shaping the spore ornamentation. Both species studied by HECKMAN (1970) belong to Lophocoleaceae Vanden Berghen, family also placed in Lophocoleineae, as is Plagiochilaceae.

In *P. disticha*, two light electron dense layers were identified, corresponding to intine. Intine is the last layer to be formed and it is directly related to spore germination (BROWN & LEMMON, 1988; MOGENSEN, 1983; NEIDHART,1979). MOGENSEN (1983) pointed that intine may have little stratification, as we observed here; HECKMAN (1970), studying the spore wall structure in Jungermanniales, presented a similar configuration of a stratified intine in *L. heterophylla*. Even though, she has not made comments of this configuration in the text, the stratification is easy recognized analyzing the image provided by the paper.

MCCLYMONT & LARSON (1964) also described a multistratified intine for *Archidium alternifoliuim* (Dicks.) Schimp; NILSSON (1990) observed it in pollen grains of Apocynaceae Juss. Estebánez *et al.* (1997) observed one to three-layered intine in *Grimmia* Hedw.; LUIZI-PONZO & MELHEM (2006) described a stratified intine on mature spores of *Helicophyllum torquatum* (Hook.) Brid.; and finally, MEDINA & ESTEBÁNEZ (2014) have reported intine stratification on *Orthotrichum ibericum* F. Lara & Mazimpaka and *Orthotrichum striatum* Hedw. Spores, and argued that an intine stratification may be due to environmental and development spore condition.

The different exine element deposition observed in certain spores may be related to the early contact during spore wall formation, at the proximal pole. BROWN & LEMMON (1991) observed that in liverworts spore wall formation, especially in Jungermanniidae, the exine at proximal face is generally thinner and less ornamented in comparison with other spore wall areas, and might be a region for germination, when it is the time.

Some authors have described some *Plagiochila* spores as ornate by bacula (HEINRICHS & Grastein, 2000; HEINRICHS et al., 2001; HEINRICHS, 2002;

HEINRICHS et al., 2004a; HEINRICHS et al., 2005a, 2005b) or verrucate, even vermiculate (GROTH et al., 2003; HEINRICHS, 2002, 2004b; INOUE, 1982; MULLER, et al. 1999;). SCHUSTER (1980), in his treatment of Plagiochilaceae of North America, described the spore's ornamentation as "fine granulose". HEINRICHS (2002), in a compilation of literature data, described two basic patterns to *Plagiochila* spore's ornamentation: (1) verrucate-vermiculate structures and (2) bacula structures (varying from "bacula *sensu strictu*" to pila). Observing the images provided by these authors, we can conclude that these ornamentation patterns are, actually, granules in a different and variable shape and morphology. PUNT et al. (2007) defined *bacula* as any "free standing exine ornamentation process with more than 1 μm in length, and less than this in diameter". In the same way, *verrucate* as an element with more than 1 μm wide; *vermiculate* is used to describe *rugulate* pollen and spores. Thus, none of these term fits *Plagiochila* spore ornamentation.

In the present study, we observed elements with less than 1μm length and wide, when bigger than this, the elements were clearly united, forming groups of processes. HEINRICHS (2002) stated that *Plagiochila* spores are, necessarily, trilete with a weakly developed laesure, under SEM observation. Due to their thin and delicate spore wall, spores of *Plagiochila* species are easily damaged under the SEM processes, giving the aspect of an irregular trilete mark. But, when spores are observed under different kinds of preparation, this affirmative is clearly denied. We could observe spores under LM (Figs. 1A, B, D, E; 2A, B, G), before the acetolysis, and we can affirm they are not trilete, but tremendously fragile and easily molded. The fragility is related to the thin sporoderm, and especially concerned to the nexine delicacy, which is folded under SEM preparations (Figs. 1F, H; 2C, E, H), however, it is important to note that, in spite of the

fragility, the sporoderm tolerates the acetolysis method, due its sporopollenin content (BROWN & LEMMON, 1988; MOGENSEN, 1983).

The hierarchical clustering interpretation and taxonomic implications

The hierarchical clustering analysis revealed five groups with cophenetic correlation coefficient higher than 0.8, which represents low distortion and representatively reliable data (ROHLF & FISHER, 1968). These five groups, G1 to G5, did not correspond to the current infrageneric classification. Considering the binary matrix and the common characteristics shared by species at the same group, for G1 to G4, most of these characteristics are related to gametophyte morphology. Besides, species of the same ornamentation type described in this study were also separated in cluster analysis. To G1 and G4, spore ornamentation was a shared variable. In G2 and G3, surface ornamentation was variable into groups. Although these groups did not correspond directly either to infrageneric circumscription or spore ornamentation types, the palynological information added to gametophyte morphology clearly formed new groups, suggesting that spore data may contribute for understanding the phylogeny of *Plagiochila*. Spore morphology is a genetic marked characteristic (CLARKE, 1979) and carry evolutive information that can be used to improve phylogenetic analyses.

Palynological approach contributes to taxonomic studies, especially to taxa that present determination problems. In our study, *Plagiochila* (Dumort.) Dumort. species studied are quite homogeneous regarding gametophyte characteristics, but different in spore structure, size, sporoderm strata, and surface ornamentation. As demonstrated here, different ornamentation processes were observed, and they can be used to ensure species description.

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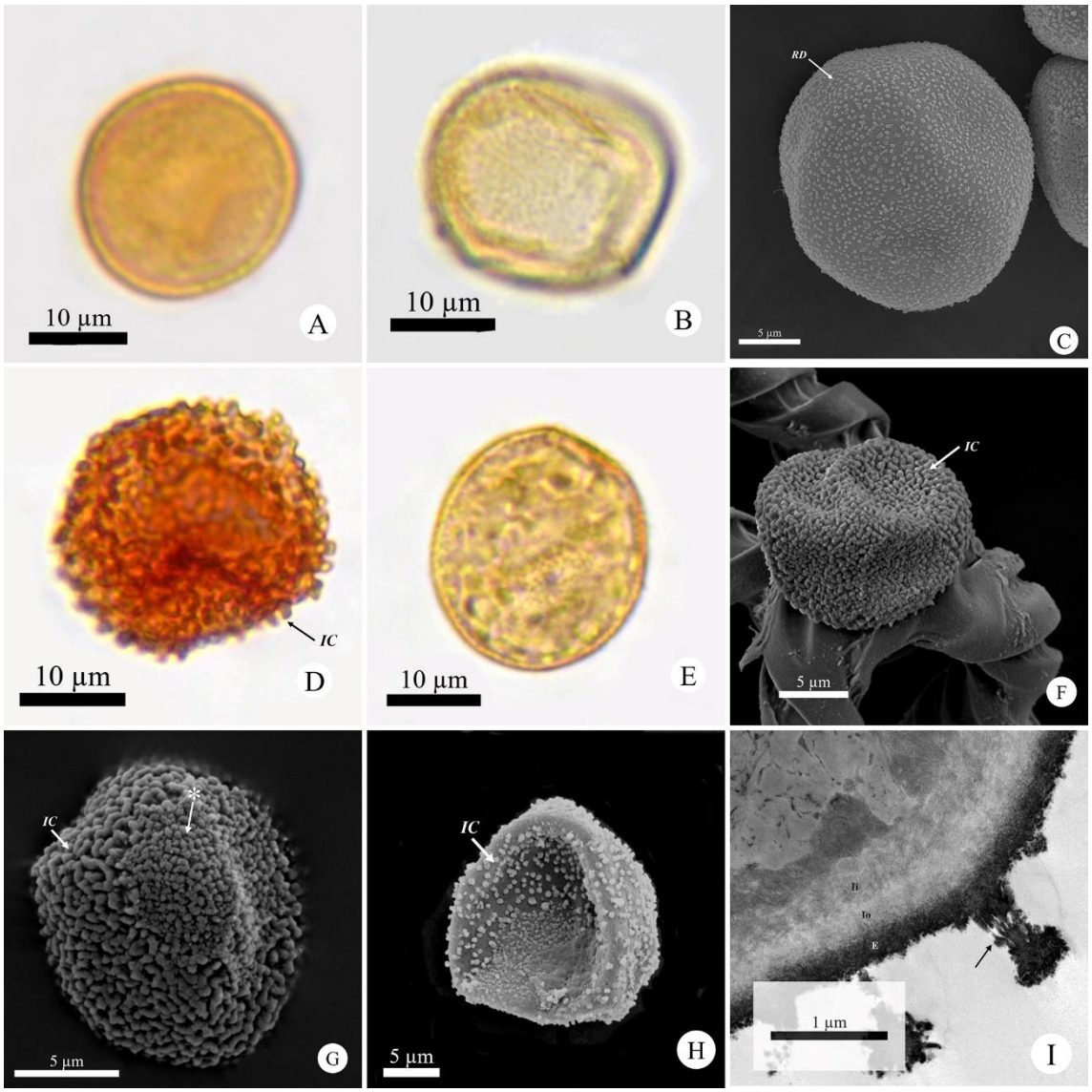

Figure 1. Photomicrographs and electron micrographs of *Plagiochila* (Dumort.) Dumort. Ornamentation type I - A: *Plagiochila asplenioides*, LM; B: *P. patula*, LM; C: *P. patula*, SEM; Ornamentation type II – D: *P. gymnocalycina*, LM; E: *P. laetevirens*, LM; F – G: *P. porelloides*, SEM – arrow with asterisks: proximal face; H: *P. raddiana*, SEM; I: *P. disticha*, TEM – arrow: lamellar slips. RD = regular and delicate granule; IC = irregular and coarse granule. In I: In = inner intine, Io = outer intine, E = exine.

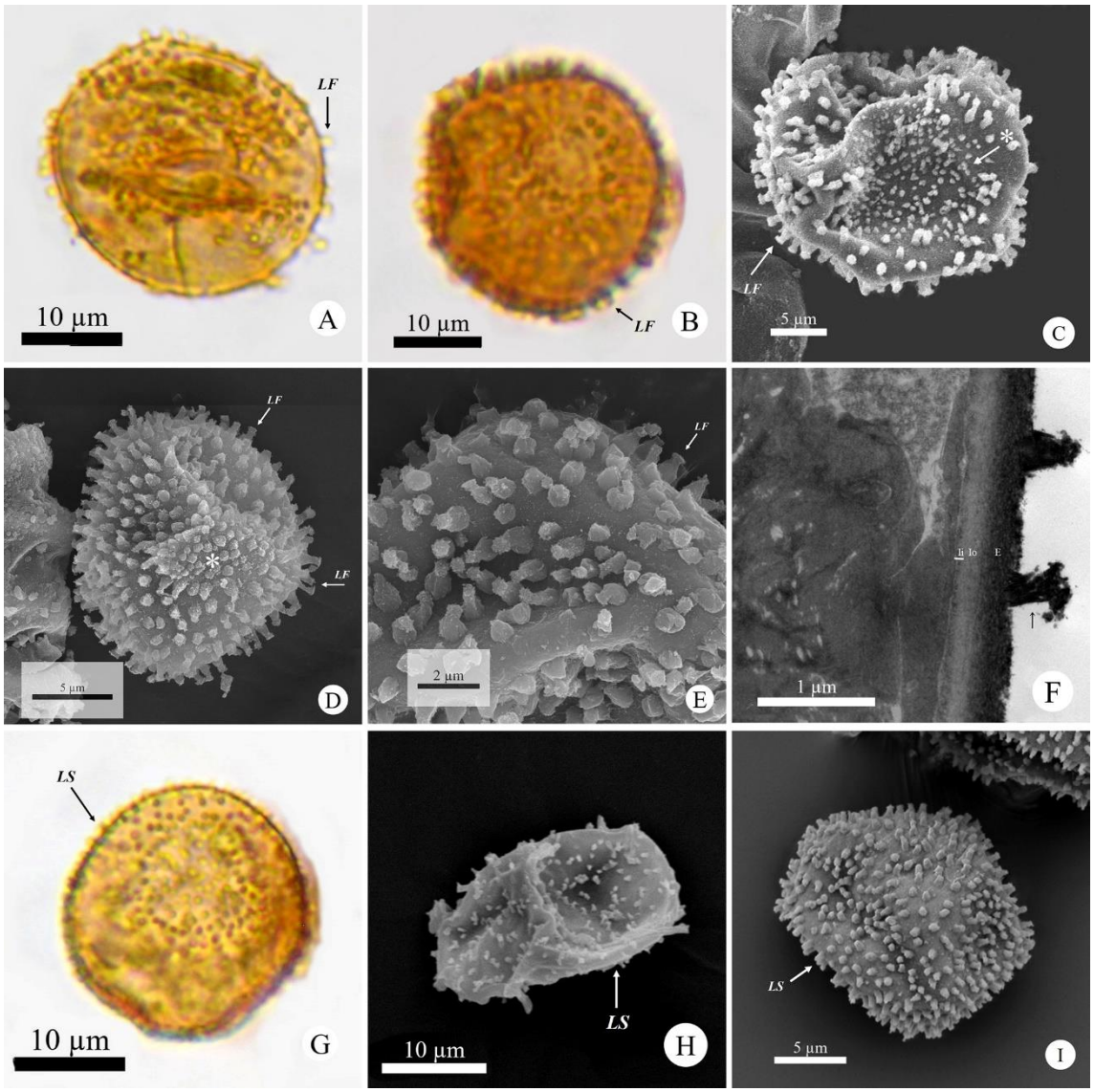

Figure 2. Photomicrographs and electron micrographs of *Plagiochila* (Dumort.) Dumort. Ornamentation type III – A: *Plagiochila crispabilis*, LM; B: *P. simplex*, LM; C: *P. crispabilis*, SEM – arrow: long granules with a flattened apex; D-E: *P. simplex*, SEM – arrow: long granules with a flattened apex, asterisks: proximal face; F: *P. simplex*, TEM; G: *P. trichostoma*, LM; H: *P. corrugata*, SEM; I: *P. trichostoma*, SEM. In F: In = inner intine, Io = outer intine, E = exine, arrow – lamellar slips.

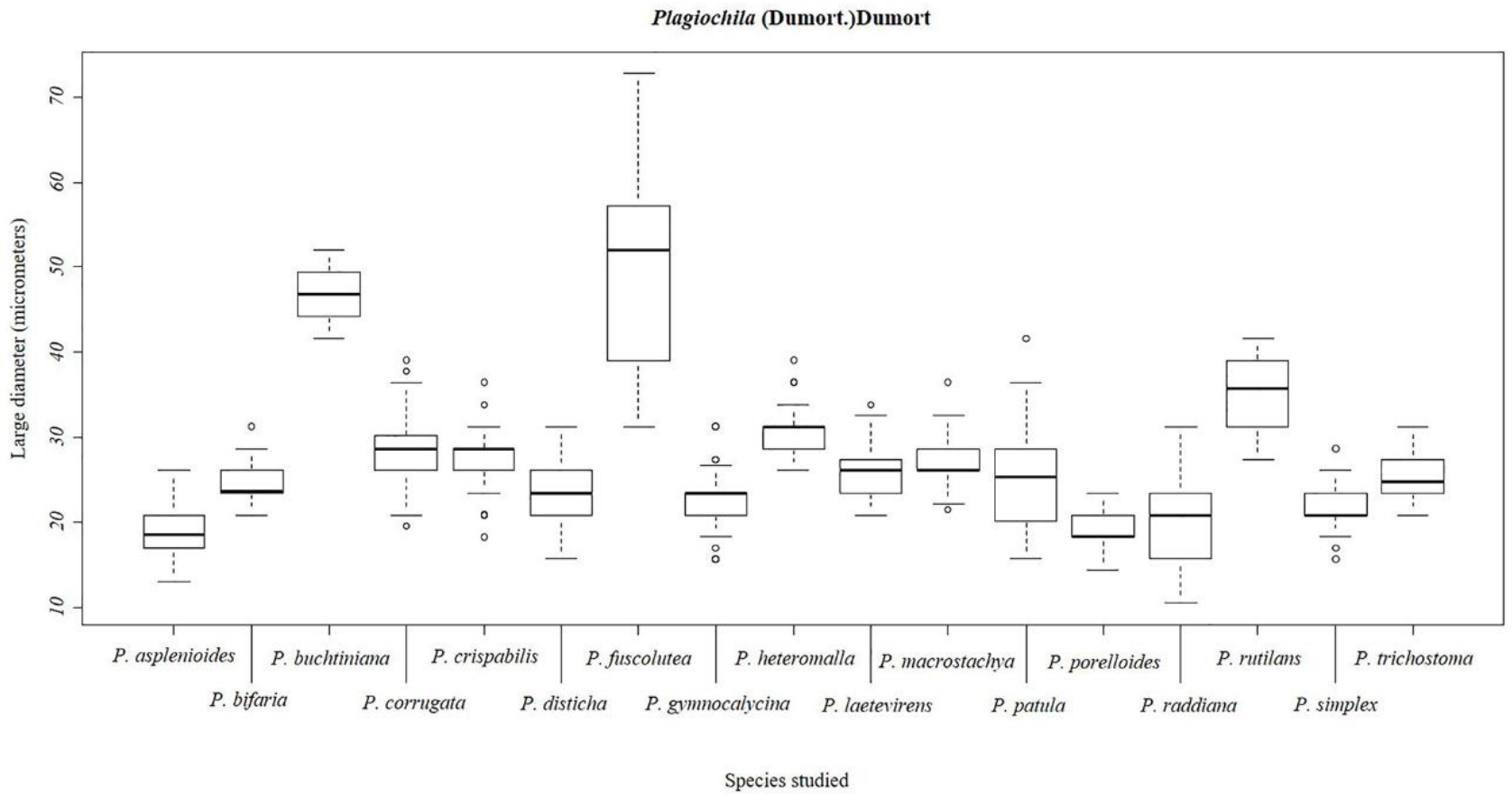

Figure 3. Boxplots representing the spore size distribution in *Plagiochila* (Dumort.) Dumort. Error bars above and below the box indicate the 90th and 10th percentiles, and white circles represent the outliers.

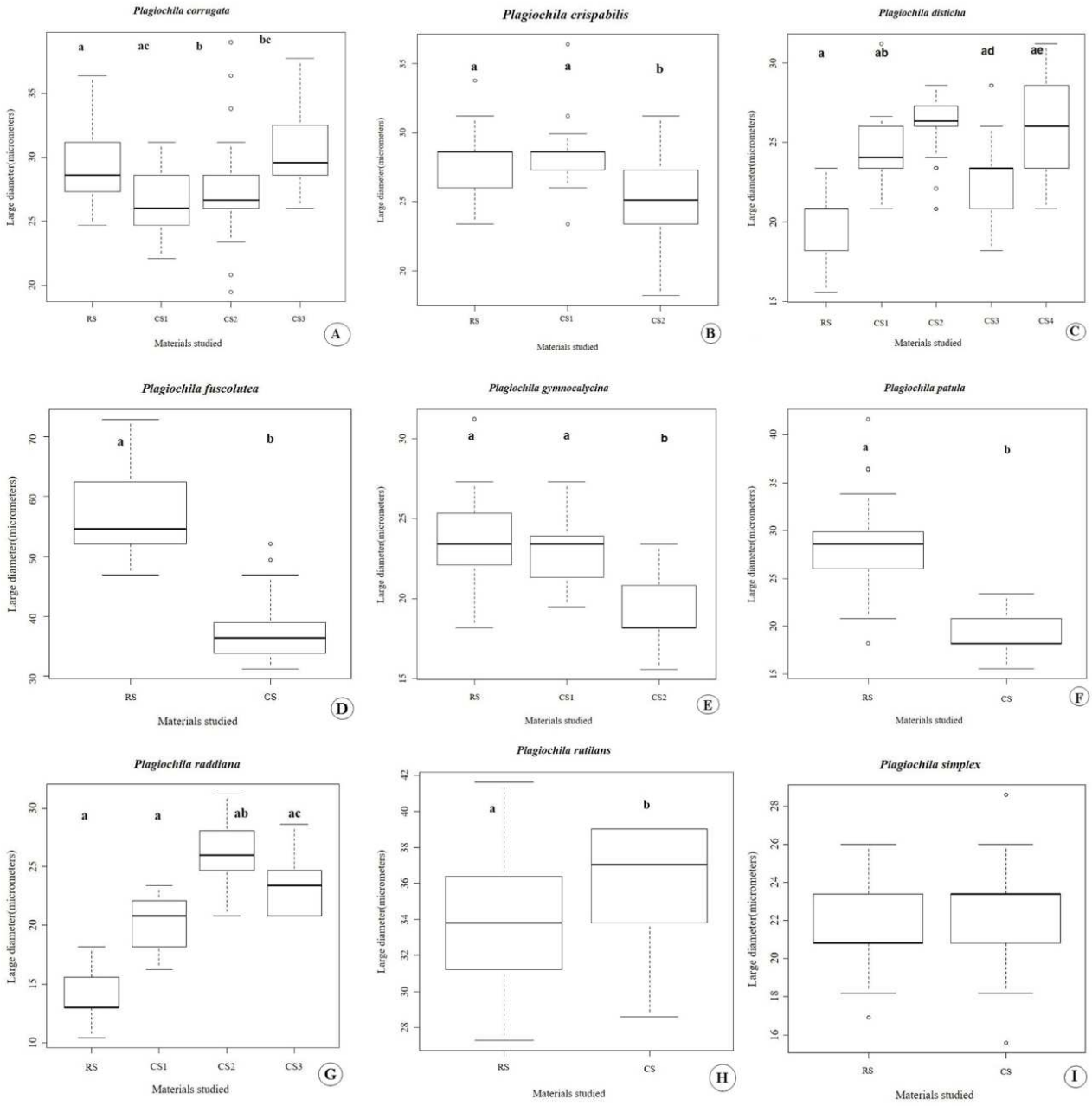

Figure 4. Boxplots representing the spore size distribution in those species which RS and CS were observed. Error bars above and below the box indicate the 90th and 10th percentiles, and white circles represent the outliers. Different letters (a, b, c, d, e) represent statistical differences among treatments (Krukal -Wallis test, Dunnett's test post hoc, $p < 0.05$).

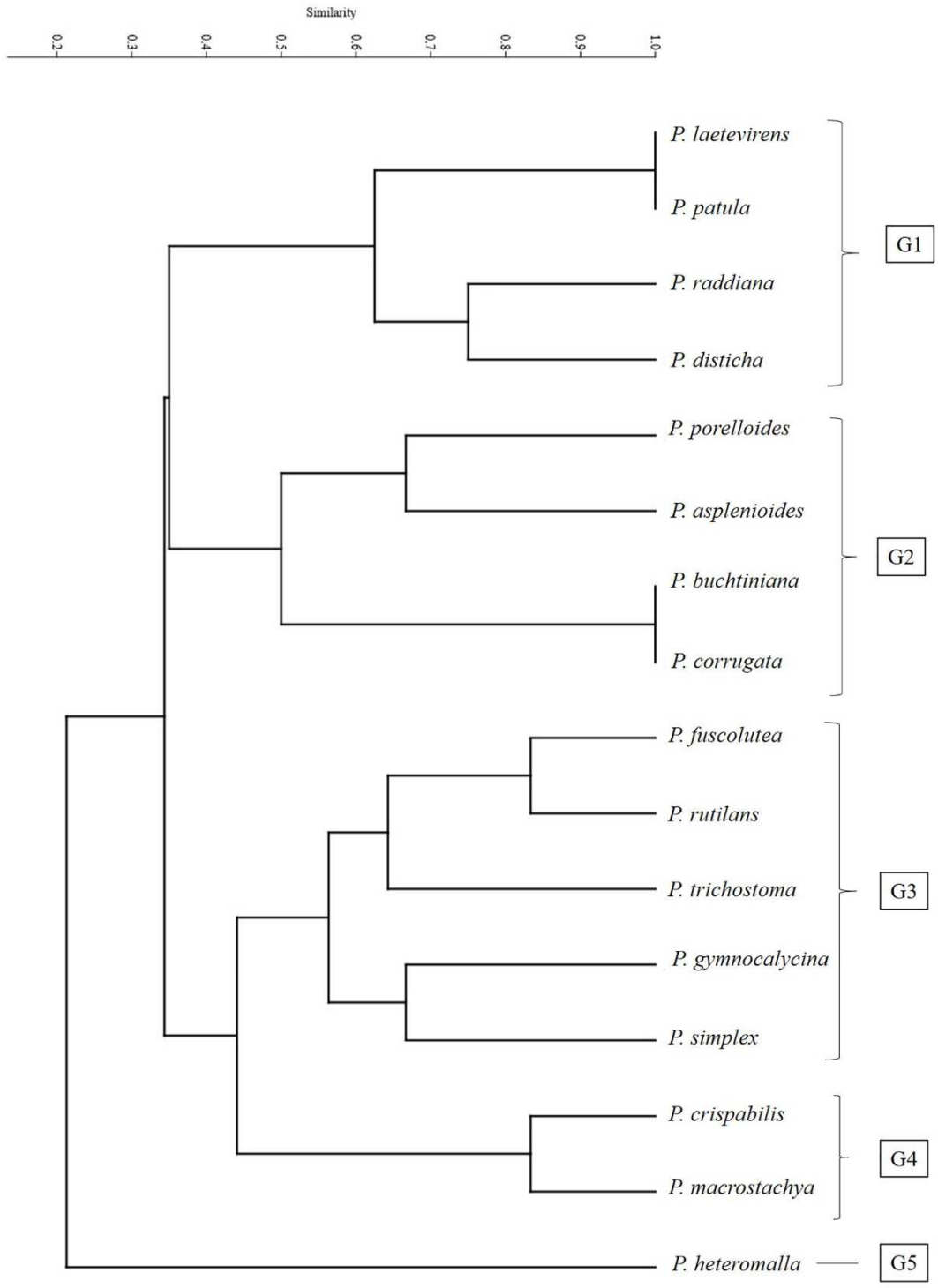

Figure 5. Hierarchical clustering analysis representation, showing the five groups of species, in *Plagiochila* (Dumort.) Dumort. UPGMA algorithm, Jaccard similarity index, Cophenetic correlation = 0.8130.

Table 1. Morphometric data of acetolyzed spores of *Plagiochila*. In Reference Specimen, indicated by an asterisk, n=50; Comparison Specimens, n=30.

Material	(X_{min}-X_{max})	$X \pm S_x$	S	95% IC	CV (%)
Plagiochila asplenioides (L.) Dumort. Hanner, 5020 (MN) *	13.00 – 26.00	18.79 ± 0.36	2.60	18.06 – 19.52	13.84
Plagiochila bifaria (Sw.) Lindenb. M.C. Vaughan Bandeira (166934/RB)*	20.80 – 31.20	24.50 ± 0.32	2.28	23.85 – 25.15	9.30
Plagiochila buchtiniana Steph. J. HEINRICHS *et al*., 4128 (RB) *	41.60 – 52.00	46.54 ± 0.38	2.74	45.77 – 47.31	5.88
Plagiochila corrugata (Nees) Nees & Mont.					
L.A. Paiva, 320 (CESJ) *	24.70 – 36.40	29.22 ± 0.38	2.70	28.46 – 29.98	9.24
A. Sehnem, 2407 (PACA)	22.10 – 31.20	26.21 ± 0.42	2.34	25.37 – 27.05	8.92
A. Sehnem, 1103 (PACA)	19.50 – 39.00	27.84 ± 0.71	3.92	26.38 – 29.30	14.08
A. Sehnem, 4742 (PACA)	26.00 – 37.70	30.57 ± 0.58	3.20	29.38 – 31.76	10.46
Plagiochila crispabilis Lindenb.					
D.P. COSTA *et al*., 5100 (RB) *	23.40 – 33.80	27.89 ± 0.30	2.18	27.27 – 28.5	7.81
D.M. Vital, 4946 (RB)	23.40 – 36.40	28.21 ± 0.39	2.19	27.40 – 29.02	7.76
P. Occhioni, s/n (166931/ RB)	18.20 – 31.20	25.22 ± 0.59	3.27	24.00 – 26.44	12.96
Plagiochila disticha (Lehm. & Lindenb.) Lindenb.					
D.P. COSTA *et al*., 2895 (RB) *	15.60 – 23.40	20.18 ± 0.23	1.68	19.70 – 20.66	8.32
D.P. COSTA *et al*., 2769 (RB)	20.80 – 31.20	24.32 ± 0.39	2.11	23.52 – 25.12	8.67
D.P. COSTA *et al*., 2685 (RB)	20.80 – 28.60	25.93 ± 0.41	2.22	25.09 – 26.77	8.56
D.P. COSTA *et al*., 2615 (RB)	18.20 – 28.60	22.99 ± 0.44	2.41	22.09 – 23.89	10.48
D.P. COSTA *et al*., 2623 (RB)	31.20 – 20.80	25.87 ± 0.48	2.67	24.88 – 26.86	10.32
Plagiochila fuscolutea Taylor					
H. Anton & J. HEINRICHS (373220/RB)	46.80 – 72.80	57.05 ± 0.89	6.35	55.25 – 58.85	11.13
J. Henrichs, 3915 (RB)	31.20 – 52.00	37.65 ± 0.92	4.99	35.76 – 39.54	13.25
Plagiochila gymnocalycina (Lehm. & Lindenb.) Lindenb.					
N.D. Santos *et al*.,840 (RB) *	18.20 – 31.20	23.46 ± 0.41	2.91	22.63 – 24.29	12.40
D.P.COSTA, 3890 & S.R. GRADSTEIN (RB)	19.50 – 27.30	23.27 ± 0.36	1.99	22.52 – 24.02	8.55
D.P.COSTA, 3876 & S.R. GRADSTEIN (RB)	15.60 – 23.40	19.34 ± 0.45	2.45	18.41 – 20.27	12.66
Plagiochila heteromalla Lehm. & Lindenb.					
N.D. Santos *et al*., 475 (RB) *	26.00 – 39.00	30.94 ± 0.38	2.71	30.17 – 31.71	8.75
Plagiochila laetevirens Lindenb.					
M. Bandeira (223901/RB) *	20.80 – 33.80	26.17 ± 0.37	2.66	25.42 – 26.92	10.16
Plagiochila macrostachya Lindenb.					
E. T. Amorim, P2 (CESJ) *	21.45 – 36.40	26.87 ± 0.38	2.3	26.10 – 27.64	10.16
Plagiochila patula (Sw.) Lindenb.					

D.P. COSTA *et al*., 5054 (RB) *	18.20 – 41.60	28.16 ± 0.61	4.30	26.94 – 29.38	15.26
D.P. COSTA *et al*., 2885 (RB)	15.60 – 23.40	19.24 ± 0.48	2.63	18.26 – 20.22	13.66

Table 1. continuance

Material	(X_{min}-X_{max})	X ± S_x	S	95% IC	CV (%)
Plagiochila porelloides (Torr. ex. Ness) Lindenb.					
D.N. McLetchie, s/n (UKY) *	14.30 – 23.40	19.43 ± 0.36	2.56	18.71 – 20.15	13.17
Plagiochila raddiana Lindenb.					
E.C. Rente, 426 (MN) *	10.40 – 18.20	13.80 ± 0.24	1.70	13.32 – 14.28	12.31
D.P. COSTA *et al*., 2659 (RB)	16.25 – 23.40	20.29 ± 0.40	2.17	19.47 – 21.11	10.69
D.P. COSTA *et al*., 5062 (RB)	20.80 – 31.20	26.06 ± 0.52	2.86	25.00 – 27.12	10.97
D.P. COSTA *et al*., 2596 (RB)	20.80 – 28.60	23.44 ± 0.37	2.06	22.68 – 24.20	8.78
Plagiochila rutilans Lindenb.					
M.C. Vaughan Bandeira (166936/RB) *	27.00 – 41.60	34.16 ± 0.48	3.45	33.18 – 35.14	10.09
D. Sucre 2453. & P.I.S. Braga (RB)	28.60 – 39.00	36.31 ± 0.52	3.23	35.11 – 37.51	8.89
Plagiochila simplex (Sw.) Lindenb.					
P. Dusén, 450 (MN) *	16.90 – 26.00	21.36 ± 0.27	1.95	20.81 – 21.91	9.12
D.P. COSTA *et al*., 4863 (RB)	15.60 – 28.60	22.44 ± 0.45	2.50	21.51 – 23.37	11.14
Plagiochila trichostoma Gottsche					
J. HEINRICHS *et al*., 4323 (RB)*	20.80 – 31.20	25.15 ± 0.38	2.69	24.39 – 25.91	10.69

Table 2. Morphometric data of sporoderm thickness of spores in *Plagiochila* (n=10).

Species	Sporoderm thickness (µm)
P. asplenoides	1.5327
P. buchtiniana	1.2636
P. corrugata	1.3923
P. crispabilis	1.6497
P. disticha	1.2519
P. fuscolutea	1.5444
P. gymnocalycina	2.0241
P. heteromala	1.4742
P. laetevirens	1.3221
P. macrostachya	1.8369
P. patula	1.17
P. porelloides	1.1457
P. raddiana	0.9594
P. rutilans	1.8837
P. simplex	1.5561
P. trichostoma	1.2168

Table 3. Palynological, gametophytic and ecological aspects of the studied species of *Plagiochila* (Dumort.) Dumort.

Species	Spore Size	Spore Ornamentation	Sporoderm thichness	Branching type	Substratum occupation	Androecia shape	Androecia position	Perianth base	Asexual reproduction	Section
P. asplenioides	Small	RD	1.5327	*Frullania*	Exclusive	Single	Intercalary	Bracts	Absent	*Plagiochila*
P. buchtiniana	*Medium*	LS	1.2636	*Frullania*	Exclusive	Single	Intercalary	Bracts	Absent	*Glaucescentes*
P. corrugata	*Medium*	LS	1.3923	*Frullania*	Exclusive	Single	Intercalary	Bracts	Absent	*Vagae*
P. crispabilis	Medium	LF	1.6497	*Frullania*	Generalist	Fan-Shaped	Terminal	Bracts	Propagules	*Vagae*
P. disticha	Medium	RD	1.2519	*Frullania*	Generalist	Single	Terminal, Intercalary	Bracts	Propagules	*Vagae*
P. fuscolutea	Large	LS	1.5444	*Plagiochila*	Generalist	Single	Intercalary	Bracts	Absent	*Fuscolutea*
P. gymnocalycina	Small	IC	2.0241	*Plagiochila*	Generalist	Single	Intercalary	Naked	Caducous leaves	*Rutilantes*
P. heteromalla	Medium	IC	1.4742	*Plagiochila*	Exclusive	Single	Terminal	Naked	Caducous leaves and fragments	*Rutilantes*
P. laetevirens	Medium	IC	1.3221	*Frullania*	Generalist	Sngle	Terminal, Intercalary	Bracts	Propagules	*Vagae*
P. macrostachya	Medium	LS	1.8369	*Plagiochila*	Generalist	Fan-Shaped	Terminal	Bracts	Caducous leaves	*Hylacoetes*
P. patula	Medium	RD	1.1700	*Frullania*	Generalist	Single	Intercalary	Bracts	Propagules	*Vagae*
P. porelloides	Small	IC	1.1457	*Plagiochila*	Exclusive	Single	Terminal, Intercalary	Bracts	Absent	*Plagiochila*
P. raddiana	Medium	IC	0.9594	*Frullania*	Generalist	Single	Terminal	Bracts	Propagules	*Vagae*
P. rutilans	Medium	LS	1.8837	*Plagiochila*	Generalist	Single	Intercalary	Bracts	Caducous leaves	*Rutilantes*
P. simplex	Small	LF	1.5561	*Plagiochila*	Generalist	Single	Intercalary	Naked	Absent	*Vagae*
P. trichostoma	Medium	LS	1.2168	*Plagiochila*	Generalist	Single	Intercalary	Bracts	Caducous leaves and fragments	*Rutilantes*

Table 4. Binary matrix showing the arrangement of palynological, gametophytic and ecological aspects of *Plagiochila* (Dumort.) Dumort. Spore size: <26 µm = 1, ≥ 26 µm =0; Spore ornamentation: Types RD and IR = 0, Types LF and LS = 1; Sporoderm thickness: ≥1.4900 µm = 1; < 1.4900 µm = 0; Branching type: *Plagiochila*-type = 1; *Frullania*-type = 0; Substratum occupation: exclusive = 1, generalist = 0; Androecia shape: fan-shaped = 1, single = 0; Androecia position: intercalary = 1, terminal = 0; Perianth base: bracts present = 1, naked = 0; Asexual reproduction: present = 1, absent = 0.

Species	Size	Ornamentation	Sporoderm Thickness	Branching Type	Substrum Occupation	Androecia Shape	Androecia Position	Perianth Base	Asexual Reproduction
P. asplenioides	1	0	1	0	1	0	1	1	0
P. buchtiniana	0	1	0	0	1	0	1	1	0
P. corrugata	0	1	0	0	1	0	1	1	0
P. crispabilis	0	1	1	0	0	1	0	1	1
P. disticha	1	0	0	0	0	0	1	1	1
P. fuscolutea	0	1	1	1	0	0	1	1	0
P. gymnocalycina	1	0	1	1	0	0	1	0	1
P. heteromalla	0	0	0	1	1	0	0	0	1
P. laetevirens	0	0	0	0	0	0	1	1	1
P. macrostachya	0	1	1	1	0	1	0	1	1
P. patula	0	0	0	0	0	0	1	1	1
P. porelloides	1	0	0	1	1	0	1	1	0
P. raddiana	1	0	0	0	0	0	0	1	1
P. rutilans	0	1	1	1	0	0	1	1	1
P. simplex	1	1	1	1	0	0	1	0	0
P. trichostoma	1	1	0	1	0	0	1	1	1

Chapter Two

Variation in population sex ratio and evaluation of sexual reproduction and environmental factors in *Plagiochila porelloides*

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*This chapter is under preparation for publication.

Variation in population sex ratio and evaluation of sexual reproduction and environmental factors in *Plagiochila porelloides*.

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ABSTRACT

Successful sexual reproduction requires both sexes and can be limited by the rarer sex. Variation in sex ratio has been correlated with numerous environmental factors in vascular plants, but there are few studies on bryophytes, where population sex ratios can be extreme. Using the liverwort *Plagiochila porelloides*, we tested for sex ratio heterogeneity among populations, a relationship between sex ratio and fertilization success, and correlations between sex ratio and light environment (light quantity and canopy openness), topography and species richness. We assessed sex ratios and fertilization success using a 50 cm transect in 29 populations. For environment factors we 1) estimated light conditions with hemispherical photographs, 2) measured slope and aspect, and 3) calculated species richness via identification of bryophyte species present in the transects of each population. The expressed sex ratios in *P. porelloides* populations are heterogeneous varying froam all male to all female but was overall male-biased (0.375 ♀:1 ♂, an unexpected pattern in bryophytes), and associated with fertilization. Sex ratio was not related to any of the environmental factors. The male biased sex ratio found in this species, which belongs to an ecologically understudied yet the most specious liverwort genus, may challenge the general expectation of female biased sex ratios in non-vascular plants.

Key-Words: biased sex ratio; bryophyte population; community characteristics; light quantity; reproductive biology; species richness.

INTRODUCTION

Sexual reproduction is critical for population persistence and to maintain evolutionary potential (FISHER, 1930; LLOYD, 1980; WEISMANN, 1889); and requires a male and a female to occur in close proximity to ensure fertilization, especially in taxa with limited sperm dispersal distance (KNIGHT et al., 2005; LONGTON, 1976; LONGTON & SCHUSTER, 1983; RYDREN et al., 2006; VAN DER VELDE et al., 2001). An unbalanced population sex ratio, especially if the distance between individuals is relatively large, can negatively impact sexual reproduction and consequently population maintenance, and genetic variation (e.g. VANDEPITTE et al., 2010).

In plants species with unisexual individuals (dioecious species) population sex ratios vary and can be characteristically biased against one sex or the other across major plant taxa. Among seed plants, the sex ratio is generally male biased (ABE et al., 2002; ANDERSON & LEVINE, 1982; BARRET et al., 2010; GARCIA & ANTOR 1995; GLODLEY 1964; LENZA & Oliveira 2006; NICROTA, 1998; OPLER & BAWA, 1978; SINCLAIR et al., 2012) but it can be female biased in herbaceous and willow species (FIELD et al., 2013a; LLOYD, 1974; PUCHOLT et al., 2017). In seed plant species, variation in sex ratios can be associated with environmental gradients including moisture (FIELD et al., 2013b; SOLDAAT et al., 2000), light (VANDEPITTE et al., 2010), and landscape fragmentation (YU & LU, 2011).

Alternatively, in bryophytes, sex ratios are mostly female biased (BISANG & HEDENAS, 2005; BOWKER et al., 2000; GLIME & BISANG, 2017; HAIG, 2016; RYDGREN et al., 2010;). Evidence of a relationship between sex ratios and environmental gradients is subtle (BENASSI et al., 2011; BOWKER et al., 2000; STARK et al., 2005; among others) but see Pettet (1967) for differential exposure to light and moisture, Groen et al. (2010b) for sex

differential response to canopy openness, and MACIEL-SILVA et al. (2012) for altitudinal responses.

While bryophyte sex ratio studies have been increasing, most empirical studies on bryophyte sex ratios use mosses, the most specious of the bryophyte group (GOFFINET et al., 2009; GRADSTEIN et al., 2001). When liverworts are used, they are generally thalloid with very few studies on leafy liverworts (see BISANG & HEDENAS, 2005 for a review and references therein) and rarely are hornworts used (see RENZAGLIA & MCFARLAND, 1999 for an exception). There is no detailed sex ratio study on the most specious liverwort group, the leafy liverwort genus *Plagiochila* (Dumort.) Dumort (GRADSTEIN et al., 2001; HEINRICHS, 2002) with ca. 700 species worldwide (HEINRICHS, 2002; SO & GROLLE, 2000; SÖDERSTRÖM et al., 2016).

Studies on this genus have focused primarily on taxonomy, systematics and phylogenetics (GRADSTEIN, 2015, 2016; HEINRICHS, 2002; HEINRICHS et al., 2002, 2004a, 2006; PATZAK et al., 2016; among many others), with some biogeography (GROTH et al., 2003; HEINRICHS et al. 2004b) and reproductive biology works (MACIEL-SILVA & MARQUES-VÁLIO, 2011; PIIPO, 1992). Based on herbarium collections, LONGTON & SCHUSTER (1983) cite *Plagiochila* species as examples of extreme sex ratio, as in *Plagiochila sullivantii* Gottsche ex A. Evans and *Plagiochila austinii* A. Evans which were only known by male specimens. MACIEL-SILVA & MARQUES-VÁLIO (2011) studied the reproductive phenology of *Plagiochila martiana* (Nees) Lindenb. in the Brazilian Atlantic Forest and found that this species is sexually plastic in response to altitudinal variation.

The effect of population sex ratios on sexual reproduction has been previously noted among bryophytes, where many species with unisexual individuals have infrequent or no sexual reproduction, while species with bisexual individuals (monoecious species) frequently

reproduce sexually (LAAKA-LINDBERG et al., 2000; LONGTON & SCHUSTER, 1983; MACIEL-SILVA & PÔRTO, 2014; REESE, 1984). The cause of infrequent or no sexual reproduction is linked either to short dispersal distance of male gametes (CRUM, 2001; MCLETCHIE, 1996; REYNOLDS, 1980; WYATT, 1977) or too few or no males in populations (female only species) (FUSELIER & MCLETCHIE, 2004; LONGTON, 1990; LONGTON & SCHUSTER, 1983; MISHLER & OLIVER, 1991;). While the expressed sex ratio patterns are well known, few studies have empirically measured the relationship between sex ratios and sexual reproduction (but see ALVARENGA et al., 2013 and references within). In populations with absence or low levels of sexual reproduction, population persistence occurs mainly by asexual reproduction via specialized propagules and plant fragmentation (see reviews by FREY & KURSCHNER, 2011 and LAAKA-LINDBERG, 2000).

In this study, we used *Plagiochila porelloides* (Torr. ex Nees) Lindenb. to test for 1) variation in population sex ratios; 2) an association between these sex ratios and sexual reproduction; 3) an association between sex ratios and environment factors: light intensity, canopy openness, and topography variables (slope and aspect), and 4) if community characteristics could be a proxy for environmental gradients. We hypothesized the presence of a female biased sex ratio. We expected a positive relationship between sex ratio (proportion of males) and sexual reproduction (sporophyte production). As mentioned above, fertilization of females is known to be sperm limited. Thus, in populations in which males are abundant, female will more likely be fertilized and produce offspring than in populations where males are not abundant.

We expect an association between sex ratios and environment gradients. We also did not stablish a prediction for a relationship between expressed sex ratio and light quantity or others environmental factors, because previous studies found weak or no relationship in bryophytes

(FUSELIER & MCLETCHIE, 2004; GROEN et al., 2010b; MCLETCHIE & PUTERBAUGH, 2000; STARK et al., 2005).

MATERIALS AND METHODS

Study system - *Plagiochila porelloides* is a leafy liverwort with unisexual individuals, in which the sexual organs are superficial, axillary or at stem apex, and protected by modified leaves. The species is well distributed in Asia (KONSTANTINOVA et al., 1992; KONSTANTINOVA & POTEMKIM, 1996; SÖDERSTRÖM et al., 1999), Europe (CRONBERG, 2000; SIM-SIM et al., 2003) and North America (STOTLER & CRANDALL-STOTLER, 1997, 2017) and has a Holarctic distribution (SCHUSTER, 1980) or a boreal and artic circumpolar distribution (SÖDERSTRÖM et al., 1999). The common substratum for *P. porelloides* is moist boulders, generally in shady and moist environments (SIM-SIM et al., 2003; SÖDERSTRÖM et al., 1999). SCHUSTER (1980) mentioned *P. porelloides* as having remarkable flexibility to edaphic conditions and wide fluctuation in moisture and light. Furthermore, he mentioned that males and females grow in separate patches, and asexual reproduction via specialized structures was absent (SCHUSTER 1980).

Plants for this study were collected from July 28th to August 2nd, 2017 from streams of Robinson Forest Research Station (Department of Forest, Agriculture College, The University of Kentucky), Clayhole, Breathitt County, Kentucky, U.S.A (Fig. 1, Fig 2A). This is a mixed deciduous forest (14,800 acres). The tree species along streams include *Tsuga canadensis* (L.) Carrière, L. *Magnolia tripetala* (L.) L. *Salix nigra* Marshall, *Ulmus americana* L., and *Ulmus rubra* Muhl (Carpenter & Rumsey 1976). For a description and history of Robinson Forest, see Reece & Krupa (2013). *Plagiochila porelloides* was found growing on large boulders along streams, in shaded environments (Fig. 2B). Populations were separated by water or inhospitable substratum (humus to sand to rocks). Plants were generally found on

very steep slopes of sandstone boulders and associated with various species of mosses and liverworts (Fig. 2C). In a few instances, plants occur on horizontal sections of the boulder (Fig. 2D). Species identification was verified using the specific key for Plagiochilaceae in North America by SCHUSTER (1980) and comparison with previously identified material collected at nearby regions. Specimens were vouchered at the Missouri Botanical Garden (St Louis, MO, USA – Silva-e-COSTA, J.C. 135 & D.N. McLetchie) (Supplementary Table 1) and at Morehead State University (Morehead, KY, USA – specimen numbers are being awaited).

Sampling methods and field collections – In order to estimate population sex ratios and test for associations of sex ratio with sexual reproduction, and with environmental variables at the population scale, we located, by walking in streams, 29 populations of *P. porelloides* on boulders that were at least 1 m at their longest length. The population were clustered in the following four geographic regions of the forest: John Carpenter (four), Falling Rock (six), Big MillSeat (ten) and Little MillSeat (nine) (Fig. 1).

Sex ratios and fertilization – To determine the current operational sex ratio of each population, a 50 cm transect was randomly placed along each *P. porelloides* population. We choose the transect location so that it occurred on one face of the boulder. In an upper corner of the population, a 25 cm measuring tape was laid down, and a random point between 0 and 25 was chosen. At this point the transect was laid down perpendicular to the measuring tape. Along the 50 cm transect, we collected at 5 cm intervals, starting at 0 cm, a one-centimeter square (1 cm^2) sample of *P. porelloides* shoots for a total of eleven 1 cm^2 shoot samples for each population. The spacing and size of the sample were chosen to increase the likelihood a collecting different genet and having a sufficient number of shoots per transect (> 20) to estimate a sex ratio. We observed that males and females occurred together but have no

information and the size of genets or the connectively of ramets within genets. Sex ratios are thus based on shoots (ramets) and not genets, similar to most sex ratio studies using clonal plants. Plants were then taken to the laboratory and identified to sex (presence of sexual reproductive structures) under a dissecting scope (Fig. 2E to H). To determine fertilization success, the presence or absence of sporophytes (which is an indication of fertilization) was noted (Fig. 2H). In our final analysis we excluded non-sexually reproductive shoots, thus the sex ratios represent current sex expressing individuals and population level patterns. In almost all plant species the sex of an individual can only be identified when an individual produces a sex structure, and most studies focus on individuals that are currently sexually reproductive as an estimate of the operational sex ratio. For example, in a recent meta-analysis, only eight of 217 seed plant studies included non-reproductive individuals (FIELD et al. 2013b).

Population sex ratios used in the present study are the sex ratios of individuals currently producing sex structures. In clonal plants species with unisexual individuals, clone mates (ramets) of the same genetic sexual offspring (genet) cannot be readily identified, thus populations sex ratio is usually a ramet sex ratio (see FIELDs et al., 2013a and references therein for seed plants and BISANG & HEDENÄS 2005 and references therein for bryophytes.

Environmental variables

*Light environment***:** To test for associations between sex ratios and light environment (percentage of canopy openness and light intensity (photosynthetic flux density (PPFD, mol m^{-2} day^{-1})), we took hemispherical canopy photographs on August 2nd to 9th 2017 and on November 22nd to 29th 2017 using a Coolpix 4500, (Nikon, Tokyo, Japan) with a 180^{o} fisheye lens attached. These digital photographs were then analyzed with WinSCANOPY (Regent

Instruments, Ville de Québec, Québec, Canada) to assess the light environment. Canopy openness is an estimate of the percentage of open sky. WinSCANOPY uses the known path of the sun (daily and seasonally) and the location of the tree canopy in hemispherical photographs to calculate PPFD for the location the picture was taken. To estimate PPFD, we use the period from May 1st to July 1 st, that represents the period where the canopy was leafed out, and the period of November 1st to March 31st, when the deciduous trees were without leaves (the USA National Phenology Network, www.usanpn.org).

Topography – To test if sex ratio was associated with the topography of the population, aspect and slope were taken for each population where the transect was laid down, using a Suunto ® mirror sighting compass with a clinometer. Aspect was adjusted for true north.

Bryophyte community (species richness) – To test if sex ratio can be associated with the biotic environment (the community characteristic of species richness), samples of mosses and liverworts that co-occur within the 50 cm transect were recorded. We focused on species richness and not species diversity because estimating abundances of each bryophyte species in each population was beyond the scope of this study. Specimens were also vouchered at the herbariums listed above.

Statistical analyses – We used a combination of Microsoft Excel (2016), SAS 9.4 (SAS Institute, Cary, North Carolina, USA and JMP 12 (SAS Institute, Cary, North Carolina, USA) to analyze the data. We consider $p < 0.05$ as significant, and $0.05 < p < 0.1$ as a tendency that is worthy of discussion in this field base study.

Sex ratios – To evaluate the expressed sex ratio, we used the proportion of males in each population (total of male expressed individuals divided by the total number of individuals expressing sex – males, females, and females with sporophyte). We used G test (Goodness-

of-fit test) to evaluate the frequency of males and females in each population, and a heterogeneity test was used to test for significant variation in sex ratios among populations (SOKAL & ROHLF, 1995). The expected sex ratio used was 1:1 (0.5 male proportion). For more conservative tests, populations with 10 and less individuals with sex structures were dropped from the analysis to test for variation in sex ratios, and to test for association of sex ratios with the independent variables.

Fertilization – To test if fertilization success (proportion on females with sporophytes) is associated with sex ratios, we used Pearson correlation. Only populations containing female shoots and that had more than 10 individuals with sex structures were used. Both sex ratio and fertilization success were arcsine transformed to improve normality.

Environmental variables – The environmental variables were grouped into three sets of explanatory variables (light, topography and community). The association between sex ratio and these groups was analyzed separately.

Light conditions – We used ANOVA to test if sex ratios were associated with light environment (canopy openness and PPFD).

Topography – We first used the Rayleigh test to determine if the populations where non-uniformly distributed with respect to aspect (ZAR, 1999). To test if sex ratios were associated with aspect, we used a linear-circular regression ($Y_i = b_0 + b_1 \cos a_i + b_2 \sin a_i$, where Y_i is the sex ratio of population I, b_0 is the Y-intercept, b_1 and b_2 are the partial regression coefficients and, a_i is the aspect of population I, ZAR, 1999). We included slope in this analysis.

Community – We used ANOVA to test if sex ratio was associated with the number of species of moss and liverworts found in the populations. We also tested if sex ratio was associated

with mosses and liverwort species richness combined. Sex ratio was arsine transformed and species richness was square root transformed to improve normality.

RESULTS

Sex ratio and fertilization success – Number of shoots in each transect ranged from 55 to 462, out of a total of 6,254, and 19% had sex structures. Five of the 29 populations did not have any individuals with sex structures. Seven of the remaining 24 populations were single sex (Supplementary Fig. 1). Twenty-one populations (containing more than 10 individuals with sex structures) were used to analyze population sex ratios, which ranged from 0 (all female) to 1 (all male). Five were female-biased ($p < 0.01$), ten were male-biased ($p < 0.0001$), one had a tendency ($0.1 < p < 0.05$) to be male biased, and five showed no bias (the proportions of males and females shoots were not significantly different from 0.5). The populations showed significant heterogeneity ($p < 0.0001$), the pooled sex ratio was male-biased (0.727 - proportion of males or 0.375 ♀:1 ♂, $p < 0.0001$) and the overall mean sex ratio across all 21 populations was 0.64 ± 0.08. In populations with females and with 10 or more individuals with sex structures, sex ratio ranged from 0.0 to 0.986, and fertilization success ranged from 0.07 to 1; sex ratio and fertilization were positively associated ($n = 17$, $r^2 = 0.3127$, $p = 0.01965$; Fig. 3A).

Environmental factors

Light environment and topography – During the closed canopy period, canopy openness (%) ranged from 4.85 to 11.3 with a mean of 6.2 ± 0.235 (n = 29, mean ± standard error) and PPFD (mol m^{-2} day^{-1}) ranged from 4.26 to 14.66 with a mean of 7.18 ± 0.408 (n = 29, mean ± standard error). There was no relationship between canopy openness and PPFD ($r^2 = 0.007$, $p = 0.6453$). During the growing season, sex ratios of the 21 populations that were included in the analyzed were not associated with canopy openness ($F_{1/20} = 0.379$, p= 0.5459) and tended to be negatively associated to PPFD ($F_{1/20} = 3.969$, 0.0617; Fig. 3B). During the winter season, canopy openness (%) ranged from 17.38 to 32.02 with a mean of 25.61 ± 0.639 (n =

29, mean ± standard error) and PPFD (mol m^{-2} day^{-1}) ranged from 1.04 to 9.55.66 with a mean of 4.71 ± 0.403 (n = 29, mean ± standard error). There was a positive relationship between canopy openness and PPFD (r^2 = 0.23, p = 0.0045). Sex ratios were not associated with light environment based on the winter season (openness $F_{1,20}$ = 1.01, p = 0.3251, and PPFD $F_{1,20}$ = 0.1132, p = 0.7405).

Overall, populations were found to be uniformly distributed with respect to aspect ($z_{0.05, 29}$ = 0.701, calculated z = 0.689 (Supplementary Fig. 2). The slope of substratum ranged from 24º to 88º with a mean of 64.55º ± 3.12 º (n = 29, mean ± standard error). Sex ratio was not associated with aspect parameters (cos $F_{1/20}$ = 0.05 p = 0.820, sin $F_{1/20}$ = 0.07, p = 0.7933,) or slope ($F_{1/20}$ = 0.2 p = 0.6598).

Species richness – Thirty-four different species of mosses and liverworts were found co-occurring with *P. porelloides*: 25 mosses and nine liverworts (one thalloid and eight leafy liverworts). The range of frequency was 20 (one species) to one occurrence (16 species, see Supplementary Table 1 for species list by population). Populations (Fig. 1) BM2, BM4, and FR5 were the richest ones, with 11 species; population LM2 had the lowest richness with two species. For all populations, bryophyte species richness ranged from two to 11. For mosses this range was one to 10 species and for liverworts this range was zero to four species. Only populations with 10 or more individuals of known sex were used to test for an effect of species richness on *P. porelloides* sex ratio. Population sex ratios were not associated to moss species richness ($F_{1,20}$ = 0.0729, p = 0.7901; Fig. 4A), neither to liverwort species richness ($F_{1,20}$ = 2.1589, p = 0.1581; Fig. 4B).

DISCUSSION

This study represents the first systematic assessment of expressed sex ratios in *Plagiochila* and contains both expected and novel findings. As predicted, we found a positive relationship between the proportion of fertile shoots expressing male gametangia and probability of sporophyte production. Population sex ratios were found to be overall male-biased, in contrast to the female bias sex ratios, generally reported for bryophytes. Although we detected no association, but a tendency of an association between sex ratio and light quantity (PPFD), this connection is not well documented for bryophytes.

Causes and consequences of male-biased sex ratio in bryophytes - *Plagiochila porelloides* had an overall male sex ratio bias with populations ranging from only male to only female. Sex ratios in both mosses and liverworts are generally found to be female biased (BISANG & HEDENAS, 2005; GLIME & BISANG ,2017; HAIG, 2016; among many others). Explanations proposed for female bias include sex differences in germination percent (MCLETCHIE, 1992), life history (HAIG, 2016; SLATE, 2017), stochastic events during establishment process (RYDGREN & ØKLAND, 2002), small refugia (LONGTON, 1990), realized cost of reproduction (MCLETCHIE, 1992; STARK et al., 2000), and sex differences in water use to achieve fertilization (MOORE et al., 2016). However, a few studies found male-biased populations, including *Lophozia silvicola* Buch, 0.63♀: 1♂ (LAAKA-LINDBERG, 2005), *Crossomitrium patrisiae* (Brid.) Müll. Hal., 0.43♀: 1♂ (Alvarenga et al. 2013), and *Scapania undulata* (L.) Dumort., 0.33♀: 1♂ (Holá et al. 2014).

The above authors suggested that successful fertilization events, and therefore, offspring production lead to male biased sex ratio. Female reproductive cost is associated with maturating offspring and is possibly manifested as a lower survival, or lower growth rates in females relative to males. ALVARENGA et al. (2013) suggested that rarity of females of *C.*

patrisiae might be a phenotypic response: a high fertilization success and high sporophyte production resulted in higher resource allocation to sexual reproduction for females due to sporophyte maturation. HOLÁ et al. (2014) also accredited the male bias to high sporophyte production. LAAKA-LINDBERG (2005) suggested that the strong male bias found in *L. silvicola* was due to higher female resource investment in reproductive organ production compared to males, and populations also experience high fertilization success. For our results, the fertilization success and sporophyte production are consistent with this explanation.

A male and variable sex ratio and fertilization success - A positive relationship between sex ratio and fertilization success was detected in the studied population os *P. porelloides*. Although dioicious bryophytes are known by low sporophyte production (sperm short dispersal distance – CUM (2001); REYNOLDS (1980); WYATT (1977); or absence of one of the sexes – LONGTON, 1990), proximally 50% of the female plants of *P. porelloides* was found fertilized. GARCIA-RAMOS et al. (2007), MCLETCHIE et al. (2002), and RYDGREN et al. (2010) have developed mathematical models for bryophytes that predict a male bias when fertilization success is high and female bias when success is low. Thus, that the male bias detected here might be a result of the high proportion of fertilized females and fertilization success.

In addition to being male bias, our study found sex ratios on *P. porelloides* to be very variable among populations (all male to all female), suggesting a meta-population system. BRZYSKI et al (2018), CRONBERG et al. (2003), and CRONBERG et al. (2006) also detected sex ratio variation among populations in other bryophyte species. The overall male bias sex ratio couple with variation in sex ratios is consistent with a mathematical meta-population models developed to explain variation in sex ratio in *Marchantia inflexa*, a species with discrete population along streams (GARCIA-RAMOS et al., 2007; STIEHA et al., 2016)

analogous to *P. porelloides*. In these models, variation in sex ratios among populations were consistent with sex specific life histories differences coupled with population disturbances and population extinction events: spores of both sexes colonize a substrate resulting in a bisexual population able to produce spores that can colonize other substrates (MCLETCHIE et al., 2002). During the existence of a population from colonization to extinction a population can be subjected to disturbance events affecting the sex ratio; more frequent disturbances result in a more male bias population because males can have higher asexual reproductive levels relative to females in this tllose liverwort (MCLETCHIE et al,. 2002). Given sufficient time one sex will replace the other within a population depending on the frequency of the disturbance. If populations go extinct at different times, then the resulting empty substrate can be recolonized by spores producing new bisexual populations. Thus, populations in the meta-populations will be at different stages from colonization to single sex to extinct. This pattern will be manifested as variable sex ratios among populations. Besides the universal expectation that male and female function differ and can lead to life history differences, there are no specific studies of life history sex differences in *P. porelloides* except for shoot size noted in species descriptions (SCHUSTER, 1980).

The environmental parameters analyzed and their potential contribution to the study of sex **-** We found that the proportion of sex expressing males tended to be negatively associated with light quantity ($p < 0.1$) while no statistical relationship between those and moss or liverwort species richness. Physiological sex difference response to environmental conditions has been demonstrated by PETTET (1967) and NEWTON (1972); STIEHA et al. (2014) have detected a sex difference in response to canopy openness. Similar patters might be occurring among the populations of *P. porelloides*, leading to a female rarity and male dominance. Relantionships between plant community characteristics and sex ratio are expected to occur, once sex ratio can vary along envrironmental gradients, as well as plant community aspects

(CUEVAS et al., 2017; GENTRY, 1988; PUTWAIN & HARPER, 1972; PICKUP & BARRET, 2013; SANDERSON et al., 2016; STEHLIK, 2008). Althoug a significant associated between sex ratio and the community characteristics species richnees, we argue that this is a potencial valuable approach to explore and understand the patters of sex ratio variation: the community may be an indicator for a yet to be detected environmental variable or, alternatively, plant communities can alter the local environment during succession where moisture, light and nutrients change as shown by GARNIER (2004), JIANG & DEANGELIS (2013), and ULRICH et al. (2014), and these changes can affect the sexes of a species differently.

CONCLUSION

Plagiochila porelloides presents an excellent opportunity for comparative studies to investigate the causes of sex ratio variation. The overall male-biased sex ratio and the positive association between sex ratio and fertilization success detected here highlight the diversity of sex ratio patterns among taxa, and the link between sex ratios and sexual reproduction. Further, this study is one of the few studies on sex ratios in a leafy liverwort.

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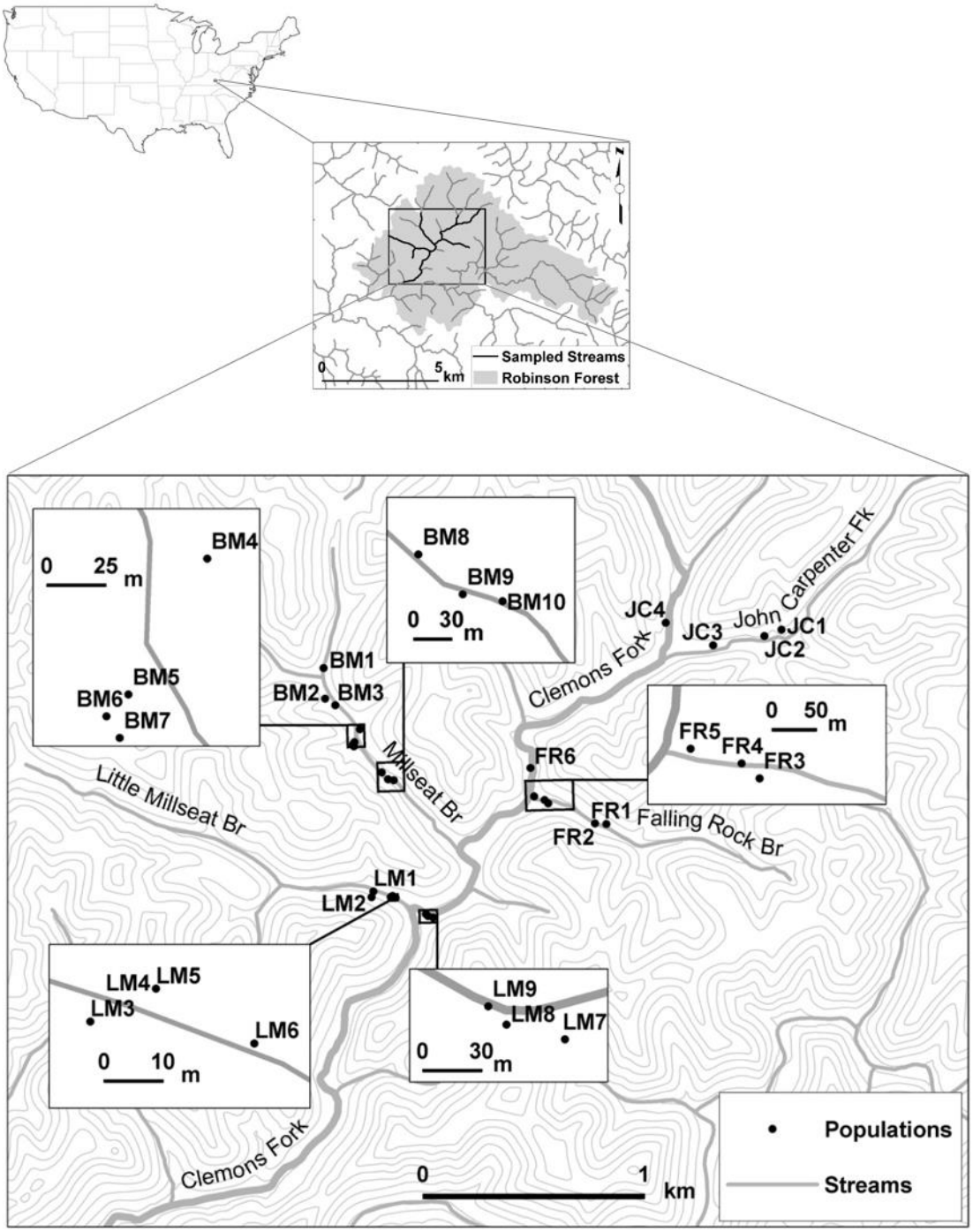
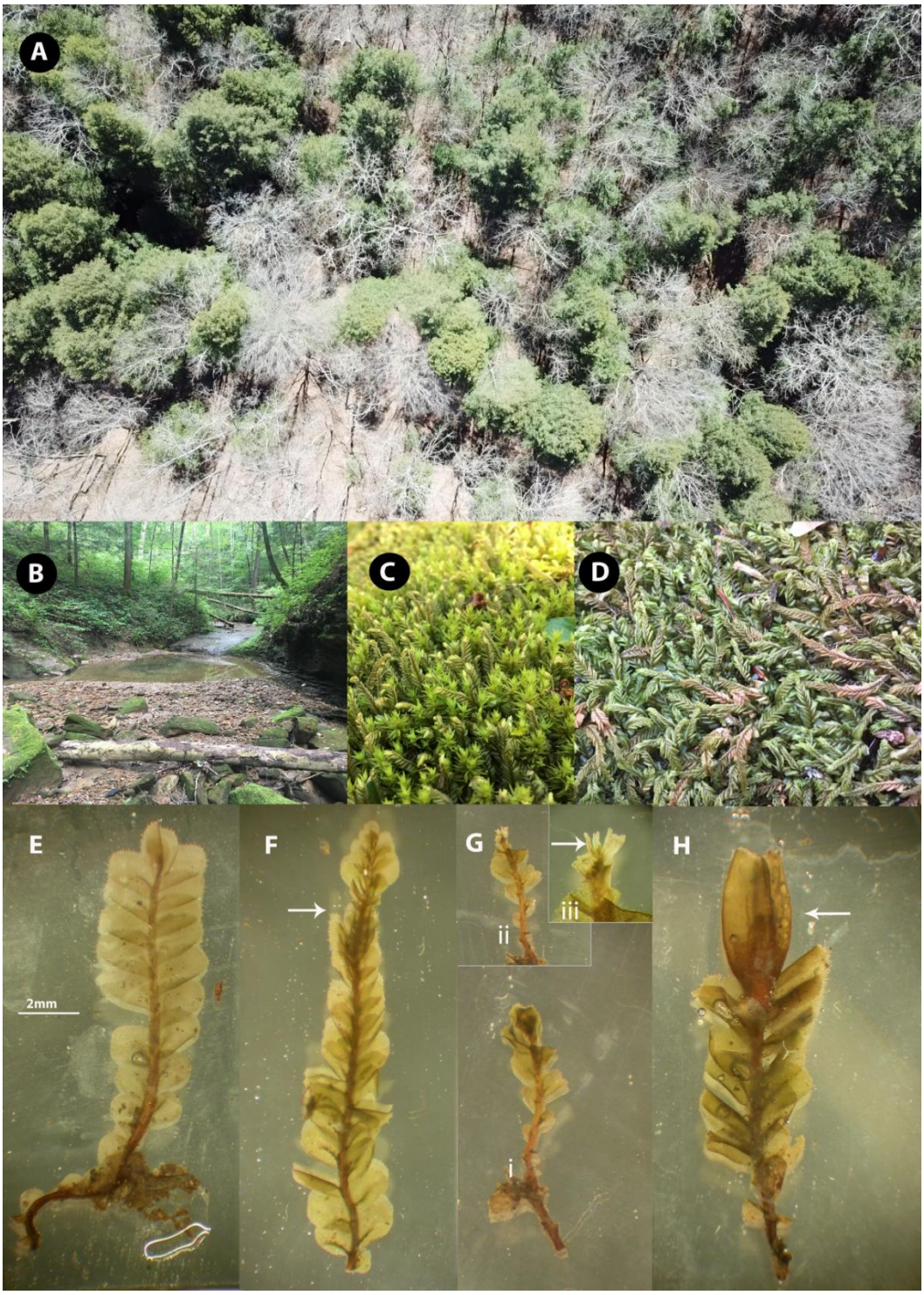


FIGURE 1. Map of Robinson Forest Area, showing the streams and each population's location. BM: Big MillSeat Stream Region; FR: Falling Rock Stream region; JC: John Carpenter Stream Region; LM: Little MillSeat Stream Region.


A
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H

FIGURE 2. From above the canopy to the different Plagiochila porelloides shoot sexes. (A) Image of a section of John Carpenter Fork taken before leafing out in spring. The green trees are the evergreen *Tsuga canadensis* (Eastern hemlock) and is one of the common tree species found along the streams of Robinson Forest. (B) An understory view of Little Millseat Branch Region. One of the populations is on the boulder in the lower left-hand corner. (C) An example of the bryophyte community on one of the populations. (D) A clump of P. porelloides. A few shoots have developed perianths containing sporophytes. (E) A vegetative shoot. The lack of sex structures is obvious throughout this shoot. (F) A male shoot with sex structures immediately below new growth, indicates with the arrow. (G): i) A female shoot with archegonia within modified leaves; ii) The same shoot with the leaves removed to show a cluster of archegonia; iii) A close up of this cluster, indicated with an arrow. (H) A female shoot with a developing perianth and an immature sporophyte within the perianth, indicated with an arrow. The scale in E is the same for F, Gi and H.

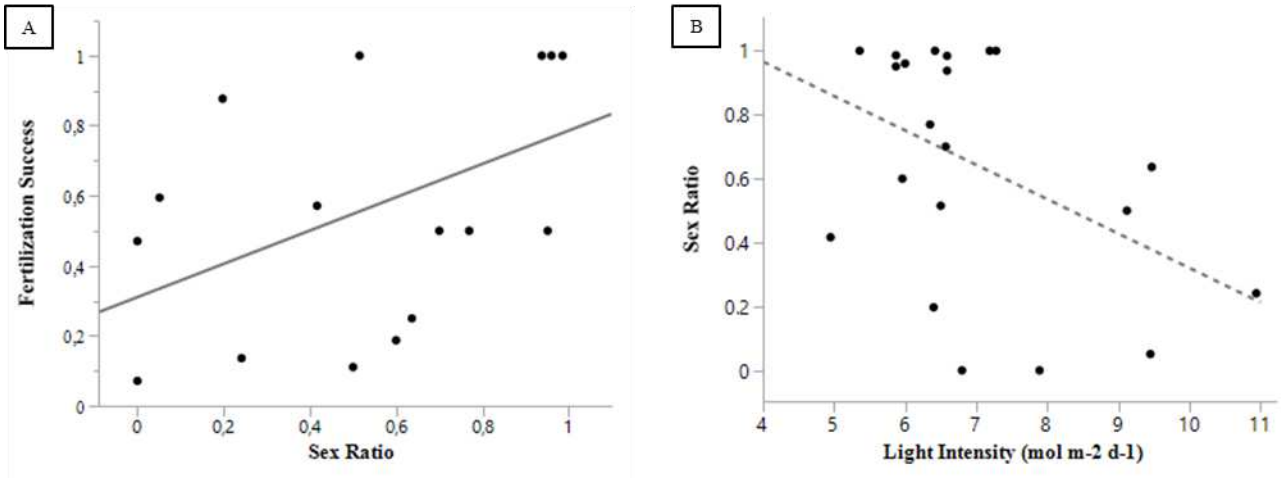


FIGURE 3. Relationship between sex ratio and fertilization success (A) and light intensity (B). (A) The positive association between fertilization success and sex ratio (proportion of males) in Plagiochila porelloides populations where at least ten shoots had sex structures and ten females were present. (B) The tendency of a negative association between sex ratios of *P. porelloides* and light intensity (mol m^{-2} d^{-1}) in *P. porelloides* populations where at least ten shoots had sex structures. Data are untransformed values. Solid line indicates significance ($p < 0.05$), dashed line indicates a tendency (p value <0.1 and > 0.05).

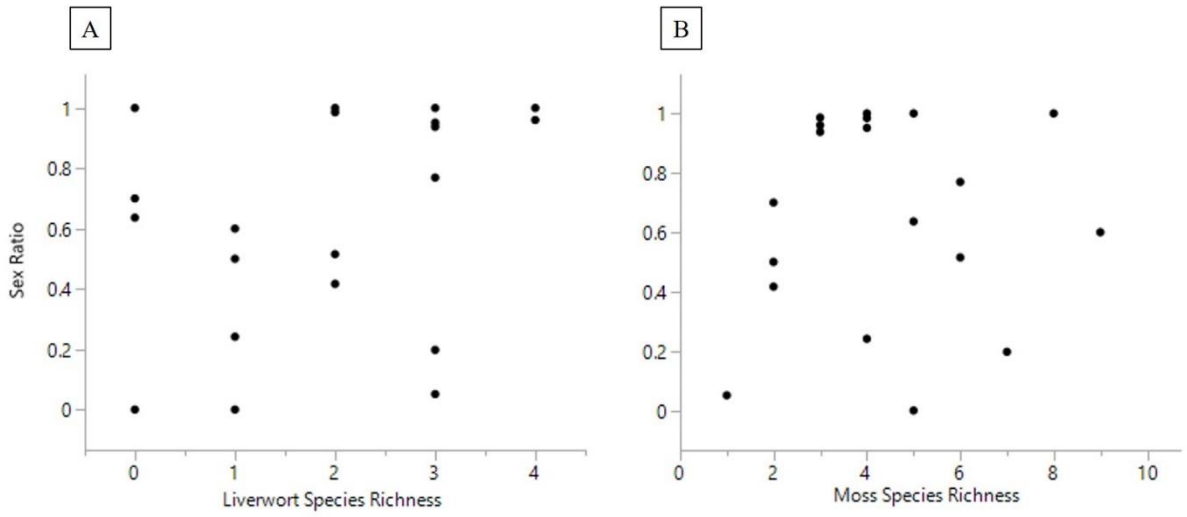


FIGURE 4. Relationship between sex ratio and liverwort and moss species richness in 21 populations of *Plagiochila porelloides* where at least ten plants in each population had sex structures. (A) There is an absence of an association between proportion of males of *P. porelloides* and liverwort species richness. (B) There is an absence of a relationship between moss species richness and the proportion of males of *P. porelloides*. Data are untransformed value.

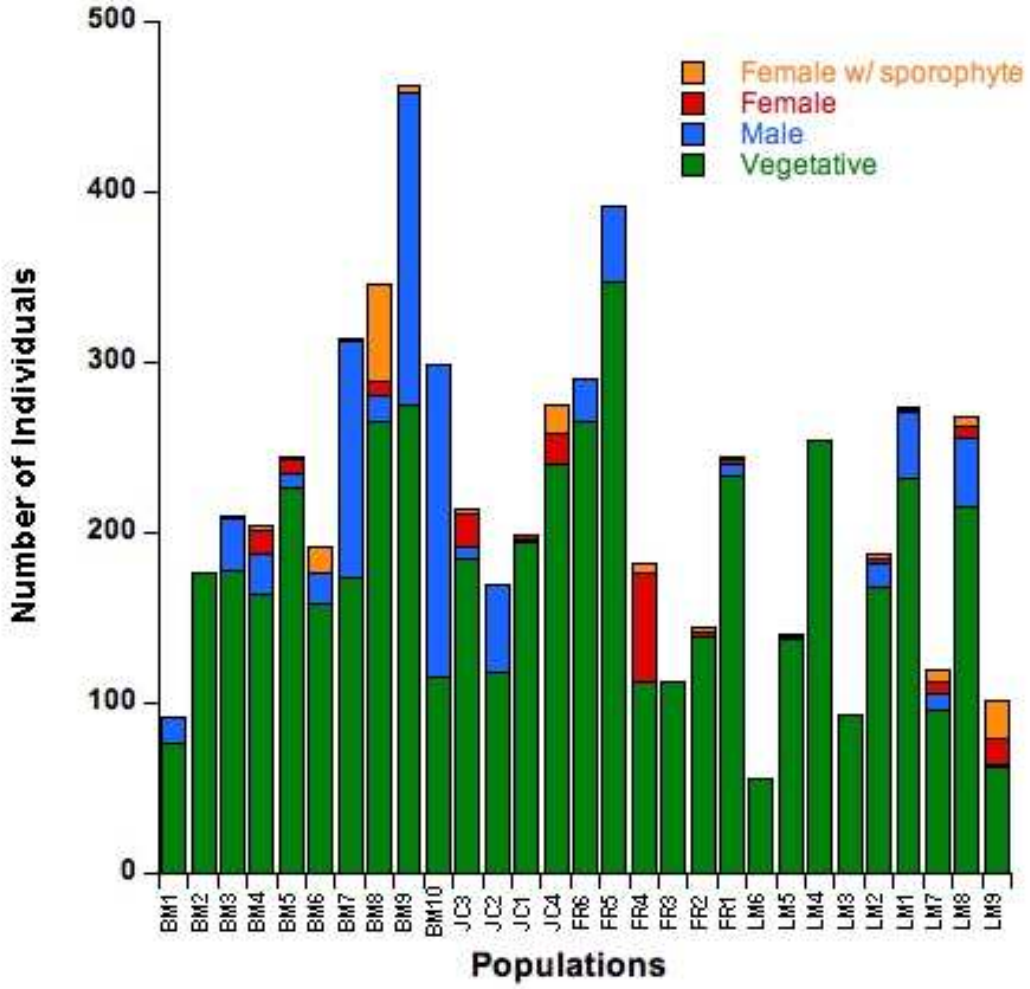
SUPPLEMENTARY MATERIAL

Supplementary Table 1: Species of mosses and liverworts presented in each population studied. 0 = absence; 1 = presence.

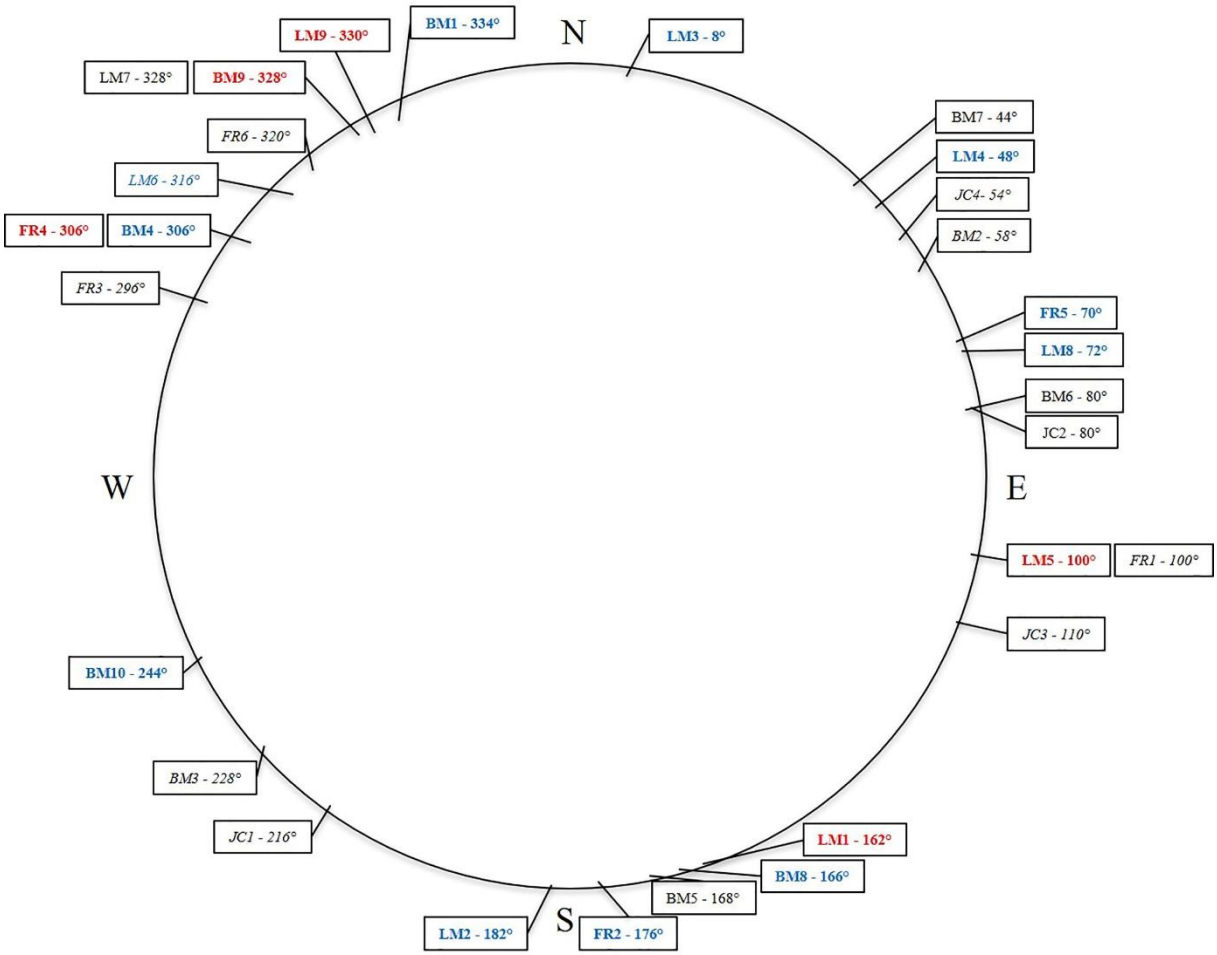
Species	Bryophyte Lineage	Populations																												
		Big MillSeat										John Carpenter				Falling Rock						Little MillSeat								
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9
Anomodon attenuates (Hedw.) Huebener	M	0	1	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0
Atrichum angustatum (Brid.) Bruch & Schimp.	M	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atrichum undulatum (Hedw.) P. Beav.	M	1	1	0	1	0	1	1	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0
Aulacomnium heterostichum (Hedw.) Bruch & Schimp.	M	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
Bazzania trilobata (L.) Gray	L	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachythecium campestre (Müll. Hal) Schimp.	M	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachythecium rutabulum (hedw.) Schimp	M	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BryoANDERSONia illcebra (Hedw.) Robins	M	1	1	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	1	0	0	0
Calypogeia fissa (L.) Raddi	L	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cephalozia bicuspidata (L.) Dumort.	L	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Climacium americanum Brid.	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Conocephalum salebrosum (Steph.) A. Evans	L	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	0	1	1	1	0	1	1	1	1	1	1	1
Dicranum montanum (Hedw.)	M	0	0	0	0	0	1	0	1	1	0	1	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
Diphyscium foliosum (Hedw.) D. Mohr.	M	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
Fissidens osmundioides Hedw.	M	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hookeria acutifolia Hook. & Grev.	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Hypnum Hedw.	M	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Hypnum curvifolium Hedw.	M	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0
Isopterygiopsis pulchella (Hedw.) Z. Iwats.	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jubula pennsilvanica (Steph.) A. Evans	L	1	1	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
Leptodictyum (Schimp.) Warnst.	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table 1: continuance

Species	Bryophyte Lineage	Populations																												
		Big MillSeat										John Carpenter				Falling Rock						Little MillSeat								
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9
Leucobryum albidum (Brid. Ex P. Beauv.) Lindb.	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucobryum glaucum (Hedw.) Ångstr.	M	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiomnium cilliare (Müll. Hal) T. J. Kop.	M	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiomnium medium (Bruch & Schimp) T. J. Kop.	M	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Plagiothecium denticulatum (Hedw.) Schimp.	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0
Radula obconica Sull.	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Radula tenax Linb.	L	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizomnium punctatum (Hedw.) T. J. Kop.	M	1	1	0	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	0
Rhodobryum ontariense (Kindb.) Paris	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Scapania nemorea (L.) Grolle	L	1	0	0	0	1	1	1	1	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1
Tetraphis pellucida Hedw.	M	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thuidium delicatum (Hedw.) Schimp.	M	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	0	1	1	0	0	0	0	0
Trichocolea tomentella (Ehrh.) Dumort.	M	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1



Supplementary Figure 1: Number of individulas in each population in accordance with their reproductive condition: vegetative, male, female, and female bearing sporophyte.



Supplementary Figure 2. Population location in accordance with the aspect.

Chapter Three

The sexes can differ in desiccation tolerance in the liverwort *Plagiochila porelloides*

*This chapter is under preparation for publication.

The sexes can differ in desiccation tolerance in the liverwort *Plagiochila porelloides*

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ABSTRACT

Background: Water scarcity is a common stress factor that affects all living organisms, and is considered an evolutionary driver for life on land. For plants, water deficit can negatively impact their productivity and survival. Strategies of water stress tolerance such as desiccation tolerance (DT) evolved to endure dry intervals. Desiccation tolerance is an important evolutionary and ecological feature widespread amongst land plants, influencing plant fitness. DT ability in both vegetative and reproductive phases was crucial to land plant colonization and vegetative desiccation tolerance is particularly found among bryophytes. Surprisingly, few studies have documented variation in desiccation tolerance within a species. Here we used the leafy liverwort *Plagiochila porelloides* to investigate for desiccation tolerance responses and sex differences under controlled relative humidity and temperature.

Methods: Seventy-two plants from three different populations were field collected and, after dehardening over a week, plants were subjected to a desiccation tolerance assay, in four desiccation chambers at 40% of relative humidity for a period of 22 h. Plant water status and photosynthetic rates were checked to verify tissue desiccation. For recovery proxy, efficiency of photosystem II was evaluated.

Key Results: *Plagiochila porelloides* shoots survived the low relative humidity of 40%, demonstrating that the species is desiccation tolerant. Plant water status showed a water content by dry weight varying between 8% and 10%. Net carbon loss was detected in recently desiccated plants (< 3 days from rehydration) while net carbon gain occurred after 30 days from rehydration. Relative to males, females recovered faster and this was driven by one of three population.

Conclusions: Sex differences in desiccation tolerance occurs reflecting intraspecific variation. Population level variation might be due to plastic or genetic effects.

INTRODUCTION

Deficiency of water is amongst the most common abiotic stress factors that influence plant productivity (OSAKABE et al., 2011; RHODES & ORCZYK, 2001). It is an important ecological aspect and an evolutionary driver in land environments (ALPERT, 2005). Plants may experience water stress condition when water supply availability is limited or even when transpiration rates become intense (LISA et al., 2012). Understanding whether plants can adapt or acclimate to increasing water scarcity is important in predicting ecological changes due to forthcoming climate projection (OSAKABE et al., 2011; PARDOW & LAKATOS, 2013).

In order to cope with the adverse situation of water deficit, several strategies have evolved, including desiccation tolerance (DT, or desiccation tolerant), a widespread condition in many living organisms, especially in plants. DT is one mechanism of water stress survival (ALPERT, 2005; ZHANG & BARTELS, 2018). Many authors have studied the DT phenomenon and various definitions have been formulated. In general, DT can be physiologically defined as the ability to dry out to equilibrium with surrounding air (that may be moderately to extremely dry) and then return to normal metabolic activity after rewetting (ALPERT, 2005; OLIVER, 2009; VANDERPOORTEN & GOFFINET, 2009). Thus, this strategy demands reversible metabolic management and development of a protective mechanism at cellular level, during periods of drying (ALPERT & OLIVER, 2002). For an ecological definition, DT is the ability to survive under intermittent water scarcity (PROCTOR, 2009).

Another form of water stress occurs when plants tolerate mild water lost, namely dehydration tolerance (DhT). Thus, water stress tolerance includes plants that are DT when they can tolerate a water potential of < - 100 MPa (PROCTOR & PENCE, 2002) and DhT –

when plants can tolerate a water potential of – 10MPa (MARKS et al., 2016; OLIVER, 2009; OLIVER et al., 2010; ZHANG & BARTELS, 2018). In the former case water content (WC) can be as low as 0.1 g H_2O g^{-1} dry weight or 10% (STARK, 2017).

Desiccation tolerance is considered a very ancient characteristic allowing plants to survive in terrestrial habitats (ALPERT, 2000; ALPERT, 2005 and references within; LEVITT, 1980; PROCTOR et al., 2007), being found in bryophytes and vascular plants (ALPERT, 2000; 2005; DINAKAR & BARTELS, 2013). However, the ability to tolerate desiccation in both vegetative and reproductive phases is extensively found in bryophytes, whose ancestors were the first plants to colonize the terrestrial environment, and it is widely found among extant species (OLIVER, 2009; OLIVER et al., 2000; PROCTOR et al., 2007; STARK, 2017). Bryophytes are poikilohydric plants, which means that these plants can gain and lose water rapidly, without control over water loss (PROCTOR et al., 2002), and their hydration condition is regulated by the ambient humidity (VANDERPOORTEN & GOFFINET, 2009). Although DT is very common among them, it is not a characteristic feature (PROCTOR, 1990; PROCTOR, 2009; PROCTOR & PENCE, 2002; PROCTOR et al., 2007; WOOD, 2007), and the degrees and levels of tolerance can vary greatly between species (PROCTOR & TUBA, 2002).

The vegetative ability to cope with desiccation in bryophytes has been widely studied. Attempts to understand DT in bryophytes have been made since early 1900's (e.g. CAMPELL, 1904), using field observations of plants in a dry season subsequent development in the following wet season. Latter studies on liverwort and moss included comparison of DT (CLAUSEN, 1964), regeneration of vegetative tissues (NEWTON, 1972), immediate physiological recovery after drying event (DILKS & PROCTOR, 1974) or metabolic recovery (OLIVER & BEWLEY, 1984). More recently, experimental and empirical studies include investigation of survival and regeneration after a desiccation period (STARK et al., 2005;

STIEHA et al., 2014, STARK & BRINDA, 2015; STARK et al., 2016), photosynthetic responses and chlrorophyll fluorescence (ZHANG et al., 2011), and protein synthesis and genetic responses (GAO et al., 2017; OLIVER, 1991; OLIVER & BEWLEY, 1984; OLIVER et al., 2000, 2005; PROCTOR et al., 2007a).

Bryophyte response to desiccation also comprises various and sophisticated processes (PROCTOR et al., 2007a; PROCTOR, 2009). Protection/repair mechanisms is a common apparatus to endure dry conditions (OLIVER & BEWLEY, 1997). Cellular protection and repair efficiency during the dehydration and rehydration processes determine the intensity of the desiccation that a species can bear (OLIVER, 2009). Most metabolic activity including respiration and photosynthesis reaction reduce during these desiccation events (DILKS & PROCTOR, 1979). A study on *Anomodon viticulosus*, a moss species, showed that the longer the desiccation periods is, the greater is the net carbon loss, and a more difficult recovery to pre-desiccation conditions (HINSHIRI & PROCTOR, 1971).

Photosynthetic rates of full recovery can take around 24 hours, while chloroplasts, organelles, and other cellular structures are reorganized in the meantime (PROCTOR et al., 2007a). Generally, while there is an extensive body of literature of DT in bryophytes as indicated by the large number recent reviews and books (ALPERT, 2000; 2005; BLACK & PRITCHARD, 2002; LÜÜTTGE et al., 2011; OLIVER et al., 2000; PROCTOR & TUBA, 2002; PROCTOR et al., 2007b; STARK, 2017; VITT et al., 2014; WOOD, 2007), there are very few studies evaluating sex differences in DT recovery and its constrains to population dynamics among bryophytes (NEWTON, 1972; MARKS et al., 2016; STARK et al., 2005; STIEHA et al., 2014).

Recently, the importance of DT to plant fitness has been suggested (STARK, 2017). In fact, due to sex function, male and female individuals in unisexual species will have different

physiologies (DELPH, 1999) and these might include (either directly or indirectly) different DT responses. Sex differences in water stress are found in seed plants but the sex that is more tolerant varies greatly across species (FREEMAN & MC ARTHUR, 1982; JUVANY & MUNNÉ-BOSCH, 2015; SINCLAIR et al., 2012). Among bryophytes, females were found to be more water stress tolerant than male plants (MARKS *et al*. 2016; NEWTON, 1972; SLATE et al., 2017; STIEHA et al., 2014). These studies suggested that this difference in water stress tolerance might affect factors at the population level.

A DT female advantage might contribute to a female bias sex ratio, and this bias is very common in bryophytes (BISANG & HEDENAS, 2005; GLIME & BISANG, 2017; HAIG, 2016). However, in bryophytes, there are few studies testing for sex differences in DT to support an association between sex specific DT and population sex ratios. For example, *Syntrichia caranervis* is a desiccation tolerant moss species and has extreme female bias, but a sex specific DT have not been reported (STARK et al., 2005). Nevertheless, a reasonable expectation for female DT advantage relative to male is that due to female gametophyte function to bear and nutritionally support the embryo after a successful fertilization, (HAIG, 2016; LIGRONE et al., 1993; VANDERPOORTEN & GOFFINET, 2009) a DT advantage might ensure survival during sporophyte development and spore production.

Here we use a species of the most specious liverwort genus *Plagiochila* (Dumort.) Dumort., *P. porelloides* (Torr. ex Nees) Lindenb., to test for DT and sex differences in DT. *Plagiochila* is a world widespread plant group, with more than 700 species recognized (SÖDERSTRÖM et al., 2016). While most studies have focused on solving various taxonomic problems within *Plagiochila* (e.g. GROTH et al., 2003; HEINRICHS, 2002; JAMY et al., 2016; PATZAK et al., 2016; SÖDERSTRÖM et al., 2016), few efforts have been applied aiming a better understanding the ecological constrains that some species might

face. The questions investigated in this study are: (1) is *P. porelloides* a DT species? and (2) are there any differences in DT between male and female plants?

We predict that the species studied will possess desiccation tolerance mechanisms and that females will have a greater DT level that males due to female physiological requirements to bear and sustain the sporophyte.

MATERIAL AND METHODS

Study organism, sampling conditions and field characteristics - *Plagiochila porelloides* (Torr. ex Nees) Lindenb. is a leafy liverwort with unisexual individuals, found in Asia (SÖDERSTRÖM et al., 1999), Europe (CRONBERG, 2000; SIM-SIM et al., 2003) and North America (STOTLER & CRANDALL-STOTLER, 1997, 2017). Plants are variable with respect to size (1.8 – 6 mm wide, 1-10 cm long) (GRADSTEIN et al., 2001), and heterothallism is reported for the sexes with males smaller than females (SCHUSTER, 1980). The leaves are unistratose and arranged in three rows: two lateral rows have quite variable leaves, varying from spreading to strongly curved into the ventral face; the third row is composed of the underleaves, which are minute and inconspicuous, frequently not noticeable (SCHUSTER, 1980).

The common substratum for *P. porelloides* is moist boulders, generally in mesic (shady and moist) environments (SIM-SIM et al., 2003; SÖDERSTRÖM et al., 1999). Plants are attached to the substratum by a rhizome-like creeping stem, configuring a tail life form (GRADSTEIN et al., 2001; MAGDEFRAU, 1989; SCHUSTER, 1980). The photosynthetic aerial shoot arises from the creeping stem. SCHUSTER (1980) mentioned *P. porelloides* as having remarkable flexibility to edaphic conditions and wide variation under moisture and

light but it is often identified as a shade-loving species (GRADSTEIN et al., 2001; MAGDEFRAU, 1982).

Plants for this study were collected from streams of Robinson Forest Research Station (Department of Forest, Agriculture College, The University of Kentucky), Clayhole, Breathitt County, Kentucky, USA, in two different locations: Clemons Fork and Big MillSeat. *Plagiochila porelloides* was found growing on large boulders along streams, in shaded environments. After collection, plants were maintained in growth chamber conditions at the University of Kentucky, Lexington, KY, USA. Species identification was verified using the specific key for Plagiochilaceae in North America by SCHUSTER (1980) and comparison with previously identified material collected at nearby regions. Specimens were vouchered at the Missouri Botanical Garden (St Louis, MO, USA – Silva-e-COSTA, J.C. 135 & D.N. McLetchie) and duplicates were sent to Morehead State University (Morehead, KY, USA).

Male and female plants were collected from three different populations, physically separated from the other to increase the likelihood that they were genetically different from each other. These populations were chosen because they had enough male and female shoots to allow for experimental replication. Populations were geopositioned and marked: P1: 37º47’895” N 083º14’89” W– Big MillSeat region, P2: 37º47’172” N 83º14’51” W– Clemons Fork region, and P3: 37º47’179” N 83º14’54 W– Clemons Fork region.

To assess field environment for water conditions, we collected field data of temperature and relative humidity (RH), using a humidity sensor HOBO ^{TM} attached to a data logger (Onset Computer Corporation, Bourne, MA, USA). Data were recorded at 5 min intervals between 10:00 to and 15:00 h (GTM-5) on various between August 8th and November 30th, 2017. Because we were interested in levels of water stress, we report only the means of temperature and RH between 12:00 and 15:00 h.

Desiccation tolerance assay and recovery of photosystem II- In order to determine a lower relative humidity (RH) limit to test for DT, we completed a pilot experiment using salt solutions to achieve 75% (Sodium Chloride), 55% (Magnesium Nitrate) and 33% (Magnesium Chloride) RH (Greenspan, 1977). Sufficient salt was added to each salt solution conteiners so salt cristals were visible in the solution during the period of the experiment. We also tested rewetting protocols for recovery using (1) free water or a (2) pre-hydration period of 6 h in high RH, then addition of free water. Pre-hydration conditions were created by removing the desiccationsolution and adding free water inside the DT chamber (a quantity sufficient to cover the botton of the chamber), while the plants were kept on the dried filter paper on Petri dishes. *Plagiochila porelloides* was able to survive at the lowest RH tested and recovered better in pre-hydration conditions compared to direct rewetting to achieve rehydration. Thus, we used 33% RH and the recovery protocol of a pre-hydration to test for sex differences in DT.

Twelve males and 12 female plants were collected from each of the three populations for a total of 72 plants and they were maintained in environmental chamber Percival® with a 12h light/ 12h dark cycle period, and light condition ranging between 24 to 44 μmol m^{-2} s^{-1}, and a constant temperature of 14º C. Plants were kept for at least one week after collection in these conditions to allow for DT dehardening to occur (STARK, 2017).

Before the DT assays, plants were placed in full hydrated conditions for 24 h (Fig. 1A). After, full hydration, plants were then blotted to remove external water, using filter paper (No. 1 Whatman® filter paper) and immediately placed in 35 x 10 mm Falcon ™ brand Petri dishes with 125μl of distilled water added to a disc of filter paper. This condition provides a slowly drying process that, according to SCHONBECK & BEWLEY (1981), PROCTOR et al. (2007b), and STARK (2017), avoids severe physiological damage.

Following the experimental design of MARKS et al. (2016), Petri dishes containing male and female shoots were placed inside four desiccation chambers, each one containing three males and three females from each population, for a total of 18 plants in each desiccation chamber. Assignment to a desiccation chamber was random. The desiccation chamber consists in a plastic box (24 x10x 32 cm), where dishes were arranged peripherally inside; an open container of saturated salt solution was placed at the center of the chamber. To ensure air mixing, a battery-powered fan was placed above the salt solution. RH of the chambers during desiccation was verified using the humidity sensor HOBO ^{TM} attached to a data logger (Onset Computer Corporation, Bourne, MA, USA). Plants were kept in desiccation conditions for 22 h.

To test for the status of the plants we assayed for maximal quantum yield (F_v/F_m) of photosystem II (PSII) as a proxy for recovery, using a OS5-FL modulated chlorophyll fluorometer (Opti-Sciences, Tyngsboro, Massachusetts, USA) with measurement parameters set to a saturation intensity of 100 and a pulse duration of 0.8 seconds; plants were dark-adapted (20 min), using clips for dark-adaption. F_v/F_m (efficiency of photosystem II) and the fluorescence of the chlorophyll have been routinally used to assay and indicate recovery in DT studies (PROCTOR et al., 2007 and references therein; STARK 2017 and references therein; MARKS et al., 2016). We measured an initial F_v/F_m of each shoot before desiccation. After the desiccation period of 22 h, shoots were placed in pre-hydration chamber, similar to the desiccation chamber but with water instead of saturated salt solution, for a period of six hours. Immediately after this high humidity exposure, free water was added and, again, efficiency of PSII were accessed at various intervals: 0 h, 4 h, 12 h, 24 h, 48 h, 72 h, 144 h, and 216 h after addition of free water.

Evaluation of water content by dry weight - To evaluate shoot water content (WC) at 40% RH (measured value with humidity sensor) and to establish the rate of drying, ten vegetative tissues of *P. porelloide*s maintained in the growth chamber were subjected to a desiccation experiment as described above. To assay WC and plant water lost, we weighed each plant to the nearest nanogram using a Chan 29 electrobalance, after 14 h, 18 h, and 22 h of desiccation in a 40% RH chamber. To obtain dry weight (DW), plants were then oven dried at 70º C for 48 h. The first time of weighting (14 h) was determined by observing the leaves desiccated morphology (curled or contorted leaves, Fig. 1B) (STARK, 2017). If the mass did not change in value during the weights, we considered that the plant tissue was equilibrated to RH in the desiccation chamber. The WC was calculated on a dry weight basis (STARK, 2017):

$$WC(\%) = \frac{(Shoot\ mass\ at\ RH - DW)}{DW} \times 100$$

Gas exchange responses to water stress - To confirm that desiccation reduced key physiological activities and that recovery resulted in a return of these activities we measured gas exchange (in light and in the dark) in three different sets of plants: G1 - seven groups of (healthy) plants, G2 - three groups of desiccated plants, measured three hours after rewetting, and G3 - threes groups of plants measured three days (72 hours) after rewetting. Finally, the groups of plants that were subjected to desiccation (G2 and G3) were re-assayed one month after desiccation. Thus, all groups of plants were measured once, except for the desiccated groups, which were assayed twice.

Gas exchange was measured with an open-flow system (LI-6800, Li-Cor, Lincoln, Nebraska, USA) equipped with a multiphase flash fluorometer as the light source. In the sample chamber, air temperature was set to 24°C and relative humidity at 75% (vapor pressure deficit (VPD = 0.749 kPa). Airflow rate was set at 125 μmol s^{-1}, and the reference

CO_2 was set at 450 µmol mol^{-1}. In a pilot trial we determined that A_{max} (maximum assimilation) for *P. porelloides* occurred by a light level of 100 µmol m^{-2} s^{-1}. Thus, physiological activity in the light was tested at 100 µmol m^{-2} s^{-1}. The infrared gas analyzers (sample and reference) were matched prior to assays.

We used three shoots of *P. porelloides* in each assay to improve the gas exchange signals. Sample area was estimated by measuring length and width of each shoot, assuming the shoot is rectangular, and the leaves cover this area (Fig. 1A). Plants were placed on acetate and held in place with a thin fishing line. To standardize water status across assays plants were blotted dry with tissue paper to remove free water and 5 µl of water were added to ensure that plants had enough moisture to prevent drying out before the completion of the assay. CO_2 assimilation rates (µmol CO_2 m^{-2} s^{-1}) were taken after equilibrium (approximately 6 minutes after the beginning of the assay) and at 100 µmol m^{-2} s^{-1} (net photosynthesis) and 0 µmol m^{-2} s^{-1} (respiration in the dark). Generally, three readings were then taken within one minute to obtain a mean estimate for that sample. These means were then used for statistical analysis.

Statistical Analyses

We used a combination of Microsoft Excel (2016) and JMP® 12 (SAS Institute, Cary, North Carolina, USA) to analyze the data. We consider $p < 0.05$ as significant.

Desiccation tolerance assay – To access sex and population variation in desiccation tolerance we estimated initial F_v/F_m of healthy tissues of each sex (collected from the three populations) using 36 shoots for each sex, 24 per population. The initial F_v/F_m for each shoot was considered the value for healthy and hydrated plants. Subsequently F_v/F_m was measured

in each shoot after desiccation and rewetting through time. These values were used to calculate percent recovery using the following equation:

$$\text{Percent recovery} = (F_v/F_m \text{ at time t} / \text{Initial } F_v/F_m) \times 100.$$

We chose using percent recovery by standardizing initial F_v/F_m due to females having higher initial F_v/F_m compare to males (p=0.0268). Initial F_v/F_m for populations were not different (p=0.2267). Percentage recovery was arcsine transformed to improve normality (SOKAL & ROHLF, 1995).

Using percent recovery, we evaluate sex and population effect over time, performing a repeated measures analysis using multivariate analysis of variance (MANOVA) with full factorial design. To perform the test, we used sex and population as independent variables in the full factorial model effect (Sex, Population, and Sex by Population); and evaluated Time, Time*Sex, Time*Population, and Time*Sex*Population as possible interactions. Time was evaluated to test if plants increased in recovery, a significant sex effect would indicate the sexes differed in recovery. A significant population effect would suggest population differences. Interaction of these main effects over time would indicate that differences in the main factor depended on time (the slopes differed).

Evaluation of water content by dry weight - Descriptive statistics were performed to assess water content.

Gas exchange responses to water stress in the light – To assess recovery via gas exchange we used an ANOVA with comparisons between mean of photosynthesis at 100 μmol m^{-2} s^{-1} of healthy plants with mean of photosynthesis of plant that were desiccated within three hours, 72 hours and one month of recovery in hydrated conditions.

Gas exchange responses to water stress in the dark - To assess respiration in the dark, we used ANOVA with comparisons between mean of respiration at 0 μmol m^{-2} s^{-1} of healthy plants and mean of respiration of plant that were desiccated within three hours, 72 hours and one month of recovery in hydrated conditions.

RESULTS

Field water conditions - From August 8th to November 30th, *P. porelloides* experienced field RH ranging from 75.97 % to 94.2 % (a mild and high humidity levels); and an average temperature ranging from 4.55ºC, in late autumn, to 19.71ºC, during the summer. These RH and temperatures translated to VPD ranged from 0.1053 to 0.2700 kPa.

Desiccation tolerance recovery – Plagiochila porelloides showed ability to survive the desiccation assay performed, confirmed by recovery ofPSII activities recovery. There was an overall time effect, indicating significant increases in plant recovery ($F = 35.5669_{7/20}$, $p < 0.0001$). Females had a higher percentage of recovery than males, demonstrated by an overall sex effect ($F = 4.7408_{1/26}$, $p = 0.0387$). The overall percent recovery among the populations did not differ ($F = 0.2353_{2/26}$, $p = 0.1983$) (Fig. 2A).

Changes in rates of recovery for males and females over time did not differ as indicated by a non-significant sex by time interaction ($F = 1.1083_{7/20}$, $p = 0.3958$). Changes in rates of recovery for populations over time differed ($F = 2.5559_{14/40}$, $p < 0.01$). However, there was a significant sex by population by time interaction ($F = 2.0737_{14/40}$, $p = 0.0360$), suggesting that the difference among the populations over time depended on sex). To further explore this interaction, each population was analyzed separately to test for sex differences. Changes in rates of recovery for males and females did not differ in populations P1 ($F=1.4956_{7/5}$, $p= 0.4882$) and P2 ($F=29.1519_{7/2}$, $p= 0.1113$), but did differ in P3($F=39.8416_{1/7}$, $p < 0.001$), with females increasing in recovery faster than males (Fig. 2B).

Water content by dry weight – After six hours, RH in desiccation chambers reached 40%, and remained stable until the end of the experiment (Fig. 3A) At this RH and temperature 14 ºC (growth chamber temperature), VPD value was 0.9309 kPa. Turgid tissue had a WC of 442.28

± 136.49 % (Fig. 1A); tissue equilibrated to 40% RH in desiccation conditions had a WC of 9.87 ±1.24% by 14 h, and 9.27± 0.50% by 18 h in the desiccation chamber. After 22 h of desiccation, plant tissue had a water content of 8.81±0.33% (Fig. 3B).

Gas exchange responses to water stress – The photosynthetic rates of healthy plants were 0.3205± 0.1047 μmol CO_2 s^{-1} m^{-2}. Desiccated plants in the recovering process for three and 72 h had a net carbon loss, with photosynthetic rate of -0.0938 ± 0.0922 μmol CO_2 s^{-1} m^{-2}, and -0.0805 ± 0.0912 μmol CO_2 s^{-1} m^{-2}, respectively. These rates were significantly lower than the rate for healthy plants ($t= 2.4367$, $p = 0.0278$ and $t = 2.358$, $p = 0.0324$ for comparisons between healthy and recovering plants at 3 h and 72 h respectively). After a month of recovering in hydrated conditions, the photosynthetic rates of plants that were recovering was 0.2021 ± 0.1079 μmol CO_2 s^{-1} m^{-2}. This assimilation rate was not different from the photosynthetic rate of healthy plants ($t= 0.8636$, $p = 0.4014$) (Fig. 4). The dark respiration rates of plants did not differ among healthy and desiccated plants (p values range from 0.3152 to 0.4634).

DISCUSSION

The mesic liverwort *Plagiochila porelloides* is desiccation tolerant (DT), and this tolerance differed by sex and varied by population. Photosystem II recovered after a few days, but this recovery did not reflect carbon gain.

Plagiochila porelloides is a DT liverwort – In spite of growing in a mesic habitat *P. porelloides* is DT. During the period of the study, plants in the field during the driest part of the day were exposed to a RH that varied from 75% to 92%, a mild to high humid levels. However, in the laboratory, plants were exposed to 40% RH which resulted in plants having less than 0.1 g H_2O g^{-1} dry mass or 10 % of water content (WC); and they were still able to recover, demonstrating that *P. porelloides* is desiccation tolerant. PROCTOR et al. (2007b) established that, in general, bryophyte DT species demonstrate a best recovery when they are dry out in RH ranging between 20% and 50%, considering a temperature of 20 ºC, and having a WC between 5 – 10% of their dry weight. PROCTOR (2003) demonstrate that, for long term survival, DT species showed best survival after desiccated in 20 – 45% of RH. Our findings are consistent with DT previous definitions by OLIVER (2009), PROCTOR et al. (2007b), OLIVER et al. (2010), and STARK (2017).

Here, we demonstrated that a species found in a mesic habitat is DT. Thus, the ecological descriptor of a plant as mesic does not disqualify being DT. The links between DT and habitat have been reviewed elsewhere (ALPERT, 2000; PROCTOR ET AL., 2007b); there are examples at the species level where plants found with the same habitat differ in DT, based on microhabitat (epiphytic overstory versus epiphytic understory; Pardow and Lakatos (2013)). There are reports of species surviving more extreme drying events as is the case of *Syntrichia ruralis* and *Racomitrium lanuginosum*, which have an ecological descriptor as xeric (expose and sunny) habitats (surviving WC as low as 5% of their dry weight)

(PROCTOR, 2000) and are considered species highly desiccation tolerant (DILKS & PROCTOR, 1974). On the order hand, *Anomodon viticulosus* and *Plagiothecium undulatum*, species found in shady WOODland or grassland habitats, were considered less desiccation tolerant (surviving WC of 15 – 20%) (DILKS & PROCTOR, 1974; PROCTOR, 2000). However, despite the rich history of studies examining variation in DT among plants, they do not explore this tolerance within a species nor its ecological features as seasonal variation, population dynamics, and development (but see FARRANT & MOORE (2001) and FARRANT et al. (2009) for vascular plants, and OLIVER et al. (1993) and STARK et al. (2007) for bryophytes.

Sex differences on water stress – Relative to males, female plants of *P. porelloides* were found to recover more from desiccation event, as indicated by the fluorescence measured data. This result corroborates the few previous reports on female DT advantage. Female advantage in water strees tolerance relative to males was first suggested in the liverwort *Riccia frostii* (Pettet, 1967) and later studies of NEWTON (1972), STIEHA et al. (2014), MARKS et al. (2016), and Slate et al. (2017). However, in the moss *Syntrichia caninervis*, sex differences in DT was not found (STARK et al., 2005).

We suggest that females are subjected to different water requirements relate to males because of female function (gamete production, interception of male gametes, and offspring maturation). Considering that female function can occur over a longer time interval than male function (gamete production and release), female plants will experience more drying events during their life span, including the critical sexual reproductive period relative to males. Moore et al. (2016) demonstrate that *Bryum argentum* female clumps have a greater water holding capacity than male clumps, indicating sex differences in water use.

In seed plants, sex differences in water stress tolerance is dependent on species and environmental conditions (JUVANY & MUNNÉ-BOSCH, 2015). In general, studies have demonstrated female sensitivity under dry conditions relative to males (LEIGH & NICOTRA, 2003; LI et al., 2004), in addition to a lower photosynthetic capacity under drought stress (CHEN et al., 2010; ZHANG et al., 2010). In contrast, females showed higher photosynthetic rates than males in the shrub *Corema album* in dry conditions (ÁLVARES-CANSINO et al., 2010).

Population patterns - We found that there was variation in population in recovery over time that depended on sex. Specifically, based on the chlrorophyll fluorescence, females had a higher percentage of recovery in one population compared to the other two. The population variation might be genetic or a plastic response. Plants in this study were dehardened for at least 7 days and this process would have removed some field effects. If these effects were removed, then the patterns would be due to genetic differences among the populations. However, desiccation tolerance can be environmentally induced, gaining or losing the ability to cope with desiccation depending on environmental stimuli (PROCTOR et al., 2007b).

Some authors suggest that bryophyte responses to desiccation are a phenotypic response to previous drought intervals and different intensities of water deficit (PROCTOR, 2009; PROCTOR et al., 2007b). The scale of a bryophyte, including size, microclimate events of humidity, temperature and windspeed is critical to bryophyte physiology and ecology (PROCTOR et al., 2007b). These might easily vary with populations, modulating the phenotypic responses of DT in a species (PROCTOR et al., 2007b and references within).

Physiological responses to recovery on rehydration process – Our results show that assessment of recovery based on quantum yield versus carbon gain differed with quantum recovery occurring by three days, but carbon gain occurs sometime after. Thus, initial

photosystem II recovery in its functions does not reflect a recovered plant in terms of carbon gain. Bryophytes often experience many drying/rewetting cycles during their life span (MISHLER & OLIVER, 2009; OLIVER, 2009). In these cycles, assuming that the dried plants will have a zero-carbon balance due to metabolism suspense, there is a net carbon loss after rehydration (MISHLER & OLIVER, 2009). Although the photosystems can recovery within minutes of rewetting (PROCTOR & SMIRNOFF, 2000; PROCTOR, 2001), respiration recommences even quicker (OLIVER, 1991), causing an initial carbon loss for recovering plants. Similar results were found in the moss *Anomodon viticulosus*, in which net photosynthesis were negative immediately after rewetting; and this net carbon loss was directly proportional to desiccation period (HINSHIRI & PROCTOR, 1971). In *Syntrichia caninervis*, another moss species, a significant longer desiccation period increases the respiration rates by 50%, indicating a respiration cost of hydration extending to carbon deficit state (COE et al., 2012).

CONCLUSION

We found *Plagiochila porelloides*, a mesic species belonging to the largest liverwort genus, to be a desiccation tolerant liverwort, supporting the expectation that many bryophytes are DT. We observed a differential sex response on recovery of photosystem II, indicating that females hada better recovery and that, ultimately, they were more desiccation tolerant than males; and a population difference in sex recovery, indicating a sex difference associated with some genetic and possible plastic response. Photosynthetic rates of recovering desiccated plants indicate a deficit of carbon gain extending through 72 h, demonstrating that shoots took more than three days to recovery, despite chlorophyll fluorescence indicated initial recovery. Our study adds important information to sex-differences in DT in plants.

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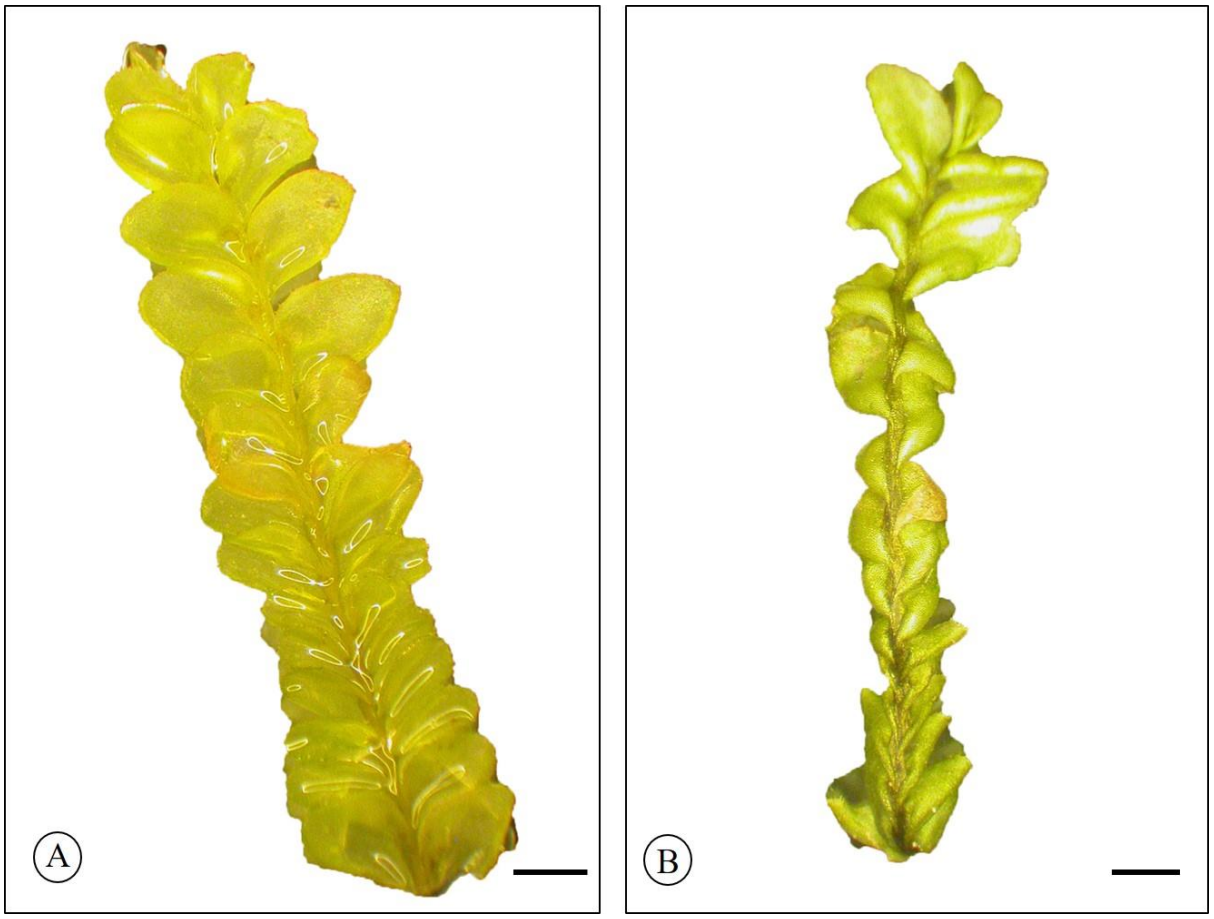

Figure 1. Dorsal view of the gametophyte of *Plagiochila porelloides* aspects in (A) full hydrated condition and (B) dry condition. Scale bar: 1 mm.

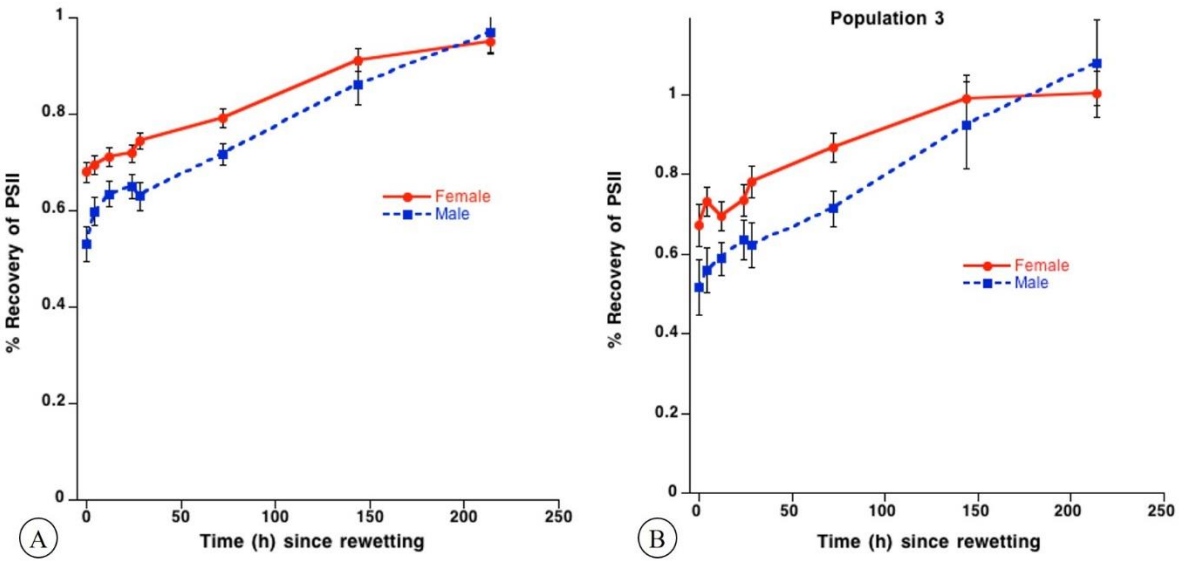

Figure 2. Percent recovery of photosystem II assayed by chlorophyll fluorescence. A. Overall sex difference in recovery B. Recovery in population 3 showing change the sex difference in recovery over time.

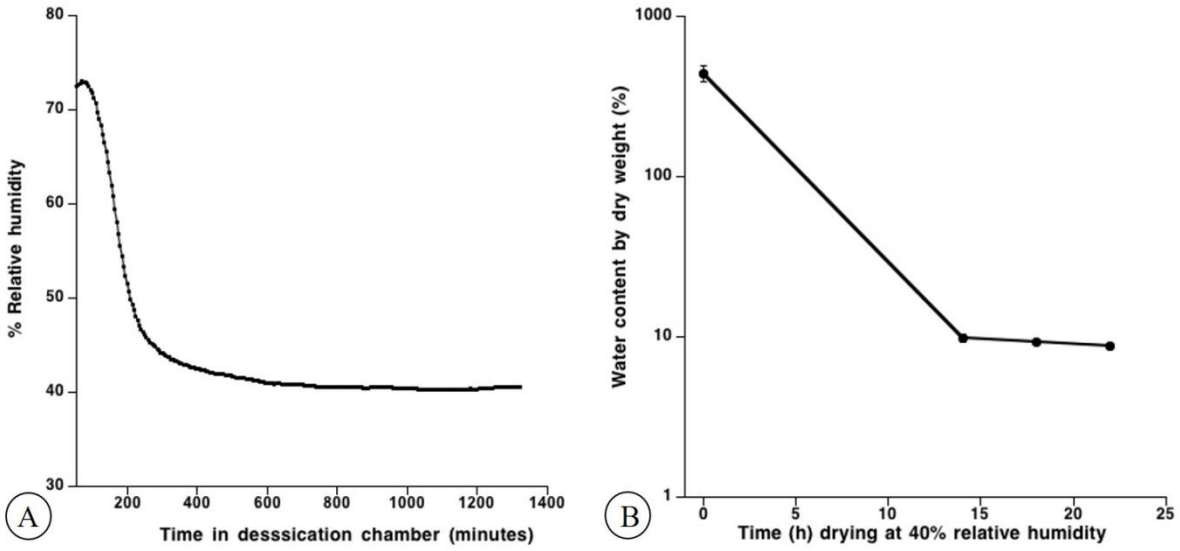

Figure 3. A: Relative humidity inside desiccation chamber during the assay: after the first 6 h, RH stabilized in 40%; B: Water plant status showing water content loss during the desiccation assay at 40% of relative humidity.

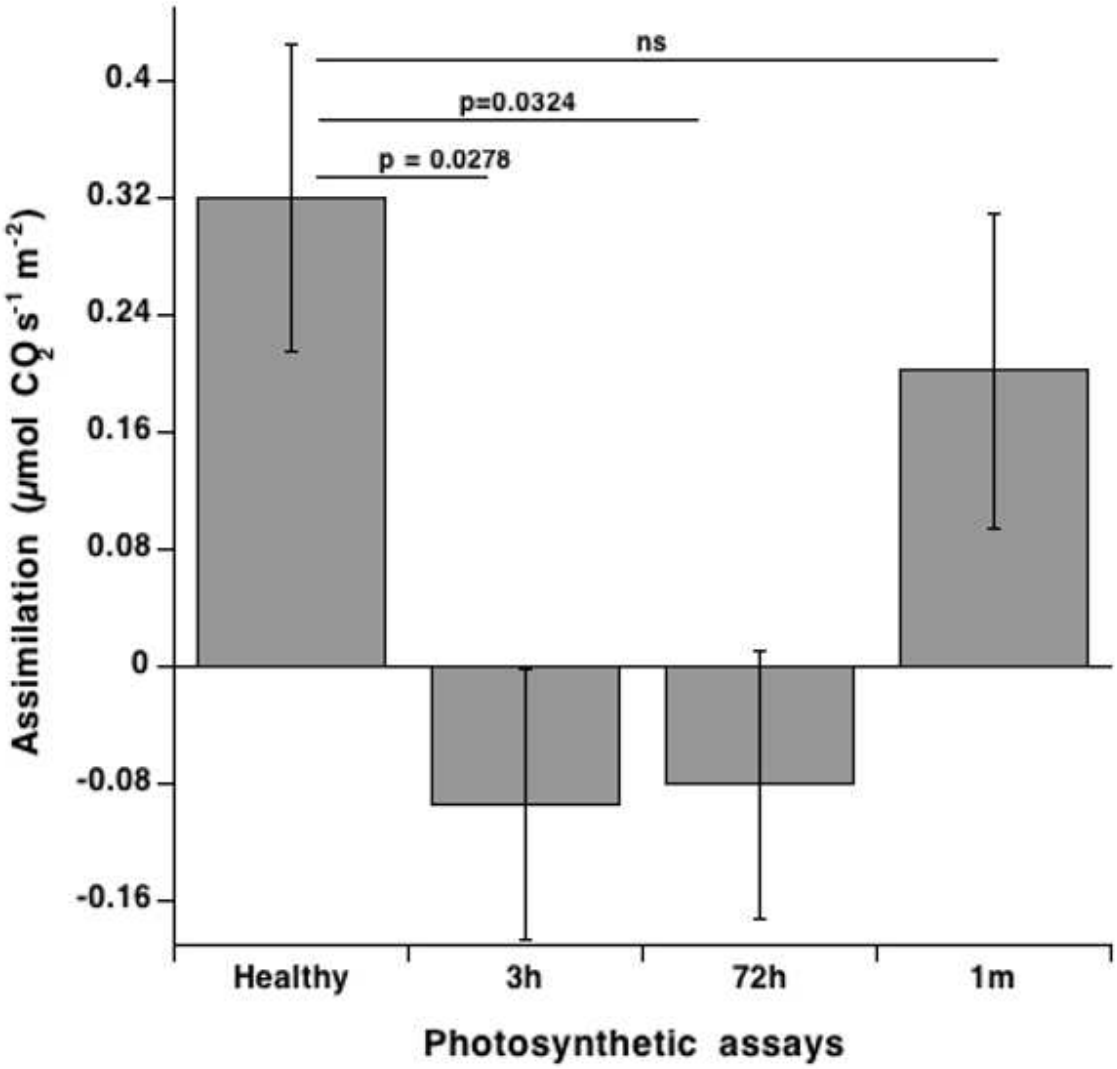

Figure 4. Carbon net assimilation evaluated through a photosynthetic assay in three groups of plants, in four different water status conditions: healthy, three hours and 72 in recovery after 22 h of desiccation, and one month of recovery after desiccation. A significant difference was detected between carbon net assimilation in healthy plants and three hours of recovery (p = 0.0278) and 72 hours of recovery (p=0.0324). No differences were found between healthy plants and those in a month of recovery. 1m = one month.

FINAL CONCLUSION

Plagiochila is a notable genus among leafy liverworts and bryophytes, with a great richness and species diversity, and remains a challenge for taxonomists. This dissertation contains novel information about the genus *Plagiochila* regarding to spore morphology, reproductive biology, and ecological aspects, that have potential to contribute to both taxonomic and ecological interpretation:

1. Spores were found to have a granulate ornamentation, previously observed in some phylogenetic and taxonomic works, but mistaken interpreted as verrucate or baculate ornamentation;
2. Spore analyses under electron microscopy revealed characteristics of the sporoderm found in other spores of liverworts (exine divided into nexine and sexine) and mosses (stratified intine), demonstrating the importance of spore morphology knowledge to understand how these plants had evolved;
3. The inclusion of spore morphology information in a matrix containing taxonomic information as gametophyte features, reproduction and substrata occupation resulted in species aggregation into groups that do not confirm the current taxonomic arrangement;
4. The male-biased sex ratio detected in populations of *Plagiochila porelloides* was an interesting find among a dominance of female-biased sex ratio species in plants; and its correlates to fertilization success demonstrated the importance of the study to improve the understanding of the biology of sexual reproduction;
5. Although no significative relationships were detected between sex ratios and the environmental factors studied here for *P. porelloides*, light, canopy exposure, and community composition have already been related to the sexual reproduction in other bryophytes and vascular plants. Thus, it is worth to further investigations;

6. A mesic leafy liverwort was found to be desiccation tolerance in laboratorial conditions;
7. Physiological requirements vary between the sexes, as observed that females were more desiccation tolerant than males, a pattern also found to few other liverworts and some vascular plants;
8. As demonstrated here, *Plagiochila porelloides* has different sex responses to water stress tolerance and also has populations with an unbalanced sex ratio. The different tolerance between the sexes can potentially influence the rates of survival, and consequently, population dynamics.

REFERENCES

ABE, T. et al. Sex ratio variation of *Bischofia javanica* Bl. (Euphorbiaceae) between Native Habitat, Okinawa (Ryukyu Islands), and Invaded Habitat, Ogasawara (Bonin Islands). **International Journal of Plant Sciences**, n. 163:1011–1016, 2002.

ALPERT, Peter. The discovery, scope and puzzle of desiccation tolerance in plants. **Plant Ecology** n. 151: 5–17, 2000.

ALPERT, Peter. The limits and frontiers of desiccation-tolerant life. Integrative and **Comparative Biology** n. 45: 685–695, 2005.

ALPERT, Peter.; OLIVER, Melvin J. Drying without dying. In: BLACK, Michael; PRITCHARD, Hugh W. **Desiccation and Survival in Plants**: Drying without Dying. Wallingford: CABI Publishing, 2002. 3-43.

ALVARENGA, Lisi D.; PÔRTO, Kátia C.; ZARTMAN, Charles E. Sex ratio, spatial segregation, and fertilization rates of the epiphyllous moss *Crossomitrium patrisiae* (Brid.) Müll.Hal. in the Brazilian Atlantic rainforest. **Journal of Bryology** 35(2):88-95, 2013.

ÁLVAREZ-CANSINO, Leonor et al. Physiological performance and xylem water isotopic composition underlie gender-specific responses in the dioecious shrub *Corema album*. **Physiologia Plantarum** n. 140 (1): 32–45, 2010.

AMORIM, Eduardo T.; GOMES, Helen C. S; LUIZI-PONZO, Andrea P. Plagiochilaceae of Southeastern Brazilian Region (Parque Estadual do Ibitipoca, Minas Gerais, Brazil). **Boletim do Instituto de Botânica (São Paulo)** n. 21: 173-183, 2011.

ANDERSON, Gregory; LEVINE Deborah A. Three taxa constitute the sexes of a single dioecious species of *Solanum*. **Taxon** n. 31:667-672, 1982.

ARTEAGA-VAZQUEZ, Mario A. Land Plant Evolution: Listen to Your Elders. **Current Biology**, n. 26, 2016. Disponível em <http://dx.doi.org/10.1016/j.cub.2015.12.001>.

BARRET, Spencer C. H. et al. Ecological genetics of sex ratios in plant populations. **Philosophical Transactions of the Royal Society B** n. 365: 2549–2557, 2010.

BENASSI, Michael et al. Plant size, sex expression and sexual reproduction along an elevation gradient in a desert moss. **The Bryologist** n. 114(2):277-288, 2011.

BERRIE, Geoffrey K. Sex chromosomes of *Plagiochila praemorsa* Stephani and the status of structural sex chromosomes in Hepatics. **Bulletin de la Société Botanique de France** n. 121(2): 129-135, 1974.

BISANG, Irene et al. Family affiliation, sex ratio and sporophyte frequency in unisexual mosses. **Botanical Journal of the Linnean Society** n. 174 (2): 163–172, 2014.

BISANG, Irene; HEDENÄS, Lars. Sex ratio patterns in dioicous bryophytes re-visited. **Journal of Bryology** n. 27:207–219, 2005.

BLACK, M.; PRITCHARD, H. W. **Desiccation and Survival in Plants**: Drying without Dying. Wallingford: CABI Publishing, 2002.

BLACKMORE, Stephen; BARNES, Susan H. Embryophye spore walls: Origin, development, and homologies. **Cladistics** n. 3: 185 -195, 1987.

BOWKER, Matthew A. et al. Sex expression, skewed sex ratios and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). **American Journal of Botany** n. 87: 517-526, 2000.

BROWN, Roy C.; LEMMON, Betty E. Ultrastructure of sporogenesis in a moss: *Ditrichum pallidum*. III. Spore wall formation. **American Journal of Botany** n. 67(6): 918-934, 1980.

BROWN, Roy C.; LEMMON, Betty E. Ultrastructure of sporogenesis in moss *Amblystegium riparium* II. Spore wall development. **Journal of Hattori Laboratory** n. 57:139-152, 1984a.

BROWN ,Roy C.; LEMMON, Betty E. Spore wall development in *Andreaea* (Musci: Bryopsida). **American Journal of Botany** n. 71:412-420, 1984b.

BROWN, Roy C.; LEMMON, Betty E. Sporogenesis in Bryophytes. **Advances in Bryology** n. 3: 159-223, 1988.

BROWN, Roy C.; LEMMON, Betty E. Sporogenesis in simple land plants. In: BLACKMORE, Stephen; BARNES, Susan H. (eds.) **Pollen and Spores**. Patterns of Diversification. Oxford: Clarendon Press, 1991, 9-24

BROWN, Roy C. et al. Spores of relictual bryophytes: Diverse adaptations to life on land. **Review of Palaeobotany and Palynology** 216: 1-17, 2015.

BRZYSKI, Jessica R.; STIEHA, Christopher; MCLETCHIE, D. Nicholas. The impact of asexual and sexual reproduction in spatial genetic structure within and between populations of the dioecious plant *Marchantia inflexa* (Marchantiaceae). **Annals of Botany** n. 122: 993-1003, 2018.

CALDEIRA, Isabela C.; GONÇALVES-ESTEVES, Vânia.; LUIZI-PONZO, Andrea P. Morfologia dos esporos das espécies de Leucobryaceae Schimp. (Bryophyta) do Parque Estadual de Ilha Grande, Município de Angra dos Reis, Estado do Rio de Janeiro. **Revista Brasileira de Botânica** n. 29: 301-307, 2006.

CALDEIRA, Isabela C.; GONÇALVES-ESTEVES, Vânia.; LUIZI-PONZO, Andrea P. Morfologia dos esporos de Sematophyllaceae Broth. ocorrentes em três fragmentos de Mata Atlântica, no Rio de Janeiro, Brasil. **Revista Brasileira de Botânica** n. 32: 299-306, 2009

CALDEIRA, Isabela C.; GONÇALVES-ESTEVES, Vânia.; LUIZI-PONZO, Andrea P. Palynology of selected species of *Fissidens* (Hedw.). **Plant Systematics and Evolution** n. 299: 187-195, 2013.

CAMPELL, Douglas H. Resistance of Drought by Liverworts. **Torreya** n. 4(6): 81-86, 1904.

CARL, Helmut. Die Arttypen und die systematische Gliederung der Gattung *Plagiochila* Dum. **Annales Bryologici**, Supplement 2: 1-170, 1931.

CARPENTER, Stanley B.; RUMSEY, Robert L. Trees and Shrubs of Robinson Forest Breathitt County, Kentucky. **Castanea** n. 41 (4): 277-282, 1976.

CHEN, Lianghua et al. Sex-related adaptive responses to interaction of drought and salinity in *Populus yunnanensis*. **Plant, Cell and Environment** n. 33: 1767–1778, 2010.

CID-BENEVENTO, Carmen R. Relative Effects of Light, Soil Moisture Availability and Vegetative Size on Sex Ratio of Two Monoecious Woodland Annual Herbs: *Acalypha rhomboidea* (Euphorbiaceae) and *Pilea pumila* (Urticaceae). **Bulletin of the Torrey Botanical Club** n. 114 (3): 293-306, 1987.

CLARKE, Giles Colin S. Spore Morphology and Bryophyte Systematics. In: CLARKE, Giles Colin S.; DUCKETT, Jeffrey G. **Bryophyte Systematics**. Systematic Association Special Volume n. 14. London and New York: Academic Press, 1979, 231-250.

CLAUSEN, Eva. The Tolerance of Hepatics to Desiccation and Temperature. **The Bryologist** n. 67 (4): 411-417, 1964.

COE, Kirsten K.; BELNAP, Jayne; SPARKS, Jed P. Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. **Ecology** n. 93: 1626–1636, 2012.

CORREA, Sônia Maria B.B. **Probabilidade e estatística**. 2.ed. Belo Horizonte: PUC Minas Virtual, 2003. 116p.

COX, Cymon J. Land Plant Molecular Phylogenetics: A Review with Comments on Evaluating Incongruence Among Phylogenies. **Critical Reviews in Plant Sciences**, DOI: 10.1080/07352689.2018.1482443, 2018.

CRANDALL-STOTLER, Barbara; STOTLER, Raymond E.; LONG, David G. Morphology and Classification of the Marchantiophyta. In: SHAW, Jonathan A., GOFFINET, Bernard. **Bryophyte Biology**. Cambridge, Cambridge University Press, 2009, 1-54.

CRONBERG, Nils. Absence of genetic variation in populations of the liverwort *Plagiochila porelloides* from northern Greece and southern Scandinavia. **Lindbergia** n. 25: 20-24, 2000.

CRONBERG, Nils. Clonal distribution, fertility and sex ratios of the moss *Plagiomnium affine* (Bland.) T. Kop. in forests of contrasting age. **Journal of Bryology** n. 25:155-62, 2003.

CRONBERG, Nils; RYDGREN, Knut; ØKLAND, Rune H. Clonal structure and genet-level sex ratios suggest different roles of vegetative and sexual reproduction in the clonal moss *Hylocomium splendens*. **Ecography** n. 29:95-103, 2006.

CRUM, Howard. **Structural diversity of bryophytes**. Ann Arbor: University of Michigan Press Herbarium, 2001. 379 p.

CUEVAS, Eduardo; PÉREZ, María Ángeles; SEVILLANO, Lucero. Population size, sex ratio, and sexual dimorphism in *Fuscia parviflora* (Onagraceae) an endemic dioecious shrub. **Botanical Sciences** n. 95 (3): 401-408, 2017.

DILKS, Thomas J. K.; PROCTOR, Michael C. F. The pattern of recovery of bryophytes after desiccation. **Journal of Bryology** 8: 97–115, 1974.

DINAKAR, Challabathula; BARTELS, Dorothea. Desiccation tolerance in resurrection plants: new insights from transcriptome, proteome and metabolome analysis. **Frontiers in Plant Sciencies** 4:482, 2013. DOI: 10.3389/fpls.2013.00482

DUGAS, Marguerite. Contribution à l'étude du genre "*Plagiochila*" Dum. **Annales des Sciences Naturelles; Botanique**, Série 10 11: 1–199, 1929.

ELLISON, Aaron. M., et al. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. **Frontiers in Ecology and The Environment** n. 3(9): 479 – 486, 2005.

ERDTMAN, Gunnar. **Pollen Morphology and Plant Taxonomy**. Angiosperms. An Introduction to Palynology I. Stockholm: Almqvist and Wiksell, 1952.

ERDTMAN, Gunnar. **The acetolysis method**. A revised description. Svensk Bot Tidskr n. 39: 561-564, 1960.

ERDTMAN, Gunnar. **Pollen and Spore Morphology/ Plant Taxonomy**. Gymnospermae, Bryophyta. An Introduction to Palynology III. Stockholm: Almqvist and Wiksell, 1965.

ESTÉBANEZ, Belén; ALFAYATE, Carmen; RON, Eugenia. Observations on spore ultrastructure in six species of *Grimmia* (Bryopsida). **Grana** 36: 347-357, 1997.

FARRANT, Jill M.; MOORE, John P. Programming desiccation-tolerance: From plants to seeds to resurrection plants. **Current Opinion in Plant Biology** n. 14:340–345, 2011.

FIELD, David L.; PICKUP, Melinda; BARRETT, Spencer C. H. Comparative analyses of sex-ratio variation in dioecious flowering plants. **Evolution** n. 67: 661-672, 2012.

FIELD, David L.; PICKUP, Melinda; BARRETT, Spencer C. H. Comparative analyses of sex-ratio variation in dioecious flowering plants. **Evolution** n. 67: 661-672, 2013a.

FIELD, David L.; PICKUP, Melinda; BARRETT, Spencer C. H. Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations. An**nals of Botany** n. 111: 917–923, 2013b.

FISHER, Ronald A. **The Genetical Theory of Natural Selection**. Oxford: Oxford University Press, 1930, 308 p.

FORD, Chelcy R. et al. Forest dynamics following eastern hemlock mortality in the southern Appalachians. **Okios** n. 121: 523-536, 2012.

FREEMAN, D. Carl; MCARTHUR, E. Durant. A comparison of twig water stress between males and females of six species of desert shrubs. **Forest Science** n. 28: 304–308, 1982.

FREY, Wolfgang; KÜRSCHNER, Harald. Asexual reproduction, habitat colonization and habitat maintenance in bryophytes. **Flora** n. 206: 173–184, 2011.

FUSELIER, Linda; MCLETCHIE, D. Nicholas. Microhabitat and sex distribution in *Marchantia inflexa*, a dioicous liverwort. **The Bryologist** n. 107: 345-356, 2004.

GAO, Bei et al. Desiccation tolerance in bryophytes: The dehydration and rehydration transcriptomes in the desiccation-tolerant bryophyte *Bryum argenteum*. **Scientific Reports** 7: 7571 | DOI:10.1038/s41598-017-07297-3, 2017.

GARCIA, María B.; ANTOR, Ramón. Sex ratio and sexual dimorphism in the dioecious *Borderea pyrenaica* (Dioscoreaceae). **Oecologia** n. 101:59-67, 1995.

GARCIA-RAMOS, Gisela. et al. Persistence of the sexes in metapopulations under intense asymmetric competition. **Journal of Ecology** n. 95: 937-950, 2007.

GARNIER, Eric et al. Plant functional markers capture ecosystem properties during secondary succession. **Ecology** n. 85(9): 2630–2637, 2004.

GENTRY, Alwyn H. Changes in Plant Community Diversity and Floristic Composition on Environmental and Geographical Gradients. **Annals of the Missouri Botanical Garden** n. 75(1): 1-34, 1988.

GLIME, Janice M. Water Relations: Physiological Adaptations. In: GLIME, Janice M. **Bryophyte Ecology**. Volume 1. Physiological ecology. Chapt. 7-5. 2017. E-book sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 7 March 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>

GLIME, Janice M.; BISANG, Irene. Sexuality: Sex Ratio and Sex Expression. In: GLIME, Janice M. **Bryophyte Ecology**. Volume 1. Physiological ecology. Chapt. 3-2. 2017. E-book sponsored by Michigan Technological University and the International Association of Bryologists. Last updated 19 April 2017 and available at <http://digitalcommons.mtu.edu/bryophyte-ecology/>.

GODLEY, Eric J. Breeding systems in New Zealand plants. 3. Sex ratios in some natural populations. **New Zealand Journal of Botany** n. 2(2): 205-212, 1964.

GOFFINET, Bernard; BUCK W. R.; SHAW Jonathan A. 2009. Morphology, anatomy, and classification of the Bryophyta. In: GOFFINET, Bernard; SHAW Jonathan A. **Bryophyte Biology**, 2nd ed. New York: Cambridge University Press, 2009. 55 – 138.

GOFFINET, Bernard; SHAW, Jonathan. 2009. **Bryophyte Biology**, 2nd ed. New York: Cambridge University Press, 2009. 580 p.

GRADSTEIN, S. Robert; CHURCHILL, Steven P.; SALAZAR-ALLEN, Noris. **Guide to the Bryophytes of Tropical America**. New York: Memoirs of the New York Botanical Garden, 2011. 577 p.

GRADSTEIN, S. Robert; COSTA, Denise P. **The Hepaticae and Anthocerotae of Brazil**. New York: Memoirs of the New York Botanical Garden, 2003. 318 p. 87 v.

GRADSTEIN, S. Robert; REINER-DREHWALD, Elena. *Szweykowskia*, a new genus of Plagiochilaceae (Hepaticae) from tropical America. **Fragmenta Floristica et Geobotanica** n. 40 (1): 31 -38, 1995.

GRADSTEIN, S. Robert. New Synonyms and New Lectotypifications in Neotropical *Plagiochila* (Marchantiophyta). **Cryptogamie, Bryologie**, n. 36(4):369-379, 2015a.

GRADSTEIN, S. Robert. Annotated key to the species of *Plagiochila* (Marchantiophyta) from Brazil. **Pesquisas, Botânica** n. 67:23-36, 2015b.

GRADSTEIN, S. Robert. The genus *Plagiochila* (Marchantiophyta) in Colombia. **Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales** n. 40(154):104-136, 2016.

GREENSPAN, Lewis. Humidity Fixed Points of Binary Saturated Aqueous Solutions. Journal of Research of the National Bureau of Standards- A. **Physics and Chemistry** n. 81 A (1): 89 – 96, 1977.

GROEN, Kristen E. et al. Sex-specific plant responses to two light levels in the liverwort *Marchantia inflexa* (Marchantiaceae). **The Bryologist** n. 113 (1):81-89, 2010a.

GROEN, Kristen E. et al. Sex-specific plant responses to light intensity and canopy openness: implications for spatial segregation of the sexes. **Oecologia** n. 162: 561-570, 2010b.

GROLLE, Riclef; HEINRICHS, Jochen. Redescription and synonymy of *Plagiochila aerea* Täylor 1846 (Hepaticae), first describe as *Lycopodium pinnatum* by Lamarck 1792. **Nova Hedwigia** n. 68 (3-4): 551-525, 1999.

GROTH, Henk et al. Biogeography of Plagiochila (Hepaticae): natural species groups span several floristic kingdoms. **Journal of Biogeography** n. 30: 965-978, 2003.

HAIG, David. Living together and living apart: the sexual lives of bryophytes. **Philosophical Transactions of the Royal Society B** n. 371:1-9, 2016.

HAMMER, Øyvind; HARPER, David A. T.; RYAN Paul. PAST: Paleontological Statistics Software Package for Education and Data Analysis. **Palaeontologia Electronica** 4(1): 1-9, 2001.

HÄSSEL, Gabriel G. Andinopatagonian Species of *Plagiochila* (Plagiochilaceae, Marchantiophyta). I. Sectio *Robustae* Carl Emend. Hässel and II. Sectio *Equitantes* Carl Emend. Hässel. **Journal of the Hattori Botanical Laboratory** n. 96: 245-260, 2004.

HÄSSEL, Gabriela G. Andinopatagonian species of *Plagiochila* (Plagiochilaceae, Marchantiophyta). I sectio *Hirtae* and II sectio *Angulatae*. **Journal of the Hattori Botanical Laboratory** n. 100: 101–118, 2006.

HECKMAN, Caroline A. Spore Wall Structure in the Jungermanniales. **Grana** n. 10 (2): 109-119, 1970.

HEINRICHS, Jochen et al. Revision of the Neotropical species of *Plagiochila* (Dumort.) Dumort. (Hepaticae) described by Olof Swartz. **The Journal of The Hattori Botanical Laboratory** n. 85: 1-32, 1998.

HEINRICHS, Jochen; GRADSTEIN, S. Robert. On *Plagiochila longiramea* Steph., a poorly known species of Bolivia. **Candollea** n. 54: 73-81, 1999.

HEINRICHS, Jochen et al. Systematics of *Plagiochila* sect. *Glaucescentes* Carl (Hepaticae) from tropical America: a morphological and chemotaxonomical approach. **Plant Systematics and Evolution** n. 220: 115-138, 2000.

HEINRICHS, Jochen. *Plagiochila rutilans* (Hepaticae): A Poorly Known Species from Tropical America. **The Bryologist** n. 104 (3): 350-361, 2001.

HEINRICHS, Jochen. A taxonomic revision of *Plagiochila* sect. *Hylacoetes*, sect. *Adiantoideae* and sect. *Fuscoluteae* in the Neotropics with a preliminary subdivision of Neotropical Plagiochilaceae into nine lineages. Bryophytorum Bibliotheca; Bd. 58. Berlin; Stuttgart: Cramer in der Gebr.-Bomtraeger-Veri.-Buchh, 2002.

HEINRICHS, Jochen et al. The Systematic Position of *Plagiochila moritziana*, *P. trichostoma*, and *P. deflexa* Based on ITS Sequence Variation of Nuclear Ribosomal DNA, Morphology, and Lipophilic Secondary Metabolites. **The Bryologist** n. 105(2): 189 – 203, 2002.

HEINRICHS, Jochen et al. Intercontinental distribution of *Plagiochila corrugata* (Plagiochilaceae, Hepaticae) inferred from nrDNA ITS sequences and morphology. **Botanical Journal of the Linnean Society** n. 146: 469–481. 2004a.

HEINRICHS, Jochen; LINDNER, Melanie; GROTH, Henk. Sectional Classification of the North American *Plagiochila* (Hepaticae, Plagiochilaceae). **The Bryologist** n. 107(4): 489 496, 2004b.

HEINRICHS, Jochen. Distribution and synonymy of *Plagiochila punctata* (Taylor) Taylor, with hypotheses on the evolutionary history of *Plagiochila* sect. *Arrectae* (Plagiochilaceae, Hepaticae). **Plant Systematics and Evolution** n. 250: 105–117, 2005a.

HEINRICHS, Jochen et al. Origin and subdivision of *Plagiochila* (Jungermanniidae: Plagiochilaceae) in tropical Africa based on evidence from nuclear and chloroplast DNA sequences and morphology. **Taxon** n. 54 (2): 317–333, 2005b.

HINSHIRI, Hussein N.; PROCTOR, Michael C. F. The effect of desiccation on subsequent assimilation and respiration of the bryophytes *Anomodon viticulosus* and *Porella platyphylla*.

New Phytologist n. 70: 527–538, 1971.

HOLÁ, Eva et al. Sex ratio, sex-specific pattern in vegetative growth and gemma production in an aquatic liverwort, *Scapania undulata* (Marchantiophyta: Scapaniaceae). **Botanical Journal of the Linnean Society** n. 175: 229–241, 2014.

INOUE, Hiroshi; SCHUSTER, Rudolf M. A monograph of the New Zealand and Tasmanian Plagiochilaceae. **Journal of the Hattori Botanical Laboratory** n. 34:1-225, 1971.

INOUE, Hiroshi. Notes on the Plagiochilaceae, XI. A Review of the Species of *Plagiochila* (Dum.) Dum. From Samoa. **The Journal of The Hattori Botanical Laboratory** n. 49: 335-357, 1981.

INOUE, Hiroshi. Scanning electron microscopy in hepatic taxonomy. **Nova Hedwigia** n. 71: 75-80, 1982.

INOUE, Hiroshi. Notes on the Plagiochilaceae, XVI. Studies on Some Plagiochila Species in the Neotropics. **Bulletin of the National Science Museum** n. 15(2): 35 – 47, 1989.

ITO, Takuya et al. Arabidopsis MALE STERILITY1 Encodes a PHD-Type Transcription Factor and Regulates Pollen and Tapetum Development. **The Plant Cell** n. 19: 3549–3562, 2007.

JAMY, Mahwash et al. Reinstatement of *Plagiochila* sect. *Abietinae* (Plagiochilaceae, Jungermanniopsida). **Cryptogamie, Bryologie** n. 37(4):351-360, 2016.

JIANG, Jiang; DEANGELIS Donald L. Strong species-environment feedback shapes plant community assembly along environmental gradients. **Ecology and Evolution** n. 3(12): 4119–4128, 2013.

JUVANY, Marta; MUNNÉ-BOSCH, Sergi. Sex-related differences in stress tolerance in dioecious plants: a critical appraisal in a physiological context. **Journal of Experimental Botany** n. 66: 6083–6092, 2015.

KNIGHT, Tiffany M. et al. Pollen Limitation of Plant Reproduction: Pattern and Process. **Annual Review of Ecology, Evolution, and Systematics** n. 6: 467–97, 2005.

KONSTANTINOVA, Nadezhda. A.; POTEMKIM, Alexey; SCHLJAKOV, Roman N. Checklist of the Hepaticae and Anthocerotae of the former USSR. **Arctoa** n. 1: 87 – 127, 1992.

KONSTANTINOVA, Nadezhda. A.; POTEMKIM, Alexey. Liverworts of the Russian Artic: an annotated check-list and bibliography. **Arctoa** n. 6: 125 – 150, 1996.

LAAKA-LINDBERG, Sanna. Substrate preference and reproduction in *Lophozia silvicola* (Hepaticopsida) in southern Finland. **Annales Botanici Fennici** n. 37 (2): 85-93, 2000.

LAAKA-LINDBERG, Sanna. Reproductive phenology in the leafy hepatic *Lophozia silvicola* Buch in southern Finland. **Journal of Bryology** n. 27: 253–259, 2005.

LAAKA-LINDBERG, Sanna; HEDDERSON, Terry; LONGTON, Royce E. Rarity and Reproductive Characters in the British Hepatic Flora. **Lindbergia** n. 25 (2/3): 78-84, 2000.

LEIGH, Andrea; NICOTRA, Adrienne B. Sexual dimorphism in reproductive allocation and water use efficiency in *Maireana pyramidata* (Chenopodiaceae), a dioecious, semi-arid shrub. **Australian Journal of Botany** n. 51(5):509-514, 2003.

LENZA, Eddie; OLIVEIRA, Paulo Eugênio. Biologia reprodutiva e fenologia de *Virola sebifera* Aubl. (Myristicaceae) em Mata Mesofítica de Uberlândia, MG, Brasil. **Revista Brasileira de Botânica** n. 29 (3): 443-451, 2006.

LEVITT, Jacob. **Responses of Plants to Environmental Stress**. 2nd edn. New York: Academic Press, 1980. 698 p.

LI, Chunyang et al. Sex-specific physiological and growth responses to water stress in *Hippophae rhamnoides* L. population. **Acta Physiologiae Plantarum** n. 26: 123–129, 2004.

LIGRONE, Roberto; DUCKETT, Jeffrey G.; RENZAGLIA, Karen S. The gametophyte-sporophyte junction in land plants. **Advances in Botanical Research** n. 19: 231–317, 1993.

LINDENBERG, Johann B. W. Species hepathicarum, fasc. 1. Henry & Cohen, Bonn, 1–36, 1839.

LISAR, Seyed Y. et al. Water Stress in Plants: Causes, Effects and Responses, Water Stress. In: RAHMAN, Ismail M. M.; HASEGAWA, Hiroshi. **Water stress**, 2012. ISBN: 978-953-307-963-9. 1 -16.

LLOYD, David G. Female-predominant sex ratios in angiosperms. **Heredity** n. 32 (1): 35-44, 1974.

LLOYD, David G. Benefits and Handicaps of Sexual Reproduction. In: HECHT, Max K.; STEERE, W. C.; WALLACE, Bruce. **Evolutionary Biology**. New York: Plenum Press, 1980. 69 – 112.

LONGTON, Royce E.; SCHUSTER, Rudolf M. Reproductive biology. In: SCHUSTER, Rudolf M. **New manual of bryology**. Nichinan: Hattori botanical Laboratory, 1983. 386–462

LONGTON, Royce E. Reproductive biology and evolutionary potential in bryophytes. **The Journal of the Hattori Botanical Laboratory** n. 41:205–223, 1976.

LONGTON, Royce E. 1990. Sexual reproduction in bryophytes in relation to physical factors of the environment. In: CHOPRA, R. N.; BHATLA, Satish C. **Bryophytes development, physiology and biochemistry**. Boca Raton: CRC Press, 1990. 139-146.

LUIZI-PONZO, Andrea P.; BARTH, Ortrud M. Spore morphology of some Bruchiaceae species (Bryophyta) from Brazil. **Grana** n. 37:222-227, 1998.

LUIZI-PONZO, Andrea P.; BARTH, Ortrud M. Spore morphology of some Dicranaceae species (Bryophyta) from Brazil. **Grana** n. 38(1): 42-49, 1999.

LUIZI-PONZO, Andrea P.; MELHEM, Therezinha S. Spore morphology and ultrastructure of the tropical moss *Helicophyllum torquatum* (Hook.) Brid. (Helicophyllaceae) in relation to systematics and evolution. **Cryptogamie, Bryologie** n. 27:413–420, 2006.

LÜTTGE, Ulrich; BECK, Erwin; BARTELS, Dorothea. **Plant Desiccation Tolerance**. German: Springer-Verlag Berlin Heidelberg, 2011. 389 p.

Maciel-Silva, Adaíses S.; MARQUES- VÁLIO, Ivan F. Reproductive phenology of bryophytes in tropical rain forests: the sexes never sleep. **The Bryologist** n. 114(4): 708-719, 2011.

Maciel-Silva, Adaíses S.; PÔRTO, Kátia C. Reproduction in Bryophytes. In: RAMAWAT, Kishan G.; MÉRILLON, Jean-Michel; SHIVANNA, K. R. **Reproductive Biology of Plants**. 1st ed. London: CRC Press, 2014. 57- 84.

Maciel-Silva, Adaíses S.; MARQUES- VÁLIO, Ivan F.; RYDIN, Ha°kan. Altitudinal affects the reproductive performance in monoicous and dioicous bryophytes: examples from a Brazilian Atlantic rainforest. **AoB Plants**, pls016, 2012.

MÄGDEFRAU, Karl. Life-forms of Bryophytes. In: SMITH, A. J. E. **Bryophyte Ecology**. Dordrecht: Springer, 1982. 45 – 58.

MARKS, Rose; BURTON, James; MCLETCHIE, D. Nicholas. Sex differences and plasticity in dehydration tolerance: insight from a tropical liverwort. **Annals of Botany** n. 118: 347-56, 2016.

MCCLYMONT, John W.; LARSON, Donald A. An Electron-Microscopic Study of Spore Wall Structure in the Musci. **American Journal of Botany** n. 51(2):195-200, 1964.

MCLETCHIE, D. Nicholas; PUTERBAUGH, Mary N. Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort, *Marchantia inflexa*. **Oikos** n. 90: 227-237, 2000.

MCLETCHIE, D. Nicholas. Sex ratio from germination through maturity and its reproductive consequences in the liverwort *Sphaerocarpos texanus*. **Oecologia** n. 92: 273-278, 1992.

MCLETCHIE, D. Nicholas. Sperm limitation and genetic effects on fecundity in the dioecious liverwort *Sphaerocarpos texanus*. **Sexual Plant Reproduction** n. 9: 87-92, 1996.

MCLETCHIE, D. Nicholas, GARCIA-RAMOS, Gabriela; Crowley, Phillipe H. Local sex-ratio dynamics: a model for the dioecious liverwort *Marchantia inflexa*. **Evolutionary Ecology** n. 15: 231-254, 2002.

MEDINA, Nagore G.; ESTÉBANEZ, Belén. Does Spore Ultrastructure Mirror Different Dispersal? A Study of Seven Iberian *Orthotrichum* Species. PLoS ONE 9 (11): e112867. doi:10.1371/journal.pone.0112867, 2014.

MISHLER, Brent D.; OLIVER, Melvin J. The phenology of *Tortula ruralis*. A desiccation-tolerant moss, in the Organ Mountains, southern New Mexico. **The Bryologist** n. 94(2):143–153, 1991.

MOGENSEN, Gert Steen. The spore. In: SCHUSTER, Rudolf M. **New Manual of Bryology**. Nichinan: The Hattori Botanical Laboratory. 1983. 324-342.

MOORE, Jonathan D.; KOLLAR, Leslie; MCLETCHIE D. Nicholas. Does selection for gamete dispersal and capture lead to a sex difference in clump water-holding capacity? **American Journal of Botany** n. 103: 1449-1457, 2016.

MÜLLER, Jochen; HEINRICHS, Jochen; GRADSTEIN, S. Robert. A revision of *Plagiochila* sect. *Plagiochila* in the Neotropics. **The Bryologist** n. 102(4): 729-746, 1999.

NEIDHART, H. B. 1979. Comparative studies of sporogenesis in bryophytes. In: CLARKE, Giles Colin S., DUCKETT, Jeffrey G. **Bryophyte Systematics**. London: Academic Press, 1979. 251-280.

NEWTON, Martha E. Sex-ratio differences in *Mnium hornum* Hedw. and *M. undulatum* Sw. in relation to spore germination and vegetative regeneration. **Annals of Botany** n. 36: 163–178, 1972.

NICROTA, Adrielle B. Sex ratio variation and spatial distribution of *Siparuna grandiflora*, a tropical dioecious shrub. **Oecologia** n. 115:102-113, 1998.

NILSSON, Siwert. Taxonomic and evolutionary significance of pollen morphology in the Apocynaceae. In: HESSE, M.; EHRENDORFER, F. **Morphology, Development, and Systematic Relevance of Pollen and Spores**. Plant Systematics and Evolution. Vienna: Springer, 1990. vol 5. 91-102.

NISHIYAMA, Tomoaki et al. Chloroplast Phylogeny Indicates that Bryophytes are Monophyletic. **Molecular Biology and Evolution** n. 21(10): 1813-1818, 2004.

OLESEN, Peter; MOGENSEN, Gert Steen. Ultrastructure, histochemistry and notes on germination stages of spores in selected mosses. **The Bryologist** n. 81:493-516, 1978.

OLIVER, Melvin J. Influence of protoplasmic water loss on the control of protein synthesis in the desiccation-tolerant moss *Tortula ruralis*: Ramifications for a repair-based mechanism of desiccation tolerance. **Plant Physiology** n. 97:1501–1511, 1991.

OLIVER, Melvin J. Biochemical and molecular mechanisms of desiccation tolerance in bryophytes. In: GOFFINET, Bernard; SHAW Jonathan A. **Bryophyte Biology**, 2nd ed. 2009. New York: Cambridge University Press. 269-298.

OLIVER, Melvin J.; BEWLEY, J. Derek. Desiccation and ultrastructure in bryophytes. **Advances in Bryology** n. 2:91–131, 1984

OLIVER, Melvin J.; BEWLEY, J. Derek. Desiccation-tolerance of plant tissues: a mechanistic overview. **Horticultural Reviews** n. 18: 171–214, 1997.

OLIVER, Melvin J.; CUSHMAN, John C.; KOSTER, Karen L. Dehydration tolerance in plants. **Methods in Molecular Biology** n. 639: 3–24, 2010.

OLIVER, Melvin J.; TUBA, Zoltán; MISHLER, Brent D. The evolution of vegetative

desiccation tolerance in land plants. **Plant Ecology** n. 151:85–100, 2000.

OPLER, B. A.; BAWA, K. S. Sex ratio in tropical forest trees. **Evolution** n. 32(4): 812-821, 1978.

OSAKABE, Yuriko et al. Responses to environmental stresses in woody plants: key to survive and longevity. **Journal of Plant Research** 125: 1. DOI 10.1007/s10265-011-0446-6, 2011.

PARDOW, Alexandra; LAKATOS, Michael. Desiccation tolerance and global change: implications for tropical bryophytes in lowland forests. **Biotropica** 45: 27-36, 2013.

PATZAK, Simon D. F. et al. A phylogeny of Lophocoleaceae-Plagiochilaceae-Brevianthaceae and a revised classification of Plagiochilaceae. **Organisms Diversity & Evolution** n. 16(3): 481-495, 2016.

PETTET, Antony. Sex Ratios of *Riccia frostii* Aust. in Khartoum Province, Sudan. **Journal of Bryology** n. 5(2): 332-337, 1967.

PICKUP, Melinda; BARRET, Spencer C. H. The influence of demography and local mating environment on sex ratios in a wind-pollinated dioecious plant. **Ecology and Evolution** n. 3 (3): 629 – 639. 2013.

PIIPO, Sinikka. 1992. On the asexual reproduction of *Plagiochila pluma* Steph. **Tropical Biology** n. 5(1): 7-10

PROCTOR, Michael C. F. The physiological basis of bryophyte production. **Botanical Journal of the Linnean Society** n. 104: 61–77, 1990.

PROCTOR, Michael C. F. The bryophyte paradox: tolerance of desiccation, evasion of

drought. **Plant Ecology** n. 151: 41–49, 2000.

PROCTOR, Michael C. F. Patterns of desiccation tolerance and recovery in bryophytes. **Plant Growth Regulation** n. 35: 147–156, 2001.

PROCTOR, Michael C. F. Experiments on the effect of different intensities of desiccation on bryophyte survival, using chlorophyll fluorescence as an index of recovery. **Journal of Bryology** n. 25: 215–224, 2003.

PROCTOR, Michael C. F. Physiological Ecology. In: SHAW, Jonathan A., GOFFINET, Bernard. **Bryophyte Biology**. Cambridge, Cambridge University Press, 2009, 237 - 268.

PROCTOR, Michael C. F.; SMIRNOFF, Nicholas. Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. **Journal of Experimental Botany** n. 51: 1695–1704, 2000.

PROCTOR, Michael C. F.; PENCE, V. Vegetative Tissues: bryophytes, vascular resurrection plants and vegetative propagules. In: BLACK, M.; PRITCHARD, H. Desiccation and Survival in Plants: Drying without dying. Oxford: CABI Publishing, 2002. 207–238.

PROCTOR, Michael C. F.; TUBA, Zlotán. Poikilohydry and Homoihydry: antithesis or spectrum of possibilities? **New Phytologist** n. 156: 327 – 349, 2002.

PROCTOR, Michael C. F.; DUCKETT, Jeffrey G.; LIGRONE, Roberto. Desiccation tolerance in the moss *Polytrichum formosum* Hedw.: physiological and fine-structural changes during desiccation and recovery. **Annals of Botany** n. 99: 75–93, 2007a.

PROCTOR, Michael C. F. et al. Desiccation tolerance in bryophytes: a review. **The Bryologist** n. 110: 595-621, 2007b.

PUCHOLT, Pascal; HALLINGBÄCK Henrik R.; BERLIN Sofia. Allelic incompatibility can explain female biased sex ratios in dioecious plants. **BMC Genomics** n. 18: 251, 2017.

PUNT, Willem et al. Glossary of pollen and spore terminology. **Review of Palaeobotany and Palynology** n. 143: 1–81, 2007.

PUTTICK, Mark N. et al. The Interrelationships of Land Plants and the Nature of the Ancestral Embryophyte. **Current Biology** n. 28: 1-13, 2018.

PUTWAIN, Phil D.; HARPER, John L. Studies in the Dynamics of Plant Populations: V. Mechanisms Governing the Sex Ratio in *Rumex acetosa* and *R. acetosella*. **Journal of Ecology** n. 60(1): 113-129, 1972.

R Development Core Team. **R: A language and environment for statistical computing**. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org. 2018.

REECE, Erik; KRUPA, James J. **The Embattled Wilderness**: The Natural and Human History of Robinson Forest and the Fight for Its Future. Athens: University of Georgia Press, 2013.

REESE, William D. Reproductivity, fertility, and range of *Syrrhopodon texanus* Sull. (Musci: Calymperaceae), a North American endemic. **The Bryologist** n. 87:217–222, 1984.

RENZAGLIA, Karen S. et al. Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. **Philosophical Transactions of the Royal Society B**: Biological Sciences n. 355: 769-793, 2000.

RENZAGLIA, Karen S.; MCFARLAND, Ken D. Antheridial plants of *Megaceros*

aenigmaticus in the southern Appalachians: anatomy, ultrastructure and population distribution. **Haussknechtia Beiheft** n. 9: 307–316, 1999.

RETUERTO, Rubén; SÁNCHEZ-VILAS, Júlia; VARGA, Sandra. Sexual dimorphism in response to stress. **Environmental and Experimental Botany** n. 146: 1-4, 2018.

REYNOLDS, Edward D. The use of lead citrate at high Ph as an electron-opaque stain in electron microscopy. **Journal of Cell Biology** n. 17(1): 208-212, 1963.

REYNOLDS, Douglas N. Gamete dispersal in *Mnium ciliare*. **The Bryologist** n. 83: 73-77, 1980.

RHODES, David; ORCZYK, Anna N. **Plant Stress Physiology**. Encyclopedia of Life Sciences DOI: 10.1038/npg.els.0001297, 2001.

ROCHA, Lucas M.; GONÇALVES-ESTEVES, Vânia; LUIZI-PONZO, Andrea P. Morfologia de esporos de espécies de Polytrichaceae Schwägr. (Bryophyta) do Brasil. **Revista Brasileira de Botânica** n. 31: 537-548. 2008.

RODRIGUES, Raquel S.; LUIZI-PONZO, Andrea P. Palinologia de espécies selecionadas da família Pottiaceae (Bryophyta). **Pesquisas, Botânica** n. 67: 303-317, 2015.

ROHLF, James; FISHER, David R. Tests for Hierarchical Structure in Random Data Sets. **Systematic Zoology** n. 17 (4): 407-412, 1968.

RYDGREN, Knut; ØKLAND, Rune H. Sex distribution and sporophyte frequency in a population of the clonal moss *Hylocomium splendens*. **Journal of Bryology** n. 24 (3): 207–214, 2002.

RYDGREN, Knut; CRONBERG, N.; ØKLAND, Rune H. Factors influencing reproductive success in the clonal moss, *Hylocomium splendens*. **Oecologia** n. 147: 445–454, 2006.

RYDGREN, Knut; HALVORSEN, Rune; CRONBERG, Nils. Infrequent sporophyte production maintains a female-biased sex ratio in the unisexual clonal moss *Hylocomium splendens*. **Journal of Ecology** n. 98: 1224–1231, 2010.

SANDERSON, Brian J. et al. Scale dependence of sex ratio in wild plant populations: implications for social selection. **Ecology and Evolution** n. 6(5): 1411–1419. 2016.

SAVAROĞLU, Filiz. Spore morphology of some Orthotrichaceae Arn. species (Bryophyta) from Turkey. **Bangladesh Journal of Botany** n. 44 (4): 499-506, 2015.

SAVAROĞLU, Filiz, ERKARA, I. Potoglu, KOYUNCU, Onur. Observations of spore morphology of some species of Hypnaceae Schimp. (Bryophyta) in Turkey. Bangladesh Journal of Botany 46 (1): 9-17, 2017.

SCHIFFNER, Victor Felix. Die Hepaticae der Flora von Buitenzorg. I. Leiden: Band. E. J. Brill, 1900. 220 p.

SCHONBECK, Mark W.; BEWLEY, J. Derek. Responses of the moss *Tortula ruralis* to desiccation treatments. II. Variations in desiccation tolerance. **Canadian Journal of Botany** n. 59: 2707–2712, 1981.

SCHUSTER, Rudolf M. A monograph of the Nearctic Plagiochilaceae. Part. I. Introduction and section I. *Asplenioides*. **American Midland Naturalist** n. 62: 1–166. 1959.

SCHUSTER, Rudolf M. A monograph of the Nearctic Plagiochilaceae. Part. III. Sectio *Contiguae* to conclusion. **American Midland Naturalist** n. 63: 1–130. 1960.

SCHUSTER, Rudolf M. **The Hepaticae and Anthocerotae of North America**. IV. New York: Columbia University Press, 1980. 1334 p.

SILVA-E-COSTA, Juliana C. et al. Spore germination, early development and some notes on the effects of in vitro culture medium on *Frullania ericoides* (Nees) Mont. (Frullaniaceae, Marchantiophyta). **Acta Botanica Brasilica** n. 31(1): 19-28, 2017.

SILVA-E-COSTA, Juliana C. **Palinologia de Frullaniaceae Lorch (Marchantiophyta) do Brasil**. 2015. 55 f. Dissertação de Mestrado – Programa de Pós-graduação em Ecologia - Universidade Federal de Juiz de Fora, Juiz de Fora, 2015.

SILVEIRA, Marina. Preparo de amostras para Microscopia Eletrônica de Varredura. In: SOUZA, Wanderley. **Técnicas de Microscopia Eletrônica Aplicadas às Ciências Biológicas**. Rio de Janeiro: Sociedade brasileira de Microscopia e Microanálise, 2007. 47-59.

SIM-SIM, Maria et al. *Plagiochila porelloides* (Torr. ex Nees) Lindenb. in mainland Portugal and Madera archipelago. New records and the threatened status. **Portugaliae Acta Biologica** n. 21: 231-238, 2003.

SINCLAIR, Jordan; EMLEN J.; FREEMAN, D. C. Biased Sex Ratios in Plants: Theory and Trends. **Botanical Review** n. 78:63–86, 2012.

SLATE, Mandy; ROSENSTIEL, Todd N.; EPPLEY, Sarah M. Sex-specific morphological and physiological differences in the moss *Ceratodon purpureus* (Dicranales). **Annals of Botany** n. 120: 845–854, 2017.

SO, May Ling; GROLLE, Riclef. 2000. Checklist of *Plagiochila* (Hepaticae). **Journal of The Hattori Botanical Laboratory** n. 88: 199-243, 2000.

SO, May Ling. *Plagiochila* (Hepaticae, Plagiochilaceae) in China. **Monographs in Systematic Botany** n. 60: 1–214, 2001.

SÖDERSTRÖM, Lars et al. 2016. World checklist of hornworts and liverworts. PhytoKeys 59: 1–828.

SÖDERSTRÖM, Lars et al. Notes on Early Land Plants Today. 36. Generic treatment of Lophocoleaceae (Marchantiophyta). **Phytotaxa** n. 97 (2): 36–43, 2013.

SÖDERSTRÖM, Lars et al. *Plagiochila porelloides* (Plagiochilaceae, Hepaticae) form Changbai Mountain, new to China, with chemical characterization and chromosomes measurements. **Bryobrothera** n. 5: 195-201, 1999.

SÖDERSTRÖM, Lars et al. World checklist of hornworts and liverworts. **PhytoKeys** 59: 1–828, 2016.

SOKAL, Robert R.; ROHLF, F. James. **Biometry**: the principles and practice of statistics in biological research. 3rd ed. New York: W.H. Freeman and Company, 1995.

SOLDAAT, Leo L.; LORENZ, Helge; TREFFLICH, Annette. The effect of drought stress on the sex ratio variation of *Silene otites*. **Folia Geobotanica** n. 35: 203-210, 2000.

SPRUCE, Richard. Hepaticae amazonicae et andinae. II. **Transactions and Proceedings of the Botanical Society of Edinburgh** n. 15: 309-588, 1885.

STARK, Lloyd. R.; MISHLER, Brent D.; MCLETCHIE, D. Nicholas. The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. **American Journal of Botany** n. 87: 1599-1608, 2000.

STARK, Lloyd. R. Ecology of desiccation tolerance in bryophytes: A conceptual framework and methodology. **The Bryologist** n. 120(2):130-165, 2017.

STARK, Lloyd. R. et al. Generational Differences in Response to Desiccation Stress in the Desert Moss *Tortula inermis*. **Annals of Botany** n. 99 (1): 53–60, 2007.

STARK, Lloyd. R.; BRINDA, John C. Developing sporophytes transition from an inducible to a constitutive ecological strategy of desiccation tolerance in the moss *Aloina ambigua*: effects of desiccation on fitness. **Annals of Botany** n. 115: 593–603, 2015.

STARK, Lloyd. R.; BRINDA, John C.; GREENWOOD, Joshua L. Propagula and shoots of *Syntrichia pagorum* (Pottiaceae) exhibit different ecological strategies of desiccation tolerance. **The Bryologist** n. 199: 181–192, 2016.

STARK, Lloyd. R.; MCLETCHIE, D. Nicholas; MISHLER, Brent D. Sex Expression, Plant Size, and Spatial Segregation of the Sexes Across a Stress Gradient in the Desert Moss *Syntrichia caninervis.* **The Bryologist** n. 108(2):183-193, 2005.

STEHLIK, Ivana; FRIEDMAN, Jannice; BARRETT, Spencer C. H. Environmental influence on primary sex ratio in a dioecious plant. Proceedings of the National **Academy of Sciences** n. 105 (31): 10847–10852, 2008.

STEINKAMP Myrna P.; DOYLE, W. T. Spore Wall Ultrastructure in four species of the liverwort Riccia. **American Journal of Botany** n. 66 (5): 546 – 556, 1979.

STEPHANI, Franz. 1902. Species hepaticarum 2. Bulletin de l'Herbier Boissier, Série 2 2: 657–688.

STIEHA, Christopher et al. The dispersal process of asexual propagules and the contribution

to population persistence in *Marchantia* (Marchantiaceae). American **Journal of Botany** n. 101:348-356, 2014.

STIEHA, Christopher et al. Maintenance of the Sexes and Persistence of a Clonal Organism in Spatially Complex Metapopulations. **Evolutionary Ecology** n. 31:363–386, 2016.

STOTLER, Raymond E.; CRANDALL-STOTLER, Barbara. A Checklist of the Liverworts and Hornworts of North America. **The Bryologist** n. 80 (3): 405-428, 1997.

STOTLER, Raymond E.; CRANDALL-STOTLER, Barbara. A Synopsis of the liverwort flora of north America north of Mexico. **Annals of Moussuri Botanical Garden** n. 102(4):574-709, 2017.

THIERS, Barbara. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Disponível em <http://sweetgum.nybg.org/science/ih/>. Acessado em: 01 fev 2019.

TIEDJENS, Victor A. Sex ratios in cucumber flowers as affected by different conditions of soil and light. **Journal of Agricultural Research** n. (8): 721 – 746, 1928.

TROPICOS.ORG. Missouri Botanical Garden. Disponível em: <http://www.tropicos.org.> Acessado em: 01 fev 2019.

ULRICH, Werner et al. Small-scale spatial variability in phylogenetic community structure during early plant succession depends on soil properties. **Oecologia** n. 175:985–995, 2014.

VAN DER VELDE, Marco et al. The reproductive biology of *Polytrichum formosum*: clonal structure and paternity revealed by microsatellites. **Molecular Ecology** n. 10:2423–2434, 2001.

VANDEPITTE, Katrien et al. Patterns of sex ratio variation and genetic diversity in the dioecious forest perennial *Mercurialis perennis*. **Plant Ecology** n. 206: 105-114, 2010.

VANDERPOORTEN, Alain.; GOFFINET, Bernard. Introduction to Bryophyte Biology. Cambridge: Cambridge University Press, 2009. 389 p.

VITT, Dale. H.; CRANDALL-STOTLER, Barbara.; WOOD, Andrew. J. Survival in a dry world through avoidance and tolerance. In. RAJAKARUNA, Nishanta; BOYD, Robert; HARRIS, Tanner. **Plant Ecology and Evolution in Harsh Environments**. Nova Publishers, 2014. 267-295.

VOJTKKÓ, Andras. The spore morphology of Hepaticae species. **Acta Biologica Szegediensis** n. 39: 59-69, 1993.

WALLACE, Simon et al. Evolutionary development of the plant spore and pollen wall. AoB PLANTS. p. 1-18. doi:10.1093/aobpla/plr027, 2011.

WEISMANN, Auguste. 1889. The significance of sexual reproduction in the theory of natural selection. In: POULTON, Edward B.; SCHONLAND, Selma; SHIPLEY, Arthur E. **Essays upon Heredity and Kindred Biological Problems**, 2nd ed. Oxford: Clarendon Press, 1889. 257-342

Wellman, Charles H. Origin, function and development of the spore wall in early land plants. In: HEMSLEY, Alan R.; POOLE, Imoge. **The Evolution of Plant Physiology: From whole plants of ecosystems**. Linnean Society of London. Paleobotany Specialist Group Elsevier Academic Press, 2004. 43 – 60.

WICKETT, Norman J. Phylotranscriptomic analysis of the origin and early diversification of land plants. **PNSA PLUS** www.pnas.org/cgi/doi/10.1073/pnas.1323926111, 2014.

WODEHOUSE, Roger P. **Pollen grains**: Their structure, identification and significance in science and medicine. New York: McGraw-Hill, 1935.

WOOD, Andrew J. The nature and distribution of vegetative desiccation-tolerance in hornworts, liverworts and mosses. **The Bryologist** n. 110: 163–177, 2007.

WYATT, Robert. Spatial pattern and gamete dispersal distances in *Atrichum angustatum*. **The Bryologist** 80: 284-291, 1977.

YANO, Olga; LUIZI-PONZO, Andrea P. *Chonecolea doellingeri* (Chonecoleaceae, Hepaticae), taxonomia e distribuição geográfica no Brasil. **Acta Botanica Brasilica** n. 20: 783-788, 2006.

YANO, Olga; LUIZI-PONZO, Andrea P. *Dumortiera hirsuta* (Dumortieraceae, Marchantiophyta), taxonomy, palynology and geographic distribution. **Boletim do Instituto de Botânica** n. 21: 9-18, 2011.

Yu, Li; Lu, Jiambo. Does Landscape Fragmentation Influence Sex Ratio of Dioecious Plants? A Case Study of *Pistacia chinensis* in the Thousand-Island Lake Region of China. **PLoS ONE** 6(8): e22903, 2011.

ZAR, Jerrold M. **Biostatistical Analysis**, 4th ed. New Jersey: Prentice Hall, 1999.

ZHANG, Jn et al. Photosynthetic and cytological recovery on remoistening *Syntrichia caninervis* Mitt., a desiccation-tolerant moss from Northwestern China. **Photosynthetica** n. 49 (1): 13-20, 2011.

ZHANG, Sheng et al. Comparative physiological, ultrastructural and proteomic analyses reveal sexual differences in the responses of *Populus cathayana* under drought stress.

Proteomics n. 10: 2661–2677, 2010.

ZHANG, Qingwei; BARTELS, Dorothea. Molecular responses to dehydration and desiccation in desiccation-tolerant angiosperm plants. **Journal of Experimental Botany** n. 69 (13): 3211–3222, 2018.

ZHAO, Dong-Ping; BAI, Xue-Liang; WANG, Li-Hong. Observations of spore morphology of some hepatics species (Marchantiophyta) in China. **Arctoa** n. 20: 205-210, 2011.