

# The consequences of mating system and dispersal potential on the genetic structure of leaf-inhabiting bryophyte metapopulations in a fragmented Amazonian landscape

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## Abstract

Patch size and connectivity are the main predictors of population demographic and genetic stability. Habitat fragmentation continues at unprecedented rates justly affecting plant functional connectivity worldwide. However, few terrestrial plant groups have sufficiently foreshortened generation times in which to empirically disentangle the demographic and genetic consequences of reduced patch size and connectivity. Herein, we combine evidence from long-term (15 yr.) population censuses of two epiphyllous bryophytes and population genetic estimates in an experimentally fragmented Amazonian landscape to create a profile of the eco-evolutionary impacts of reduced habitat connectivity. Single nucleotide polymorphisms (SNPs) derived from Genotyping by Sequencing were used to analyze genetic patterns among fragmented (1-, 10- and 100-ha) and continuous forests. In the context of the world's most diverse biome, fragmentation induces marked effects on plant population demography and genetic differentiation. Declines in colonization events in small patches (1- and 10-ha), associated with reduced colony densities, led to accelerated genotypic differentiation (genetic drift) compared to 100-ha fragment and continuous forests. Furthermore, the mating system was related to differential sensitivities to the intensity of fragmentation. Unexpectedly, the unisexual species (hypothesized to be more challenged in terms of spore output) was characterized by higher migration rates among smaller patches than its bisexual counterpart. These results point to the complex and unpredictable patterns revealed when life history strategies, demography, and plant genetic structure are simultaneously evaluated in the context of the long-term effects of reduced landscape connectivity.

## INTRODUCTION

Habitat (patch) size is a fundamental determining factor of an organism's population size, as it is inversely related to local extinction probability (Rosenzweig, 1995; Tjørve & Tjørve, 2017). Likewise, the degree of isolation influences a species' colonization rate as it is directly related to the degree of connectivity across a landscape (Thrall et al., 2000; Wang & Altermatt, 2019). Both Island biogeography theory (IBT) (MacArthur & Wilson, 1967; Whittaker et al., 2008) and the metapopulation theory (Levins, 1969; Hanski & Gaggiotti, 2004) predict population dynamics across the spatial variation of patch size and connectivity. In metapopulation regional survivability depends on threshold levels of migration events among subpopulations of spatially isolated habitat patches as unoccupied patches are constantly (re)colonized from dispersal events (Levins, 1969; Hanski & Gaggiotti, 2004). Adopting the metapopulation concepts offers a fundamental framework from which to study population demographic and evolutionary processes in fragmented habitats (Hanski & Gaggiotti, 2004; Hanski, 2012; Hanski et al., 2017) and for landscape conservation planning (Thrall et al., 2000; Fahrig, 2019). Indeed, preserving the effective dispersal of organism individuals and their genes among

habitat patches (i.e., *plant functional connectivity*) is crucial for long-term metapopulation persistence across a fragmented landscape (Auffret et al., 2017; Hanski et al., 2017; Vellend et al., 2017).

Anthropogenic deforestation is radically transforming landscape configurations worldwide (Haddad et al., 2015; Taubert et al., 2018; Fischer et al., 2021). In biodiverse hotspots such as the Amazon, projections point out that deforestation is increasing at a 0.5% rate per year, thus dramatically augmenting the density of small tropical forest patches (Taubert et al., 2018). To date, the Amazon Forest has lost an estimated 17% of its original forest in the past half century, while more than 50% of the remaining forests are characterized as degraded (Lovejoy & Nobre, 2018; Matricardi et al., 2020; Lapola et al., 2023; Albert et al., 2023). Such rapid ecosystem-wide changes continue to preclude species' ability to adapt to such spatial disturbances over short time scales (Albert et al., 2023) resulting in impaired global biodiversity and ecosystem functions (Sala et al., 2000; Morris, 2010; Gomes et al., 2019; Daskalova et al., 2020). The effects of habitat fragmentation are separated into three components, outright habitat loss, reduced remnant size, and increased isolation, all of which effectively drive metapopulations into a non-equilibrium state (Thrall et al., 2000). Ecosystem decay in fragments demonstrably accelerates biodiversity loss (Hanski et al., 2013; Chase et al., 2020) as predicted by the species-area relationship (SAR): smaller habitats harbor fewer species (Hanski et al., 2013). However, the compounding effects of forest fragmentation (such as edge effects, neighborhood density, lowered dispersal, or demographic stochasticity) further exacerbate demographic stability by increasing extinction risk and/or decreasing recolonization potential (Lovejoy et al., 1984; Chase et al., 2020; Scott et al., 2021).

The demographic dynamics (colonization/extinction) among isolated metapopulation patches are fundamental in determining standing genetic diversity (Aycrigg & Garton, 2014; Buza et al., 2000; Vellend & Geber, 2005; Wang & Altermatt, 2019) but it remains difficult to predict (Vranckx et al., 2012). For oceanic island species, genetic diversity generally declines with few exceptions (see Laenen et al., 2011) as patch size decreases and isolation increases (Costanzi & Steifetten, 2019; Hill et al., 2017; Whittaker et al., 2017; Hamabata et al., 2019). These studies assume that processes influencing island species diversity are reflected in the genetic structure allowing for opportunities to predict micro-evolutionary outcomes in light of community-level patterns (Losos & Ricklefs, 2009; Vellend, 2003; Vellend & Geber, 2005). Conservation genetic theory posits that stochastic extinction events, disproportionately experienced by smaller-sized populations, will lead to an increased loss of alleles, and genetic differentiation through genetic drift (Lowe et al., 2005; Aguilar et al., 2008). Interruptions to migration subsequently impede (re)colonization of unoccupied patches precluding the renewal of locally extinct genotypes (Lowe et al., 2005; Auffret et al., 2017).

The effects of patch size and isolation on genetic diversity depend on the time since isolation (Young et al., 1996; Aguilar et al., 2008). Due to the long generation times of most plants, integrating the demographic and genetic consequences of the recent exponential increase in forest fragmentation over multiple generations is scarce, particularly in tropical regions harboring the greatest plant diversity (Hamilton, 1999; Aldrich et al., 1998; Côrtes et al., 2013). Since the effects of human-induced habitat loss are relatively recent in an evolutionary context, long-lived species will take decades to reveal extinction debts as characterized by a loss of genetic variability through genetic drift (Morris et al., 2008; Vranckx et al., 2012). Terrestrial plant groups characterized by sufficiently accelerated demographic parameters (such as colonization, extinction, and local growth) are ideal systems to examine habitat fragmentation consequences in a tractable time frame (Pharo & Zartman, 2007; Spagnuolo et al., 2007; Zartman et al., 2006).

Bryophytes stand out as a model system to bridge demography and population genetics in the context of fragmentation (Pharo & Zartman, 2007). Bryophytes are characterized by widely divergent reproductive strategies (Snäll et al., 2004; Wang et al., 2013; Holá et al., 2015; Sierra et al., 2019a; Alonso-García et al., 2021; Lang et al., 2021) thus providing opportunities from which to generate predictions of dispersal trait related long-term gene-flow patterns in metapopulations (Obbard et al., 2006; Patiño et al., 2013). Moreover, habitat-dependent reproductive performance observed in bryophytes (Maciel-Silva et al., 2012) will help in the understanding of the species' different sensitivity to habitat isolation (Sierra et al., 2019b).

Bryophyte metapopulations inhabiting the leaves of vascular plants (epiphyllous bryophytes) present short generation times (~6 months), where colonization is mainly carried out through the dispersal of microscopic

spores and asexual propagules (Sierra et al., 2019a; Mežaka et al., 2019). Local patch occupancy is determined by the immediate environment (Sonnleitner et al., 2009), and neighboring colony densities (Zartman et al., 2012). Epiphyllous bryophytes are ideal bellwethers of biodiversity associated with landscape changes in diverse ecosystems in the Amazon and Atlantic Forests (Zartman, 2003; Alvarenga et al., 2009). At the Biological Dynamics of Forest Fragmentation Project (BDFFP) in the Amazonian basin, populations of epiphyllous bryophytes have persisted at low local abundances for decades (>40 years) in small forest fragments <10-ha (Sierra et al., 2019b) governed by suppressed colonization (Zartman & Shaw, 2006). In these small fragments after 20 years of isolation, Zartman et al., (2006) observed linkage disequilibrium between loci from amplified fragment length polymorphisms (AFLPs) markers, but it was no observed sign of genetic drift. However, a multi-species assessment of Amazonian bryophytes suggested a high genetic structure of populations across a large spatial scale (Ledent et al., 2020; Campos et al., 2022). Such contrasting results suggest a priori that the degree of functional connectivity among Amazonian bryophyte metapopulations is not universal and is dependent on the interaction of landscape configuration and species dispersal capacity (Auffret et al., 2017).

In this study, we focus on the demographic and genetic basis of metapopulation persistence of two epiphyllous bryophyte species distributed across a 10 000-km<sup>2</sup> experimentally fragmented Amazonian landscape in the BDFFP. Herein, we combine evidence from long-term censuses of two ephemeral epiphyllous bryophytes to create a comprehensive profile of the ecological genetic impacts of fragmentation at a medium-term (half-century) time scale. Single nucleotide polymorphisms (SNPs) identified from Genotyping by Sequencing (GBS) were used to analyze the genetic diversity and differentiation in fragmented (1-, 10- and 100-ha) and continuous forests. Specifically, we addressed the following questions: Are demographic changes associated with the intensity of fragmentation (patch size and isolation) reflected in their genetic structures? Subsequently, does a species' mating system confers different sensitivity at the genetic level to the effects of habitat fragmentation? Considering these questions, we hypothesized: (H1) reduced colony densities in smaller fragments over 40 years of isolation leads to population genetic drift (lower diversity and higher differentiation) when compared to continuous forests. Secondly (H2), these fragmentation impacts are less evident in bisexual as compared to unisexual species due to the former's demonstrably greater reproductive performance and dispersal capacities (Maciel-Silva et al., 2012; Laenen et al., 2016). Consequently (H3), the landscape connectivity network of the bisexual species will present high migration (Figure 1: patchy metapopulation), with all patches exchanging migrants among them, irrespective of size and isolation. While the unisexual species, we expect to observe only migration from the continuous forest towards nearby small forest fragments at a low rate (Figure 1: island-mainland metapopulation).

## METHODS

### *Study area*

The Biological Dynamics of Forest Fragmentation Project (BDFFP), is located in central Amazonia (2°30 S, 60° W) along the BR-174 highway, ~80 km north of Manaus, Brazil. The BDFFP consists of *terra firme* (non-flooded), lowland rainforest of nutrient-poor soils, with elevations ranging between 50 and 150 masl. (Gascon & Bierregaard, 2001). The rainy season of the region occurs from November to June, and rainfall in nearby climatic stations at the ZF2 and Reserva Ducke ranges from 1900 to 2550 mm annually (Ferreira et al., 2005; Aleixo et al., 2019). Eleven forest fragments that were isolated in 1980 are distributed among three adjacent cattle ranches (Dimona, Porto Alegre, and Colosso in the Esteio ranch). Forest fragments vary in size with replicates of 1-, 10-, and 100-ha, separated 70 to 1000 m from the mature continuous forest (Figure S1). The matrix in the BDFFP landscape was composed of cattle pasture from 1980 to 1995. As pasture creation slowed down in the region through the 1980s and ceased in the 1990s, areas of pastures and young scrub vegetation have been overtaken by secondary forests dominated by woody species of *Vismia spp.* and *Cecropia spp.* by the year 2015 (Laurance et al., 2011; Laurance et al., 2018; Stouffer, 2020).

### *Estimates of epiphyllous population size dynamics*

Data from the epiphyllous metacommunity sampling collected in the year 2000 (Zartman, 2003) and 2016

(Sierra et al., 2019b) were used as baseline information on demographic changes accountable to fragmentation in this study. The two focal leafy liverworts species selected, *Radula flaccida* Lindenb. & Gott. (Radulaceae) and *Cololejeunea surinamensis* Tixier (Lejeuneaceae), are regionally and locally abundant across the landscape (Sierra et al., 2019b). At BDFFP, both species predominantly occur on understory leaves of young trees and shrubs (Figure S2A, E). Colonies of *R. flaccida* and *C. surinamensis* are typically found with sexual reproductive structures (male: androecia or/and female: gynoecia/sporophytes), and specialized asexual propagules (gemmae). Nevertheless, the two species differ in the mating system where *R. flaccida* is unisexual and *C. surinamensis* is bisexual (Figure S2). These species were chosen due to their: (i) comparatively higher local population densities relative to the other 74 epiphyllous species known from BDFFP (Zartman, 2003; Sierra et al., 2019b); (ii) distinct patterns in radial growth allowing for clear spatial delimitation of colonies; (iii) the negative effect of fragmentation on their local abundances (a characteristic this species shares with a vast majority of the other epiphylls at the BDFFP, see Sierra et al., 2019b); and (iv) empirical data for demographic patterns over a 15-year interval (Sierra et al., 2019b) as well as for associations between colonization and extinction with fragmented patches from transplant experiments (Zartman & Shaw, 2006).

The number of colonies was estimated for a total of 22 study plots (1-ha) distributed in 1-, 10-, 100-ha forest fragments, and in continuous forest (Table S1); see Sierra et al., (2019b) for further detail on the sampling design. The number of colonies was estimated for each study plot by calculating the percentage of occupied host leaves by the species (occupied patches) and multiplying by the total number of host plants with epiphyllous colonies within the respective study plot (suitable patches). This approach led us to accurately compare population size among plots in the fragmented and continuous forest avoiding sampling size bias. For each species, differences in the number of colony estimates were compared among small (1- and 10- ha), and large patches (100- ha fragments and continuous forests) over time from the years 2000 and 2016 using the non-parametric Wilcoxon rank sum exact test. Likewise, was compared tested if the increase colony density observed in small patches in the year 2016 reached similar values when compared to large patches.

#### *DNA sampling*

Between April and May 2017, a total of 142 colonies of *R. flaccida* and 135 of *C. surinamensis* were sampled in twelve local populations inside the permanent study plots (Table S1), in six small patches: 1-ha ( $n = 3$ ) and 10-ha ( $n = 3$ ); and six large patches: 100-ha ( $n = 1$ ) and continuous forests ( $n = 5$ ). Each study plot is mapped into twenty-five subplots of  $20 \times 20$  m ( $400 \text{ m}^2$ ). Within each  $400 \text{ m}^2$  subplot, host leaves occupied by at least one colony were collected at regularly spaced intervals of 10 m minimum distance. Single-species colonies growing on the leaf were prioritized. In the laboratory using a dissecting scope, 0.5 – 1 g of the bryophyte gametophyte was carefully separated from the leaf surface avoiding contamination from host plant leaf tissue or other epiphyllous organisms. Plant tissue was dried using silica gel after collection. Colonies sampled varied from eight to fifteen in each reserve. Specimens detailed information are presented in Table S2.

#### *DNA extraction, and Genotyping by sequencing*

Samples were frozen in liquid nitrogen and total genomic DNA was extracted using an E.Z.N.A. Plant DNA DS Mini kit (Omega Bio-tek, Inc, Norcross, GA, USA). A double digest Genotyping by sequencing (GBS) library with the enzymes PstI/MspI (Abed et al., 2019) was prepared and sequenced on the Ion Proton instrument by the Plateforme d'analyses génomiques (Institut de Biologie Intégrative et des Systèmes (IBIS), Laval University, Quebec City, QC, Canada). Each sample was identified using a unique forward and reverse 13-15 base-pairs barcode at the 5' and 3' end to identify each individual when multiplexing.

#### *Sequences assembly bioinformatics pipelines*

Raw sequence reads were trimmed to 130 base pairs representing the best quality length distribution observed in FastQC v0.11.3 using the default parameter 'phred33' (Andrews, 2010). The Stacks v2.4 pipeline was used to demultiplex the libraries and cluster loci using the function `process_radtags` with a minimal percentage of identity of 85% within and among individuals (Rochette et al., 2019). A *de novo* assembly was run for the complete dataset for each species separately. The pipeline was run with the optimal parameters as described

in the supplementary methods (Appendix A).

The program *populations* in the software Stacks (Rochette & Catchen, 2017) was used to produce two filtered genomic datasets for each species that differed in the percentage of missing data and the number of individuals by population (Table S2). The first dataset filtered the loci present in at least 15% of the individuals (filter parameter:  $-R=0.15$ ), with sample sizes of  $n=105$  individuals for *R. flaccida*, and  $n=108$  individuals for *C. surinamensis*. A second dataset was generated by filtering the loci that were present in at least 20% of the individuals (filter parameter:  $-R=0.20$ ), considering  $n=80$  individuals for *R. flaccida*, and  $n=71$  individuals for *C. surinamensis*. Both datasets present a similar number of SNPs after filtering, yet a different amount of missing data. Heretofore, these two datasets will be referred to as the complete dataset which presents over  $>80\%$  of missing data, and the reduced dataset with  $>75\%$  of missing data. Tests were done to check if the amount of missing data or reduced sample size of our two datasets, will bias downstream analysis. A full detailed description of the methods used is shown in the supplementary methods (Appendix A).

### Genetic summary statistics

Genetic summary statistics were calculated for each of the twelve local populations. Variant sites, polymorphic sites, private alleles, and nucleotide diversity ( $\pi_T$ ) were calculated using the *population* program (Catchen et al., 2013). Overall summary statistics per loci and gene diversity ( $h$ ) by population were calculated using the function `basic.stats`, as well as allelic richness estimates ( $A_r$ ) by rarefying allelic counts per population with the `allelic.richness` with the *hierfst* version 0.5-11 R package (Goudet & Jombart, 2022). Genotype diversity (MLG) and the richness indexes: Shannon-Weiner Diversity index (H), Stoddard and Taylor's Index (G), and Simpson's index (lambda) were calculated using the function `poppr` with the *poppr* 2.9.3 R package (Kamvar et al., 2014, 2015). Linear regressions were fitted with the function `lm` in the *stats* package (R Core Team, 2022) to explore differences between the genetic diversity metrics calculated above and with forest fragment sizes (1-, 10-, 100-ha and continuous forests) and size class (small and large patches).

### Population structure with individual-based clustering

A multivariate statistical approach was used to infer the genetic relatedness of the individuals within the study plots in small patches (1- and 10-ha) and large patches (100-ha and continuous forests). Discriminant Analysis of Principal Components (DAPC) partitions the genetic variance into between-group and within-group components, to maximize discrimination between groups without making assumptions of panmixia (Jombart et al., 2010). This approach is more convenient for populations that are assumedly partially clonal and genetically related due to relatively recent isolation events. DAPC uses both principal component analysis (PCA) that are identified using discriminant analysis (DA), to infer the number of clusters in the metapopulation. To select the optimal number of PCs that should be retained in the following DAPC, stratified cross-validation of DAPC was performed by sampling variable numbers of PCs from a subset of the observations in each population (while the number of discriminant functions remained fixed). The number of principal components (PCs) with the best score was considered as the optimal number to include most sources of variation. A DAPC was run using population IDs ( $n=12$ ) corresponding to their geographical site as priors for population clusters, with the optimal number of PCs axes and using the five first axes retained in the DA. Following the same workflow, a DAPC was run grouping the twelve populations to their corresponding fragment sizes (1-, 10, 100-ha, and continuous forests) as cluster population priors.

To compare whether populations in small and large patches present significant genetic differentiation within and between populations, the pairwise genetic differentiation index ( $F_{ST}$ : Weir and Cockerham, 1984) was computed for each population pair using the package *hierfstat* (Goudet & Jombart, 2022). Further, hierarchical analysis of molecular variance (AMOVA) was applied to the  $F_{ST}$  calculations of the total genetic variation observed among forest habitat size (small vs. large patches), and populations within small patches, and large patches separately. Significances were determined using 999 permutations. All calculations were done using the software R with the packages *hierfst* version 0.5-11 R package (Goudet & Jombart, 2022), *poppr* (Kamvar et al., 2014, 2015), and *pegas* (Paradis, 2010).

### *Spatial autocorrelation*

Spatial genetic differences as a result of isolation by distance (IBD) were analyzed by computing a Mantel test Pearson’s product-moment correlation (Mantel, 1967) between a pairwise population genetic distances matrix [ $F_{ST}/1-F_{ST}$ ; Rousset, 1997] and geographical distance between sampling localities [log(Euclidean distance)]. Genetic and geographic distances were computed using the functions `glibd` from the *dartR* package (Gruber et al., 2018), and the statistical Mantel test using the functions from the package *vegan* (Oksanen et al., 2016). For this test, the significance values were calculated from 999 random permutations. Isolation by distance was examined for all population pairs, and within pairs of populations in small and large patches separately.

### *Network analysis of migration pattern*

To quantify the proportion and direction of recent migration patterns among populations in small and large patches, relative migration levels ( $Nm$ ) were estimated between populations calculated based on neutral SNPs using the  $G_{ST}$  and  $Nm$  parameters (Sundqvist et al., 2016) with the *divMigrate* function of the R package *diveRcity* (Keenan et al., 2013). This approach permits the determination of migration directional differences between genetically structured populations, where deterministic extinction and density-dependent processes govern source-sink population dynamics (i.e., metapopulation structure). Relative migration network analysis was estimated using only the complete dataset, as the probability of assigning the correct migration direction is directly related to the increase in the number of samples and loci in the dataset (Sundqvist et al., 2016). The direction and magnitude of recent migration patterns were summarized as a network from the significant estimated effective number of migrants ( $Nm$ ) based on 1000 bootstrap interactions and with a filter threshold of 0.35. Network graphics were generated using the *qgraph* R package (Epskamp et al., 2012).

## RESULTS

### *Change of epiphyllous population occupancy patterns in fifteen years*

For both species (*R. flaccida* and *C. surinamensis*) we observed changes in the estimated number of colonies censused per 1-ha plot in a 15-year interval (Figure 2A-B; Table 1; Table S3). Specifically, the number of colonies of *R. flaccida* increased significantly in small patches (1- and 10-ha) across all study plots: average increases from 53.03 to 176.89 ( $W = 2$ ,  $p$ -value = 0.002). Among large patches, the number of colonies either increased or decreased over time, with a non-significant overall average increase from 284.29 to 338.42 ( $W = 47$ ,  $p$ -value = 0.16). Similarly, the number of colonies of *C. surinamensis* increased in small patches in all study plots, with a significant average increase from 33.04 to 198.27 ( $W = 0$ ,  $p$ -value = 0.0005). In large patches, the number of colonies presented a slightly significant average increase from 250.83 to 397.72 ( $W = 33$ ,  $p$ -value = 0.02). In the year 2000, the estimated number of colonies in all small patches (1- and 10-ha) was significantly reduced when compared to large patches (Figure 2C in 2000, *R. flaccida* :  $W = 86$ ,  $p$ -value = 0.0004; *C. surinamensis* :  $W = 85$ ,  $p$ -value = 0.0007). The estimated number of colonies in small patches (1- and 10-ha) remained reduced in 2016 when compared to large patches of 100-ha and continuous forests (Figure 2C in 2016; *R. flaccida* :  $W = 112$ ,  $p$ -value = 0.006; *C. surinamensis* :  $W = 113$ ,  $p$ -value = 0.005). When only 10-ha patches were compared to larger patches they harbored in 2000 significantly fewer epiphyllous colonies (*R. flaccida* :  $W = 49$ ,  $p$ -value = 0.005; *C. surinamensis* :  $W = 47$ ,  $p$ -value = 0.015). In the year 2016, these 10-ha patches present a similar number of colonies compared with larger patches (*R. flaccida* :  $W = 53$ ,  $p$ -value = 0.19; *C. surinamensis* :  $W = 53$ ,  $p$ -value = 0.19) (Table 1).

### *Assembled loci and SNPs summary*

The number of raw reads generated as well as the number of assembled loci and SNPs for each specimen are summarized in Table S2. An average of 918813 (35940 – 3186367) reads per individual were obtained for both species and on average 639727 (24930 – 2393023) reads were retained after demultiplexing. A total of 1218974 loci were genotyped for *Radula flaccida* (ranging by sample between 3706 – 79145), and mean sample coverage was 5.4x (3.9x, to 11.6x, stdev=1.2x). *Cololejeunea surinamensis* showed a similar range number

of genotyped loci 1306155 (ranging by sample between 3706 – 79145) and with mean sample coverage 4.9x (3.6x to 9.04x, stdev=0.9x). After excluding samples with a high percentage of missing data (>90–98%), the clustering of allele diverging by a maximum of 15% or 20% among individuals resulted in 9562 or 8575 filtered loci for *R. flaccida*, and 4428 or 3258 loci for *C. surinamensis*, for the two respective datasets. These matrices present a relatively similar number of SNPs as follows (Table S2): *R. flaccida* (427 SNPs in  $n = 105$ , and 316 SNPs in  $n = 80$ ); and *C. surinamensis* (415 SNPs in  $n = 108$ , and 456 SNPs in  $n = 71$ ). The number of genotyped individuals did not bias allelic richness estimates when comparing the two datasets (Figure S3). The number of loci assembled (>400) in the two datasets for both species was enough to discriminate individuals within and between populations with further analyses (Figures S4 & S5).

#### *Population genetic summary statistics and its relationship to patch size*

A contrasting pattern in genetic diversity metrics in small and large patches was observed between the two species (Figures S6 & S7; Table S4). *Radula flaccida* in small patches (1-ha and 10-ha), presented a similar proportion of variant sites, polymorphic sites, and allelic richness when compared to large patches (100-ha and continuous forest) ( $p$ -value = >0.05, Table S6). Private alleles ( $R^2 = 0.20$ ,  $p$ -value = >0.05) and nucleotide diversity ( $R^2 = 0.15$ ,  $p$ -value = >0.05) decreased towards larger patches when the complete dataset was considered. For *Cololejeunea surinamensis*, populations in small patches (1-ha and 10-ha) present lower genetic diversity in all five calculated metrics than populations in large patches (100-ha and continuous forests) (Table S6). This difference was more evident and statistically significant for polymorphic sites ( $R^2 = 0.52 - 0.67$ ,  $p$ -value = <0.05) and nucleotide diversity ( $R^2 = 0.50 - 0.58$ ,  $p$ -value = 0.01) with both datasets (Table S6). The observed genotype diversity (MLG) showed similar patterns. An overall similar genotype diversity was observed between small and large patches for *R. flaccida* populations ( $R^2 = 0.08$ ,  $p$ -value = >0.05). A lower genotype diversity in small patches compared to populations in large patches of *C. surinamensis*. This pattern was only significant with the reduced dataset ( $R^2 = 0.49$ ,  $p$ -value = <0.05).

The richness index used, Shannon-Weiner Diversity index (H), Stoddard and Taylor's Index (G), and Simpson's index (lambda) suggest that *R. flaccida* population genetic diversity did not differ between small and large patches ( $p$ -value = >0.05; Figure S7). Populations in 1-ha fragments show a slightly lower richness compared to populations in 10-, 100-ha, and continuous forests when the complete dataset was considered (Table S5). On the contrary, for the species *C. surinamensis*, the three richness indexes were reduced towards smaller patches when the reduced dataset was considered (Figure S7). The Shannon-Weiner Diversity index (H) ( $R^2 = 0.43$ ,  $p$ -value = <0.05) and Stoddard and Taylor's Index (G) ( $R^2 = 0.49$ ,  $p$ -value = 0.01) were statistically significant, while the reduced diversity in small patches observed with the Simpson's index (lambda) was not significant ( $R^2 = 0.30$ ,  $p$ -value = >0.05) (Table S6).

#### *Population structure: individual-based clustering and pairwise differentiation*

The DAPC of posterior genotype probabilities assigned to their respective population provenance revealed high overlap between populations from different forest patch sizes in the BDFFP landscape in both species (Figure S5). When the DAPCs was performed assigning populations to patch size groups (1-, 10, 100-ha, and continuous forests), we observed that populations of *R. flaccida* in 1-ha fragments tend to diverge from the main cluster along Axis 1, which explained >60% of the genetic variation (Figure 3A). With the reduced dataset, the population in the 1-ha fragment showed little overlap with the population clusters of 10-, 100-ha, and continuous forests. The population of *C. surinamensis* in the 100-ha fragment using the complete dataset, genotypes diverged along the Axis 1 from the clusters with the populations in 1-, 10-ha, and continuous forests. Between the 1-, 10-ha, and continuous forests clusters, genotypes tend to diverge along Axis 2 which explained 25.9% of the genetic variation. When we analyzed the reduced dataset the four clusters that correspond to different forest patch sizes, diverged along both Axis, with little overlap (Figure 3B).

Pairwise  $F_{ST}$  comparison of population and  $F_{ST}$  comparison of the population over loci are presented in Tables S7 & S8. Global  $F_{ST}$  measures across populations in small and large patches were 0.064 for *R.*

*flaccida* and 0.191 for *C. surinamensis* with the complete dataset. With the reduced dataset  $F_{ST}$  measures were lower: 0.014 for *R. flaccida* and 0.128 for *C. surinamensis*. For populations of *R. flaccida*, mean pairwise comparison  $F_{ST}$  values tend to be higher among small patches, than between small-large patches and within large patches (Figure 4). Higher values of  $F_{ST}$  were also observed for pairwise comparison among small patches for *C. surinamensis*, while between small-large patches and within large patches tended to be lower (Figure 4).

Using the statistical test of AMOVA we group populations of the two species by patch size (1-, 10-, 100-ha, and continuous) and by size class (small and large patches). In both species, the major proportion of the genetic variation observed was explained by variation within populations. The geographic location of the populations across the landscape explained to a minor extent the genetic variation observed (Table 2). The species *R. flaccida*  $F_{ST}$  values tend to be slightly higher among small patches comparison and between small-large patches comparison, indicating some degree of differentiation. However, this genetic difference was not significant (Table 2). On the contrary, genetic differences between small and large patches were significant for the species *C. surinamensis* (Table 2).

### *Spatial autocorrelation and migration patterns*

Results for the spatial autocorrelation of all population pairs in small patches (1- and 10-ha), and large patches (100-ha, and continuous forests), for the complete dataset, are summarized for the two species (Figure 5; Table S9). When we examine all population pairs, irrespective of the patch size, pairwise genetic and geographic distances show no correlation based on the Mantel test for both species (*R. flaccida*:  $R^2 = 0.074$ ,  $p\text{-value} = 0.269$ ; *C. surinamensis*:  $R^2 = -0.014$ ,  $p\text{-value} = 0.514$ ). Using the dataset with 20% of missing data, the same pattern was observed (*R. flaccida*:  $R^2 = 0.006$ ,  $p\text{-value} = 0.5$ ; *C. surinamensis*:  $R^2 = 0.175$ ,  $p\text{-value} = 0.079$ ). Correlation within pairs of populations of *R. flaccida* in small patches showed a positive slope indicating that populations in small patches as they are farther distant geographically, are more genetically dissimilar (Figure S8A). This was more pronounced when we use the reduced dataset (Figure S8D) with a significant correlation based on the computed Mantel test ( $R^2 = 0.46$ ,  $p\text{-value} = 0.02$ ). For *C. surinamensis*, a non-significant positive slope was observed ( $R^2 = 0.389$ ,  $p\text{-value} = 0.068$ ) within populations in small patches. When pairwise comparisons were computed for populations within large patches, the genetic distances were not correlated to geographic distances in both species (Figure S8B, E, H, K). *R. flaccida* presents a slightly positive slope (*R. flaccida*:  $R^2 = 0.186$ ,  $p\text{-value} = 0.341$ ), while populations of *C. surinamensis* showed a non-significant negative slope (*C. surinamensis*:  $R^2 = -0.11$ ,  $p\text{-value} = 0.65$ ). The reduced dataset with >75% of missing data supports the same pattern (*R. flaccida*:  $R^2 = 0.056$ ,  $p\text{-value} = 0.44$ ; *C. surinamensis*:  $R^2 = -0.055$ ,  $p\text{-value} = 0.56$ ).

The relative migration levels ( $Nm$ ) estimated between populations based on neutral SNPs using the  $G_{ST}$  and  $Nm$  parameters showed similar patterns (Table S10). The network graph using the  $Nm$  parameters suggests a high migration between *R. flaccida* populations irrespective of patch size and isolation (Figure 5C, Table S10). Populations in small patches act as source populations with high relative migration to other forest fragments and continuous forests across the landscape. While populations in large patches showed signs of asymmetrical migration among them, significant symmetrical migration was observed between the populations in the Florestal continuous forests and the Dimona 100-ha fragment. The migration network of the species *C. surinamensis* was less complex with fewer patches connected by network vertices, suggesting low or no migration between populations across the landscape (Figure 5D, Table S10). Population in small patches showed significant relative migration among them, at a higher rate than with large forest patches. Populations in large patches were completely isolated (Dimona 100-ha and Dimona continuous), where no migration was observed. Significant migration was observed but at a relatively low rate from the Florestal continuous forest to the closest Km 41 and Cabo Frio continuous forests.

## DISCUSSION

We investigated the habitat fragmentation-related effects (e.g., the consequences of reduced size and increased isolation) on the demographic and genetic processes shaping the metapopulation of two epiphyllous

bryophyte species with contrasting mating systems. We showed that fragmentation induces marked effects on the functional connectivity of both plant populations in less than half a century of experimental size and connectivity reduction. The long-term reduced population density in small patches (1- and 10-ha) (Sierra et al., 2019b), has led to accelerated genotypic differentiation (genetic drift) compared to the populations in large patches (100-ha fragment and continuous forests). This result supports our hypothesis (H1) that spatial variation in patch size and isolation are important determinants of the metapopulation genetic process (stochastic loss of genotypes) in a fragmented forest, despite the notion of bryophytes as being highly vagile organisms (Vanderpoorten et al., 2019)

We observed that species' mating system confers different sensitivity to the effects of habitat fragmentation, where a shift in the genetic structure (diversity and allele frequency:  $F_{ST}$ ) of the unisexual plant was less negatively impacted by forest fragmentation than of the bisexual counterpart, contrary to our expectations (H2). However, this should be taken with caution since is expected that genetic diversity meltdown will take a longer time to be apparent than changes in allele frequency in response to habitat fragmentation (Lowe et al., 2005). In the case of the unisexual species the observed differentiation in 1-ha might indicate initial signs of diversity loss in the following generations under the current landscape fragmented scenario.

The metapopulation migration network of the two species shows differences in functional connectivity, with the unisexual species more inter-connected among patches than the bisexual species, contrary to our predictions (H3). For *R. flaccida* (unisexual) we observed a patchy metapopulation with a highly interconnected migration network, with all patches exchanging migrants among them, irrespective of size and isolation. However, positive isolation by distances in small patches suggests that migrants are from nearby source populations. While *C. surinamensis* (bisexual) present symmetrical migration only between small patches, and no sign of isolation by distance among populations. Stochastic extinctions are expected to increase in small patches, but genetic diversity might be maintained through colonization from nearby source populations if functional connectivity is maintained (Auffret et al., 2017). Despite the patch size effect on the metapopulation demography and genetics of both epiphyllous bryophyte species, small patches remain valuable for conservation assessment, as they can act as a source of migrants to other forest fragments and continuous forests, as observed for Macronesian oceanic islands (Laenen et al., 2011; Patiño et al., 2015).

#### *Demographic and genetic consequences of forest fragmentation*

Our results go in line with studies addressing the impact of patch size and isolation on the genetic diversity of cryptogamic organisms in temporal similar historically fragmented landscapes to our study (~30–50 years). For example, reduced genetic diversity has been observed related to small population size, patch size isolation, and habitat degradation (Holá et al., 2015; Patiño et al., 2010; Wang et al., 2013; Otálora et al., 2011). As well our results in an experimental Amazonian fragmented landscape support theoretical predictions of island biogeography theory and metapopulation theory on the genetic diversity (Whittaker et al., 2008; Vellend, 2003; Vellend & Geber, 2005) observed for oceanic islands (Costanzi & Steifetten, 2019; Hill et al., 2017; Whittaker et al., 2017; Hamabata et al., 2019), and other insular landscape (Aycrigg & Garton, 2014; Hepenstrick et al., 2022). However, climate and habitat fragmentation synergistic effects on biodiversity might take a long time to be apparent (Chase et al., 2020; Scott et al., 2021). For example, some species might become extinct immediately after habitat disturbance, whereas others persist to eventually become locally extinct (extinction debts) years after deforestation isolates local populations (Tilman et al., 1994; Kuussaari et al., 2009). During this time lag, species fitness decreases due to low reproduction and local recruitment which will further exacerbate under future climate extremes, such as droughts (Scott et al., 2021); therefore, affecting species' long-term survival probability (Figueiredo et al., 2019). Species experiencing time-delayed extinctions (extinction debts) are not necessarily doomed to extinction, as new colonization events could pay for such debts (Kuussaari et al., 2009).

Under future climate scenarios, the ability of bryophyte species to escape local extinction by tracking suitable habitats is of high concern (Zanatta et al., 2020) despite their high dispersal capacities (Vanderpoorten et al., 2019). Even though a diverse genetic pool of individuals can reach a disturbed local population, effective dispersal would be mediated by the local environmental conditions (Hedenäs et al., 2021). Amazon lowland

bryophyte communities were thought to be composed of highly dispersive species (Schuster, 1983) with low floristic dissimilarity mainly driven by niche process (Mota de Oliveira & ter Steege, 2015). Yet spore traps on the 325 m height Amazon Tall Tower Observatory (ATTO) capture few bryophyte diaspores, suggesting a low frequency of airborne propagules in the atmosphere (Mota de Oliveira et al., 2022). The high spatial genetic structure of Amazonian plant species across a regional spatial scale (Nazareno et al., 2019; Ledent et al., 2020; Campos et al., 2022), combined with non-random deforestation events in the Amazon (Taubert et al., 2018; Matricardi et al., 2020) will deplete important source populations for the genetic diversity maintenance (Lowe et al., 2005). Herein, our results offer evidence in support of our H1 for genetic drift over the 40 years that populations have been isolated in the present fragmented landscape. This establishes a premise that dispersal limitation in bryophyte metapopulations (Ledent et al., 2020) will be susceptible to stochastic demographic and genetic processes, which may accelerate even more the pace of extinction as ecological barriers rapidly increase with non-random deforestation in the Amazon (Taubert et al., 2018; Fischer et al., 2021).

### *Consequences of mating system and dispersal potential*

Species with different reproductive strategies might present different sensitivity to small patch sizes and to isolation to maintain genetic diversity (Honnay et al., 2005; Obbard et al., 2006; Nazareno et al., 2013). Bisexual bryophytes are presumed to have high fertilization rates and consequently higher spore production, which ensure successful colonization (Laenen et al., 2016). In contrast, unisexual species will present lower dispersal potential as the probability of fertilization will depend on that male and female plants are found in the same colony (Snäll et al., 2004; Maciel-Silva et al., 2012; Alvarenga et al., 2016).

We observed high migration of *R. flaccida* among patches across the fragmented landscape irrespective of patch size. Functional connectivity between patches recovered in migration network analysis explained the ability of the species to maintain genetic diversity despite population density decline. Colonization rates of *R. flaccida* (unisexual), albeit low, were sufficient to curtail genetic drift as suggested by Zartman et al., (2006). On the other hand, the maintenance of genetic diversity observed in *R. flaccida* in small patches could be attributed to other two reasons. Firstly, genetically diverse populations have been maintained in small patches (although declining) due to their adaptative potential to cope with environmental change (Lowe et al., 2005); which is corroborated by the maintenance of linkage disequilibrium in small forest fragments (Zartman et al., 2006). Second, the standing genetic variation has not yet been lost through stochastic demographic events due to constant local recruitment from asexual propagules when sexual reproduction is limited (Honnay & Bossuyt, 2005). Limited sexual reproduction has been reported for *R. flaccida* majorly during the dry season, which is expected to exacerbate under future climate change scenarios (Zartman et al., 2015) affecting long-term demographic stability (Scott et al., 2021). Clonal reproduction represents an alternative recruitment strategy when environmental conditions suppress sexual reproduction in unisexual species, maintaining genotypes before fragmentation effects become apparent (Honnay & Bossuyt, 2005). In bryophytes, as observed herein for *R. flaccida*, asexual reproduction plays an important role in maintaining genetic diversity over time by preventing local genotype extinction (Honnay & Bossuyt, 2005) and assuring successful sexual reproduction (Alvarenga et al., 2016). Evidence from unisexual bryophytes found that genetic diversity is maintained when species stakes on asexual reproduction for their survival (Pohjamo et al., 2008; Wang et al., 2013; Holá et al., 2015; Escolástico-Ortiz et al., 2023), but contrasting evidence was found for a hornwort species (Alonso-García et al., 2020).

Regarding *C. surinamensis* (bisexual), we expected a higher dispersal potential due to the higher probability to produce sporophytes since both androecial and gynoecia branches are produced in the same colony (Maciel-Silva et al., 2012; Laenen et al., 2016). Contrary to our expectations (H3), this species shows restricted dispersal among small patches (1- and 10-ha) where significantly lower genetic diversity and significant changes in allele frequency were observed. The stochastic genetic loss with declining populations of *C. surinamensis* in small patches, suggests an extinction debt has been already paid during the 40 years of patch isolation. A possible explanation for the lower dispersal potential would be that the genus *Cololejeunea* presents low spore output per capsule (250–900 spores) when compared to other genera in the family Lejeu-

neaceae (He & Zhu, 2011). Even if the sporophyte frequency of the bisexual and unisexual species studied here are similar in a population, the lower spore output per capsule of *C. surinamensis* might limit the dispersal across the landscape (Snäll et al., 2004). Demographic surveys of epiphyllous bryophytes showed that species differ in the threshold size to initiate sexual expression, where higher size requirements might limit sporophyte frequency under unsuitable environmental conditions (Zartman et al., 2015; Alvarenga et al., 2016). Environmental conditions in degraded forest fragments might affect reproductive performance, by limiting sexual expression and sporophyte frequency of colonies growing in unsuitable habitat conditions (Maciel-Silva et al., 2012), which will further determine the assembly rate of individuals and genotypes (Sierra et al., 2019a; Mežaka et al., 2019).

### *Conservation implications for metapopulation genetics in the Amazon Forest*

Anthropogenic activities are the main cause of forest conversion associated with the change in land use in Amazonia over recent decades (Curtis et al., 2018). Forest fragmentation due to commodity-driven deforestation represents the most immediate threat to the Amazonia, the synergist effects of forest loss and forest degradation (edge effects, logging, fires, and droughts) put in threaten forest ecosystems and services with nearly 38% of the forests in the region currently degraded (Chase et al., 2020; Lapola et al., 2023). Even if conservation policies with objectives of zero deforestation in the Amazon are reached by 2030, a landscape of isolated patches will remain for decades (Fischer et al., 2021).

Research efforts have been focused on determining the habitat amount, in terms of patch size and isolation, that fragmented landscape allows for biodiversity maintenance of a region (Fahrig et al., 2019, 2022; Watling et al., 2020). Conservation management debates whether efforts should be given to preserving a single large patch or several small patches (SLOSS: single large or several small) (Diamond, 1975; Tjørve, 2010). The BDFFP has focused on addressing this question, with a myriad of studies targeting different organisms, indicating an Amazonian biodiversity loss after a threshold habitat size and isolation (Laurance et al., 2011, 2018). From the organisms that have been studied at the BDFFP, few have related the demographic consequences on the evolutionary processes (drift and migration) that impact the population genetic structure in a fragmented landscape (Hamilton, 1999; Côrtes et al., 2013; Zartman et al., 2006). This has left a gap when addressing the amount of habitat for conservation genetics. In long-lived organisms, the distribution of genotypes observed in fragmented habitats is expected to reflect the historical landscape configuration, demographic patterns, and population connectivity (Carley et al., 2022). Consequently, in most long-lived organisms this association is challenging, but annual plants such as understory *Heliconia* (Côrtes et al., 2013) and patch-tracking epiphyllous metapopulation (Zartman et al., 2006) have been important in our understanding of the genetic consequence of habitat fragmentation.

Herein, we integrate demographic and genetic data of epiphyllous metapopulation to quantify threshold patch size for population viability and genetic maintenance for regional conservation management taking into consideration the functional connectivity. Our results support the maintenance of genetic diversity in small patches (>10-ha) when asymmetric migration allows for rescue effects of extinct genotypes among patches in a fragmented landscape (Hufbauer et al., 2015). This has large implications for conservation where growing knowledge points to biodiversity managers prioritizing demographic and genetic rescue effects of populations when designating conservation areas and targeting declining populations in degraded habitats (Hufbauer et al., 2015; Hanski et al., 2015; Auffret et al., 2017; Carley et al., 2022). Evidence-based conservation strategies in the Amazon Forest require spatial configuration of (>100–10 000-ha) large patches of non-degraded forests (Laurance et al., 2018), that will allow for the preservation of long-term evolutionary processes (Carley et al., 2022).

## **CONCLUSION**

Our results in an epiphyllous metapopulation, confirm that forest fragmentation has induced marked effects on both plant population demography and genetic differentiation over half a century. Declines in colonization events in 1- and 10-ha fragment replicates (Zartman & Shaw, 2006), associated with reduced colony densities, led to accelerated genetic drift altering the allelic frequency in smaller fragments, more evident

in 1-ha patches when compared to large patches (100-ha fragment and continuous forest). Furthermore, the mating system (unisexual vs. bisexual) was related to differential sensitivities to the intensity of fragmentation with a contrasting response on the genetic diversity and allelic frequency. Specifically, the unisexual species was characterized by higher migration rates which maintained similar genetic diversity between small patches and large patches, when compared to the bisexual species. Further research should expand sampling considering species with different traits (Sierra et al., 2019b), and consider habitat-dependent reproductive performance (Maciel-Silva et al., 2012) to formulate universal predictions of species sensitivity to habitat fragmentation. Also, how the synergistic effects of habitat fragmentation and climate change will impact the mid- to long-term demographic evolutionary process is of high concern (Scott et al., 2021). Finally, further research should target if genetic variations are subject to adaptive evolution in species experiencing the effects of habitat fragmentation (Hanski et al., 2017). An integrative eco-evolutionary approach will improve conservation strategies for maintaining among-population processes for the persistence of metapopulation in future landscape and climate scenarios.

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## Data accessibility

Genetic data: Raw sequence data were deposited in the NCBI Sequence Read Archive (SRA) with their respective accession numbers.

Individual genotype data are available on DataDryad (XX)

The metadata and script used for the analyses that support the findings of this study are openly available in: <https://github.com/adrielsierra/Epiphyllous-bryophyte-demography-and-genetics>.

## Benefit-Sharing Statement

The present work is the result of an international scientific partnerships that was developed with scientists from the countries providing data and genetic samples, included as co-authors. The work was done under partnership between research institutions and results will be shared with the provider communities and the broader scientific community.

## AUTHOR CONTRIBUTIONS

AMS, CEZ, and JCVA conceived and designed the study. AMS and CEZ sampled and carry out fieldwork. AMS and JCVA prepared the specimens and carried out the DNA extraction. AMS, MAG, and JCVA generated and analyzed the genomic data. AMS carried out the statistical analyses. AMS wrote the manuscript with the assistance of all co-authors.

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## Legends

### Tables

Table 1. Summary statistics results of Wilcoxon rank sum exact test of differences in estimated number of colonies between categories considering 1- & 10-ha forest fragments as small patches and 100-ha & continuous forest as large patches and compared between the two years sampled (2000 and 2016). Significant differences with  $p$ -value  $<0.01$  and  $<0.001$  are highlighted in bold.

Table 2. Analysis of Molecular Variance (AMOVA) of epiphyllous population in forest fragment and continuous forest for the two filtered dataset by the minimum percentage of individuals across populations required to process a locus. Significant differences with  $p$ -value  $<0.01$  and  $<0.001$  are highlighted in bold.

### Figures

Figure 1. Expected population genetic outcomes under different metapopulation structure models with variable degree of isolation (connectivity) and patch size. In the non-equilibrium metapopulation patches of equal

size are highly isolated, that there is no exchange of migrants between populations. In the classical metapopulation model, patches of equal size present high probability of extinction, but have enough connectivity to allow (re)colonization of unoccupied patches allowing metapopulation persistence. The mainland-island metapopulation present small and large patches, where unidirectional migration to small patches is dependent of the mainland population where extinction rate is low (Island-Mainland). Distances from the mainland will determine the rate of migration represented by the arrow size. In patchy metapopulation, patches of variable size are highly connected (patchy) with high rate of migration among all populations. In the metapopulation models following a patch size and connectivity continuum, a high degree of genetic differentiation will be observed in highly isolated small patches, and in highly connected patches of varying size, the genetic differentiation will be lower (Aycrigg & Garton 2014). Figure is adapted from Aycrigg & Garton 2014.

Figure 2. Population size as the estimated number of epiphyllous bryophyte colonies of the species *Radula flaccida* and *Cololejeunea surinamensis* per 1-ha study plot. Temporal variation in population size between the year 2000 and 2016 per 1-ha study plots in A. Small Forest patches (1- and 10-ha), and B. in large forests patches (100-ha and continuous forest). C. Population size density distribution comparison between small and large forests patches for the year 2000 and 2016. Mean population size are depicted as follow: orange solid line for 1-ha, orange dashed line for 10-ha, green solid line for 100-ha and green dashed line for continuous forests.

Figure 3. Fine-scale population genetic structure of the species *Radula flaccida* and *Cololejeunea surinamensis* in an experimental Amazonian fragmented landscape for: A. *R. flaccida* (Dataset  $n = 105$ ,  $-R = 15$ ); B. *R. flaccida* (Dataset  $n = 70$ ,  $-R = 20$ ); C. *C. surinamensis* (Dataset  $n = 108$ ,  $-R = 15$ ); and D. *C. surinamensis* (Dataset  $n = 71$ ,  $-R = 20$ ). Ordination plot of Discriminant Analysis of Principal Components (DAPC) and density plot depicting the two-ordination axis showing the genotype similarity of populations in different forest fragment size and continuous forest. Points represent individuals assigned to their respective size category and 95% ellipse showing confidence interval.

Figure 4. Population genetic differentiation of the species *Radula flaccida* and *Cololejeunea surinamensis* in an experimental Amazonian fragmented landscape with the two datasets. Mean  $F_{ST}$  pairwise comparison of populations within small patches, between small and large patches, and within large patches for: A. *R. flaccida* (Dataset  $n = 105$ ,  $-R = 15$ ); B. *R. flaccida* (Dataset  $n = 70$ ,  $-R = 20$ ); C. *C. surinamensis* (Dataset  $n = 108$ ,  $-R = 15$ ); and D. *C. surinamensis* (Dataset  $n = 71$ ,  $-R = 20$ ).

Figure 5. Isolation by distance relationship ( $F_{ij} F_{ST}/1-F_{ST}$ ) of epiphyll populations and geographic distance (log-transformed) for A. *R. flaccida* (Dataset  $n = 105$ ,  $-R = 15$ ); and B. *C. surinamensis* (Dataset  $n = 108$ ,  $-R = 15$ ). Regression lines correspond to pairwise comparison among populations in small forest patches (1- and 10-ha), populations in large forest patches (100-ha and Continuous Forest), and between small and large forest patches for both species. Symmetrical relative migration network graph using the  $N_m$  parameter among small forest patches (1- and 10-ha), and large forest patches (100-ha and Continuous Forest) for C. *R. flaccida* (Dataset  $n = 105$ ,  $-R = 15$ ); and D. *C. surinamensis* (Dataset  $n = 108$ ,  $-R = 15$ ) in an experimental Amazonian fragmented landscape. Filter threshold for the asymmetric values was set to 0.35. Colors corresponds to the patch size as in Figure 3.

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