

Effects of forest successional status on microenvironmental conditions, diversity, and distribution of filmy fern species in a temperate rainforest

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Abstract

The vertical distribution of Hymenophyllaceae species has been related to microenvironmental variations around host trees. We addressed the questions: Do the vertical microenvironmental conditions within forest stands of differing successional statuses vary significantly? Does the diversity of Hymenophyllaceae species differ between forest successional statuses? Are the vertical distribution and diversity of Hymenophyllaceae species related more to humidity or light availability? Are there any interspecific differences in the desiccation tolerance of these species which can be related to their vertical distribution? We characterized the microhabitat conditions (vapor pressure deficit [VPD], air relative humidity [RH], and light availability [PAR]) and the vertical distribution of Hymenophyllaceae species in host trees, in both a secondary forest and an old-growth temperate rainforest in Chile. Chlorophyll fluorescence was used to monitor the integrity of the photosynthetic apparatus during desiccation experiments. The stand basal area, tree height, and leaf area index were all significantly greater in the old-growth forest stands, but VPD, RH, and PAR showed no significant differences between the two forests. Both successional statuses showed the same amount of filmy fern species in terms of both abundance and diversity. In both successional statuses VPD and RH decreased while PAR increased with the height of the hosts. Regardless of the forest's age, abundance and diversity of filmy ferns were greater in microsites of greater humidity and less light availability. Desiccation tolerance differed significantly among Hymenophyllaceae species. The distribution pattern could be better explained by the specific microenvironmental requirements and desiccation tolerance rather than the forest's successional status.

Keywords: desiccation tolerance, Hymenophyllaceae species, microhabitat preferences, successional status, vertical distribution.

Received 25 May 2012; revision received 27 March 2013; accepted 4 April 2013

Introduction

Hymenophyllaceae is one of the largest fern families in terms of number of species (Dubuisson *et al.* 2003). It corresponds to a widely distributed family, associated with both tropical and temperate rainforests, presenting a remarkable diversity in terms of morphology and habitats

in which they thrive (Dubuisson 1996, 1997). Given that species of the Hymenophyllaceae family normally lack cuticles, present no differentiated epidermis, and have no stomata, they therefore depend greatly on environmental humidity because they have no mechanisms to prevent water loss from their tissues (Krömer & Kessler 2006). These anatomical characteristics have been proposed as adaptations to humid environments (Proctor 2003). Most Hymenophyllaceae species (hereafter filmy ferns) are

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considered poikilohydric organisms because they present a rudimentary control of water loss and depend on environmental humidity (Kappen & Valladares 2007). Additionally, some filmy fern species have been regarded as desiccation tolerant species (Richards & Evans 1972, Proctor 2012). Desiccation tolerance is the ability to dry to equilibrium with air that is moderately to extremely dry and then regain normal function after rehydration (*sensu* Alpert 2005). Filmy fern species' ecological distribution has been related to interspecific differences in tolerance to desiccating conditions such as high irradiance and low humidity (Proctor 2003, 2012). In this context, filmy ferns are an excellent study model in order to understand the functional ecology of vascular plants with epiphytic climbing habits (Putz & Mooney 1991; Proctor 2012) because they present a local distribution associated with a vertical gradient of environmental variation (Parra *et al.* 2009).

Most studies that have assessed both distribution and abundance of epiphytic plants in temperate forests have restricted their evaluations to the lowest few meters of the tree height (Diaz *et al.* 2010). These studies do not describe the microenvironmental conditions of the full vertical gradient where epiphytic plants are established on the trunk of the host tree. Additionally, aside from the basic characterization of habitats where filmy ferns occur in temperate forests, there is scarce quantitative information regarding this fern group's functional response to desiccation (Proctor 2012). In the Chilean temperate rainforest the microenvironmental variation along the vertical gradient of a second growth forest stand showed changes in light availability and humidity that reflect the microhabitat preferences of epiphytic Hymenophyllaceae species (Parra *et al.* 2009). However, Parra *et al.* (2009) evaluated vertical distribution and microenvironmental variation up to a height of only 1.8 m, while some filmy fern species in the Chilean temperate forest can easily reach heights of 9 to 10 m on the host tree trunks (Clement *et al.* 2001; Diaz *et al.* 2010). Parra *et al.* (2009) did not consider a higher vertical gradient because the secondary forest stand chosen for the study showed no filmy ferns distributed higher along the host trunks. Subsequently, the authors realized that forest structural variation could be one of the factors that generates a higher variability in the vertical distribution pattern (Parra, pers. comm. 2011). Thus, these ferns' maximum height could differ with the age and/or structure of the forest. Additionally, information on vertical microenvironmental variation in old-growth stands of this forest type is seriously lacking in Chile.

The Hymenophyllaceae family is one of the main epiphytic components of the Chilean temperate rainforest, including 23 species (Marticorena & Rodríguez 1995; Parra 2005). This study aimed at characterizing the microhabitats and the vertical distribution of epiphytic filmy

fern species along host trees at different successional stages of forest stands (old-growth forest versus secondary forest) in the temperate rainforest of southern Chile. Additionally, we assessed if the fern specific desiccation tolerance is consistent with that distribution pattern. In order to identify if composition, abundance, and vertical distribution of an assemblage of epiphytic ferns of the Hymenophyllaceae family change in relation to the microenvironmental variation (humidity and light availability) and forest successional status, structural and microenvironmental differences were quantitatively determined from two stands of evergreen temperate forest which a priori were believed to be of different successional stages. Forest stand structure is defined as the physical and temporal distribution of trees in a stand, and includes within the description the distribution of species, vertical and horizontal spatial patterns, size of trees, and tree ages, or combinations of these (Oliver & Larson 1990; Stone & Porter 1998). In the present study, the forests' structural differences were assumed to reflect the age difference between stands, because the site under study corresponds to a successional chronosequence in a forest matrix belonging to the same vegetation type (Katalapi, Región de Los Lagos, Chile). We evaluated the hypotheses that: (i) microenvironmental conditions (humidity and light availability) vary significantly between an old-growth forest and a secondary forest; (ii) filmy fern species abundance, richness, and diversity differ between these two forest stand ages; (iii) vertical filmy ferns' diversity and distribution are more related to humidity than light availability, independent of forest successional status; and (iv) that differences in desiccation tolerance in filmy fern species explain their distribution and habitat preferences on the host trees.

Material and methods

Study site and forest stands' structure

This study was performed in four forest stands that differ in successional status (two old-growth forest stands and two secondary forest stands) within the Katalapi Park in Pichiquillaie, south-central Chile (41°31' S, 72°45' W), in the Cordillera de Quillaie foothills 1 km from Seno de Reloncaví. This area has no clear influence from prevailing winds (Luebert & Pliscoff 2006). Its climate is characterized by short and relatively dry summers and long, wet, rainy winters (Luebert & Pliscoff 2006). The mean annual air temperature is 11°C. The mean minimum monthly air temperature (5°C) occurs in August, while the mean monthly maximum temperature (20°C) occurs in February. The climate in Pichiquillaie is considered temperate and highly humid with an oceanic tendency (Di Castri & Hajek 1976), although winter frosts do occur. Annual

precipitation is around 2200 mm or more (Armesto *et al.* 1995). Even though the daily mean relative humidity throughout the year is high, there are periods of lower humidity (Reyes-Díaz *et al.* 2005). The second-growth rainforest at this altitude of the Andes western foothills (350 m a.s.l.) is composed exclusively of broad-leaved evergreens (Saldaña & Lusk 2003; Luebert & Plissock 2006).

We determined the forest structure in both secondary and old-growth forest stands by measuring stand basal area, maximum tree height, light availability (direct site factor, DSF) and leaf area index (LAI) at plot scale, pooling five plots per forest successional status (see below for details about plot size). The DSF and LAI in each plot were quantified by hemispherical photography. Comparisons of methods confirmed that hemispherical photography was highly accurate in the description of understory light availability (Bellow & Nair 2003). The photographs were taken using a horizontally leveled digital camera (CoolPix 995, Nikon, Tokyo, Japan), mounted on a tripod and aimed at the zenith, using a fish-eye lens with a 180° field of view (FCE8, Nikon). Photographs were analyzed for canopy openness using Hemiview canopy analysis software Version 2.1 (1999, Delta-T Devices Ltd, UK). The DSF was computed using Hemiview and accounting for the geographic features of the site. This factor is an estimate of the fraction of direct radiation expected to reach the spot where the photograph was taken (Anderson 1966).

Species abundance and microenvironmental measurements

A total of 18 filmy fern species had been previously described for the study area (Parra *et al.* 2009). Of these, we found 12 species (Table 1) that were commonly present in both forest successional states. The dominant host tree species were *Amomyrtus luma* (Mol.) Legr. *et* Kausel and *Rhaphyathamnus spinosus* (A.L. Juss) Mold in the secondary forest stands; and *Laurelia philippiana* (Looser) Schodde and *Drimys winteri* J.R *et* G. Foster in the old-growth forest stands. We measured the abundance of epiphytic filmy ferns and the microenvironmental parameters in both second-growth and old-growth forest stands. We randomly selected five plots (each plot = 225 m²; 15 × 15 m) within each forest successional status, totaling 10 plots. Sampling was restricted to low sites (about 100 m a.s.l.) in order to minimize possible topographic or edaphic effects. Species richness, abundance, and diversity (Shannon-Weiner index, *H'*) of the filmy fern species were determined at different heights on three host trees (> 25 cm diameter at breast height; DBH) of the dominant species, which were randomly selected in each plot.

Table 1 The Hymenophyllaceae species that were commonly present in both forest successional states

Species	Abbreviated name
<i>Hymenophyllum caudiculatum</i> var. <i>productum</i> (K. Presl) C. Chr.	HCAU
<i>Hymenoglossum cruentum</i> (Cav.) K. Presl	HCRU
<i>Hymenophyllum cuneatum</i> Kunze	HCUN
<i>Hymenophyllum dentatum</i> Cav.	HDEN
<i>Hymenophyllum dicranotrichum</i> (K. Presl) Hook. ex Sadeb.	HDIC
<i>Hymenophyllum krauseanum</i> Phil.	HKRA
<i>Hymenophyllum pectinatum</i> Cav.	HPEC
<i>Hymenophyllum peltatum</i> (Poirot) Desv.	HPEL
<i>Hymenophyllum plicatum</i> Kaulf.	HPLI
<i>Hymenophyllum secundum</i> Hook. <i>et</i> Grev.	HSEC
<i>Hymenophyllum tortuosum</i> Hook. <i>et</i> Grev.	HTOR
<i>Serpillopsis caespitosa</i> (Gaudich.) C. Chr.	SCAE

Vertical sampling on the host trees was done at 1.5 m intervals from the bottom (0 m) up to the maximum height where there were filmy ferns (ca 5–10 m). We estimated the filmy fern species' abundances from the cover (% per m²) of each species in six quadrants (25 cm × 25 cm) at each height on the host tree. In this sampling we used a telescopic ladder to reach heights from 2 to 9 m. Given that filmy fern species generally present vegetative propagation by rhizomes (Marticorena & Rodríguez 1995), we measured the abundance of each filmy fern species by estimating the presence and percent of area on a quadrant occupied by the fronds of each species. Frond sizes of filmy ferns species ranged from 2.5 ± 0.1 cm (*Serpillopsis caespitosa*) to 9.0 ± 0.2 cm (*Hymenophyllum cruentum*) (Parra *et al.* 2009).

Vapor pressure deficit (VPD), relative humidity (RH), and light availability (PAR radiation) variation were recorded using leaf temperature, humidity, and PAR sensors connected to dataloggers (HOBO-microstations and sensors; Onset Computer Corporation, MA, USA), installed in each forest stand. We sampled five trees with the sensors and dataloggers in each type of forest, and these devices were installed in the same trees from which the filmy ferns were sampled. These sensors recorded this data every 15 min during both the day and night in the season of relatively lower rainfall in the southern temperate rainforest (*sensu* Luebert & Plissock 2006), from October 2010 through March 2011 (from spring until late summer). We measured these microenvironmental parameters up to the maximum height that filmy ferns reach in the host: at three trunk heights (< 1.0 m, 4.5 m, and 9.0 m above ground level) in the old-growth forest stand, and at two heights (< 1.0 m and 4.5 m above ground level) in the secondary forest stand. We decided not to

install sensors at 9 m in the second growth forest, because filmy ferns did not reach that height in this forest type and to optimize the number of devices that we had available.

Desiccation tolerance

Fern species can be propagated by rhizome separation (Rodríguez 1973; Saldaña *et al.* 2005; Saldaña *et al.* 2007). Hence, we separated isolated modules (i.e. units composed by fronds, rhizomes, and roots) from filmy fern mother plants of 11 out of the 12 Hymenophyllaceae species occurring in the old-growth forest stands. Mother plants were collected from the microsites where each species exhibited its greatest abundance. We decided to discard *Hymenophyllum secundum* from this experiment because of its low abundance in the collection sites. Between five and eight isolated modules of each species were submitted to desiccation and rehydration kinetics (until samples reached a constant weight) inside a growth chamber with controlled environmental conditions (temperature $15 \pm 2^\circ\text{C}$, relative humidity 80%, photoperiod 0/24 h light/dark). The experiments were performed in a growth chamber with this capability. Relative humidity was continuously monitored and was $80 \pm 7\%$ during the desiccation period. The relative water content (RWC) was calculated as $\text{RWC} = ([\text{FW} - \text{DW}] / [\text{TTW} - \text{DW}]) \times 100$, where FW is fresh weight, DW is dry weight and TTW is total turgor weight. We developed a comparative assay of the specific stress response to desiccation using chlorophyll fluorescence (F), measuring the variation of maximal photochemical efficiency F_v/F_m as a proxy of functional integrity of photosystem II (PSII) during desiccation. These measurements were used to calculate the degree of photoinactivation (PhI), which is a relative measure of the effects of stress, where higher values indicate a higher sensitivity to the strain of the photosynthetic apparatus (Larcher 2000; Neuner & Pramsohler 2006). We determined the degree of PhI at a given RWC, in order to define the critical RWC for each species, as an indirect measurement of the desiccation tolerance. We adapted the methodological approach implemented by Larcher (2000) for the freezing stress response of PSII. Thus, the critical RWC threshold of PSII, where PhI starts to increase during progressive desiccation, was then graphically determined from the PhI in order to generate RWC plots for each filmy fern species (Fig. 1). To obtain the PhI -RWC curves, we measured the PSII maximum quantum yield in the hydrated state $(F_v/F_m)_h$ and after some degree of dehydration $(F_v/F_m)_d$, with a modulated fluorometer (FMS 2, Hansatech Instruments, UK). Each species was evaluated during 48 h, in a 24 h cycle of desiccation and 24 h cycle of rehydration. The weight and the maximum quantum yield of PSII were monitored every 30 min until fronds reached a constant weight. It was possible to

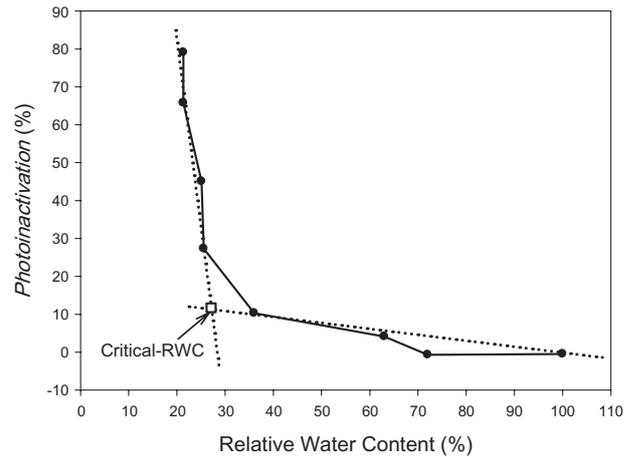


Fig. 1 Example of the PhI -RWC curve constructed to find the critical RWC threshold for *Hymenophyllum caudiculatum*, as an indirect measurement of the desiccation tolerance. The critical RWC threshold was calculated as the intersection of a line fitted visually at the level at which the decrease in PhI begins to stabilize and of a line fitted visually to the rising phase of PhI (open square).

measure two species per week, in total the experiment was achieved during 6 weeks. Photoinactivation was calculated as $PhI = 1 - [(F_v/F_m)_d / (F_v/F_m)_h]$.

Data analysis

Given the low number of replicates for the forest structure parameters (five plots per forest successional status), we used a non-parametric analysis (Mann-Whitney U test) to test differences in basal area, maximum tree height, DSF, LAI, and diversity (H') between the secondary and old-growth forest stand plots. To test differences between the microenvironmental parameters (VPD, RH, and PAR for daylight hours) of the two successional statuses we used a repeated measures one-way ANOVA. We used two-way ANOVAs to test the effects of forest successional status (old-growth versus secondary forest), filmy fern species, height in the host trees, and their bivariate combinations on filmy fern abundance. To test interspecific differences in the desiccation tolerance (critical RWC) we used one-way ANOVA. For all these parametric analyses, the distribution of the dependent variables (microenvironmental parameters, filmy fern species abundances, and desiccation tolerance data, respectively) were normalized by log 10 transformation before each analysis.

Results

Forest structural parameters differed significantly between the secondary forest and the old-growth forest

Table 2 Mean values (\pm SE) of forest structural parameters, microenvironmental characteristics, Hymenophyllaceae species richness, and diversity of the secondary and old-growth forest stands

Variable	Secondary forest ($n = 5$ plots)	Old-growth forest ($n = 5$ plot)
Basal area (m^2/ha)***†	12.40 \pm 0.98	45.10 \pm 0.92
Maximum tree height (m)***†	14.44 \pm 0.81	25.83 \pm 0.90
Direct light radiation (DSF)***†	0.15 \pm 0.01	0.06 \pm 0.01
Leaf area index (m^2/m^2)***†	1.58 \pm 0.16	3.18 \pm 0.15
Vapor pressure deficit (kPa) ^{NS} ‡	0.18 \pm 0.01	0.19 \pm 0.01
Relative humidity (%) ^{NS} ‡	88.36 \pm 0.21	88.41 \pm 0.15
PAR radiation ($\mu\text{mol m}^2/\text{s}$)***†	131.68 \pm 14.96	244.69 \pm 12.22
Total filmy fern species number	11	12
Species diversity (H') ^{NS} †	1.62 \pm 0.10	1.63 \pm 0.14

*** $P < 0.001$, significant differences; NS, not significant.
† Mann-Whitney U test. ‡ Repeated measures one-way ANOVA.

(Table 2). Stand basal area, maximum tree height and LAI were significantly greater in the old-growth forest stand (Table 2). On average, there was significantly higher light availability at plot scale (DSF) in the secondary forest (Table 2). When we averaged VPD and RH over all heights for each plot, we found that average VPD and relative humidity did not differ between the two forest ages, but average light quantity was significantly higher in the secondary forest stands (Table 2). Forests of both successional statuses showed significant differences in average VPD and RH at different heights along the host trees' trunks (one way ANOVA, $P < 0.05$, and $P < 0.05$, respectively). While VPD increased along the vertical microenvironmental gradient, relative humidity decreased (Fig. 2a–d). The PAR radiation also showed a significant increase between heights in host trees in both forest types (one way ANOVA, $P < 0.05$, Fig. 2e,f).

We found a total of 12 filmy fern species in the selected areas of the study site (Table 1). All 12 of these species occurred in the old-growth forest, and only *Hymeophyllum cruentum* was not present in the secondary forest (Table 2). The total abundance of all filmy fern species did not differ between the forests of differing ages (Table 3). The abundance of each filmy fern species differed significantly, regardless of the forest stands' age (Table 3, Fig. 3a,b). Total filmy fern abundance differed significantly with height along the host trees' trunks (Table 3). There were significant interspecific differences in abundance between the forest stands of differing ages (Table 3, Fig. 3a,b). The statistical interaction between the effects of forest successional statuses and height along the host trees' trunks indicated that the vertical distribution of filmy fern abundance differed significantly between the old-growth forest and the secondary forest (Table 3, Fig. 3a,b). Addi-

Table 3 Effects of forest successional status (old-growth vs secondary forest), filmy fern species, and height in the host trees on the abundance of each filmy fern species (% cover per m^2)

Source	Degrees of freedom	SS	F	P
Forest age (F)	1	0.524	3.556	0.064
Species	11	4.887	3.015	0.003
Height in host trunk (H)	6	9.675	10.944	< 0.001
$F \times$ species	11	4.415	2.724	0.006
$F \times$ height	6	1.984	2.244	0.049
Species \times H	66	22.312	2.294	< 0.001

SS, sum of squares; $n = 168$; squared multiple r of two-way ANOVA model = 0.82; $P < 0.01$.

tionally, there were significant interspecific differences in abundances among heights along the host trees' trunks (Table 3, Fig. 3a,b). Total filmy fern species abundance did not differ between tree host species in the secondary forest ($F_{1,122} = 1.30$, $P = 0.256$), nor did it differ between tree host species in the old-growth forest ($F_{1,191} = 0.02$, $P = 0.874$). Average species diversities were almost the same in both forest successional statuses (Table 2). In both forest stands, species richness and diversity were markedly higher in microhabitats with higher relative humidity and lower VPD (Fig. 4a,b,d,e). Light quantity (PAR radiation) showed a marked negative relationship with species richness only between < 1.0 m and 4.5 m in both forest ages; the number of filmy fern species was greater in the microhabitats of lower light availability (Fig. 4c). Additionally, in both successional statuses, filmy fern species diversity showed a marked decrease in relationship to greater light availability along their vertical distribution, (Fig. 4f).

The diverse filmy fern species showed significant interspecific differences in critical RWC ($F_{10,72} = 5.762$, $P < 0.001$; Fig. 5). Some of the species associated with less humid microhabitats (e.g. *H. plicatum*, *H. cuneatum*) showed significantly greater desiccation tolerance (i.e. lower critical RWC) than those species associated with more humid microhabitats (e.g. *H. caudiculatum*, *H. pectinatum*) (Tukey test, $P < 0.05$; Fig. 5). We observed that the best recovery after 60 min being rehydrated was presented in *H. tortuosum*, *H. dentatum*, *H. caudiculatum*, and *H. cruentum*, but only minor recovery was presented by *H. plicatum* and *S. caespitosa*. The minor recovery of *H. plicatum* agrees with the lower value of the critical RWC and the lower time to reach the critical RWC (Table 4).

Discussion

Our results indicate that in this forest there is an environmental variation that potentially determines

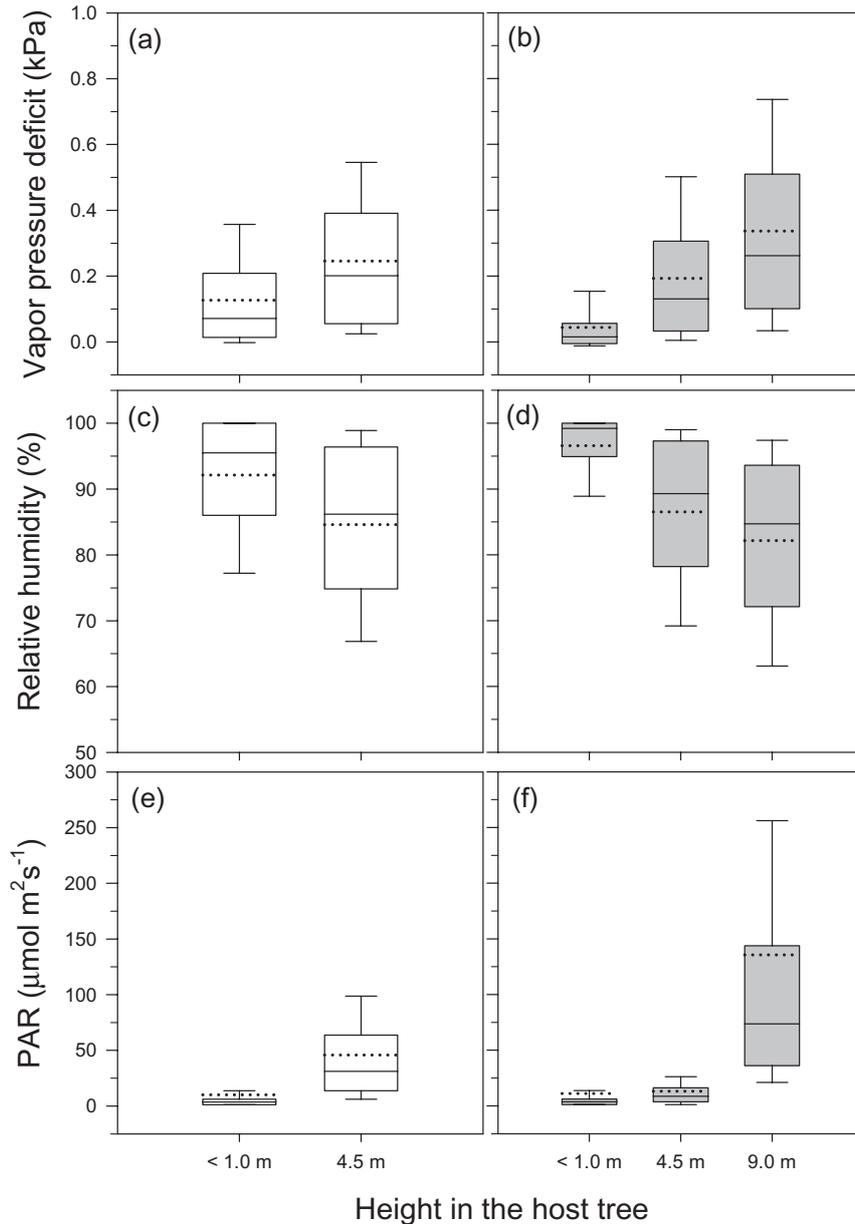


Fig. 2 Mean (dotted line), median (solid line), 10th, 25th, 75th and 90th percentiles of (a, b) vapor pressure deficit distribution, (c, d) relative humidity distribution, and (e, f) light availability distribution at different heights along the host-trees' trunks in the secondary forest (white boxes) and old-growth forest stands (grey boxes).

differences in vertical distribution as well as composition and abundance of the Hymenophyllaceae assemblage (Table 2). We acknowledge that a more realistic description of the forest successional statuses would have required dendrochronological dating of the trees of larger diameters and heights in each forest. However, the assessed forest structural parameters, which included the physical arrangement and characteristics of the forest (Stone & Porter 1998), reflected the highly visible differences between the forest stands of different successional statuses (Table 2). The differences in the filmy ferns' vertical distribution in the two forests of different successional statuses are clearly reflected by the

fact that in the old-growth forest, the average maximum height at which the Hymenophyllaceae species is distributed is significantly greater than that in the secondary forest (Table 2, Fig. 2). In both types of forest the abundance pattern reflects three habitat preferences of filmy ferns: (i) those species which are distributed mainly at the trunk base, for example *Hymenoglossum cruentum*, *Hymenophyllum caudiculatum* var. *productum*, *H. pectinatum*; (ii) species distributed up to a medium height, between 4 and 4.5 m., for example *H. peltatum*, *H. dicranotrichum*; and (iii) species distributed up to the greatest heights found (9 m), for example *H. dentatum*, *H. tortuosum* (Fig. 1).

Fig. 3 Vertical Hymenophyllaceae species distribution along the host-trees' trunks in (a) the old-growth forest and (b) secondary forest stands. Codes represent abbreviated names of filmy fern species (see Table 1). ■, HCAU; ■, HCRU; ■, HCUN; ■, HDEN; ■, HDIC; ■, HKRA; ■, HPEC; ■, HPEL; ■, HPLI; ■, HSEC; ■, HTOR; ■, SCAE.

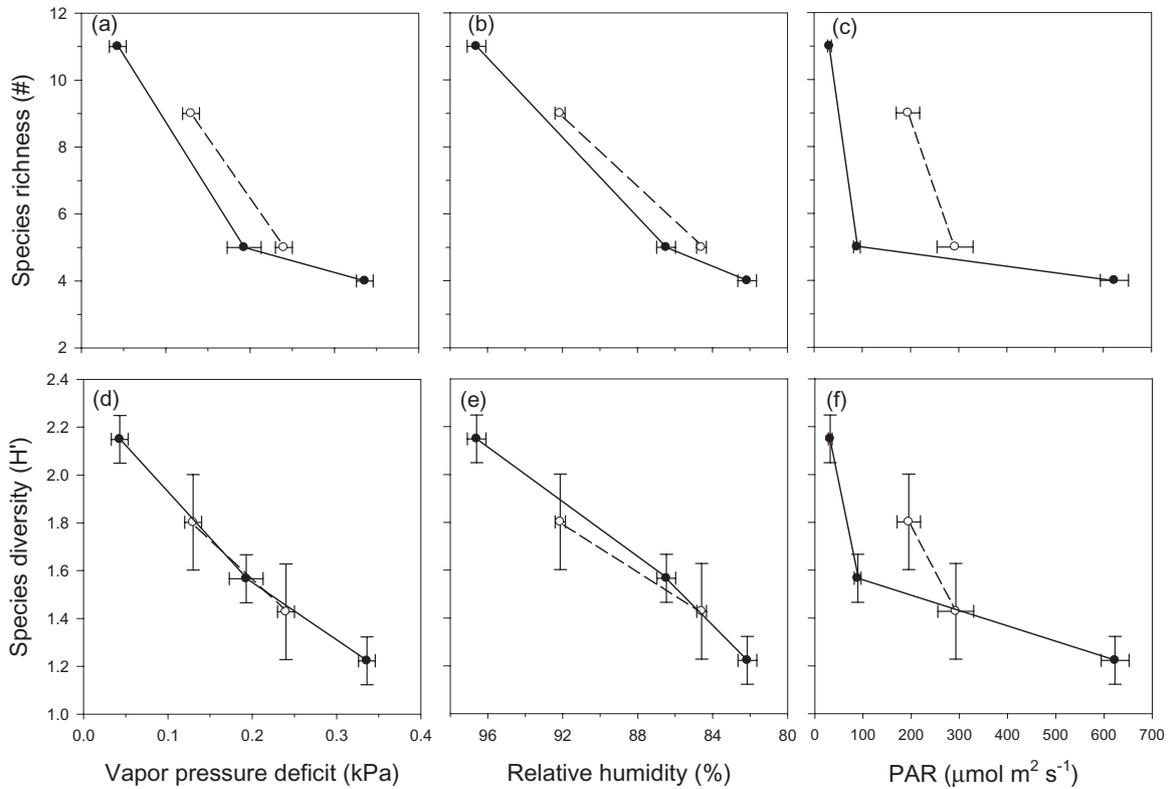
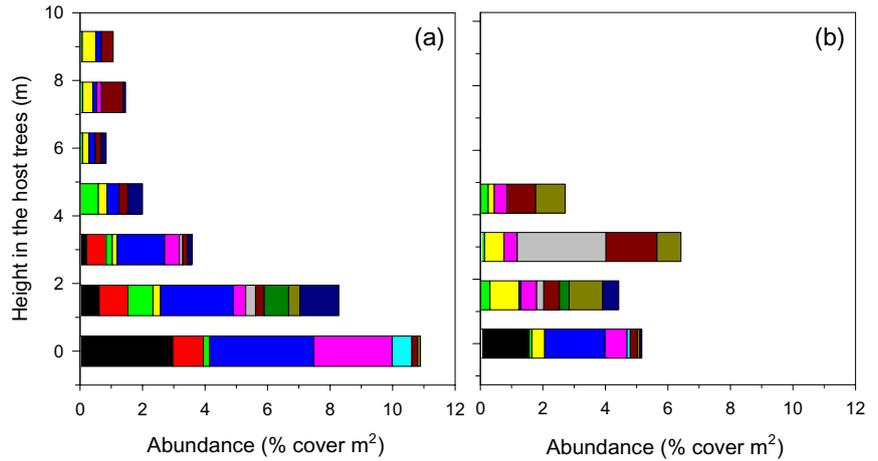


Fig. 4 Scatterplots for Hymenophyllaceae species richness and microenvironmental parameters: means (\pm SE) of (a) vapor pressure deficit, (b) relative humidity, and (c) light availability at three heights along the host-trees' trunk. Scatterplots for Hymenophyllaceae species diversity means (\pm SE) and environmental parameters: means (\pm SE) of (d) vapor pressure deficit, (e) relative humidity, and (f) light availability at three heights along the host-trees' trunk. Old-growth forest, closed circles; secondary forest, open circles. Symbols from left to right represent microenvironmental sensors placed at heights of 1 m and 4.5 m respectively, in the secondary forest stands; and sensors placed at heights of 1 m, 4.5 m, and 9 m respectively, in the old-growth forest stands. ●, old-growth forest; ○, secondary forest.

The functional mechanism underlying this distributional pattern could be the fact that filmy fern species tend to display differing degrees of drought tolerance (Proctor 2003, Proctor 2012), which would allow certain species to

occur in the upper part of the forest canopy (Zotz & Buche 2000). We found that desiccation tolerance was higher in three of the filmy fern species that persisted in exposed microhabitats (*H. cuneatum*, *H. plicatum*, and *S. caespitosa*),

and relatively low in three of the species that exhibited a vertical distribution restricted to shady or moist microsites (*H. caudiculatum*, *H. cruentum*, and *H. pectinatum*) (Fig. 5). For filmy fern species that showed the former distribution pattern, the ability to tolerate desiccation during periods with less rainfall or lower RH has been reported as a relevant underlying mechanism (Proctor 2003; Proctor 2012). Filmy fern species can tolerate drought stress and desiccation and are able to recover from desiccation at very low water potentials (Alpert 2000). Mechanisms such as recovery of water content, quantum efficiency (Fv/Fm), photosynthetic pigment and chloroplast ultrastructure, as well as prevention of damage to the plasma membrane have been related to the

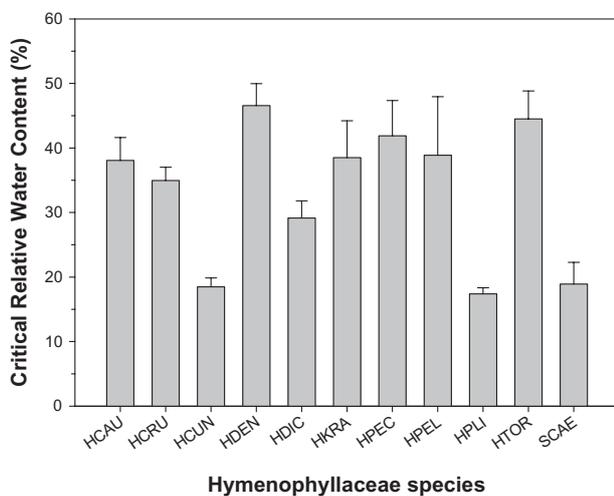


Fig. 5 Average critical relative water content (\pm SE) of 11 filmy fern species from a Chilean temperate rain forest. Codes represent abbreviated names of fern species (see Table 1).

above desiccation tolerance (Sherwin & Farrant 1996). Surprisingly, one of the species found to cover the broadest vertical microenvironmental range (*H. dentatum*) showed a significantly low desiccation tolerance (high critical RWC) (Fig. 5). Proctor (2012) pointed out that trade-offs between light adaptation and desiccation tolerance, as well as those between stem conduction and water absorption through the leaf surface underlie adaptive radiation and niche differentiation of species within the family. In the case of *H. dentatum*, Parra *et al.* (2009) reported that on average this species showed a low rate of evapotranspiration along its vertical distribution. This fact suggests that *H. dentatum* is able to occur across a wide vertical range, being less susceptible to desiccation, and likely displaying a functional strategy different than desiccation-tolerance to cope with drought stress.

Interspecific differences in abundance indicate that almost all species were present in stands of both ages (Fig. 3), while some species were clearly dominant in the old forest, and others were more abundant in the secondary forest (Fig. 3). The most notorious difference was the great dominance of *Hymenophyllum dicranotrichum* in the old stands, and the comparative dominance of *H. dentatum* in the young stands (Fig. 2). The evaluation of the forest structural descriptors together with ecological data on the abundance and vertical distribution of epiphytic Hyemnophyllaceae species in the two successional statuses of this evergreen temperate rainforest, suggests that variations in the microenvironmental factors that change with forest age (humidity and light availability) can determine the pattern of both richness and abundance of the species found. Several previous studies have reported that filmy fern distribution is mainly linked to water availability (Dickinson *et al.* 1993; Andrade & Nobel 1997; Muñoz *et al.* 2003; Parra *et al.* 2009) and, to a lesser

Table 4 Critical RWC with time taken to reach the threshold (\pm SE) and maximum quantum yield of PSII (Fv/Fm) (\pm SE) in the hydrated state (after 12 h submerged in distilled water), desiccated state (until the frond reached a constant weight without being sprinkled with distilled water), rehydrated state (after 60 min being sprinkled with distilled water and after being subjected to a desiccation period), and the recovery in the percentage of Fv/Fm reached after 60 min of rehydration related to the hydrated state

Species	Time to reach critical	Maximum quantum yield of PSII (Fv/Fm)			
	RWC (min)	Hydrated	Desiccated	Rehydrated	Recovery (%)
<i>H. caudiculatum</i>	109.1 \pm 15.4	0.794 \pm 0.007	0.216 \pm 0.028	0.712 \pm 0.013	89.719 \pm 1.273
<i>H. cruentum</i>	300.5 \pm 23.3	0.807 \pm 0.005	0.462 \pm 0.029	0.710 \pm 0.009	87.902 \pm 0.653
<i>H. cuneatum</i>	129.9 \pm 8.4	0.813 \pm 0.005	0.318 \pm 0.027	0.628 \pm 0.011	77.276 \pm 1.450
<i>H. dentatum</i>	103.5 \pm 26.4	0.788 \pm 0.007	0.229 \pm 0.013	0.716 \pm 0.017	90.970 \pm 2.565
<i>H. dicranotrichum</i>	62.2 \pm 5.2	0.778 \pm 0.006	0.344 \pm 0.031	0.563 \pm 0.047	72.255 \pm 5.884
<i>H. krauseanum</i>	53.4 \pm 5.1	0.811 \pm 0.005	0.206 \pm 0.024	0.647 \pm 0.032	79.652 \pm 3.627
<i>H. pectinatum</i>	83.3 \pm 13.6	0.792 \pm 0.003	0.285 \pm 0.023	0.623 \pm 0.045	78.775 \pm 5.820
<i>H. peltatum</i>	61.9 \pm 11.8	0.781 \pm 0.015	0.364 \pm 0.030	0.576 \pm 0.044	74.151 \pm 6.428
<i>H. plicatum</i>	36.2 \pm 6.6	0.777 \pm 0.022	0.429 \pm 0.023	0.515 \pm 0.031	66.609 \pm 4.518
<i>H. tortuosum</i>	67.2 \pm 11.6	0.819 \pm 0.011	0.385 \pm 0.015	0.769 \pm 0.010	94.031 \pm 1.386
<i>S. caespitosa</i>	47.7 \pm 7.3	0.825 \pm 0.008	0.318 \pm 0.073	0.550 \pm 0.092	66.371 \pm 11.062

extent, to light availability (Parra *et al.* 2009). However, it has also been discussed that a more detailed evaluation of the filmy ferns vertical distribution drivers would require the measurement of other factors, such as the nutritional quality of the host tree's bark substrate (Nadkarni 1984; Parra *et al.* 2009). Additionally, in this study we found that the total filmy fern species abundance did not differ between host-tree species, contrary to the findings of Parra *et al.* (2009) from a second-growth forest stand, who found that the abundance of filmy fern species was significantly greater in the host-tree species *D. winteri* than in the other host species. However, in this study we measured the filmy fern abundance on dominant tree species in each successional state, which would account for the bulk of the forest stands' biomass, with the result that the host species *D. winteri* was not dominant in the secondary forest plots, nor did it show a significant effect on filmy fern abundance on the old-growth forest.

On the other hand, the filmy fern species reached greater heights along the host trees' trunks in the old-growth forest than in the younger one, which could be related to the differences in the forest structure parameters and their concomitant effects on the microenvironmental conditions. This pattern must be associated with the fact that Hymenophyllaceae species were found to be more abundant in the old-growth forest, even though the species richness differed by only one species in the two successional statuses (Table 2, Fig. 3). Both species richness and diversity of filmy fern species showed a clear and positive relationship with the humidity of the microsite and decreased abruptly with the increase of solar radiation, independent of the forest successional status. This pattern coincides with results obtained by Parra *et al.* (2009), who in a vertical gradient lower than that of the present study (up to a height of only 1.8 m along the trunk) found that most of the variation in abundance and composition of filmy ferns was explained by the difference in the air RH. In this context, previous studies have shown that filmy fern species restricted to the lower part of the trunk, where the humidity is usually high, can decrease leaf-to-air vapor pressure, promoting a positive carbon balance (Martin & Siedow 1981; Callaway *et al.* 2002; Zotz 2007) and tolerating a higher VPD. Additionally, the filmy fern species that persisted in the upper part of host-tree trunks in forest stands of both ages (*e.g.* *H. dentatum*) showed lower evapotranspiration and less water loss (Parra *et al.* 2009).

This study presents a more rigorous investigation of the pattern of vertical distribution of the filmy fern species in a temperate rainforest, considering the successional status of the forest as a driver of potential environmental variation, the specific desiccation tolerance responses, and a more extensive vertical gradient. We found that in the vertical microenvironmental gradient

constituted by the host tree, Hymenophyllaceae species differ in habitat preferences and desiccation tolerance. This could reflect their coexistence along the stem and their vertical distribution. We found that the total filmy fern abundance and richness in a temperate rainforest seems to be independent of the forest successional status and the host-tree's identity. Further research on the vertical distribution of filmy fern species in the temperate rainforest should also include the persistence of specific colonization rates (*e.g.* via local recruitment, once it has established), or determine to what extent the recruitment and abundance of filmy fern sporophytes depend on the presence and biological success of gametophytes. This is particularly intriguing, given that it is known that some filmy fern species can survive out of their primary habitat as gametophytes without ever producing sporophytes (Farrar 1990). Finally, future studies could test whether the slightly higher diversity in the old-growth forest was simply the product of a longer history, which allowed for the accumulation of more individuals and species.

Acknowledgments

We are grateful to Karina Acuña and Carla Alvear for their help in the field sampling. This research was funded by FONDECYT project 1090397.

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