

# Physiological and Phenotypic Plasticity of Ferns in a Tropical Dry Forest in Mexico



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## Abstract

The study of the causes and expressions of physiological and phenotypic plasticity in plants is crucial to understanding the responses of plants to environmental heterogeneity, which may reflect central aspects of plant strategies. In this work, we analyze whether closely related species distributed in the same environment present similar strategies when faced with changes in light intensity. We evaluated the physiological and phenotypic plasticity in two species of ferns under different light intensities and constant irrigation. We assessed physiological and morphological characters and evaluated the index of plasticity (RPDI). We found significant differences in RDPI between the two species in the physiological traits, particularly in the high light intensity treatments, and for the morphological variables, the RDPI of the leaf area and the trichome area. The physiology of *Notholaena aschenborniana* acclimates more quickly to high amounts of light than that of *Cheilanthes cucullans* but is less plastic in the long term. *C. cucullans* presents a higher phenotypic plasticity at the morphological level. We found that both fern species have the capacity to adapt to contrasting light environment conditions by adjusting their physiology and morphology in a short period of time using different strategies.

**Keywords:** Chlorophyll Concentration; ETR; Morphological; NPQ; RDPI; Yield

**Abbreviations:** TDF: Tropical dry forests; NPQ: Non-Photochemical Quenching; ETR: Electron Transport Rates; RDPI: Relative Phenotypic Distance Index; LA: Leaf Area; LL: Leaf Length; LW: Leaf Width; ANOVA: Analysis of Variance Environmental Agreements;

## Introduction

As sessile organisms, plants confront high environmental heterogeneity, requiring them to adapt to changing environmental conditions [1-3]. Physiological and phenotypic plasticity allows individuals to confront this environmental heterogeneity by modifying their physiology and structure morphoanatomy to facilitate survival, establishment, and reproduction in a particular habitat, conferring advantages over less plastic individuals [2-6]. Plants can be plastic using resources such as light, water, and nutrients through various morphological traits. Examples include leaf area modification [7-11], development of indumenta such as trichomes, scales, and waxes [12-14], and even modification of the photosynthetic apparatus [11, 15,16].

Plant-environment dynamics have taken on great relevance since measuring phenotypic and physiological plasticity helps to understand how plants adapt functionally to seasonally changing environments [5,11, 16,17]. In ecosystems with an apparent alternation between wet and dry seasons, the phenotypic characteristics of plants are strongly affected by the change in seasons [16, 18]. Tropical dry forests (TDF) in Mexico have cyclical precipitation regimes and a marked dry season [18]; rains are highly seasonal, with five to eight months of drought and only four months of rain per year [18, 19]. The structure of the forest is strongly determined by the dry season, which can trigger the loss of leaves in the tree canopy and, therefore, the availability of light

[18, 20]. The seasonal patterns of TDF plants are pronounced but can be irregular, as they depend directly on microenvironmental conditions [21,22]. In this context, plasticity can play a primary role in plant survival in these heterogeneous environments, as high plasticity allows them to confront the physiological challenges of fluctuations in light intensity and water stress conditions [11,23].

Ferns dominate the forest understory and contribute to the dynamics of these ecosystems [24,25], representing 4.4% of the vascular flora of Mexico [26]. In addition, ferns are considered to be sensitive to environmental changes, such that from a climate change perspective, they can show the capacity for survival in a particular environment [27,28]. There are increasing studies on fern physiology [15, 27-33], however, there is an information gap about the physiological and morphological strategies of fern species that are able to acclimate to highly seasonal environments like TDF. We therefore hypothesize that environmental factors, such as light availability, can induce changes in species fern physiology and morphology. In particular, light conditions in the TDF are highly variable within and between seasons due to changes in the structure of the forest canopy [34]. Therefore, we expect fern species to exhibit high physiological and phenotypic plasticity to different light conditions. We tested this hypothesis by evaluating physiological and phenotypic plasticity under different controlled light intensities in terms of effective chlorophyll efficiency (Yield), Non-Photochemical Quenching (NPQ), Electron Transport Rates (ETR), and chlorophyll concentration, in addition to the Relative Phenotypic Distance Index (RPDI) in two terrestrial fern species of the TDF with similar characteristics.

## Materials and Methods

### Study Species

These two species were chosen because they have evergreen leaves and are drought-tolerant. They also coexist in the same habitat, the Tropical Dry Forest, which alternates between wet and dry seasons [22]. The identification of species was carried out using keys and descriptions [35].

*Cheilanthes cucullans* Fée belongs to the family Pteridaceae. Presents a long-creeping, horizontal rhizome with linear-lanceolate scales, with a brownish-orange coloration and ciliate margins; leaf length is 15 to 40 cm, the petiole is chestnut to black in color, shiny, with brownish-orange filiform scales, the leaves are pinnate-pinnatifid to 3-pinnate, oblong-lanceolate, chartaceous, the underside of the blade is densely scaly and with brownish-orange catenate hairs, the rachis and ribs have dense filiform adpressed scales; the sori are covered by the segment margins; spores are trilete and brown in color. Its distribution extends from northern Mexico through Oaxaca. It grows particularly in rocky areas like cliffs and roadsides in TDF at altitudes between 500 and 2350 m [35].

*Notholaena aschenborniana* Klotzsch.- belongs to the family Pteridaceae. It presents a compact, horizontal rhizome with

linear-lanceolate scales 3 mm in length, margin ciliate, with fronds 21 to 28 cm long and 4 to 6 cm wide; stipe black terete, covered with brown scales with ciliate margin, 3 mm long; leaf blades lanceolate bipinnate to bipinnate-pinnatifid, indumentum of the abaxial surface composed of white farina and dense brown scales, linear, margin ciliate; adaxial surface composed of dense stellate hairs, rachis black terete, venation not conspicuous; sori submarginal; spores chestnut-orange in color. It is distributed from the southern United States in Arizona and Texas to central Mexico [35].

### Experimental Design

Sixty individuals of both *Notholaena aschenborniana* Klotzsch and *Cheilanthes cucullans* Fée were collected from natural habitats between September 2016 and January 2017 from a Tropical Dry Forest in the ejido of San Andres de la Cal, Tepoztlan, in the state of Morelos (18°57'22.2 N - 99°06'50.2 E, at an altitude of 1500 m asl). The climate is semi-warm subhumid [(A) Cw2 (w) ig], with a mean annual temperature > 18°C and mean annual precipitation between 800 and 1000 mm [36]. The 120 individuals were collected, transplanted, and maintained for three months in acclimation under homogeneous environmental conditions in a nursery garden. After acclimation, 15 individuals of *N. aschenborniana* and 15 individuals of *C. cucullans* were assigned to each of four light treatments in a random design, which varied in photosynthesis photon flux density (PPFD) —low (30%), medium (40%), high (60%), and fully exposed (100%)— covering a range of light variation in natural populations of the TDF. The PPFD treatments were achieved using different grades of shade mesh. The plants were watered constantly through a drip irrigation system twice a day for six months. In each light treatment, a sensor to record PPFD (S-LIA-M003) and one to record relative humidity and air temperature (S-THB-002) were installed and connected to a data logger (HOBO H21-002 microstation). The sensors recorded light and humidity data for 10 sec once every 30 min during the experiment.

### Physiological Measurements

For each plant under light treatments, the chlorophyll fluorescence (Yield), Electron Transport Rate (ETR), and Non-Photochemical Quenching (NPQ) were measured with a portable actinic light fluorometer with a clip that holds the optical fiber (MINI-PAM II-Walz - Germany) in a diurnal cycle at 09:00, 12:00 and 15:00 h. We used predawn (6:00h) maximum fluorescence (Fm) to calculate NPQ. All calculations were performed as suggested by [37]. The values for ETR and NPQ were registered to obtain values at the end of the sampling period when plants had already presented pronounced changes in response to the light treatments. Chlorophyll concentration (Chl) was measured with a sensor (MC 100 - Apogee) at 06:00, 12:00 and 18:00 h. All measurements were taken from mature expanded leaves from five individuals of each species from whom the physiological measurements were taken

every 15 days from February to June 2017. It is worth noting that the fluorescence parameter  $F_v/F_m$  was not included because we obtained inconsistent measurements.

**Morphological Measurements**

To determine the effect of light on the morphological changes of the leaves, the leaf area (LA), leaf length (LL) and leaf width (LW) of a total of 104 expanded leaves (10 to 14 leaves per species per treatment) were calculated by scanning each leaf with a CI-202 Portable Leaf Area Meter. Moreover, the cuticle thickness (CT) in a 3 cm<sup>2</sup> section of each leaf at the apical, middle, and basal region was measured using a digital caliper (CALDI-6MP). In addition, for each section, we counted the trichome number and measured the scale length (TL, SL, respectively) of the leaf’s abaxial and adaxial sides for each individual in the different PPFd treatments. The density of trichomes (TD) and scales per cm<sup>2</sup> were summed and multiplied by the leaf area to estimate the number of trichomes and scales per leaf area. To quantify the density and size of the trichomes and scales, we took photographs with a Nikon stereoscopic microscope at 10x with a 1 mm scale and later measured them using the program ImageJ.

**Physiological Plasticity Index**

The index of plasticity was calculated based on the relative phenotypic distances (RPDI), which range from 0 (no plasticity) to 1 (high plasticity; 23). The index defined as:  $RPDI = \frac{\sum(d_{ij} \rightarrow i'j')}{(\sum(x_{i'j'} + x_{ij}))}/n$ . Where  $(d_{ij} \rightarrow i'j')/(x_{i'j'} + x_{ij})$ , is the relative distance defined by all pairs of individual leaves exposed to the different light treatments, and n is the number of repetitions [23,16]. We calculated the RPDI for the physiological variables (Yield, ETR, NPQ, and chlorophyll concentration) and the morphological variables (leaf area, trichome density and size, and scale size) of the two species of ferns under the different PPFd treatments. For the evaluation of the physiological and morphological RPDI,

the data from the last month of measurement (July) were used to obtain the most pronounced changes and from the measurement of the highest light intensity (12:00 pm).

**Statistical Analysis**

The study was realized in a completely random design with three repetitions, four light treatments, and two fern species. The interactive effects between the physiological variables were evaluated using a factorial analysis of variance (ANOVA), and significant ANOVAs were followed by Student’s t-tests to determine pairwise differences among groups. All of the statistical analyses were carried out in RStudio [38]. To analyze the variance of physiological responses for both species and response changes under different light intensities for each species, a Principal Component Analysis (PCA) for quantitative characters (Yield, ETR, NPQ, Chl, LA, CT, TD, and TL) was conducted in R Stats Package [38].

**Results**

The maximum photon flux density (PPFD) measured by the data loggers for each of the light intensity treatments was 32.87 mol m<sup>-2</sup> d<sup>-1</sup> for the 30% treatment, 40.30 mol m<sup>-2</sup> d<sup>-1</sup> for the 40% treatment, 57.06 mol m<sup>-2</sup> d<sup>-1</sup> for the 60% treatment, and 67.24 mol m<sup>-2</sup> d<sup>-1</sup> for the 100% light treatment.

**Physiological and Morphological Responses**

Only the physiological variables ETR and Chl showed significant differences among species-light treatment interaction ( $F_{(3,1442)} = 10.36$ ;  $P < 0.05$ , and  $F_{(3,592767)} = 59.95$ ;  $P < 0.05$ , respectively); while that Yield and NPQ were non-significant ( $F_{(3,0.016)} = 0.53$ ;  $P > 0.05$ , and  $F_{(6,7.1)} = 1.74$ ;  $P > 0.05$ , respectively) (Table 1). When comparing light treatments or species, most physiological or morphological variables showed significant differences, except NPQ comparisons between species, which did not show significant differences (Table 1).

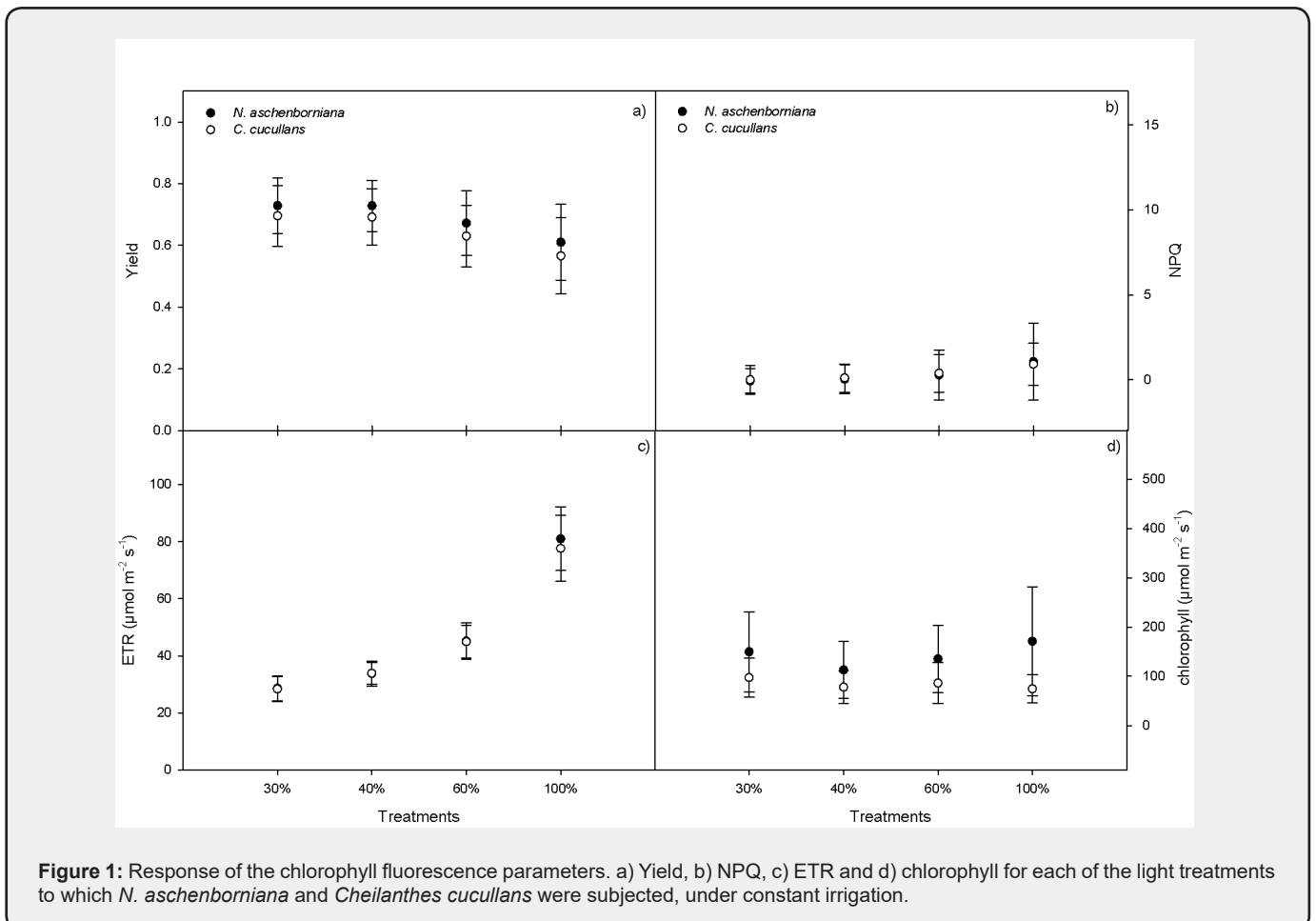
**Table 1:** ANOVA table indicating significant differences of species and physiological responses. The number of asterisks shows the degree of significance (\*\* - 0.001, \* - 0.01, \* - 0.05).

Effect	Physiological variable	Df	F	P
Specie	Yield	1	144.09	0.00***
	NPQ	1	0.86	0.35
	NPQ	1	11.52	0.00***
	Chl	1	1224.68	0.00***
Time	Yield	2	23.04	0.00***
	NPQ	2	0.15	0.85
	ETR	2	7.99	0.00***
	Chl	2	6.33	0.00**
Light treatment	Yield	3	349.77	0.00***
	NPQ	3	114.77	0.00***
	ETR	3	8969.25	0.00***
	Chl	3	47.6	0.00***

Specie:Time	Yield	2	3.52	0.02*
	NPQ	2	0.92	0.39
	ETR	2	0.49	0.62
	Clh	2	3.16	0.04*
Specie:Light treatment	Yield	3	0.53	0.65
	NPQ	3	1.74	0.15
	ETR	3	10.36	0.00***
	Clh	3	59.95	0.00***
Time: Light treatment	Yield	6	12.21	0.00***
	NPQ	6	0.14	0.99
	ETR	6	1.92	0.07
	Clh	6	0.54	0.77
Specie: Time: Light treatment	Yield	6	0.69	0.65
	NPQ	6	1.01	0.41
	ETR	6	3.51	0.00**
	Clh	6	1.97	0.06

Low Yield values were found in high light intensity treatments (100%) in both *N. aschenborniana* ( $0.61 \pm 0.12$ ) and *C. cucullans* ( $0.56 \pm 0.12$ ). Highest values for this variable were found in 30%

and 40% light treatments, where *N. aschenborniana* showed a Yield of ( $0.72 \pm 0.09$ ) and *C. cucullans* ( $0.69 \pm 0.09$ ) (Figure 1a; Table S1).



**Figure 1:** Response of the chlorophyll fluorescence parameters. a) Yield, b) NPQ, c) ETR and d) chlorophyll for each of the light treatments to which *N. aschenborniana* and *Cheilanthes cucullans* were subjected, under constant irrigation.

**Table S1:** Means and standard deviations of physiological variables in *Notholaena aschenborniana* and *Cheilanthes cucullans* under four different light intensity treatments.

Variable	Specie	Light treatment			
		30%	40%	60%	100%
Yield	<i>N. aschenborniana</i>	0.72 ± 0.09	0.72 ± 0.08	0.67 ± 0.10	0.61 ± 0.12
	<i>C. cucullans</i>	0.69 ± 0.09	0.69 ± 0.09	0.63 ± 0.10	0.56 ± 0.12
NPQ	<i>N. aschenborniana</i>	-0.07 ± 0.71	0.02 ± 0.86	0.27 ± 1.46	1.06 ± 2.26
	<i>C. cucullans</i>	-0.005 ± 0.84	0.09 ± 0.82	0.38 ± 1.11	0.91 ± 1.23
ETR	<i>N. aschenborniana</i>	28.56 ± 4.34	33.74 ± 4.41	45.14 ± 6.32	81.01 ± 11.15
	<i>C. cucullans</i>	28.31 ± 4.30	33.86 ± 3.82	44.92 ± 5.73	77.64 ± 11.59
Chl	<i>N. aschenborniana</i>	149.85 ± 81.34	112.90 ± 57.92	135.22 ± 68.64	171.16 ± 110.51
	<i>C. cucullans</i>	97.43 ± 39.53	77.87 ± 33.42	86.37 ± 41.93	74.45 ± 28.77

Moreover, both species had the lowest NPQ values under 30% light treatment and the highest under full light (100%) (Figure 1b) (Table S1). Specifically, in the 30% light treatment, the lowest values were presented for both *N. aschenborniana* (-0.07±0.71) and for *C. cucullans* (-0.005±0.84) while the highest values were in the treatment of 100% light intensity, for both species *N. aschenborniana* (1.06±2.26) and *C. cucullans* (0.91±1.23) (Figure 1b) (Table S1).

The ETR of both species showed an increase with light treatment (Figure 1c), with significant differences in both species and light treatments (S1), even though the highest differences were observed under the 100% light treatment for *N. aschenborniana* (81.01 ± 11.15) in comparison of *C. cucullans* (77.64 ± 11.59) (Figure 1c) (Table S1).

*Notholaena aschenborniana* showed higher Chl values under higher light treatments (171.16 ± 110.51), while the lowest value was found under 40% light intensity (112.90 ± 57.92) (Figure 1d). In *C. cucullans*, the highest Chl average values were recorded in both 30% (97.43 ± 39.53) and 40 % (86.37 ± 41.93) light

treatments, while the lowest Chl values were observed in 100% light treatment. Comparatively, *N. aschenborniana* showed greater variation in Chl response (mean of all treatments) than *C. cucullans* (mean of all treatments) (Figure 1d) (Table S1).

Regarding morphological responses, most variables were significantly different between LA species (F(1,3649.6) = 12.15; P<0.05), LW (F(1,17.87) = 9.78; P<0.05), (F(1,0.33) = 276.24; P<0.05) TD (F(1, 456892861) = 47.18; P<0.05), except LL (F(1,27.64) = 0.80; P>0.05). The morphological responses among the light treatments that showed significant differences were LW (F(3,2.99) = 0.54; P>0.05), CT (F(3,0.01) = 3.43; P<0.05), TD (F(3, 407239880) = 14.01; P<0.05). Finally, when comparing the interaction between species and light treatment, LA (F(3,1696.5) = 1.88; P<0.05), CT (F(3,0.01) = 3.78; P<0.05), and TD (F(3, 358188738) = 12.32; P<0.05) showed significant differences (Table 2). In *N. aschenborniana* there were no significant differences in SL between light treatments (F(3, 155118933521) = 0.84; P>0.05), but SL showed significant variation (Table 2; Table S2).

**Table 2:** ANOVA table indicating significant differences of factors between levels for the morphological characters of *Notholaena aschenborniana* and *Cheilanthes cucullans*. The number of asterisks shows the degree of significance (\*\*\* - 0.001, \*\* - 0.01, \* - 0.05).

Physiological variable	Specie	Light treatment (%)	Df	P
Yield	<i>N.aschenborniana</i>	30	495	0.000
	<i>N.aschenborniana</i>	40	429	0.000
	<i>N.aschenborniana</i>	60	141	0.000
	<i>N.aschenborniana</i>	100	301	0.000
	<i>C.cucullans</i>	30	676	0.000
	<i>C.cucullans</i>	40	646	0.000
	<i>C.cucullans</i>	60	698	0.000
	<i>C.cucullans</i>	100	631	0.000

NPQ	<i>N.aschenborniana</i>	30	406	0.012
	<i>N.aschenborniana</i>	40	387	0.180
	<i>N.aschenborniana</i>	60	126	0.024
	<i>N.aschenborniana</i>	100	256	0.004
	<i>C.cucullans</i>	30	547	0.849
	<i>C.cucullans</i>	40	551	0.004
	<i>C.cucullans</i>	60	604	0.004
	<i>C.cucullans</i>	100	469	0.004
ETR	<i>N.aschenborniana</i>	30	406	0.000
	<i>N.aschenborniana</i>	40	335	0.000
	<i>N.aschenborniana</i>	60	111	0.000
	<i>N.aschenborniana</i>	100	256	0.000
	<i>C.cucullans</i>	30	545	0.000
	<i>C.cucullans</i>	40	554	0.000
	<i>C.cucullans</i>	60	615	0.000
	<i>C.cucullans</i>	100	523	0.000
Chl	<i>N.aschenborniana</i>	30	652	0.000
	<i>N.aschenborniana</i>	40	484	0.000
	<i>N.aschenborniana</i>	60	413	0.000
	<i>N.aschenborniana</i>	100	391	0.000
	<i>C.cucullans</i>	30	813	0
	<i>C.cucullans</i>	40	788	0
	<i>C.cucullans</i>	60	839	0
	<i>C.cucullans</i>	100	806	0

**Table S2:** Means and standard deviations of morphological variables in *Notholaena aschenborniana* and *Cheilanthes cucullans* under four different light intensity treatments.

Variable	Specie	Light treatment			
		30%	40%	60%	100%
LA	<i>N. aschenborniana</i>	21.03±14.15	25.14±11.44	19.32±13.47	20.65±7.98
	<i>C. cucullans</i>	49.38±32.72	31.03±14.87	33.40±10.82	26.37±20.40
LW	<i>N. aschenborniana</i>	3.62±1.47	3.90±1.25	3.22±1.15	3.43±1.09
	<i>C. cucullans</i>	5.19±1.80	4.53±1.12	4.18±0.69	4.09±1.82
LL	<i>N. aschenborniana</i>	13.85±7.07	16.61±5.47	11.74±5.21	13.27±2.30
	<i>C. cucullans</i>	17.87±9.20	14.50±5.63	16.26±4.82	11.55±4.90
CT	<i>N. aschenborniana</i>	0.17±0.04	0.15±0.02	0.21±0.05	0.19±0.05
	<i>C. cucullans</i>	0.05±0.02	0.06±0.02	0.06±0.01	0.04±0.02
TD	<i>N. aschenborniana</i>	10392.38±4402.29	8261.54±1778.58	3397.54±1915.64	15053.25±52773
	<i>C. cucullans</i>	6671.26±3956.37	3893.64±1366.97	3767.86±1988.47	3653.51±1391.11
SD	<i>N. aschenborniana</i>	20122.82±16328.71	127076.4±261584.3	148588.5±418719.8	8829.77±4274.16

LA was higher in *C. cucullans* (49.38 ± 32.72) in 30% light treatment, while the lower leaf area was shown in *N. aschenborniana* (19.32 ± 13.47) in 60% light treatment. LW was wider in *C. cucullans* in the 30% light treatment (5.19 ± 1.80), while *N. aschenborniana* did

not present significant differences between light treatments (3.54 ± 1.24) (Table S2). The highest LW was recorded in *C. cucullans* in 30% light treatment (5.19 ± 1.80), while *N. aschenborniana* did not present significant differences in all light treatments (3.54

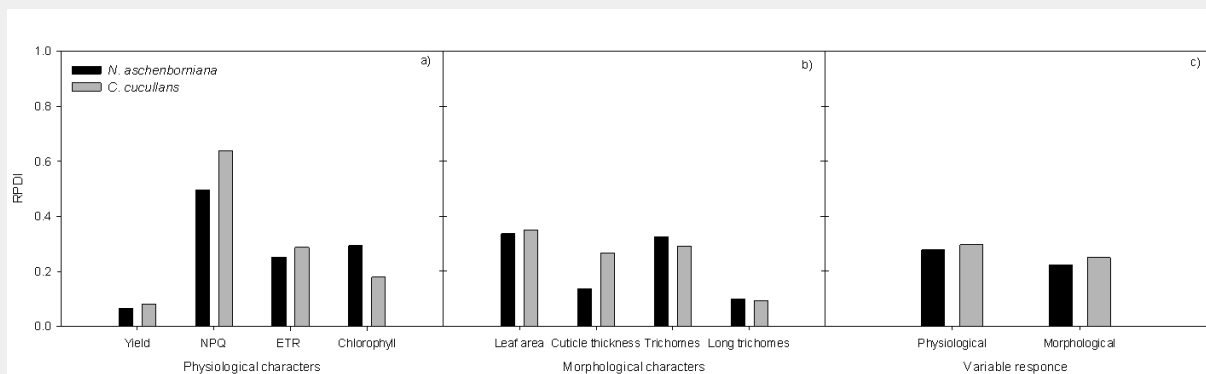
$\pm 1.24$ ). The highest LL occurred in *N. aschenborniana* ( $16.61 \pm 5.47$ ) in 40% light treatment, and the lowest LL was in 60% light treatment ( $11.74 \pm 5.21$ ), while the highest LL in *C. cucullans* occurred in 30% light treatment ( $17.87 \pm 9.20$ ), and the lowest LL in 100% light treatment ( $11.55 \pm 4.90$ ) (Table S2).

The CT was highest in *N. aschenborniana* in 60% light treatment ( $0.21 \pm 0.05$ ) and the lowest CT in 40% light treatment ( $0.15 \pm 0.02$ ), while *C. cucullans* presented greater CT in 40% light treatments ( $0.06 \pm 0.02$ ) and the lowest in 100% light treatment ( $0.04 \pm 0.02$ ) (Table S2). In *N. aschenborniana*, the highest amount of TD occurred in 30% light treatments ( $10392.38 \pm 4402.29$ ) and 100% light treatment ( $15053.25 \pm 5277.03$ ), while the lowest values were shown in 60% light treatment ( $3397.54 \pm 1915.64$ ). For *C. cucullans*, the largest amount occurred in 30% light treatment ( $6671.26 \pm 3956.37$ ), while 40%, 60% and 100% treatments showed little variation. Finally, SD in *N. aschenborniana* also presented scales on the abaxial side of the leaf, where the highest

amount was found in 60% light treatment ( $148588.5 \pm 418719.8$ ) and the lowest values in 100% light treatment ( $8829.77 \pm 4274.16$ ) (Table S2).

### Index of Plasticity of the Chlorophyll Fluorescence and Morphological Parameters

The RPDI values of the two fern species differed among the variables. The lowest RDPI values were for Yield (*N. aschenborniana*: 0.06, *C. cucullans*: 0.08) (Figure 2a). The NPQ was the most plastic variable; *N. aschenborniana* had an RPDI value of 0.49, while *C. cucullans* had a value of 0.63 (Figure 2a). The RPDI values for the ETR were similar between species (*N. aschenborniana*: 0.25, *C. cucullans*: 0.28). The chlorophyll concentration also had low plasticity values for both species (*N. aschenborniana*: 0.29, *C. cucullans*: 0.17) (Figure 2a). The average RPDI values among all physiological traits were similar between the species (*N. aschenborniana*: 0.27, *C. cucullans*: 0.29) (Figure 2c).



**Figure 2:** Relative plasticity difference index (RDPI) for physiological (a), morphological (b) and general response of variables (c) of *Cheilanthes cucullans* and *Notholaena aschenborniana*.

Each species showed plasticity for different morphological variables. Both species had high RDPI values for leaf area (*N. aschenborniana*: 0.34, *C. cucullans*: 0.35). Meanwhile, *N. aschenborniana* had lower leaf thickness plasticity (RDPI=0.14) compared to *C. cucullans* (RDPI= 0.27; Figure 2b). The number of trichomes per leaf area of *N. aschenborniana* presented a plasticity value of 0.32 in comparison with *C. cucullans* with a value of 0.29. Trichome length showed very low plasticity in both species (RDPI=0.09 for both *N. aschenborniana* and *C. cucullans*). With respect to the scales, *N. aschenborniana* had low plasticity for scale length (0.13). Still, the number of scales per leaf area presented a high RDPI (0.43), showing a change in the indumentum of this species under different amounts of light (Figure 2b). The average RDPI value for both the physiological and morphological characters showed low plasticity since, in both species, the values ranged from 0.24 for *N. aschenborniana* to 0.25 in *C. cucullans* (Figure 2c).

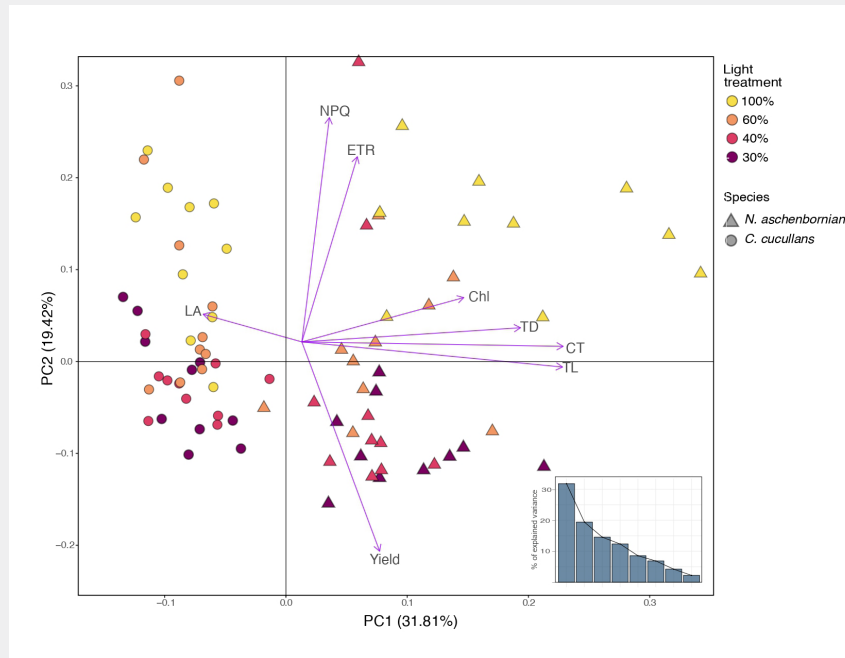
In the PCA, the first two principal components (PC) explained 51.23% of the variance (Figure 3). PC1 explains differences between species, mainly due to CT, TL, TD, and LA variance. PC2 explains the different responses to light treatments, mainly due to the loadings of the physiological variables NPQ, Yield, and ETR (Figure 3), showing similar responses in both species.

### Discussion

In this study, we demonstrated the ability of two terrestrial fern species to adapt to contrasting environmental light conditions by adjusting their physiology and morphology in a short time. The ecophysiological response of different species of ferns to changes in light intensity indicates differential responses [27,28,30,39]. In this sense, the two terrestrial fern species showed similar responses of effective chlorophyll efficiency (Yield) under different light treatments. The low Yield of *N. aschenborniana* (0.61) and *C. cucullans* (0.56) in 100% light treatment could indicate that plants were stressed at higher light intensities [37, 40], reducing the

quantum yield of photosynthesis caused by exposure to radiation excess [41]. This reveals the direct effect of light treatments on leaf photochemistry, especially in the environmental conditions in which these species are found, such as the dry tropical forest. It

has been mentioned that in three species of tree ferns the Yield decreased during a leaf desiccation experiment [30]. Therefore, the decrease in Yield of both species at high light intensities can negatively affect photosynthetic processes.



**Figure 3:** PCA for physiological and morphological variables of *Notholaena aschenborniana* and *Cheilanthes cucullans* under different light treatments.

The non-photochemical quenching (NPQ) in *N. aschenborniana* and *C. cucullans* was similar, although *N. aschenborniana* showed a slight increase under 100% light treatment. Higher NPQ values in plants facilitate colonization of different habitats since photoprotective processes prevent photoinactivation, decreasing excess excitation of the PSII reaction center to avoid irreversible damage in plants [41, 42]. Moreover, high NPQ values are generally found in plants that inhabit areas with high light levels (sun plants), as those that inhabit the TDF [43]. Similar results were reported by [29], who found an increase in NPQ under high light intensity conditions at midday in three epiphytic ferns, which allowed them to avoid damage to the photosynthetic apparatus due to excess light and therefore gives these species the ability to adapt to different light conditions under the forest canopy. Ferns from dry forests showed a large capacity to grow in sunny environments thanks to their unique adaptive characteristics, including photoprotection with pigments, antioxidants, dense indument, leaf curling, and drought avoidance by shedding leaves in the dry season, allowing them to colonize a diverse range of habitats [15,21,33].

The ETR in both fern species studied increased under the higher light intensity treatments, which coincides with the high NPQ values, since at higher energy transport higher the quenching capacity [44,45], which indicates that although the fern species were in conditions of energy excess, they compensated by dissipating energy and heat at the same time; therefore, these ferns were able to protect their photosynthetic apparatus against light excess. Moreover, it has also been reported that under conditions where light is less intense (i.e., cloud forests), ferns can maintain low ETR values [30].

Many species can alter the composition of their photosynthetic apparatus to optimize photosynthesis for the light environment in which they are growing [46]. Therefore, having low chlorophyll concentrations is considered a strategy that helps plants establish and/or grow in a wide range of environments with light heterogeneity [47]. Regarding chlorophyll, *N. aschenborniana* showed an increase in Chl values under the higher light intensity treatment, unlike *C. cucullans* (Figure 1d). It is generally stated that in pteridophytes, the content of chlorophylls is qualitatively



and quantitatively similar to that of higher plants [48]. Higher chlorophyll values have been suggested to represent a degree of adaptation or acclimatization in plants [49]. This trend has been documented in other taxonomic groups, such as angiosperms, particularly in *Calendula officinalis* leaves (Asteraceae), where a higher concentration of chlorophyll a/b was found in shaded conditions compared to those exposed to full exposure to light [50].

### Physiological Plasticity Under Different Amounts of Light

It has been demonstrated that ferns present physiological and phenotypic plasticity under different light conditions [32]. For the physiological variables Yield, NPQ, and ETR, we found lower plasticity values under light changes in *C. cucullans* than in *N. aschenborniana* (Figure 2a). However, these values were low compared to other plant groups; for example, low plasticity values have been reported in epiphytic orchid species in the dry tropics, possibly because environmental conditions are more stressful due to constant drought conditions [51]. The high plasticity value of NPQ for *C. cucullans* indicates a high adaptive capacity to survive under light-stress conditions. It is important to note that both plants and animals have integrated development, in which environmental change can generate changes in a wide variety of physiological and morphological characters, which go hand in hand with developmental processes since they are not isolated characters [52].

### Morphological Plasticity Under Different Amounts of Light

A common response of plants to light heterogeneity within a habitat is to change the expression of morphological traits such as the size, shape, structure, or function of different organs [23]. The morphological characters measured in this study showed that *C. cucullans* exhibits more plasticity under light treatments than *N. aschenborniana*. An expression of phenotypic plasticity in plants is the modification of leaf characteristics through development and under different light conditions [11,28]. Plants can decrease the number of leaves when light intensities are low and physiological activity decreases [11,23, 28]. Another strategy documented in plants is the increase in leaf area, allowing each leaf to intercept greater amounts of light, maximizing the photosynthetically active area [53]. Similarly, in our study, the leaf area of *N. aschenborniana* was high in plasticity (Figure 2c), since this species doubled the size of its pinnae in the 30% light treatment, the same as *C. cucullans*, suggesting a capacity to respond to light limitation. These results corroborate the *Sinapis arvensis* findings, which, under low light conditions, doubled its specific leaf area while the leaf area stabilizes under full sun [3].

Another key morphological trait that changes with light intensity is the thickness of the leaf blade. The leaf thickness in *C. cucullans* was more plastic than in *N. aschenborniana*, suggesting an important capacity of *C. cucullans* to modify itself under

variable conditions. Leaves found at high light intensities have a more developed epidermis, spongy mesophyll, and vascular systems than shade leaves [54,55]. Generally, shade leaves have a larger surface area but are thinner because they have fewer palisade layers in the mesophyll, more grana per chloroplast, and a higher number of thylakoid stacks per grana, giving them a higher chlorophyll concentration [54, 55]. *Cheilanthes cucullans* presents characteristics of a leaf adapted to shade (leaves with a greater leaf area and greater leaf thickness), although with lower chlorophyll concentrations. The low plasticity could be attributed to adaptation to its original natural habitat, given that it is commonly found in the BTC understory and is exposed to high light intensities during the dry season.

However, *N. aschenborniana* presents characteristics of sun plants since this species is mostly exposed to high amounts of light. Lack of plasticity in the variable cuticle thickness among light treatments could be related to the presence of abundant pubescence (trichomes), which can reduce incident radiation on the surface of the leaf; therefore, water loss through transpiration is prevented [56]. Studies that corroborate these results in other taxonomic groups, such as woody angiosperms, show that leaves in shadier conditions tend to decrease in thickness [57,58] and that leaf thickness is strongly correlated with rates of light in photosynthesis. Moreover, little has been studied about the fern indumenta plasticity; however, it has been documented in other species that trichomes act as a photoprotectant against high light intensity and protect against water loss [59]. In this study, we observed that in *N. aschenborniana* the trichome density was higher according to increases in light intensity (100% light treatment) in response to immediate leaf photoprotection; however, *N. aschenborniana* was not plastic at a general level compared to *C. cucullans*. Although *C. cucullans* did not present a higher average trichome density in the treatments with higher light intensity, it was more plastic in morphological variables such as trichomes, which provided leaf protection and greater capacity for long-term energy dissipation under conditions of high light intensity, which is corroborated with our results.

Finally, we conclude that *N. aschenborniana* physiologically responds immediately in the variables studied (e.g., ETR and NPQ) under unfavorable high light intensities. This response is probably a function of the morphological variables studied (e.g., TD). In this sense, *C. cucullans* show highly plastic responses to light changes in both the physiological and morphological variables studied (Figure 2c), and this plasticity allows the species to take advantage of specific environmental conditions. Fern plasticity deserves further research, as it represents an important aspect of the future success of these fern species.

## 8. Conclusion

Ferns play an important ecological role in tropical dry forests characterized by heterogeneous environmental conditions. Both studied species showed plasticity associated with exposure to

different light intensities. Although the species studied are closely related and grouped in the Chelantoid complex, they present different responses to light changes. While *N. aschenborniana* presents greater physiological plasticity, a greater morphological response was found in *C. cucullans*. Characterizing the physiological responses of ferns to environmental heterogeneity is relevant for ecological restoration programs. Understanding the phenotypic response of ferns to aridity will improve conservation programs considering the future effects of climate change.

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