

Diversity and distribution of ferns and clubmosses in the eastern canyons of Isalo National Park, Madagascar

Rivoharifara Randrianarimanana^{1,3}, France Rakotondrainibe², Elodie Boucheron-Dubuisson²,
Lovanomenjanahary Marline^{3,4}, Mijoro Rakotoarinivo¹, Catherine Reeb²

¹ University of Antananarivo, Department of Plant Biology and Ecology, Antananarivo 101, Madagascar

² Institut de Systématique Evolution Biodiversité, Sorbonne Université, MNHN, CNRS, EPHE, UMR 7205, MNHN, Paris

³ Kew Madagascar Conservation Centre, Ambodivoanjo, Ivandry, Antananarivo 101, Madagascar

⁴ Association Vahatra, Antananarivo 101, Madagascar

Corresponding author: Catherine Reeb (catherine.reeb@mnhn.fr)

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Abstract

Background and aims – In contrast to the flowering plants, the pteridophyte flora of Madagascar is still understudied. While several studies have been published on the eastern and central parts of the island, there are currently few works dedicated to the pteridophytes of southwestern Madagascar. The aim of this work is to increase the knowledge of the pteridophyte flora of the Isalo massif in southwestern Madagascar. It presents a checklist of Isalo's pteridophytes and an analysis of the diversity and distribution patterns of pteridophyte communities across ecological gradients in the eastern canyons of Isalo.

Material and methods – Eighty plots were placed in six canyons. In each plot, pteridophyte species abundance was inventoried, as well as several ecological and geomorphological variables. A census in the field and observations on specimens in herbaria were carried to make a checklist of all the pteridophyte species known for Isalo. Statistical analysis was carried out to understand the pteridophyte diversity and distribution patterns in the Isalo's canyons: (1) exploratory analysis (FAMD and HCPC) highlights the general patterns of ecological gradients, (2) a rarefaction curve was used to compare species diversity, and (3) co-inertia analysis investigated the relationship between ecological gradient and pteridophyte communities.

Key results – In total, 60 species of ferns and lycophytes have been recorded in the massif, including ten endemic species to Madagascar and 11 species reported for the first time in the Isalo massif. Species diversity is especially high in the northern canyons (Anjofo, Andramanero, Antsifotra) in contrast to the middle (Maki and Rats) and southern (Namaza) canyons. Fern distribution patterns were correlated to a combination of environmental factors, highlighting species-specific ecological preferences.

Keywords

ecological factors, habitat diversity, Madagascar, pteridophytes, species diversity

INTRODUCTION

The two lineages monilophytes (ferns and horsetails) and lycophytes (clubmosses, spike mosses, and quillworts) were the first vascular plants to colonise land (Ranker and Haufler 2008). They are traditionally referred to as “pteridophytes”, or ferns and allies, a group that cannot be

recognised as a taxon due to its paraphyly but is used for convenience (PPG I 2016). Pteridophytes are the second most diverse group of vascular plants, comprising around 12,000 species. They are reliable bioindicators, closely linked to environmental factors, readily recognizable and monitored, particularly in riparian ecosystems (Paixão et al. 2013; Della and Falkenberg 2019; Bonari et al. 2022).

Madagascar is recognised as one of the world's biodiversity hotspots (Myers et al. 2000) displaying over 90% endemism for various animal taxa (e.g. 100% for amphibians, 92% for reptiles; Vences et al. 2009) and 82% for vascular plants. Endemism is lower for spore-dispersed plants, e.g. 50% for ferns (Rakotondrainibe et al. in press). Madagascar hosts the most diversified pteridophyte flora in the African region, with a species richness equivalent to that of the entire continental Africa, which is approximately 50 times larger in area (Aldasoro et al. 2004; Bauret et al. 2017; Rouhan and Gaudeul 2021). Rakotondrainibe (2003) reported 586 taxa of ferns and lycophytes, including 38 infraspecific taxa, in Madagascar with 40% endemism. A new version of the “Flora of the Pteridophytes of Madagascar”, led by F. Rakotondrainibe, reports 739 taxa with more than 50% endemism (Rakotondrainibe et al. in press). The distribution of the Malagasy pteridophyte species across the five defined bioclimatic domains in Madagascar (Humbert 1955) shows that approximately 85% of the pteridophytes found in Madagascar were reported from the Central and Eastern domains (Rakotondrainibe and Raharimalala 1996, 1998; Rakotondrainibe 2000a, 2000b, 2002, 2003; Reeb et al. 2012), which are characterized by humid or subhumid forests and a high altitudinal range (Humbert and Cours Darne 1965; Cornet 1973; Du Puy and Moat 1996). However, little is known about the

diversity and endemism of pteridophytes in the Western and Southern domains of Madagascar, subject to arid or subarid conditions (Humbert and Cours Darne 1965; Cornet and Guillaumet 1976; Du Puy and Moat 1996).

This study was conducted in Isalo National Park, which is located in the southwestern part of Madagascar. Isalo National Park (Fig. 1A) experiences a subhumid climate similar to the Central domain (Fig. 1B), which is an exception in the Southwest region of Madagascar, exposed to semi-arid and hot climate. The Isalo massif is a combination of ruiniform sandstone formations, sand domes, and tabular structures (Sourdat 1970). The summits are mainly covered by sclerophyllous woodland and shrubs, pseudo-steppes, and rocky vegetation, and in the damper and shadier canyons of the ruined reliefs, a narrow evergreen humid forest may grow and develop (Świerkosz 2012; Rakotomalala et al. 2022). A total of 47 species of pteridophytes were recorded by Lebigre (2010) for the entire of southwestern Madagascar (Tulear province to north of Tsingy de Bemaraha and east to Isalo). Several herbarium specimens of pteridophytes from Isalo massif are held in various herbaria (TAN, P, MO) but there has been no dedicated investigation of the pteridological flora of the area. Documenting pteridophyte diversity is crucial for the Isalo National Park since it will enhance its value and potentially open up research avenues such as ethnobotany or studies on ecosystem services.

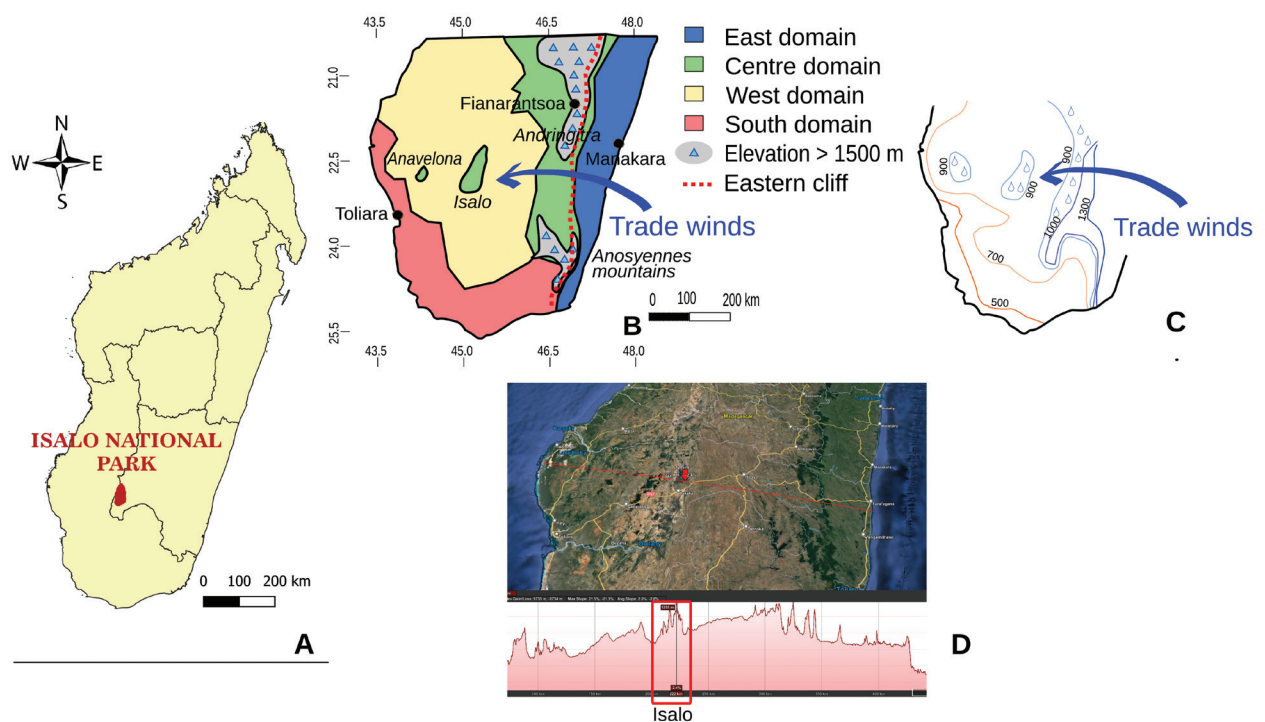


Figure 1. A. Location of Isalo National Parc in southwestern Madagascar. B. Distribution of the phytogeographic domain in the southern Madagascar showing the incursion of the Central domain (subhumid) in the Isalo massif and Anavelona area; the elevation gap (< 1500 m) between northern and southern mountains let the trade winds blow from east to west. C. Simplified isohyets (mm/year) for the southern Madagascar, from the eastern escarpment to the western coast, showing the highest humidity in Isalo National Park (and Anavelona area) (modified from Guillaumet et al. 2008). D. Profile view of a west-east transect passing through the Isalo massif to the Indian Ocean (Google Earth profile view).

In order to determine the richness of the pteridophytes of the Isalo massif and how those communities are organised in the canyons, this study aims to achieve the following objectives: (1) produce a checklist of ferns and lycophytes found in Isalo National Park using newly collected samples and herbarium records, (2) investigate the diversity and distribution patterns of pteridological communities of the eastern canyons of Isalo, depending on the ecological gradient.

MATERIAL AND METHODS

Study area

Isalo National Park is located in southwestern Madagascar, lying between 22°13'–22°45'S and 45°13'–45°29'E, and belongs to the Ihorombe region (Ihosi district) and the Atsimo-Andrefana region (Ankazoabo district) (Fig. 1A). Covering an area of 815.4 km², the Isalo National Park is the fourth largest protected area in Madagascar, and is managed by Madagascar National Parks since 2017 (Madagascar National Park 2017). It was one of the first protected areas in Madagascar, since 1962, and was declared a national park in 1991 (Rakotomalala et al. 2022).

The Isalo massif belongs to the youngest series (Triassic–early Jurassic) of the Malagasy Karoo formation (Sourdat 1970; Wescott and Diggins 1998), and consists of crossbedded sandstones and conglomerates up to 6000 m thick, which are considered a fluvial origin (Geiger et al. 2004). The deposits have been sculpted by both water and wind, creating a majestic ruiniform relief, where the plateau grassland is dissected by a network of deep canyons, up to 200 m deep (Fig. 2A, B). The elevation of the Isalo National Park ranges from 510 to 1268 m (Świerkosz 2012) and the Park has two alternating contrasting seasons, a wet season (October–March) and a dry season (April–September), hence its subhumid climate (Koechlin et al. 1974) (Fig. 1B, C). On the coldest days, fog can form in the canyons, buffering the effects of the dry season (Fig. 2C).

The vegetation of the Isalo massif belongs to the western slopes of the Central domain (Humbert 1955), in a regional subarid climate (Cornet and Guillaumet 1976). Świerkosz (2012) recognised six vegetation types, based on a general transect of the different geomorphological microreliefs of the Isalo: secondary bush on the lowest slopes accessible to anthropogenic activities, rock communities (*Pachypodium*, *Aloe*) on the top of the highest ruiniform relief, sclerophyllous woodland (*Uapaca*) and pseudosteppe (*Aristidia* group) on the plateau, gallery forest (*Pandanus*) or evergreen humid

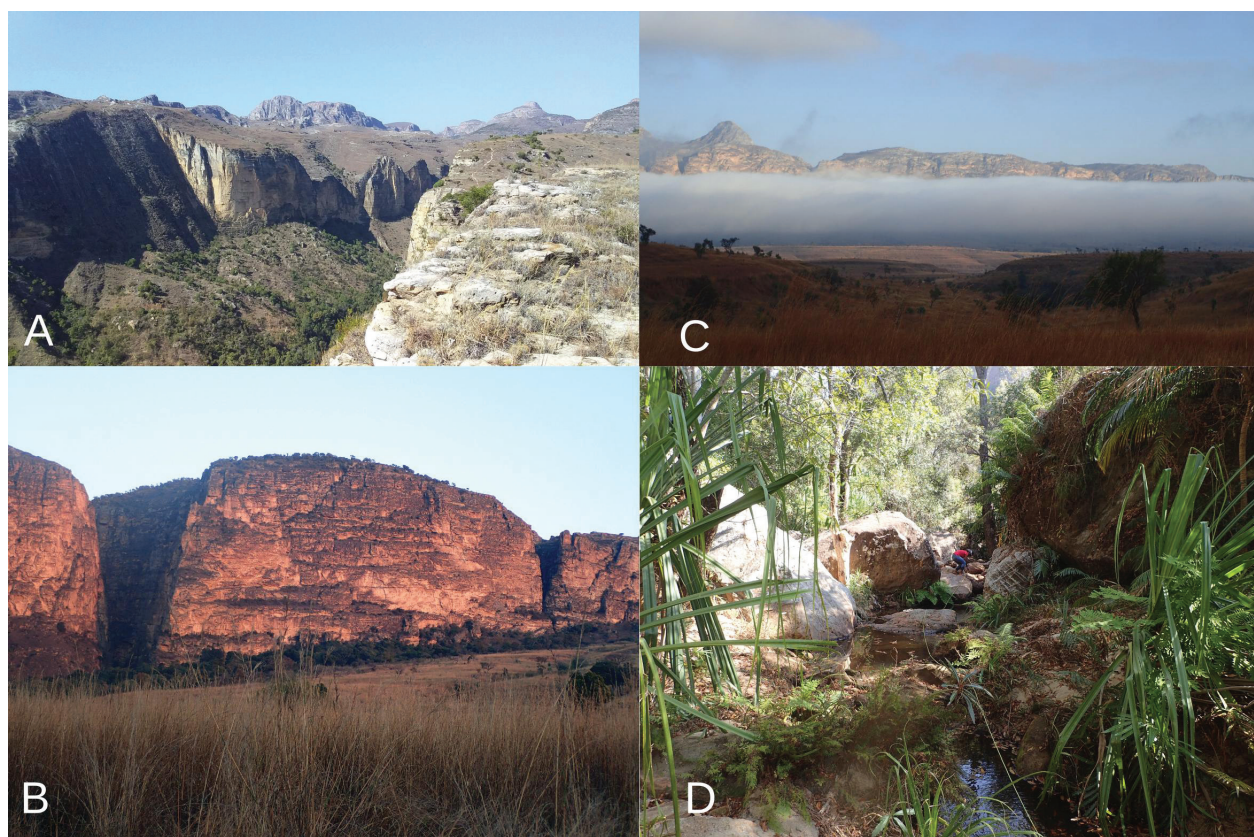


Figure 2. Landscapes of Isalo National Park. **A.** Andramanero canyon seen from the summit. **B.** Entrances of the Makis and Rats canyons on the eastern side of Isalo. **C.** Fog in the early morning along the eastern side of the massif. **D.** View of a plot in Andramanero canyon.

Table 1. List of ecological factors considered and their description.

Ecological factor	Description
L index	Percentage value: the proportion of the sky not obscured by cliffs and canopies
River regime in the dry season	Ordinal: (1) water completely evaporated, (2) still stagnant river, (3) running water without ripples, (4) running water with ripples or small rapids
Distance D	Numeric: D = major riverbed width - minor riverbed width
Percentage of sand / total surface of the major riverbed	Ordinal: (1) no sand 0–10%, (2) 10–25%, (3) 25–50%, (4) 50–75%, (5) 75–100% (estimation)
Rocks in the riverbed	Ordinal: (1) no rocks, (2) rocks < 0.1 m, (3) 0.1 m < rocks < 0.5 m, (4) 0.5 m < rocks < 1.5 m, (5) rocks > 1.5 m
Trees and <i>Pandanus</i> density	Ordinal: (1) no trees in the canyon, (2) 2–3 scattered trees, (3) > 3 trees with a diameter > 10 cm; (1) no <i>Pandanus</i> in the plot, (2) 2–3 scattered <i>Pandanus</i> , (3) > 3 <i>Pandanus</i>
Herbaceous layer density	Ordinal: (1) no herbaceous layer, (2) scattered to discontinuous herbaceous layer, (3) continuous herbaceous layer
Seepage presence, used as a supplementary variable	Binary: (1) present, (2) absent
Orientation	Discrete, not ordinal: (1) N–S, (2) NW–SE, (3) W–E, (4) SW–NE, (5) S–N
Slope	Numeric
Distance to opening	Numeric (metres): the position of the origin is the opening of the cliff; negative values are considered in the plain, positive values are considered in the canyon

forest in the canyons or river valley (Rakotomalala et al. 2022) (Fig. 2A, D).

This study explored six canyons that open on the southern part of the eastern side of the Isalo massif, which is a very steep cliff abruptly cut by a few east–west canyons. From south to north, these canyons are named as Namaza canyon, Maki canyon, Rats canyon, Anjofo canyon, Andramanero canyon, and Antsifotra canyon (Supplementary material 1).

Taxonomic sampling and analysis

The checklist of the pteridophytes from Isalo was compiled from all herbarium specimens held at the Malagasy National Herbarium Antananarivo, Madagascar (TAN) and the MNHN herbarium, Paris, France (P), the Missouri Botanical Garden, St Louis, USA (MO), new collections from this study, and online databases (Tropicos, Sonnerat, JSTOR). Family nomenclature follows PPG I (2016). Species nomenclature follows Rakotondrainibe et al. (in press) and Rakotondrainibe (2003) for distributional accuracy.

Ecological survey

Sampling design

Ecological surveys were adapted from Rakotondrainibe (1989), Rakotondrainibe and Raharimalala (1996, 1998), and Reeb et al. (2012). A plot is an area established in the riverbed of the canyon. Its central axis was determined by a 20 m string placed in the centre of the riverbed (Fig. 2D). The lateral limits of the plot are defined by the highest flood marks. Abiotic characteristics and fern abundance were recorded (see below). Four plots of 20 m long were successively sampled in a homogeneous part

of the canyon, both in terms of vegetation and topology. In total, an area of at least 800 m² with similar conditions is studied, which is considered the minimal area for fern studies, even along a river (Rakotondrainibe 1989; Reeb et al. 2012).

Plot type definition

The openness of a canyon can influence the variation in shading of pteridophyte community patterns due to differences in preferences between heliophilous and sciaphylous species (Dai et al. 2020). Five types of plots were defined geomorphologically, from open plots (type 1) to semi-open plots (types 2, 3) and closed plots (types 4, 5) (Supplementary material 2).

Fern abundance

The number of individuals of each fern species was recorded for each plot. In the case of ferns with long creeping rhizomes, each frond was considered as an individual, as the “true” individual is born from the development of a spore, data which is not accessible in the field. To limit observer bias, individuals of each species in each plot were counted 3 to 4 four times by 3 to 4 pairs of observers. The mean of the observers’ counts was then calculated to estimate the number of individuals for each species in each plot.

Ecological factors

To understand the factors that shape the diversity and distribution of pteridophyte communities, we considered ecological factors related to the vegetation, morphology, and riverbed characteristics of a canyon (Table 1). The riverbed is the area permanently or temporarily inundated by water. Two main parts can be defined in a riverbed (Piegay 2019): the minor riverbed which is permanently

occupied by flowing water in the lowest parts of the canyon, and the major riverbed which is only filled with water during floods. These higher limits are generally easy to detect in the canyons because debris from the highest floods is still embedded in the terrestrial vegetation and because of the change in terrestrial vegetation between flood affected/non-affected zones.

To assess the effect of light penetration in the canyon on pteridophyte diversity, we developed an L index that is related to the combination of canopy and rock/cliff cover that act as barriers for light penetration in the canyon. To measure this L index, we developed a simple and repeatable method based on the analysis of canopy images using ImageJ (Supplementary material 3) (Schindelin et al. 2012).

Descriptions of the ecological factors are given in Table 1. The data analysed in this paper are presented in two different data tables (Supplementary materials 4, 5). The plots are the individuals (rows); in Supplementary material 4, the ecological factors are the variables, quantitative and qualitative; in Supplementary material 5, the abundance of fern species is the variable.

Statistical analysis

The analysis of the pteridophyte community and its relationships with ecological factors is mainly based on ordination methods. These methods allow the transformation of multivariate data into an interpretable form that revealing patterns and relationships within the data (Thioulouse et al. 2018a). As fern abundances ranged from 0 to 6406 (due to a few very common tufted species or to small Hymenophyllaceae), the range of the variation in species abundance was reduced by applying a square root transformation to the abundance table (David 2017).

Analysis of similarity between plots according to ecological factors

An exploratory analysis of the ecological data was first carried out using a Hierarchical Clustering on Principal Components (HCPC). The HCPC is a clustering method that we use to generate a dendrogram that groups plots into a class according to the similarity of their ecological gradients (Gardner 2014). We first performed a Factor Analysis for Mixed Data (FMAD), which is an ordination method belonging to the family of principal components used to identify patterns in data containing both numerical and categorical variables (Kassambara 2017). This FMAD reduces the dimension in the data to produce a matrix output that can be used by the HCPC to generate clusters. The FMAD and the HCPC were performed using the R packages *factoextra* v.1.0.7 and *FactomineR* v.2.8 (Lê et al. 2008) with a maximum category $\max = 6$, as there were six geographical catchments for the canyons. The geographical localisation of each plot (the canyon watershed) was plotted against the tree obtained.

Pteridophyte community analysis

First, we defined the general pattern of species composition in the canyon catchment and then explored the abundance of each species in each plot. To do this, we performed a Correspondence Analysis (CA) from the R package *ade4* v.1.7-22 (Thioulouse et al. 2018b) on the fern abundance dataset (Supplementary material 5). CA is an ordination method that maximises the correspondence between species score and plot scores (Gardner 2014) revealing patterns of plot-species affinities. The abundance dataset was first reordered according to the coordinates of the first CA axes to produce a figure showing the distribution of each species in each plot.

Second, we measured the similarity of species composition at two levels: at the 6 canyons catchment level and at the plot types level (Supplementary materials 1, 2) by using Non-Metric Multidimensional Scaling (NMDS). This produced ordination graphs that highlight dissimilarities between classes (i.e. dissimilarities between the 6 canyon catchments on one hand and between plot types on the other hand in terms of species composition). NMDS was performed on a matrix of Bray-Curtis distance metrics. Species abundances was used to generate a Bray-Curtis distance metrics matrix which is then used for NMDS. Bray-Curtis distance is a dissimilarity metric suitable for species abundance data collected at different sampling locations (Gardner 2014). The NMDS process was performed using the *metaMDS* function from the R package *vegan* v.2.6-4 (Oksanen et al. 2022), with 999 numbers of random starts and three dimensions were conserved to obtain a meaningful stress value. A non-parametric test, one-way analysis of similarity (ANOSIM, with 9999 permutations) was performed to test the significance of NMDS ordination result.

Canyons' flora diversity comparison

To understand the distribution of species in the pteridophyte community, we use diversity indices to compare species diversity in the six canyons (Maki, Rats, Andramanero, Namaza, Anjofo, Antsifotra). The sample size differs from canyon to canyon, due to the difference in sampling effort. A rarefaction method was used because it provides a standardisation of the sample size to overcome the influence of sample size differences when comparing species diversity (Gotelli and Colwell 2001; Colwell et al. 2012). An individual-based abundance rarefaction curve for each canyon was performed, using the R package *iNextPD* v.3.0.0 (Hsieh et al. 2016). Three Hill Numbers q (effective number of species) were calculated: species richness ($q = 0$), Shannon diversity ($q = 1$, the exponential of Shannon entropy), and Simpson diversity ($q = 2$, the inverse of Simpson concentration) (Chao et al. 2014). These three Hill numbers allow us to compare community diversity by considering species richness, species evenness, and species dominance in the community.

Investigation of the links between the pteridophyte communities and the ecological factors of the canyons

Co-inertia analysis was used to explore the relationship between fern community patterns and the factors that may control them. Co-inertia analysis takes two multivariate datasets as an input and searches for the pair of new axes that maximises the concordance between the two datasets. The optimisation criterion in co-inertia analysis is that the resulting sample scores (ecological scores and floristic scores) are the most covariant (Dolédéc and Chessel 1994). The ecological dataset contains both quantitative and qualitative data, so a Hill-Smith analysis was used (Dray and Dufour 2007). A PCA was performed on the fern abundance dataset. We maximise covariance between the two data tables and calculate the correlation coefficient R (between 0 and 1; the closer R is to 1, the stronger the correlation between the tables). The test of the significance of co-inertia was performed using a 100 co-inertia analysis after random permutation of the rows in both tables. For each run, the R^2 value was calculated and the frequency distribution of these values for the two first co-inertia axes was projected. If the observed value was higher than 95% of the values obtained for the permuted datasets, then the co-inertia was considered significant (Dolédéc and Chessel 1994). Data analyses were performed using the R package *ade4* v.1.7-22.

RESULTS

Species composition

Sixty (60) species of ferns (Table 2, Supplementary material 6) and lycophytes (Table 3, Supplementary material 6) are recorded for the Isalo massif including ten fern species endemic to Madagascar (16% of endemism for the Isalo massif). Thirty-eight (38) species were inventoried during the fieldwork of which 11 species are reported for the first time in the Isalo massif: *Asplenium erectum*, *Asplenium* aff. *aethiopicum*, *Doryopteris kitchingii*, *Equisetum ramosissimum*, *Lygodium kerstenii*, *Macrothelypteris torresiana*, *Nephrolepis biserrata*, *Sticherus flagellaris*, *Thelypteris arbuscula*, *Thelypteris dentata*, and *Thelypteris unita*. The families Pteridaceae, Thelypteridaceae, and Dennstaedtiaceae are the most represented in terms of richness (Supplementary material 7A). At the genus level, *Pellea* and then *Thelypteris* are the two most important by their number of species (Supplementary material 7B).

The pteridophyte flora of the Isalo Park shows a high affinity with that of the Central Domain. (Tables 2, 3): 92% of Isalo's fern communities are found in the Central Domain, 42 % in the eastern dense rainforests and Sambirano; 38% in the Western Domain with dense dry forests; 10% in the Southern Domain with scrub.

Comparison of ecological gradient at the level of canyon and at plot (or station) scale

Five clusters are identified by hierarchical clustering of plots according to ecological factors (Fig. 3A), which are well separated when individuals are projected (Fig. 3B). Most of the series of successive plots are in the same clade (e.g. [7–10], [24–28], [62–65]), confirming the choice of similar conditions of successive plots, in order to reach the 800 m² minimum area. Most of the plots are grouped by canyon: the majority of the plots within the Maki and Rats canyons are grouped together (Fig. 3A cluster 4) as well as the Namaza plots (Fig. 3A cluster 5). Series [24–28] from Rats canyon and series [68–71] from Antsifotra canyon are open plots (type 1) outside of the cliff area (Supplementary materials 1, 2). However, a few plots are not grouped with their neighbours (e.g. plot 2 regarding [1, 3, 4]).

Ordination of pteridophyte species abundance

Different patterns of fern abundance emerge with respect to plot ordination (Fig. 4). The pteridophyte flora of the eastern canyons of Isalo is mainly dominated by *Odontosoria chinensis* and *Stenochlaena tenuifolia*. However, some canyons differ from this pattern: this is the case of Antsifotra's canyon that is dominated by *Thelypteris interrupta* and *Nephrolepis biserrata* and in which *Stenochlaena tenuifolia* and *Odontosoria chinensis* have very low abundances (see Discussion). Some species are restricted to a single canyon: *Osmundilindaea squamata* and *Thelypteris arbuscula* occur only in the southern Namaza canyon; *Didymoglossum erosum* and *Asplenium buettneri* only in the Maki canyon; *Doryopteris kitchingii* in Anjofo.

Comparison of fern communities at the level of canyon and at plot scale

The similarities between the composition of the fern communities at the scale of the six canyons catchments is analysed with NMDS (Fig. 5A). The stress value $s = 0.11$ (< 0.2) for $k = 3$, indicates that the ordination is not random. The ANOSIM value for the similarity analysis with the grouping by canyon is $R = 0.2486$, $p = 0.0001$; the p value (significant value) is lower than 0.05, and R is higher than p , which means that there are significant statistical differences between the fern communities of the six canyons. Some superposition is observed, but the all the centres of gravity of each catchment are distinct.

In a second analysis, when the composition of the fern communities is projected with NMDS according to plot type (Fig. 5B), the open plots (type 1; Supplementary material 2) clearly host different communities from plots in the internal parts of canyons. The ANOSIM value for analysis of similarities with grouping by type of plots is $R = 0.2533$, $p = 0.0001$; here again, there is significant statistical differences between the fern communities

Table 2. Checklist of ferns from the Isalo massif. The nomenclature of species and family is based on Rakotondrainibe et al. (in press) using PPG I (PPG I 2016). When possible, the corresponding nomenclature from Rakotondrainibe (2003) is given, together with the distribution in Madagascar, outside the Isalo massif (data from 2003). END = endemic to Madagascar.

Family	Species	Species (Rakotondrainibe 2003)	Endemic to Madagascar	W domain	C domain	E domain	S domain
Pteridaceae	<i>Actiniopteris dimorpha</i> Pic. Serm.	=		X	X		X
Pteridaceae	<i>Adiantum capillus-veneris</i> L.	=		X	X		X
Cyatheaceae	<i>Alsophila dregei</i> (Kunze) R.M.Tryon	<i>Cyathea dregei</i> Kunze		X	X		
Cyatheaceae	<i>Alsophila hyacinthi</i> R.M.Tryon var. <i>hyacinthi</i>	<i>Cyathea isaloensis</i> C.Chr.			X		
Anemiaceae	<i>Anemia madagascariensis</i> C.Chr.	=	END		X		
Tectariaceae	<i>Arthropteris orientalis</i> (G.F.Gmel.) Posth. var. <i>orientalis</i>	=			X	X	
Aspleniaceae	<i>Asplenium</i> aff. <i>aethiopicum</i> (Burm.f.) Bech.	<i>Asplenium</i> <i>aethiopicum</i> (Burm.f.) Bech.			X		
Aspleniaceae	<i>Asplenium blastophorum</i> Hieron	=		X	X	X	
Aspleniaceae	<i>Asplenium buettneri</i> Hieron. ex Brause	=			X		
Aspleniaceae	<i>Asplenium erectum</i> Bory ex Willd.	=			X		
Aspleniaceae	<i>Asplenium formosum</i> Willd.	=		X	X		
Aspleniaceae	<i>Asplenium pseudostuhlmanii</i> Viane	<i>Asplenium</i> <i>stuhlmannii</i> Hieron.		X	X		
Blechnaceae	<i>Blechnum attenuatum</i> (Sw.) Mett.	=			X	X	
Blechnaceae	<i>Blechnum tabulare</i> (Thunb.) Kuhn	=			X		
Dennstaedtiaceae	<i>Blotiella isaloensis</i> (Tardieu) P.Roux	=	END		X		
Pteridaceae	<i>Cheilanthes bonapartei</i> P.Roux	<i>Adiantopsis</i> <i>linearis</i> Bonap.	END		X		
Pteridaceae	<i>Cheilanthes hirta</i> Sw.	<i>Cheilanthes hirta</i> Sw.			X		
Pteridaceae	<i>Cheilanthes perrieri</i> P.Roux	not recorded	END		X		X
Gleicheniaceae	<i>Dicranopteris linearis</i> (Burm.f.) Underw.	=			X	X	
Hymenophyllaceae	<i>Didymoglossum erosum</i> (Willd.) P.Roux	<i>Trichomanes</i> <i>erosum</i> Willd.			X	X	
Pteridaceae	<i>Doryopteris kitchingii</i> (Baker) Bonap. ex C.Chr.	=	END		X		
Dryopteridaceae	<i>Elaphoglossum poolii</i> (Baker) Christ.	=	END		X		
Equisetaceae	<i>Equisetum ramosissimum</i> Desf.	=		X	X		X
Gleicheniaceae	<i>Gleichenia polypodioides</i> (L.) Sm.	=			X		
Cyatheaceae	<i>Gymnosphaera rouhaniana</i> (Rakotondr. & Janssen) S.Y.Dong	not recorded	END		X		
Dennstaedtiaceae	<i>Histiopteris incisa</i> (Thunb.) Sm.	=					

Table 2 (continued). Checklist of ferns from the Isalo massif. The nomenclature of species and family is based on Rakotondrainibe et al. (in press) using PPG I (PPG I 2016). When possible, the corresponding nomenclature from Rakotondrainibe (2003) is given, together with the distribution in Madagascar, outside the Isalo massif (data from 2003). END = endemic to Madagascar.

Family	Species	Species (Rakotondrainibe 2003)	Endemic to Madagascar	W domain	C domain	E domain	S domain
Thelypteridaceae	<i>Macrothelypteris torresiana</i> (Gaudich.) Ching	=			X	X	
Marsileaceae	<i>Marsilea minuta</i> L.	=		X	X		
Dennstaedtiaceae	<i>Microlepia speluncae</i> (L.) T.Moore	=			X	X	
Nephrolepidaceae	<i>Nephrolepis biserrata</i> (Sw.) Schott	=			X	X	
Lindsaeaceae	<i>Odontosoria chinensis</i> (L.) J.Sm.	=		X	X	X	
Oleandraceae	<i>Oleandra distenta</i> Kunze	=		X	X	X	
Lindsaeaceae	<i>Osmolindsaea latisquama</i> Lehtonen & Rouhan	<i>Lindsae odorata</i> auct.			X		
Osmundaceae	<i>Osmunda regalis</i> L. var. <i>obtusifolia</i> (Kaulf.) Milde	<i>Osmunda regalis</i> L.			X	X	
Pteridaceae	<i>Pellaea angulosa</i> (Bory ex Willd.) Baker	=			X	X	
Pteridaceae	<i>Pellaea boivinii</i> Hook.	=		X	X		
Pteridaceae	<i>Pellaea calomelanos</i> (Sw.) Link	=		X	X		
Pteridaceae	<i>Pellaea dura</i> (Willd.) Hook.	=			X		
Pteridaceae	<i>Pellaea pectiniformis</i> Baker	=		X	X		
Pteridaceae	<i>Pityrogramma argentea</i> (Willd.) Domin	=			X		
Pteridaceae	<i>Pityrogramma calomelanos</i> (L.) Link	=				X	
Psilotaceae	<i>Psilotum nudum</i> (L.) P.Beauv.	=		X	X	X	
Dennstaedtiaceae	<i>Pteridium aquilinum</i> (L.) Kuhn subsp. <i>capense</i> (Thunb) C.Chr.	=			X	X	
Pteridaceae	<i>Pteris pseudolonchitis</i> Bory ex Willd.	=			X	X	
Marattiaceae	<i>Ptisana fraxinea</i> (Sm.) Murdock	<i>Marattia fraxinea</i> Sm.			X	X	
Blechnaceae	<i>Stenochlaena tenuifolia</i> (Desv.) Moore	=		X	X	X	
Gleicheniaceae	<i>Sticherus flagellaris</i> (Bory ex Willd.) Ching	=			X		
Thelypteridaceae	<i>Thelypteris arbuscula</i> (Willd.) K.Iwats.	<i>Sphaerostephanos</i> <i>arbuscula</i> (Willd.) Holtum				X	
Thelypteridaceae	<i>Thelypteris confluens</i> (Thunb.) C.V.Morton	=		X	X		
Thelypteridaceae	<i>Thelypteris dentata</i> (Forssk.) E.P.St.John	<i>Christella dentata</i> (Forssk.) Holtum			X	X	
Thelypteridaceae	<i>Thelypteris interrupta</i> (Willd.) K.Iwats.	<i>Cyclosorus</i> <i>interruptus</i> (Willd.) H.Itô		X	X	X	
Thelypteridaceae	<i>Thelypteris unita</i> (L.) C.V.Morton	<i>Sphaerosyrtophanos</i> <i>unitus</i> (L.) Holtum		X	X	X	

Table 3. Checklist of lycophytes from the Isalo massif. The nomenclature of species and family is based on Rakotondrainibe et al. (in press) using PPG I (PPG I 2016). When possible, the corresponding nomenclature from Rakotondrainibe (2003) is given, together with the distribution in Madagascar (data from 2003), outside the Isalo massif (data from 2003). END = endemic to Madagascar.

Family	Species	Species (Rakotondrainibe 2003)	Endemic to Madagascar	W domain	C domain	E domain	S domain
Lygodiaceae	<i>Lygodium kerstenii</i> Kuhn	=		X	X		
Lygodiaceae	<i>Lygodium lanceolatum</i> Desv.	=			X	X	
Lycopodiaceae	<i>Palhinhaea cernua</i> (L.) Franco & Vasc.	<i>Lycopodiella cernua</i> (L.) Pic. Serm			X	X	
Lycopodiaceae	<i>Pseudolycopodiella caroliniana</i> (L.) Holub	<i>Lycopodiella caroliniana</i> (L.) Pic.Serm.		X	X	X	
Selaginellaceae	<i>Selaginella digitata</i> Spring.	=	END	X			X
Selaginellaceae	<i>Selaginella echinata</i> Baker	=	END	X	X		X
Selaginellaceae	<i>Selaginella helicoclada</i> Alston ex Alston	=	END	X	X		

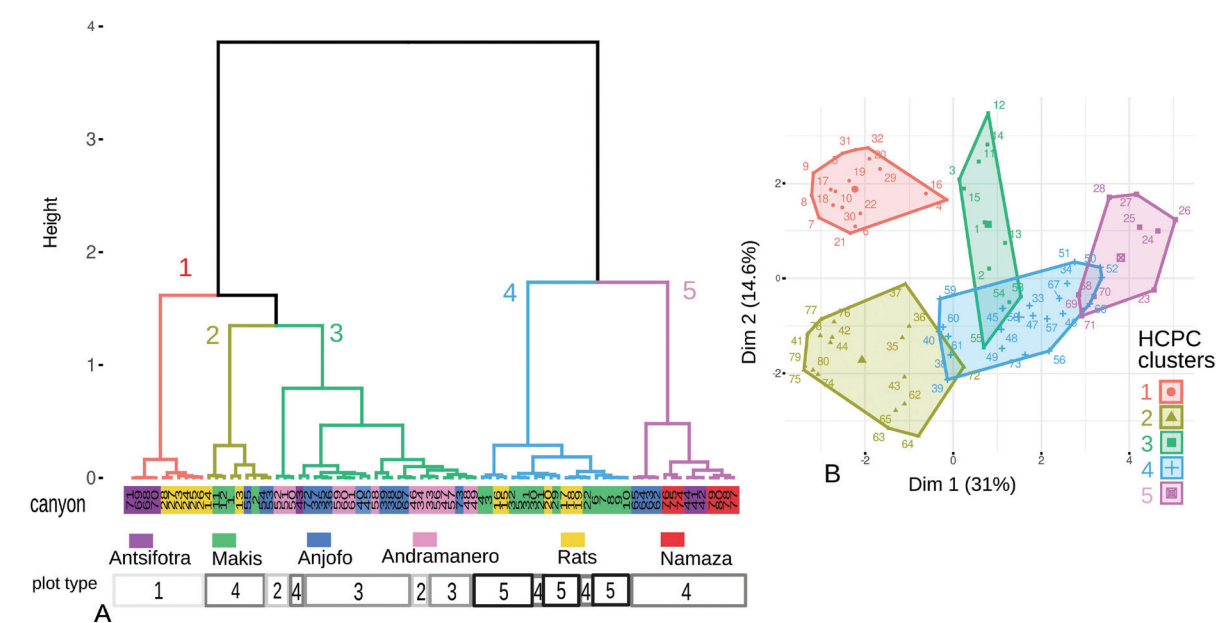


Figure 3. A. Hierarchical clustering tree of the plots according to ecological factors, 5 clades are numbered; the plots labels are highlighted according to their canyon (legend below the plots); the plot type is reported in the bottom row. B. Projection of the plots grouped by clades of the HCPC on the first two dimensions of the FMAD.

according to the plot type. The communities of type 2 and 3 communities overlap, and the communities of type 4 and 5 appear to be very similar (Fig. 5B).

Species diversity comparison

Rarefied species diversity values (Table 4) give an overview of the diversity pattern between the six canyons. The species richness accumulation curve (Fig. 6, $q = 0$) shows that Anjofo canyon has the highest number of pteridophyte species. Then, Andramanero, Maki, and Rats have almost the same species richness, and finally,

Namaza and Antsifotra canyon have the lowest number of species. However, the accumulation curve of the Shannon diversity index (Fig. 6, $q = 1$) shows that despite its low species richness, the pteridophyte community of Antsifotra canyon has a higher species evenness and is therefore more diversified than the communities of Andramanero, Maki, Rats, and Namaza. Of all of the canyons, Anjofo's pteridophyte community is the most diversified because it has the highest species evenness and species richness. Furthermore, in contrast to the central (Maki + Rats) and the southern (Namaza) canyons, the pteridophyte flora in the northern canyons (Anjofo,

Table 4. Rarefied species diversity values of the six canyons. In brackets, asymptotic diversity estimate values of the six canyons: first number = LCL, 95% associated Lower Confidence interval Limit; second number = UCL, 95% Upper Confidence interval Limit.

Canyon	Rarefied species richness q = 0	Rarefied Shannon diversity q = 1	Rarefied Simpson diversity q = 2
Andramanero	19 [19–19.60]	5.21 [5.12–5.35]	3.090 [3.031–3.0156]
Anjofo	21 [21–21.196]	7.623 [7.505–7.742]	5.371 [5.289–5.545]
Antsifotra	15 [15–15]	6,697 [6.465–6.930]	4.091 [3.900–4.289]
Maki	19 [19–20.35]	2.746 [2.716–2.776]	1.902 [1.882–1.922]
Namaza	15 [15–12.798]	3.277 [3.203–3.351]	2.524 [2.480–2.569]
Rats	17 [17–17.467]	2.9672 [2.939–3.007]	2.062 [2.037–2.087]

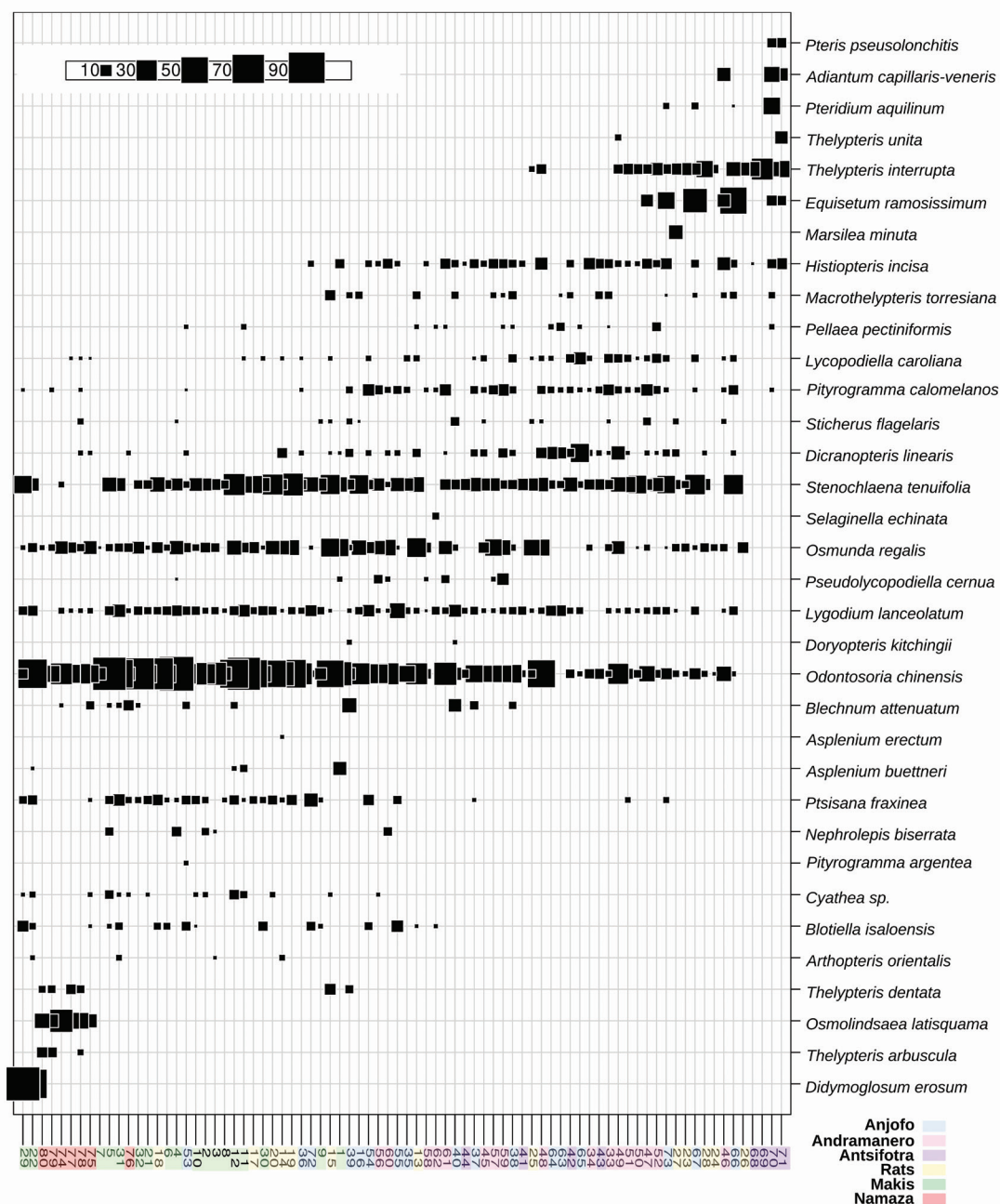


Figure 4. Ordination of the fern abundance table on the first axis of the CA analysis, the x-axis shows the species identified, the y-axis shows the number of plots, coloured according to their location (= the canyons they were sampled). The size of the black squares is proportional to species abundance.

Andramanero, and Antsifotra) is more diverse. The same pattern is seen in the accumulation curve of the Simpson diversity index (Fig. 6, $q = 2$), which indicates that the pteridophyte communities in the central and southern canyons tend to be dominated by a few species.

Exploring the links between the pteridophyte communities and the ecological factors

The co-inertia analysis (Fig. 7) measures the concordance between the two datasets and shows a covariance between

the two tables (Fig. 7C); the first axis accounts for 42% of co-inertia and the second for 22% (64% for cumulative projected inertia for the first two axes). The Monte-Carlo test shows a consistent correlation between the ecological factors of the plots and the fern communities ($RV = 0.29$, $p = 0.0001$). The correlation is significant, fern distribution is correlated with a combination of environmental factors and not specifically with just one, although the influence of a few major factors can be highlighted (Fig. 7A, B). On the first axis the influence of the canopy cover is inversely

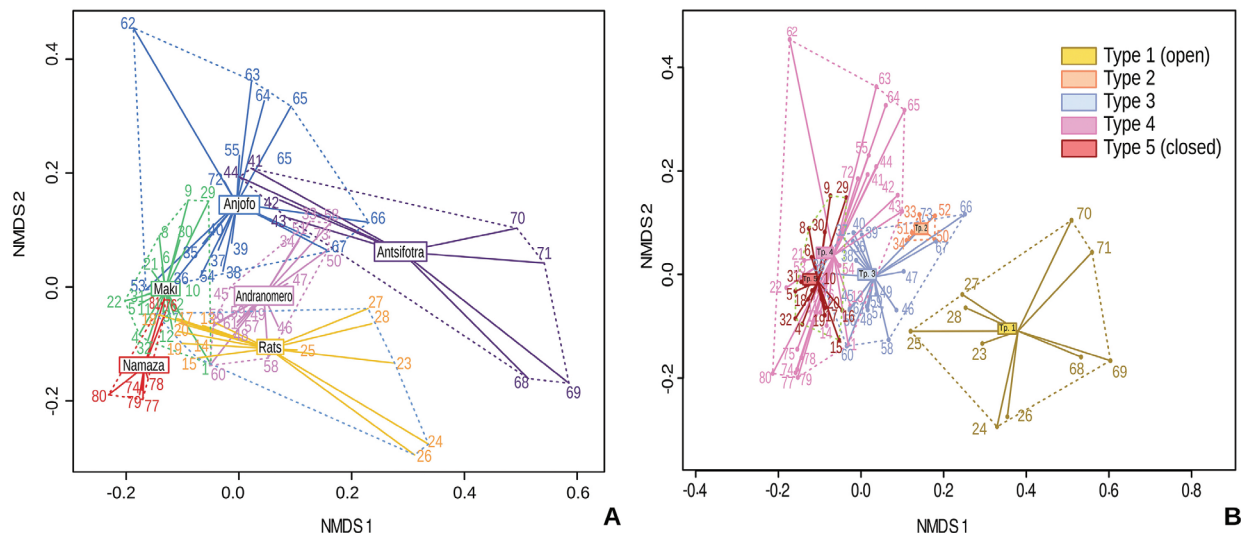


Figure 5. A. NMDS analysis according to the six canyons. B. NMDS analysis according to the type of plots. The horizontal x-axis represents the first dimension extracted by the NMDS analysis. This dimension is constructed so that the distance or dissimilarity between the points on this axis reflects as closely as possible the original dissimilarity between plots. In A coloured according to the canyons, in B coloured according to the type of plot. The vertical axis is the second dimension extracted from the NMDS analysis, also designed to reflect the dissimilarity between the plots, but it is orthogonal to the x-axis, which means that it captures another source of dissimilarity relative to the x-axis. It is important to remember that the axes in an NMDS plot are used to visualise the relative similarity or dissimilarity of the objects analysed (here plots) in a reduced dimensional space, and have no direct meaning in terms of specific variables.

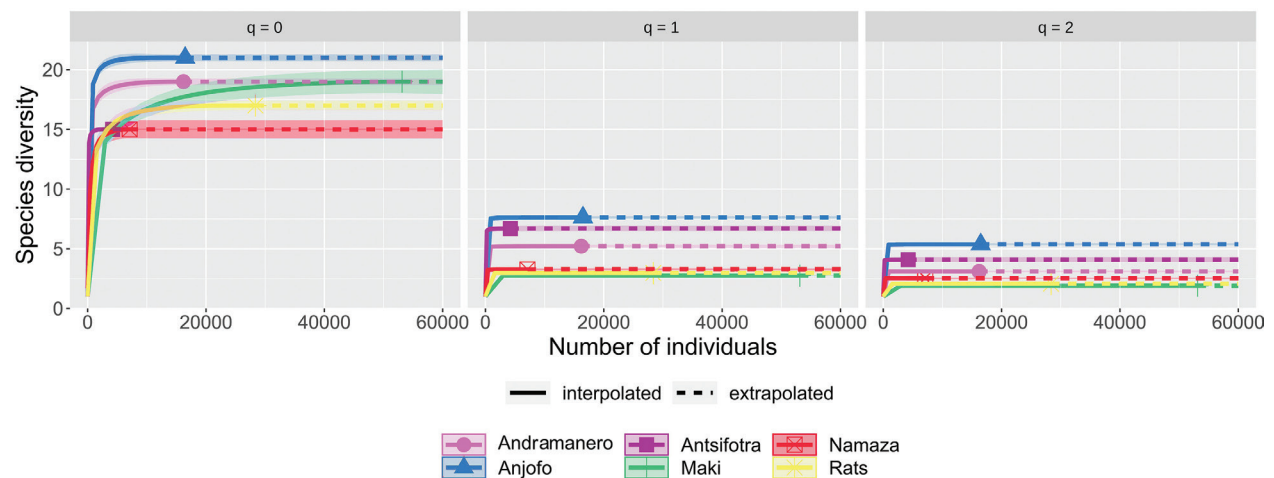


Figure 6. Rarefaction curves for the six canyons showing the diversity detected compared with the predicted total diversity. The x-axis represents the number of individuals recorded while the y-axis represents a measure of the species richness detected, estimated with the Chao1 index.

correlated with the orientation and the presence of rocks in the minor or major riverbed (Fig. 7A, B).

On the second axis, the difference D between the width of the major and minor riverbeds is inversely correlated with the distance to the mouth of the canyon. The presence of vegetation (trees, pandanus, grasses) is positively correlated on axis 1, with lower values.

In open plots, species such as *Equisetum ramosissimum* and *Thelypteris interrupta* are clearly associated with a high L index, sandy substrate, and the presence of vegetation. *Marsilea minuta* occurs in pools in open areas, and is negatively correlated with the distance from the mouth of the canyon. It means that *Marsilea minuta* becomes rarer as we move towards the bottom of the canyon.

On the other hand, on the negative part of axes 1 and 2, species such as *Ptisana fraxinea* and *Cyathea* sp. are strongly correlated with the very narrow canyons, surrounded by high vertical cliffs that shade the canopy. Also associated with the presence of rocky outcrops that provide anchorage are *Ptisana fraxinea* and *Didymoglossum erosum*. *Osmunda regalis* and *Stenochlaena tenuifolia* are associated with large major riverbeds. *Odontosoria chinensis*, one of the most common ferns, is probably more linked to rock presence. *Blotiella isaloensis* and *Lygodium lanceolatum*, two lianaceous species seem to be correlated with the presence of rocks in the riverbed, both minor and major. It confirms the sensitivity of fern species to environmental factors, such as rocks, sand, or trees, the presence of grasses, and orientation which can influence the light intensity during the day.

DISCUSSION

This study contributes to the understanding of the pteridophyte flora in southeastern Madagascar. The Isalo massif has a significant richness of 60 species compared to other geomorphologically similar areas. For example, the Makay massif, a larger sandstone massif to the north of Isalo, was reported to have only 38 species (Roubaud et al. 2018). Similarly, the Bemaraha Tsingy, a ruiniform limestone area in the mid-west of Madagascar, was reported to have only 16 species (Madacamp 2022). The low number of species recorded in these areas may be due to the lack of comprehensive surveys, with the exception of exploratory studies conducted in the Makay Massif in 2011 and 2017 (Roubaud et al. 2018; Tropicos <https://tropicos.org/person/100099987>). The high diversity of pteridophytes in Isalo can be attributed to the patchwork of ecosystems of the massif ranging from shady, cool, and humid environments in narrow canyons to disturbed riverbanks, dry and hot open savannas, and rocky slopes (Fig. 2). The lower community diversity in the southern canyon (Namaza, Maki, and Rats) might be explained by the higher human activity in these frequently visited areas.

The low rate of endemism in the Isalo massif (16% for Isalo, ~50% for the Madagascar fern flora) may be attributed to the geomorphology of the area that has an influence on the trade winds at both local and regional scales. On a regional level, the absence of significant relief (> 1500 m) between the Isalo massif and the eastern coast of Madagascar allows the trade winds blowing from the

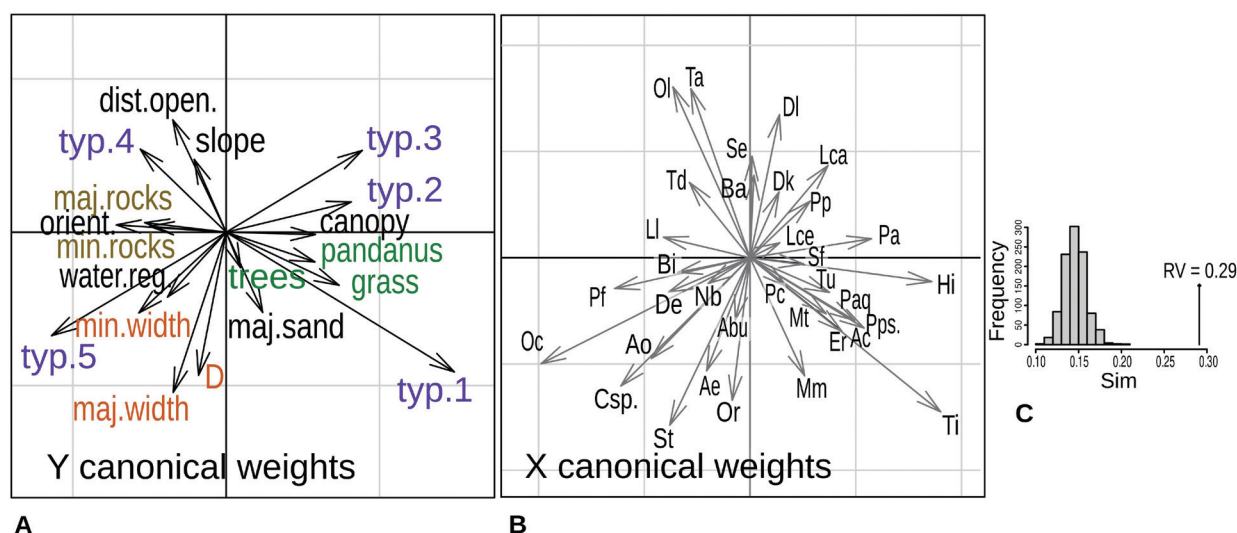


Figure 7. Co-inertia analysis. **A.** Scatter plot representing the coefficients of the combinations of the variables for ecological variables. The colours are only informative for easier reading: “green” = vegetation presence; “brown” = rocks; “orange” = riverbed dimensions; “violet” = type of plot. **B.** Scatter plot representing the coefficients of the combinations of the variables for fern species abundance. **C.** Result of the permutation test; the RV coefficient appears as different that RV expected by chance. See Supplementary material 8 for plot position in the co-inertia.

east to reach the massif, thus facilitating the wind-borne transport of pteridophyte spores even from other countries or continents (Fig. 1C, D) (Donque 1978; Guillaumet et al. 2008). The favourable moisture conditions found within the canyons of Isalo (Ferry et al. 1998) create a suitable environment for the germination of these spores. On a local scale, the low vegetation (compared to a dense forest where trees and leaves act as significant barriers) and the absence of physical barriers on the slopes, domes, and plateaus or in the through valleys of the Isalo canyons may promote dispersal of spores by wind (Karst et al. 2005; Millerón et al. 2012; Robledo-Arnuncio et al. 2014). Cyclones reaching the Isalo massif, free of any relief obstacles, dense forests, in all directions play a role in spore recruitment and dispersal (Donque 1978; Walker and Sharpe 2010). Together, these factors reduce genetic isolation, which is a major driver of speciation and endemism.

In particular, the fern communities of Isalo show a variety of affinities with the different phytogeographical domains (Tables 2, 3). About 92% of the fern communities in Isalo are found in the Central domain of the high plateaus, 42% in the eastern dense rainforests and the Sambirano region (e.g. *Didymoglossum erosum*, *Asplenium erectum*, *Gymnosphaera boivinii*, *Osmolindsaea latisquama*); 38% in the Western domain with dense dry forests (e.g. *Selaginella helicoclada*, *Selaginella digitata*); 10% in the Southern domain with bushes and shrubs (e.g. *Cheilanthes perrieri*) (Humbert 1955; Cornet 1973; Rakotondrainibe 2003; Rakotondrainibe et al. in press). The strong affinity (92%) with the Central and Eastern domains is again due to the geographical position and geomorphology of the Isalo massif, which allows the humid easterly trade winds to reach the massif and pour into the canyon valleys of east-facing canyons that we have studied, which are then subject to a fairly sub-humid climate, similar to that of the Central domain (Fig. 1B). Such an intrusion of a sub-humid climate influence into an arid regional context has also been observed in the Daraina region (Binara forest) in northern Madagascar (Goodman and Wilmé 2006; Rakotondrainibe and Andriambolantsoa 2006) or in the Sambirano region.

Apart from these strong affinities, some species such as *Odontosoria chinensis*, *Stenochlaena tenuifolia* are present from north to south in many riparian ecosystems, at the interface between the flowing water and the beginning of the major riverbed as well as in other tropical countries (Walker and Sharpe 2010; Reeb et al. 2012; Rakotondrainibe et al. in press). These riparian species belong to the azonal wetland system (Moat and Smith 2007), which means that specific local ecological conditions (soil, substrate) influence the communities more than regional ecological factors (Macintyre and Mucina 2021).

Ferns are the most abundant tracheophytes found in riverbeds, due to their widely dispersed spores and their high frequency of vegetative reproduction (Walker and Sharpe 2010). Co-inertia analysis confirms the

relationships between local ecological conditions (plots) and the presence of fern species (Fig. 7B, C), with canopy cover and riverbed width exerting a stronger influence. The L index (canopy and rocks/cliff cover) is a factor that influences the presence of pteridophytes in a plot, which contrasts with the conclusions of other studies of riparian pteridophyte communities that focused on a single river portion each (Paixão et al. 2013). This difference may be attributed to the environmental heterogeneity of plots within each canyon (Fig. 3) and their different geomorphological characteristics, where shading is provided by high cliffs surrounding narrow plots (narrow riverbeds) or by tree shade in open areas or by a combination of tree shade and cliffs.

Finally, ferns also reflect human disturbance, even in a privileged, protected environment such as the Isalo massif. The creeping fern *Pteris aquilinum*, the scrambling ferns *Dicranopteris linearis*, and *Sticherus flagellaris* are characteristic of anthropogenic disturbance (Walker and Sharpe 2010), and are favoured by activities such as slash-and-burn or deforestation (Autrey and Bosser 2008; Robinson et al. 2010; Reeb et al. 2012; Yang et al. 2021). Although these ferns are regularly present in the Isalo canyons, they occur in small populations. Monitoring the presence of such ferns can provide insights into ecosystem evolution, with an increase indicating an increase in anthropogenic disturbance. However, Yang et al. (2021) argues that the invasive colonization of barren areas by *Dicranopteris* could contribute to ecosystem recovery, providing an interesting nuance to the general perception of the negative effects of *Dicranopteris* invasion.

CONCLUSION AND PERSPECTIVES

The Isalo massif hosts a mosaic of original fern communities, including both common and rare species that exploit specific local conditions. The study confirms the complex interactions between a multiplicity of factors at different scales to explain the structure of pteridophyte communities. The regional conditions (regional climate, position of Isalo in the southwest of Madagascar), the local scale level caused by the different canyon conditions (geomorphology, orientation, etc.), the fine-scale influences at the plot level, the biology and niche requirements of each fern species, their evolutionary history (e.g. dispersal) ultimately influence the presence or absence of a fern species in a particular area.

Thanks to the protected status of Isalo National Park, the canyons of Isalo are mostly subject to natural disturbances, such as extreme flooding. The biology of ferns gives them advantages to survive in such unstable environments (Walker and Sharpe 2010), but indirect threats can affect the canyon environment, such as siltation due to erosion of domes, plateau, or slopes, after deforestation or repeated fires. We have witnessed several fires in the Andramanero area during the fieldwork.

Sand favours the establishment of species such as *Equisetum ramosissimum* and *Pteridium aquilinum*, which can be good indicators of siltation. Other species such as *Didymoglossum erosum*, a filmy fern, could be good candidates for studying the evolution of the canyon's climate and the impact of climate change on species biodiversity; this species has a low tolerance to dry atmosphere, and its potential loss could alert to such changes. Tree ferns could also be monitored, especially to control potential and illegal exploitation (no data were found on this subject).

The accessible eastern part of the Isalo National Park was investigated for this study and in those of Rakotomalala et al. (2022). Considering the geomorphology of the studied area (exposure to trade winds, foehn effect on the eastern façade), it seems necessary to explore the western façade, which is not exposed to the same conditions. The fern flora there may reveal surprises and very different patterns of diversity, as shown by a first exploratory study in 2023 (Germinal Rouhan pers. comm.). Our study focused on the valleys of the canyons: the summits, the slope grasslands, and the rocky formations were not investigated by the plot protocol, but only by exploratory collections. In the dry season, ferns in these areas are quite difficult to see and to collection and exploration during or after the rainy season will probably reveal new information about this amazing sandstone massif.

Finally, it would be very interesting to compare the fern diversity of the Isalo massif with the larger sandstone massif of the Makay north of the Mangoky River, in a very similar geomorphological and geological context. It has recently shown that the aquatic Adephaga (aquatic Coleoptera) diversity was lower in the Makay than in Isalo (Ramahandrison et al. 2022), and climatic or site considerations (Makay is much drier than Isalo, the substrate of the canyons is mostly sandy, enrichment of the water with iron bacteria) have been proposed to explain this difference. The study of the fern flora of Makay would allow interesting comparisons to be made. At the same time, the exploration of the northern and western parts of the Isalo National Park could reveal more diversity including endemic species.

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SUPPLEMENTARY MATERIALS

Supplementary material 1

Location and details of the plots in the Isalo National Park.

<https://doi.org/10.5091/plecevo.101827.suppl1>

Supplementary material 2

Plot type definition, and illustration of the different types.

<https://doi.org/10.5091/plecevo.101827.suppl2>

Supplementary material 3

Canopy image treatment: the method used to evaluate the canopy opening, with a repeatable method, using a simple camera and a binary image treatment with ImageJ.

<https://doi.org/10.5091/plecevo.101827.suppl3>

Supplementary material 4

Dataset for ecological factors for the plots analysed during this study.

<https://doi.org/10.5091/plecevo.101827.suppl4>

Supplementary material 5

Dataset for fern abundances in the 80 plots.

<https://doi.org/10.5091/plecevo.101827.suppl5>

Supplementary material 6

Check-list of the ferns from the Isalo massif, with a selection of specimens.

<https://doi.org/10.5091/plecevo.101827.suppl6>

Supplementary material 7

Taxonomic fern diversity at the family and the genus level in the Isalo massif, compared to the fern flora of the whole of Madagascar.

<https://doi.org/10.5091/plecevo.101827.suppl7>

Supplementary material 8

Co-inertia: position of the plots sampled.

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