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1 Ferns and clubmosses in the eastern canyons of Isalo National Park

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3 **Diversity and distribution of ferns (Filicophytes) and clubmosses (Lycophytes) in the**
4 **eastern canyons of Isalo National Park, Madagascar**

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17

18 **Abstract**

19

20 **Background and aims** – In contrast to the flowering plants, the pteridophyte flora of
21 Madagascar is still understudied. While several studies have been published on the eastern
22 and central parts of the island, there are currently few works or scientific publications
23 dedicated to the pteridophytes of southwestern Madagascar. The aim of the present work is to
24 increase the knowledge of the pteridophyte flora of the Isalo massif in southwestern
25 Madagascar. It presents 1) a checklist of Isalo's pteridophytes and 2) an analysis of the
26 diversity and distribution patterns of pteridophyte communities across ecological gradients in
27 the eastern canyons of Isalo.

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

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

43

44 **Keywords**

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

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INTRODUCTION

48

49 The two lineages Monilophytes (ferns and horsetails) and Lycophytes (club mosses, spike

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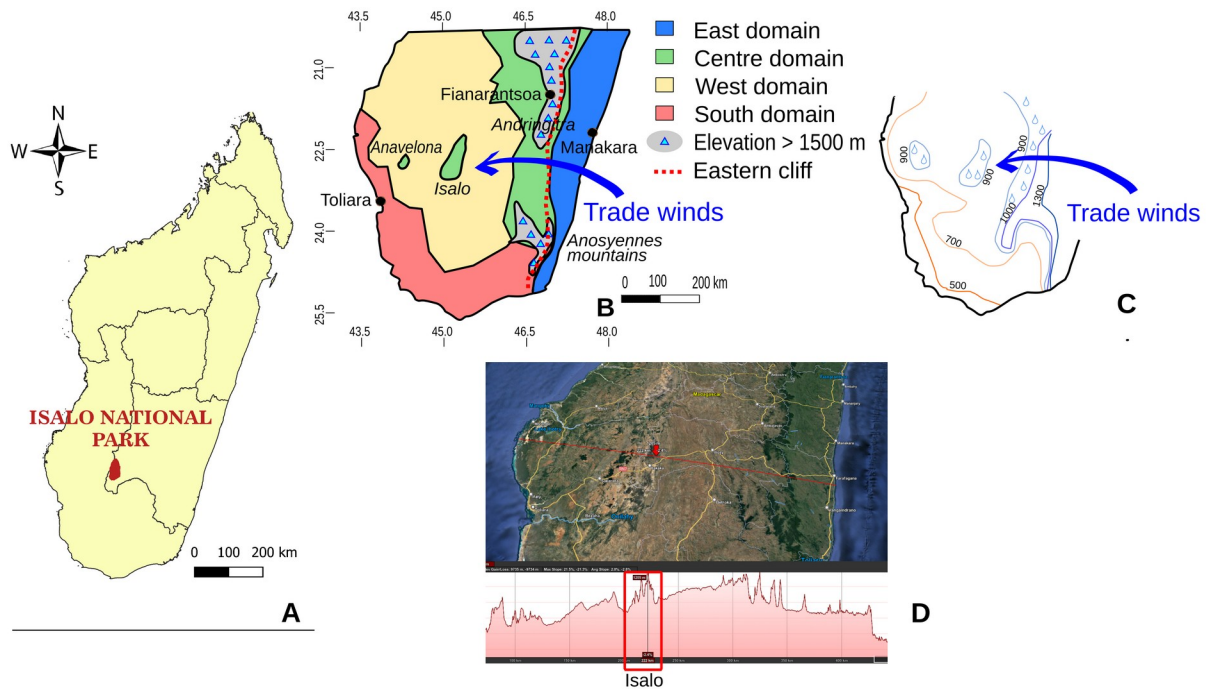
50 mosses and quillworts) were the first vascular plants to colonise land (Ranker and Haufler
 51 2008). They are traditionally referred to as “pteridophytes”, or ferns and allies, a group that
 52 cannot be recognised as a taxon due to its paraphyly but is used for convenience (PPG I
 53 2016). Pteridophytes are the second most diverse group of vascular plants, comprising around
 54 12000 species. They are reliable bioindicators, closely linked to environmental factors,
 55 readily recognizable and monitored, particularly in riparian ecosystems (Paixão et al. 2013;
 56 Della and Falkenberg 2019; Bonari et al. 2022).

57 Madagascar is recognised as one of the world’s biodiversity hotspots (Myers 2000)
 58 displaying over 90 % endemism in various animal taxa (e.g. 100 % for amphibians, 92 % for
 59 reptiles; Vences et al. 2009) and 82 % in vascular plants. Endemism is lower for spore-
 60 dispersed plants (50 % for ferns) (Rakotondrainibe et al. in press). Madagascar hosts the most
 61 diversified pteridophyte flora in the African region, with a species richness equivalent to that
 62 of the entire continental Africa, which is approximately 50 times larger in area (Aldasoro et
 63 al. 2004; Bauret et al. 2017; Rouhan and Gaudeul 2021). Rakotondrainibe (2003) reported
 64 586 taxa of ferns and lycophytes, including 38 infraspecific taxa, in Madagascar with 40 %
 65 endemism. A new version of the “Flora of the Pteridophytes of Madagascar”, led by F.
 66 Rakotondrainibe, reports 739 taxa with more than 50 % endemism (Rakotondrainibe et al., in
 67 press). The distribution of the Malagasy pteridophyte species across the five defined
 68 bioclimatic domains in Madagascar (Humbert 1955) shows that approximately eighty-five
 69 (85 %) of the pteridophytes found in Madagascar were reported from the Central and Eastern
 70 domains (Rakotondrainibe and Raharimalala 1996, 1998; Rakotondrainibe 2000a, 2000b,
 71 2002, 2003; Reeb et al. 2012), which are characterized by humid or subhumid forests and a
 72 high altitudinal range (Humbert and Cours-Danes 1965; Cornet 1972; Du Puy and Moat
 73 1996). However, little is known about the diversity and endemism of pteridophytes in the
 74 Western and Southern domains of Madagascar, subject to arid or subarid conditions
 75 (Humbert and Cours-Darne 1965; Cornet and Guillaumet 1976; Du Puy and Moat 1996).

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

91 In order to determine richness of the pteridophyte of the Isalo massif and how those
 92 communities are organised in the canyons, this study aims to achieve the following
 93 objectives: (1) produce a check-list of ferns and lycophytes found in Isalo National Park
 94 using our new collected samples and herbarium records, (2) investigate the diversity and
 95 distribution patterns of pteridological communities of the eastern canyons of Isalo, depending
 96 on canyons and the plots characteristics.

97



98
 99 **Figure 1.** **A.** Location of Isalo National Parc in Southwestern of Madagascar. **B.** Distribution of the
 100 phytogeographic domain in the Southern Madagascar showing the incursion of the Central Domain
 101 (subhumid) in Isalo Massif and Anavelona area; the elevation gap < 1500 m between northern and
 102 southern mountains let the trade winds blow from East to West. **C.** Simplified isohyets (mm/year)
 103 for the Southern Madagascar, from the eastern escarpment to the Western coast, showing the highest
 104 humidity in Isalo National Parc (and Anavelona area) (modified from Guillaumet et al 2008). **D.**
 105 Profile view of a West-East transect passing through Isalo massif to the Indian Ocean (Google earth
 106 profile view).

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110 Study area

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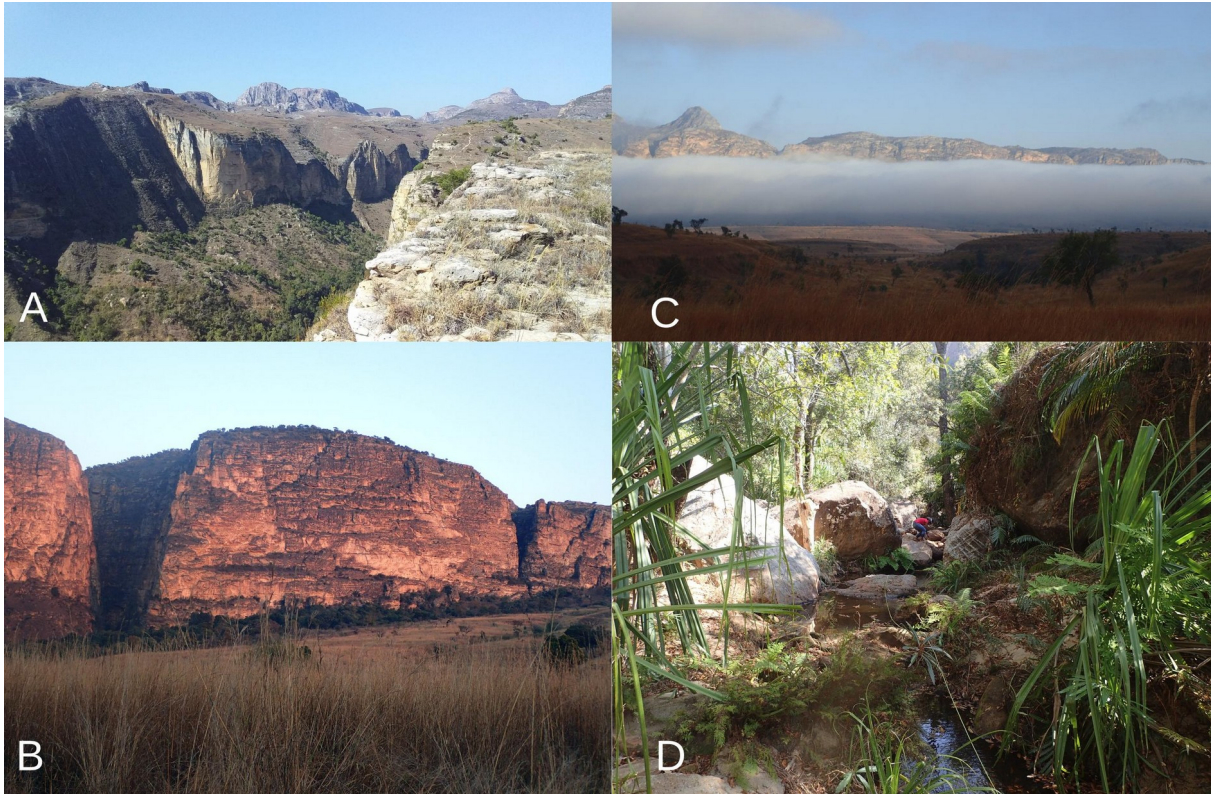
112 Isalo National Park is located in southwestern Madagascar, lying between 22°13 – 22°45
 113 South and 45°13 – 45°29 East and belongs to the Ihorombe region (Ihosa district) and the
 114 Atsimo-Andrefana region (Ankazoabo district) (Fig. 1A). Covering an area of 815,4 km², the
 115 Isalo National Park is the fourth largest protected area in Madagascar, and is managed by
 116 Madagascar National Parks since 2017 (MNP 2017). It was one of the first protected areas in
 117 Madagascar, since 1962, and was declared a national park in 1991 (Rakotomalala et al.
 118 2022).

119 The Isalo massif belongs to the youngest series (Triassic-early Jurassic) of the Malagasy
 120 Karoo formation (Sourdat 1970; Wescott and Diggins 1998), and consists of crossbedded
 121 sandstones and conglomerates up to 6000 m thick which are considered a fluvial origin
 122 (Geiger et al. 2004). The deposits have been sculpted by both water and wind, creating a
 123 majestic ruiniform relief, where the plateau grassland is dissected by a network of deep
 124 canyons, up to 200 m deep (Fig. 2A, B). The elevation of the Isalo National Park ranges from
 125 510 to 1268 m (Świerkosz 2012) with two alternating contrasted seasons, a wet season
 126 (October-March) and a dry season (April–September), hence its subhumid climate (Koechlin
 127 et al. 1974) (Fig. 1B, C). On the coldest days, fog can form in the canyons, buffering the
 128 effects of the dry season (Fig. 2C).

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

134

135 The vegetation of the Isalo massif belongs to the western slopes of the Central Domain
136 (Humbert 1955), in a regional subarid climate (Cornet and Guillaumet 1976). Świerkosz
137 (2012) recognised six vegetation types, based on a general transect of the different
138 geomorphological micro-reliefs of the Isalo: secondary bush on the lowest slopes accessible
139 to anthropogenic activities, rock communities (*Pachypodium*, *Aloe*) on the top of the highest
140 ruiniform relief, sclerophyllous woodland (*Uapaca*) and pseudosteppe (*Aristidia* group) on
141 the plateau, gallery forest (*Pandanus*) or evergreen humid forest in the canyons or river
142 valley (Rakotomalala et al. 2022) (Fig. 2A, D).

143 This study explored six canyons that open on the southern part of the eastern face of the Isalo
144 Massif, which is a very steep cliff abruptly cut by a few East-West canyons. From south to
145 north these canyons are named as Namaza canyon, Maki canyon, Rats canyon, Anjofo
146 canyon, Andramanero canyon, Antsifotra canyon (Supplementary material 1A).

147

148 **Taxonomic sampling and analysis**

149

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

156

157 **Ecological survey**

158

159 ***Sampling design***

160 Ecological surveys were adapted from Rakotondrainibe (1989), Rakotondrainibe and
 161 Raharimalala (1996 1998), and Reeb et al. (2012). A plot is an area established in the
 162 riverbed of the canyon. Its central axis was determined by a 20 m string placed in the centre
 163 of the riverbed (Fig. 2D). The lateral limits of the plot are defined by the highest flood marks.
 164 Abiotic characteristics and fern abundance were recorded (see below). Four plots of 20 m
 165 long were successively sampled in a homogeneous part of the canyon, both in terms of
 166 vegetation and topology. Finally, an area of at least 800 m² in similar conditions is reached,
 167 which is considered the minimal area for fern studies, even along a river (Rakotondrainibe
 168 1989; Reeb et al. 2012).

170 ***Plot type definition***

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

176 ***Fern abundance***

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

184 ***Ecological factors***

185 To understand the factors that shape the diversity and distribution of pteridophyte
 186 communities, we considered ecological factors related to the vegetation, morphology and
 187 riverbed characteristics of a canyon (Table 1). The riverbed is the area permanently or
 188 temporarily occupied by water. Two main parts can be defined in a riverbed (Piegay 2019):
 189 the minor riverbed which is permanently occupied by flowing water in the lowest parts of the
 190 canyon, and the major riverbed which is only filled with water during floods. These higher
 191 limits are generally easy to find in the canyons because the debris load of the highest floods is
 192 still embedded in the terrestrial vegetation and because of the change in terrestrial vegetation
 193 between flood affected/non-affected zones.

194
 195 To assess the effect of light penetration in the canyon on pteridophyte diversity, we
 196 developed an index L (L-index) which is related to the combination of canopy and rock/cliff
 197 cover that act as barriers to light penetration in the canyon. To measure this L index, we
 198 developed a simple and repeatable method based on the analysis of canopy images using Fiji-
 199 Image J (Supplementary material 3) (Schindelin et al. 2012).

200
 201 Descriptions of the ecological factors are given in Table 1. The data analysed in this paper are
 202 presented in two different data tables (Supplementary materials 4 and 5). The plots are the
 203 individuals (rows); in Supplementary material 4, the ecological factors are the variables,
 204 quantitative and qualitative; in Supplementary material 5, the abundance of fern species is the
 205 variable.

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

208

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 stream ripples, (4) running water with stream ripple 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)
Stones in the waterbed	Ordinal: (1) no stone, (2) gravels < 0.1 m, (3) 0.1 m < stones < 0, 5 m, (4) 0.5 m < stones < 1.5 m, (5) stones > 1.5 m high.
Trees and Pandanus density	Ordinal: (1) no tree in the canyon, (2) 2-3 scattered trees, (3) > 3 trees with a diameter > 10 cm. (1) no Pandanus in the plot, (2) 2-3 scattered Pandanus, (3) > 3 Pandanus
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) presence (2) absence
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 (meters); the position of the origin is just the opening of the cliff; negative values are considered in the plain, positive values are considered in the canyon

209

210

211 **Statistical analysis**

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

219

220 *Analysis of similarity between plots according to ecological factors*

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

233

234 *Pteridophyte community analysis*

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

243 Secondly, we measure the similarity of species composition at two levels: at the 6 canyons
 244 catchment level and at the plot types level (Supplementary material 1 & 2) by using Non-
 245 Metric Multidimensional Scaling (NMDS). This produces ordination graphs that highlight
 246 dissimilarities between classes (*i.e.* dissimilarities between the 6 canyon catchments on one
 247 hand and between plot types on the other hand in terms of species composition). NMDS was
 248 performed on a matrix of Bray-Curtis distance metrics. Species abundances was used to
 249 generate a Bray-Curtis distance metrics matrix which is then used for NMDS. Bray-Curtis
 250 distance is a dissimilarity metric suitable for species abundance data collected at different
 251 sampling locations (Gardner 2014). The NMDS process was performed using the
 252 metaMDS function from the R-vegan package (Oksanen et al. 2013), with 999 numbers of
 253 random starts and three dimensions were conserved to obtain a meaningful stress value. A
 254 non-parametric test, one-way analysis of similarity (ANOSIM, with 9999 permutations) was
 255 performed to test the significance of NMDS ordination result.

256

257 ***Canyons' flora diversity comparison***

258 To understand the distribution of species in the pteridophyte community, we use diversity indices
 259 to compare species diversity in the six canyons (Maki, Rats, Andramanero, Namaza, Anjofo,
 260 Antsifotra). The sample size differs from canyon to canyon, due to the difference in sampling
 261 effort. A rarefaction method was used because it provides a standardisation of the sample size
 262 to overcome the influence of sample size differences when comparing species diversity
 263 (Gotelli and Colwell 2001; Colwell et al. 2012). An individual-based abundance rarefaction
 264 curve for each canyon was performed, using iNExtPD package in R (Hsieh et al. 2016).

265 Three Hill Numbers q (effective number of species) were calculated: species richness ($q = 0$),
 266 Shannon diversity ($q = 1$, the exponential of Shannon entropy) and Simpson diversity ($q = 2$,
 267 the inverse of Simpson concentration) (Chao et al. 2014). These three Hill numbers allow us
 268 to compare community diversity by considering species richness, species evenness, and
 269 species dominance in the community.

270

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

273 Co-inertia analysis was used to explore the relationship between fern community patterns and
 274 the factors that may control them. Co-inertia analysis takes two multivariate data-sets as an
 275 input and searches for the pair of new axes that maximises the concordance between the two
 276 data-sets. The optimisation criterion in co-inertia analysis is that the resulting sample scores
 277 (ecological scores and floristic scores) are the most covariant (Dolédec and Chessel 1994).

278 The ecological dataset contains both quantitative and qualitative data, so a Hillsmith analysis
 279 was used (Dray and Dufour 2007). A PCA was performed on the fern abundance dataset. We
 280 maximise covariance between the two data tables and calculate the correlation coefficient R
 281 (between 0 and 1; the closer R is to 1, the stronger the correlation between the tables). The
 282 test of the significance of co-inertia was performed using a 100 co-inertia analysis after
 283 random permutation of the rows in both tables. For each run, the R^2 value was calculated and
 284 the frequency distribution of these values for the two first co-inertia axes was projected. If the
 285 observed value was higher than 95% of the values obtained for the permuted data-sets, then
 286 the co-inertia was considered significant (Dolédec and Chessel 1994). Data analyses were
 287 performed using the ade4 package in the R software.

288

289

RESULTS

290

291 **Species composition**

292

293 ***Checklist***

294

295 Sixty (60) species of ferns (Table 2A) and lycophytes (Table 2B) are recorded for the Isalo
 296 massif including ten fern species endemic to Madagascar (16 % of endemism for the Isalo
 297 massif). Thirty-eight (39) species were inventoried during the fieldwork of which 11 species
 298 are reported for the first time in the Isalo massif: *Asplenium erectum*, *Asplenium aff.*
 299 *aethiopicum*, *Doryopteris kitchingii*, *Equisetum ramosissimum*, *Lygodium kerstenii*,
 300 *Macrothelypteris torresiana*, *Nephrolepis biserrata*, *Sticherus flagellaris*, *Thelypteris*
 301 *arbuscula*, *Thelypteris dentata*, *Thelypteris unita*. Pteridaceae, Thelypteridaceae,
 302 Denstaediaceae families are the most represented in terms of richness (Supplementary
 303 material 7A). At the genus level, *Pellea* then *Thelypteris* are the two most important by their
 304 number of species (Supplementary material 7B).

23

24

305 The pteridophyte flora of the Isalo Park shows a high affinity with that of the Central
 306 Domain. (Table 2A, 2B): 92 % of Isalo's fern communities are found in the Central Domain,
 307 42 % in the eastern dense rainforests and Sambirano; 38 % in the Western Domain with
 308 dense dry forests; 10 % in the Southern Domain with scrub.

309
 310 **Table 2A.** Check-list of ferns from the Isalo massif. The nomenclature of species and family is based
 311 on Rakotondrainibe et al. (in press) using PPG1 (PPG1 2016). When possible, the corresponding
 312 nomenclature from Rakotondrainibe (2003) is given, together with the distribution in Madagascar,
 313 outside the Isalo massif (data 2003). Family names are from Rakotondrainibe et al. (in press). A
 314 selection of specimens is given in Supplementary material 6

Species (Rakotondrainibe et al. in press)	Endemic (END = endemic in Madagascar)	Species (Rakotondrainibe 2003)	Family (Rakotondrainibe et al, in press)
<i>Actiniopteris dimorpha</i> Pic.Serm.		<i>Actiniopteris dimorpha</i> Pic.Serm.	Pteridaceae
<i>Adiantum capillus-veneris</i> L.		<i>Adiantum capillus-veneris</i> L.	Pteridaceae
<i>Alsophila dregei</i> (Kunze) R.M.Tryon		<i>Cyathea dregei</i> Kunze	Cyatheaceae
<i>Alsophila hyacinthaei</i> R.M.Tryon var. <i>hyacinthaei</i>		<i>Cyathea isaloensis</i> C.Chr.	Cyatheaceae
<i>Anemia madagascariensis</i> C.Chr.	END	<i>Anemia madagascariensis</i> C.Chr.	Anemiaceae
<i>Arthropteris orientalis</i> (G.F.Gmel.) Posth. var. <i>orientalis</i>		<i>Arthropteris orientalis</i> (G.F.Gmel.) Posth. var. <i>orientalis</i>	Tectariaceae
<i>Asplenium aff. aethiopicum</i> (Burm.f.) Bech.		<i>Asplenium aethiopicum</i> (Burm.f.) Bech.	Aspleniaceae
<i>Asplenium blastophorum</i> Hieron		<i>Asplenium blastophorum</i> Hieron	Aspleniaceae
<i>Asplenium buettneri</i> Hieron. ex Brause		<i>Asplenium buettneri</i> Hieron. ex Brause	Aspleniaceae
<i>Asplenium erectum</i> Bory ex Willd.		<i>Asplenium erectum</i> Bory ex Willd.	Aspleniaceae
<i>Asplenium formosum</i> Willd.		<i>Asplenium formosum</i> Willd.	Aspleniaceae
<i>Asplenium pseudostuhlmannii</i> Viane		<i>Asplenium stuhlmannii</i> Hieron.	Aspleniaceae
<i>Blechnum attenuatum</i> (Sw.) Mett.		<i>Blechnum attenuatum</i> (Sw.) Mett.	Blechnaceae
<i>Blechnum tabulare</i> (Thunb.) Kuhn		<i>Blechnum tabulare</i> (Thunb.) Kuhn	Blechnaceae
<i>Blotiella isaloensis</i> (Tardieu) P.Roux	END	<i>Blotiella isaloensis</i> (Tardieu) P.Roux	Dennstaedtiaceae
<i>Cheilanthes bonapartei</i> P.Roux	END	<i>Adiantopsis linearis</i> Bonap.	Pteridaceae
<i>Cheilanthes hirta</i> Sw.		<i>Cheilanthes hirta</i> Sw.	Pteridaceae
<i>Cheilanthes perrieri</i> P.Roux	END	not recorded	Pteridaceae
<i>Dicranopteris linearis</i> (Burm.f.) Underw.		<i>Dicranopteris linearis</i> (Burm.f.) Underw.	Gleicheniaceae
<i>Didymoglossum erosum</i> (Willd.) P.Roux		<i>Trichomanes erosum</i> Willd.	Hymenophyllaceae
<i>Doryopteris kitchingii</i> (Baker) Bonap. ex C.Chr.	END	<i>Doryopteris kitchingii</i> (Baker) Bonap. ex C.Chr.	Pteridaceae
<i>Elaphoglossum poolii</i> (Baker) Christ.	END	<i>Elaphoglossum poolii</i> (Baker) Christ.	Dryopteridaceae
<i>Equisetum ramosissimum</i> Desf.		<i>Equisetum ramosissimum</i> Desf.	Equisetaceae

<i>Gleichenia polypodioides</i> (L.) Sm.		<i>Gleichenia polypodioides</i> (L.) Sm.	Gleicheniaceae
<i>Gymnosphaera rouhaniana</i> (Rakotondr. & Janssen) S.Y.Dong	END	not recorded	Cyatheaceae
<i>Histiopteris incisa</i> (Thunb.) Sm.		<i>Histiopteris incisa</i> (Thunb.) Sm.	Dennstaediaceae
<i>Macrothelypteris torresiana</i> (Gaudich.) Ching		<i>Macrothelypteris torresiana</i> (Gaudich.) Ching	Thelypteridaceae
<i>Marsilea minuta</i> L.		<i>Marsilea minuta</i> L.	Marsileaceae
<i>Microlepia speluncae</i> (L.) T.Moore		<i>Microlepia speluncae</i> (L.) T.Moore	Dennstaediaceae
<i>Nephrolepis biserrata</i> (Sw.) Schott		<i>Nephrolepis biserrata</i> (Sw.) Schott	Nephrolepidaceae
<i>Odontosoria chinensis</i> (L.) J.Sm.		<i>Sphenomeris chinensis</i> (L.) Maxon	Lindsaeaceae
<i>Oleandra distenta</i> Kunze		<i>Oleandra distenta</i> Kunze	Oleandraceae
<i>Osmolindsaea latisquama</i> Lehtonen & Rouhan		<i>Lindsae odorata</i> auct.	Lindsaeaceae
<i>Osmunda regalis</i> L. var. <i>obtusifolia</i> (Kaulf.) Milde		<i>Osmunda regalis</i> L.	Osmundaceae
<i>Pellaea angulosa</i> (Bory ex Willd.) Baker		<i>Pellaea angulosa</i> (Bory ex Willd.) Baker	Pteridaceae
<i>Pellaea boivinii</i> Hook.		<i>Pellaea boivinii</i> Hook.	Pteridaceae
<i>Pellaea calomelanos</i> (Sw.) Link		<i>Pellaea calomelanos</i> (Sw.) Link	Pteridaceae
<i>Pellaea dura</i> (Willd.) Hook.		<i>Pellaea dura</i> (Willd.) Hook.	Pteridaceae
<i>Pellaea pectiniformis</i> Baker		<i>Pellaea pectiniformis</i> Baker	Pteridaceae
<i>Pityrogramma argentea</i> (Willd.) Domin		<i>Pityrogramma argentea</i> (Willd.) Domin	Pteridaceae
<i>Pityrogramma calomelanos</i> (L.) Link		<i>Pityrogramma calomelanos</i> (L.) Link	Pteridaceae
<i>Psilotum nudum</i> (L.) P.Beauv.		<i>Psilotum nudum</i> (L.) P.Beauv.	Psilotaceae
<i>Pteridium aquilinum</i> (L.) Kuhn subsp. <i>capense</i> (Thunb) C.Chr.		<i>Pteridium aquilinum</i> (L.) Kuhn subsp. <i>capense</i> (Thunb) C.Chr.	Dennstaediaceae
<i>Pteris pseudolonchitis</i> Bory ex Willd.		<i>Pteris pseudolonchitis</i> Bory ex Willd.	Pteridaceae
<i>Ptisana fraxinea</i> (Sm.) Murdock		<i>Marattia fraxinea</i> Sm.	Marattiaceae
<i>Stenochlaena tenuifolia</i> (Desv.) Moore		<i>Stenochlaena tenuifolia</i> (Desv.) Moore	Blechnaceae
<i>Sticherus flagellaris</i> (Bory ex Willd.) Ching		<i>Sticherus flagellaris</i> (Bory ex Willd.) Ching	Gleicheniaceae
<i>Thelypteris arbuscula</i> (Willd.) K.Iwats.		<i>Sphaerostephanos arbuscula</i> (Willd.) Holttum	Thelypteridaceae
<i>Thelypteris confluens</i> (Thunb.) C.V.Morton		<i>Thelypteris confluens</i> (Thunb.) C.V.Morton	Thelypteridaceae
<i>Thelypteris dentata</i> (Forssk.) E.P.St. John		<i>Christella dentata</i> (Forssk.) Holttum	Thelypteridaceae
<i>Thelypteris interrupta</i> (Willd.) K.Iwats.		<i>Cyclosorus interruptus</i> (Willd.) H.Itô	Thelypteridaceae
<i>Thelypteris unita</i> (L.) C.V.Morton		<i>Sphaerosytophanos unitus</i> (L.) Holttum	Thelypteridaceae

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316

317 **Table 2B.** Check-list of Lycophytes from the Isalo massif. The nomenclature of species and family is

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318 based on Rakotondrainibe et al. (in press) using PPG1 (PPG1 2016). When possible, the
 319 corresponding nomenclature from Rakotondrainibe (2003) is given, together with the distribution in
 320 Madagascar (data 2003), outside the Isalo massif (data 2003). Family names are from Rakotondrainibe
 321 et al. (in press). A selection of specimens is given in Supplementary material 6

Species (Rakotondrainibe et al. in press)	Endemic (END = endemic in Madagascar)	Species (Rakotondrainibe 2003)	Family (Rakotondrainibe et al, in press)	Western Domain
<i>Lygodium kerstenii</i> Kuhn		<i>Lygodium kerstenii</i> Kuhn	Lygodiaceae	X
<i>Lygodium lanceolatum</i> Desv.		<i>Lygodium lanceolatum</i> Desv.	Lygodiaceae	
<i>Palhinhaea cernuea</i> (L.) Franco & Vasc.		<i>Lycopodiella cernua</i> (L.) Pic.Serm	Lycopodiaceae	
<i>Pseudolycopodiella caroliniana</i> (L.) Holub		<i>Lycopodiella caroliniana</i> (L.) Pic.Serm.	Lycopodiaceae	X
<i>Selaginella digitata</i> Spring.	END	<i>Selaginella digitata</i> Spring.	Selaginellaceae	X
<i>Selaginella echinata</i> Baker	END	<i>Selaginella echinata</i> Baker	Selaginellaceae	X
<i>Selaginella helicoclada</i> Alston ex Alston	END	<i>Selaginella helicoclada</i> Alston ex Alston	Selaginellaceae	X

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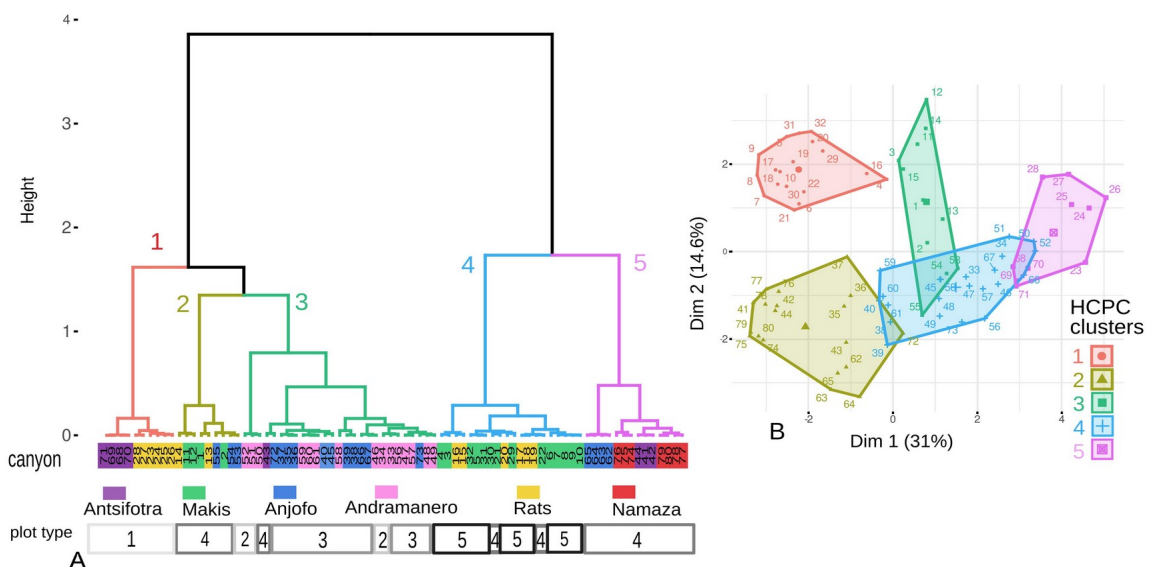
328

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

330

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

340



341

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

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347 **Ordination of pteridophyte species abundance**

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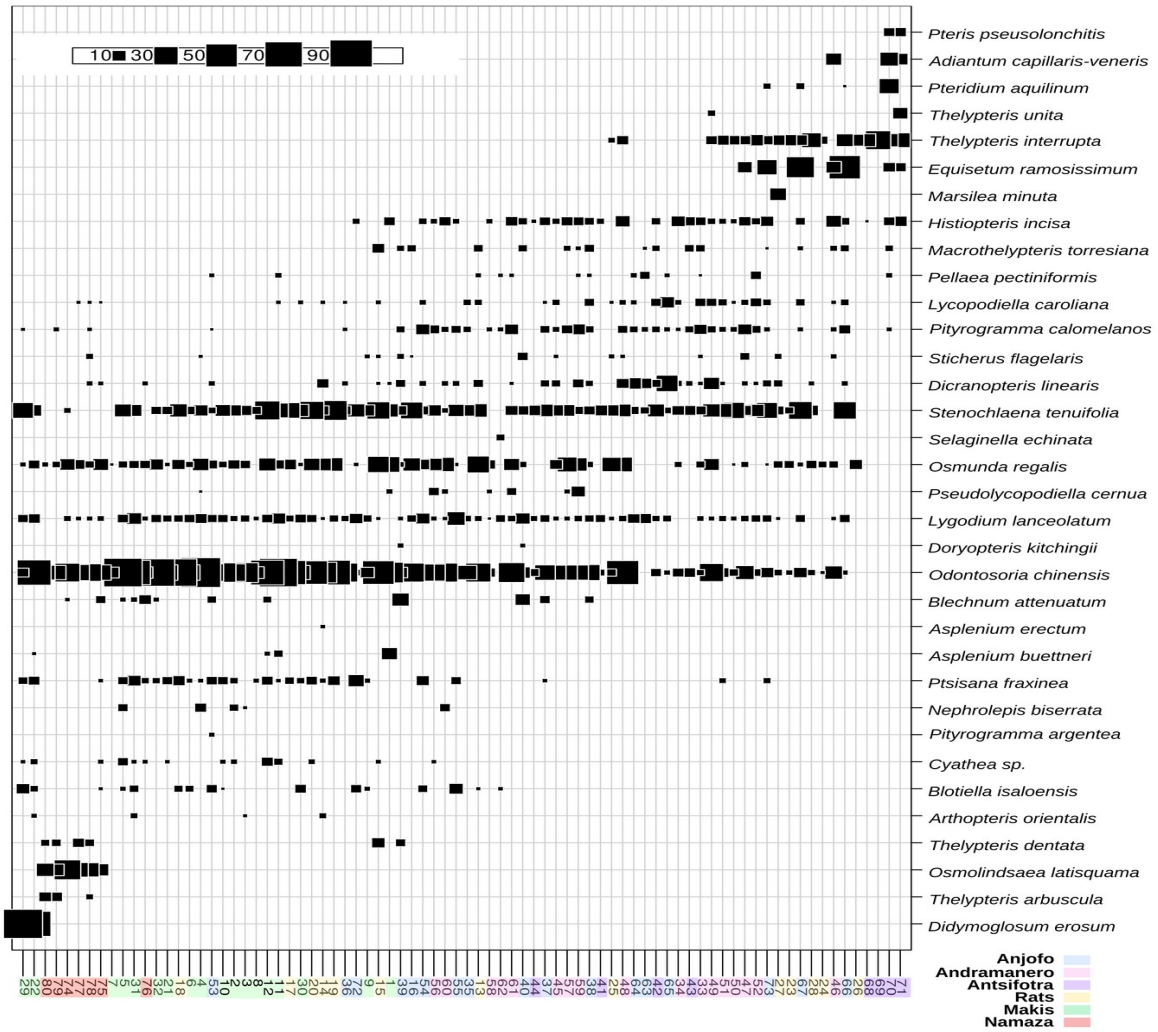
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359 **Comparison of fern communities at the level of canyon and at plot scale**

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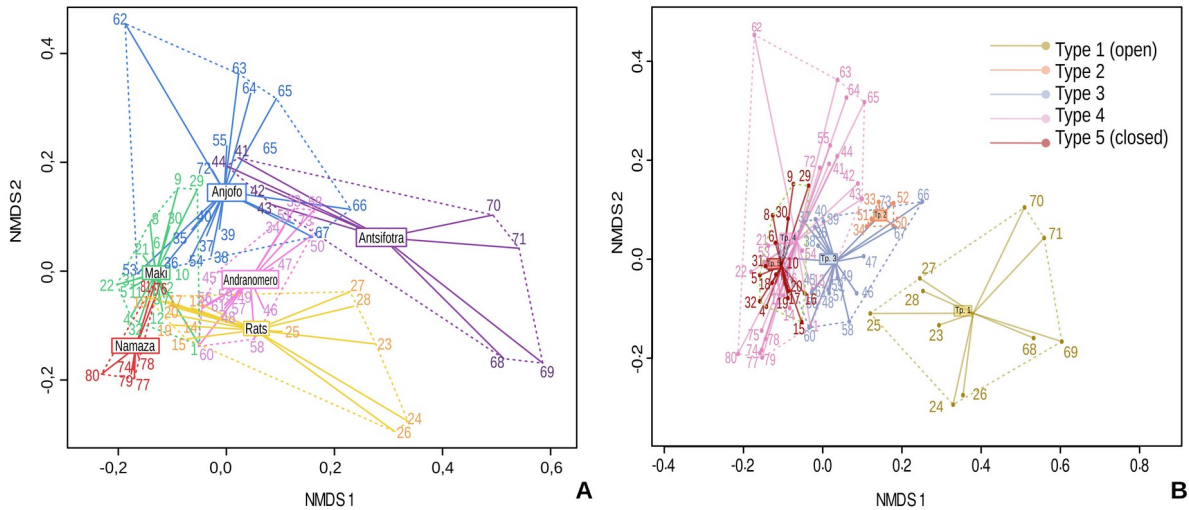
40



361
 362 **Figure 4.** Ordination of the fern abundance table on the first axis of the CA analysis, the x-axis shows
 363 the species identified, the y-axis shows the number of plots, colored according to their location (= the
 364 canyons they were sampled). The size of the black squares are proportional to species abundance.
 365

366 The similarities between the composition of the fern communities at the scale of the six
 367 canyons catchments is analysed with NMDS (Fig. 5A). The stress value $s = 0,11 (< 0,2)$ for k
 368 $= 3$, indicates that the ordination is not random. The ANOSIM value for the similarity
 369 analysis with the grouping by canyon is $R = 0.2486$, $p = 0, 0001$; the p-value (significant
 370 value) is lower than 0,05, and R is higher than p, which means that there are significant
 371 statistical differences between the fern communities of the six canyons. Some superposition
 372 is observed, but the all the centres of gravity of each catchment are distinct.

373 In a second analysis, when the composition of the fern communities is projected with NMDS
 374 according to plot type (Fig. 5B), the open plots (type 1, Supplementary material 2) clearly
 375 host different communities from plots in the internal parts of canyons. The ANOSIM value
 376 for analysis of similarities with grouping by type of plots is $R = 0,2533$, $p = 0,0001$; here
 377 again, there is significant statistical differences between the fern communities according the
 378 plot type. The communities of type 2 and 3 communities overlap, and the communities of
 379 type 4 and 5 appear to be very similar (Fig. 5B).
 380



381
 382 **Figure 5. A.** NMDS analysis according to the six canyons. **B.** NMDS analysis according the type of
 383 plots. The horizontal axis x represents the first dimension extracted by the NMDS analysis. This
 384 dimension is constructed so that the distance or dissimilarity between the points on this axis reflects as
 385 closely as possible the original dissimilarity between plots. In A colored according to the canyons, in
 386 B colored according to the type of plot. The vertical axis is the second dimension extracted from the
 387 NMDS analysis, also designed to reflect the dissimilarity between the plots, but it is orthogonal to the
 388 X axis, which means that it captures another source of dissimilarity relative to the X axis. It is
 389 important to remember that the axes in an NMDS plot are used to visualise the relative similarity or
 390 dissimilarity of the objects analysed (here plots) in a reduced dimensional space, and have no direct
 391 meaning in terms of specific variables.

392
 393 **Species diversity comparison**

394
 395 Rarefied species diversity values (Table 3) give an overview of the diversity pattern between
 396 the six canyons. The species richness accumulation curve (Fig. 6, $q = 0$) shows that Anjofo’s
 397 canyon has the highest number of pteridophyte species. Then, Andramanero, Maki and Rats
 398 have almost the same species richness, and finally, Namaza and Antsifotra canyon have the
 399 lowest number of species. However, the accumulation curve of the Shannon diversity index
 400 (Fig. 6, $q = 1$) shows that despite its low species richness, the pteridophyte community of
 401 Antsifotra canyon has a higher species evenness and is therefore more diversified than the
 402 communities of Andramanero, Maki, Rats and Namaza. Of all of the canyons, Anjofo’s
 403 pteridophyte community is the most diversified because of it has the highest species evenness
 404 and species richness. Furthermore, in contrast to the central (Maki + Rats) and the southern
 405 (Namaza) canyons, the pteridophyte flora in the northern canyons (Anjofo, Andramanero,
 406 Antsifotra) is more diverse.. The same pattern is seen in the accumulation curve of the
 407 Simpson diversity index (Fig. 6, $q = 2$), which indicates that the pteridophyte communities in
 408 the central and southern canyons tend to be dominated by a few species.

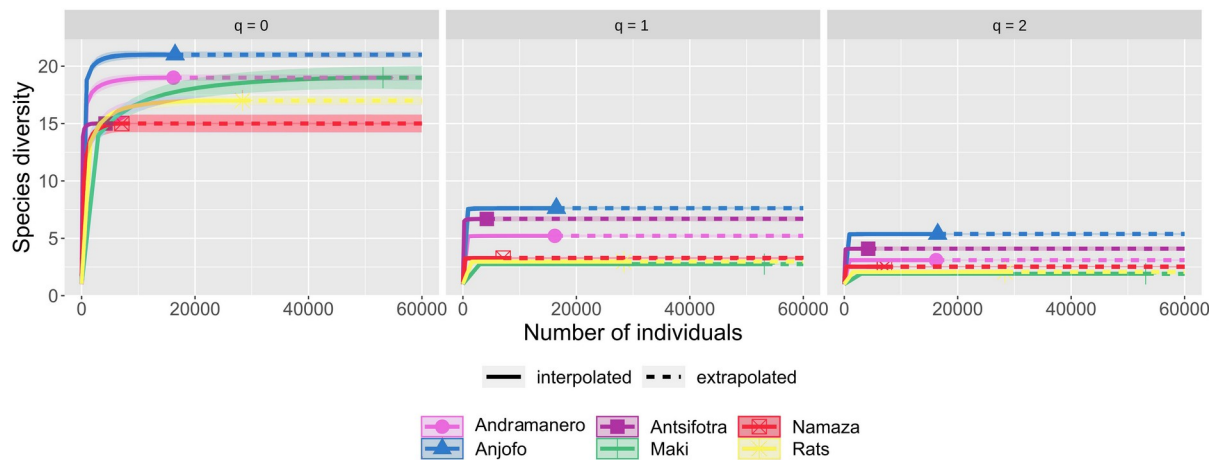
409 **Table 3.** Rarefied species diversity values of the 6 canyons. In brackets, asymptotic diversity
 410 estimate values of the 6 canyons: first number = LCL, 95% associated Lower Confidence
 411 interval Limit; second number = UCL: 95% Upper Confidence interval Limit.

Canyon	Rarefied species richness $q = 0$	Rarefied Shannon diversity $q = 1$	Rarefied Simpson diversity $q = 2$
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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]

412

413



414

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

418

419 **Exploring the links between the pteridophyte communities and the ecological factors**

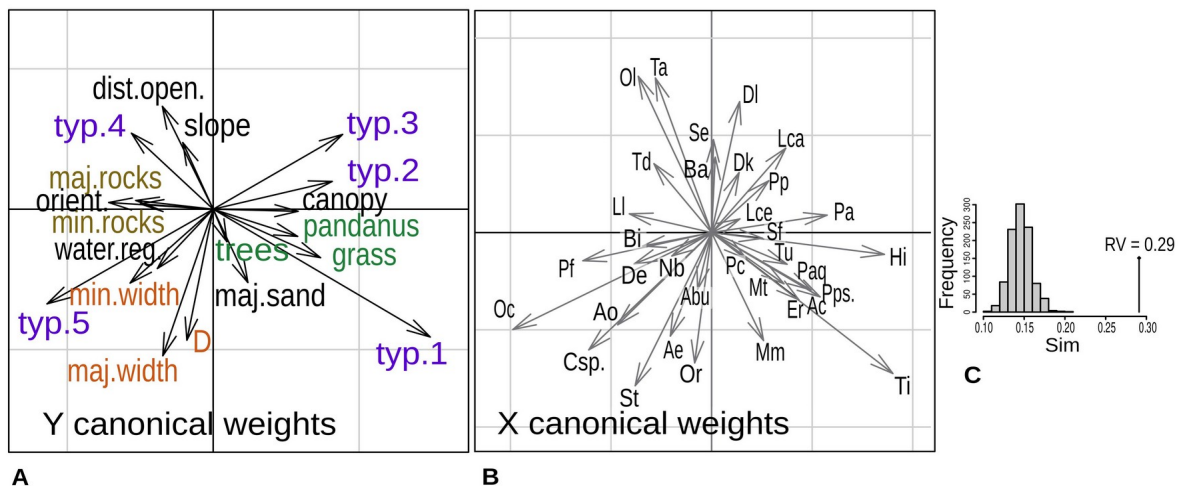
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421 The co-inertia analysis (Fig. 7) measures the concordance between the two datasets and
 422 shows a covariance between the two tables (Fig. 7D); the first axis accounts for 42 % of co-
 423 inertia and the second for 22 % (64 % for cumulative projected inertia for the first two axes).
 424 The Monte-Carlo test shows a consistent correlation between the ecological factors of the
 425 plots and the fern communities (RV = 0.29, p = 0.0001). The correlation is significant, fern
 426 distribution is correlated with a combination of environmental factors and not specifically
 427 with just one, although the influence of a few major factors can be highlighted (Fig. 7A, B).

48

49

428 On the first axis the influence of the canopy cover is inversely correlated with the orientation
 429 and the presence of rocks in the minor or major riverbed (Fig. 7A, B).
 430 On the second axis, the difference D between the width of the major and minor riverbeds is
 431 inversely correlated with the distance to the mouth of the canyon. The presence of vegetation
 432 (trees, pandanus, grasses) is positively correlated on axis 1, with lower values.
 433 In open plots, species such as *Equisetum ramosissimum* and *Thelypteris interrupta* are clearly
 434 associated with a high L-index, sandy substrate and the presence of vegetation. *Marsilea*
 435 *minuta* occurs in pools in open areas, and is negatively correlated with the distance from the
 436 mouth of the canyon. It means that *Marsilea minuta* becomes rarer as we move further
 437 towards the bottom of the canyon.
 438 On the other hand, on the negative part of axes 1 and 2, species such as *Ptisana fraxinea*,
 439 *Cyathea sp.* are strongly correlated with the very narrow canyons, surrounded by high
 440 vertical cliffs that shade the canopy. Also associated with the presence of rocky outcrops
 441 that provide anchorage are *Ptisana fraxinea* and *Didymoglossum erosum*. *Osmunda regalis*
 442 and *Stenochlaena tenuifolia* are associated with large major riverbeds. *Odontosoria chinensis*,
 443 one of the most common ferns, is probably more linked to rock presence. *Blotiella isaloensis*
 444 and *Lygodium lanceolatum*, two lianeceous species seem to be correlated with the presence of
 445 rocks in the riverbed, both minor and major. It confirms the sensitivity of fern species to
 446 environmental factors, such as rocks, sand or trees, the presence of grasses, and orientation
 447 which can influence the light intensity during the day.
 448



449 **Figure 7.** Co inertia analysis. **A.** Scatter plot representing the coefficients of the combinations of the
 450 variables for ecological variables. The colors are only informative for easier reading: “green” =
 451 vegetation presence; “brown”= riverbed dimensions; “violet” = type of plot. **B.** Scatter plot
 452 representing the coefficients of the combinations of the variables for fern species abundance. **C.**
 453 Result of the permutation test; the RV coefficient appears as different that RV expected by chance.
 454 See Supplementary material 8 for plot position in the coinertia.
 455

DISCUSSION

456
 457
 458
 459 This study contributes to the understanding of the pteridophyte flora in south-eastern
 460 Madagascar. The Isalo massif has a significant richness of 60 species compared to other
 461 geomorphologically similar areas. For example, the Makay massif, a larger sandstone massif
 462 to the north of Isalo, was reported to have only 38 species (Roubaud et al. 2018). Similarly,
 463 the Bemaraha Tsingy, a ruiniform limestone area in the mid-west of Madagascar, was
 464 reported to have only 16 species (Tsingy de Bemaraha National Park

465 https://www.madacamp.com/Tsingy_de_Bemarahana_National_Park [accessed 20.12.2022]).
 466 The low number of species recorded in these areas may be due to the lack of comprehensive
 467 surveys, with the exception of exploratory studies conducted in the Makay Massif in 2011
 468 and 2017 (Roubaud et al. 2018, Tropicos <http://legacy.tropicos.org/Person/100099987>). The
 469 high diversity of pteridophytes in Isalo can be attributed to the patchwork of ecosystems of
 470 the massif ranging from shady, cool and humid environments in narrow canyons to disturbed
 471 riverbanks, dry and hot open savannas, and rocky slopes (Fig. 2). The lower community
 472 diversity in the southern canyon (Namaza, Maki, Rats) might be explained by the higher
 473 human activity in these frequently visited areas.

474
 475 The low rate of endemism in the Isalo massif (16 % for Isalo, ~ 50 % for the Madagascar
 476 fern flora) may be attributed to the geomorphology of the area which has an influence on the
 477 tradewinds at both local and regional scales. On a regional level, the absence of significant
 478 relief (> 1500m) between the Isalo massif and the eastern coast of Madagascar allows the
 479 trade winds blowing from the east to reach the massif, thus facilitating the wind-borne
 480 transport of pteridophyte spores even from other countries or continents (Fig 1C, 1D)
 481 (Donque 1978; Guillaumet et al. 2008). The favorable moisture conditions found within the
 482 canyons of Isalo (Ferry et al., 1998) create a suitable environment for the germination of
 483 these spores. On a local scale, the low vegetation (compared to a dense forest where trees and
 484 leaves act as significant barriers) and the absence of physical barriers on the slopes, domes,
 485 and plateaus or in the through valleys of the Isalo canyons may promote dispersal of spores
 486 by wind (Karst et al. 2005; Millerón et al. 2012; Robledo-Arnuncio et al. 2014).
 487 Cyclones reaching the Isalo massif, free of any relief obstacles, dense forests, in all directions
 488 play a role in spore recruitment and dispersal (Donque 1978; Walker and Sharpe 2010).
 489 Together, these factors reduce genetic isolation, which is a major driver of speciation and
 490 endemism.

491
 492 In particular, the fern communities of Isalo show a variety of affinities with the different
 493 phytogeographical domains (Table 2). About 92 % of the fern communities in Isalo are found
 494 in the Central Domain of the high plateaus, 42 % in the eastern dense rainforests and the
 495 Sambirano region (e.g. *Didymoglossum erosum*, *Asplenium erectum*, *Gymnosphaera boivinii*,
 496 *Osmonlinsaea latisquamata*); 38 % in the Western domain with dense dry forests (e.g.
 497 *Selaginella helicoclada*, *Selaginella digitata*); 10 % in the Southern Domain with bushes and
 498 shrubs (e.g. *Cheilanthes perrieri*)-(Humbert 1955; Cornet 1972; Rakotondrainibe 2003;
 499 Rakotondrainibe et al., in press). The strong affinity (92%) with the central and eastern
 500 domains is again due to the geographical position and geomorphology of the Isalo massif,
 501 which allows the humid easterly trade winds to reach the massif and pour into the canyon
 502 valleys of east-facing canyons that we have studied, which are then subject to a fairly sub-
 503 humid climate, similar to that of the Central Domain (Fig. 1B). Such an intrusion of a sub-
 504 humid climate influence into an arid regional context has also been observed in the Daraina
 505 region (Binara forest) in northern Madagascar (Goodman and Wilmé 2006; Rakotondrainibe
 506 and Andriambolantsoa 2006) or in the Sambirano region.

507
 508 Apart from these strong affinities, some species such as *Odontosoria chinensis*,
 509 *Stenochlaena tenuifolia* are present from north to south in many riparian ecosystems, at the
 510 interface between the flowing water and the beginning of the major riverbed as well as in
 511 other tropical countries (Walker and Sharpe 2010; Reeb et al. 2012; Rakotondrainibe et al. in
 512 press). These riparian species belong to the azonal wetland system (Moat and Smith 2007)
 513 which means that specific local ecological conditions (soil, substrate) influence the

514 communities more than regional ecological factors (Macintyre and Mucina 2021).

515

516 Ferns are the most abundant Tracheophytes found in riverbeds, due to their widely dispersed
517 spores and their high frequency of vegetative reproduction (Walker and Sharpe 2010). Co-
518 inertia analysis confirms the relationships between local ecological conditions (plots) and the
519 presence of fern species (Figs. 7B, 9C), with canopy cover and riverbed width exerting a
520 stronger influence. The L-index (canopy and rocks/cliff cover) is a factor that influences the
521 presence of pteridophytes in a plot, which contrasts with the conclusions of other studies of
522 riparian pteridophyte communities that focused on a single river portion each (Paixo et al.
523 2013). This difference may be attributed to the environmental heterogeneity of plots within
524 each canyon (Fig. 3) and their different geomorphological characteristics, where shading is
525 provided by high cliffs surrounding narrow plots (narrow riverbeds) or by tree shade in open
526 areas or by a combination of tree shade and cliffs.

527

528 Finally, ferns also reflect human disturbance, even in a privileged, protected environment
529 such as the Isalo massif. The creeping fern *Pteris aquilinum*, the scrambling ferns
530 *Dicranopteris linearis* and *Sticherus flagellaris* are characteristic of anthropogenic
531 disturbance (Walker and Sharpe 2010), and are favoured by activities such as slash-and-burn
532 or deforestation (Autrey & Bosser 2008; Robinson et al. 2010; Reeb et al. 2012; Yang et al.
533 2021). Although these ferns are regularly present in the Isalo canyons, they occur in small
534 populations. Monitoring the presence of such ferns can provide insights into ecosystem
535 evolution, with an increase indicating an increase in anthropogenic disturbance. However,
536 Yang (2021) argues that the invasive colonization of barren areas by *Dicranopteris* could
537 contribute to ecosystem recovery, providing an interesting nuance to the general perception
538 of the negative effects of *Dicranopteris* invasion.

539

540 CONCLUSION AND PERSPECTIVES

541

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

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

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

563 The accessible eastern part of the Isalo Park was investigated for this study and in those of
 564 Rakotomalala et al. (2022). Considering the geomorphology of the studied area (exposure to
 565 trade-winds, foehn effect on the eastern facade), it seems necessary to explore the western
 566 façade, which is not exposed to the same conditions. The fern flora there may reveal surprises
 567 and very different patterns of diversity, as shown by a first exploratory study in 2023
 568 (Rouhan, pers. com.). Our study focused on the valleys of the canyons: the summits, the slope
 569 grasslands and the rocky formations were not investigated by the plots protocol, but only by
 570 exploratory collections. In the dry season, ferns in these areas are quite difficult to see and to
 571 collection and exploration during or after the rainy season will probably reveal new
 572 information about this amazing sandstone massif.
 573 Finally, it would be very interesting to compare the fern diversity of the Isalo massif with the
 574 larger sandstone massif of the Makay north of the Mangoky river, in a very similar
 575 geomorphological and geological context. It has recently shown that the aquatic Adephegata
 576 (aquatic Coleoptera) diversity was lower in the Makay than in Isalo (Ramahandrison et al.
 577 2022), and climatic or site considerations (Makay is much drier than Isalo, the substrate of
 578 the canyons is mostly sandy, enrichment of the water with iron bacteria...) have been
 579 proposed to explain this difference. The study of the fern flora of Makay would allow
 580 interesting comparisons to be made. At the same time, the exploration of the northern and
 581 western parts of the Isalo National Park could reveal more diversity including endemic
 582 species.

583

584

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585

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 599 guides and our cooks.

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