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1 **Recording earthworm diversity on the tropical island of Martinique using DNA barcoding**  
2 **unveiled endemic species in bromeliad plants**

3

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15

16 **Abstract**

17           In a context of accelerating biodiversity loss, some species may become extinct before  
18 taxonomists have time to collect and describe them. This is an even more critical problem for  
19 taxa with poor taxonomic knowledge, such as earthworms in the tropics. To overcome this  
20 taxonomic impediment, we investigated earthworm diversity on the tropical island of  
21 Martinique using DNA barcoding. Firstly, we sampled earthworms at 81 sites evenly  
22 distributed in two categories corresponding to natural and anthropized environments. We  
23 obtained a total of 684 sequences of a fragment of the COI gene. MOTU delimitation and  
24 morphological identification suggested that this dataset corresponded to 49 putative species, of  
25 which 16 were known exotic species. The rarefaction curve suggested that the sampling effort  
26 was not sufficient to capture total earthworm diversity in the natural environment and that many  
27 more species could be discovered. Secondly, we focused on a heterogeneous landscape around  
28 the town of Morne Rouge, where arboreal earthworms were sampled using a standardized  
29 protocol. We found 8 different species in epiphytic plants, three of which were found almost  
30 exclusively in bromeliad plants that were sampled above 1.4 metres from the ground. The  
31 presence of these species, probably endemic to Martinique and new to science, was significantly  
32 correlated to the proportion of forest land cover. The most common earthworm in these habitats  
33 was *Dichogaster andina*, an exotic species found in bromeliads and *Heliconia* plants that were  
34 sampled at every height from the bottom of the tree up to 6.8 metres. Its presence was correlated  
35 to the proportion of total river length in the landscape, suggesting that rivers may act as a  
36 dispersal vector. This study therefore highlights the great diversity of earthworms in  
37 Martinique, while emphasizing the main threats to this biodiversity, namely the alteration of  
38 habitats and the presence of invasive species.

39

40 **Keywords**

41 Arboreal earthworms, DNA barcode library, landscape variables, molecular identification,

42 peregrine species, tropical island

43

44

## 45        **1. Introduction**

46

47        Despite the acknowledged critical role of earthworms in ecosystems (Blouin et al., 2013),  
48 their species diversity in the tropics is not well known, which is mainly attributable to low  
49 sampling intensity (James et al. , 2021) and the global taxonomic impediment (Decaens et al.,  
50 2016). As describing biological diversity with traditional approaches takes place at a much  
51 slower rate than that of species loss caused by human activities (Hubert and Hanner, 2015),  
52 some earthworm species may become extinct even before it has been possible for them to be  
53 collected and described by taxonomists (Lavelle and Lapied, 2003). This is particularly true on  
54 islands, which are often considered as biodiversity hotspots due to a higher rate of endemic and  
55 threatened species, where smaller population sizes and greater vulnerability to disturbance  
56 events make species more prone to extinction (Fernández-Palacios et al., 2021).

57        The 1128 km<sup>2</sup> volcanic island of Martinique belongs to one of the 36 biodiversity hotspots  
58 in the world (i.e. the Caribbean Islands Biodiversity Hotspot, Mittermeier et al., 2004) and is  
59 typical of many mountainous islands in the Lesser Antilles arc, with a volcanic geological  
60 history and a diverse microclimate resulting in pronounced habitat zonation (Ogden and  
61 Thorpe, 2002). While the South of the island is characterized by low altitude hills covered by  
62 semi-deciduous tropical forest and a large proportion of agricultural areas, the North of the  
63 island is mountainous and dominated by ancient rainforests and cloud forest. Such natural  
64 habitat heterogeneity usually contributes to species diversity through an increase in available  
65 niche spaces, provision of refuges and opportunities for isolation and divergent adaptation  
66 (Stein et al., 2014). Human activities, such as urbanization and conversion of pristine  
67 ecosystems into agricultural lands may break up natural habitats into smaller habitats that may  
68 no longer support many species, resulting in a loss of biodiversity (Wilson et al., 2016). We

69 assumed that landscape fragmentation due to human activities might affect the species diversity  
70 of earthworms in Martinique (e.g. Vanbergen et al., 2007).

71 This may be particularly true for rare species living in unexpected habitats, such as epiphytic  
72 soils present inside bromeliads. Earthworms have already been recorded inside bromeliads in  
73 several countries of the Caribbean and Central America, such as Costa Rica (Righi and Fraile  
74 Merino, 1987), Dominica (Richardson et al., 2006), Guadeloupe (James, 1996; James and  
75 Gamiette, 2016), Mexico (Fragoso and Rojas-Fernández, 1996), Nicaragua (Sherlock et al.,  
76 2011) and Puerto Rico (Richardson et al., 2006), but the presence of earthworms in bromeliads  
77 is probably much more widespread than currently documented. Studies on the ecology of  
78 invertebrates living in bromeliads have focused mainly on the aquatic fauna living in the  
79 phytotelma reservoir (Dézerald et al., 2014; Céréghino et al., 2018), but the outer leaf axils  
80 often harbour an accumulation of organic matter highly suited to the development of  
81 earthworms. Studies of earthworm communities from French Guiana (Decaens et al., 2016;  
82 Maggia et al., 2021) using DNA barcoding (i.e. use of the DNA sequence of a 658-bp fragment  
83 of the mitochondrial gene cytochrome c oxidase I (COI) to make species-level identification,  
84 Hebert et al., 2003), revealed that arboreal microhabitats can harbour a wide diversity of  
85 earthworm species, some of which are not observed in soils. While French Guiana is covered  
86 by a vast Amazonian forest that is still well preserved, Martinique is an island territory  
87 undergoing strong anthropic pressures, where agricultural areas are intermingled with natural  
88 environments. The issue of the conservation of arboreal earthworm biodiversity in such a  
89 fragmented island landscape is particularly sensitive because it requires patches of forest  
90 vegetation sheltering particular epiphytic vegetation. Furthermore, the difficulties related to  
91 taxonomy and recognition of neotropical earthworms is a real obstacle to studies on arboreal  
92 earthworms.

93 DNA barcoding can be considered as a solution for studying earthworm biodiversity in such  
94 a context. Indeed, it has proved to be a powerful approach when dealing with hyper-diverse  
95 tropical fauna by facilitating the delineation of new evolutionary lineages representing instances  
96 of new species, sometimes at unexpected rates (Hubert and Hanner, 2015). Species delineation  
97 was not originally conceived as a primary purpose of DNA barcoding (Hebert et al., 2003), the  
98 principle of which was based on two basic steps: (1) building the DNA barcode library of known  
99 species and (2) matching the barcode sequence of the unknown sample against the barcode  
100 library for identification. Species identification can thus be acquired if a barcode sequence is  
101 compared to sequences of conspecifics deposited in a database for which the taxonomic name  
102 is already known (i.e. DNA barcode library, Hebert et al., 2003). However, in poorly studied  
103 taxonomic groups with an incomplete DNA barcode library, DNA barcoding can be carried out  
104 before conventional taxonomic work to quickly sort specimens into genetically divergent  
105 groups (i.e. Molecular Operational Taxonomic Units or MOTUs), thus providing a quick start  
106 for the taxonomic process. Moreover, with DNA barcoding morphologically unidentifiable  
107 specimens, such as juvenile earthworms or cocoons, as well as cryptic species, can be taken  
108 into account, unlike with the traditional taxonomy identification method (Maggia et al., 2021).  
109 The term “cryptic species” refers to species that cannot readily be distinguished  
110 morphologically but can be distinguished with molecular data (Struck et al., 2018). In some  
111 cases, it has been shown that “cryptic” species can be distinguished morphologically *a*  
112 *posteriori*, after a molecular study such as a DNA barcoding approach, thereby becoming  
113 “pseudo-cryptic” species (Korshunova et al., 2019). Several complexes of cryptic earthworm  
114 species have been revealed over the last ten years or more, thanks to molecular studies (e.g.  
115 King et al., 2008; Dupont et al., 2011; Taheri et al., 2018a), but in-depth morphological  
116 description work to succeed in distinguishing them morphologically has generally not been  
117 carried out. Molecular identification using DNA barcoding, in addition to an intensive sampling

118 strategy, therefore remains the only way to gain access to the true species diversity of  
119 earthworms and thereby avoid underestimating it.

120 The main aim of this study was to use barcode-based species delimitation to reveal the  
121 diversity of earthworms in Martinique. Specifically, the objectives of the study were to (i)  
122 construct a DNA barcode library of earthworms from Martinique that can be used as a reference  
123 in future studies of earthworm biodiversity in the Caribbean islands, (ii) assess the overall  
124 diversity of earthworm species in Martinique using DNA barcoding and (iii) focus on the impact  
125 of human activities on earthworm biodiversity, and more particularly arboreal earthworms, on  
126 the scale of Martinique.

127

128



129        **2. Material and methods**

130

131        **2.1 Earthworm sampling**

132            Two distinct sampling strategies were used for the study. Firstly, to construct the DNA  
133 barcode reference library, the objective was to collect as many different specimens as possible  
134 throughout the island. The sampling effort was greater in the north of the island, characterized  
135 by the presence of forests, and where the presence of endemic species was expected. For this  
136 first aim of the study, samples were opportunistically collected at 81 sites (Fig. 1). Because  
137 complementary sampling methods may improve biodiversity estimates (Bosch et al., 2017;  
138 Adao et al., 2022), various sampling schemes that were not standardized were used and took  
139 place at different time periods. Specimens were searched in all microhabitats usually colonized  
140 by earthworms in tropical regions. The soil was explored at depths ranging from 10 to 40 cm  
141 depending on the depth of the bedrock; and the other main microhabitats prospected were, in  
142 order of importance: bromeliads, dead wood on the ground, moss mantles covering trunks,  
143 decaying non-woody plant pseudostems (e.g. banana, heliconia), aerial dead wood, the axils of  
144 palm leaves and any accumulations of soil and aerial litter (Fig. 2). A few samples were  
145 collected in canopy habitats at more than 30 m above ground level, but the majority of the  
146 arboreal habitats surveyed were at a height between ground level and 7m as was the case in the  
147 second part of the study (see below). Earthworms were sampled by active collection in  
148 preference to repellent extraction methods which are often ineffective in many tropical regions.  
149 The sampling sites were classed in two categories: 38 sites were situated in a natural  
150 environment (i.e. without anthropogenic influence in a circle with a radius of approximately  
151 200 m), and 42 sites were situated in an anthropized environment (i.e. near dwellings, houses,  
152 roads or agricultural areas). The list of locations and sampling dates is available in the  
153 supplementary data (SI Table 1).

154 For the second aim of the study, which was to investigate arboreal earthworm  
155 community structures in disturbed habitats, we focused on 18 sites situated around the town of  
156 Morne Rouge in northern Martinique, which extends from the Pitons du Carbet (six peaks  
157 exceeding 1000 m) on the southern limit up to Mount Pelée (1398 m) on the northern limit, and  
158 under various levels of anthropogenic pressure. Eight sites were situated in natural  
159 environments (i.e. characterized by an absence of field, house, or road) and the other 10 sites  
160 were situated in anthropized environments. The sites were pre-selected beforehand by checking  
161 for the presence of habitats favouring the existence of epiphytic vegetation (i.e. tropical  
162 rainforest, linear hedges, gullies bordered by forest patches). At these sites we used a  
163 standardized sampling protocol. Each sampling point consisted of a 10 m-radius circle centred  
164 on a geolocated point in which earthworms were sought in the following arboreal microhabitats  
165 over a fixed period of one researcher-hour: phytotelma plants (bromeliads and decomposing  
166 *Heliconia* inflorescences), axils of tree branches, tree fern petioles and epiphytic moss. No  
167 discrimination was made between phytotelma plant species, but the bromeliad species were  
168 mainly *Vriesea ringens*, *Guzmania lingulata* and *Glomeropitcairnia penduliflora*, and the  
169 *Heliconia* species were mainly *H. caribea* and *H. bihai*. Adults and juvenile specimens were  
170 collected in environments accessible to humans or reachable using a pole to reach habitats up  
171 to 7 m from the ground. The specimens were stored in a box containing organic matter from  
172 the micro-habitat in which they were found. In the laboratory, the earthworms were cleaned up  
173 in water and anaesthetized in 10% alcohol, before being fixed in a 70% ethanol solution and  
174 stored at -20°C for molecular analysis.

175

## 176 **2.2 Morphological identification**

177 We used a process of iterative taxonomy (Yeates et al., 2011) to define and refine  
178 species boundaries using morphological and molecular evidence. Earthworms were

179 morphologically identified in three steps. Firstly, the sampled earthworms were grouped into  
180 34 morphotypes based on external morphological data. These were adults and some of the  
181 juveniles. Secondly, using both external (numbers of setae per segment, distances between setal  
182 lines, location and shape of clitellum, shape of prostomium, pigmentation, visible genital pores,  
183 modified setae, and genital markings including tubercula pubertatis if appropriate) and internal  
184 characters (location and number of gizzards, numbers and types of calciferous glands, locations  
185 of hearts, male reproductive organs, spermathecae, nephridia per segment, intestinal  
186 characters), some of the morphotypes could then be assigned unambiguously to well-known  
187 species using taxonomic keys (Blakemore, 2002; Chang et al., 2016; Gabriac et al., submitted),  
188 while others could only be assigned to morphospecies. For the Glossoscolecidae and  
189 Rhinodrilidae, we used the keys of Righi (1996) and Zicsi (1995), and for the Dichogaster  
190 species we used James and Gamiette (2016) and James (1996, 2004). A genus could be assumed  
191 for some of these morphospecies, but not for all because some genera cannot be distinguished  
192 on specimens lacking sexual characters. Thirdly, morphological identification took place after  
193 the DNA barcoding step. Once the specimens had been grouped into genetically divergent  
194 groups (i.e. MOTUs), it was possible to refine the description and identification of the  
195 morphospecies by more detailed examinations and comparisons of characters among the  
196 MOTUs. This was mostly necessary for the presumed endemic species. We scored a standard  
197 set of anatomical characters, both external and internal, the latter being obtained from dorsal  
198 dissections.

199

### 200 **2.3 DNA barcoding**

201 A small piece of cutaneous tissue was collected from a total of 754 individuals for DNA  
202 extraction using the NucleoSpin® Tissue or NucleoSpin® 96 Tissue kits (Macherey-Nagel).  
203 The COI gene was amplified using the primer pair described in Folmer et al. (1994). Failed

204 samples after the first pass were amplified using the primers LEP-F1 and LEP-R1 (Hebert et  
205 al., 2004). DNA sequencing was carried out by the Eurofins Genomics company and we  
206 manually aligned the sequences using the BioEdit program (Hall, 1999). All sequences are  
207 available in the public dataset [dx.doi.org/10.5883/DS-CARMT](https://dx.doi.org/10.5883/DS-CARMT) in the Barcode of Life Data  
208 Systems, and with Genbank accession numbers OQ222189 – OQ222858, OP404345-  
209 OP404353 and OP404355-OP404359.

210

## 211 **2.4 MOTU delimitation and specimen assignments**

212 MOTU delimitation was done using the Assemble Species by Automatic Partitioning  
213 (ASAP) method based on implementing a hierarchical clustering algorithm (Puillandre et al.,  
214 2021). Pairwise genetic distances (Kimura 2-parameters and p-distances were tested) were used  
215 to build a list of partition ranked by a composite score computed using the probabilities of  
216 groups to be panmictic species and the barcode gap widths. The different partitions,  
217 representing the different species delimitation hypotheses, were analysed in light of the  
218 morphological data available *a priori* and *a posteriori*.

219 Sequences were grouped into haplotypes using DNASP 6.0 software (Rozas et al., 2017)  
220 and the identification engine of BOLD (Barcode of Life Data Systems – <https://www.boldsystems.org/>) was used for taxonomic assignments. Species names were checked for validity in  
221 the DRILOBASE Taxo database (<http://taxo.drilobase.org/>) and in Nomenclatura  
222 Oligochaetologica (<https://nomenclatura-oligochaetologica.inhs.illinois.edu/>). A status of  
223 native species (i.e. species found in a certain area due to natural processes, such as natural  
224 distribution and evolution) or exotic species (i.e. species occurring in areas outside their natural  
225 geographical range) was attributed to each MOTU, based on the assumption that most exotic  
226 species are peregrine (i.e. species with a wide distribution range, owing to human action) and  
227 therefore well-known and referenced in the databases. Unknown MOTUs in the BOLD  
228

229 database therefore have a strong chance of being native. The main limit of this method is that  
230 exotic species new to science, or still little studied for the moment, could be present in  
231 Martinique and would therefore artificially increase the proportion of native species. This  
232 specific point will be discussed later.

233 For visualisation of the MOTUs, phylogenetic trees were reconstructed using both  
234 Bayesian inference and distance-based methods. The best-fitting model (TVM+ I + G) was  
235 identified with the Bayesian Information Criterion (BIC) implemented in jModelTest2  
236 (Guindon and Gascuel, 2003; Darriba et al., 2012). Bayesian inference analysis was performed  
237 with the more complex model GTR + I + G (which also had a low BIC score) because the TVM  
238 + I + G model is not implemented in MrBayes v. 3.2.2 (Ronquist et al., 2012). In MrBayes,  
239 each analysis consisted of two four-chained MCMC runs for 1 million generations, with  
240 sampling of model parameters occurring every 100 generations. The initial 25% of generations  
241 were discarded as burn-in. In addition, a distance-based tree was built using the Neighbour-  
242 Joining method, Kimura 2 parameter distance and 1000 bootstrap replicates such as  
243 implemented in MEGA 11 software (Tamura et al., 2021). Trees were visualized using the  
244 iTOL v5 online tool (Letunic and Bork, 2021).

245

## 246 **2.5 Overall richness estimation**

247 Rarefaction and extrapolation curves were calculated on the basis of standardized  
248 sample size using the iNEXT package in R project (Hsieh et al., 2016). We used this approach  
249 to test whether the sampling effort was enough to cover the vast majority of MOTUs or putative  
250 species, and to estimate species richness based on the Chao1 index. This analysis was performed  
251 for all sites grouped together, and for the natural and anthropized sites separately.

252

## 253 **2.6 Analysis of the diversity of arboreal earthworm species in a disturbed system**

254 Landscape and environmental patterns in relation to the diversity and abundance of  
255 arboreal earthworm communities were analysed using data collected at 18 sites located around  
256 the town of Morne Rouge (Fig 1B). In order to assess how the landscape affected the structure  
257 of arboreal earthworm communities, landscape variables were analysed in buffers ranging from  
258 100 to 1000 metres in radius, centred on each of the 18 sampling sites. We used 2004-2017  
259 databases from the National Institute of Geographic and Forest Information (IGN) and from  
260 Géomartinique in order to calculate the total length of rivers and the proportion of agricultural  
261 areas and forest cover within the buffers. We characterised forest fragmentation by computing  
262 the mean shape index of forest patches within each buffer (McGarigal et al., 2012). This index  
263 corresponds to a ratio between the actual perimeter of patches and their hypothetical minimum  
264 perimeter; its value is 0 if all patches are squares and increases as the shapes of patches become  
265 more complex. We first computed several fragmentation indices (number of patches, patch  
266 density, edge density, clumpiness, normalized landscape shape index, mean fractal dimension,  
267 mean perimeter-area ratio, mean shape index; McGarigal et al., 2012), and eventually adopted  
268 the shape index because it was highly variable across sites and only slightly correlated to forest  
269 areas (mean Spearman's rho across spatial scales = -0.49). The buffers and the different  
270 landscape layers were visualized with QGIS 3.12.1 software (<http://qgis.osgeo.org>).  
271 Fragmentation indices were computed using the landscapemetrics R package (Hesselbarth et  
272 al., 2019).

273

274 We explored the relationship between the abundance of native species and two landscape  
275 features: the proportion of forests and the shape index, including their interaction, as well as the  
276 altitude of samples because we suspected that some species might occupy different altitudinal  
277 ranges. Furthermore, we tested the relationship between the abundance of exotic species and  
278 the proportion of agricultural areas, as well as the total length of rivers. Models were fitted

279 using negative binomial generalized linear models (nbGLM), because we found evidence of  
280 overdispersion when modelling data with a Poisson distribution, using the MASS R package  
281 (Venables and Ripley, 2002). For each model, the buffer radius that best fitted the landscape  
282 data was selected using the Akaike Information Criterion corrected for small sample size  
283 (AICc). Once the best buffer radius had been selected, we simplified the models when  
284 appropriate, based on the set of variables that provided the lowest AICc.

## 285 3. Results

286

### 287 3.1 Assembly and evaluation of the DNA barcode reference library

288 A total of 684 COI sequences was obtained. The best ASAP score was obtained for a  
289 clustering of the sequences into 50 MOTUs whatever the substitution model used (ASAP score  
290 of 2 and 1.5 with Kimura and p-distances, respectively), of which 11 MOTUs were singletons.  
291 Two MOTUs corresponded to the same species (identified using morphological characters),  
292 namely *Dichogaster andina*, and one of these MOTUs was represented by only one individual  
293 (singleton). Overall, the grouping in MOTUs corresponded well to the clades observed in the  
294 phylogenetic trees. The trees obtained with Bayesian inference and the Neighbour-joining  
295 method gave similar results, except that the singleton corresponding to *D. andina* was grouped  
296 in the main clade of this species in the Bayesian tree, whereas it was separated in the NJ tree  
297 (Fig. 3). As a consequence, we decided that these two *D. andina* MOTUs belonged to the same  
298 species and we therefore considered that the dataset comprised 49 putative species. Of them,  
299 16 were identified at species level using morphological data and assignment under BOLD.  
300 According to the DRILOBASE Taxo database, they were peregrine species and were thus  
301 considered as exotic in this study. This status attribution was confirmed by expert knowledge  
302 of the biogeography of major Caribbean earthworm taxa. Identification at species level was not  
303 possible for the other 33 MOTUs. This absence of taxonomic information suggested that these  
304 species are rarely or never encountered by specialists and that they are therefore probably native  
305 species.

306 The rarefaction and extrapolation curve obtained for the whole data set (Fig. 4) indicated  
307 that the 49 putative species observed in the samples may have represented ca. 86% of the real  
308 diversity of the study region, and that up to ca. 57 putative species may occur in Martinique  
309 (95% CI: 50.93-78.49). Although our sampling effort was approximately sufficient to capture



310 most of the species richness in the anthropized habitats, as shown by the rarefaction curve  
311 obtained for the anthropized sites (24 observed species; estimated richness = 29 [95% CI: 25.14-  
312 48.16]), the rarefaction curves obtained for the natural sites revealed that the sampling effort in  
313 natural environments was far from sufficient (Fig. 4), and that many more species probably  
314 occur (37 observed species; estimated richness = 73 [95% CI: 44.67-205.39]).

315

### 316 3.2 Diversity of arboreal earthworms in Martinique

317 We found 7 putative species in the global dataset that were sampled more than 90% of  
318 the time in the trees and that were thus considered as truly arboreal. Two of them were exotic  
319 species (i.e. *Dichogaster annae* and *Dichogaster andina*).

320 When we focused on the region around the town of Morne Rouge, we found that eight  
321 earthworm species could be distinguished among the 212 individuals collected in an arboreal  
322 habitat, including 5 exotic species (*Dichogaster andina*, *D. annae*, *Perionyx excavatus*,  
323 *Amyntas rodericensis* and *Eudrilus eugeniae*) and 3 native species (*Dichogaster* spp.) (Table  
324 1). Of these species, only 5 could be classified as truly arboreal, with the species *Perionyx*  
325 *excavatus*, *Amyntas rodericensis* and *Eudrilus eugeniae* being considered as marginally  
326 arboreal, as they have also been collected in soil or litter in other studies. In this area, the  
327 abundance of native species was significantly correlated to the proportion of forest land cover  
328 in buffers with a 500 m radius that best fitted the landscape data (nbGLM;  $\beta = 11.1358$ , SE =  
329 4.9370,  $p = 0.0241$ , Fig. 5A). Of these 8 species collected in an arboreal habitat, the peregrine  
330 species *D. andina* was the most abundant species and accounted for 82% of individuals. It was  
331 found at every height above ground level from the bottom of the tree up to 6.8 metres. The other  
332 peregrine species were found below 2 metres, while the three native species were found  
333 between 1.4 and 5.5 metres (Table 1). The species found in *Heliconia* plants were only exotic

334 species (*D. andina*, *D. annae*, *P. excavatus*, *A. rodericensis*, *E. eugeniae*), except one  
335 *Dichogaster* sp6 (Table 1). Moreover, the abundance of *D. andina* was significantly correlated  
336 to the total river length in the 500 m-radius buffer and (nbGLM;  $\beta = 0.0005$ , SE = 0.0002,  $p =$   
337 0.0211  $p < 0.05$ , Fig. 5B).

338

#### 339 4. Discussion

340

341 Earthworm biodiversity on tropical islands is most likely underestimated, because of a lack  
342 of sampling effort and because species identification based on external morphology is difficult  
343 (Rodriguez et al., 2007). Here, thanks to a DNA barcoding approach, we were able to build a  
344 library of 684 COI sequences of earthworms from Martinique, corresponding to an estimation  
345 of 49 putative species. Thus, like Decaens et al. (2016) and Maggia et al. (2021) in French  
346 Guiana, we showed that DNA barcodes can be an efficient way of overcoming the taxonomic  
347 impediment and speed up the description of biodiversity patterns for earthworms. Carrying out  
348 this type of survey on earthworm MOTU diversity in areas of known data deficiency, such as  
349 tropical islands, may likely result in the discovery of species new to science, helping to address  
350 the Linnean shortfall (i.e. referring to the fact that most species living on Earth are not formally  
351 described, Brito, 2010).

352 Of the 49 MOTUs, 33 could not be assigned to a known species, either by morphological  
353 analysis, or by comparison of COI sequences with international databases. It is very likely that  
354 most of these 33 putative species were new to science and native to the Caribbean region,  
355 although it is not totally ruled out that a poorly known exotic species was collected outside its  
356 native range. Earthworm species of the genera *Dichogaster* (Acanthodrilidae family) and  
357 *Glossodrilus* (Glossoscolecidae family) have numerous representatives in the Caribbean region,  
358 and we thus believe that the 23 species collected in Martinique belonging to these genera (those  
359 in the lower part of the tree in Fig. 3) are natives. The *Periscolex* sp1 species (Rhinodrilidae  
360 family) could also reasonably be considered as native. There are several MOTUs grouped with  
361 known Asian taxa whose origin is more questionable. Even if we consider only the 23 potential  
362 new species that are certainly native, our results still confirm that the native earthworm  
363 community of Martinique is highly diverse. Similar results were obtained in two others

364 Caribbean islands : Guadeloupe has at least 12 species of *Dichogaster* and several other  
365 undescribed endemics from *Glossodrilus* and the Rhinodrilidae (James, 1996; James and  
366 Gamiette, 2016) while the nearby island Dominica has several undescribed endemics of  
367 *Dichogaster* and *Glossodrilus* (Fragoso et al., 1995). This confirms that a high rate of  
368 endemism is often reached on islands, which may act simultaneously as cradles of evolutionary  
369 diversity and museums of formerly widespread lineages (Fernández-Palacios et al., 2021).  
370 Moreover some habitats, such as those founded in epiphytic plants, may particularly facilitate  
371 allopatric speciation and favour a high diversification of native or endemic arboreal earthworms  
372 (Ladino et al., 2019).

373 In tropical America, the leaves of many epiphytic bromeliad species (Bromeliaceae) overlap  
374 at the base and form water-filled (i.e. by rainwater) central tanks (also called phytotelmata) in  
375 which litter accumulates and soil forms, thus providing a rich habitat for soil-dwelling  
376 invertebrates (Paoletti et al., 1991). In Central and South America, Schmelz et al (2015)  
377 reviewed 25 described species belonging to 5 families or sub-families (Acanthodrilidae -  
378 Benhamiinae; Acanthodrilidae–Acanthodrilinae; Megascolecidae, Glossoscolecidae and  
379 Ocnodrilidae) found in bromeliad water tanks. Paoletti et al (1991) showed that at least eight  
380 species of arboreal earthworms, not identified at species level but presumably belonging to the  
381 Rhinodrilidae family, formed the dominant fraction of the invertebrate biomass in the arboreal  
382 soil of one site of the Parque Nacional Henri Pittier in North-Central Venezuela. Of the 13  
383 earthworm species most recently described in Guadeloupe, another Caribbean island, seven  
384 were found exclusively in bromeliads (James, 1996; Csuzdi and Pavlicek, 2009; James and  
385 Gamiette, 2016) and belonged to the Acanthodrilidae family and Benhamiinae subfamily  
386 (*Dichogaster* or *Eutrigaster* genus). Similarly, in Martinique, we recorded a total of seven  
387 species considered as arboreal (i.e. found more than 90% of the time in trees) and belonging to  
388 the Acanthodrilidae family. Two of them, namely *Dichogaster annae* and *Dichogaster andina*

389 were peregrine arboreal species. *D. annae* was already recorded in bromeliads of Guadeloupe  
390 (Csuzdi and Pavlicek, 2009). The other five had never been described before and were probably  
391 endemic to Martinique, although it remains possible that they are present in other islands where  
392 no COI records exist. Other species were more opportunistically found in epiphytic habitats,  
393 while they were more frequent in other habitats such as litter or soil. This opportunistic  
394 occupation of the epiphytic habitat was also found in French Guiana where, out of a total of 17  
395 putative species collected in epiphytic microhabitats, only 3 putative species of earthworms  
396 were found exclusively in epiphytic plants (Decaens et al., 2016). These results suggest that  
397 epiphytic niches may be colonized by individuals dispersing from ground level populations  
398 confirming that the canopy may provide an attractive habitat for soil-dwelling invertebrates.  
399 Paoletti et al. (1991) indeed revealed that these epiphytes can trap amounts of leaf litter that  
400 would otherwise enrich the terrestrial soils. It is therefore particularly important to integrate  
401 epiphytic habitats in studies of earthworm communities in tropical forests. The fact that some  
402 earthworm families are largely found in these habitats has a major implication for studies of  
403 phylogenetic community structure that aim to understand how evolutionary and ecological  
404 factors have shaped present-day earthworm communities (Brussaard et al., 2012).

405 Here, we found that the abundance of the native and potentially endemic species in  
406 epiphytic habitats in the Morne-Rouge region of Martinique was significantly correlated to the  
407 proportion of forest land cover, highlighting that anthropogenic pressures, such as land use  
408 changes as well as climate change, have the potential to alter arboreal earthworm communities  
409 by modifying vegetation. Introduction of some tree species (as cocoa or rubber) in tree  
410 plantations, although having the potential to maintain some of the natural vegetation, will also  
411 promote the invasion of exotic species and the probable disappearance of arboreal endemic  
412 earthworm species. Thus, another threat to the diversity of earthworms in Martinique revealed  
413 in this study was the presence of at least 16 exotic species on this island, of which 11 (i.e.

414 *Dichogaster affinis*, *D. annae*, *D. bolaii*, *Amyntas rodericensis*, *Metaphire houlleti*, *Perionyx*  
415 *excavatus*, *Polypheretima elongata*, *Pontodrilus litoralis*, *Eudrilus eugeniae*, *Pontoscolex*  
416 *corethrurus*, *Po. spiralis*) have also been reported in Guadeloupe, the nearby Caribbean island  
417 (Csuzdi and Pavlicek, 2009; James and Gamiette, 2016). Most of these exotic species were  
418 probably introduced by human activities such as gardening and fishing as only a few species  
419 can withstand seawater (Eijsackers, 2010). A notable exception is the euryhaline earthworm  
420 *Pontodrilus litoralis* which is known to survive long periods of immersion in seawater (Chen  
421 et al., 2021). These capacities could be shared by the two other species of *Pontodrilus* detected  
422 in Martinique (Seesamut et al., 2022). Suggested dispersal mechanisms of *P. litoralis* dispersal  
423 were natural rafting of cocoons or adults and transport by boats (i.e. on wooden vessels or in  
424 sand- ballast; Blakemore, 2007; Chen et al., 2021). These exotic species with worldwide  
425 distribution may replace the many local species that are unable to compete successfully in such  
426 altered environments (Lavelle et al., 2022). In this case, they can be considered as invasive  
427 species which, through their own presence or by influencing the composition of native species,  
428 increase measures of community similarity as a consequence of global biotic homogenisation  
429 (Muthukrishnan and Larkin, 2020). Successfully introduced pan-tropical species, such as  
430 *Pontoscolex corethrurus*, are believed to show a superior ability to exploit local resources when  
431 compared to native residents (Sakai et al., 2001; Taheri et al., 2018b). They generally show a  
432 wide ecological plasticity (Fragoso et al., 1999). For instance, in this study we showed that  
433 exotic arboreal species were able to occupy the epiphytic soil inside *Heliconia* plant species  
434 while native species were specialized on bromeliad plants and were very occasionally found  
435 inside *Heliconia*.

436 The most frequent of these arboreal exotic species was *Dichogaster andina* (Cognetti de  
437 Martiis, 1904). This tropical peregrine earthworm was previously recorded in Brazil, Ecuador  
438 and French Guiana (Cognetti de Martiis, 1904; Brown and James, 2007; Zicsi, 2007; Csuzdi

439 and Pavlicek, 2010). The dispersal of these introduced species into habitats such as bromeliad  
440 phytotelmata, which might at first glance be considered isolated, is intriguing. Although these  
441 arboreal epiphyte bromeliads and their associated soils are patchily distributed in trees, they are  
442 linked by climbing vegetation, percolating rainwater and probably by movement of animals.  
443 They could therefore be considered tenuously interconnected islands (Paoletti et al., 1991).  
444 Active dispersal of earthworms between phytotelmata is therefore not unrealistic. For instance,  
445 in French Guiana, *D. andina* was observed climbing on humid trunks during rainy days  
446 (Decaens et al., 2016). Moreover, passive dispersal could also play an important role in the  
447 dispersal of these species. We found a correlation between the abundance of *D. andina* and the  
448 proportion of total river length in the landscape, suggesting that rivers may act as vectors of  
449 dispersal for this species. Indeed, bromeliads containing earthworms, or directly worms or  
450 cocoons, may fall into rivers or streams and be carried away by the current.

451 To our knowledge, there are almost no data on the biology of *D. andina* in the literature.  
452 Our results suggest that this species may be parthenogenetic, or at least that we have  
453 encountered a parthenogenetic morph of the species. Indeed, out of the 204 COI sequences of  
454 *D. andina* obtained in the global dataset, only 2 haplotypes were obtained. One haplotype was  
455 observed in 203 specimens, while the other was observed in only one specimen and was  
456 assigned to a distinct MOTU in the ASAP and Neighbour-Joining analyses. It could be a  
457 genetically differentiated clone, while the main haplotype would seem to correspond to a more  
458 generalistic clone. For instance, in the parthenogenetic peregrine species *Aporrectodea*  
459 *trapezoides*, one clone represented one third of the specimens and was present in 11 different  
460 countries, while the rest of the clones showed geographically restricted distributions (Fernandez  
461 et al., 2011). Parthenogenesis and stable and humid conditions of phytotelmata may promote a  
462 high rate of reproduction and, as a consequence, high colonisation success. Indeed, while soil  
463 earthworms only reproduce in the wet season in tropical rainforests and savannas, bromeliads

464 offer the opportunity for continuous reproduction throughout the year as both food and moisture  
465 remain available (Fragoso and Rojas-Fernández, 1996). Moreover, parthenogenesis  
466 predisposes a species to invasiveness. The ability of a single individual to establish a population  
467 is indeed an important characteristic of many invasive species (e. g. Dybdahl and Drown, 2011).  
468 In the context of the Lesser Antilles, where many native endemic species live in bromeliads,  
469 the peregrine species *D. andina* could be a serious threat to earthworm biodiversity. *D. andina*  
470 should be considered as an invasive alien species, and further studies on its harmfulness should  
471 be conducted.

472

## 473 **5. Conclusion**

474 This study revealed that the island of Martinique harbours a great diversity of earthworms,  
475 but that biodiversity is potentially threatened by the alteration of natural habitats and  
476 colonization by ubiquitous peregrine species. Actually, the earthworm diversity in Martinique  
477 was not fully estimated here for several reasons. Firstly, most of the earthworm specimens could  
478 not be identified at species level. The only earthworm species that could be identified at species  
479 level using recorded morphological data, or uploaded molecular data, were peregrine species  
480 (16 in total) that are well known in the tropical regions. This result highlights both the lack of  
481 morphological and molecular taxonomic data allowing the identification of native species in  
482 the Caribbean Islands Biodiversity Hotspot. Thus, for most DNA barcodes, no species name  
483 could be associated. This lessens the use of this DNA barcoding library in the context of other  
484 studies on earthworms from Martinique and highlights the need for integrative taxonomic work  
485 on earthworms from the tropics in general and the island of Martinique in particular, combining  
486 morphological description (internal and external), DNA barcoding and phylogenetic analysis.  
487 Indeed, accurate identification of species using DNA barcoding requires reliable sequence  
488 reference libraries of known taxa, but also taxonomically comprehensive coverage (e.g. Geiger



489 et al., 2021). This meets the second limitation of our study. The rarefaction curves obtained for  
490 the natural sites revealed that the sampling effort in natural environments is not yet sufficient  
491 and that several more species have yet to be collected in Martinique.

492

493

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731 **Table 1:** Abundance of the different arboreal earthworm species sampled at 18 different sites  
732 around the town of Morne Rouge, with the type of epiphytic microhabitat (bromeliads or  
733 *Heliconia* plants, or other) in the anthropized (A) and natural (N) sites and the mean height in  
734 the tree where each species was sampled (with standard deviation and minimum and maximum  
735 height when appropriate).

Species	Status	Nb	Microhabitat						Height (cm) ± SD [ min – max]
			Bromeliad		<i>Heliconia</i>		Other		
			A	N	A	N	A	N	
<i>Amyntas rodericensis</i>	exotic	1	-	-	-	1	-	-	160 ± 0
<i>Dichogaster andina</i>	exotic	174	24	55	78	12	4	1	249 ± 165 [ 0 – 680]
<i>Dichogaster annae</i>	exotic	5	-	-	4	1	-	-	128 ± 72 [ 0 – 160]
<i>Dichogaster sp6</i>	native	15	-	14	-	1	-	-	281 ± 125 [ 144 – 470]
<i>Dichogaster sp11A</i>	native	12	-	11	-	-	-	1	395 ± 117 [170 – 550]
<i>Dichogaster sp18</i>	native	2	-	2	-	-	-	-	280 ± 0
<i>Perionyx excavatus</i>	exotic	2	-	-	2	-	-	-	140 ± 0
<i>Eudrilus eugeniae</i>	exotic	1	-	-	1	-	-	-	100 ± 0

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739 **Figure captions**

740

741 **Figure 1:** Sampling map. Orange circles indicate the sites in a natural environment and blue  
742 circles indicate the sites in an anthropized environment. Colours on the map indicate ground  
743 cover: in pink, anthropized surfaces; in blue natural areas without vegetation (bare soil and  
744 water surface); in light green, non-woody vegetation and in dark green, woody vegetation. A.  
745 Location of all the sampling sites where specimens used for the construction of the earthworm  
746 DNA barcode library were collected. B. Location of the arboreal earthworm sampling site in  
747 the Morne Rouge region.

748

749 **Figure 2:** Photograph showing two of the major arboreal microhabitats sampled in this study:  
750 bromeliads (A-B) and decaying *Heliconia* stems (C-D). The bromeliads support predominantly  
751 native species of the genus *Dichogaster* and the *Heliconia* flowering stems support mostly an  
752 exotic species (*Dichogaster andina*).

753

754 **Figure 3:** Neighbour-joining tree of the 684 COI sequences obtained from the analysis of the  
755 earthworms collected in Martinique. MOTUs are represented by triangles whose longer and  
756 shorter lateral edges represent maximum and minimum intra-divergence. MOTUs are  
757 considered as putative species and the name of the identified species is indicated when possible,  
758 otherwise only a genus name is proposed or the species is considered undetermined. Putative  
759 species names coloured in red correspond to exotic species; putative species branches coloured  
760 in green correspond to arboreal species (i.e. with a specimen found more than 90% of the time  
761 in trees). Singletons are represented in small font size.

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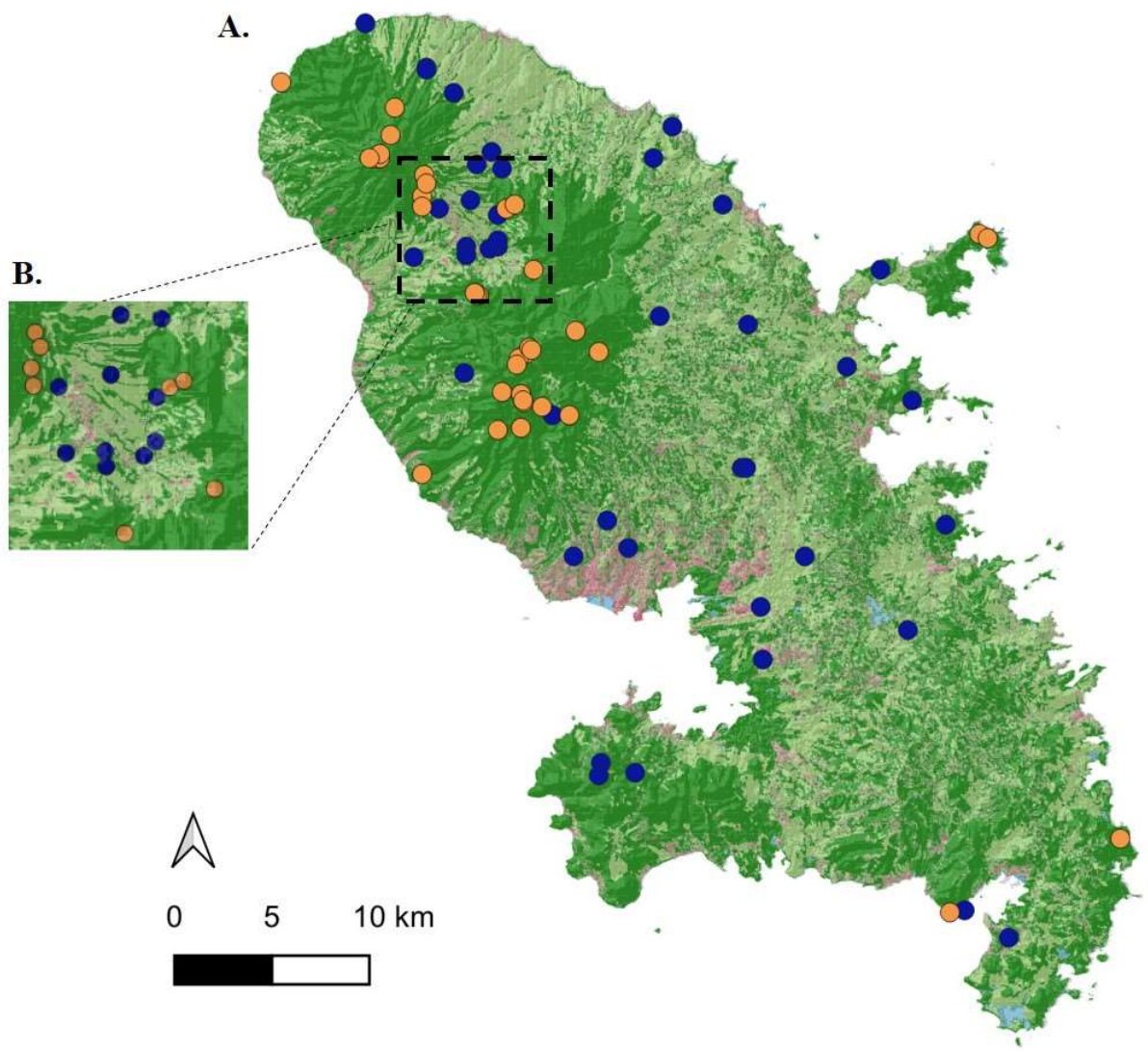
763 **Figure 4:** Rarefaction and extrapolation curves for the whole dataset (brown), and separately  
764 for the sites in the anthropized (blue) and natural (orange) environments. The shapes at the end  
765 of the solid line are the number of observed species. The dotted lines represent the species  
766 number obtained by extrapolation.

767

768 **Figure 5:** Effect of landscape features on the abundance of native and peregrine earthworm  
769 species in the Morne Rouge region. (A) Relationship between the abundance of native species  
770 and the proportion of forest cover in a 500-m radius. (B) Relationship between the abundance  
771 of the peregrine species *Dichogaster andina* and the total length of rivers in a 500-m radius.  
772 We show only the effect of significant variables; statistical models were fitted as negative  
773 binomial regressions with patch shape and altitude, and proportion of agricultural cover as  
774 covariates, respectively.

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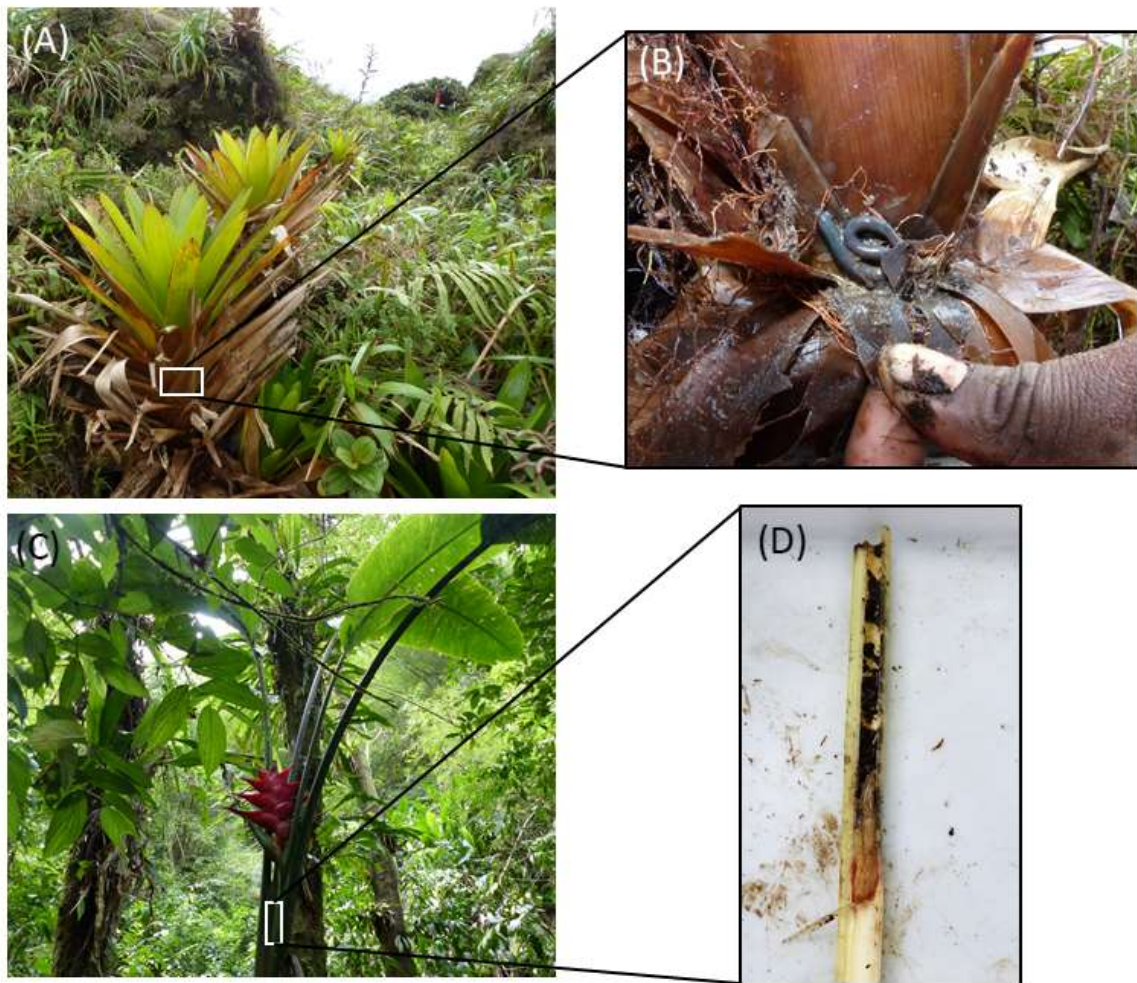
776 **Figure 1**



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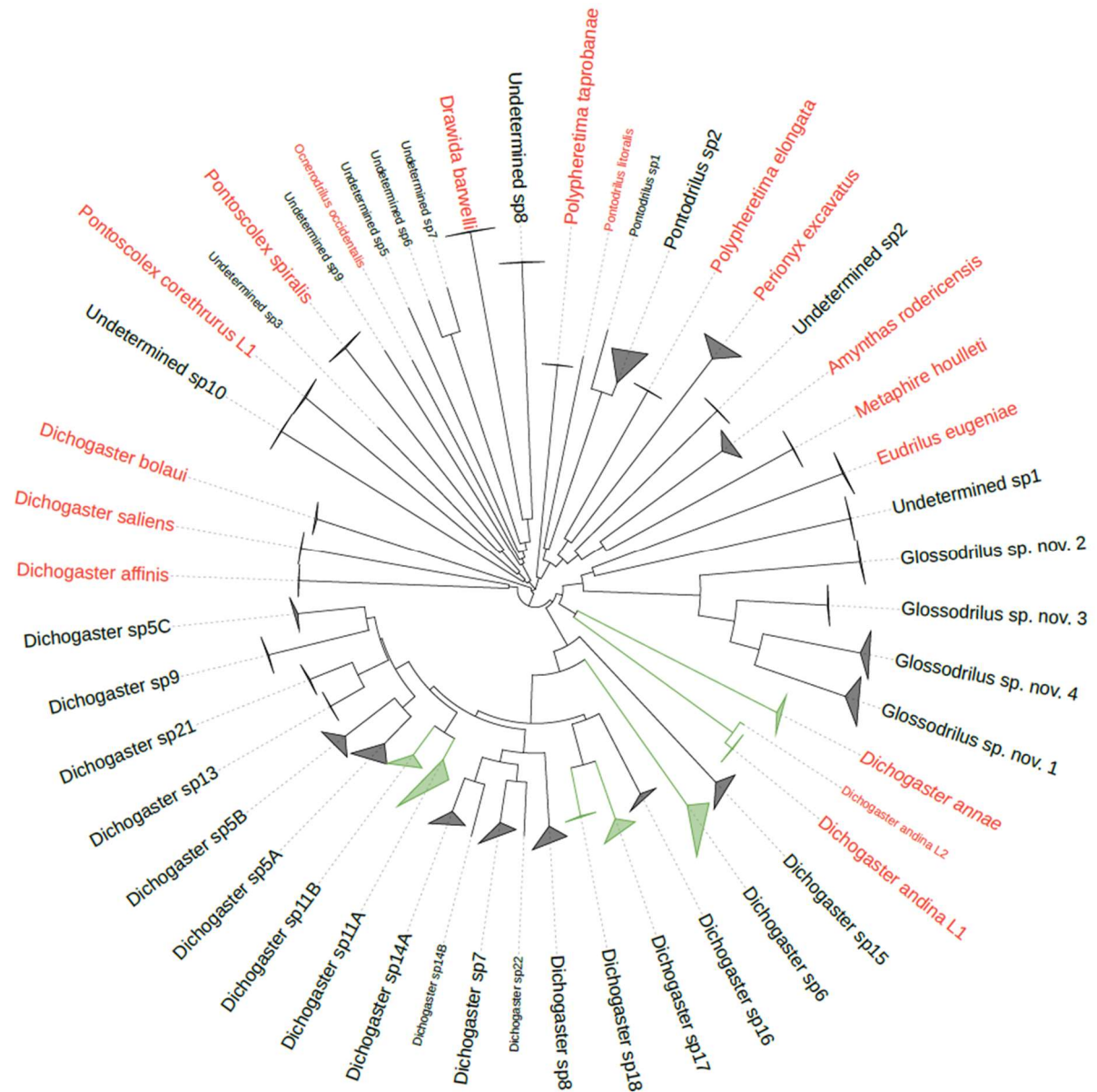
779 **Figure 2**



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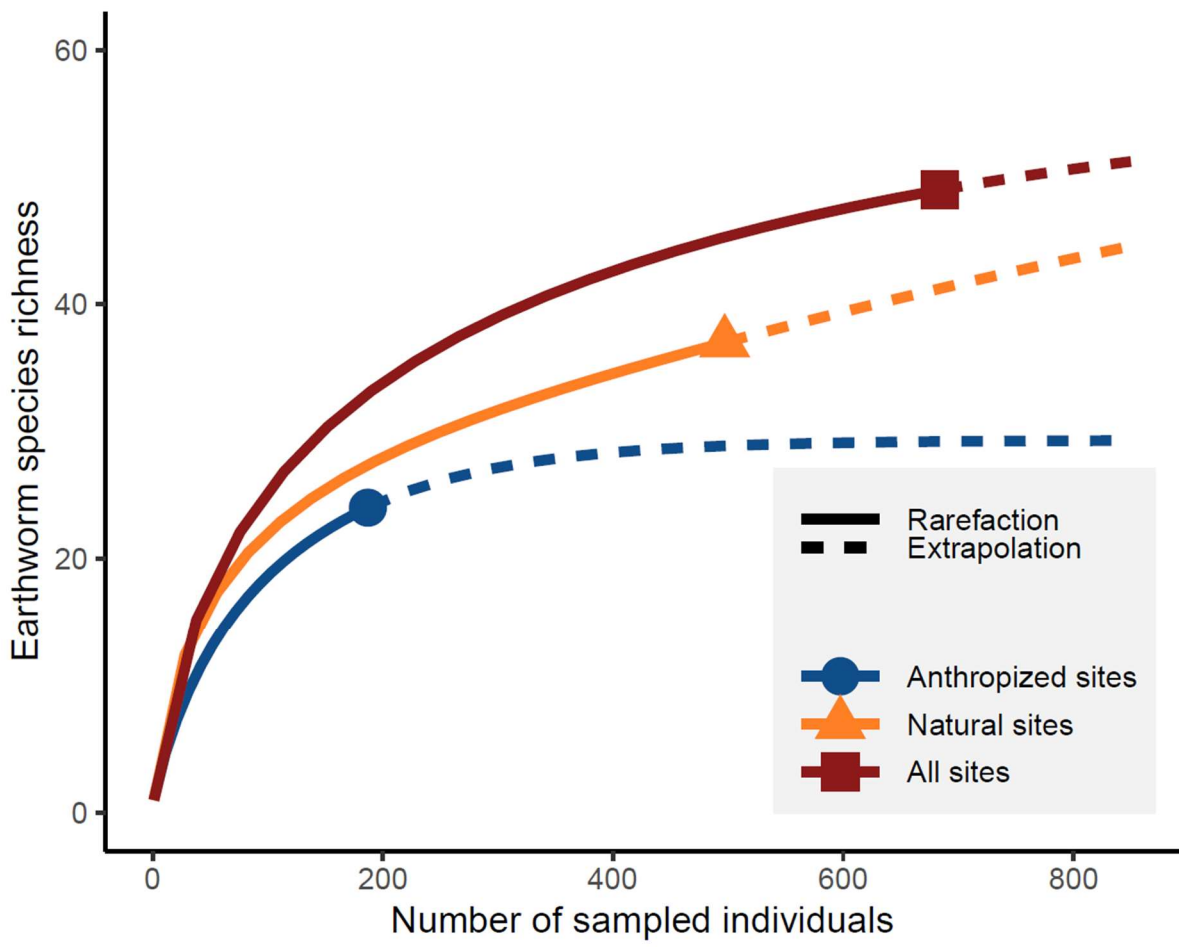
782 **Figure 3**



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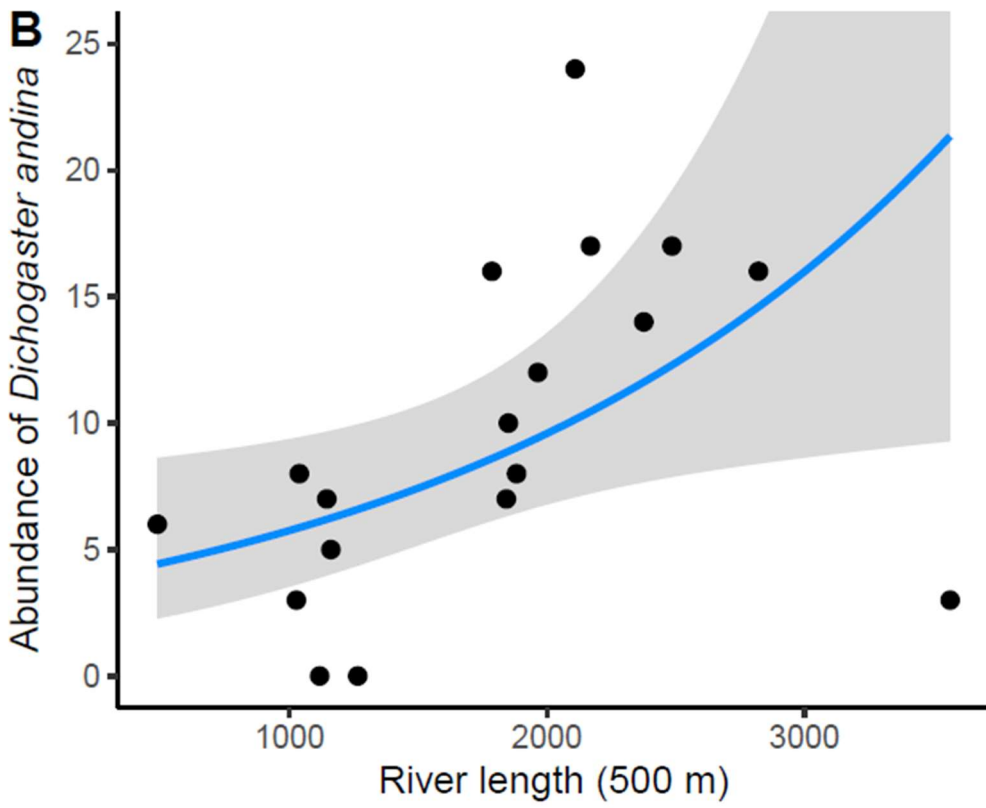
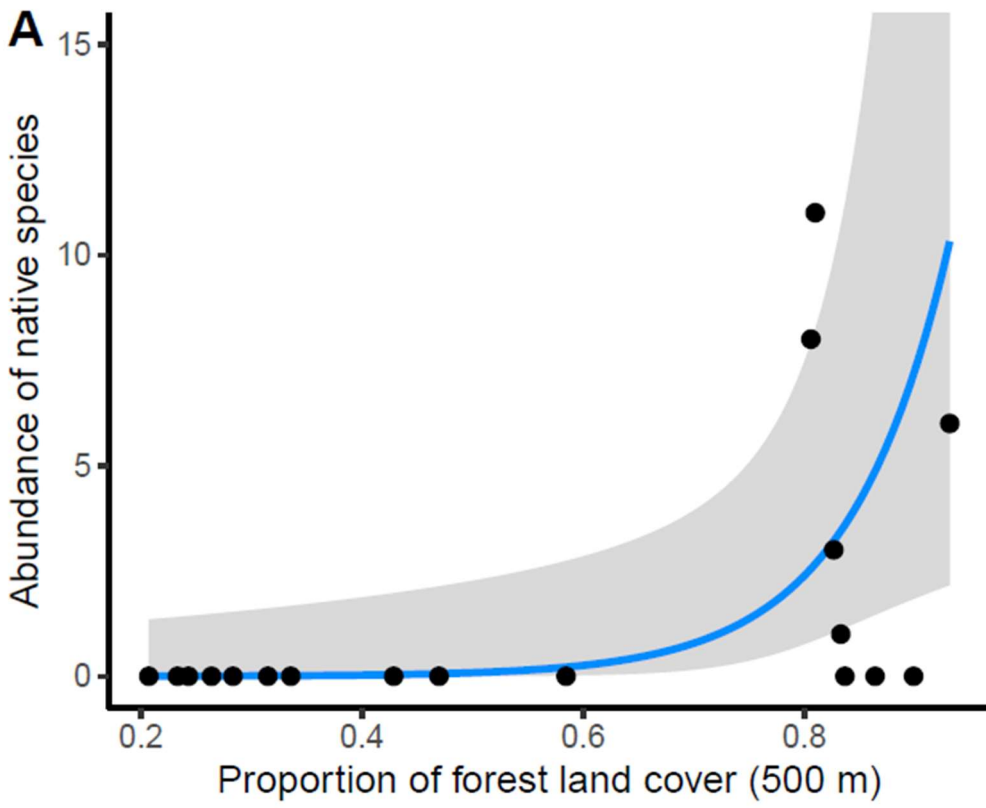
785 **Figure 4**



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788 **Figure 5**



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