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

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Abstract

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

Keywords

Arboreal earthworms, DNA barcode library, landscape variables, molecular identification, peregrine species, tropical island

1. Introduction

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

The 1128 km^2 volcanic island of Martinique belongs to one of the 36 biodiversity hotspots in the world (i.e. the Caribbean Islands Biodiversity Hotspot, Mittermeier et al., 2004) and is typical of many mountainous islands in the Lesser Antilles arc, with a volcanic geological history and a diverse microclimate resulting in pronounced habitat zonation (Ogden and Thorpe, 2002). While the South of the island is characterized by low altitude hills covered by semi-deciduous tropical forest and a large proportion of agricultural areas, the North of the island is mountainous and dominated by ancient rainforests and cloud forest. Such natural habitat heterogeneity usually contributes to species diversity through an increase in available niche spaces, provision of refuges and opportunities for isolation and divergent adaptation (Stein et al., 2014). Human activities, such as urbanization and conversion of pristine ecosystems into agricultural lands may break up natural habitats into smaller habitats that may no longer support many species, resulting in a loss of biodiversity (Wilson et al., 2016). We assumed that landscape fragmentation due to human activities might affect the species diversity of earthworms in Martinique (e.g. Vanbergen et al., 2007).

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

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

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

2. Material and methods

2.1 Earthworm sampling

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

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

2.2 Morphological identification

We used a process of iterative taxonomy (Yeates et al., 2011) to define and refine species boundaries using morphological and molecular evidence. Earthworms were morphologically identified in three steps. Firstly, the sampled earthworms were grouped into 34 morphotypes based on external morphological data. These were adults and some of the juveniles. Secondly, using both external (numbers of setae per segment, distances between setal lines, location and shape of clitellum, shape of prostomium, pigmentation, visible genital pores, modified setae, and genital markings including tubercula pubertatis if appropriate) and internal characters (location and number of gizzards, numbers and types of calciferous glands, locations of hearts, male reproductive organs, spermathecae, nephridia per segment, intestinal characters), some of the morphotypes could then be assigned unambiguously to well-known species using taxonomic keys (Blakemore, 2002; Chang et al., 2016; Gabriac et al., submitted), while others could only be assigned to morphospecies. For the Glossoscolecidae and Rhinodrilidae, we used the keys of Righi (1996) and Zicsi (1995), and for the Dichogaster species we used James and Gamiette (2016) and James (1996, 2004). A genus could be assumed for some of these morphospecies, but not for all because some genera cannot be distinguished on specimens lacking sexual characters. Thirdly, morphological identification took place after the DNA barcoding step. Once the specimens had been grouped into genetically divergent groups (i.e. MOTUs), it was possible to refine the description and identification of the morphospecies by more detailed examinations and comparisons of characters among the MOTUs. This was mostly necessary for the presumed endemic species. We scored a standard set of anatomical characters, both external and internal, the latter being obtained from dorsal dissections.

2.3 DNA barcoding

A small piece of cutaneous tissue was collected from a total of 754 individuals for DNA extraction using the NucleoSpin® Tissue or NucleoSpin® 96 Tissue kits (Macherey-Nagel). The COI gene was amplified using the primer pair described in Folmer et al. (1994). Failed samples after the first pass were amplified using the primers LEP-F1 and LEP-R1 (Hebert et al., 2004). DNA sequencing was carried out by the Eurofins Genomics company and we manually aligned the sequences using the BioEdit program (Hall, 1999). All sequences are available in the public dataset dx.doi.org/10.5883/DS-CARMT in the Barcode of Life Data Systems, and with Genbank accession numbers OQ222189 – OQ222858, OP404345-OP404353 and OP404355-OP404359.

2.4 MOTU delimitation and specimen assignments

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

Sequences were grouped into haplotypes using DNASP 6.0 software (Rozas et al., 2017) 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 the DRILOBASE Taxo database (http://taxo.drilobase.org/) and in Nomenclatura Oligochaetologica (https://nomenclatura-oligochaetologica.inhs.illinois.edu/). A status of native species (i.e. species found in a certain area due to natural processes, such as natural distribution and evolution) or exotic species (i.e. species occurring in areas outside their natural geographical range) was attributed to each MOTU, based on the assumption that most exotic species are peregrine (i.e. species with a wide distribution range, owing to human action) and therefore well-known and referenced in the databases. Unknown MOTUs in the BOLD database therefore have a strong chance of being native. The main limit of this method is that exotic species new to science, or still little studied for the moment, could be present in Martinique and would therefore artificially increase the proportion of native species. This specific point will be discussed later.

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

2.5 Overall richness estimation

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

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

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

We explored the relationship between the abundance of native species and two landscape features: the proportion of forests and the shape index, including their interaction, as well as the altitude of samples because we suspected that some species might occupy different altitudinal ranges. Furthermore, we tested the relationship between the abundance of exotic species and the proportion of agricultural areas, as well as the total length of rivers. Models were fitted using negative binomial generalized linear models (nbGLM), because we found evidence of overdispersion when modelling data with a Poisson distribution, using the MASS R package (Venables and Ripley, 2002). For each model, the buffer radius that best fitted the landscape data was selected using the Akaike Information Criterion corrected for small sample size (AICc). Once the best buffer radius had been selected, we simplified the models when appropriate, based on the set of variables that provided the lowest AICc.

3. Results

3.1 Assembly and evaluation of the DNA barcode reference library

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

The rarefaction and extrapolation curve obtained for the whole data set (Fig. 4) indicated that the 49 putative species observed in the samples may have represented ca. 86% of the real diversity of the study region, and that up to ca. 57 putative species may occur in Martinique (95% CI: 50.93-78.49). Although our sampling effort was approximately sufficient to capture most of the species richness in the anthropized habitats, as shown by the rarefaction curve obtained for the anthropized sites (24 observed species; estimated richness = 29 [95% CI: 25.14-48.16]), the rarefaction curves obtained for the natural sites revealed that the sampling effort in natural environments was far from sufficient (Fig. 4), and that many more species probably occur (37 observed species; estimated richness = 73 [95% CI: 44.67-205.39]).

3.2 Diversity of arboreal earthworms in Martinique

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

When we focused on the region around the town of Morne Rouge, we found that eight earthworm species could be distinguished among the 212 individuals collected in an arboreal habitat, including 5 exotic species (*Dichogaster andina*, *D. annae*, *Perionyx excavatus*, *Amynthas rodericensis* and *Eudrilus eugeniae*) and 3 native species (*Dichogaster* spp.) (Table 1). Of these species, only 5 could be classified as truly arboreal, with the species *Perionyx excavatus*, *Amynthas rodericensis* and *Eudrilus eugeniae* being considered as marginally arboreal, as they have also been collected in soil or litter in other studies. In this area, the abundance of native species was significantly correlated to the proportion of forest land cover in buffers with a 500 m radius that best fitted the landscape data (nbGLM; $\beta = 11.1358$, SE = 4.9370, $p = 0.0241$, Fig. 5A). Of these 8 species collected in an arboreal habitat, the peregrine species *D. andina* was the most abundant species and accounted for 82% of individuals. It was found at every height above ground level from the bottom of the tree up to 6.8 metres. The other peregrine species were found below 2 metres, while the three native species were found between 1.4 and 5.5 metres (Table 1). The species found in *Heliconia* plants were only exotic species (*D. andina*, *D. annae*, *P. excavatus*, *A. rodericensis*, *E. eugeniae*), except one *Dichogaster* sp6 (Table 1). Moreover, the abundance of *D. andina* was significantly correlated to the total river length in the 500 m-radius buffer and (nbGLM; $\beta = 0.0005$, SE = 0.0002, $p = 0.0211$ $p<0.05$, Fig. 5B).

4. Discussion

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

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

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

In tropical America, the leaves of many epiphytic bromeliad species (Bromeliaceae) overlap at the base and form water-filled (i.e. by rainwater) central tanks (also called phytotelmata) in which litter accumulates and soil forms, thus providing a rich habitat for soil-dwelling invertebrates (Paoletti et al., 1991). In Central and South America, Schmelz et al (2015) reviewed 25 described species belonging to 5 families or sub-families (Acanthodrilidae - Benhamiinae; Acanthodrilidae–Acanthodrilinae; Megascolecidae, Glossoscolecidae and Ocnerodrilidae) found in bromeliad water tanks. Paoletti et al (1991) showed that at least eight species of arboreal earthworms, not identified at species level but presumably belonging to the Rhinodrilidae family, formed the dominant fraction of the invertebrate biomass in the arboreal soil of one site of the Parque Nacional Henri Pittier in North-Central Venezuela. Of the 13 earthworm species most recently described in Guadeloupe, another Caribbean island, seven were found exclusively in bromeliads (James, 1996; Csuzdi and Pavlicek, 2009; James and Gamiette, 2016) and belonged to the Acanthodrilidae family and Benhamiinae subfamily (*Dichogaster* or *Eutrigaster* genus). Similarly, in Martinique, we recorded a total of seven species considered as arboreal (i.e. found more than 90% of the time in trees) and belonging to the Acanthodrilidae family. Two of them, namely *Dichogaster annae* and *Dichogaster andina* were peregrine arboreal species. *D. annae* was already recorded in bromeliads of Guadeloupe (Csuzdi and Pavlicek, 2009). The other five had never been described before and were probably endemic to Martinique, although it remains possible that they are present in other islands where no COI records exist. Other species were more opportunistically found in epiphytic habitats, while they were more frequent in other habitats such as litter or soil. This opportunistic occupation of the epiphytic habitat was also found in French Guiana where, out of a total of 17 putative species collected in epiphytic microhabitats, only 3 putative species of earthworms were found exclusively in epiphytic plants (Decaens et al., 2016). These results suggest that epiphytic niches may be colonized by individuals dispersing from ground level populations confirming that the canopy may provide an attractive habitat for soil-dwelling invertebrates. Paoletti et al. (1991) indeed revealed that these epiphytes can trap amounts of leaf litter that would otherwise enrich the terrestrial soils. It is therefore particularly important to integrate epiphytic habitats in studies of earthworm communities in tropical forests. The fact that some earthworm families are largely found in these habitats has a major implication for studies of phylogenetic community structure that aim to understand how evolutionary and ecological factors have shaped present-day earthworm communities (Brussaard et al., 2012).

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

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

The most frequent of these arboreal exotic species was *Dichogaster andina* (Cognetti de Martiis, 1904). This tropical peregrine earthworm was previously recorded in Brazil, Ecuador and French Guiana (Cognetti de Martiis, 1904; Brown and James, 2007; Zicsi, 2007; Csuzdi and Pavlicek, 2010). The dispersal of these introduced species into habitats such as bromeliad phytotelmata, which might at first glance be considered isolated, is intriguing. Although these arboreal epiphyte bromeliads and their associated soils are patchily distributed in trees, they are linked by climbing vegetation, percolating rainwater and probably by movement of animals. They could therefore be considered tenuously interconnected islands (Paoletti et al., 1991). Active dispersal of earthworms between phytotelmata is therefore not unrealistic. For instance, in French Guiana, *D. andina* was observed climbing on humid trunks during rainy days (Decaens et al., 2016). Moreover, passive dispersal could also play an important role in the dispersal of these species. We found a correlation between the abundance of *D. andina* and the proportion of total river length in the landscape, suggesting that rivers may act as vectors of dispersal for this species. Indeed, bromeliads containing earthworms, or directly worms or cocoons, may fall into rivers or streams and be carried away by the current.

To our knowledge, there are almost no data on the biology of *D. andina* in the literature. Our results suggest that this species may be parthenogenetic, or at least that we have encountered a parthenogenetic morph of the species. Indeed, out of the 204 COI sequences of *D. andina* obtained in the global dataset, only 2 haplotypes were obtained. One haplotype was observed in 203 specimens, while the other was observed in only one specimen and was assigned to a distinct MOTU in the ASAP and Neighbour-Joining analyses. It could be a genetically differentiated clone, while the main haplotype would seem to correspond to a more generalistic clone. For instance, in the parthenogenetic peregrine species *Aporrectodea trapezoides*, one clone represented one third of the specimens and was present in 11 different countries, while the rest of the clones showed geographically restricted distributions (Fernandez et al., 2011). Parthenogenesis and stable and humid conditions of phytotelmata may promote a high rate of reproduction and, as a consequence, high colonisation success. Indeed, while soil earthworms only reproduce in the wet season in tropical rainforests and savannas, bromeliads offer the opportunity for continuous reproduction throughout the year as both food and moisture remain available (Fragoso and Rojas-Fernández, 1996). Moreover, parthenogenesis predisposes a species to invasiveness. The ability of a single individual to establish a population is indeed an important characteristic of many invasive species (e. g. Dybdahl and Drown, 2011). In the context of the Lesser Antilles, where many native endemic species live in bromeliads, the peregrine species *D. andina* could be a serious threat to earthworm biodiversity. *D. andina* should be considered as an invasive alien species, and further studies on its harmfulness should be conducted.

5. Conclusion

This study revealed that the island of Martinique harbours a great diversity of earthworms, but that biodiversity is potentially threatened by the alteration of natural habitats and colonization by ubiquitous peregrine species. Actually, the earthworm diversity in Martinique was not fully estimated here for several reasons. Firstly, most of the earthworm specimens could not be identified at species level. The only earthworm species that could be identified at species level using recorded morphological data, or uploaded molecular data, were peregrine species (16 in total) that are well known in the tropical regions. This result highlights both the lack of morphological and molecular taxonomic data allowing the identification of native species in the Caribbean Islands Biodiversity Hotspot. Thus, for most DNA barcodes, no species name could be associated. This lessens the use of this DNA barcoding library in the context of other studies on earthworms from Martinique and highlights the need for integrative taxonomic work on earthworms from the tropics in general and the island of Martinique in particular, combining morphological description (internal and external), DNA barcoding and phylogenetic analysis. Indeed, accurate identification of species using DNA barcoding requires reliable sequence reference libraries of known taxa, but also taxonomically comprehensive coverage (e.g. Geiger et al., 2021). This meets the second limitation of our study. The rarefaction curves obtained for the natural sites revealed that the sampling effort in natural environments is not yet sufficient and that several more species have yet to be collected in Martinique.

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

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

Figure captions

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

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

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

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

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

Figure 1

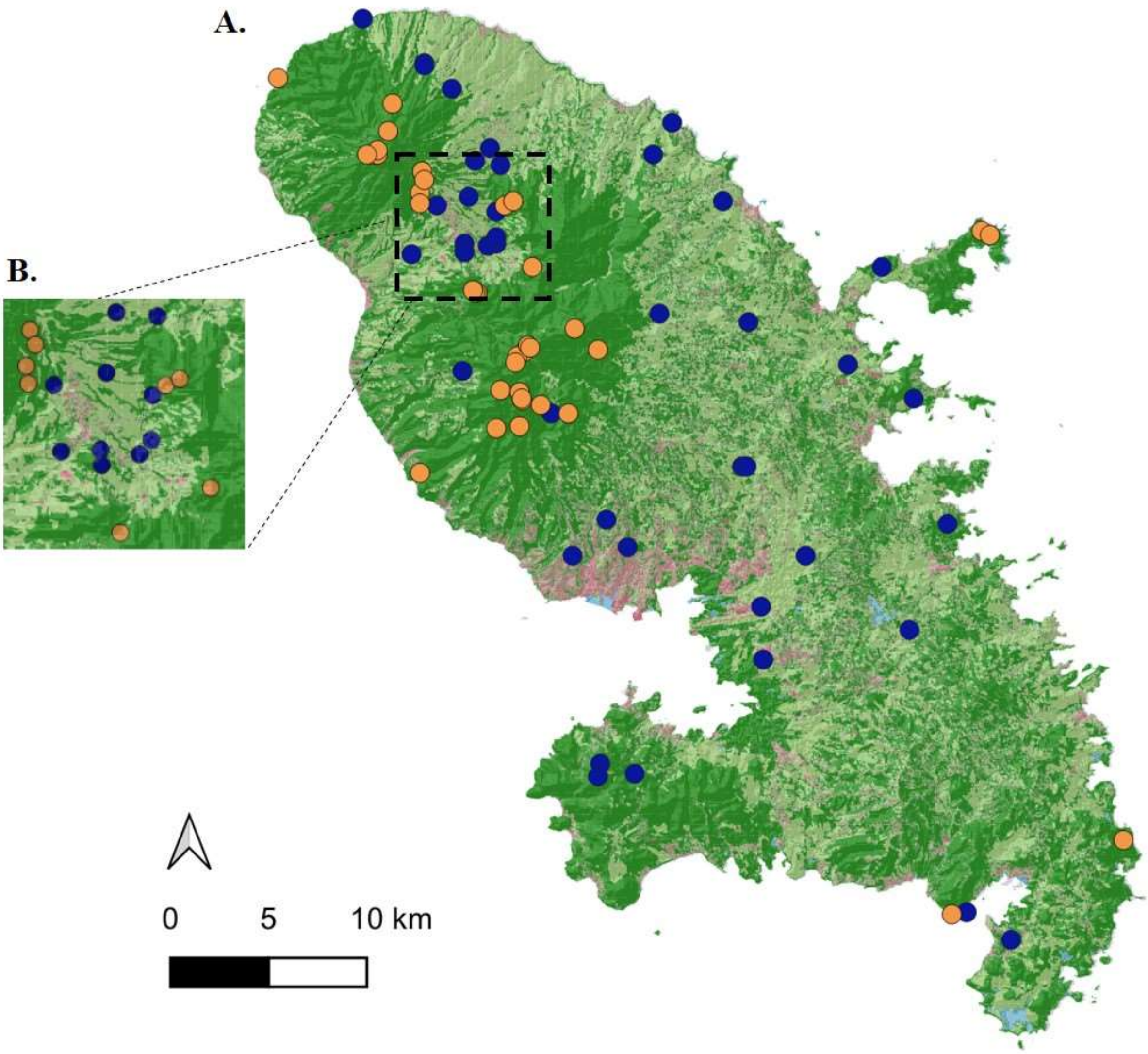


Figure 2

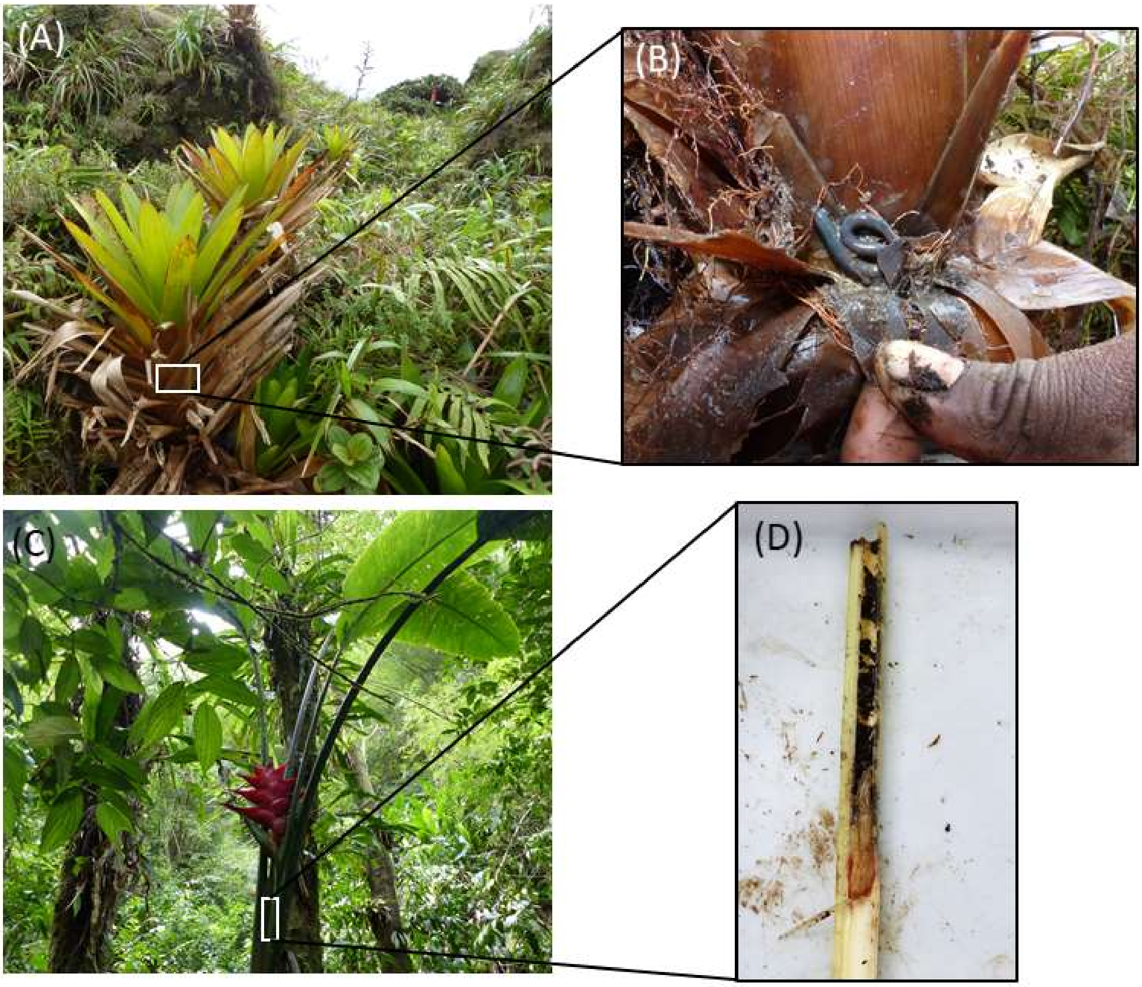


Figure 3

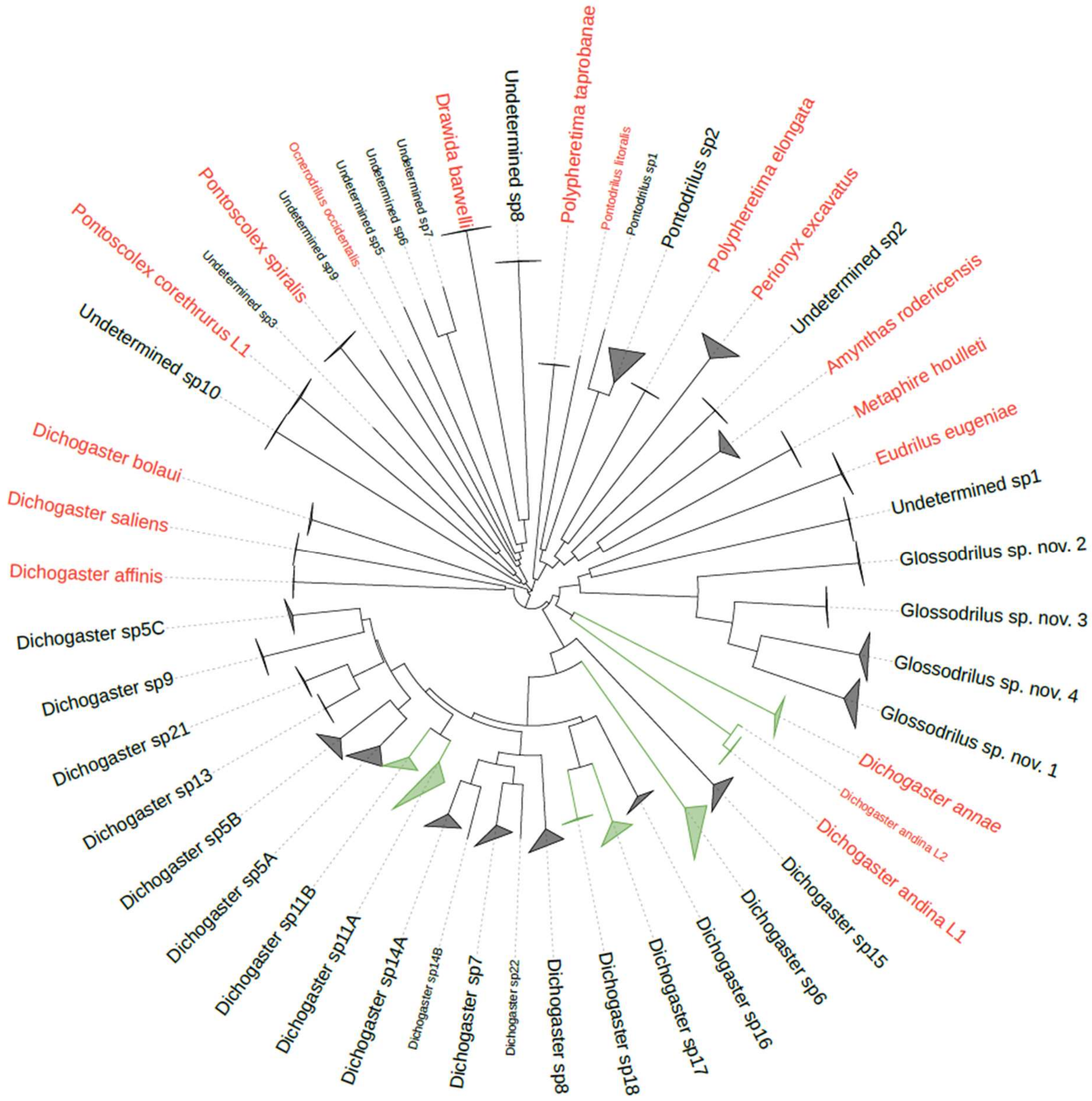
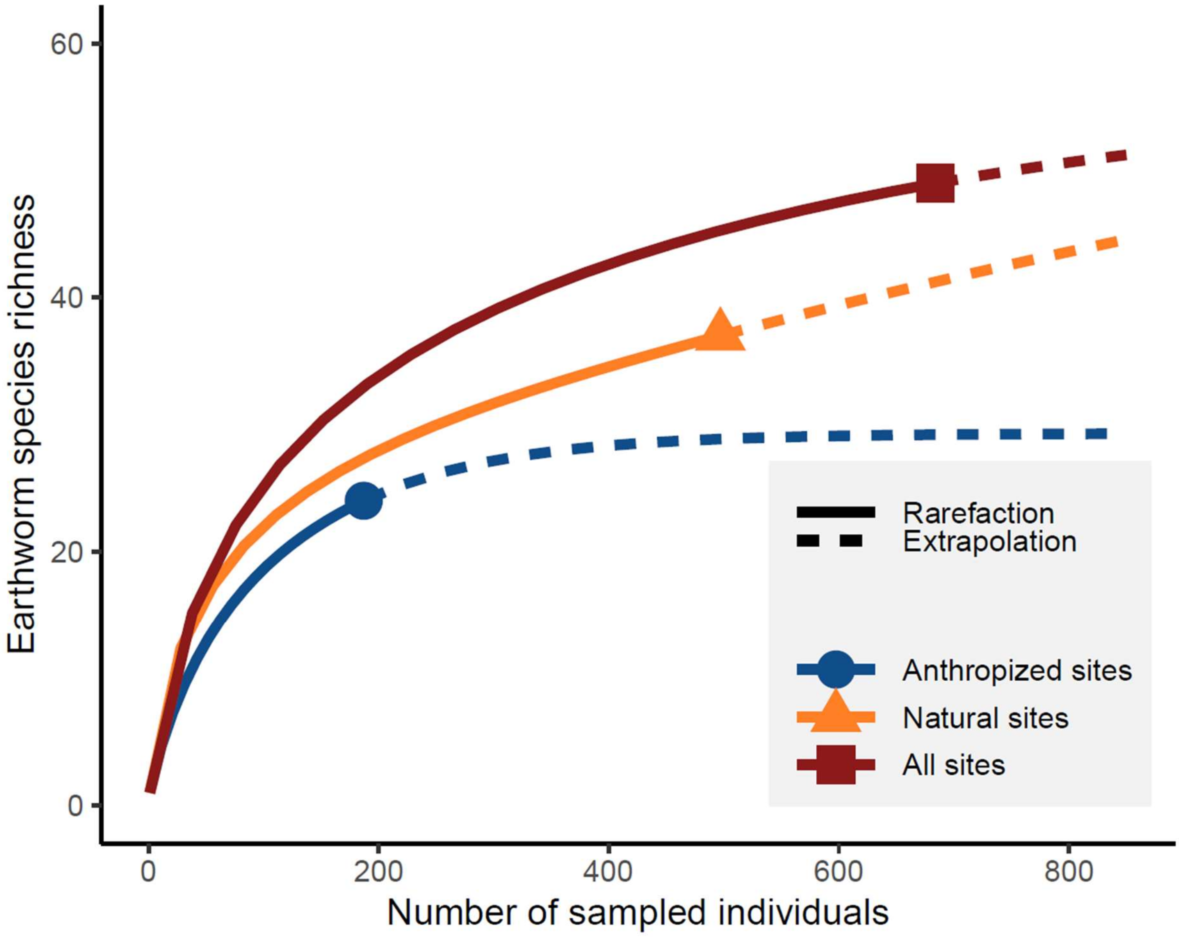
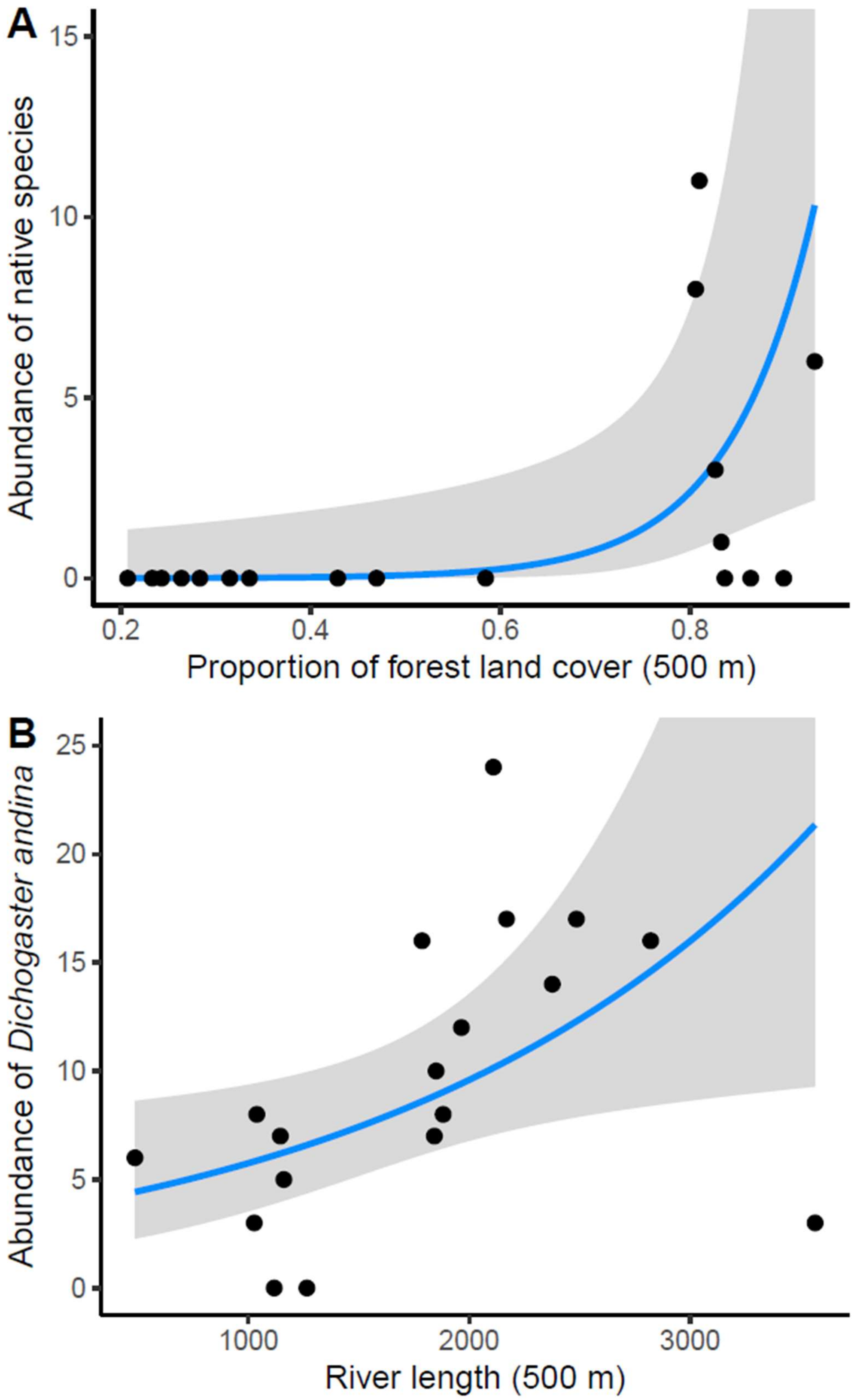

Dichogaster bolaui
Dichogaster saliens
Dichogaster affinis
Dichogaster sp5C
Dichogaster sp9
Dichogaster sp21
Dichogaster sp13
Dichogaster sp5B
Dichogaster sp5A
Dichogaster sp11B
Dichogaster sp11A
Dichogaster sp14A
Dichogaster sp14B
Dichogaster sp7
Dichogaster sp22
Dichogaster sp8
Dichogaster sp18
Dichogaster sp17
Dichogaster sp16
Dichogaster sp6
Dichogaster sp15
Dichogaster andina L1
Dichogaster andina L2
Dichogaster annae
Glossodrilus sp. nov. 1
Glossodrilus sp. nov. 4
Glossodrilus sp. nov. 3
Glossodrilus sp. nov. 2
Undetermined sp1
Eudrilus eugeniae
Metaphire houlleti
Amynthas rodericensis
Undetermined sp2
Perionyx excavatus
Polypheretima elongata
Pontodrilus sp2
Pontodrilus sp1
Pontodrilus litoralis
Polypheretima taprobanae
Undetermined sp8
Drawida barwelli
Undetermined sp7
Undetermined sp6
Undetermined sp5
Ocnerodrilus occidentalis
Undetermined sp9
Pontoscolex spiralis
Undetermined sp3
Pontoscolex corethrurus L1
Undetermined sp10

Figure 4



60
40
20
0
Earthworm species richness
0
200
400
600
800
Number of sampled individuals
Rarefaction
Extrapolation
Anthropized sites
Natural sites
All sites

Figure 5


A
Abundance of native species
0
5
10
15
0.2
0.4
0.6
0.8
Proportion of forest land cover (500 m)
B
Abundance of *Dichogaster andina*
0
5
10
15
20
25
1000
2000
3000
River length (500 m)