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DNA barcoding reveals diversity patterns of earthworm communities in remote tropical forests of French Guiana

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ABSTRACT

Despite representing a key component of terrestrial biota, soil invertebrates in tropical rainforests have been poorly studied from both a taxonomic and an ecological perspective when compared to other groups of terrestrial animals. We sampled earthworm communities in a range of sampling locations in two different study sites of the Nouragues Natural Reserve in French Guiana, focusing on lowland to plateau and hilltop forests as well as on savannah-like vegetation of the Nouragues granitic inselberg. We used the barcode region of the COI gene to delimit Molecular Taxonomic Units (MOTUs), further validated using species-level diagnostic morphological characters. A total of 651 sequences was obtained, most of them corresponding to juveniles that cannot be identified to the species level from morphology alone. We found a total of 48 MOTUs, and both rarefaction curves and diversity estimators (Chao1 and ACE) suggested that 60 species could occur in the study area, representing the highest earthworm richness ever recorded worldwide. Beta-diversity analyses highlighted a strong species turnover between sampling locations. Except in a few specific cases, species richness was usually limited to 12 species at the scale of a given location, which likely indicates the influence of competitive interactions during community assembly process. Community structure was dominated by species living in the upper soil layers and in surface microhabitats, with some of them able to colonize epiphytic soils up to more than 40 m above ground level. These results suggest the importance of long-term diversification processes and current ecological factors for the structuring and the diversity of earthworm communities in tropical rainforests of French Guiana.

1. Introduction

Tropical rainforests, whilst only covering 6–7% of the worldwide continental surfaces, have been popularized as the most emblematic ecosystems from a biodiversity viewpoint (Wilson, 1988). Understanding the patterns and drivers that govern tropical biodiversity inspired generations of ecologists, allowing the emergence of new concepts leading to fundamental advances in theoretical and evolutionary ecology (Chazdon and Whitmore, 2002).

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However, tropical biodiversity studies historically focused on conspicuous vertebrates and higher plants, neglecting most invertebrate taxa that represent the vast majority of the world's eukaryotic diversity (May, 2011). Studying invertebrate community patterns in tropical rainforests is currently challenged by the huge number of locally co-occurring species, and by the weakness of our taxonomic knowledge in many groups (Godfray et al., 1999; Adams et al., 2014). In this context, considering soil communities represents a major challenge, as they harbor a vast diversity of species (Decaëns, 2010) which activities are essential for a large range of key ecological processes (Lavelle et al., 2006). Paradoxically, soil invertebrates have been given poor taxonomic and ecological coverage by comparison with aboveground organisms (Decaëns, 2010), resulting in a global taxonomic impediment for soil biodiversity studies (André et al., 2001). As a consequence, we know little about tropical soil invertebrate community structure and species richness along key environmental gradients.

DNA barcoding, using a fragment of the mitochondrial gene cytochrome c oxidase I (COI) as a standard genetic marker for species detection and identification in animal kingdom (Hebert et al., 2003), represents a potential solution to remove this taxonomic impediment. Given the limitations and the potential pitfalls of defining species boundaries through a single-gene approach (Rubinoff and Holland, 2005; Wiemers and Fiedler, 2007), several authors advocated for the use of an integrative taxonomic approach combining different sources of evidence (e.g. morphological characters, ecological features or geographic distributions) in addition to molecular data (Dayrat, 2005; Puillandre et al., 2012). Nevertheless, since the seminal paper of Hebert et al. (2003), a growing number of studies have demonstrated the potential of DNA barcodes, alone or combined with other taxonomic data, as a tool to alleviate the taxonomic impediment and increase the pace of biodiversity pattern description (Puillandre et al., 2012; Smith et al., 2013; Lees et al., 2014). DNA barcoding can also be useful in describing community patterns in poorly studied regions, and/or for groups of organisms with poorly resolved taxonomy or with strong identification difficulties (Tanzler et al., 2012; Young et al., 2012; Porco et al., 2013). Obtained systematically for a large number of individuals collected across an ecologically meaningful sampling design, it can be used to delimit Molecular Operational Taxonomic Units (MOTUs) usable as reliable species proxy to estimate taxonomic richness and to describe community patterns at different spatial scales (Blaxter et al., 2005). In soil communities, this approach has now been used in several studies focusing on e.g. collembolans (Porco et al., 2013), ants (Smith et al., 2005) and mites (Young et al., 2012).

In this study, we used DNA barcodes to describe earthworm assemblages in a remote forest area of French Guiana. Despite being the focus of a prolific literature documenting their contribution to key ecosystem processes, earthworms are characterized by a strong taxonomic deficit which represents a severe bottleneck to develop basic studies of their community ecology (Decaëns et al., 2013). In the tropics, only a few studies have described the structure of earthworm assemblages in natural ecosystems (Lavelle, 1978; Nemeth, 1981; Fragoso, 1985; Jiménez, 1999; Feijoo, 2001). We conducted two successive earthworm surveys in a range of habitat types in the Nouragues Nature Reserve to describe species richness levels and community patterns at different scales. Samples were analyzed using a combination of DNA barcoding, morphological and ecological data. The operational species concept is based on the assumption of separately-evolving populations leading to the emergence of divergent lineages (de Queiroz, 2007). Our surrogate of that is an integrative combination of molecular species (MOTUs) delimited using COI sequences and morphology based on species-level diagnostic characters. These surrogate taxa were used to describe the structure of earthworm communities and to explore their assembly rules.

2. Material and methods

2.1. Study sites

Sampling was conducted in June 2011 in the Pararé and in January and June 2011 in the Inselberg research stations (RS) of the Nouragues Nature Reserve, in French Guiana. Climate is tropical humid with mean annual rainfall of 3000–3250 mm, mainly distributed during the wet season between December and June, and average maximum and minimum monthly temperatures are 20.3 °C (19.7–21 °C) and 33.5 °C (32.1–35.8 °C), respectively.

Inselberg RS is located at the piedmont of the Nouragues granitic inselberg mountain culminating at 411 m above sea level (base camp WGS84 coordinates: N4°05′17.73″/W52°40′47.90″). Vegetation around the base camp consists of a patchwork of different types of tropical rainforest and 'rocky savannah' vegetation on the slopes of the inselberg. Pararé RS is 6,400 m from the Inselberg RS, and is located on the edge of the Arataye River (base camp WGS84 coordinates: N4°2′17.30″/W52°40′22.31). Vegetation around the base camp is dominated by tropical rainforest on flooded to well-drained soils depending on topography. In the vicinity of each of the two base camps (SI Fig. 1), we sampled earthworms in a total of 11 distinct sampling locations representing the main types of vegetation available. The main characteristics of these locations are given in Table 1 and SI Table 1.

2.2. Earthworm sampling

For each sampling location, we sampled earthworms at one to six sampling points, depending on the relative representation of the corresponding habitat in the landscape. Overall, our sampling design allowed for a consistent survey of the range of ecosystems found in the vicinity of each of the two research stations. Each sampling point consisted of a 50 m-radius circle centered on a geolocated point and in which earthworms were sought in all available and attainable microhabitats during a fixed period of six researcher-hours. All life stages (i.e. adults, juveniles and cocoons) were collected, in four main types of microhabitats considered as suitable for earthworms (Fragoso and Rojas-Fernandez, 1996): (1) organo-mineral and holorganic soil layers, and (2) sandy to muddy sediments of stream banks were dug out with a spade and hand-sorted; (3) litter accumulations and decaying trunks on the soil surface were prospected by carefully sorting them with a small spade or a machete; (4) 'epiphytic soils' (i.e. organic matter accumulation in epiphytic plants and hollow trees) up to 40 m above the surface were brought to ground level to be hand-sorted. Specimens collected at a given point were kept alive in separate boxes corresponding to the location and microhabitat in which they were found (i.e. soil, river bank sediments, decaying trunks or epiphytic soils).

2.3. DNA barcoding

Specimens were cleaned with water before being killed and fixed in 100% ethanol. For large specimens, the solution was changed once after 24 h in order to insure an efficient fixation. Once fixed for at least 24 h, the specimens collected at a given point and microhabitat were broadly sorted into 'morpho-groups' based on external morphological characters, mainly size, pigmentation, and clitellum and genital markings positions and shapes in adults. We then selected up to 5 specimens per morpho-group for DNA barcoding. Although these groups were imperfectly defined

Table 1
Main characteristics of the eleven sampling locations in Nouragues Natural Reserve of French Guiana, where earthworm communities were sampled. Elevation in meters; mean coordinates XY in meters under the Datum WGS84, UTM 22N.

Code	Locations	Vegetation type	# of sampling points	Elevation	Mean X	Mean Y	Mean Lat	Mean long
Inselberg Research Station								
HF	Hill-top forest	Primary tropical rainforest with low canopy height on shallow soils of Inselberg hill-top	2	390	313272	453021	4° 5′48.41″N	52°40′55.58″W
TE	Terraces	Patchwork of Clusia woods, rocky savannahs and nude granitic areas	1	220	313723	452715	4° 5′38.45″N	52°40′40.94″W
TF	Transition forest	Primary tropical rainforest with low canopy height on shallow soils of the Inselberg slopes	3	150	313799	452511	4° 5′31.84″N	52°40′38.46″W
IC	Inselberg camp	Anthropic area, mainly open vegetation on deep and well drained soils	2	110	313507	452079	4° 5′17.76″N	52°40′47.90″W
LiF	Liana forest	Old secondary rainforest on deep and well drained soils	2	110	313771	451486	4° 4′58.47″N	52°40′39.30″W
SP	Small plateau	Primary tropical rainforest on deep and well drained soil	7	104	313238	451725	4° 5′06.22″N	52°40′56.60″W
SB	Stream banks	Riparian tropical rainforest on shallow soils and sandy sediments along streams	6	74	313319	451538	4° 5′00.14″N	52°40′53.96″W
GP	Great plateau	Primary tropical rainforest on deep and well drained soils	6	60	313993	452077	4° 5′17.73″N	52°40′32.14″W
Pararé Research Station								
PP	Pararé plateau	Primary tropical rainforest on deep and well drained soil	1	140	313529	447370	4° 2′44.46″N	52°40′46.87″W
PF	Palm forest	Palm vegetation on swampy lowland soils	1	50	312802	446897	4° 2′29.02″N	52°41′10.41″W
LF	Lowland forest	Primary tropical raintropical forest on hydromorphic lowland soils	5	40	313738	446300	4° 2′29.64″N	52°40′40.02″W

taxonomically (e.g. adults and juveniles were usually placed in different groups), such sorting allowed for a representative sampling of all life stages and most putative species, and thus was assumed to result in the best approximation of the taxonomic diversity of each sample.

A small piece of cutaneous tissue (c.a. 1 mm^2) was collected from each individual and transferred to a well of a 96-well plate. DNA extraction, PCR reactions and sequencing of the 5′ region of the COI gene were done at the Canadian Centre for DNA Barcoding (CCDB) following standard automated protocols of the International Barcode of Life project (http://ibol.org/). We used a primer cocktail combining the pairs of M13 tailed primers LCO1490/HCO2198 (Folmer et al., 1994) and LepF1/LepR1 (Hebert et al., 2004). Failed samples after this first pass were amplified using the internal primers MLepR1 and MLepF1 along with the LCO/HCO pair, respectively (Hajibabaei et al., 2006). All sequences are available within the public dataset "Earthworms diversity – Nouragues" [DS-EWNOU] (http://dx.doi.org/10.5883/DS-EWNOU) in the Barcode Of Life Data systems, and with Genbank accession numbers JN260446–JN260768 and KM527504–KM527831.

2.4. Delimitation of molecular operational taxonomic units

Distance analyses were performed with MEGA5 (Tamura et al., 2011), using a Neighbor-Joining (Saitou and Nei, 1987) algorithm with the Kimura-2 parameter model (Kimura, 1980) to estimate genetic distances. The K2p model was chosen in order to allow a consistent comparison with most barcoding studies where this model is set as a default. The use of uncorrected p-distances as recommended in Srivathsan and Meier (2012) was tested and had no impact on the assignation of sequences to each MOTU and on the community composition results (data not shown). The robustness of nodes was evaluated through bootstrap re-analysis of 1000 pseudoreplicates. Trees were re-plotted using the online utility iTOL v2.2.2 (Letunic and Bork, 2007). MOTUs were defined with the software 'mothur' v1.34.2 using 'hcluster' command with the option 'furthest neighbor' (Schloss et al., 2009).

We first plotted various threshold values against the number of MOTUs acquired from the application of these thresholds to the dataset (SI Fig. 2). We obtained a characteristic plateau representing the insensitivity to changes in cut-off value; in this threshold value interval, most MOTUs delineated could be valid biological species (Plaisance et al., 2009). Within the threshold range of this plateau (11–17%), we chose the value corresponding to the lowest interspecific/inter MOTUs divergence from the distribution of the frequencies of sequences pairwise comparison (SI Fig. 2). This value was 14% in our dataset and this conservative cutoff threshold was applied to delineate MOTUs (the same cutoff was found using the ABGD online software [Puillandre et al., 2012 – data not shown]). This cutoff value and the barcode gap associated are not to be taken as universal for earthworms in the area; indeed these values might exhibit variations as more data are added into the analysis. This strategy was then compared to three other methods available for MOTU delineation (i.e. BINs, GMYC, and PTP). All the results obtained were then contrasted to the morphological, ecological and distributional data in order to figure out which method produced the more congruent MOTUs delineations under the requirements of integrative taxonomy (Puillandre et al., 2012), i.e. MOTUs that fitted the best with morphological units and that did not separate into different units morphologically identical specimens occurring in the same locality and microhabitats (see SI Table 2 and SI Fig. 3 for a summary of the results of this comparative analysis). As a conclusion to these additional analyses, our method of barcode gap identification proved to produce the more reliable MOTUs delineations, i.e. it produced the MOTUs that were the most congruent with the morphological, ecological and distributional information available from our study.

2.5. Morphological analyses

Individual specimens were selected for morphological examination based on the MOTUs defined by the Neighbor-Joining tree and clustering process described above. We examined morphologically all individuals of MOTUs with less than six specimens, whereas a selection of up to 15 individuals was examined when MOTUs had a larger numbers of specimens (Table 3). Exceptions were made when a given MOTU had subclusters below the MOTU designation threshold, separated by total branch lengths of at least 2% divergence. Here we examined specimens from each subcluster. In a few MOTUs, fewer specimens were examined because most or all specimens were juveniles or damaged beyond complete

presentation of morphological characters. In total 310 specimens were examined for morphology, representing 47% of the barcoded specimens.

For all MOTUs we recorded external data on setal relations (distance 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). Internal characters appropriate to the various earthworm supra-specific taxa encountered were recorded. For all taxa we recorded locations and form of calciferous glands, shape and orientation of the gizzard, muscularity of anterior septa, form of typhlosole (a dorsal inward extension of the intestinal wall), spermathecal characters, condition of male reproductive organs, and location of the intestinal starting point. The number of caecal pouches was recorded in genus *Nouraguesia*. For the family Benhamiidae the numbers and forms of nephridia were recorded, as well as relative sizes of penial setae, and the characteristics of the acanthodriline male fields represented. Explanations of earthworm morphological characters used here are in the supplemental information (SI text) and additional information is available in Csuzdi (2010) and Righi (1996).

Morphological diagnoses followed a standard taxonomic practice for earthworms (cf Gates, 1972): If two MOTUs showed consistent differences in more than one character traditionally deemed of specific value, we assigned the two to different morphological units (hereafter referred to as 'morpho-species').

2.6. Data analyses

2.6.1. Richness estimations

Rarefaction curves were calculated using EcoSim 7.71 (Gotelli and Entsminger, 2001) with a 95% confidence interval and plotted with R 2.15.0 (R Development Core Team, 2004) using the package 'Plotrix' (Lemon, 2006). We used the 'Vegan' package for R 2.15.0 (Oksanen et al., 2008) to compute several diversity indices: observed richness was calculated as the total number of MOTUs observed in a given locality (i.e. Inselberg or Pararé RS), habitat or microhabitat; theoretical species richness was estimated using the Chao1 and ACE diversity estimators. We also performed bootstrap estimates of species richness following the procedure described in Manly (1997). This estimated a correction factor accounting for species rarity that was used to compute a corrected value of species richness (S.Cor).

2.6.2. Community structure

Community structure was assessed by analyzing the composition of MOTUs in the different sampling locations and microhabitats using different community ecology packages for R 2.15. Distributions of MOTUs among locations and microhabitats were represented using the 'heatmap' function on log-transformed occurrence data. We used a euclidean distance measure to compute the dendrograms representing species and location/microhabitats clustering.

Variation of MOTUs composition between sampling locations (beta-diversity) was assessed using the package 'Vegan' by calculating the average Sørensen's index of dissimilarity: $\beta_{BC} = (b + c)/(2a + b + c)$, where a is the number of MOTUs shared between two sites B and C, and b and c are the numbers of unique MOTUs (not shared) for sites B and C. Beta-diversity was then decomposed into components of spatial turnover and nestedness according to Baselga (2010). Nestedness of species assemblages occurs when the composition of communities with smaller numbers of species are subsets of the richer communities (Wright and Reeves, 1992; Ulrich et al., 2009), reflecting non-random processes of species loss (Gaston and Blackburn, 2000), while spatial turnover implies the replacement of some species by others as a consequence of environmental sorting or spatial and historical constraints (Qian et al., 2005). Singletons (i.e. MOTUs represented by a single specimen in the dataset) are by definition unique to a single collecting site or sample, and it is hard to draw any reliable conclusion regarding their distribution. To avoid any overestimation of beta-diversity as a consequence of a high proportion of these singletons, analyses were done using successively the whole dataset and a subset without singletons.

Additionally, composition overlap among study sites and microhabitats were represented by drawing venn diagrams (using the 'VennDiagram' package) in order to highlight the proportions of components *a* (MOTUs shared by two sites or microhabitat), *b* and *c* (unique MOTUs) as suggested by Koleff et al. (2003).

3. Results

3.1. Earthworm diversity at a regional scale

In total, 729 specimens (237 adults, 426 juveniles, 42 cocoons and 24 fragmented specimens) were selected for DNA analyses. We obtained 651 COI sequences (i.e. 87% sequencing success), which clustered in a total of 48 MOTUs (Figs. 1 and 2). Mean intra-MOTU divergence was 1.27% (range: 0%–5.25%) and mean inter-MOTU was 23.33% (range: 13.68%–31.01%), highlighting a clear barcode gap that supported the relevance of the MOTU clustering obtained with the 14% threshold (SI Fig. 2).

The rarefaction curve obtained for the whole data set (Fig. 3A) agreed with richness indices (Table 2) by indicating that the 48 MOTUs observed in the samples may represent ca. 80% of the real diversity of the study region, and that up to 60 putative species may occur in the neighboring habitats of the two research stations. The proportion of rare MOTUs found in the sample set was quite high, with singletons representing as much as 27% of the total (SI Fig. 4).

A total number of 39 and 27 MOTUs were observed within the 440 and 213 specimens sequenced from the Inselberg and Pararé RS, respectively (Table 2). Rarefaction curves and diversity indices computed for each locality (Table 2, Fig. 3A) suggested that both observed and estimated richness were substantially higher in Inselberg than in Pararé RS. A total of 18 MOTUs were shared by both study sites, while 21 and 9 MOTUs were unique for Inselberg and Pararé, respectively. The Sørensen's index of beta diversity calculated between study sites was of 0.45 when calculated for the whole dataset and 0.32 when calculated without singletons (Fig. 4A). In both cases, beta-diversity mostly corresponded to spatial turnover (i.e. 60.59 and 82.55%, respectively), with only a small proportion related to assemblage nestedness (i.e. 39.41 and 17.45%, respectively).

A high proportion of individuals (c.a. 60%) and MOTUs (13 out of 48, i.e. 27% of the total pool) was represented by juvenile specimens which lack the necessary morphological characteristics to allow species level identification (Fig. 1). Coarse taxonomic assignments at the family to genus levels were however possible for most of the MOTUs (Table 3, SI Table 3). These were assigned to five families and 12 genera, except for two MOTUs that were only represented by young juveniles without any diagnostic characters and therefore unidentifiable even at these taxonomic levels. Morphological diagnoses were also globally congruent with the delimitations of the MOTUs (Fig. 1), with the exception of four MOTUs assigned to genera *Neogaster* and *Wegeneriona*, which were subdivided into two to four morpho-species based on species-level diagnostic characters (Table 3). Four MOTUs were formally identified to species level: *Pontoscolex corethrurus* (Müller, 1857), a well-known peregrine species originating from the Guyana Shield (Righi, 1984; Dupont et al., 2012), *Martiodrilus tenkatei* (Horst, 1887), a

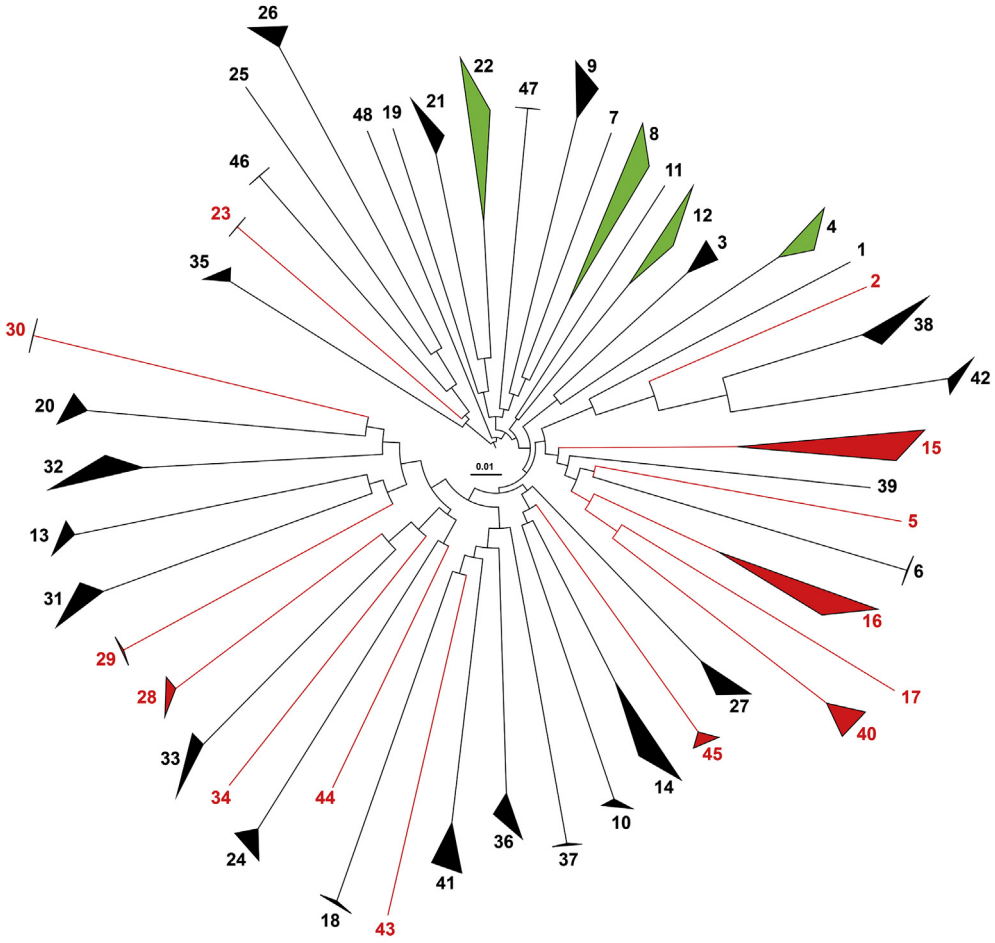

Fig. 1. Neighbor-joining tree of the 651 COI sequences obtained from the analysis of earthworms collected in the Nouragues Natural Reserve. MOTUs are represented by triangles whose longer and shorter lateral edges represent maximal and minimal intra-divergence. MOTUs colored in red were represented only by juveniles in the samples; MOTUs colored in green were polymorphic, i.e. subdivided into at least two morpho-species (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. A few representative species of Nouragues' earthworm communities: A) *Nouraguesia* sp1 (EW-NOU-0099, MOTU # 28); B) *Martiodrilus* sp3 (EW-NOU-0094, MOTU # 29); C) *Martiodrilus tenkatei* (EW-NOU-0007, MOTU # 31); D) *Pontoscolex corethrurus* (EW-NOU-0178, MOTU # 26); white scale = 1 cm.

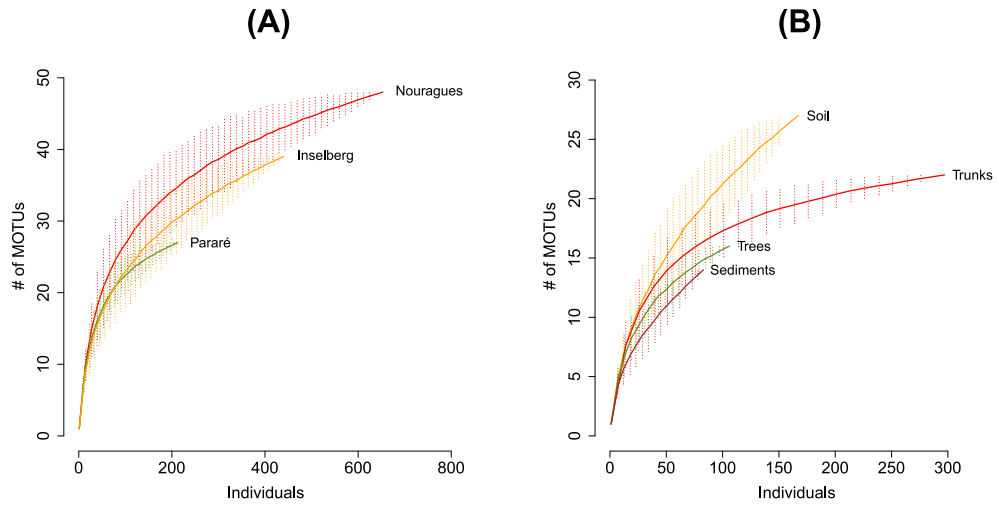

Fig. 3. Individual-based species rarefaction curves computed for: A) the two study sites (Pararé and Inselberg RS) and the whole Nouragues Natural Reserve; B) the main four types of microhabitats (soils, river bank sediments and surface microhabitats). Error bars represent 95% confidence intervals.

Table 2
Number of specimens collected and diversity indices of earthworm communities in the Pararé and Inselberg research stations of the Nouragues Natural Reserve, sorted by habitats (see Table 1 for habitat codes) and microhabitats (i.e. mineral soil, sediment, decaying trunks and epiphytic soil). Diversity indices were calculated using MOTUs as species proxy. # ind = number of specimens; S.obs = observed richness; se.obs = standard error for the average richness from 1000 bootstrap simulations; S.Cor = bias-corrected species richness; S.chao1 = Chao1 index of estimated richness; se.Chao1 = standard error for Chao1; S.ACE = ACE index for estimated richness; se.ACE = standard error for ACE; Samp. Eff = sampling efficiency (observed to mean estimated richness ratio).

Locations/microhabitats	# ind	S.obs	se.obs	S.Cor	S.ACE	se.ACE	Samp. Eff
Nouragues	651	48	1.93	53.75	61.03	3.89	81.9
Inselberg RS	439	39	1.91	44.59	55.32	4	75.7
Pararé RS	212	27	1.37	29.7	30.68	2.5	88.9
GP	102	10	0.74	10.88	12.01	1.68	89.8
HF	17	8	0.93	9.54	10.23	1.52	84.1
IC	17	10	1.07	12.39	17.77	2.04	66.4
LF	132	19	0.93	20.24	19.72	1.86	96.3
LiF	30	10	0.92	11.43	11.69	1.22	87.9
PF	49	12	1.16	14.19	19.09	2.56	74.0
PP	23	7	0.9	8.33	10.08	1.57	79.5
SB	145	25	1.73	29.68	46.23	4.33	67.0
SP	87	12	0.64	12.54	12.24	1.51	97.9
TE	9	2	0.07	2.01	2	0	99.8
TF	32	4	0.68	4.7	8	1.19	67.8
Organo-mineral soils	166	26	1.86	32.21	43.99	3.71	62.8
Decaying trunks	296	22	1.13	23.91	24.54	2.38	91.7
Epiphytic soils	106	16	1.19	18.18	20.96	2.26	82.1
Sediments	83	14	1.34	16.85	32.32	3.38	59.9

species recently reported from several localities in French Guiana, including the Nouragues' reserve (Csuzdi and Pavlicek, 2011), and two species of *Dichogaster* – *D. bolaui* (Michaelsen, 1891) and *D. andina* Cognetti, 1904 – which are both invasive species originating from Africa (Csuzdi et al., 2008). The remaining samples likely corresponded to undescribed taxa, giving a net contribution of our study to earthworm biodiscovery of at least 42 new species.

3.2. Earthworm diversity and composition across different habitats

Table 2 gives the different richness indices calculated individually for the different sampling locations. More MOTUs were observed in the stream banks and the lowland forests (25 and 19 MOTUs, respectively). Local richness did not exceed 12 MOTUs in the other habitats, with the lower values observed in the terrace rocky savannas and in the transition forest (two and four MOTUs, respectively). Inselberg camp, lowland forests, palm forest and stream banks had high richness estimates, with S.Cor values ranging from 12 to 30 MOTUs, and ACE predicting up to 46 MOTUs in the stream banks.

Pairwise comparisons of assemblage composition highlighted that the proportion of MOTUs shared by two different sampling locations was almost always lower than 60% and often lower than 40%, even after deleting singletons from the data set (SI Fig. 5). The average Sørensen's index calculated between all possible pairs of locations was 0.69 for the whole dataset and 0.64 when calculated without singletons (Fig. 4B). This high beta diversity was mainly explained by spatial turnover when considering the complete data set (i.e. 60.81 and 39.19% explained by turnover and nestedness, respectively), but deleting singletons resulted in a higher relative contribution of assemblage nestedness (i.e. 52.11 and 47.89%, respectively). Fig. 5A presents the distribution of the 48 MOTUs among the different habitats. The clustering of sampling locations resulted into six main groups: (1) the three habitats of the Inselberg

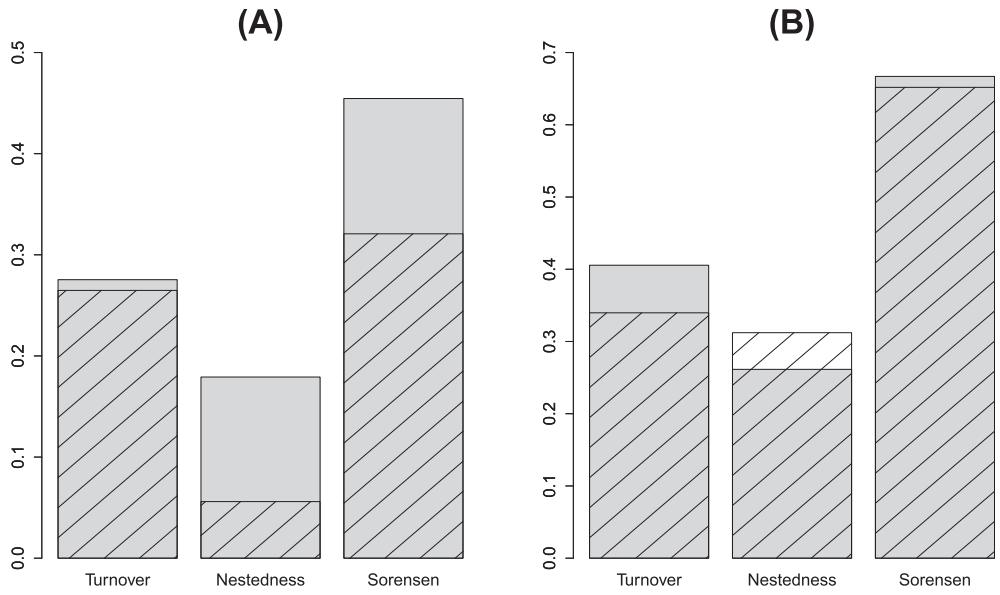

Fig. 4. Partition of beta-diversity into nestedness and turnover components: A) between the two study sites (Pararé and Inselberg RS); B) between the 11 sampling locations. Gray bars and hatched areas represent the results obtained for the whole data set and after deleting singletons, respectively.

Table 3
Taxonomic composition of the earthworms collected, and an estimate of the number of new earthworm species discovered at the Nouragues Natural Reserve of French Guiana.

Family/genus	# of MOTUs	# of specimens analyzed for morphology	# of morpho-species	Estimated # of sp new for science	Already known sp diversity in the genus
Rhinodrilidae					
Rhinodrilus	2	5	2	2	56
Pontoscolex	3	20	3	2	16
Urobenus	1	15	1	1	2
Glossoscolecidae					
Atatina	1	1	1	1	2
Glossodrilus	4	18	4	4	55
Martiodrilus	8	62	8	7	89
Nouraguesia	1	15	1	1	2
Righiodrilus	6	16	6	6	26
Acanthodrilidae (Benhaminae)					
Dichogaster	3	17	3	1	190
Neogaster	7	41	11	7–11	4
Wegeneriona	6	68	9	6–9	6
Haplotaxidae					
Haplotaxis	1	9	1	1	18
Ocnerodrilidae					
Ocnerodrilidae	3	9	3	3	177
Not identified					
Not identified	2	14	2	0–2	–
Total	48	310	55	42–51	–

mountain (terraces, hilltop and transition forests); (2) a group composed of the Pararé plateau and the Inselberg camp; (3) a group composed of the palm forest and the liana forest; (4) a group composed of the high-canopy forests of the Inselberg station (great and small plateaus); (5–6) two individual locations (stream banks of the Inselberg RS and lowland forest of the Pararé RS) both branching at the root of the previous group.

3.3. Earthworm distribution into microhabitats

The rarefaction curves computed for the four main types of microhabitats show that MOTU richness was higher in the soil, followed by decaying trunks, while MOTUs found in epiphytic soils and in sediments were seemingly less diversified (Fig. 3B). This was confirmed by the richness indices (Table 2). Earthworms were found in three different types of epiphytic soils, i.e. in bromeliads, in *Philodendron* roots and in *Heliconia* flowers (SI Fig. 6A). MOTU richness decreased in the different microhabitats as their elevation above the ground level increased, from 37 MOTUs at the ground level to 22 in decaying trunks, and from 14 to one in the different strata of tree canopy (SI Fig. 6B). The similarity in MOTU composition among microhabitats was clearly related to their location in the vegetation column (Fig. 5B).

Each MOTU was assigned to ecological guilds based on those proposed by Bouché (1977) (Fig. 6A) and considering their main morphological features (mainly size and pigmentation, SI Table 3) and their distribution in the different microhabitats (Fig. 5B; SI Fig. 7): endogeics were defined as non-pigmented worms that were found exclusively below the soil or the sediment surface (12 MOTUs, representing 25% of total richness, and 11% of total number

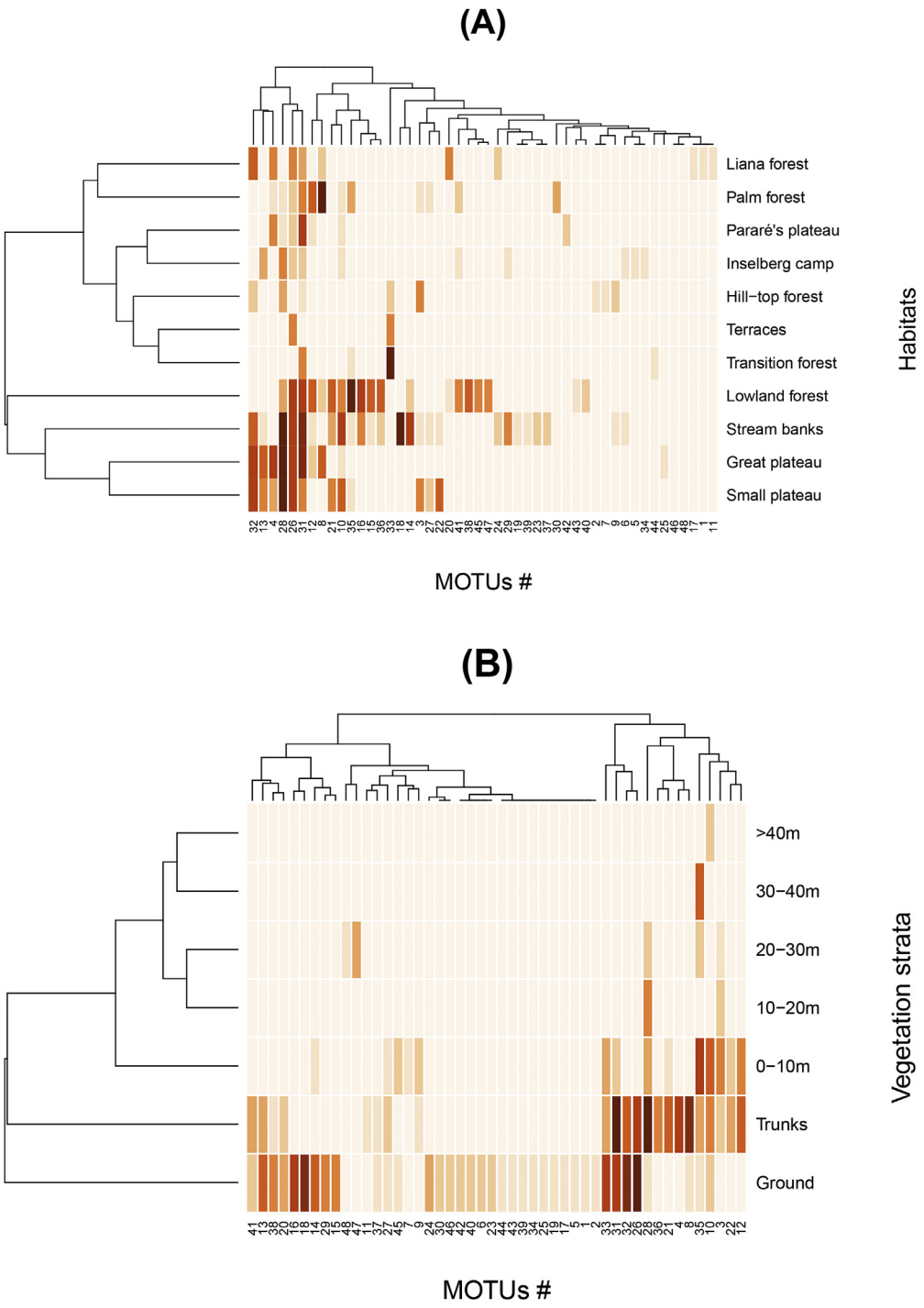

Fig. 5. Heatmaps showing the distribution of the 48 MOTUs among sampling locations (A) and among the vertical stratification of microhabitats (B). Color intensity is proportional to the log-transformed number of individuals corresponding to each MOTU. Dendrograms (Euclidean distances) are grouping locations/microhabitats according to their assemblage composition, and MOTUs according to their distribution in the samples (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of barcoded specimens); anecics were large pigmented worms also found in the soil or crawling at the surface during heavy rain events (one MOTU represented by two specimens); epigeics were assumed to group of small to medium size worms that presented marked body pigmentation (32 MOTUs, i.e. 67% of total richness and 77% of total of barcoded specimens). A few MOTUs presenting mixed characteristics were placed at the boundary between two guilds: *Martiodrilus* sp3 (MOTU # 29) and *Rhinodrilus* sp2 (MOTU # 24) had the size of anecics but were also probably related to endogeics due to their slight pigmentation; *P. corethrurus* (MOTU # 26, representing 10% of the total of barcoded specimens) is a typical endogeic which was however also found in decaying trunks (SI Fig. 7A). Epigeics were further subdivided into litter dwelling (collected in the superficial soil layers), corticolous (collected in decaying trunks) and arboricolous (collected in epiphytic soils) (SI Table 3).

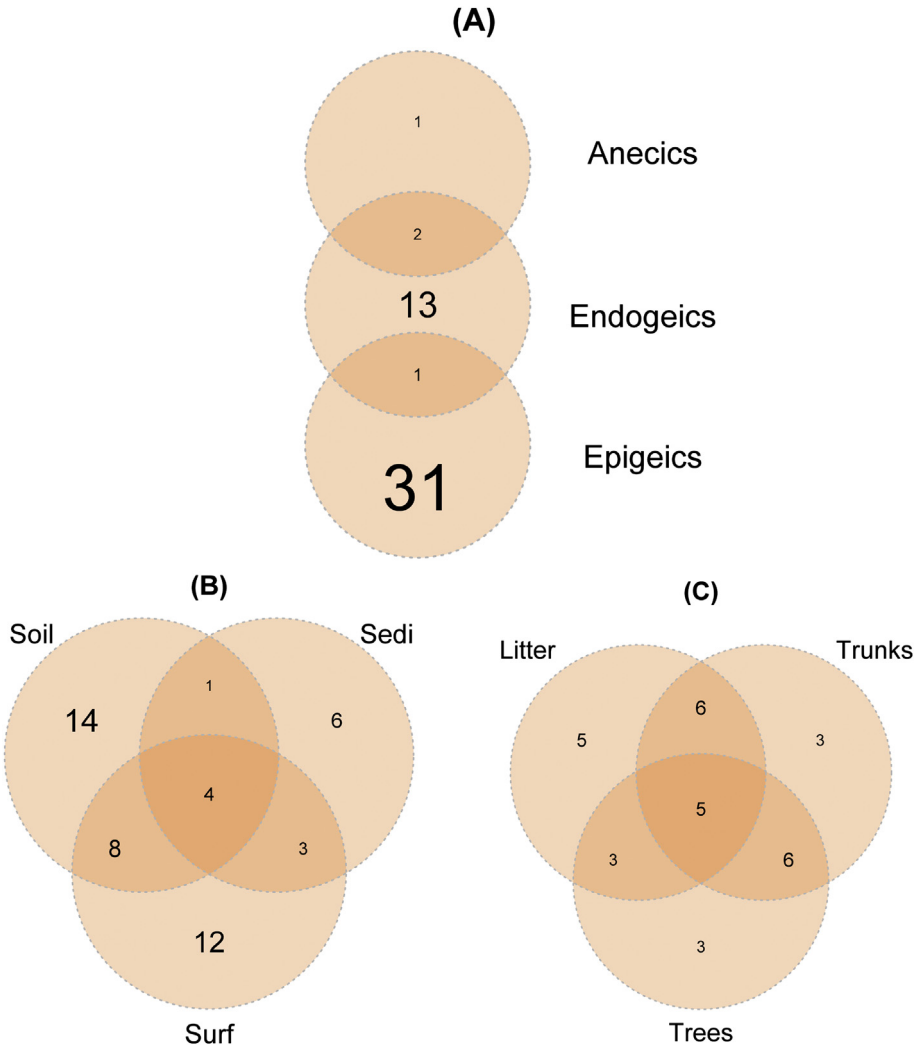

Fig. 6. Venn diagrams showing the distribution: A) of the 48 MOTUs among ecological guilds; B) of the 48 MOTUs among three main types of microhabitats (soils, river bank sediments and surface microhabitats, i.e. surface litter, decaying trunks and epiphytic soils); C) of the 31 MOTUs of epigeic earthworms among the three types of surface microhabitats.

Twenty-six MOTUs (54% of total) were restricted to a single type of microhabitat, 14 others (29% of total) were only observed in two microhabitats, and only one (*M. tenkatei*/MOTU # 31) was present in the five main types of microhabitats (i.e. soils, sediments, litter, decaying trunks and epiphytic soils). Soil and sediments presented a high specificity in their MOTU assemblages, with approximately half of their richness that was not found in other microhabitats (Fig. 6B). Surface microhabitats (i.e. epiphytic soils and decaying trunks) shared 55% of their MOTUs with ground level microhabitats (i.e. soils and/or sediments). Fig. 6C shows that epigeics were mostly generalist worms that were able to develop in different types of surface microhabitats.

4. Discussion

4.1. DNA barcoding and the census of earthworm diversity

The detection of a barcode gap at a 14% cut-off value was congruent with the range of inter-specific divergences usually observed between well-established species in better-known earthworm families such as Lumbricidae (Rougerie et al., 2009; Decaëns et al., 2013). We also found that genetic delineations matched the results inferred from morphological diagnoses, except for four surface dwelling MOTUs that were divided into two or more morpho-species separated by shallow genetic divergences. This finding is in contrast to other studies that found a high frequency of cryptic species in tropical earthworm collections, due to the relative morphological simplicity in these organisms, and to soil environmental constraints that may favor conservation of morphological traits over evolutionary time scale (King et al., 2008; James et al., 2010; Novo et al., 2010). That the only cases for which morphology split MOTUs into several entities were observed for surface dwelling earthworms suggests that morphological characters may evolve more rapidly in these surface microhabitats, maybe as a consequence of higher levels of competition (e.g. for scarce humus accumulation in hollow trees and epiphytes) and higher diversity of organic substrates that may enhance long-term morphological differentiation as a consequence of niche partitioning (Ferriere, 1980). Assessing whether these morpho-species correspond to different biological species or relate to intra-specific phenotypic variability will require further analysis and the use of other genetic markers. In the absence of such additional information, we adopted a conservative position, i.e. we considered MOTUs as objective proxies

for biological species to describe species richness and assemblage patterning.

The systematic barcoding of a large amount of samples allowed us to include in our study the juveniles and cocoons that constituted 60% of sampled specimens, highlighting the presence of 13 MOTUs (i.e. 30% of total) only represented by juveniles that would have been missed with a traditional identification approach. Almost nothing is known to date regarding earthworm population dynamics in tropical rainforests, but one can expect seasonal cycles with a dominance of juvenile cohorts, and adults being present only during the short periods of reproduction (Jiménez, 1999). The probability of sampling only juveniles in a given population could thus be important, especially for the less abundant MOTUs. In our results, seven out of 13 of the strictly juvenile MOTUs were singletons, and all but two were represented by less than four specimens. These results provide an empirical support to the findings of Richard et al. (2010) who suggested that DNA barcoding, by allowing the integration of juveniles into species surveys, could significantly improve the reliability of any study addressing earthworm diversity or based on diversity data.

Our survey of Nouragues' habitats detected 48 species-level MOTUs belonging to four families and 12 genera. This is a considerable increase compared to the 22–33 species listed in currently available check lists for French Guiana (Brown and Fragoso, 2007; Pavlicek and Csuzdi, 2012). However, after morphological examination, a species name could only be assigned to four MOTUs, among which three species already recorded by the authors listed above, while an other (*Dichogaster* sp./MOTU # 5) was likely an invasive species observed for the first time in the region. Most of the remaining 44 MOTUs are probably newly discovered species, leading to a spectacular reassessment of species richness for a number of taxa (e.g. *Nouraguesia*, *Neogaster*, *Wegeneriona*) in which the number of MOTUs was equivalent to or even higher than the current number of described species of these genera.

The diversity of earthworms observed in our study is also much higher than what was previously reported from other tropical forests of Caribbean Islands (3–10 species), Meso-America (5–17 species), South America (7–14 species) and West Africa (40 species at Mt. Nimba, Guinea) (Omodeo, 1958; Fragoso and Lavelle, 1992; Brown and Fragoso, 2007; Csuzdi et al., 2009; Jiménez et al., 2012). So far, the Nouragues' reserve may thus represent the highest local richness recorded to date for earthworms. It is however worth mentioning that none of the previously published studies had used DNA barcoding for species discrimination, which probably resulted in a lower efficiency in species detection compared to our work. This is exemplified by the results of Pavlicek and Csuzdi (2012) whose 21-days survey of the Nouragues' area resulted in a list of only 22 species based on a classical morphological approach.

4.2. Earthworm diversity estimations in the Nouragues' reserve

Adding to this remarkable level of observed species richness, the high proportion of singletons in our results suggests the undersampling of rare species (Coddington et al., 2009; Lim et al., 2012), and both rarefaction curves and richness estimators highlight that up to 60 species could occur in the study area. We need to consider how current environmental conditions and historical factors converge in the Nouragues Natural Reserve to create a hotspot for earthworm biodiversity.

First, as most tropical rainforests, Nouragues' habitats are providing optimal conditions for the maintenance of soil fauna diversity. Their high productivity results in abundant and diverse organic matter substrates, supporting complex detritus food webs (Wardle, 2002). Climatic conditions are also supposed to increase the efficiency of mutualism between soil fauna and microbiota, thus enhancing the range of soil resources usable by earthworms and allowing higher levels of ecological diversity and niche partitioning within communities (Lavelle, 1983). Finally, the Nouragues' region is characterized by a relatively high topographic heterogeneity and an important diversity of habitats, which may result in high ecological and spatial segregation.

Additionally, historical biogeography may also explain the wealth of earthworm diversity in the Nouragues' forests. For instance, the presence in the French Guiana' species pool of six of eight South-American earthworm families (James and Davidson, 2012) suggests that the region may represent a 'museum' (*sensu* Stenseth, 1984) for earthworm diversity, i.e. a place where low extinction rates allowed the conservation of long-divergent lineages across evolutionary times. On the other hand, the high species richness found in some genera, as well as the polymorphism observed in some of them, advocates for recent and/or ongoing events of radiation, which is congruent with predictions of the 'evolutionary cradle' hypothesis (Stenseth, 1984). Testing these scenarios will require large-scale data sets and time-calibrated phylogenies not currently available for earthworms in the Neotropics. However, our results at least suggest that a combination of both hypotheses, as observed for other groups of organisms (McKenna and Farrell, 2006), could be proposed in addition to the influence of current local environment to explain the impressive earthworm diversity in the study area.

The presence of two recognized and one supposed invasive species in this remote and pristine region (i.e. *D. bolaui*, *D. andina* and *Dichogaster* sp.) was a quite unexpected result. However, invasive earthworms are common in French Guiana, representing as much as 40% of the species in some disturbed ecosystems (Lavelle and Lapied, 2003; Brown and Fragoso, 2007). Many invasives are adapted to passive dispersion and are spread by human activities. As an example, Dupont et al. (2012) illustrated how human activities in the Nouragues' reserve may trigger long-distance population dispersal for the species *P. corethrurus*. Similarly, the establishment of *Dichogaster* species in the Nouragues' reserve may have resulted from early introduction near the research stations (e.g. transport with soil, plants, etc), followed by progressive spread in the nearby ecosystems thanks to passive dispersal (e.g. transport of cocoons by streams and surface water, phoresy by birds, mammals and again humans). This hypothesis of recent and human-mediated introduction is supported by the fact that *Dichogaster* sp. and *D. bolaui* were observed in a single place corresponding to current or past research base camps (i.e. the Inselberg RS and the now abandoned 'Museum' camp in Pararé' lowland forest). Conversely, *D. andina* was found in a number of habitats in both Pararé and Inselberg RS, suggesting a more ancient introduction in one or several points, followed by population dispersal in the surrounding area.

Our results suggest a substantially higher richness in the Inselberg RS than in Pararé RS. Even if not very distant from each other, both study sites may host quite different earthworm communities: more than 60% of the MOTUs were observed in a unique site and beta-diversity was mostly explained by spatial turnover and not assemblage nestedness. This can be partly due to the substantial differences in landscape composition at each site. Inselberg RS is in fact characterized by an important topographic heterogeneity, with habitats dominated by well-drained plateaus isolated by small river valleys, and the presence of specific habitats

on the Inselberg mountain. By contrast, Pararé RS exhibits more homogeneous habitats dominated by lowland forests. Alternatively, spatial turnover could be explained by the predominance of species with narrow geographical ranges, as proposed by Lavelle and Lapied (2003) who suggested that local endemism may explain the low ratio of local to regional richness observed for earthworm communities of the Guyanan Shield.

4.3. Diversity patterns across habitats and microhabitats

Alpha diversity in earthworm communities is commonly assumed to be limited to 12 species irrespectively to latitudinal position and to the size of the regional species pool (Wardle, 2002). This has been attributed to the relatively low diversity of resource types in soils and to the ecological plasticity of most earthworm species where juveniles are often 10 to 100 times smaller than fully-grown adults, therefore occupying quite different niches (Lavelle, 1983). These factors lead to a rapid saturation of the ecological space available for the community and limit the number of coexisting species during assembly processes (Decaëns et al., 2008). Our results broadly support this idea, as both observed and estimated richness remained below this limit in most of the habitats, despite the huge richness recorded at the scale of the whole study area. Higher levels of diversity were however observed in the lowland forests (19 MOTUs) and in the stream banks (25 MOTUs), which are both characterized by a higher availability of organic resources and a higher microhabitat diversity compared to other habitats (e.g. different types of sediments and organic matter accumulation in the stream banks). This may result in a slower saturation of the ecological space during community assembly process, thus allowing a higher number of species to coexist locally in these habitats.

Assemblage composition substantially differed from one habitat to another, with beta diversity mainly explained by spatial turnover, suggesting that differences in assemblage composition among habitats are mainly explained by MOTU replacement, and to a lesser extent by assemblage nestedness indicating that MOTU composition in less diversified communities was a subset of MOTU composition in more diversified ones. This points out how local assemblages are composed of species originating from the regional pool, with generalists mainly constrained by distance and dispersal abilities, and specialists by local scale environmental filtering (Belyea and Lancaster, 1999). This is exemplified by habitat clustering (Fig. 6A) in which a high assemblage similarity was found between the three habitats of the inselberg mountain, which all differ from other habitats due to the superficial granitic bedrock. Another grouping was found between the great and small plateaus, which present similar environmental characteristics and are relatively close to each other. The two clusters formed by the palm forest, the liana forest, the Pararé plateau and the Inselberg camp may further be related to the high level of disturbance that characterize these habitats and/or by the abundance of decaying wood observed at the soil surface. For the same reasons as presented previously, lowland forest and stream banks significantly differed from the surrounding habitats for the MOTUs composition of their communities. Similar results were found by Fragoso (1985) in the Mexican Lacandon forest.

Earthworm assemblages in the Nouragues' forests are dominated by specialist species, with only one generalist observed in the five main types of microhabitats, while 54% and 29% of MOTUs restricted to a single or two type of them, respectively. Earthworm assemblages are also clearly dominated by the epigeic guild, both in terms of diversity and number of specimens. This supports the results of previous studies pointing out that earthworm communities in oligotrophic tropical rainforests of South America are usually dominated by epigeic/anecic assemblages, whereas endogeics might be dominant in nutrient-rich ecosystems of Meso-America and Africa (Fragoso and Lavelle, 1992).

Another interesting result of our study was the observation of 17 MOTUs living in epiphytic microhabitats. Arboricolous earthworms have been already described by a few authors such as Lavelle and Kohlmann (1984) and Rodriguez et al. (2007) who mentioned their presence in bromeliads of Mexican and Caribbean forests without any indication of the number of species involved. Lavelle (1978) observed five species in the heads of palm trees in Ivory Coast savannas, and Fragoso and Rojas-Fernandez (1996) reported the existence of four species in bromeliads of the Chajul forest in south-eastern Mexico. Our results highlight that earthworms living in epiphytic microhabitats maybe more diversified than previously thought, and may colonize bromeliads, but also root networks of *Philodendron* and decaying organs of *Heliconia* flowers. Three of these MOTUs were only found in epiphytic soils, while the others were observed in at least one other type of microhabitat, suggesting that epiphytic niches are likely colonized by individuals dispersing from ground level populations. This supposition is supported by several direct observations of specimens of e.g. *Nouraguesia* sp. (MOTU # 28) and *D. andina* (MOTU # 35) climbing on humid trunks during rainy days. Such a behavior may result from random displacement of individuals for resource foraging, or from active dispersal triggered by adverse conditions (e.g. resource depletion, environmental stress, excessive population density) occurring in the source population (Mathieu et al., 2010).

5. Conclusion

Our study exemplified that DNA barcodes can represent an efficient way to by-pass the taxonomic impediment and increase the pace of biodiversity pattern description for understudied invertebrate taxa and/or geographic areas. A similar study using a classical morphological approach for species discrimination would have probably been extremely difficult and more time consuming, due to the high proportion of juveniles present in the samples, and to the predominance of new taxa in the Nouragues' species pool.

Repeating this approach in a standardized way for a range of localities can further represent a promising way to allow rapid and reliable inter-site comparisons for macroecological studies, far beyond what could be expected from traditional identification approaches. Aggregating compatible dataset at such a regional scale would for instance allow reliable beta-diversity analysis of earthworm communities. Our findings support that large scale spatial turnover might be considerable in the Amazonian region (Lavelle and Lapied, 2003), but more information is still needed to test this pattern and understand its environmental and historical determinants. Another output of such a regional analysis will be the re-evaluation of broad-scale species richness. Our results represent a first step in this direction, suggesting that the species pool for French Guiana could be much higher and more spatially structured than initially expected.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.10.009.

References

Adams, M., Raadik, T.A., Burridge, C.P., Georges, A., 2014. Global biodiversity assessment and hyper-cryptic species complexes: more than one species of elephant in the room? Systematic Biology 63, 518–533.

André, H.M., Ducarme, X., Anderson, J.M., Crossley, D., Koehler, H., Paoletti, M., Walter, D., Lebrun, P., 2001. Skilled eyes are needed to go on studying the richness of the soil. Nature 409, 761.

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography 19, 134–143.

Belyea, L.R., Lancaster, J., 1999. Assembly rules within a contingent ecology. Oikos 86, 402–418.

Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., Abebe, E., 2005. Defining operational taxonomic units using DNA barcode data. Philosophical Transactions of the Royal Society B: Biological Sciences 360, 1935–1943.

Bouché, M.B., 1977. Statégies lombriciennes. In: Lohm, U., Persson, T. (Eds.), Soil Organisms as Component of Ecosystems. Ecological Bulletin, Stockholm, pp. 122–132.

Brown, G.G., Fragoso, C., 2007. Minhocas na América Latina: biodiversidade e ecologia. Embrapa, Curitiba, p. 545.

Chazdon, R.L., Whitmore, T.C., 2002. Foundations of Tropical Forest Biology. The University of Chicago Press, Chicago, p. 863.

Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M., Hormiga, H., 2009. Undersampling bias: the null hypothesis for singleton species in tropical arthropod surveys. Journal of Animal Ecology 78, 573–584.

Csuzdi, C., 2010. A Monograph of the Paleotropical Benhamiinae Earthworms (Annelida: Oligochaeta, Acanthodrilidae). Hungarian Natural History Museum, Budapest, 348 pp.

Csuzdi, C., Guei, M.A., Tondoh, J.E., 2009. New and little known earthworm species from the Mt. Nimba, Guinea (Oligochaeta, Acanthodrilidae: Benhamiinae). Zootaxa 2141, 56–68.

Csuzdi, C., Pavlicek, T., 2011. New and little known species of the genus *Martiodrilus* Michaelsen, 1936 from French Guiana (Oligochaeta, Glossoscolecidae). Zootaxa 3099, 57–64.

Csuzdi, C., Pavlicek, T., Nevo, E., 2008. Is Dichogaster bolaui (Michaelsen, 1891) the first domicole earthworm species? European Journal of Soil Biology 44, 198–201.

Dayrat, B., 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society 85, 407–415.

de Queiroz, K., 2007. Species concepts and species delimitation. Systematic Biology 56, 879–886.

Decaëns, T., 2010. Macroecological patterns in soil communities. Global Ecology and Biogeography 19, 287–302.

Decaëns, T., Margerie, P., Aubert, M., Hedde, M., Bureau, F., 2008. Assembly rules within earthworm communities in North-Western France a regional analysis. Applied Soil Ecology 39, 321–335.

Decaëns, T., Porco, D., Rougerie, R., Brown, G.G., James, S.W., 2013. Potential of DNA barcoding for earthworm research in taxonomy and ecology. Applied Soil Ecology 65, 35–42.

Dupont, L., Decaëns, T., Lapied, E., Chassany, V., Marichal, R., Dubs, F., Maillot, M., Roy, V., 2012. Genetic signature of accidental transfer of the peregrine earthworm *Pontoscolex corethrurus* (Clitellata, Glossoscolecidae) in French Guiana. European Journal of Soil Biology 53, 70–75.

Feijoo, A., 2001. Impacto del uso de la tierra en áreas de laderas sobre comunidades de macro-fauna del suelo (Caldono, Cauca, Colombia). Universidad Nacional de Colombia, Palmira.

Ferriere, G., 1980. Fonctions des Lombriciens. VII. Une méthode d'analyse de la matière organique végétale ingérée. Pedobiologia 20, 263–273.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3, 294–299.

Fragoso, C., 1985. Ecología general de las lombrices terrestres (Oligochaeta: Annelida) de la región Boca del Chajul, Selva Lacandona (Chiapas, México). Universidad Autonoma de México, p. 133.

Fragoso, C., Lavelle, P., 1992. Earthworm communities of tropical rain-forests. Soil Biology and Biochemistry 24, 1397–1408.

Fragoso, C., Rojas-Fernandez, P., 1996. Earthworms inhabiting bromeliads in Mexican tropical rainforests: ecological and historical determinants. Journal of Tropical Ecology 729–734.

Gaston, K.J., Blackburn, T.M., 2000. Pattern and Process Inmacroecology. Blackwell Science, Oxford.

Gates, G.E., 1972. Burmese earthworms. Transactions of the American Philosophical Society 62, 1–326.

Godfray, H.C.J., Lewis, O.T., Memmott, J., 1999. Studying insect diversity in the tropics. Philosophical Transactions of the Royal Society London B 354, 1811–1824.

Gotelli, N.J., Entsminger, G.L., 2001. Ecosim: Null Models Software for Ecology – Version 6.10. Acquired Intelligence Inc. & Kesey-Bear.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., Hebert, P.D.N., 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences 103, 968–971.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B 270, 313–321.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., Hallwachs, W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings of the National Academy of Sciences 101, 14812–14817.

James, S.W., Davidson, S.K., 2012. Molecular phylogeny of earthworms (Annelida: Crassiclitellata) based on 28S, 18S and 16S gene sequences. Invertebrate Systematics 26, 213–229.

James, S.W., Porco, D., Decaëns, T., Richard, B., Rougerie, R., Erséus, C., 2010. DNA barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): resurrection of *L. herculeus* (Savigny, 1826). PLoS One 5, e15629.

Jiménez, J.J., 1999. Dinamica de las poblaciones y estructura de las comunidades de lombrices de tierra de las sabanas naturales y perturbadas de los Llanos Orientales de Colombia. Universidad Complutense de Madrid.

Jiménez, J.J., Decaëns, T., Rossi, J.P., 2012. Soil environmental heterogeneity allows spatial co-occurrence of competitor earthworm species in a gallery forest of the Colombian 'Llanos'. Oikos 121, 915–926.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. Journal of Molecular Evolution 16, 111–120.

King, R.A., Tibble, A.L., Symondson, W.O.C., 2008. Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Molecular Ecology 17, 4684–4698.

Koleff, P., Gaston, K.J., Lennon, J.J., 2003. Measuring beta diversity for presence-absence data. Journal of Animal Ecology 72, 367–382.

Lavelle, P., 1978. Les vers de terre de la savane de Lamto (Côte d'Ivoire): peuplements, populations et fonctions dans l'écosystème. University of Paris VI.

Lavelle, P., 1983. The structure of earthworm communities. In: Satchell, J.E. (Ed.), Earthworm Ecology: from Darwin to Vermiculture. Chapman & Hall, London, pp. 449–466.

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology 42, S3–S15.

Lavelle, P., Kohlmann, B., 1984. Etude quantitative de la macrofaune du sol dans une forêt tropicale humide du Mexique (Bonampak, Chiapas). Pedobiologia 27, 377–393.

Lavelle, P., Lapied, E., 2003. Endangered earthworms of Amazonia: an homage to Gilberto Righi. Pedobiologia 47, 419–427.

Lees, D.C., Kawahara, A.Y., Rougerie, R., Oshima, I., Kawakita, A., Bouteleux, O., De Prins, J., Lopez-Vaamnonde, C., 2014. DNA barcoding reveals a largely unknown fauna of Gracillariidae leaf mining moths in the Neotropics. Molecular Ecology Resources 14, 286–296.

Lemon, J., 2006. Plotrix: a package in the red light district of R. R-News 6, 8–12.

Letunic, I., Bork, P., 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23, 127–128.

Lim, G., Balke, M., Meier, R., 2012. Determining species boundaries in a world full of rarity: singletons, species delimitation methods. Systematic Biology 61, 165–169.

Manly, B.F.J., 1997. Randomization and Monte Carlo Methods in Biology. Chapman & Hall.

Mathieu, J., Barot, S., Blouin, M., Caro, G., Decaëns, T., Dubs, F., Dupont, L., Jouquet, P., Nai, P., 2010. Habitat quality, conspecific density, and habitat pre-use affect the dispersal behaviour of two earthworm species, *Aporrectodea icterica* and *Dendrobaena veneta*, in a mesocosm experiment. Soil Biology and Biochemistry 42, 203–209.

May, R.M., 2011. Why worry about how many species and their loss? PloS Biology 9, e1001130.

McKenna, D.D., Farrell, B.D., 2006. Tropical forests are both evolutionary cradles and museums of leaf beetle diversity. Proceedings of the National Academy of Sciences 103, 10947–10951.

Nemeth, A., 1981. Estudio ecológico de las lombrices de tierra (Oligochaeta) en ecosistemas de bosque húmedo tropical en San Carlos de Río Negro, Territorio Federal Amazonas. Universidad Central de Venezuela.

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., Díaz Cosín, D.J., 2010. Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. Molecular Phylogenetics and Evolution 56, 507–512.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Stevens, M.H.N., Wagner, H., 2008. vegan: Community Ecology Package. R Package Version 1.13-1.

Omodeo, P., 1958. Oligochetes. La reserve naturelle integrale du Mont Nimba. In: Memoire de l'Institut francais d'Afrique noire, vol. 53, pp. 9–109.

Pavlicek, T., Csuzdi, C., 2012. Earthworm fauna of French Guiana (Oligochaeta). Zoology in the Middle East S4, 107–110.

Plaisance, L., Knowlton, N., Paulay, G., Meyer, C., 2009. Reef-associated crustacean fauna: biodiversity estimates using semi-quantitative sampling and DNA barcoding. Coral Reefs 28, 977–986.
Porco, D., Skarżyński, D., Decaëns, T., Hebert, P.D.N., Deharveng, L., 2013. Barcoding the Collembola of Churchill: a molecular taxonomic reassessment of species diversity in a sub-Arctic area. Molecular Ecology Resources 14, 249–261.
Puillandre, N., Modica, M.V., Zhang, Y., Sirovich, L., Boisselier, M.C., Cruaud, C., Holford, M., Samadi, S., 2012. Large-scale species delimitation method for hyperdiverse groups. Molecular Ecology 21, 2671–2691.
Qian, H., Ricklefs, R.E., White, P.S., 2005. Beta diversity of angiosperms in temperate floras of eastern Asia and eastern North America. Ecology Letters 8, 15–22.
R Development Core Team, 2004. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Richard, B., Decaëns, T., Rougerie, R., James, S.W., Porco, D., Hebert, P.D.N., 2010. Reintegrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. Molecular Ecology Resources 10, 606–614.
Righi, G., 1984. Pontoscolex (Oligochaeta, Glossoscolecida), a new evaluation. Studies on Neotropical Fauna and Environment 19, 159–177.
Righi, G., 1996. Colombian earthworms. Studies on Tropical Andean Ecosystems 4, 485–607.
Rodriguez, C., Borges, S., Martinez, M.A., Fragoso, C., James, S.W., Gonzalez, G., 2007. Biodiversidad y ecologia de las lombrices de tierra en las islas caribeñas. In: Brown, G.G., Fragoso, C. (Eds.), Minhocas na América Latina: biodiversidade e ecologia Embrapa, Curitiba, pp. 79–108.
Rougerie, R., Decaëns, T., Deharveng, L., Porco, D., James, S.W., Chang, C.-H., Richard, B., Potapov, M., Suhardjono, Y., Hebert, P.D.N., 2009. DNA barcodes for soil animal taxonomy. Pesquisa Agropecuaria Brasileira 44, 789–801.
Rubinoff, D., Holland, B.S., 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology 54, 952–961.
Saitou, N., Nei, M., 1987. The neighbor-joining method – a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.
Schloss, P.D., Westcott, S.L., Ryabin, T., et al., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.
Smith, M.A., Fernandez-Triana, J.L., Eveleigh, E.S., Gomez, C.G., Hallwachs, W., Hebert, P.D.N., Hrcek, J., Huber, J.T., Janzen, D.H., Mason, P.G., Miller, S.E., Quicke, D.L., Rodriguez, J., Rougerie, R., Shaw, M.R., Varkonyu, G., Ward, D.F., Whitfield, J.B., Zaldivar-Riveron, A., 2013. DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): impacts after 8 years and nearly 20 000 sequences. Molecular Ecology Resources 13, 168–176.
Smith, M.A., Fisher, B.L., Hebert, P.D.N., 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. Philosophical Transactions of the Royal Society London B 360, 1825–1834.
Srivathsan, A., Meier, R., 2012. On the inappropriate use of kimura-2-parameter (k2p) divergences in the DNA-barcoding literature. Cladistics 28, 190–194.
Stenseth, N.C., 1984. The tropics: cradle or museum? Oikos 43, 417–420.
Tamura, K., Peterson, D., Peterson, N., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731–2739.
Tanzler, R., Sagata, K., Surbakti, S., Balke, M., Riedel, A., 2012. DNA barcoding for community ecology – how to tackle a hyperdiverse, mostly undescribed Melanesian fauna. PLoS One 7, e2883.
Ulrich, W., Almeida-Neto, M., Gotelli, N.J., 2009. A consumer's guide to nestedness analysis. Oikos 118, 3–17.
Wardle, D.A., 2002. Communities and Ecosystems – Linking the Aboveground and Belowground Components. Princeton University Press, Princeton, 392 pp.
Wiemers, M., Fiedler, K., 2007. Does the DNA barcoding gap exist? A case study in blue butterflies (Lepidoptera: Lycaenidae). Frontiers in Zoology 4, 8.
Wright, D.H., Reeves, J.H., 1992. On the meaning and measurement of nestedness of species assemblages. Oecologia 92, 416–428.
Wilson, E.O., 1988. Biodiversity. National Academy of Sciences, Washington DC.
Young, M.R., Behan-Pelletier, V.M., Hebert, P.D.N., 2012. Revealing the hyperdiverse mite fauna of subarctic Canada through DNA barcoding. PLoS One 7, e48755.