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ECOSYSTEMS

First record on enchytraeids in a Savanna Tall Woodland (Cerradão) and Upper Montane Atlantic Forest in Brazil

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Abstract: Brazil is considered a megadiverse country, but the soil fauna is still very poorly known. The aim of this study was to report, for the first time, the abundance and genus composition of terrestrial enchytraeids (Enchytraeidae, Oligochaeta) in Savanna Tall Woodland (Cerradão) and a pasture in Cerrado Biome and in Upper Montane Atlantic Forest and a grassland in Atlantic Forest Biome. The enchytraeid density in Pasture and Cerradao was 2,036 and 18,844 (204 and 2,094, on average) individuals per square meter, respectively. At the Atlantic forest and Grassland, density was 9,666 and 12,242 individuals per square meter (1,075 and 1,471 on average). About genus composition for the studied areas, *Enchytraeus* and *Hemienchytraeus* were found in the four ecosystems evaluated, while *Tupidrilus* and *Fridericia* were found only in Cerradão and Atlantic Forest, respectively. *Achaeta* was absent in Upper Montane Atlantic Forest, but dominant in pasture, while *Guaranidrilus* was absent in Pasture, but predominant in the other ecosystems.

Key words: Atlantic forest, cerrado, soil ecology, potworms, enchytraeid abundance, genus composition.

INTRODUCTION

Enchytraeids (Enchytraeidae, Oligochaeta, Annelida) are often small, whitish worms without pigment, ranging in body length from about 2 to 2 to 40 millimeters. They are distributed worldwide, in soils with appropriate moisture, oxygen and nutrient supply (Jänsch et al. 2005, Schmelz et al. 2013). At the local scale, distribution and composition of species are strongly influenced by factors such as pH, organic matter content, moisture, and land-use system (Didden 1993, Jänsch et al. 2005).

Enchytraeids are known to be an important group of soil invertebrates in temperate regions, but few studies on their biology and ecology have been carried out in the tropics (Schmelz et al. 2013, Römbke 2007). According to Schmelz & Collado (2015), there are 710 species within 33 genera with valid descriptions in the world, among which 62 species of 15 genera have been reported from Latin America (Schmelz et al. 2013). There is a hypothesis that enchytraeids may have originated in South America (Coates 1989), but knowledge about the species diversity of enchytraeids in typical ecosystems of this continent is so incomplete that it is not yet possible to estimate the number of existing species.

The occurrence of enchytraeids has been sporadically reported in studies of macrofauna in several land use systems in South America

(Silva et al. 2006, Manetti et al. 2010, Portilho et al. 2011, Domínguez & Bedano 2016a, b). However, the methodology to assess the population on these small oligochaetes was not standardized and neither revealed species diversity. Studies on their usefulness as bioindicators in the southern hemisphere are needed because of their importance in the maintenance of soil ecosystem services and their broad distribution, but knowledge on their ecology and diversity are still very poor (Schmelz et al. 2013).

In Brazil, after Righi´s remarkable contributions to enchytraeid taxonomy (Römbke 2003), more recent ecological and taxonomic information has been made available on enchytraeids from the Amazon region (Römbke & Meller 1999, Schmelz & Römbke 2005) and the Atlantic Forest in Paraná State (Römbke et al. 2005, 2007, 2015, Schmelz et al. 2008, 2009, 2011, Niva et al. 2015). These studies revealed that species composition responds to soil type and land use and, above all, revealed many new species, and even a new genus. Studies on new tropical and subtropical ecosystems are important to broaden the ecological database on this group of organisms. Even though Brazil is considered a megadiverse country, soil fauna is still very poorly known and needs more attention (Lewinsohn et al. 2005).

Here we present a pioneer study on abundance and genus distribution of enchytraeids in four hitherto almost completely neglected vegetation types in the biomes of Cerrado and Atlantic Forest. Data on abundance and genus composition were assessed and compared in a fragment of Upper Montane Atlantic Forest and a contiguous grassland in Atlantic Forest biome in Santa Catarina State and a fragment of “Cerradão” and a pasture of Cerrado biome in The Federal District of Brazil.

MATERIALS AND METHODS

Study sites and soil sampling

Four areas with contrasting vegetation were studied, two of them in Planaltina, Brasília, Federal District, within the Cerrado biome, and further two in the municipality of Urupema, State of Santa Catarina, Southern Brazil, within the Atlantic Forest biome (Figure 1).

In Planaltina (Figure 1), the sampling sites were both located at 1007 m asl in an experimental area of Embrapa at Brazilian Cerrado (15°36’S 47°42W), one in a fragment of Savanna Tall Woodland - “*Cerradão*” (CER) and the other in a continuous pasture of *Urochloa brizantha* cv. BRS Piatã (Almeida et al. 2009) with frequent animal grazing, implemented six years prior to sampling (PAST6). The climate is Aw according to Köppen’s classification, with minimum and maximum yearly average temperatures of 16.7 °C and 28.5 °C, respectively, and an annual average precipitation of 1,500 mm, with a pronounced dry season in July to September and a rainy season in October to April. The soils in CER and PAST6 are classified as Oxisols.

In Urupema (Figure 2), the sampling sites were both located at 1,540 m asl, one of them in a fragment of the upper montane Atlantic Forest (HAF), in primary succession stage, without anthropic intervention and livestock entry for at least ten years, and characterized by *Araucaria angustifolia* associated with *Drimys angustifolia* and *Ilex paraguariensis*, (27°57’55,52” S 49°50’22.77” W). The other site was a native grassland (GRA) dominated by native species of *Andropogum* and *Paspalum* (27°57’44,24” S 49°50’67” W). The climate at the Urupema sites is subtropical Cfb, according to Köppen’s classification, with evenly distributed rainfall during the year and annual average precipitation of 1,789 mm. It is considered one of the coldest places in Brazil. The annual average

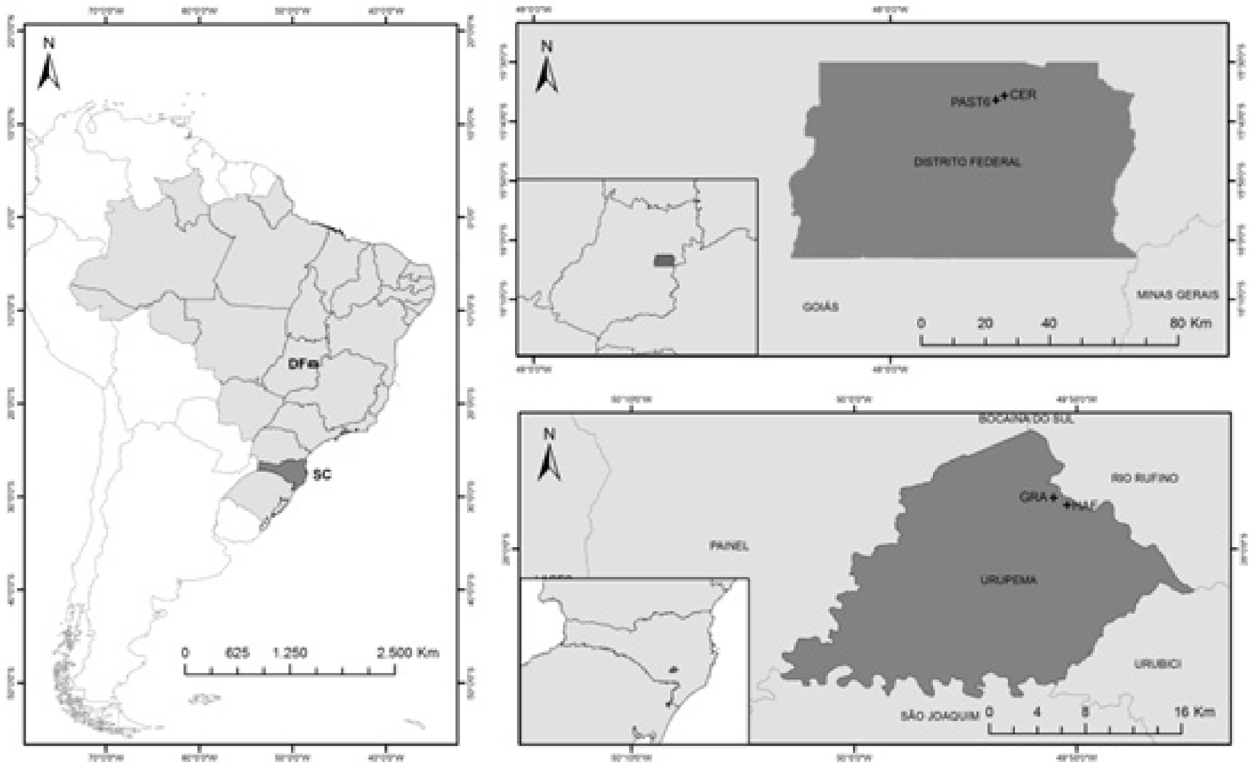

Figure 1. **Map of Brazil, with Santa Catarina State (SC) and Federal District (DF) highlighted, on the left. Geographical localization of Planaltina Federal District (Distrito Federal) and sampling points on Pasture (PAST6) and Savanah Tall Woodland (CER) on the upper-right. Geographical localization of Urupema at Santa Catarina State and sampling points on Upper Montane Atlantic Forest (HAF) and grassland (GRA), on the lower right.**

temperature is 13 °C, snow commonly occurs in July and August. The soil in GRA is classified as an Inceptisol while the soil in HAF is an Entisol.

The chemical and physical characteristics of the soils from the four sites were determined at Embrapa Cerrados and UDESC Laboratories, following standard analyses based on methods described in Embrapa (1997), and are presented in Table I.

Soil cores of 5.0 cm of diameter and 5.0 cm depth were collected following the ISO guideline 23611-3 (ISO 2007). At each site, samples were collected along an imaginary line of approximately 100 m, where 10 sampling points were established with a minimum of 10 meters distance from each other. The sampling was performed in the central area of the sites leaving about 30 meters from the border of each site to avoid border effects. The samples were transported to the laboratory and kept refrigerated at 18 ± 2 °C until further processing. Sampling in Cerrado was performed at the end of rainy season, in May 2015, and in Atlantic Forest biome was performed in summer, in February 2017.

Diversity assessment

The enchytraeids were extracted by O'Connor's hot wet extraction method (O'Connor 1955) following the adaptations of Niva et al. (2010, 2015) for subtropical regions. The identification was performed *in vivo*, through the observation of internal and external morphological structures of the individuals under a light microscope according to guidance given in in Schmelz & Collado (2010).

Total and average population density in each site were calculated eliminating outliers if

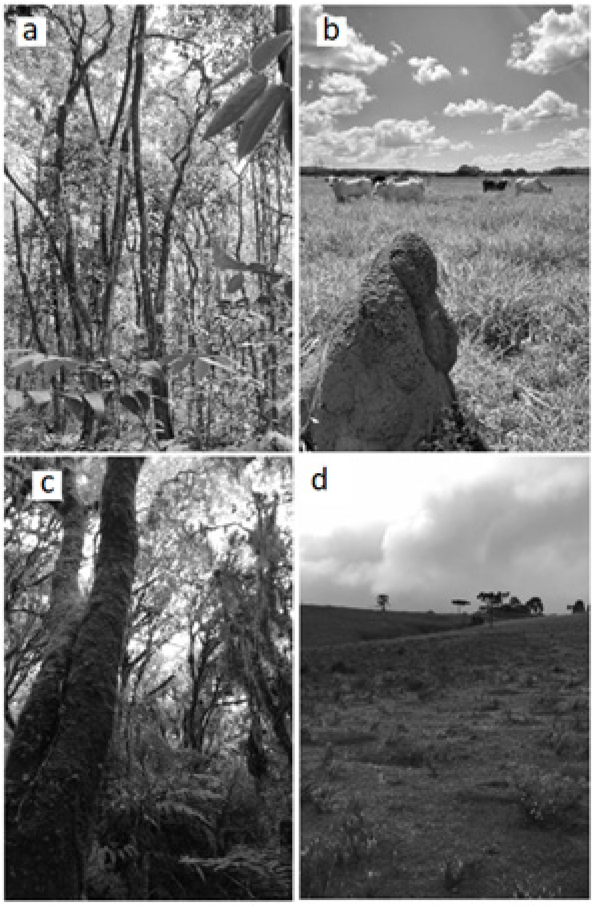

Figure 2. View of the sites. Savanna Tall Woodland – Cerradão (a) and pasture with a termite mound (b) in Cerrrado biome, Planaltina, Federal District. Upper Montane Atlantic Forest (c) and grassland (d) in Atlantic Forest biome, Urupema, Santa Catarina State.

needed. The diversity of enchytraeid communities was calculated by the Shannon-Wiener diversity index (H'), as well as the Simpson's index (S) and the Pielou evenness index (J). The diversity indexes for each system were estimated using total abundance values of each genus with the software Past 3.0. The relative abundance of each genus was also estimated.

RESULTS

The calculated total population density varied from 2,000 to almost 19,000 ind.m^{-2} distributed in six different genera in the studied sites (Table II). The lowest average value was found in PAST6 and the highest in CER. Enchytraeid densities in CER varied from 0 to 3,500 while in Past6 from 0 to 500, in HAF from 0 to 2,000 and in GRA from 0 to 4,500 ind.m^{-2}. The genus richness was also highest in CER and the lowest in PAST6, both within the Cerrado biome, while the population density and genus richness in the other sites were intermediate. The diversity indices did not show striking differences between the sites, although the highest Shannon-Wiener index found in HAF and the lowest Simpson's index and Pielou evenness found in CER, suggest dominance of some genus especially in CER.

The relative abundance of genera is shown in figure 3. In CER, *Guaranidrilus* (62.2%) predominated over *Hemienchytraeus* (13.5%), *Achaeta* and *Hemienchytraeus* (10.8% both) and *Tupidrilus* (2.7%) while in PAST6 *Achaeta* (50%) was most abundant, followed by *Enchytraeus* and *Hemienchytraeus* (25% both). In HAF, *Enchytraeus* and *Guaranidrilus* (36.8% both) were predominant, followed by *Hemienchytraeus* (21%) and *Fridericia* (5.3%). In GRA, the predominant genus was *Guaranidrilus* (53.8%), followed by *Enchytraeus* (19.2%), *Achaeta* (15.4%) and *Hemiechytraeus* (11.5%). *Tupidrilus* occurred exclusively in CER, while *Fridericia* occurred only in HAF.

DISCUSSION

The values of abundance found on this study (average 200 to 2,000 ind.m^{-2}) are within the range found in Mata Atlantica in Antonina and Guaraqueçaba (Römbke et al. 2015) (Table II). However, pilot quantitative samplings in Cerradao in previous months, when rainfall was more intense (December, 2014 and April, 2015), revealed 5,600-13,000 ind.m^{-2}, meaning that enchytraeid abundance can be much higher. In Central Amazonia, Römbke & Meller (1999)

Table I. Soil chemical and physical attributes at the four study sites. CER: Savanna Tall Woodland – Cerradão. PAST6: Pasture (Cerrado biome). HAF: Upper montane Atlantic Forest. GRA: Grassland (Atlantic forest biome).

	CER	PAST6	HAF	GRA
pH $_{water}$	4.36	5.01	3.7	4.6
Ca (cmol.dm^{-3})	0.13	1.20	0.001	2.07
Mg (cmol.dm^{-3})	0.25	0.38	0.35	1.71
Al (cmol.dm^{-3})	2.79	0.56	6.09	2.87
Effective cation exchange capacity (cmolc^{-3})	3.30	2.33	6.61	6.85
Organic Matter (%)	5.64	2.79	5.7	6.0
Clay (%)	52.1	53.7	14	15
P Mehlich (mg.dm^{-3})	1.80	4.30	0.1	0.1
K (mg.dm^{-3})	56.30	76.30	77.00	61.00

found, on average, 3,900 – 4,600 ind.m^{-2}. In Atlantic Forest in the State of Paraná, Römbke et al. (2007) reported a maximum of 4,945 ind.m^{-2}, at a primary forest on Cambisol, and in advanced secondary forest on Gleysol 2,457 ind.m^{-2}. Lowest abundance was recorded from a pasture on Cambisol and a medium secondary forest on Gleysol with 498 ind. m^{-2} and 591 ind. m^{-2} respectively. Niva et al. (2015), evaluated two methods of enchytraeids extraction (cold and hot extraction) in an Araucaria mixed Forest also in Paraná state. The obtained average abundances were between 2,000 – 12,600 ind. m^{-2} with cold extraction and 5,000 – 12,600 ind.m^{-2} with hot extraction.

Silva et al. (2006, 2008) & Aquino et al. (2008), in studies carried out in Dourados – MS, in the Cerrado biome during summer and winter cropping seasons, found abundance of enchytraeids reached in average 120 ind.m^{-2} in soils under cropping systems and pastures, while in a natural Cerrado they found 7,758 ind.m^{-2}. According to the authors, the highest values of abundance found in natural systems are probably due to the greater supply of energy resources and diversity of microhabitats provided by the plant diversity of natural systems. However, these authors used the Tropical Soil Biology and Fertility method (Anderson & Ingram 1993) with hand sorting of the specimens, a method typically used for macrofauna studies, which underestimates the values of enchytraeid abundance due to the small size of these worms (Niva et al. 2015). Domínguez & Bedano (2016a,b), who applied the same method, found similarly low numbers of enchytraeids in grassland and farmland of differing management intensity in the Argentinean Pampas. Compared to other macrofauna groups studied by Silva et al. (2008), the strong dominance of the enchytraeids in the Cerrado (more than half of the total community density) was attributed to a more stable environment in terms of temperature, humidity and better quality and abundance of organic matter.

Edaphoclimatic factors can influence the taxon-specific distribution of enchytraeids. Römbke et al. (2007) observed that enchytraeids in the Atlantic Forest of the Paraná State grouped differently depending on soil type and vegetation succession stage. Pelosi & Römbke (2016) point out that changes in land use management influence total abundance and community composition, nevertheless the

Table II. **Abundance (total and average ± standard error of mean), genus richness and diversity indexes of enchytraeids in Cerradão (CER), Pasture (PAST6), Upper Montane Atlantic Forest (HAF) and Grassland (GRA). Shannon-Wiener diversity index (H'); Simpson's index (S) and Pielou evenness index (J).**

	Sites			
	CER	**PAST6**	**HAF**	**GRA**
Total density (ind.m^{-2})	18,844	2,036	9,677	13,242
Average Density (ind.m^{-2})	2,094 ± 344	204 ± 83	1,075 ± 232	1,471 ± 452
Genus richness	5	3	4	4
H'	1.144	1.040	1.219	1.188
S	0.571	0.625	0.681	0.636
J	0.711	0.946	0.879	0.857

community structure is probably more sensitive. As we can see by the difference in the genera composition in each site shown in figure 2, community structure can be influenced by local conditions. In PAST6, the only studied site which had undergone land use change from a natural Cerrado to a crop-livestock system for 3 years and then to a continuous pasture for six years, the change in community structure seems to be drastic.

It was remarkable that the genus *Guaranidrilus* was predominant in natural ecosystems, especially in CER. On the other hand, *Hemienchytraeus* and *Enchytraeus* were present in all the systems studied. Although diversity indexes were not so different among the studied sites, the lowest Simpson's index and Pielou evenness in CER might have been due to *Guaranidrilus* predominance. The difference between the pasture and grassland was expected since PAST6 is a non-natural pasture with an introduced grass largely cultivated in Brazil and with regular cattle grazing pressure. In addition, soil attributes of both sites are also different (Table I). Organic matter was higher in GRA, while soil pH in PAST6 was more alkaline due to a liming some years prior to the sampling date. These conditions could impact the survival and maintenance of some species changing the community structure. It is known that different enchytraeid species are associated to different pH ranges in the soil (Graefe & Schmelz 1999, Graefe & Beylich 2003). It is notable that in the present study, *Guaranidrilus* was the only genus absent in PAST6, the site with highest pH and lowest organic matter content compared to the other studied sites. Perhaps pH condition and lower organic matter content of soil are critical for the survival of species of the genus *Guaranidrilus*.

Römbke et al. (2015) published data referring to the diversity of species in the Atlantic Forest reporting a pattern of species dominance belonging to the genus *Guaranidrilus* (30 species), followed by *Hemienchytraeus* (9 species), *Xetadrilus* (5 species), *Fridericia* (2 species), *Tupidrilus* (1 species) and *Enchytraeus* (1 species). Niva et al. (2015) found a similar situation studying a fragment of Araucaria Mixed Forest where *Guaranidrilus* was followed by *Hemienchytraeus* in abundance. In the present study, the results are mostly in agreement with the above mentioned reports, however, CER seems to have a higher proportion of the

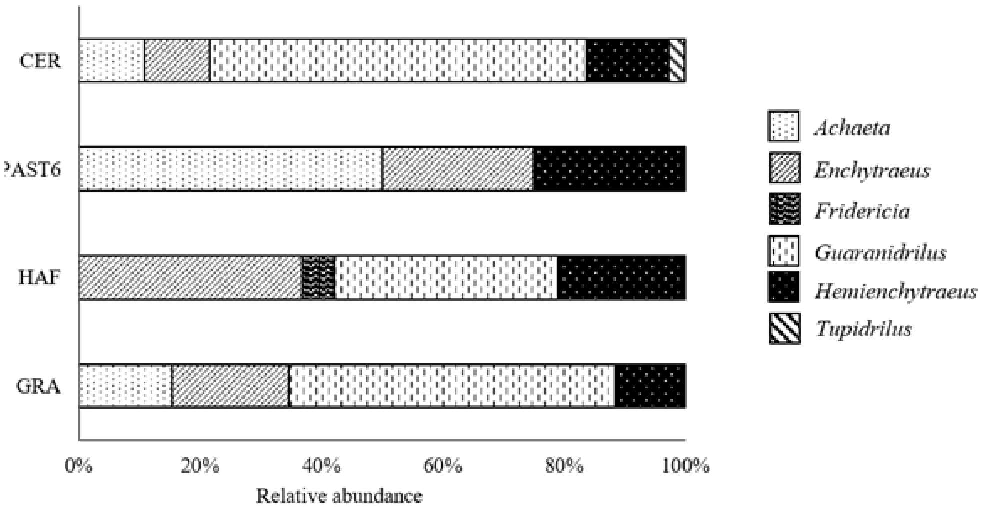

Figure 3. Relative abundance of enchytraeid genera in the Savanna Tall Woodland – Cerradão (CER), Pasture (PAST6), Upper Montane Atlantic Forest (HAF) and Grassland (GRA).

community formed by *Guaranidrilus* species. *Hemienchytraeus* was a common genus among the studied sites (Figure 3) and is a species rich group with a wide distribution in South America (Römbke et al. 2015). Furthermore, *Tupidrilus* was not found in HAF and GRA in the present study, but previous reports suggest it is a less frequent genus so it can be a matter of increasing sampling effort to find it.

In a study carried out in the Atlantic Forest Biome, at Paraná State (Römbke et al. 2007), the genus *Hemienchytraeus* and *Guaranidrilus* occurred in similar abundances independently of soil type, while *Achaeta*, *Enchytraeus* and *Fridericia* were more present in Cambisol than in Gleysol. In the same study, the genus *Achaeta* was more abundant in pastures and forests in the initial stage and absent in old-growth forests. *Fridericia* occurred almost exclusively in pastures and *Enchytraeus* abundance decline in old-growth-forests. Identifiable specimens of *Friderica* belonged to subtropical peregrine species, whereas the individuals of *Enchytraeus* belonged to a group of species with the ability to reproduce by fragmentation which, together with their ability to exploit easily accessible food sources, qualify them as a r-strategists and colonizers (Graefe & Schmelz 1999, Römbke et al. 2007). On this background the authors suggested that abundance and occurrence of *Enchytraeus* and *Fridericia* were negatively correlated with the biological quality of the soils, understood as a state as closely as possible to the natural state. Apparently, these criteria are not easily applicable to our sites, since the Upper montane *Araucaria* forest (site HAF) would receive lowest quality scores: it is the one with highest percentage of *Enchytraeus* spp. and the only one where *Fridericia* spp. was found. However, the taxic composition of this forest site in state of primary succession could be interpreted as a legacy effect of previous cultivation. Absence of *Fridericia* in the strongly degraded pasture (PAST6) may also be due to the fact that too little time has passed for immigration of species of this taxon.

Guaranidrilus, on the other hand, is a genus native to South America, with highest species richness on this subcontinent (Schmelz et al. 2013), and with high and mostly unexplored diversity at natural or semi-natural sites (Römbke et al. 2015). We recorded highest relative and absolute abundance of this genus at the Cerradão (CER) and the native grassland (GRA), which are the least disturbed among the four sites studies and may therefore harbor a rich and possibly endemic enchytraeid fauna. The present report is only a first approach to know the diversity of enchytraeids in Cerrado and in a colder region of Mata Atlantica biome. Substantial sampling effort is still needed in the biomes to confirm potential differences in the community composition of enchytraeids and to allow an estimation of the expected number of species. Above all, the species level has to be addressed. The first studies in Paraná State at Mata Atlantica (Römbke et al. 2007, 2015) for example, revealed more than 60 new species, among which 39 belonged to genus *Guaranidrilus*. There is still a huge gap in the knowledge of enchytraeids diversity in South America and tropics in general. Samplings with standard methods for regional, biome and ecosystem characterizations and comparisons are highly needed.

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Author contributions

Each author contributed individually and in significant form for the development of the study. A.P.B, R.G.S, C.C.N, D.A, R.M and A.G contributed on sampling and processing of the samples. D.A and C.N.N performed the identification of the organisms and lead in writing the manuscript. RMS confirmed identification of some difficult specimens. O.K.F, C.C.N, D.A, R.M.S, R.M.S and R.M contributed to the interpretation of the results and writing the manuscript. All authors approved the final version of the manuscript.