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β-DIVERSIDADE DAS COMUNIDADES DE OLIGOQUETAS DA ÁREA DE ENDEMISMO DE BELÉM.

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β-DIVERSIDADE DAS COMUNIDADES DE OLIGOQUETAS DA ÁREA DE ENDEMISMO DE BELÉM.

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Orientador: Prof. Dr. Guillaume Xavier Rousseau

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ATA DE DEFESA DE TESE

Aos 28 dias do mês de Fevereiro de 2019, às 8:30 **h,** compareceu ao Auditório do Programa de Pós-Graduação em Agroecologia/PPGA/UEMA, o aluno **Luis Manuel Hernández García** em **nível de Doutorado,** para apresentar e defender o trabalho de tese intitulado **"B-Diversidade das comunidades de oligoquetas da Área de Endemismo de Belém",** perante a Banca Examinadora abaixo relacionada. Após a apresentação do trabalho e arguição pelos membros da Banca, **o aluno foi considerado:**

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São Luís (MA), 28 **de** fevereiro **de 2019.**

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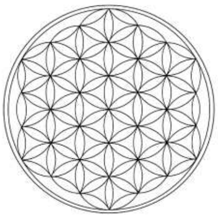
Dedico

A minha amada filha SOFÍA HERNÁNDEZ RODRÍGUEZ e a minha esposa
LESBIA NEYLESS RODRIGUEZ GODOY.



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MATERIAL SUPLEMENTAR

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SUMÁRIO

β-DIVERSIDADE DAS COMUNIDADES DE OLGOQUETAS DA ÁREA DE ENDEMISMO DE BELEM.

RESUMO

A Área de Endemismo de Belém (AEB) é atualmente a área mais desmatada da amazônia, nela existe um mosaico de usos de solos com diferentes graus de degradação que permitem a avaliação complexas interações ecológicas e de uma grande quantidade de nichos edáficos ainda desconhecidos. As minhocas representam uns dos grupos mais importantes da fauna do solo, porem são susseptíeveis as mudanças do uso do solo, sendo as espécies nativas mais vulneráveis e podendo serem extintas se a perturbação é prolongada e se a área afetada tem dimensões espaciais no nível de paissagem. Neste trabalho caracterizou-se as comunidades de oligoquetas em diferentes condições de uso de solo, paisagem e relevo, localizados na Reserva Biológica do Gurupi e assentamentos rurais vizinhos nos municípios de Centro Novo do Maranhão, Itinga do Maranhão e Bom Jesus do Maranhão. Amostras existentes coletadas no período 2010-2015 nas localidades de Tome Açú, São Luís, São José do Ribamar e Alcântara foram consideradas para completar registros taxonômicos de espécies novas na AEB. As minhocas foram coletadas durante o período chuvoso (2015-2017) usando o método do TSBF modificado (3 monolitos até 20cm) complementado com uma busca ativa durante 4h nos diferentes microhabitats susceptíveis a terem minhocas. Como resultados principais temos a descripção de 8 espécies novas para a ciência sendo dois delas pertencentes a dois gêneros novos. Outras espécies provavelmente novas estão atualmente em processo de descripção. Observamos que muitas espécies consideradas nativas sobrevivem o desmatamento, no entanto a relação entre a comunidade de minhocas e usos do solo depende do tipo de paisagem. Apesar de não haver mudanças na diversidade local de minhocas, existe uma alta taxa de substituição (18-19%) de espécies dentro de cada tipo de uso de solo. O modelo de β-diversidade mostra que o clima, e as variáveis físico-químicas estão relacionados com a taxa de substituição de espécies de minhocas e o gradiente geográfico só é importante na fase clímax da floresta.

Palavras-chave: Minhocas, Taxonomia, Macrofauna, Conservação.

β-DIVERSITY OF OLIGOCHAETA COMMUNITIES FROM THE BELÉM ENDEMISM AREA.

ABSTRACT

The Belém Endemism Area (BEA) is actually the most deforested area from the Amazon, there are a mosaic of land use with different levels of degradation that allow to study complex ecological interactions and also has an important quantity of unknown edaphic niches. Earthworms represent one of the most important groups of soil fauna, but they are succeptible to changes in land-use, especially native earthworms which can also be extint over long time disturbance effect, and when the affected area has large dimension i.e landscape level. In this work we studied the earthworm communities in different conditions of land-use, landscape and relief, located in the Gurupi Biological Reserve and neighboring settlements of the Centro Novo do Maranhão, Itinga do Maranhão, and Bom Jesus do Maranhão counties. Others samples collected in 2010-2015 in Tome Açú, São Luís, São José do Ribamar, and Alcântara counties were used to complete the new species taxonomic descriptions of the BEA. Earthworms were collected in the raining season (2015-2017) using the modified TSBF method (3 monolith, 20cm in deepth) complemented whit an active search over 4h/research on those microhabitats supceptivel to had earthworm. As results, we found 8 new species to science, two of that belonged in two new genera. Another species, also potentially new to science, are over description. Some of the native species can survive after deforestation events, however the relation between earthworm communities and land-use depend on the landscape type. Despite there is not has local changes of earthworm diversity, we estimate a high turnover (17-18%) of species by land-use type. The β-diversity model showed there are different factors influencing the earthworm turnover, and the geographical distance is only important at the final stage of succession. The loss of native earthworms after deforestation events was minimal because of survival, of species habiting the ancient forest, in pastures. Therefore, the biodiversity recuperation is fast, but the composition changes depends on the landscape type and the history back of landsuse type.

Keywords: Earthworms, Taxonomic, Macrofauna, Conservation.

1 INTRODUÇÃO

Na busca por uma melhor compreensão dos processos que controlam a distribuição das espécies e a dinâmica das comunidades nos ecossistemas, um grande esforço tem sido feito para descrever os padrões de variação da composição de espécies que existem entre comunidades locais, conhecida comumente como “diversidade Beta” ou β-diversidade (WHITTAKER, 1960, 1972).

Os estudos em comunidades vegetais tem mostrado que na determinação da β-diversidade das áreas temperadas os fatores ambientais são mais importantes que o gradiente espacial, enquanto que no trópico o espaço é determinante, limitando a dispersão (MYERS et al., 2013). Resultados semelhantes têm sido encontrados tanto em comunidades de invertebrados aquáticos, quanto em comunidades de macrofauna do solo em ecossistemas tropicais (AL-SHAMI et al., 2013; DYER et al., 2007; RUIZ-COBO; BUENO-VILLEGAS; FEIJOO-MARTÍNEZ, 2010).

Dentro do grupo da macrofauna do solo, os oligoquetas apresentam uma maior limitação na dispersão, portanto se espera uma alta afinidade de nichos e uma alta diversidade. No entanto, estudos prévios em florestas tropicais confirmaram uma alta sobreposição de nicho quando considerada a escala de distribuição espacial horizontal, e enfatizam como mecanismo mais importante a distribuição vertical (BLANCHART; JULKA, 1997; FRAGOSO; LAVELLE, 1992). Esta mesma tendência foi observada mais recentemente na floresta Amazônica em um gradiente espacial curto, menos de 10 km (DECAËNS et al., 2016).

Até agora, tem sido reportadas 124 espécies de minhocas nativas no bioma Amazônico brasileiro, distribuídas em quatro famílias: Glossoscolecidae, Rhinodrilidae, Ocnerodrilidae e Acanthodrilidae (BROWN; JAMES, 2007). Embora exista um progresso recente em análises e modelagem de diversidade de espécies (ELITH et al., 2011; LEGENDRE et al., 2009; LEGENDRE; BORCARD; PERES-NETO, 2005; PAVOINE; BONSALL, 2011; ROSINDELL; HUBBELL; ETIENNE, 2011), poucos cientistas tem acompanhado as tendências recomendadas para melhorar a compreensão sobre os possíveis padrões espaciais que governam a composição de comunidades de oligoquetas na floresta amazônica (DECAËNS et al., 2016). No caso do Brasil, não existem estudos robustos referentes à β-diversidade de minhocas, sendo estes de grande importância para a análise das áreas de ocupação de cada espécie, e a taxa de substituição de espécies dependendo de gradientes geográficos. Na Área de Endemismo Belém (AEB), onde remanentes de florestas amazônicas maranhenses, entre eles a Reserva Biológica do Gurupi e as Terras Indígenas, há um grande potencial de diversidade, considerando a quantidade de nichos ambientais encontrados nessa região, e o baixo esforço amostral realizado até o momento, na área.

Portanto, o objetivo geral deste trabalho foi estimar a diferentes padrões de diversidade dos Oligoquetas em diferentes ecossistemas localizados dentro da AEB e determinar as relações com diferentes gradientes espaço-ambientais.

2 REVISÃO BIBLIOGRÁFICA

2.1 Minhocas

As minhocas são organismos invertebrados pertencentes ao grupo dos anelídeos (*PHYLUM ANNELIDA*, do latim *annellus*: anel pequeno), distribuídas majoritariamente nos solos úmidos do mundo, algumas de apenas poucos centímetros e outras com até três metros de comprimento, conhecidas no Brasil como “minhocuçú” (SCHIEDECK et al., 2010). O corpo dos indivíduos é cilíndrico, metamerizado (segmentos corporais ou anéis) e alongado, com a boca e o ânus em extremos opostas, Figura 1 (MADIGAN et al., 2010; RUPPERT; FOX; BARNES, 2005).

Uma característica distintiva dos anelídeos é a presença de cerdas ou estruturas epidérmicas quitinosas (HICKMAN, JR. ; ROBERTS, L; LARSON, 2014), cuja disposição e quantidade por metâmeros permite diferenciar dos grandes grupos de anelídeos: os *Poliquetas* apresentam muitas (“*poli*”) cerdas por segmento e pódios, mas carecem de engrossamento epidérmico anterior. Já os *Oligoquetas* apresentam poucas (“*oligo*”) cerdas por segmento e clitelo (engrossamento epitelial anterior) nos indivíduos adultos, mas carecem de pódios.

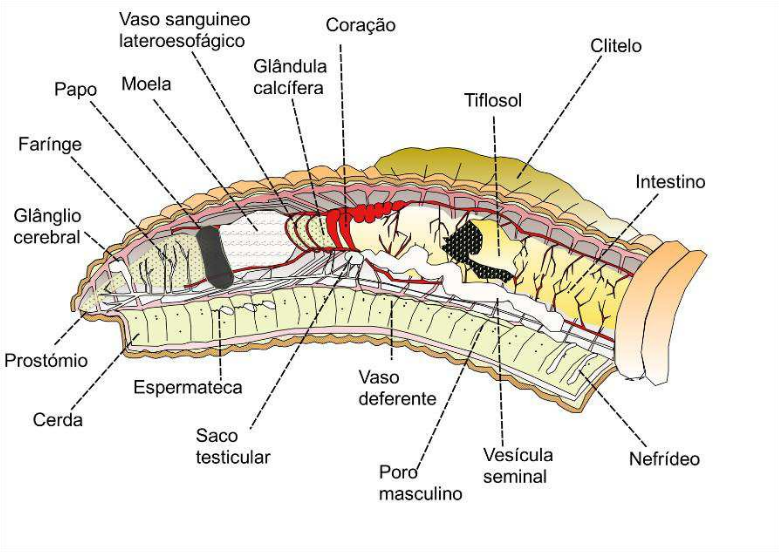

Figura 1:Representação esquemática da anatomia de *Pontoscolex corethrurus*. Produzida por HERNÁNDEZ-GARCÍA, LM.

Embora algumas espécies de minhocas possam ocupar diferentes microhabitats epígeos (DECAËNS et al., 2016) a maioria dos indivíduos são principalmente subterrâneos e constroem constantemente galerias e canais enquanto buscam abrigo e comida em forma de detritos que podem misturar com diferentes quantidades de solo. Por apresentarem um sistema digestivo simples o material excretado pelas minhocas é basicamente solo medianamente modificado.

Em condições ótima de umidade e temperatura as minhocas ocupam principalmente as camadas superficiais do solo até profundidades de 30 a 50 cm (BROWN; JAMES, 2007) onde podem participar direta ou indiretamente nas funções ecológicas do solo (HENDRIX; BOLHEN, 2002). O efeito físico destes indivíduos pode ser tão significativo que junto com cupins, besouros e formigas foram consideradas “engenheiros do ecossistema” (LAVELLE, 2011; WRIGHT; JONES, 2006).

Os oligoquetas são organismos invertebrados frágeis e existem poucos registros fósseis referentes à sua origem. Alguns registros de câmaras de estivação construídas por minhocas, foram datados até uma era próxima ao paleozoico inferior, 570-635 milhões de anos atrás (VERDE et al., 2007), onde inicialmente estavam mais adaptadas ao ambiente aquático e posteriormente foram migrando para ambientes terrestres (BROWN; JAMES, 2007). As análises moleculares evidenciameste fato para famílias de minhocas terrestres emparentadas com famílias habitantes de ambientes aquáticos (JAMES; DAVIDSON, 2012). Desta forma os oligoquetas conseguiram colonizar uma ampla variedade de habitats terrestres onde as condições permitissem seu estabelecimento (LEE, 1985).

2.2 Ciclo de vida das minhocas

Atualmente o ciclo de vida para as minhocas sul-americanas não está bem definido, a maioria dos estudos foram desenvolvidos na Europa ressaltando ciclos de vida para as espécies mais importante nessa região. As minhocas são indivíduos hermafroditas podendo apresentar órgãos reprodutivos femininos e masculinos simultaneamente (HICKMAN, JR. ; ROBERTS, L; LARSON, 2014). Após a minhoca ter se tornado adulta passa a ser considerada como individuo reprodutor, algumas espécies podem apresentar padrões de reprodução continua ou quase contínua (OLIVE; CLARK, 1978), Figura 2.

A formação dos casulos envolve diferentes etapas começando com a fertilização cruzada, onde há uma troca de espermatozoides entre indivíduos das mesmas espécies que é armazenado em estruturas especiais denominadas espérmatecas (RIGHI, 1966). Estas estruturas variam em número e graus de desenvolvimento, dependendo da espécie, e em ocasiões podem estar ausentes, o que dificulta ainda mais o entendimento do ciclo de vida de esse tipo especícico de minhocas. As espermatecas podem armazenar espermatozoides de um ou vários indivíduos da mesma espécie (OLIVE; CLARK, 1978).

Uma vez garantidos os espermatozoides, iniciasse o processo de desenvolvimento da ooteca (casulo) no redor do clitelo que terá como função inicial o recebimento dos óvulos da própria minhoca (RIGHI, 1996). Na maioria das espécies sul-americanas os poros ováricos encontram-se em disposição pareada e no segmento 14 coincidindo muitas vezes com o inicio dá área clitelar. Seguidamente, a ooteca desloca-se no sentido anterior da minhoca onde encontram-se as espermatecas para a posterior fecundação dos óvulos. Na Reserva Biológica do Gurupi e Alcântara *Righiodrilus gurupi* apresenta as espermatecas na região clitelar (SANTOS et al., 2017) indicando que a fecundação poderia ser realizada diretamente no clitelo. A ooteca pode armazenar entre um a dois ovos fecundados que serão depositados posteriormente em áreas específicas do solo.

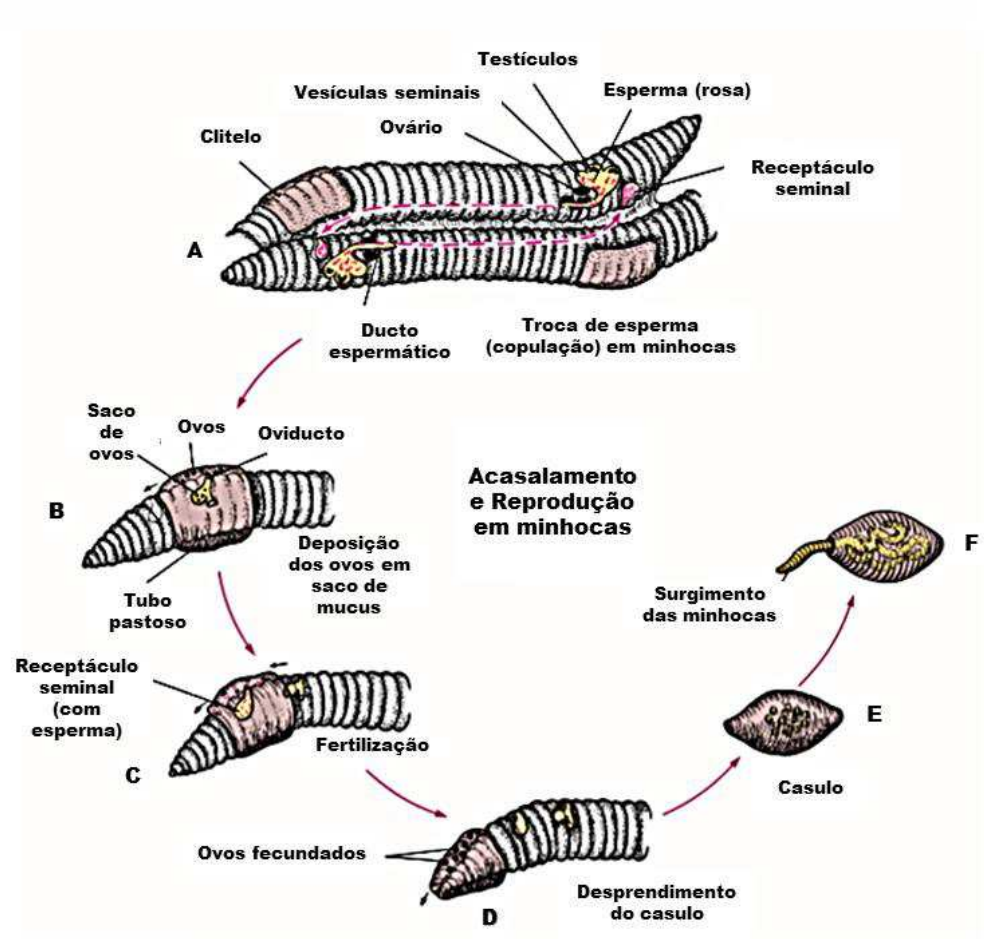

Figura 2: Esquema básico da copulação de uma minhoca típica europeia (*Eisenia foetida*) e formação de casulos. A. Inseminação mútua; o espermatozóide do poro genital (somito 15) passa pelos sulcos seminais para os receptores seminais (somitos 9 e 10) de cada parceiro. B, C. Depois que as minhocas se separam, um tubo pastoso formado sobre o clitelo passa adiante para receber óvulos de ovidutos e espermatozoides de receptores seminais. D. O casulo desliza sobre a extremidade anterior e suas extremidades se fecham e selam. E. O casulo é depositado perto da entrada da galeria. F. As minhocas jovens emergem entre 2 a 3 semanas (HICKMAN, JR. ; ROBERTS, L; LARSON, 2014).

Dependendo da espécie algumas ootecas serão colocadas nas primeiras camadas do solo e outras em áreas mais profundas (JIMÉNEZ et al., 1998).

Em áreas tropicais onde há uma marcada sazonalidade o padrão de deposição de casulos pode mudar dependendo da espécie, por exemplo em pastagens da Colômbia *Martiodrilus carimaguensis* deposita os casulos meses antes de entrar na fase de estivação, que casualmente coincide com uma diminuição drástica na umidade do solo, no entanto, uma espécie nova da mesma área pertencente ao gênero *Glossodrilus* prefere colocá-los na época seca (JIMENEZ et al. 1998). Os casulos depositados ficam em estado de desenvolvimento até chegar a época de chuva, quando nasce e inicia o processo de crescimento para repetir o ciclo.

2.3 Caracterização e identificação de espécies

A identificação tradicional de minhocas até o nível de espécies requer expertise taxonômica e é geralmente complicada pela carência de caracteres estáveis, fáceis e pontuais de diagnóstico provenientes da variabilidade ambiental induzida sobre os aspectos morfológicos (RICHARD et al., 2010). Esta caracterização externa é denominada caracterização morfológica e são realizados em exemplares previamente fixados em formol (4 a 10%) ou em etanol (100%), os quais são mergulhados na solução de interesse (água ou álcool 70%), em cubas para dissecação, onde são presos com alfinetes entomológicos (RIGHI, 1966).

Os trabalhos taxonômicos exigem o conhecimento dos caracteres externos como cor, forma, comprimento, diâmetro, segmentos, forma do prostômio e do clitelo, sulcos intersegmentares, forma e localização das cerdas, tipos de poros, marcas genitais, traves pubertais, sulcos seminais, papilas e zona caudal e internos, como septos, moela, esôfago, glândulas calcíferas, intestino, cecos intestinais, tiflosole, nefrídeos, sistema circulatório, testículos, funis seminais, sacos testiculares, vesículas seminais, canais deferentes, próstatas, câmaras copulatórias, ovários, ovisacos, funis ovulares, espermatecas e glândulas das marcas pubertais (BOUCHÉ, 1977; RIGHI, 1966; SIMS; GERARD, 1985).

Como os principais caracteres externos aparecem no estágio adulto, os casulos e indivíduos juvenis dificilmente são levados em conta em estudos de campo, prejudicando fortemente a avaliação da riqueza de espécies.

Atualmente são poucos os taxonomistas que existem no mundo e dado a velocidade acelerada de desmatamento e extinção de espécies é probable que o ritmo de perda de espécies seja maior ao ritmo de descripção.

2.4 Categorias ecológicas.

As diferentes espécies de minhocas podem ser classificadas em três grupos funcionais ou categorias ecológicas, relativas à estratificação vertical: epigéicas, endogéicas e anécicas (BOUCHÉ, 1977; LEE, 1985; JAMES, 2000), Figura 3. As espécies epigéicas ou epígeas vivem na superfície dos solos, podem habitar a serrapilheira, quanto os solos epifíticos e em áreas suficientemente úmidas podem ser encontradas nas folhas de bromélias ou palmeiras (BROWN; JAMES, 2007; DOMÍNGUEZ et al., 2009). São minhocas pigmentadas, pequenas e com estratégia de reprodução tipo “r”, porem tem expectativa de vida curta. A fonte de alimento é principalmente matéria orgânica em decomposição, portanto seus coprólitos são ricos em carbono (BROWN; JAMES, 2007).

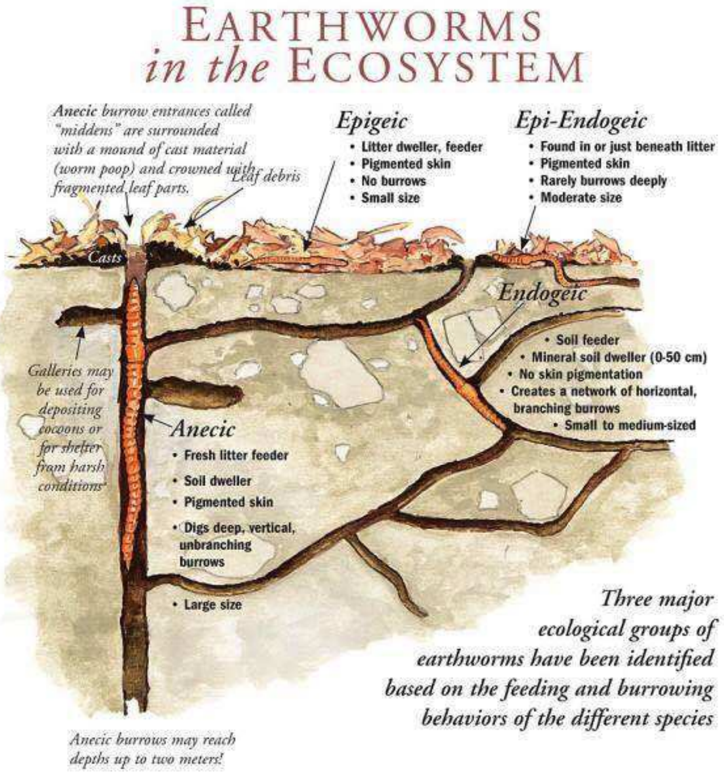

Figura 3: Categorias ecológicas das minhocas. Fonte: www.br.printerest.com

As espécies endogéicas permanecem geralmente dentro do solo, onde constroem galerias semipermanentes e permanentes. Apresentam tamanhos variáveis, geralmente sem pigmentação e com estratégia de reprodução tipo “K”. Devido ao maior consumo de solo os coprólitos produzidos por estas minhocas tem maior conteúdo de minerais, mas enriquecidos diferencialmente com diferentes teores de matéria orgânica. No trópico, grande parte das espécies de minhocas pertence a esta categoria, portanto têm sido consideradas como responsáveis pela agregação e estabilização da matéria orgânica do solo (LAVELLE; SPAIN, 2001; BROWN; JAMES, 2007).

As espécies anécicas representam uma categoria intermediaria, são minhocas grandes que vivem em galerias verticais permanentes que podem ultrapassar 1m de profundidade. Esta categoria recentemente está sendo redefinida devido ao hábito de certas minhocas tropicais de terem comportamentos intermediários. Estas espécies alimentam-se de material orgânico na superfície do solo (JAMES, 2000; BROWN; JAMES, 2007; DOMÍNGUEZ et al., 2009).

2.5 Minhocas como prestadoras de serviços ecossistêmicos nas florestas tropicais

O serviço ecossistêmico mais importante fornecido pelas minhocas é a criação e manutenção do solo (DARWIN, 1881) mediante o processo de formação de agregados e modificação do solo pela produção de coprólitos (Figura 4). No processo de ingestão do solo as minhocas participam ativamente na criação de macro e micro galerias que contribuem diretamente no aumento da macro e microporosidade do solo, porem são as protagonistas no processo de descompactação do solo, que no longo prazo tem um efeito positivo na aereação, infiltração e retenção de agua no solo (BLANCHART et al. 1999; LAVELLE et al. 2006).

O segundo serviço mais importante é a regulação do carbono e de nutrientes do solo. A minhoca favorece os processos de ciclagem de nutrientes devido à estimulação da atividade microbiana do solo quando este passa pelo seu intestino e é posteriormente depositado como dejeções ricas em nutrientes, disponíveis para serem assimilados pelas raízes das plantas (EDWARDS, 2004).

Através das suas interações com as plantas, as minhocas estão envolvidas no fornecimento de alimentos, madeira e fibras. Também influenciam diretamente os principais serviços tais como o clima, regulação de enchentes, purificação de água e podem desempenhar um papel importante na reabilitação e recuperação das áreas degradadas (BROWN et al., 2018). As minhocas também fornecem serviços culturais, por exemplo, isca para pesca e proteção de artefatos arqueológicos.

No caso das minhocas, os serviços ecossistêmicos podem ser estudados em duas linhas de abordagens: uma baseada no impacto econômico negativo que poderia ter a perda ou substituição das espécies nativas de determinada área; e a outra, propõe estimar os ganhos obtidos pela utilização de produtos de engenharia de minhocas em sistemas de produção semi-industrial (BLOUIN et al. 2013). A comercialização de produtos de minhoca, tais como vermicomposto, pertence a esta última categoria.

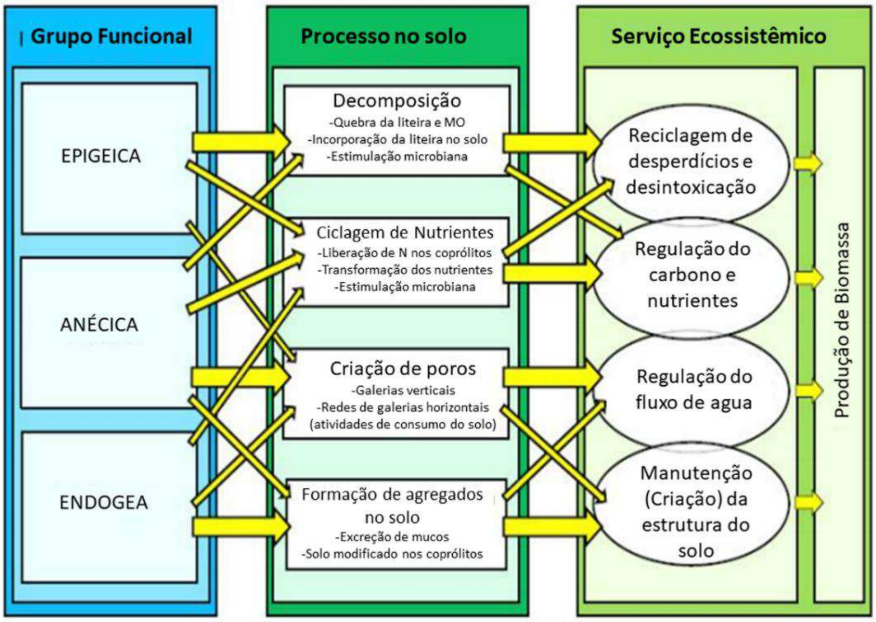

Figura 4: Rotas importantes através das quais grupos funcionais de minhocas influenciam os processos no solo e serviços ecossistêmicos. A espessura das setas representa a importância relativa. Modificado de (KEITH; ROBINSON, 2012).

A inoculação de minhocas diretamente no campo também tem sido reportada como mecanismo eficiente para aumentar a produtividade das pastagens. Uma quantidade de 645 indivíduos/m^2 da minhoca *A. calaginosa* aumentou 70% da produtividade dos pastos no Hindon, Otago (MARTIN; STOCKDILL, 1976; STOCKDILL, 1959, 1966).

Outro método mais recente de inoculação de minhocas propõe duas etapas: um cultivo inicial e logo a inoculação em campo (BUTT; FREDERICKSON; MORRIS, 1997). Na primeira fase se selecionam minhocas de diferentes categorias ecológicas e se colocam em pequenas unidades de solo. Após certo tempo de reprodução, as unidades contêm minhocas com diferentes etapas de desenvolvimento, incluindo adultos, juvenil e casulos.

A importância do uso aplicado das minhocas nos serviços que estas fornecem para os ecossistemas e agroecossistemas está relacionado a um benefício econômico direto. Em 1999 o valor monetário que as minhocas aportavam na produtividade agrícola na Escocia foi estimado entre US$ 0,11 e 0,63 por kg de minhoca no solo, para culturas de baixo e alto custo respectivamente (BOAG; NEILSON, 2006). Nessa mesma área, por exemplo, a média de minhocas é 75g/m^2 e a área de pastagem é 4.5 milhões de hectares. Se consideramos a pastagem como cultura de baixo custo (US$ 0,11 /kg de minhocas) e que as minhocas aparecem agregadas em 12% da área total (BOAG et al., 1999), então elas aportariam aproximadamente US$ 21,12 milhões.

2.6 Mudanças do uso do solo em ambientes tropicais e efeitos nas comunidades de minhocas.

Os ecossistemas tropicais são provavelmente os mais vulneráveis às mudanças do uso do solo, principalmente quando as florestas primárias são transformadas em pastagens. Tem sido reportado que a transformação de florestas tropicais em culturas ou pastagens é acompanhada pela perda da diversidade de minhocas (FEIJOO; QUINTERO; FRAGOSO, 2006; FRAGOSO et al., 1999). Contudo, as pastagens nativas tem uma importante riqueza de espécies de minhocas e *Pontoscolex corethrurus* é a principal minhoca invasora peregrina que coloniza as áreas perturbadas (BROWN et al., 2004; MARICHAL et al., 2010, 2012).

Na Tabela 1 apresenta-se resumidamente alguns valores de abundância, biomassa, riqueza e diversidade de minhocas em diferentes tipos de uso do solo no Bioma Amazônico. Pode-se observar que a maioria dos trabalhos não determinam todos os parâmetros básicos para populações de oligoquetas.

Na Amazônia oriental brasileira dois estudos contrastantes mostram diferentes respostas às mudanças do uso do solo: o primeiro relata que não há diferenças significativas nos valores de riqueza quando as florestas conservadas são transformadas em pastagens ou culturas (MARICHAL et al., 2010); já o segundo mostra que a riqueza de minhocas é maior nas florestas conservadas do que em áreas de pastagens (CASTILHO et al., 2016).

Tabela 1: Valores comparativos para parâmetros ecológicos de minhocas estimados para o bioma amazônico. S: Riqueza, H`: Shannon-Weiner.

País/Estado	Uso do Solo	Densidade (Indivíduos/m^2)	Biomassa (g/m^2)	S	H	Autor
Venezuela	Floresta Primaria LAT	54.8 ± 22.5	15.8 ± 8	5	1.82	(NEMETH; HERRERA, 1982)
	Floresta Primária POD	68.4 ± 25	16.6 ± 10.5	5	1.75	
	Floresta Primária YE	42.2 ± 20.2	10.3 ± 9	5	1.51	
	Total	42.2-68.4	10.3-16.6	5-6	1.51-1.82	
Perú	Pastagem "úmido"	573 ± 59.2	116.4 ± 22	23	-	(LAVELLE; PASHANASI, 1989)
	Pastagem "seco"	474 ± 109.7	78 ± 17.1	20	-	
	Floresta Secundária (15 anos)	85 ± 39	11.3 ± 4	27	-	
	Floresta Primária	120 ± 39.6	28.2 ± 8.9	41	-	
Rondônia, Brasil	Pastagem 1	374 ± 141	146.01 ± 48.62	9	1.34	(BARROS et al., 2002)
	Pastagem 2	80 ± 33	12.54 ± 5.68	10	2.57	
	Floresta Secundária 1	13 ± 5	1.35 ± 0.89	15	1.96	
	Floresta Secundária 2	3 ± 1	0.08 ± 0.03	17	1.7	
	Floresta Primária	26 ± 12	0.89 ± 0.57	16	2.26	
Acre, Brasil	Pastagem	10 ± 2	0.02 ± 0	10	1.27	
	Floresta Secundária	106 ± 47	0.91 ± 0.23	14	2.75	
	Floresta Primária 1	109 ± 61	18.31 ± 10.56	13	2.37	
	Floresta Primária 2	6 ± 2	0.12 ± 0.05	9	2.02	
Pará, Brasil	Floresta Secundária	177-350	-	1.7 -2.3	0.25 - 0.72	(ROUSSEAU; SILVA; CARVALHO, 2010)
Pará e Maranhão, Brasil	Pastagem	440	-	-	-	(ROUSSEAU et al., 2014)
	Floresta Secundária	227	-	-	-	
	Floresta Primária	200	-	-	-	
Guiana, Nouragues	Floresta Secundária	-	-	10	-	(DECAËNS et al., 2016)
	Floresta Primária	-	-	7-12	-	
Pará, Brasil	Pastagem	289	-	11	1.40	(CASTILHO et al., 2016)
	Floresta Primária	165	-	15	1.64	

Estes resultados podem responder a diversos fatores, entre eles está o fato de minhocas nativas poderem resistir certa intensidade de perturbações sem desaparecerem (FRAGOSO et al., 1997, 1999), mas também a existência de fragmentos florestais relativamente grandes e adjacentes às áreas de pastagens que podem favorecer a migração de espécies (LAURANCE et al., 2002).

2.7 Ecologia de paissagem e relação com a diversidade de minhocas.

Quando as mudaças do uso do solo afetam diferentes escalas espaciais podemos aceitar que existe um efeito a nível de paissagem. A escala de paissagem é medida segundo dois fatores: o tamanho do "grão" e a extensão. O grão representa em termos simples o tamanho do pixel que define a mínima resolução para o trabalho da matriz de dados, enquanto que a extensão é definida pela área total amostrada de interesse (GERGEL; TURNEL, 2002). Varias medidas são adoptadas para classificação de determinados tipos de paissagem sendo a categoria ou estatus de conservação uma das mais básicas que pode ser interpretada como fator binário, em caso de comparação de duas paissagens contrastantes, ou gradiente em caso de ter mais de três tipos de paissagens (MARICHAL et al. 2017). Os diferentes usos do solo dentro da área considerada como a unidade de paissagem, assim como a sua quantidade e formato da forma são utilizados para determinar o grau de heterogeneidade dentro dessa paissagem a partir de métricas conhecidas nas análises ecológicas (Shannon, Simpson, etc) ajustadas para este tipo de estudo (GERGEL; TURNEL, 2002). A relação de perímetro por unidade de área da uma ideia do estado de fragmentação do habitat, dada a sua relação direta com o efeito de borda e o risco que implica para determinado grupo de espécies se aproximar as áreas mais perigosas e poucos favoráveis para obtenção de alimento e recursos de qualidade. No caso das minhocas poucos são os estudos que abordam a relação entre parâmetros populacionais e tipo de paissagem, a maioria dos trabalhos só enfatiza o efeito do uso do solo mostrando que ambientes específicos degradados (como o caso das pastagem) podem tem uma influencia negativa na diversidade de oligoquetas e a composição da comunidade (FEIJOO; QUINTERO; FRAGOSO, 2006; FRAGOSO et al., 1999). Marichal el al. 2017 mostram em um trabalho recente que o tipo de paissagem influencia a presença de minhocas com certos traços funcionais em áreas degradadas e que estes diferem significativamente quando as considera-se áreas conservadas. Por outra parte Dupont et al. (2017) encontraram que a fragmentação de habitat impacta a diversidade genética de *A. chlorótica* e que a distância genética está influenciada pela conectividade da paissagem. Contudo, há uma relação sinérgistica onde o tipo de paissagem influemcia a estrutura de comunidade de minhocas e por outra parte uma alta densidade de minhocas pode influenciar mudanças no nível de paissagem (LAVELLE et al. 2002).

2.8 Diversidade de minhocas

O termo biodiversidade aparece frequentemente tanto na fala popular quanto na literatura científica, mas geralmente é usado sem uma definição clara, simplesmente porque associamos biodiversidade com riqueza de espécies. A biodiversidade pode ser vista em pequena escala (diversidade genética) ou em grande escala, onde além das espécies pode-se incluir na biodiversidade a variedade de tipos de comunidades presentes em determinadas regiões (desertos e florestas em diferentes etapas de sucessões). Já que o termo pode ter muitos significados é necessário ser específico na definição a ser utilizada para seu uso prático (BEGON; TOWNSEND; HARPER, 2006).

Existem diversos fatores que influenciam a abundância das diferentes populações, ocasionando que exista muita heterogeneidade na riqueza de espécies, portanto a determinação ecológica da "biodiversidade" envolve cálculos métricos de certos parâmetros que compõem a biodiversidade e que representam não só a diversidade biológica em termos de número de espécies da região (**"riqueza"**), mas também, resgatam o valor do impacto ou dominância das espécies dentro dos ecossistemas em termos de abundância relativa ou **"equidade"** (ARAUJO, 2007; BEGON; TOWNSEND; HARPER, 2006).

Até o ano 2015, aproximadamente 6.200 minhocas haviam sido descritas globalmente (CSUZDI; SZLÁVECZ, 2016), das quais se estima que 3.200 sejam realmente válidas (CSUZDI, 2012). Os registros para o Brasil apresentam aproximadamente 336 espécies/subespécies de minhocas (BROWN et al., 2013), mas estima-se a presença de até 1.400 espécies. De fato, muitas novas espécies foram coletadas em território brasileiro nos últimos anos, as quais se encontram em coleções institucionais aguardando serem descritas.

Aproximadamente 85% das espécies de minhocas reportadas para o Brasil são nativas (BROWN; JAMES, 2007), as quais ao serem somadas as exóticas representam um total de nove famílias, algumas delas provenientes de diferentes continentes. A maioria das espécies nativas coletadas no Brasil pertence às famílias Glossoscolecidae (MICHAELSEN, 1900), Rhinodrilidae (BENHAM, 1890; Reclassificada por JAMES, 2012), Ocnerodrilidae (BEDDARD, 1891), Acanthodrilidae (CLAUS, 1880), Almidae (DUBOSCQ, 1902) e Criodrilidae (VEJDOVSKY, 1884).

Como representantes das famílias exóticas temos: Lumbricidae (13 espécies), Megascolecidae (11 espécies), algumas espécies pertencentes à família Criodrilidae (8 espécies) Ocnerodrilidae (7) e Acanthodrilidae (12) e Eudrilidae (2 espécies). Os gêneros que apresentam maior diversidade de espécies dentro da família Glossoscolecidae e Rhinodrilidae são: *Glossoscolex* (46 espécies/subespécies), *Righiodrilus* (30 espécies/subespécies) e *Rhinodrilus* (29 espécies/subespécies), respectivamente.

Os maioria dos estudos sobre diversidade de minhocas estão concentrados no Sul do Brasil (BROWN; FRAGOSO, 2007; BARTZ et al. 2014; GERUSA et al. 2018), enquanto as regiões do norte e nordeste apresentam menor frequência de estudos de levantamento de diversidade de oligoquetas (BROWN; FRAGOSO, 2007). Os primeiros estudos na região norte e nordeste do Brasil começaram com relatos de ocorrências, descrições de espécies novas e estudos específicos sobre fatores limitantes na distribuição de oligoquetas (AYRES, GUERRA, 1981; RIGHI, 1972, 1985; RIGHI; AYRES; BITTENCOURT, 1978; ZICSI; CSUZDI, 1999) em pontos específicos dos estados Amazonas, Pará e Maranhão. Na Amazônia oriental brasileira existem apenas três trabalhos com a estimação da diversidade de oligoquetas (CASTILHO et al., 2016; MARICHAL et al., 2010; ROUSSEAU; SILVA; CARVALHO, 2010). Considerando as últimas publicações de espécies novas (HERNÁNDEZ-GARCÍA et al., 2018a, 2018b, 2018c; SANTOS et al., 2017) existem onze gêneros de minhocas reportados para a Área de Endemismo de Belém (AEB): *Pontoscolex, Dichogaster, Glossodrilus, Urobenus, Atatina, Diaguita*, *Righiodrilus, Andiorrhinus, Holoscolex, Brasilisia e Arraia* incluídos em quatro famílias (Glossoscolecidae, Rhinodrilidae, Acanthodrilidae e Ocnerodrilidae).

A lista de invertebrados do solo Maranhense está sendo construída, portanto, há muito ainda a ser feito na área de levantamento e identificação de minhocas, visando aumentar os conhecimentos sobre a diversidade de espécies presentes.

2.9 β-diversidade.

A mudança na composição de espécies entre comunidades locais é conhecida como β-diversidade. A β-diversidade é resultado das respostas das espécies às variações entre locais e, portanto, pode ser vista como o grau de especialização das espécies quanto ao hábitat (JANKOWSKI et al., 2009; MELO; RANGEL; DINIZ-FILHO, 2009). As condições e a disponibilidade de recurso no habitat influenciam a distribuição e a coexistência das espécies, assim espera-se que a β-diversidade seja maior em ambientes mais heterogêneos.

A intuição do grau de heterogeneidade ambiental pode surgir a partir dos princípios básicos da teoria de biogeografia de ilhas proposta inicialmente por Mac Arthur e Wilson (1967) e que atualmente é aplicável em diferentes condições. Uma das condições adotadas em ambientes terrestres, na ausência de barreiras geográficas, é a migração limitada, o que condiciona a troca de espécies a razões puramente espaciais, tendo uma alta correlação quando o gradiente é suficientemente longo.

Por outro lado, estas limitações de dispersão espacial podem ser restritas à presença de pequenos obstáculos. As principais barreiras regionais que podem influenciar a distribuição e especiação de oligoquetos são os rios, sendo que a mesma analogia feita por Mac Arthur e Wilson (1967) para ilhas oceânicas pode ser aplicada: quanto mais largo é o rio, maior a dificuldade de uma minhoca cruzá-lo, aumentando o endemismo das minhocas nessas condições (LAVELLE; LAPIED, 2003).

Outras barreiras são as topográficas: quanto mais alto for a montanha maior é a probabilidade de encontrar minhocas diferentes no seu topo. Contudo, a dificuldade de migração para o topo pode também impedir o incremento de riqueza nas regiões altas, portanto espera-se que exista maior riqueza nas zonas baixas onde ocorrem condições de recrutamento de oligoquetos provenientes de enchentes ou deslizamentos de terras (DECAËNS et al. 2016).

Existem diferentes índices para medir a β-diversidade, entre eles o mais clássico estabelece uma relação entre a riqueza total (Ɣ = índice gamma) e a diversidade local (α) de cada ambiente avaliado, β=Ɣ/α (WITTAKER, 1960). Para valores de α baixos β aumenta consideravelmente, por isso esta expressão é sensível à baixa diversidade local. Já os índices de Jaccard, Bray-Curtis e Sorensen-Dice refletem o grau de similaridade ou dissimilaridade entre duas comunidades baseado no compartilhamento de espécies (Figura 5), dando uma medida do valor de β-diversidade, que pode ser usado para obter modelos de regressão em função de diferentes tipos de gradientes ou matrizes ambientais de interesse (ANDERSON et al., 2011).

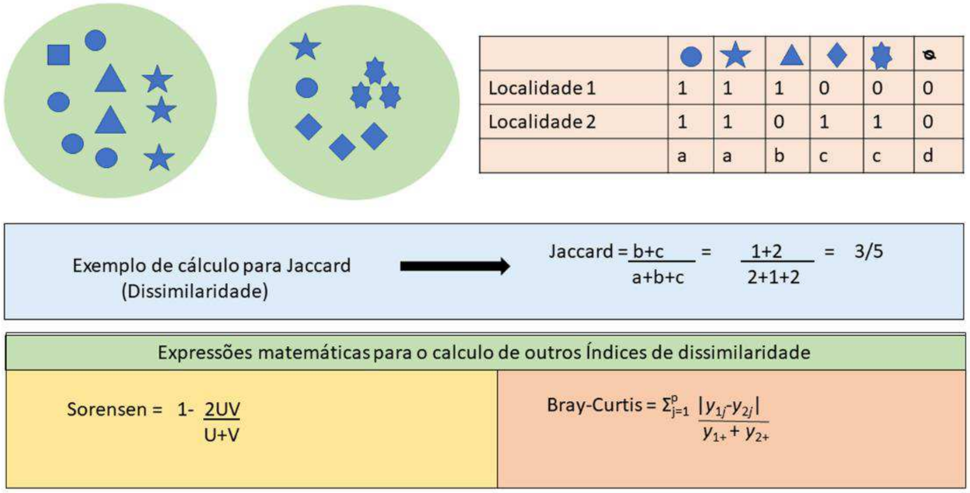

Figura 5: Índices de similaridades comumente usados para o cálculo de β diversidade. O Índice de Jaccard é expresso em uma matriz de presença e ausência (CHAO et al., 2006) e Bray-Curtis para abundância (ODUM, 1950). Para mais detalhes da derivação de U e V ver Chao et al. (2006).

Há duas formas de abordar as análises de β-diversidade: 1) considerando o fator de troca de espécies; ou 2) avaliando a variação espacial em uma área determinada. Qualquer uma das escolhas permite obter modelos que expliquem a distribuição e a mudança de espécies em gradientes específicos (ANDERSON et al., 2011). Outras abordagens propõem particionar as causas da β-diversidade total nos diferentes fatores causadores da variação, principalmente mediante o uso isolado das matrizes de variáveis ambientais (físico-química) e espaciais (LEGENDRE et al., 2009; LEGENDRE; CÁCERES, 2013; WANG et al., 2019).

Esta partição pode ser mais completa quando consideramos o desdobramento da β-diversidade total nos seus diferentes componentes “substituição” (turnover) e “aninhamento” (nestedness) e associamos os fatores causadores a cada grupo (BASELGA, 2010). Um exemplo disto é exemplificado na Figura 6, onde apresenta-se um modelo hipotético de áreas com padrões de composição de espécies diferentes e com taxas de aninhamento e substituição de espécies variáveis.

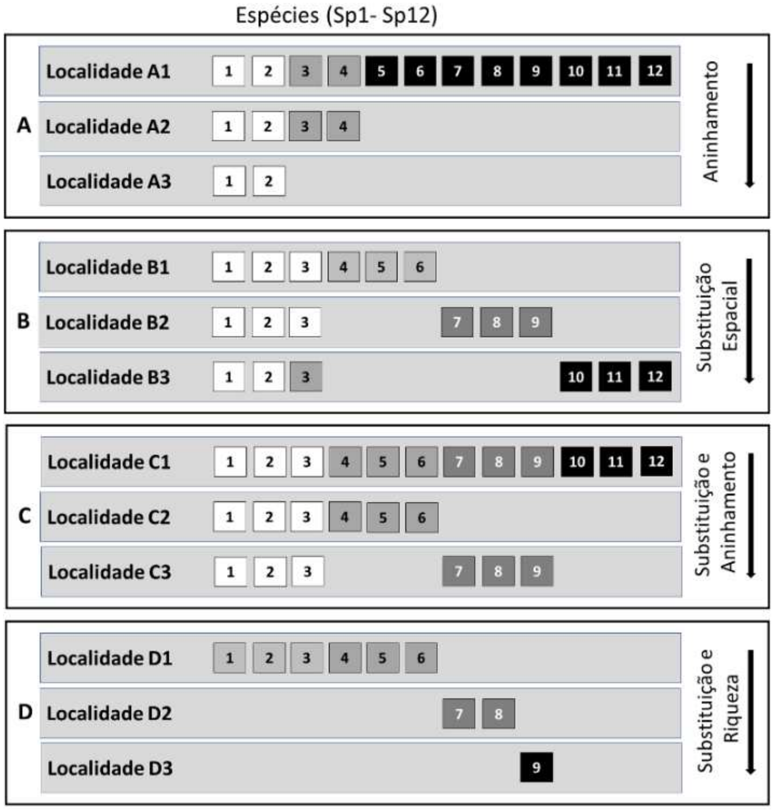

Figura 6: Exemplo hipotético envolvendo 4 ilhas (A-D) com três localidades amostradas em cada uma. As biotas A1-A3 estão completamente aninhadas, as biotas mais pobres são subconjuntos da mais rica. Localidades B1-B3 tem a mesma riqueza (seis espécies cada uma) com três espécies em comum às três localidades e três espécies exclusivas de cada área. Localidades C1-C3 apresentam ambos padrões, porque C2 e C3 são subconjuntos de C1 (aninhamento), mas algumas espécies são substituidas entre C2 e C3, as quais são exclusivas dessa áreas. As localidades D1-D3 apresentam substituição espacial e não estão aninhadas, mas apresentam diferenças de riqueza. Modificado de Baselga (2010).

O mesmo modelo enfatiza a possibilidade de existirem regiões com valores de riqueza semelhantes mas com mudanças na composição das mesmas, isto já foi demostrado para comunidades de formigas em Paragominas na AEB, próximo á Reserva Biológica do Gurupi (SOLAR et al., 2016).

Em florestas tropicais, os poucos estudos que existem mostram que há baixa correlação espacial horizontal no solo para o grupo de oligoquetas, indicando que o grau de substituição de espécies de uma localidade para outra é elevado (FRAGOSO; LAVELLE, 1992; BLANCHART; JULKA, 1997). Esta mesma tendência foi observada mais recentemente na floresta Amazônica em um gradiente espacial curto, menos de 10km de distância (DECAËNS et al., 2016). Já outras substituições no gradiente temporal são menos estudadas, especialmente aquelas relacionadas a processos competivivos nas florestas clímax. A substituição temporal mais estudada é relacionada com a sucessão onde as espácies exóticas presentes em áreas degradadas são substituídas gradualmente por espécies nativas uma vez aparecem os nichos naturais de estas espécies (LEON et al. 2003)

As principais causas das altas taxas de substituição respondem a microhabitats diferenciados na matriz espacial e outros fatores que ainda não estão confirmados. No processo de partição dos fatores causadores da β-diversidade as variáveis físico-químicas podem ser desdobradas e submetidas a análises para determinar quanto efeito elas têm para explicar a β-diversidade. No caso de serem responsáveis por uma elevada taxa de substituição de espécies, análises específicas devem ser realizadas nos gradientes alvos. Um exemplo desta suposição está representado na Figura 7, onde o gradiente de granulometria explica a diversidade de minhocas em uma floresta secundária contínua com mínimo efeito da distância geográfica.

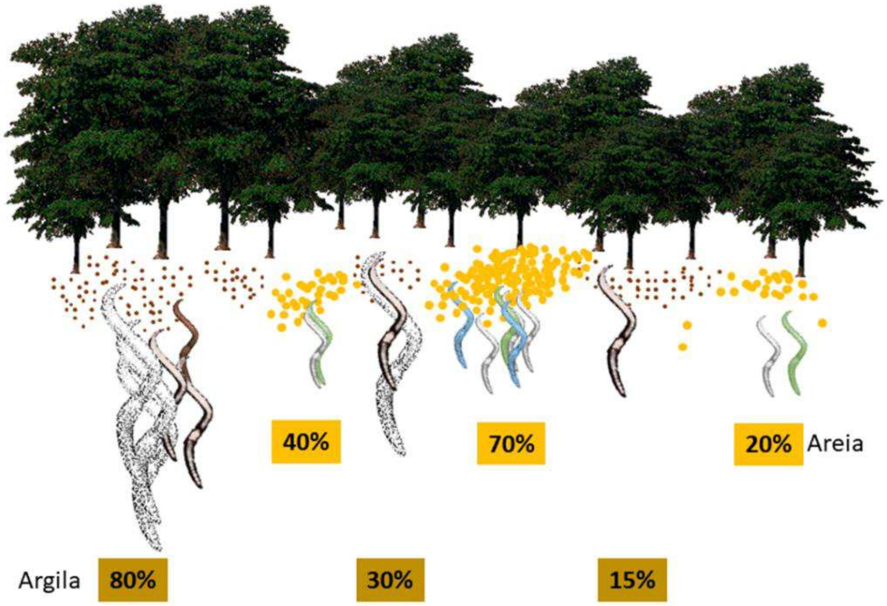

Figura 7: Modelo de β-diversidade determinada por fatores físicos do solo. O gradiente geográfico não apresenta respostas dentro de florestas secundárias.

3 HIPÓTESES

- A distribuição espacial de espécies de oligoquetas na AEB apresenta uma alta taxa de substituição (alta β-diversidade) de espécies.
- O desmatamento do remanescente de floresta amazônica na AEB ocasiona perda de riqueza de espécies nativas de oligoquetas.
- Ambientes degradados dentro de paisagens conservadas hospedam tanta riqueza quanto ambientes conservados em paisagens degradadas.

4 OBJETIVO GERAL

- Estimar o efeito da degradação sobre a comunidade de minhocas na escala de paissagem na Área de endemismo de Belém.

5 OBJETIVOS ESPECÍFICOS

- Produzir a primeira lista de minhocas do estado do Maranhão
- Estimar a riqueza e diversidade de minhocas nos principais usos de solos da Área de Endemismo de Belém
- Determinar os fatores causadores da β-diversidade de minhocas nos diferentes usos de solo mediante um modelo de correlação de MANTEL.
- Comparar a riqueza e diversidade de minhocas em fragmentos florestais com outros usos de solos.

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7 LISTA DE PUBLICAÇÕES

7.1 CAPITULO 1: New earthworm species of *Righiodrilus* (Clitellata, Glossoscolecidae) from Eastern Amazonia

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Abstract

Three new species of the glossoscolecid earthworm genus *Righiodrilus* are described from material collected in northern Pará and Maranhão States, Brazil. *Rhigiodrilus gurupi* n. sp. is characterized by four pairs of post-testicular spermathecae in xiv–xvii. *Rhigiodrilus viseuensis* n. sp. is distinguished by tubercula pubertatis in xix–xxiii and clitellum in xvi–xxiii. *Rhigiodrilus moju* n. sp. is the only species in the genus that lacks tubercula pubertatis. We provide an updated key and a distribution map for all species of *Righiodrilus*.

Key words: Haplotaxida, Oligochaeta, Annelida, taxonomy, Amazonia, geographic distribution

Introduction

Righiodrilus Zicsi, 1995 includes 27 species, which are distributed principally in South America's Amazonia region. An important compilation of species descriptions for this genus was made by Righi (1995) who placed all of them in *Glossodrilus*. The same year, in a revision of *Glossodrilus*, Zicsi (1995) erected the new genus *Righiodrilus* for species of *Glossodrilus* that possess a membranous reservoir on the surface of the calciferous gland, visible as a white area due to the calcium carbonate granules within. This reservoir is absent in *Glossodrilus*, and the structure of the calciferous gland is the only difference between the two genera (Zicsi 1995). *Righiodrilus* is distributed in Brazil, Ecuador, Colombia and Venezuela (Celis & Rangel 2015). Feijoo and Celis (2010) included three new species and presented a key for the species of *Righiodrilus*. In 2015, another new species was found in Colombia (Celis & Rangel 2015).

The Belém Center of Endemism (Da Silva et al. 2005) covers 243.000 km^2 in the Brazilian eastern Amazon, divided between eastern Pará State and western Maranhão State; it includes 147 municipalities, 62 in Pará and 85 in Maranhão (Almeida & Vieira 2010). Our work in the Belém Center of Endemism is to explore the earthworm diversity and to find new species. The many areas of endemism in the Amazon basin mean that biodiversity is geographically structured even in a region of relatively low topographic complexity (Da Silva et al. 2005). In earthworms, there are indications that their diversification is driven in part by low rates of dispersal and strong niche limitation (James & Brown 2006).

In this work we describe three new species of *Righiodrilus* collected from different locations within the Belém center of endemism and we provide an updated key and a distribution map for the species of the genus.

Material and methods

Earthworms were collected using the TSBF method (Anderson & Ingram 1993); the monoliths had surface dimensions of 25x25 cm, a depth of 20 cm, and were subdivided into three layers, litter, 0–10 cm and 10–20 cm. Collected specimens were then fixed and conserved in 95% alcohol.

Thirty specimens were examined in this study. Descriptions and nomenclature follow Feijoo and Celis (2010). Measurements were taken with an eyepiece reticle attached to a Leica MZ 16 stereomicroscope and are presented in millimeters. Illustrations and photographs were made using a drawing tube and phototube, respectively, connected to theLeica MZ 16. Multiple focal plane images were stacked with Auto-Montage Pro 5.03.0061. The holotypes and twenty-seven paratypes are deposited at the Museu Paraense Emílio Goeldi, Belém, Pará, the accession numbers beginning with MPEG.

The map was established using the known records catalogued in Brown and James (2007), Feijoo and Celis (2010), and Celis and Rangel (2015).

Results and discussion

Genus *Righiodrilus* Zicsi, 1995

Type species. *Glossodrilus tico* Righi, 1982

Diagnosis (from Feijoo & Celis 2010). Setae in viii regular longitudinal lines, generally closely arranged and rarely paired, widely arranged. Gizzard in vi. One pair of intraclitellar male pores. Female pores in pairs or rarely single. One pair of calciferous glands in xi or xii with composite tubular structure and translucent membrane, which extends over two or three segments. Sexual system metandric and metagynic; seminal vesicles long in general. Spermathecae present, rarely absent, usually without seminal chamber or diverticula.

Righiodrilus gurupi Hernández-García, Rousseau & James, n. sp. (Figure 1)

Holotype. MPEG.ANL 000286, adult. Centro Novo, PPBio Gurupi Norte, Maranhão, Brazil, primary forest, 3°41'S 46°45'W, 189 masl. 20 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000294 one adult, MPEG.ANL 000290 two adults, primary forest, MPEG.ANL 000287 two adults, MPEG.ANL 000288 three adults, MPEG.ANL 000289 one adult, MPEG.ANL 000291 one adult, MPEG.ANL 000292 one adult, primary forest disturbed, same locality as holotype. MPEG.ANL 000296 one adult, mature forest, MPEG.ANL 000295 two adults, secondary forest, Alcântara, Maranhão, Brazil, 2°21'56.3"S 44°27'10.5"W, 54 masl, 19 May 2015. Hernández-García, L.M., Burgos J.E & Rousseau G.X. colls.

Etymology. The species is named for the region where it was found, Gurupi (do tupí: "Diamante puro").

Description. Holotype 65 mm by 3.0 mm at x, 3.4 mm at clitellum, 3.0 mm at xxx, 273 segments; paratypes 59–72 mm by 2.0–2.9 mm at x, 3.0–3.8 mm at clitellum and 2.0–2.9 mm at xxx, 205–267 segments. Body cylindrical and slightly flattened in clitellar region. Setae commence on ii. Setae widely paired throughout; setal formula aa:ab:bc:cd:dd = 0.6:0.6:0.5:0.4:0.8 at xxx. Prostomium prolobous open. Unpigmented. Clitellum saddle to just above b, xvi–xxiii, tubercula pubertatis xvii–xxi (Fig. 1A). Ovipores equatorial just medial to a setae and 0.9 mm apart on xiv; male pores 2.8 mm apart on xviii/xix within paired bands of tubercula pubertatis with slight long groove and associated to genital setae (Fig. 1C). Drawings of setae (Fig. 1B,C) show the size difference between somatic and genital setae, there is no difference in structure or their surface. Nephropores just above b setal line.

Septa 6/7 membranous, 7/8–10/11 equally thick and muscular, others membranous. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in chevron pattern vii–ix, valvular in xiv, intestinal origin xv; typhlosole origin xvi, xvii–xxix cupped folds, transformed to bifid lamina near xxx, completly flat bifid by xxxiv and non-bifid by cxxx. Calciferous glands paired in xii, under seminal vesicles, composite-tubular type, oval; supra-esophageal blood vessels running to the calciferous gland from basal to anterior part (Fig. 1D). Calcium carbonate reservoir conical and latero-dorsal (Fig. 1D: "M"). Holonephric, vesiculate (Fig. 1F); nephridial ducts to body wall near level of b setae. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x–xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; four pairs of small lenticular spermathecae sacs are present near b setal line in xiv–xvii, 350x500 µm in size (Fig. 1E). Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; seminal vesicles expanded in xii, penetrate septa and range posteriorly along intestine to xiv; seminal vesicles lobulated occupying xi to xiv; vasa deferentia on body wall at b setal level en route to enter body wall in segment xviii/xix.

Remarks. *R. gurupi* sp. nov. differs from other members of the genus in the presence of four pairs of post-testicular spermathecae in xiv–xvii. Spermathecae are pre-testicular in species with 1, 2 or 3 pairs, or spermathecae are absent. The only other *Righiodrilus* species with more than 3 pairs of spermathecae is *R. sucunduris* (Righi, Ayres & Bittencourt, 1976) with six pairs in ix–xi, 2 pairs per segment.

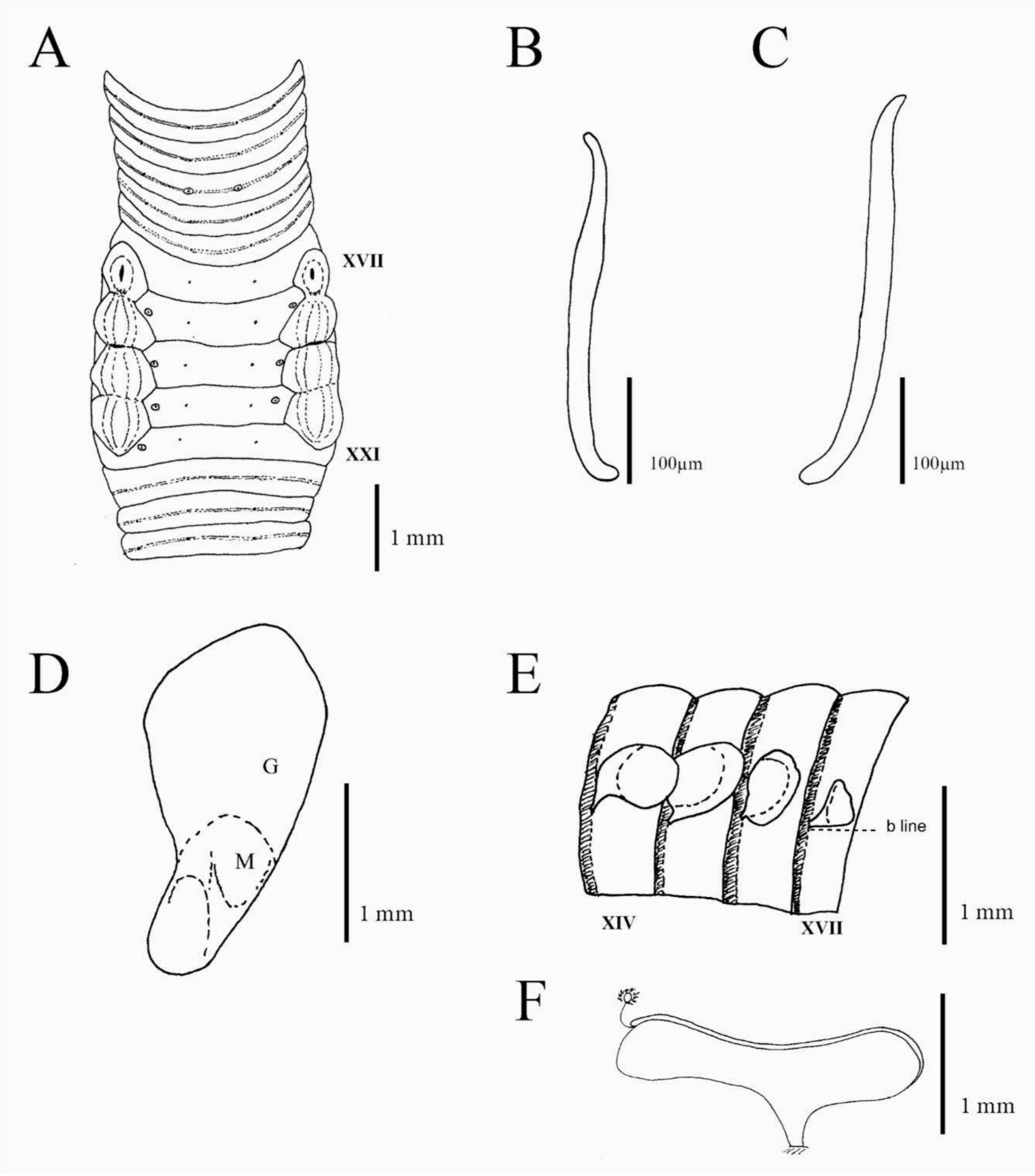

FIGURE 1. *Righiodrilus gurupi* n. sp. A. Ventral view of clitellar region. B. Somatic setae A of xxi. C. Genital setae A of xvii. D. Ventral view of calciferous gland of xii; m: membranous region; g: glandular region. E. Ventro-lateral view of spermathecae. F. Nephridium as seen in xiv–xvii.

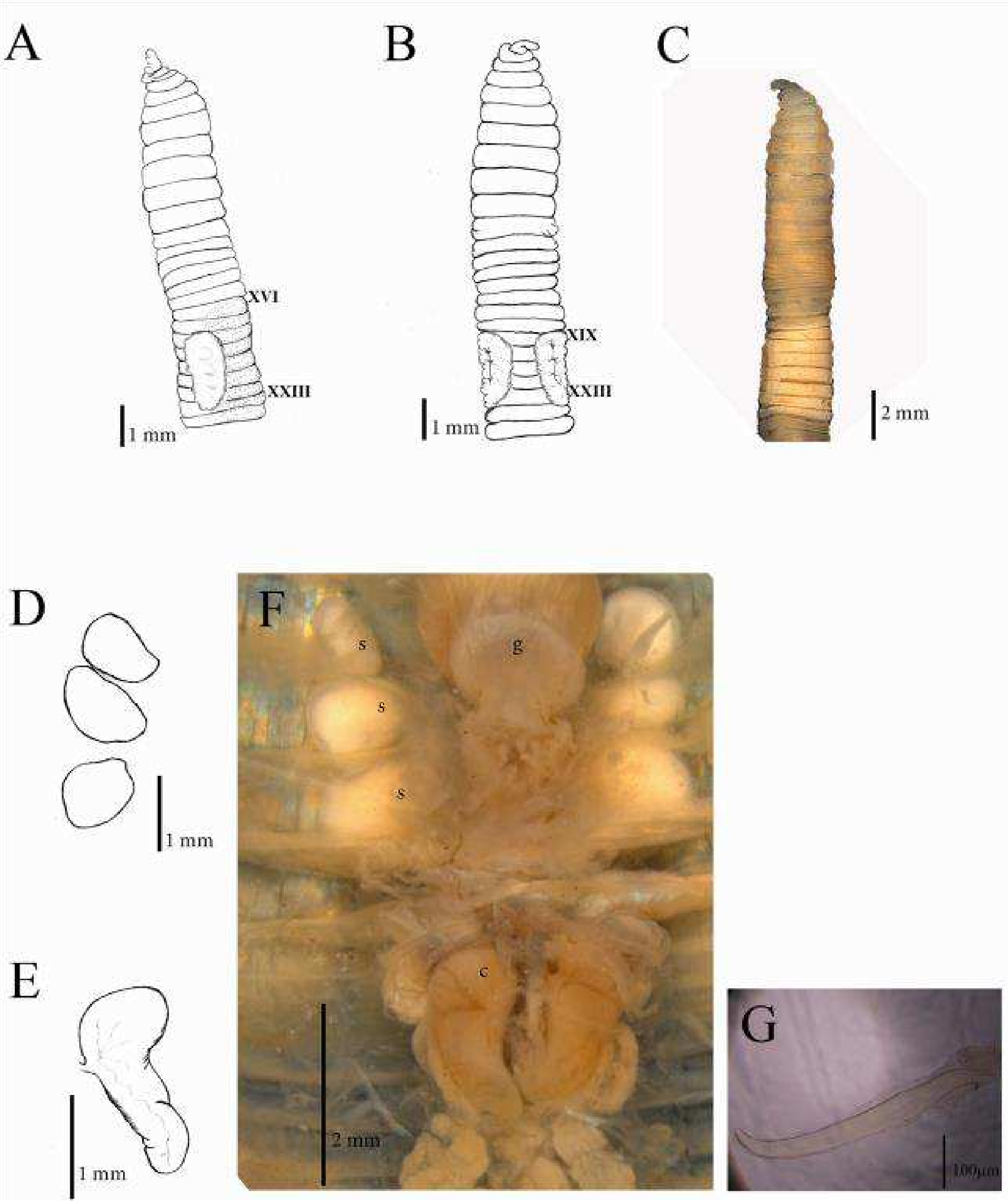

FIGURE 2. *Righiodrilus viseuensis* n. sp. A. Lateral view of anterior body with clitellum. B. Ventral view of anterior body with clitellum. C. Photography of lateral view of anterior body with clitellum. D. Spermathecae of vi–viii. E. Dorsal view of calciferous gland of xii. F. Photography of dorsal view showing gizzard (segment vi), spermathecae (segments vi, vii and viii) and calciferous gland (segment xii); g=gizzard, s=spermathecae, c=calciferous gland. G. Somatic seta A of xv.

Righiodrilus viseuensis Bartz, Santos & James, n. sp.
(Figure 2)

Holotype. MPEG 000258, adult. Type locality. Viseu, Pará, disturbed tropical rain forest, 1º35'15.37" S, 46º16'40.18"W,47 masl. 14–18 August 2014, Santos, BTS; Aguiar-Neto, M & Barros, L colls.

Paratypes. MPEG 000248 one adult, MPEG 000252 three adults, MPEG 000260 two adults. Same locality as holotype.

Etymology. The species is named for the region where it was found.

Description. Holotype 95 mm by 3 mm at x, 4 mm at clitellum, 4 mm at xxx, 284 segments; paratypes 92.25 mm by 4.5 mm at x, 4.5 mm at clitellum and 4.25 mm at xxx, 241 segments. Body cylindrical and slightly flattened. Setae ab commence on iii. Setae closely paired throughout; setal formula aa:ab:bc:cd:dd = 0.7:0.7:0.5:0.6:2 at xxx. Prostomium tentacular, post-setal secondary annulations present viii–xiv and after clitellum (Fig. 2C). Unpigmented, clitellum whitish. Ovipores in b 0.7 mm apart on xiv; male pores 3 mm apart on xix within paired oval dimpled porophores; clitellum saddle to just above b, xvi–xxiii (Fig. 2A); tubercula pubertatis in xix–xxiii (Fig. 2A, B). Nephropores within bc.

Septa 9/10, 10/11 and 11/12 thin; 6/7, 7/8 and 8/9 equally thick and muscular. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in chevron pattern vii–xi, valvular in xiv, intestinal origin xv; typhlosole origin xvi, xvii–xxxiii simple lamina in zig-zag with ventral edge bent over to form pockets, simple lamina xxxiv–xliv, after xliv becoming triple lamina. Calciferous glands paired xii, under seminal vesicles, composite-tubular type, comma-shaped, pedunculated (Fig. 2E); blood vessels to gland include large branch of dorsal vessel to approximate center of each gland. Calcium carbonate reservoir shape conical. Holonephric, vesiculate; nephridial ducts to body wall near level of b setae.

Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x– xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard, esophagus back to calciferous glands; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; three pairs of spermathecae in segments vi, vii and viii; spermathecae rounded and convex (Fig. 2D,F), size 1.24x0.23mm to 0.90x0.12mm. Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; medial to hearts of xi pass narrow tubes to seminal vesicles; seminal vesicles expanded in xii, penetrate septa and range posteriorly along intestine to xx; seminal vesicles lobulated occupying xii to xx; vasa deferentia on body wall at b en route to enter body wall in segment xix.

Remarks. The new species is unique among members of the genus for having the tubercula pubertatis in xix– xxiii and clitellum in xvi–xxiii. *Righiodrilus viseuensis* n. sp. is similar to *R. tinga* (Righi, 1971) and *R. schubarti* (Righi, Ayres & Bittencourt, 1978) in the number and position of the spermathecae. *R. schubarti* differs from the new species by the position of the clitellum at xv–xxiv, and *R. tinga* differs from the new species by male pores in xvii/xviii. *R. viseuensis* n. sp. is also similar to *R. tico* (Righi, 1982) by having the male pores in 1/2 xix; the latter species differs by the position of tubercula pubertatis in 1/2 xix–1/2 xxi.

Righiodrilus moju Bartz, Santos & James, n. sp.
(Figure 3)

Holotype. MPEG 000278, adult. Type locality. Moju-Pará, habitat disturbed tropical rain forest, 2º10"40.76"S, 48º47'36.72"W, 31 masl. 14–18 August 2014, Santos, BTS et al. colls. Paratypes. MPEG 000285, four adults. Same locality as holotype.

Etymology. The species is named for the region where it was found.

Description. Holotype 86 mm by 5 mm at x, 6 mm at clitellum, 4 mm at xxx, 230 segments; paratypes 67 mm by 4.2 mm at x, 4.3 mm at clitellum and 3.5 mm at xxx, 196 segments. Body cylindrical and slightly flattened. Setae ab commence on iii. Setae closely paired throughout; setal formula aa:ab:bc:cd:dd = 0.7:0.6:0.6:0.6:1 at xxx. Prostomium tentacular, post-setal secondary annulations present viii–xiv and after clitellum. Unpigmented, clitellum whitish. Ovipores in b setal lines 0.7 mm apart on xiv; male pores 3.5 mm apart on xx within paired oval dimpled porophores; clitellum saddle to just above b, xvi–xxiii (Fig. 3B); Four pairs of intumescences on seta c in segments xviii–xxi (Fig. 3A, B). Tubercula pubertatis absent. Nephropores between bc.

Septa 6/7,7/8, 8/9, 9/10, 10/11 equally thick and muscular, septum 11/12 absent. Alimentary canal with large cylindrical gizzard in vi; esophagus with high lamellae in radial pattern vii–xii, valvular in xiv, intestinal origin xv; typhlosole origin xvi–xxx simple lamina, xix–xxviii, zig-zag with ventral edge bent over to form pockets, after xxix becoming triple lamina. Calciferous glands paired xii (Fig. 3E, F), under seminal vesicles, composite-tubular type, comma-shaped, not pedunculated; blood vessels to gland include large branch of dorsal vessel to approximate center of each gland. Calcium carbonate reservoir shape conical. Holonephric, vesiculate; ducts to body wall near level of b setae. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in vii–ix, latero-esophageal hearts in x–xi, last pair (xi) enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventrallateral face of gizzard, esophagus back to calciferous glands; supraesophageal vessel in x–xi. Ovaries, ovarian funnels free in xiii; two pairs of spermathecae in segments ix and x (Fig. 3C, D); spermathecae cylindrical and elongate, ca. 3 mm long. Male sexual system metandric, testes and funnels in single midventral subesophageal sac in xi; medial to hearts of xi pass narrow tubes to seminal vesicles; seminal vesicles lobulated expanded in xii, penetrate septa and range posteriorly along intestine to xii–xvi; vasa deferentia on body wall between bc en route to enter body wall in segment xx.

Remarks. The difference between the new species and all other members of the genus is the absence of tubercula pubertatis, unique combination the form and quantity of spermathecae and with pair of intumescences the ventral region of body. *Righiodrilus moju* n. sp. is similar to *R. mairaro* (Righi, 1982) and *R. arapaco* (Righi, 1982) with regard to the shape, number and position of the spermathecae, and the characters of the clitellum.

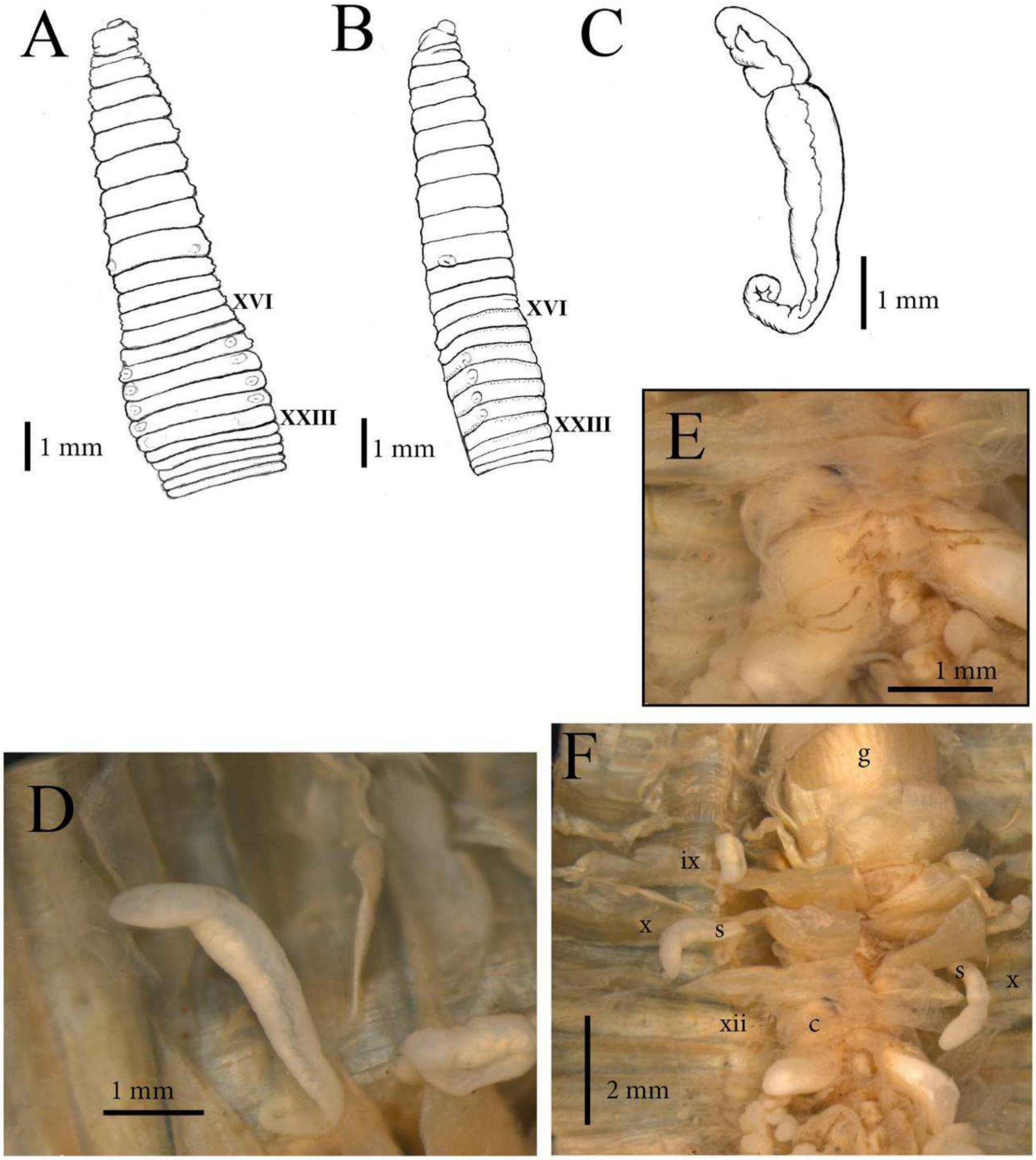

FIGURE 3. *Righiodrilus moju* n. sp. A. Ventral view of anterior body with clitellum. B. Lateral view of anterior body with clitellum. C. Spermatheca. D. Photography of the spermathecae (partly in segment ix and completely in segment x). E. Dorsal view of calciferous gland of xii. F. Photography of dorsal view showing internal structures: gizzard (segment vi), spermathecae (segments ix and x) and calciferous gland (segment xii); g=gizzard, s=spermathecae, c=calciferous gland.

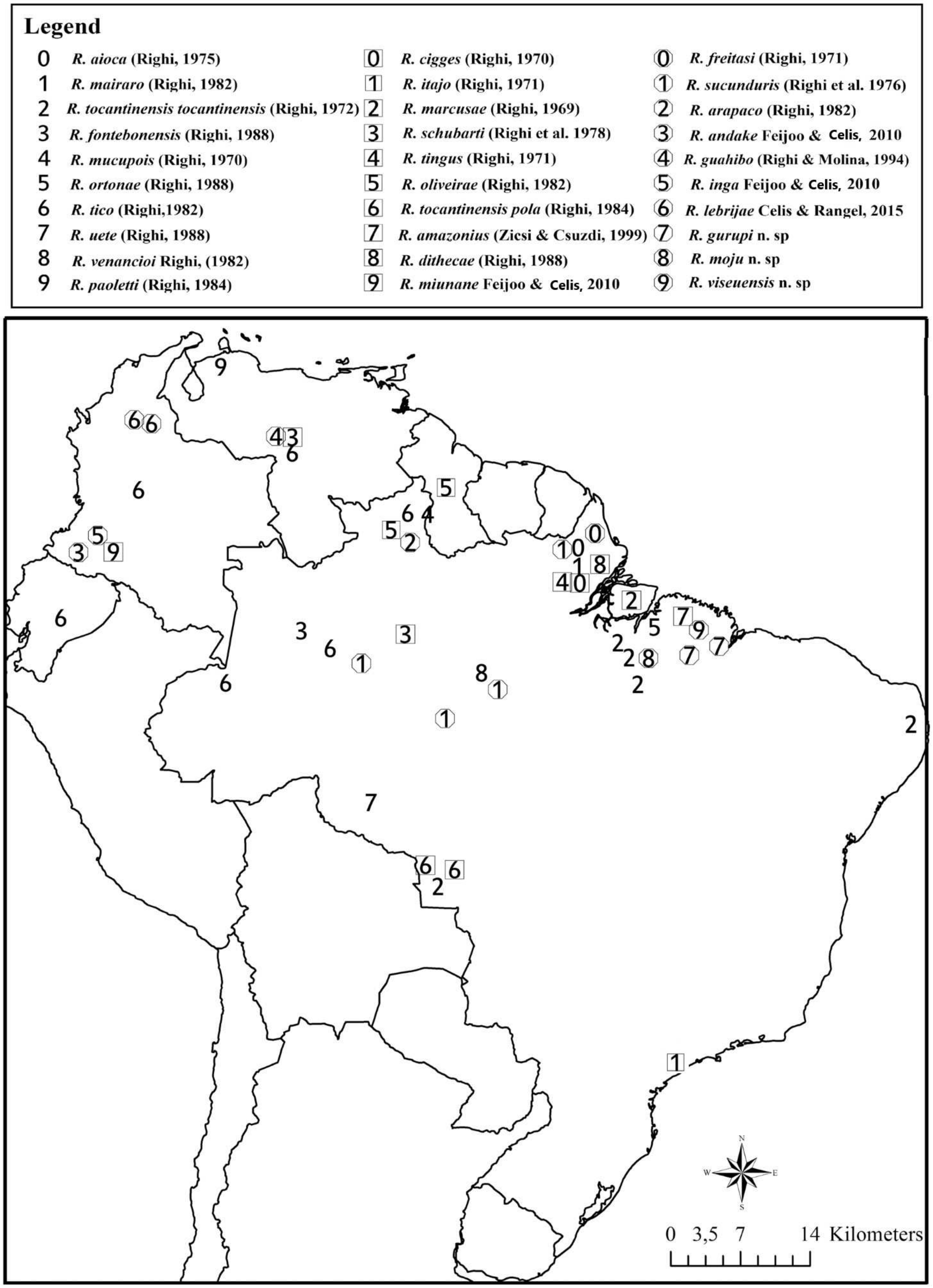

FIGURE 4. Records of species of the genus *Righiodrilus*, with data from Brown and James (2007), Feijoo and Celis (2010), and Celis and Rangel (2015).

Conclusion

With the three new species described in this paper, there are now 30 species of *Righiodrilus*. A map with finds of all species of this genus is presented in Figure 4. A key to species of *Righiodrilus* is presented below.

Key to species of *Righiodrilus* (updated and modified from Feijoo & Celis 2010)

1. Spermathecae absent 2
\- Spermathecae present 3
2. Male pores in xvii . . . . *R. muinane* Feijoo & Celis, 2010
\- Male pores with tubercula pubertatis in anterior region of xvii or middle ½ 16 – ½ 17 . . . . *R. venancioi* (Righi, 1982)
3. One pair of spermathecae 4
\- More than one pair of spermathecae 5
4. Spermathecae in viii–xix. . . . . *R. dithecae* (Righi, 1988)
\- Spermathecae in xix–x . .. . *R. guahibo* (Righi & Molina, 1994)
5. Four or six pairs of spermathecae 6
\- Two or three pairs of spermathecae 7
6. Six pairs of spermathecae in ix–xi, 2 pairs per segment; clitellum in xvi–xxi; male pores in xvii/xviii . . . . *R. sucunduris* (Righi, Ayres & Bittencourt, 1976)
\- Four pairs of spermathecae, in xiv–xvii; clitellum in xvi–xxiii; male pores in xviii/xix . . . . *R. gurupi* **n. sp.**
7. Two pairs of spermathecae 8
\- Three pairs of spermathecae 9
8. Spermathecae in 7/8 and 8/9 10
\- Not as above 11
9. Spermathecae in 7/8, 8/9, 9/10 24
\- Spermathecae in vi, vii and viii . . . . *R. viseuensis* **n. sp.**
10. Clitellum in xv–xxi 12
\- Clitellum in another position 13
11. Spermathecae in 9/10 and 10/11 . . . . *R. amazonius* (Zicsi & Csuzdi, 1999)
\- Spermathecae in another position 16
12. Tubercula pubertatis in 1/2 xix–1/2 xx. Male pores in 19/20 . . . . *R. uete* (Righi, 1988)
\- Tubercula pubertatis in xvii–xix. Male pores in 17/18 . . . . *R. cigges* (Righi, 1970)
13. Male pores in 20/21 14
\- Not as above 15
14. Clitellum in xvii–xxiii; tubercula pubertatis in xix–xxiii . . . . *R. freitasi* (Righi, 1971)
\- Clitellum in xvi–xiv; tubercula pubertatis in 1/2 xix–xxii . . . . *R. aioca* (Righi, 1975)

15. Male pores in 1/2 xix; clitellum in xvi–xxii; tubercula pubertatis in 1/2 xix–1/2 xxi . . . . .
. . . . *R. tico* (Righi, 1982)
\- Male pores in xvii; clitellum in 1/2 xv–1/2 xxiii; tubercula pubertatis in xvii – xx .
. . . . *R. andake* (Feijoo & Celis, 2010)
16. Spermathecae in 8/9and 9/10 17
\- Spermathecae in ix and x 18
17. Clitellum in xv–xxi 19
\- Clitellum in 1/2 xvi–1/2 xxii or xvi–xxii . . . . *R. arapaco* (Righi, 1982)
18. Male pores microscopic . . . . *R. oliveirae* (Righi, 1982)
\- Male pores not microscopic 21
19. Male pores in xvii 20
\- Male pores in 19/20 . . . . *R. mairaro* (Righi, 1982)
20. Tubercula pubertatis in xviii–xix . . . . *R. paolettii* (Righi, 1984)
\- Tubercula pubertatis in 1/2 xvi–1/2 xix . . . .
. . . . *R. lebrijae* (Celis & Rangel, 2015)
21. Clitellum in xv–xxii; tubercula pubertatis present 22
\- Clitellum in xvi–xxiii; tubercula pubertatis absent . . . . *R. moju* **n. sp**
22. Male pores in xvi/xvii; tubercula pubertatis in 1/2 xvi–1/2 xviii 23
\- Male pores in 17/18; tubercula pubertatis in 1/2 xvii–xx . . . .
. . . . *R. ortonae* (Righi, 1988) 23.
Spermathecae in line with setae b . . . . *R. itajo* (Righi, 1971)
\- Spermathecae in line with setae d . . . . *R. fontebonensis* (Righi, 1988)
24. Male pores in xviii 25
\- Not as above 26
25. Clitellum in xv–xxii; tubercula pubertatis in 1/2 xvii–1/2 xx 27
\- Clitellum and tubercula pubertatis in another position 28
26. Clitellum in xv–xxi; tubercula pubertatis in 1/2 xvi–1/2 xix 29
\- Clitellum in xiv–1/2 xxii; tubercula pubertatis in 1/2 xvii–1/2 xx . . . .
. . . . *R. inga* (Feijoo & Celis, 2010)
27. Papillae pubertatis absent in xxi; tubercula as grooves with dark color in xviii,
xix–xx . . . . *R. tocantinensis pola* (Righi, 1984)
\- Papillae pubertatis present in genital field 1/2 xvii–1/2 xx; papilla midventral in
xxi. . . . . *R. tocantinensis tocantinensis* (Righi, 1972)
28. Clitellum in 1/2 xiii–xxii; tubercula pubertatis in xvii–xx . . . .
. . . . *R. marcusae* (Righi, 1969)
\- Clitellum in xv–xxiv; tubercula pubertatis in xvii–xxiii . . . .
. . . . *R. schubarti* (Righi et al., 1978)
29. Male pores in 16/17 . . . . *R. mucupois* (Righi, 1970)
\- Male pores in 17/18 . . . . *R. tingus* (Righi, 1971)

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7.2 CAPITULO 2: Three new species of *Holoscolex* (Clitellata, Glossoscolecidae) from the Gurupi Biological Reserve, last forest remnant of the Belém Endemism Area, Eastern Amazon

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Abstract

We describe three new species of the earthworm genus *Holoscolex* from the Gurupi Biological Reserve in Maranhão state, Brazil. The Reserve, with the Indigenous Territories Awá, Caru, Alto Turiaçu and Alto Rio Guamá represent the last continuous forest remnants of the Belém Endemism Area, the most deforested and threatened area of Brazilian Amazonia. *Holoscolex dossantosi* sp. nov. has tubercula pubertatis looking like double bands, *Holoscolex alatus* sp. nov. presents alate tubercula pubertatis, and *Holoscolex fernandoi* sp. nov. has testis sacs and several intraclitellar atrial glands associated with genital markings between XIX and XXII. The presence of these unusual characters in *Holoscolex fernandoi* sp. nov. suggests that this species could be an evolutionary transitional stage linking Eudrilidae and more recent Glossoscolecidae genera.

Key words: Earthworm, Brazil, Maranhão, annelids, Glossoscolecidae, Oligochaeta

Introduction

The Amazon forest represents one of the most biodiverse biomes on earth and provides essential ecosystem services like regional global climate regulation (Lima et al. 2014). The Amazon biome is divided into five endemic regions according to bird and vertebrate diversity, among which dispersal is restricted by the large rivers of the Amazon basin (Silva et al. 2005). As the agriculture frontier spreads inside the South and East endemism areas (Rondonia, Tapajós, Xingu and Belém), information about the biodiversity of less-studied groups such as invertebrates is irreversibly lost. The Belém Endemism Area (BEA) is the most deforested region of the Brazilian Amazon, with only 24% of its original forest cover left (Silva et al. 2005; Almeida & Vieira 2010). The fast degradation of this region of high endemism is causing concern as the loss of biodiversity and functionality threatens the resilience of the Amazon-Cerrado ecotone, which is prone to desertification according to climate change projections (Almeida et al. 2010;

Freudenberger et al. 2012; Lees et al. 2014). This issue is particularly relevant for earthworms, whose diversity is concentrated in Amazon natural ecosystems (Brown & Fragoso 2007). Native earthworms are especially susceptible to climate (Pritchard 2011) and land-use change (Lavelle & Lapied 2003), but they are essential for the functionality of the fragile East Amazon soils (Rousseau et al. 2010). Very little information about BEA earthworms is available, especially for conserved regions like the Gurupi Biological Reserve (Fig. 1). Reports of earthworm diversity from the BEA listed the genera *Pontoscolex, Dichogaster, Glossodrilus, Rhinodrilus, Urobenus, Atatina, Righiodrilus, Diaguita* and *Ocnerodrilus* (Righi et al. 1978; Righi 1972, 1985, 1988; Zicsi & Csuzdi 1999; Rousseau et al. 2010; dos Santos et al. 2017). To date, the glossoscolecid genus *Holoscolex* Cognetti de Martiis, 1904 includes three species and one subspecies: *Holoscolex nemorosus nemorosus* Cognetti de Martiis, 1904 from Ecuador, *Holoscolex caramuru* Righi, 1975 from Brazil, *Holoscolex nemorosus tacoa* Righi et al., 1978 from Brazil, and *Holoscolex mahunkai* Zicsi &Csuzdi, 1987 from Paraguay. Further records of unidentified or undescribed *Holoscolex* species are from old forests in Colombia (Feijoo 2001) and cacao plantations in Mexico (Huerta et al. 2007). The main characters of the genus are one pair of calciferous glands in segment XI or XII, each gland bearing a calcium carbonate compartment, plus a holandric male sexual system (Cognetti de Martiis 1904). Although the genus *Holoscolex* is known from Manaus and Amapá (Righi 1975; Righi et al. 1978), no records exist for other regions of the Brazilian Amazon. As a contribution to reduce the taxonomic deficit in Amazonia, we describe three new species of *Holoscolex* from the Gurupi Biological Reserve in Maranhão, Eastern Amazon, Brazil.

Material and methods

The earthworms were collected by digging and hand-sorting in the Gurupi Biological Reserve, Maranhão State, Brazil (Fig. 1). Sampling was carried out with the quantitative modified Tropical Soil Biology and Fertility (TSBF) method (Anderson & Ingram 1993) complemented by an active search qualitative method (Decaëns et al. 2016). Each plot consisted of a 56 m-radius circle (1 ha) centered on a geolocated point where the two sampling methods were applied. For quantitative sampling, three blocks of soil 25x25 cm and 20 cm depth (divided in two 10 cm layers) located at the extremities of a 20 m equilateral triangle, were dug out and hand-sorted on a white plastic tray. Qualitative sampling was carried out, initially, by seeking earthworms in 1 m^2 of the soil surface, inside the triangle area. Then, individuals were collected by digging and hand-sorting seeing spots where large casts were visible on the soil surface. Subsequently, all life stages of earthworms (i.e. adults, juveniles and cocoons) were collected during a fixed period of four researcher-hours, in four main types of microhabitats considered as suitable for oligochaetes: (1) organo-mineral and holorganic soil layers (2) sandy to muddy sediments of stream banks (3) litter accumulations and decaying trunks on the soil surface (4) 'epiphytic soils' (i.e. organic matter accumulation in epiphytic plants and hollow trees).

Earthworms caught were anesthetized in 50% (v/v) ethanol. Posterior body fragments of the specimens were preserved in 95% (v/v) ethanol and kept in a freezer for further molecular phylogenetic studies and also considered for counting of segments and measuring of body length. The specimens were then fixed in 10% (v/v) formalin and preserved in 95% (v/v) ethanol. The specimens were examined under a Coleman NSZ-606 stereoscopic light microscope. Descriptions are based on observations of dorsal dissections of the adult specimens. Photographs were taken from specimens and drawings were edited in computer to obtain representation in highest quality. The holotypes and twenty-five paratypes were deposited at the Museu Paraense Emílio Goeldi, Belém – Pará; the accession numbers begin with MPEG and are provided with additional soil and geographical information in Supplementary Material.

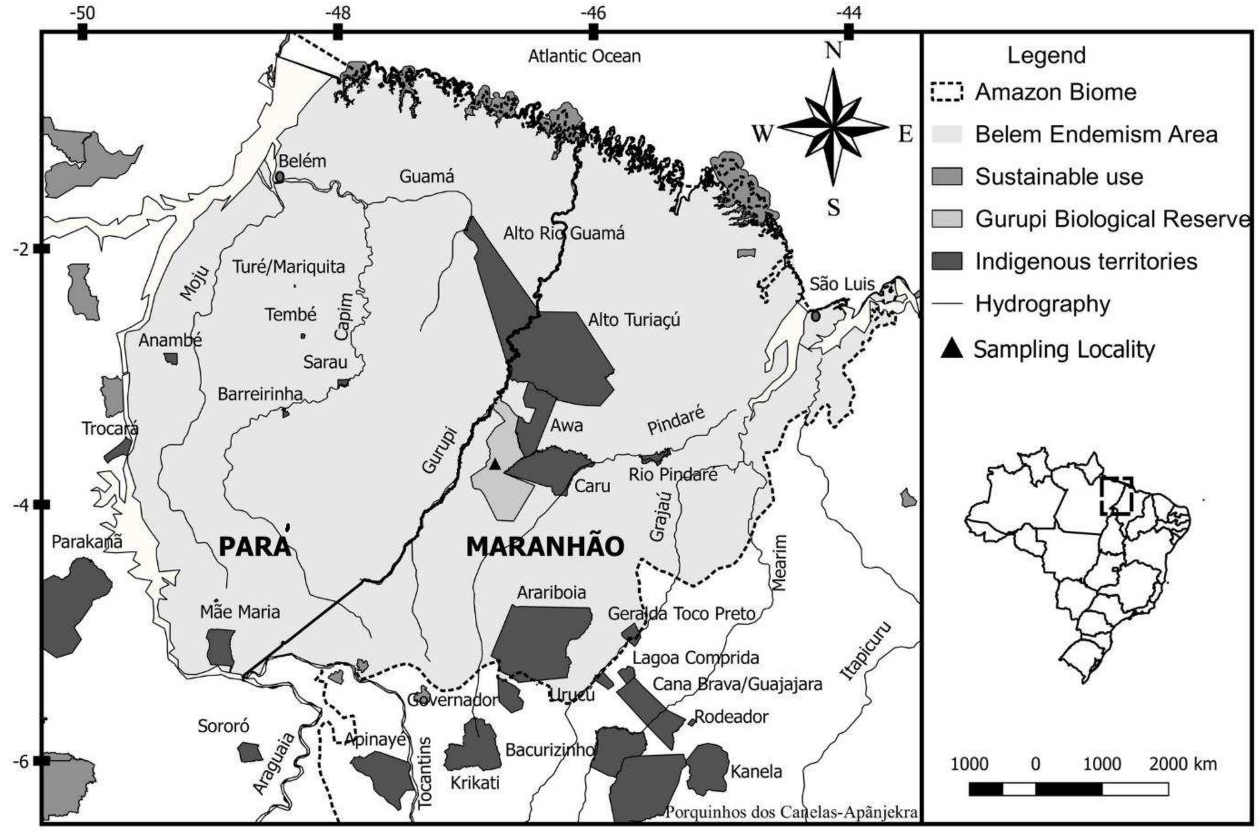

FIGURE 1. Localization of sampling site within Belém Endemism Area and Gurupi Biological Reserve in Maranhão state, Brazil.

Family Glossocolecidae Michaelsen, 1900 sensu James & Davidson (2012)

Genus *Holoscolex* Cognetti de Martiis, 1904

Type species. *Holoscolex nemorosus* Cognetti de Martiis, 1904

Diagnosis. Setae in 8 longitudinal lines. One pair of intra-clitellar male pores, paired spermathecae in one or more segments. One pair of calciferous glands in XI or XII with composite-tubular structure and non-glandular reservoir. Sexual system holandric and metagynous; seminal vesicles in general short or long.

Holoscolex dossantosi Hernández-García, Rousseau & James, sp. nov.

(Figure 2)

Holotype. MPEG.ANL 000307 one adult, clitellate, amputee. Gurupi Biological Reserve, primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'24.87"S 46°45'12.96"W, 188 masl. 26 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000308 two adults, clitellate, amputees, same locality as holotype; MPEG.ANL 000309 two adults, clitellate, fragmented, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'10.76"S 46°45'56.54"W, 158 masl. 28 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Description. Dimensions: holotype 40 mm long and 2.5 mm wide at X, 2.7 mm at clitellum, 2.5 mm at XXX, 111 segments; paratypes 43–46 mm by 2.1–2.4 mm at X, 2.4–2.6 mm at clitellum and 2.2–2.3 mm at XXX, 114–120 segments. Body cylindrical. Setae AB and CD begin on IV. Setae AB widely paired while those of CD are narrowly paired. Setal arrangement aa:ab:bc:cd:dd = 1.8:1.0:0.8:0.1:2.8 at XXX, dd < 1/2 circumference throughout. Prostomium pro-epilobous. Ovipores microscopical on small ventral elevations, equatorial approximately at the level of setae A in XIV with distance of 0.75 mm between pores. Two pairs of spermathecal pores in 7/8 and 8/9 open in B line just over intersegmental semilunar grooves associate to circular papillae. Microscopical male pores in 20/21 inside the tubercula pubertatis; milk-white clitellum saddle to BC in XVI–XXIV. Tubercula pubertatis on XIX–XXII in B line and looking like two double bands on each side, a larger and a smaller one, the smaller one from XX–XXI and with some internal wrinkles like spiral bands. Genital markings as circular papillae in VIII and IX just on AB line. Setae with papillae similar to common setae. In segment VIII genital markings are in setal line C, in IX they are in setal line B. Other small and paired genital markings on A line and becoming more widely spaced from XV to XVIII (Fig. 2A). Genital markings also on segments XXIII–XXV on A line. Setae related to genital markings of straight shape in the apical region and 300 µm in length (Fig. 2B). Common setae of post-clitellar region sigmoidal and 85–90 µm in length (Fig. 2C). Nephropores small, just dorsal to setal line C, very small and smooth valves are visible on body wall.

Septa 6/7–9/10 equally thick and muscular, 10/11 thinly muscular, other septa membranous. Alimentary canal with large cylindrical gizzard in VI; esophagus with high chevron pattern lamellae in VII–IX, longitudinal lamellae in 9–10, intestinal origin in XIII, with a dorsal typhlosole from the beginning. Thyphlosole as disk-shaped, concave folds in XV–XXVII, then wavy laminar. Calciferous glands of composite-tubular type originating in XII, with ovoid form for glandular part and conic sac form for membranous part, extending to XVI; dorsal blood vessel connects to a blood vessel on lateral face of gland part. The membranous part is a calcium carbonate reservoir on medial face of gland, towards duct (Fig. 2D). Vesiculated holonephridia; post-clitellar nephridia consisting of a thin tube arranged in irregular loops and connected directly with the bladder compartment (Fig. 2E). Vascular system with ventral trunk, single dorsal trunk, lateral vessels in VII–IX, latero-esophageal hearts in X and XI. A thin supra-esophageal vessel was observed from segment X connected to latero-esophageal hearts; the thin vessel runs along dorsal side of intestine without supra-esophageal hearts.

One pair of ovaries in segment XIII, ovarian funnels in intersegment 13/14 just on septum and with duct running to body wall opening on A line. Spermathecae in VIII and IX and opening in B line at 7/8 and 8/9 just adjacent to genital marking glands. There is not distinction between ampulla and duct. The structure is an elongate thin-walled sac, slightly tapering towards the ectal pore (Fig. 2F). Two pairs of setal glands associated to the spermathecae at A line in segment IX. Male sexual system holandric, testes and seminal funnels free in X and XI; medial to hearts of X–XI, narrow tubes from testes connect to two pairs of lobulated seminal vesicles in XI–XII; seminal vesicles of XII penetrate septa and extend posteriorly along intestine to XIV–XV; vasa deferentia on body wall at B en route to enter body wall in 20/21 within tubercula pubertatis.

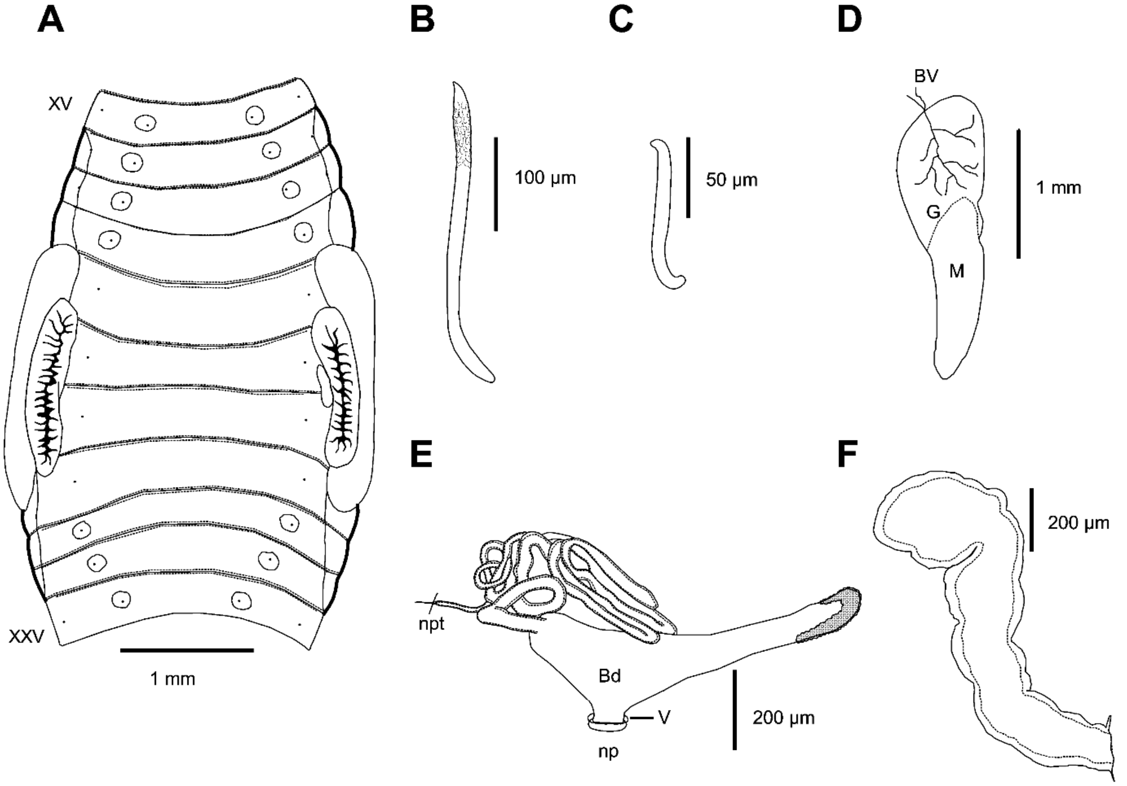

FIGURE 2. *Holoscolex dossantosi* sp. nov. (holotype). A. Ventral view of clitellar region. B. Genital setae of right papilla on A line of XVIII. C. Common seta of right A line of XXX. D. Dorsal view of right calciferous gland. BV, blood vessel; G, glandular region; M, membranous region. E. Frontal view of right post-clitellar nephridium. np, nephropore; npt, nephrostome; V, nepridium vesicle; Bd, bladder. F. Dorsal view of right spermatheca of VIII.

Etymology. The species is named in memory of Raimundo dos Santos Rodrigues, farmer and environmentalist, member of the Gurupi Biological Reserve Council, murdered August 25, 2015 by ranchers.

Remarks. *Holoscolex dossantosi* sp. nov. resembles *Holoscolex caramuru* Righi, 1975 and *Holoscolex nemorosus tacoa* Righi et al., 1978 by the presence of two pairs of spermathecae in VIII and IX, the presence of tubercula pubertatis and the beginning of clitellum on segment XVI. They are, however, separated by the following characters (*H. dossantosi* sp. nov., vs *H. nemorosus tacoa* and *H. caramuru*): i) length of tubercula pubertatis, XIX–XXII vs. XIX–XXI; ii) male pores in 20/21 vs. XX; iii) spermathecal pores in B vs. BC in *H. nemorosus tacoa* and B in *H. caramuru*. Moreover, *H. dossantosi* sp. nov. has distinctive genital markings, which are gradually more widely placed on segments XV–XVIII, and tubercula pubertatis looking like double bands. Furthermore, *H. dossantosi* sp. nov. has a distinctive setal arrangement; setae are widely paired ventrally and narrowly paired laterally.

Holoscolex alatus Hernández-García, Burgos, Rousseau & James sp. nov.

(Figure 3)

Holotype. MPEG.ANL 000310 one adult, clitellate, fragmented. Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'27.19"S 46°45'6.91"W, 209 masl. 26 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000313, two adults, clitellate, fragmented, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'16.35"S 46°45'34.74"W, 175 masl. 24 March 2015. MPEG.ANL 000315, one adult clitellate fragmented, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'10.76"S 46°45'56.54"W, 158 masl. 28 March 2015. MPEG.ANL 000314 one adult, clitellate, fragmented. Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'11.71"S 46°45'34.91"W, 189 masl. 23 March 2015. Hernández-García, L.M. & Rousseau G.X. colls.

Other Material. MPEG.ANL 000312, three adults, clitellate, fragmented, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°40'50.07"S 46°46'12.95"W, 154 masl. 24 March 2015. MPEG.ANL 000311, two adults, clitellate, fragmented, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'20.33"S 46°46'1.16"W, 172 masl. 28 March 2015. Hernández-García, L.M. & Rousseau G.X. colls.

Description. Dimensions: holotype 64 mm long and 2.7 mm wide at X, 3.2 mm at clitellum, 2.2 mm at XXX, 129 segments; paratypes 53–79 mm by 2.5–2.8 mm at X, 2.8–3.1 mm at clitellum and 2.4–2.9 mm at XXX, 188–297 segments. Body cylindrical and slightly flattened. Setae AB commence on VI, and, CD on VIII, but setae are sporadic until clitellum. Setae AB widely paired and CD narrowly paired. Setal arrangement aa:ab:bc:cd:dd = 2.0:1.0:0.4:0.1:2.8 at XXX, dd < 1/2 circumference throughout. Prostomium pro-epilobous. Two pairs of spermathecal pores are in 7/8 and 8/9 opening on very small gaps in intersegmental area at B line. Ovipores aligned to the ventral edge of genital markings on XIV, 0.8 mm apart; male pores in 20/21 inside tubercula pubertatis; clitellum slightly beige color in XVI–XXIV, saddle shaped. Wing-flat shaped tubercula pubertatis are present on XIX–XXII in B line, protruding from body wall (Fig. 3A). Small genital markings with genital setae are present in some specimens on segments VIII and IX at AB line. Other paratypes have slight genital markings on segments XI–XIV on A line, with genital markings of XIV bigger and near the female pore and large and protuberant genital markings with genital setae or internal atrial glands on the left side of XXII, sometimes on the right side. Common setae are sigmoid shape and smooth with 150μm in length, while genital setae are smooth and slightly more straight at the apical region (Fig. 3B, C). Nephropores in setal line C with very small stomata like valves seen on external body wall.

Septa 6/7–9/10 equally thick and muscular, 10/11 thinly muscular, other septa membranous. Alimentary canal with large cylindrical gizzard in VI; intestinal origin in XIV; typhlosole origin in XIV, disk-shaped, concave folds in XXXV–XL, then a simple lamina which ends in CV–CXL. Calciferous glands paired in XII, extending to XV, pedunculated sacs with a glandular region of composite-tubular type and ovoid shape, distal part membranous and with a calcium carbonate reservoir (Fig. 3D). Open vesiculated holonephridia; nematodes were found inside the bladder (Fig. 3E); two thin loops recognized in pre-septal region ending in an area with cell aggregation near the bladder. Semi-lunar valve with micro-sphincters present at nephropores, nephridia enlarged in IV–VI. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in VII–IX and latero-esophageal hearts in X and XI. Supra-esophageal vessels from X, connected to latero-esophageal hearts and running along dorsal region of intestine.

Ovaries not recognized. Ovarian funnels at 13/14. Spermathecae in VIII and IX at B line; 2.6–2.9 mm long, elongate with rounded tip and iridescent interior, ducts conical and very narrow at base (Fig. 3F). Male sexual system holandric, testes and funnels free in X and XI. Two pairs of seminal vesicles with a lobulated form in XI–XII, with those of XII penetrating septa and extending posteriorly along intestine to XVII–XVIII; vasa deferentia on body wall, extending along B to enter body wall in 20/21.

Etymology. The species name is derived from the winged shape of the tubercula pubertatis.

Remarks. *Holoscolex alatus* sp. nov. resembles *Holoscolex caramuru* Righi, 1975 and *Holoscolex nemorosus tacoa* Righi et al., 1978 by the presence of two pairs of spermathecae in VIII and IX, the presence of tubercula pubertatis and the beginning of the clitellum on segment XVI. They are however separated by the following differences (*H. alatus* sp. nov. vs. *H. nemorosus tacoa* and *H. caramuru*): i) length of tubercula pubertatis, XIX–XXII vs. XIX–XXI; ii) male pores in 20/21 vs. XX; iii) spermathecal pores in B line, vs. BC line in *H. nemorosus tacoa* and B line for *H. caramuru*. Moreover, *H. alatus* sp. nov. has alate tubercula pubertatis, unusual for this genus, protruding from the body wall and looking like a flat wing. *H. alatus* sp. nov. also has a distinctive setal arrangement, widely paired ventrally and narrowly paired laterally. More details of compared characters among species of the genus are listed in Table 1.

Holoscolex fernandoi Hernández-García, Dos Santos, Rousseau & James, sp. nov.

(Figures 4, 5)

Holotype. MPEG.ANL 000297 one adult, clitellate, entire. Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'10.76"S 46°45'56.54"W, 158 masl. 28 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

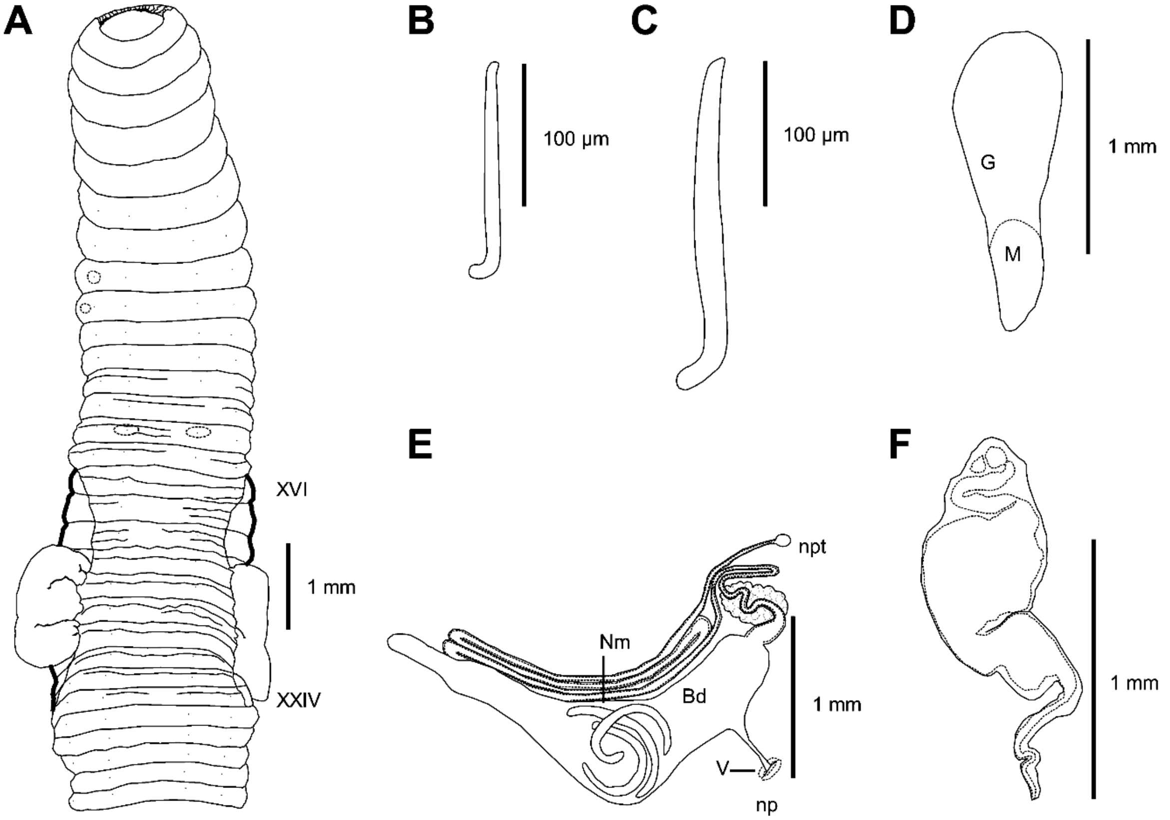

FIGURE 3. *Holoscolex alatus* sp. nov. (holotype). A. Ventral view of anterior region. B. Common seta on right A line of XXX. C. Genital seta on right A line of XXII. D. Dorsal view of right calciferous glands. G, glandular region; M, membranous region. E. Frontal view of left post-clitellar nephridium. np, nephropore; npt, nephrostome; V, nepridium vesicle; Nm, Nematoda; Bd, bladder. F. Dorsal view of right spermatheca of VIII.

Paratypes. MPEG.ANL 000300 two adults, clitellate, entire; MPEG.ANL 000298 two adults clitellate entire, same locality as holotype; MPEG.ANL 000304 two adults, clitellate, entire; MPEG.ANL 000305 one adult, clitellate, entire; MPEG.ANL 000306 one adult, clitellate, entire, Gurupi Biological Reserve, primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'24.87"S 46°45'12.96"W, 188 masl. 26 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Other material. MPEG.ANL 000299 one adult, clitellate, entire, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'20.33"S 46°46'1.16"W, 172 masl. 28 March 2015; MPEG.ANL 000301 one adult, clitellate, entire; MPEG.ANL 000302 one adult, clitellate, entire, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°40'59.74"S 46°46'1.48"W, 182 masl. 25 March 2015; MPEG.ANL 000303 one adult, clitellate, entire, Gurupi Biological Reserve, logged primary forest, Centro Novo do Maranhão, Maranhão, Brazil, 3°40'50.07"S 46°46'12.95"W, 154 masl. 24 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Description. Dimensions: holotype 61 mm long and 4.0 mm wide at X, 3.0 mm at clitellum, 3.5 mm at XXX, 219 segments; paratypes 63–104 mm by 3–4 mm at X, 4–5 mm at clitellum and 3–4 mm at XXX, 154–206 segments. Body cylindrical and slightly flattened. Setae AB commence on III, CD visible after segment X. Setae widely paired throughout; setal formula aa:ab:bc:cd:dd= 1.6:1.0:0.8:0.5:2.2 at XXX, dd < 1/2 circumference throughout. Prostomium prolobous. Unpigmented, clitellum milk-white color. Ovipores in B line on XIV, 1.2 mm apart. Two pairs of spermathecal pores in 7/8 and 8/9 on tumid lips at B line. Microscopical male pores open in 19/ 20 within B grooves and inside of tubercula pubertatis. Distance between male pores approximately 1.2 mm. Two tubercula pubertatis on each side, semi-globular and protruding, extended along AB line in XIX–XXII. Clitellum saddle in XVI–XXIV (Fig. 4A). Genital setae in A line of some segments of XIX–XXII, on papillae related to atrial glands (Figs. 4B, 5). Those setae are almost 1mm in length with straight shape and with 4–5 serial furrows at apical part. Common postclitellar setae also straight, smooth, the dimension vary between 200 and 220 µm (Fig. 4C). Nephropores near B line, very small micro sphincters are present on body wall.

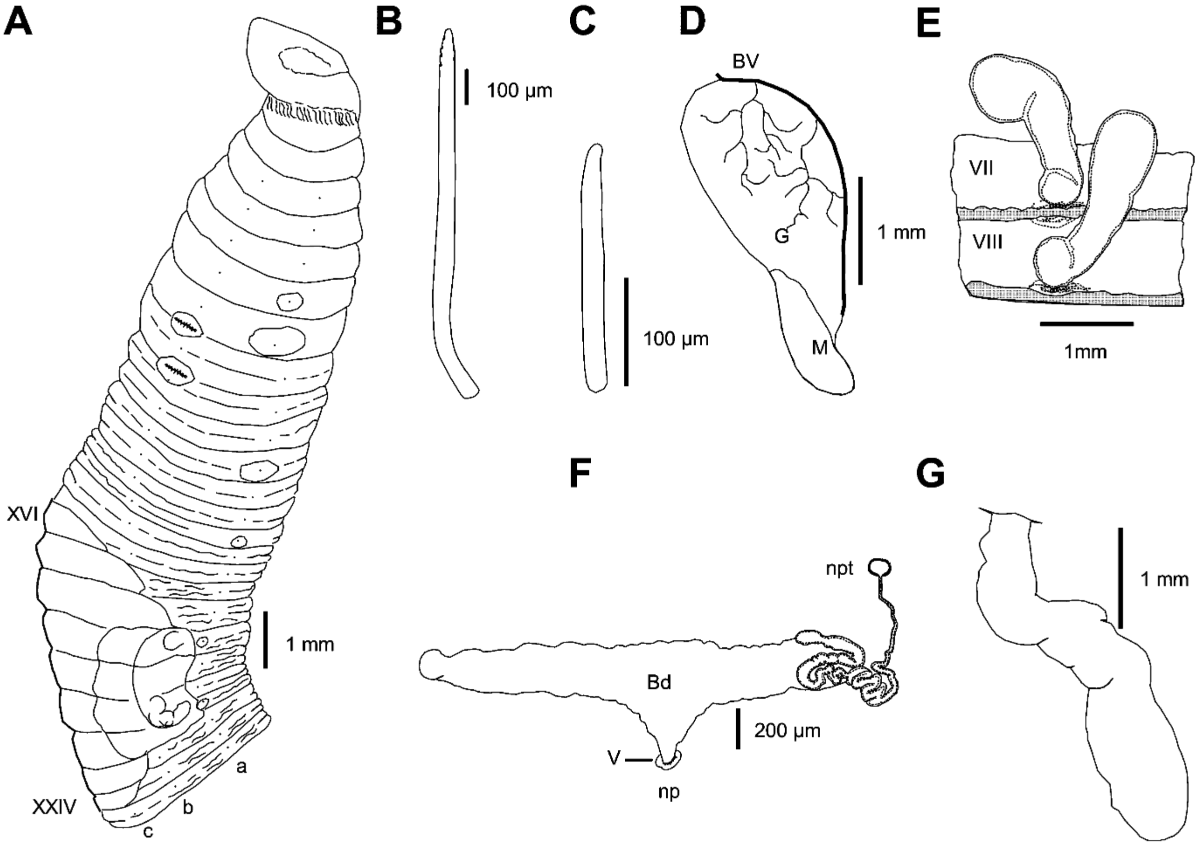

FIGURE 4. *Holoscolex fernandoi* sp. nov. (holotype). A. Latero-ventral view of anterior region. a, A setae; b, B setae; c, C setae . B. Genital seta of papillae on right A line of XX. C. Common seta on right A line of XXX. D. Dorsal view of right calciferous gland. BV, blood vessel; G, glandular region; M, membranous region. E. Dorsal view of right spermathecae of VII and VIII. F. Lateral view of left post-clitellar nephidium. np, nephropore; npt, nephrostome; V, nepridium vesicle; Bd, bladder. G. Dorsal view of a left common atrial gland of XIX.

Septa 5/6 thin, 6/7–10/11 equally thick and muscular, septum 11/12 absent. Alimentary canal with a large cylindrical gizzard in VI; esophagus with high lamellae in radial pattern VII–IX, valvular in XII, intestinal origin XVI; typhlosole origin XVI, zig-zag shaped and with the ventral edge bent over to form pockets, simple lamina after XL–XLIX. Paired ovoid-shaped calciferous glands of composite-tubular type in XII, expanded in XIII, under seminal vesicles; blood vessels to glands include a large branch of dorsal vessel to approximate anterior region of each gland. Calcium carbonate reservoir ovoid-shaped (Fig. 4D). One pair of nephridia per segment; those of postclitellar area close to intestine and with nephrostome oriented ventrally (Fig. 4F); irregular loop opening into bladder; nephropore opening in body wall through micro-sphincters surrounded by semi-lunar valves. Vascular system with ventral trunk, single dorsal trunk, lateral vessels in VII–IX, latero-esophageal hearts in X, last pair (XI) enclosed in testis sacs. A thin supra-esophageal vessel seen in X, connected to hearts, approximately half as wide as latero-esophageal hearts, running dorsally along intestine, connected to very fine lateral blood vessel.

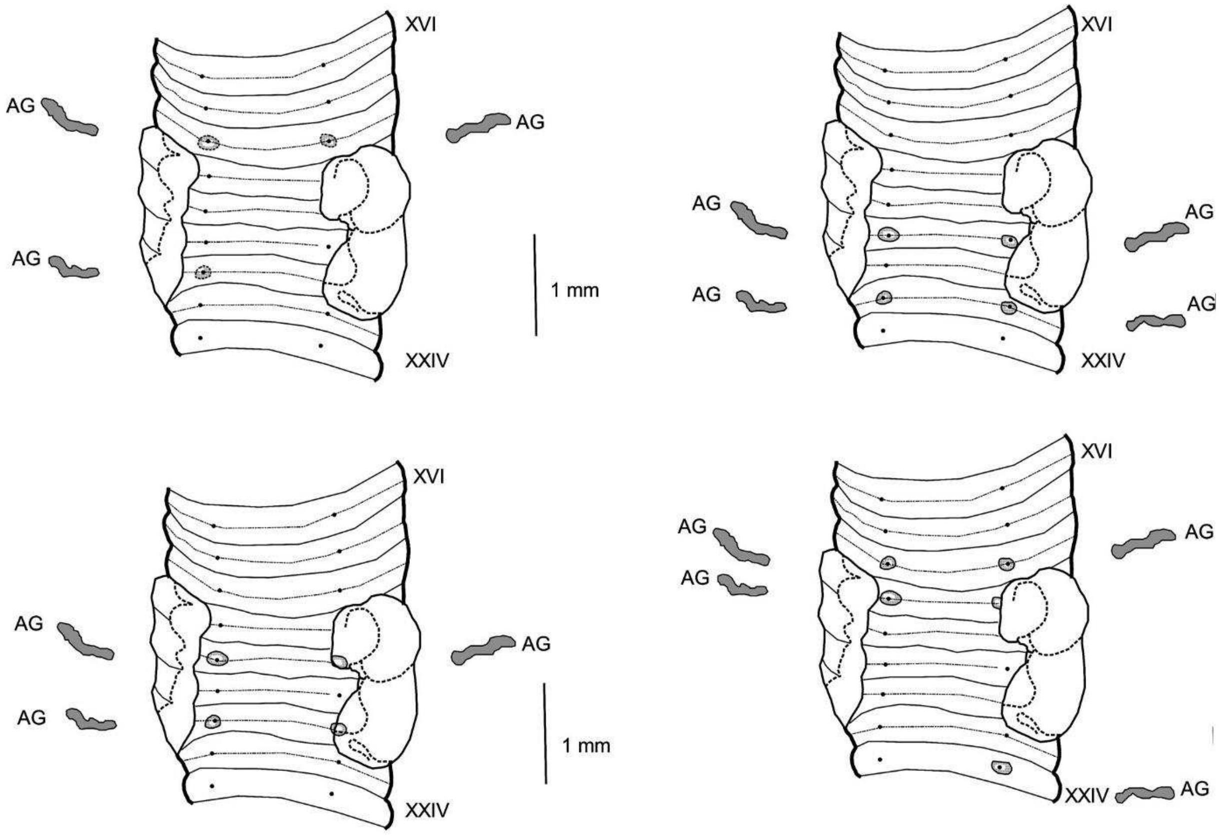

FIGURE 5. Different arrangements of atrial glands of *Holoscolex fernandoi*, schematic. AG, atrial glands.

One pair of ovaries in XIII. Ovarian funnels at 13/14, duct opening in XIV on A line. Two pairs of spermathecae in VII and VIII (Fig. 4E), elongate spermathecal sacs, no differentiation of duct and ampulla, opening posterior at inter-segment 7/8–8/9 on B line. Male sexual system holandric, testes and funnels ventral in paired sacs in X and XI; medial to hearts of XI narrow tubes from sac connect to seminal vesicles. Two pairs of lobulated seminal vesicles in XI and

XII, expanded in XIII and XIV. Vasa deferentia on body wall extend in B, entering body wall in 19/20. Atrial glands present on A line in XVIII–XXIV (Fig. 4G, 5), with different patterns: two atrial glands in XVIII and one in XXII, two atrial glands in XXI and two in XXIII, two atrial glands in XX and one in XXII and finally, two atrial glands in XVIII and one in XIX and XXIV.

Etymology. The species is named in honor of Antonio Fernando Rocha, ranger of the Reserve where the earthworms were found.

Remarks. *Holoscolex fernandoi* sp. nov. is similar to *Holoscolex nemorosus nemorosus* Cognetti de Martiis, 1904 by the presence of two pairs of spermathecae, male pore in 19/20 and the length of the clitellum (8 segments). Both species differ, however, in the following characters (*H. fernandoi* sp. nov. vs *H. nemorosus nemorosus*): i) position of the spermathecae sacs in segment VII and VIII vs in VIII and IX; ii) tubercula pubertatis present vs absent and iii) atrial glands present vs absent.

Discussion

Holoscolex is considered as an ancient genus within the Glossoscolecidae family (Cordero 1945), phylogenetically related to *Glossodrilus*, *Glossoscolex* and *Fimoscolex*, and probably present in America since the post-Pangean continental movement (Anderson et al. 2017). Species within this genus can be small to medium-sized (body length 23–120 mm), unpigmented and with intraclitellar microscopical male field. All currently known species present spermathecae and one pair of tubular calciferous glands. These ovoid glands have a membranous calcium reservoir in segment XI–XII. One of the most important characters of this genus is the presence of two pairs of testes in segments X–XI. According to Cognetti de Martiis (1904, 1906), Righi et al (1978), Righi (1975) and Zicsi &Csuzdi (1987) the genus was considered initially as gymnorchous, but in this study we found an exception for *Holoscolex fernandoi*, where testes sacs are present. Cordero (1945) and Righi (1971) suggested separating holandric of merandric earthworms considering the holandric character as ancient features. Recently, phylogenetical studies suggest a closer relationship of Glossoscolecidae with the African family Eudrilidae (James & Davidson 2012), despite strong anatomic differences, for example the presence of prostates in Eudrilidae and their absence in Glossoscolecidae. On the other hand, recent genera of Glossoscolecidae like *Fimoscolex* and *Glossoscolex* present copulatory chambers, which are intraclitellar structures approximate to atrial glands. Hence, the presence of testis sacs and intraclitellar atrial glands in *Holoscolex fernandoi* points out that this species could be an evolutionary transitional stage linking both Eudrilidae as well as recent Glossoscolecidae genera.

Our records double the species already known for the genus in the world with most occurrences in Brazil, specifically in the states of Amapá, Amazonas and the Amazon region of Maranhão. The others occurrences are located in the Chaco region of Paraguay and in the Amazon rainforest of Ecuador.

TABLE 1. Comparison of characters of *Holoscolex* species. L=body length [mm], D=body diameter [mm], S=segment number, ST=spermathecae, MP=male pores, CG=calciferous glands, IAG=intraclitellar atrial glands, TSF=testes and seminal funnels.

	L	D	S	Setal ratio XXX aa:ab:bc:cd:dd	Clitellum	Tubercula pubertatis	ST	MP	CG	IAG	Prostomium	TSF	Genital setae	Locality
H. nemorosus nemorosus Cognetti de Martiis, 1904	30	1.5	100	3.9:1.0:1.9:0.9:4.7	saddle, XV, XVI–XXII, XXIII	absent	VIII–IX *B* line	19/20	11	absent	prolobous?	free	?	Ecuador, Gualaquiza
H. nemorosus tacoa Righi *et al.*, 1978	34.5–38	0.88–1.00	94–116	2.1:1.0:1.2:0.9:1.3	saddle, XVI–XXII	XIX–XXI, ridges	VIII–IX *BC* line	20	11–12	absent	prolobous	free	?	Brazil, Amazonas, road Manaus-Itacoatira
H. caramuru Righi, 1975	23–25	1.00–1.10	91–96	1.8:1.0:1.1:0.8:2.5	saddle, XVI–XXII	(1/2)XIX–(1/2)XXI, fine ridges	VIII–IX *B* line	20	11	absent	?	free	?	Brazil, Amapa (Amazon river)
H. mahunkai Zicsi &Csuzdi, 1987	110–120	4.50–5.30	111–114	6.2:1.0:5.0:1.0:??	XV–(1/4)XXV	(2/3)XIX–(1/2)XXIV	VI–VIII *CD* line	19/20	11–12	absent	pro-epilobous	free	?	Paraguay, Acaray waterfall
H. dossantosi **sp. nov**	40–46	2.20–2.50	111	1.8:1.0:0.8:0.1:2.8	saddle, XVI–XXIV	XIX–XXII, flattened rings	VIII–IX *CD* line	20/21	12	absent	pro-epilobous	free	sigmoid, smooth	Brazil, Maranhão (Gurupi)
H. alatus **sp.nov.**	53–79	2.4–2.9	188–297	2.0:1.0:0.4:0.1:2.8	saddle, XVI–XXIV	XIX–XXII, wing-flat	VIII–IX *CD* line	20/21	12	absent	pro-epilobous	free	straight, smooth	Brazil, Maranhão (Gurupi)
H. fernandoi **sp. nov**	61–80	3.4–3.5	180–219	1.6:1.0:0.8:0.5:2.2	saddle, XVI–XXIV	XIX–XXII, voluminous ridge	VII–VIII *CD* line	19/20	12	present	prolobous	sacs	straight with excavation	Brazil, Maranhão (Gurupi)

All species reported were sampled in conserved forests or near them, which suggests that they are highly susceptible to land-use change. There is a high probability to find new species of this genus in the Brazilian Amazon; we recently collected individuals of *Holoscolex* with differing morphology—probably new species—in remnant forest from Itinga (Gurupi region) and Alcântara (North Central coastal region of the State) counties (data not shown). In recent sampling campaigns we found that *Holoscolex alatus* sp. nov. and *Holoscolex fernandoi* sp. nov. are also present in pastures inside the Gurupi Biological Reserve. The close vicinity of pasture and forest is probably necessary to maintain the presence of this species here. The Gurupi Biological Reserve is the only integral protection area of the Belem Endemism Area and was created to protect a region characterized by high biodiversity and endemic fauna like birds and mammals (Lopes & Ferrari 2008; Dornas et al. 2014; Lima et al. 2015). The record of three new *Holoscolex* species therefore confirms the importance of the reserve.

Acknowledgments

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7.3 CAPITULO 3: *Brasilisia* n. gen. and *Arraia* n. gen., two new genera of Ocnerodrilidae (Annelida, Clitellata, Oligochaeta) from Eastern Amazonia, Brazil

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Abstract

We describe two new species from two new genera of Ocnerodrilidae from Alcântara and Rosário counties of Maranhão, Brazil. They were collected in very sandy soil in the transition between the Amazonia and Cerrado biomes. One of the species, *Brasilisia punki* n. sp., is the first report of earthworms of this family with an unusual pattern of genital markings and a gizzard in segment five. The other species *Arraia nelmae* n. sp. has a gizzard in segment six and a pair of calciferous glands in nine. We also provide an updated key for all genera of Ocnerodrilidae.

Key words: Earthworms, Amazon, Cerrado, Ocnerodrilidae

Introduction

The Ocnerodrilidae were recently reviewed in Fragoso & Rojas (2009), which greatly simplified the task of working on a family of earthworms for which there is no active specialist or any other comprehensive and recent synthesis. The family has a wide distribution (Africa, the Americas, South Asia, SE Asia, and China) and contains several peregrine species, some notorious for small variations including modifications related to parthenogenetic reduction of sexual characters (Gates 1972). Many species are small and have such a simple body organization that finding distinctive characters can be difficult. In the Neotropics there are 15 genera, some only known from one or two species, which is the greatest continental genus-level diversity in the family (Fragoso & Rojas 2009).

In this work we describe two new genera of Ocnerodrilidae collected in very sandy soils (costal region) from the Amazon Biome of Maranhão State, Eastern Amazonia, Brazil, and we provide an updated key for the genera of this family.

Materials and methods

The earthworms were collected by digging and hand-sorting on Livramento Island, Alcântara County and, old secondary forest and homegarden habitats in Rosário County, Maranhão State, Brazil (Fig. 1). Sampling was carried out by the modified quantitative Tropical

Soil Biology and Fertility (TSBF) method (Anderson & Ingram 1993) for different areas and dates as described by Rousseau et al. (2014).

Earthworms caught were anesthetized in 50% (v/v) ethanol, fixed in 10% (v/v) formalin for two days and then preserved in 70% (v/v) ethanol. The specimens were examined under a Coleman NSZ-606 stereoscopic light microscope. The descriptions are based on observations of dorsal dissections of the adult specimens. The holotypes and eighteen paratypes were deposited at the Museu Paraense Emílio Goeldi, Belém - Pará, the accession numbers begin with MPEG.

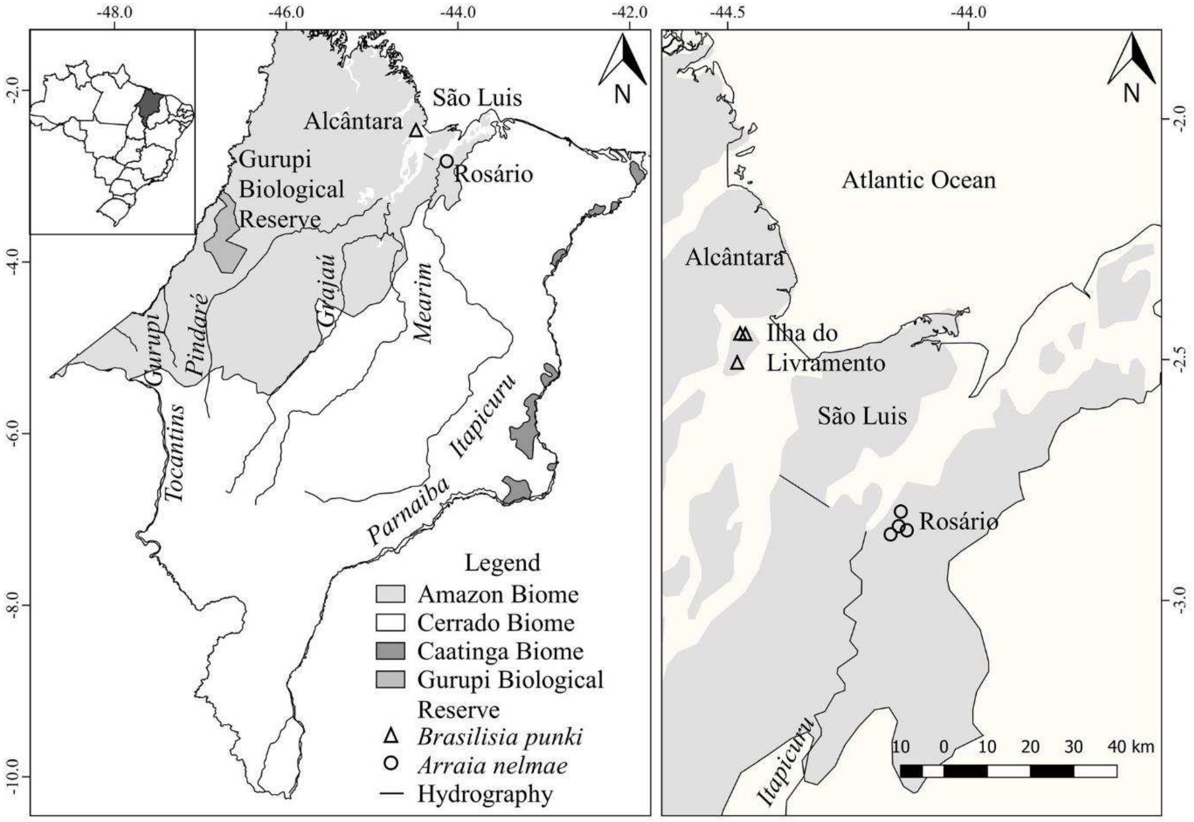

FIGURE 1. Sampling location at Alcântara and Rosário counties in Maranhão state, Brazil.

OCNERODRILIDAE Beddard, 1892

Brasilisia n. gen.

Type species. *Brasilisia punki* n. sp.

Definition. Setae eight per segment, closely paired. One gizzard in V. Esophagus with extramural calciferous glands in segment IX. Metagynous, holandric and holonephridial. Last hearts in XI. Male pores in XVII. Prostatic pores and tubular prostates in XVII. Adiverticulate spermathecae in IX. Dorsal pores present. Four paired genital markings on setae ab associated with enlarged and modified genital setae.

Etymology. The name of the new genus derives from Brasilisia de Jesus Maramaldo, the Livramento Island owner and protector.

Brasilisia punki Hernández-García & James, n. sp.

(Figure 2 A–G)

Holotype. MPEG.ANL 000329, adult, Livramento Island, Maranhão, Brazil, Restinga vegetation, 02°25'S, 44°25'W, 4 masl. June 2010. Silva, R.S. & Rousseau, G.X. colls.

Paratypes. MPEG.ANL 000330 two adults, MPEG.ANL 000331 four adults, MPEG. ANL 000332 three adults, MPEG.ANL 000333 three adults, MPEG.ANL 000334 one adult, same data as for holotype.

Etymology. The species name is derived from the nickname "Punk" of Ribamar Alves, field guide and island owner assistant.

Description. Dimensions: Holotype 41 mm by 2.6 mm at segment X, 2.6 mm at clitellum, 2.3 mm at XXX, body cylindrical, segments 135. Paratypes 32–55 mm by 2.0–3.1 mm at segment X, 2.1–3.3 mm at clitellum, 1.9–2.5 mm at XXX, body cylindrical, segments 128–172. Setae closely paired throughout, cd sub-lateral; setal formula aa:ab:bc:cd = 4:1:3:1 at X, 3:1:1.5:0.4 at XXX, DD > ½ circumference. Prostomium prolobous; some individuals with triannulate segments after XXII. Unpigmented, dorsal pores present, spermathecal pores within 8/9 approximately in b line; with tumid lips as wide as ac. Clitellum between ½ XIV, XV–XXI, saddle form, ventrolateral edges thickened in segments XVIII–XX (Fig. 2A). Microscopic ovipores in XIV just behind 13/14 in ab line; One pair of male and prostatic pores in XVII on ab line and open joint over protruding porophores. Postclitellar setae 100–110 µm in length (Fig. 2B). Setae ab in VII–XVI modified as genital setae and measuring 540–600 µm in length (Fig. 2C); genital markings of ab line in VII–XVI as papillae, those of IX–XVI are protruding like protuberances.

Anterior septa 5/6–8/9 strong, 9/10 less muscular; 10/11–12/13 thin. A strong gizzard in V measuring 1.7–2 mm large and 1.5–1.7 mm in width; esophagus valvular in XII, intestinal origin in XIII; dorsal typhlosole beginning in XV, with lamellar and sigmoidal structure, running to the posterior region, ending in LXXXI; intestinal walls lack segmental pouches. Calciferous glands paired in IX (Fig. 2D), composite-tubular type, each gland with a thin blood vessel from anterior end to extra-esophageal vessel, blood vessel from duct of gland to supra-esophageal vessel. Holonephric, first nephridia visible clearly in segment XII. Postclitellar nephridia with a short duct opening in a laminar bladder, fine folded and branched microtubes running inside the bladder (Fig. 2E). Vascular system with ventral trunk, single dorsal trunk in segments IV–IX, dorsal vessel single; lateral vessels in V–IX. Two pairs of latero-esophageal hearts present in segments X–XI.

Ovaries and funnels in XIII. One pair of adiverticulate spermathecae in segment IX, consisting of large sac widened in the middle region and narrowing at base and entally, ducts as wide as ampulla opening in 8/9, at b line (Fig. 2F). Male sexual system holandric, testes and funnels in X, XI, within sacs. Seminal grooves absent. Two pairs of deferent ducts superficial on internal body wall are visible from segments X–XI, fusing in XII and running through b line to segment XVII where deferent duct, of each side, fuses with the distal end of prostatic duct and open to the porophore in b line; seminal vesicles occupying segment IX laterally placed on body wall, X–XII large and placed ventrolaterally covering the esophagus. Tubular prostates in XVII and running in zig-zag pattern under intestine. In four specimens the prostatic tubes extended to segment L, glandular diameter between 140–150 µm, while ducts diameter are 40 µm. The prostatic ducts are 300–350 µm in length (approximately 3 segments long), slightly muscular.

Remarks. This new species is the first described from its genus. We determined it to be new based on the summary of genus characters implicit in the key of Fragoso & Rojas (2009), and here we will review the many unique anatomical features of the species, previously unknown within Ocnerodrilidae. There is no trace of seminal grooves in the male field, in which the species resembles several others, like *Gatesia unica* Jamieson, 1962, *Quechuona michaelseni* Jamieson, 1962, and *Haplodrilus tagua*, Righi, Ayres & Bittencourt, 1978 with two porophores on segment XVII, on which the prostatic and male pores open. The number of paired genital markings (10) on setae ab is unusual, if not unprecedented, and these are associated with enlarged and modified genital setae. The ventro-lateral edges of the clitellum in segments XVIII–XX are thickened to resemble tubercula pubertatis, a character which we believe is unique in the family. No other genus in the Megascolecoidea crown-group of Crassiclitellata (Megascolecidae, Acanthodrilidae, Benhamiidae, Ocnerodrilidae) has this structure, and neither does Eudrilidae, which shares a common ancestor with this crown-group (James & Davidson 2012). The strong gizzard clearly in V is very unusual, both for position and degree of development.

Among all genera of ocnerodrilids, *Dariodrilus* Righi et al., 1978 is most similar to the new genus in the presence of one gizzard, calciferous glands paired in IX, male and prostatic pores paired in XVII and two pairs of testicular sacs. The differences between the new genus and *Dariodrilus* are the position of the gizzard, which is in V for *Brasilisia* n. gen. and VII for *Dariodrilus*, dorsal pores are present for *Brasilisia* n. gen. and absent in *Dariodrilus*. The female reproductive system in *Dariodrilus* is hologynous while in *Brasilisia* n. gen. it is metagynous.

Arraia n. gen.

Type species. *Arraia nelmae* n. sp.

Definition. Setae eight per segment, closely paired. One gizzard in VI. Esophagus with extramural calciferous glands in segment IX. Holandric and holonephridial. Last hearts in XI. Male pores in XVII. Prostatic pore and tubular prostates in XVII. Adiverticulate spermathecae in IX.

Etymology. The genus name is derived from the name of Arraial Bay, one of the places in the coastal region where individuals were collected.

Arraia nelmae Hernández-García & James, n. sp.
(Figure 3A–F)

Holotype. MPEG.ANL 000325 adult, Rosário, Maranhão, Brazil, homegarden, 02°49'S 44°08'W, 38 masl. 03 June 2014. Muñoz J.A. & Cardozo E.G. colls.

Paratypes. MPEG.ANL 000326 three adults, same data as holotype, MPEG.ANL 000327 one adult, 02°51'S 44°09'W, 14 masl. 02 June 2014. MPEG.ANL 000328 one adult, old secondary forest 02°51'S, 44°09'W, 89 masl 31 May 2014. Muñoz J.A. & Rousseau G.X. colls.

Etymology. The species name is derived from Nelma Freitas, the landowner of the homegarden where the holotype was collected.

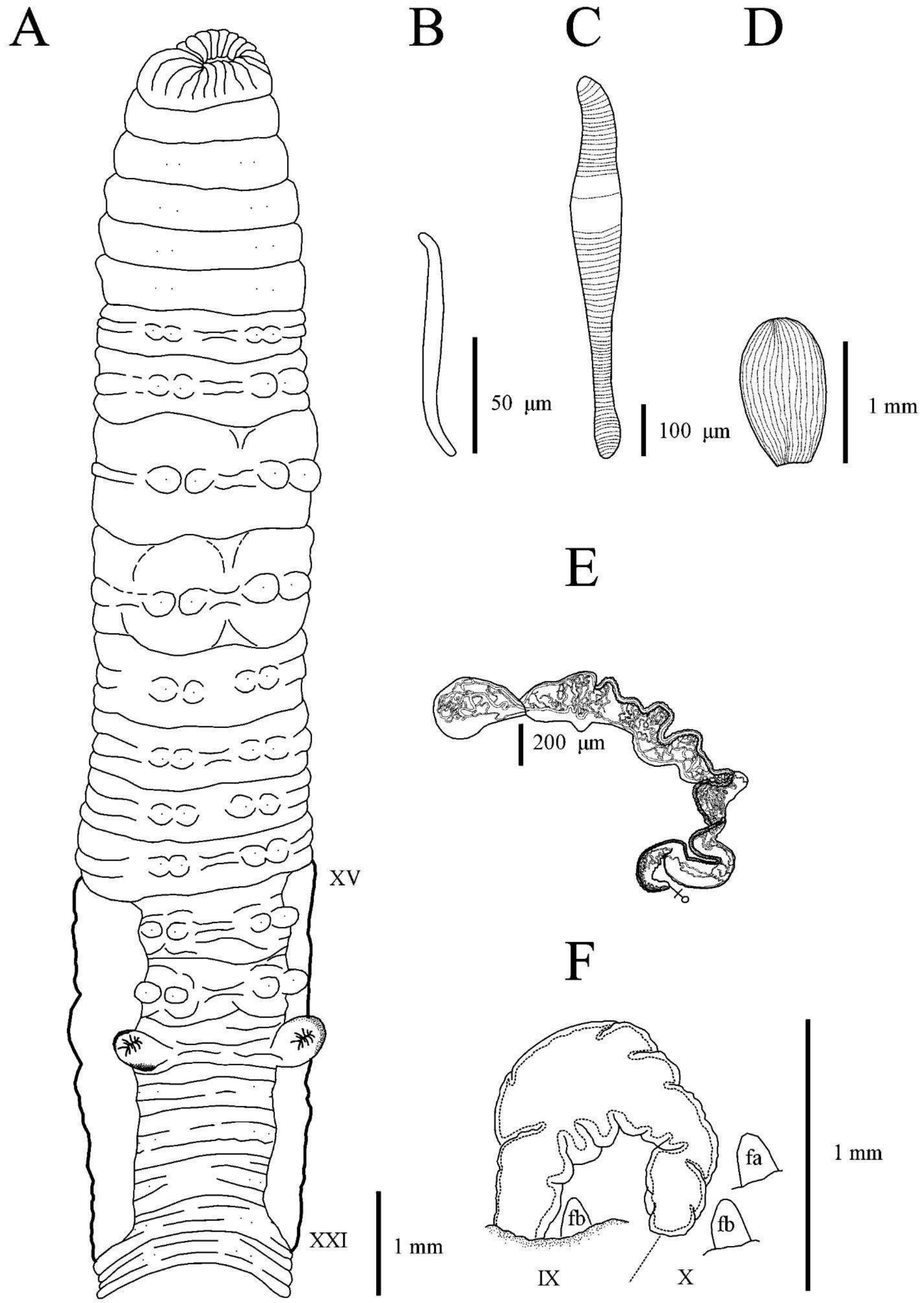

FIGURE 2. *Brasilisia punki* n. gen. A. Ventral view of anterior body, with clitellar region. B. Post clitellar seta of a line of XXXV. C. Genital seta of b line of segment XVI. D. Dorsal view of left calciferous gland of IX. E. Dorsal view of left post clitellar nephridium. F. Dorsal view of left spermatheca of IX. sa = follicle of setae a, sb = follicles of setae b.

Description. Dimensions: Holotype 346 mm by 1.9 mm at segment X, 2.2 mm at clitellum, 1.8 mm at XXX, body cylindrical, segments 100. Paratypes 210–350 mm by 1.2–1.9 mm at segment X, 1.65–2.2 mm at clitellum, 1.5–1.8 mm at XXX, body cylindrical, segments 73–112. Setae closely paired throughout, ab beginning in II and cd beginning in III; setal formula aa:ab:bc:cd = 6:1:5:1 at X, 5:1:3.5:1 at XXX, DD > ½ circumference. Prostomium tanylobous; segments lacking secondary annulations. Unpigmented, dorsal pores absent, spermathecal pores within 8/9 approximately centered in b line; with tumid lips more than ab. Ovipores in XIV just behind 13/14 in b line; male pores in 16/17, near prostatic pores in a line. Prostatic pores at ends of grooves, along b line in XVII. Setae ab of XVII unmodified. Clitellum in XIV–XVIII, saddle-shaped to b line (Fig. 3A). Post clitellar common setae 170–190 µm in length, smooth and sigmoidal form. Genital setae 200–210 µm in length, excavation absent and slightly sigmoid (Fig. 3B).

Anterior septa 5/6–9/10 slightly muscular, in following segments decreasingly muscular until remaining all thin. Gizzard in VI; esophagus valvular in XII, intestinal origin in XIII; lacking typhlosole. Calciferous glands paired in IX, tubular type (Fig. 3C,D), each gland with a thin blood vessel from anterior end to extra-esophageal vessel, blood vessel from duct of gland to supra-esophageal vessel. Holonephric, first nephridia visible in segments XI–XIII. Post clitellar nephridia with one short loop open in a laminar bladder (Fig. 3E). Vascular system with ventral trunk, single dorsal trunk V–IX, dorsal vessel single; lateral vessels in V–IX, latero-esophageal hearts X–XI.

One pair of ovaries and female funnels in XIII. One pair of small adiverticulate spermathecae in segment IX, cylindrical shape. Spermathecal ducts as wide as ampulla, open to septa 8/9 in b line (Fig. 3F). Male sexual system holandric, testes and funnels in X, XI, within sacs, vas deferens superficial on internal body wall from 9/10–10/11, to body wall in XVII where it runs through b line ending just in 16/17 near to prostatic pore; seminal vesicles occupying segments IX–XII, covering the esophagus. Tubular prostates in XVII. Four individuals with tubular prostates extended to anterior, ending in the pharynx region. Glandular diameter of prostates 100 µm, duct diameter was 60 µm; prostatic ducts 175–200 µm in length (2 to 3 segments long), muscular, enter in XVII in equatorial line at b line.

Remarks. This new species is the first described from its genus. We determined it to be new based on the summary of genus characters implicit in the key of Fragoso & Rojas (2009). *Arraia* n. gen. is similar to *Xibaro* genus (Righi 1981) by the gizzard in segment VI, calciferous glands paired in IX, male and female reproductive system holandric and metagynous, respectively, and dorsal pores absent. The principal difference between the new genus and *Xibaro* is the number of gizzards: while *Arraia* n. gen. has only one gizzard in VI, *Xibaro* has two gizzards, one in VI and another in VII. The male pores for *Arraia* n. gen. are in 16/17 while in *Xibaro* they are in XVIII. There are two pairs of prostates in segments XVII and XIX in *Xibaro*, while in *Arraia* n. gen. there is only one pair in XVII.

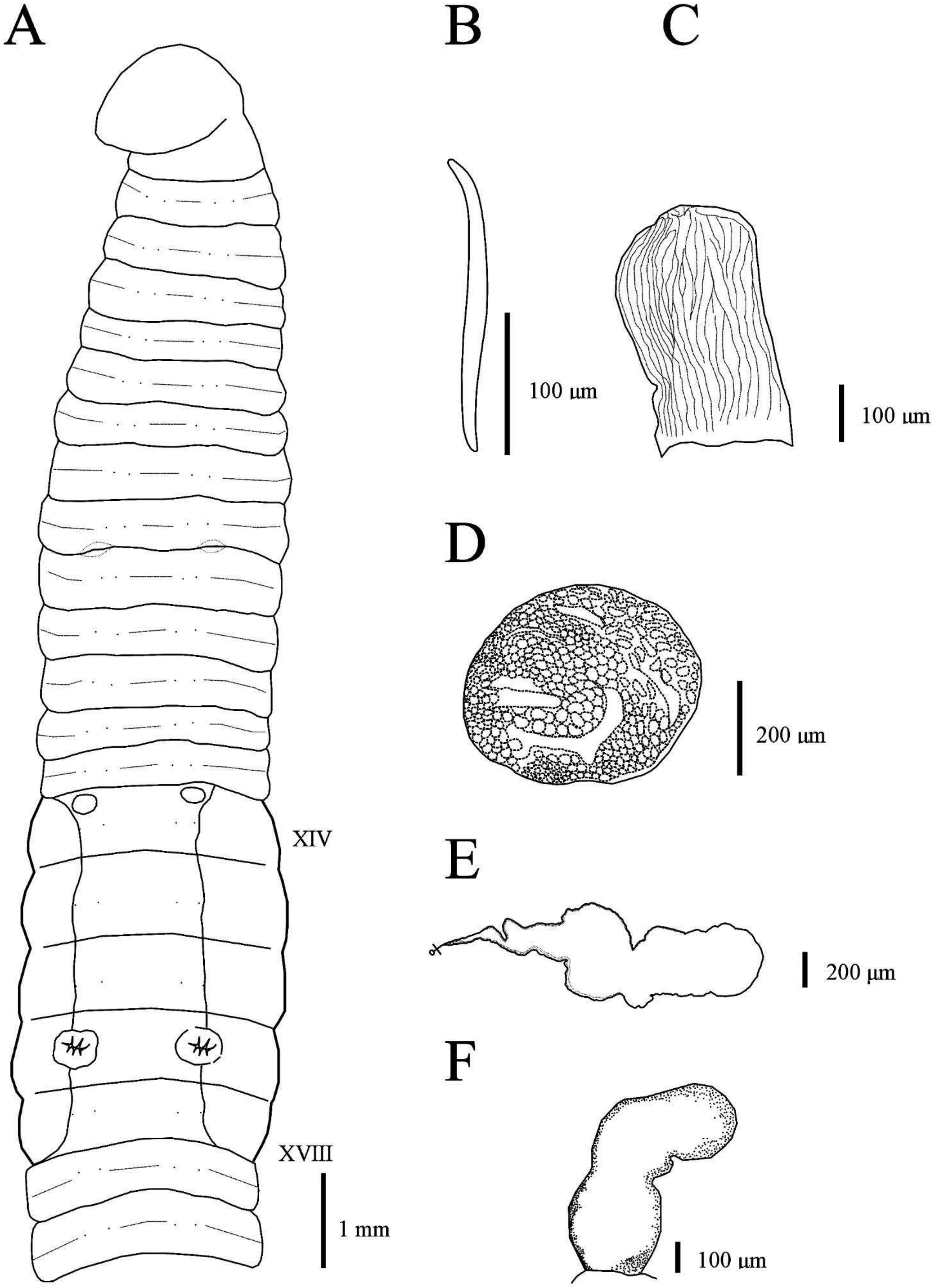

FIGURE 3. *Arraia nelmae* n. gen. A. Ventral view of anterior body, with clitellar region. B. Genital seta of a line of segment XVI. C. Dorsal view of left calciferous gland of IX. D. Transversal cut of left calciferous gland of IX. E. Dorsal view of left post-clitellar nephridium. G. Dorsal view of right spermatheca of IX.

Key to the genera of Ocnerodrilidae (from Fragoso & Rojas, 2009, extended)

1. Esophagus without extramural calciferous glands 2
- Esophagus with extramural calciferous glands. 7
2. Gizzard absent (Hawaii) . . . . *Enicmodrilus* Eisen, 1900
- Gizzard present 3
3. Two gizzards, in VI and VII 4
- One gizzard in VII. 5
4. With dorsal pores and typhlosole; metandric (testes in XI), prostates in XVII, XIX, XX, XXI (India). . . . . *Deccania* Gates, 1949
- Without dorsal pores and typhlosole; holandric (testes in X and XI) (Mexico) . . . . *Chacdrilus* Fragoso & Rojas, 2009.
5. Two pairs of prostates in XVII and XIX or three pairs of prostates in XVII, XIX and XX (India) . . . .*Thatonia* Gates, 1942
- One pair of prostates in XVII or two pair of prostates in XVII and XX. 6
6. Holandric, prostates in XVII or XVII and XX (India, China, Myanmar). . . . .*Malabaria* Stephenson, 1924
- Metandric, prostates in XVII (India). . . . . *Aphanascus* Stephenson, 1924
7. Unpaired segmental calciferous glands 8
- Paired segmental calciferous glands 10
8. Calciferous glands in IX and X (India). . . . . *Curgiona* Gates, 1941
- Calciferous gland only in IX. 9
9. Two pairs of prostatic glands in XVII and XIX; gizzard generally absent (Tropical Africa, Brazil?, some peregrines). . . . . . . *Gordiodrilus* Beddard, 1892
- Three pairs of prostatic glands in XVII–XIX (Africa) . . . .*Nannodrilus* Beddard, 1894
10. Calciferous glands in IX and X. 11
- Calciferous glands in VIII or IX or XI 14
11. Metandric (Seychelles Islands) . . . . *Maheina* Michaelsen, 1897
- Holandric. 12
12. One pair of prostates in XVII or XVIII. 13
- Two pairs of prostates, one pair in XVII and the other in XIX (Brazil) . . . .*Bauba* Righi, 1980
13. Prostates in XVII (Brazil) . . . .*Paulistus* Michaelsen, 1926
- Prostates in XVIII (Argentina, Ecuador, Peru) . . . . *Quechuona* Gates, 1941 (= *Quechua* Michaelsen, 1923)
14. Calciferous glands in VIII or XI 15
- Calciferous glands in IX 16
15. Calciferous glands VIII (Brazil) . . . . *Brunodrilus* Righi, 1971
- Calciferous glands in XI (Brazil) . . . . *Exisdrilus* Righi et al., 1978
16. With gizzards. 17
- Without gizzards 27
17. Two pairs of testes (Holandric). 18
- One pair of testes. 22
18. Two gizzards, one in VI and another in VII (Ecuador). . . . . *Xibaro* Righi, 1981
- One gizzard 19

19. Gizzard in V, genital markings as protuberances in AB line (Brazil). . . . .
. . . . *Brasilisia* Hernández-García et al., **n. gen.**
- Gizzard not in 5, no genital markings as protuberances in AB line 20
20. Gizzard in VI (Brazil) . . . . *Arraia* Hernández-García et al., **n. gen.**
- Gizzard in VII or VIII 21
21. Gizzard in VII (Brazil) . . . . *Dariodrilus* Righi et al. 1978
- Gizzard in VIII (West Africa) . . . . *Dorgiodrilus* Gates, 1962
22. Proandric (one pair of testes in X) 23
- Metandric 25
23. One pair of spermathecae 24
- Two pairs of spermathecae (South America, some peregrines) . . . .
. . . . *Eukerria* Michaelsen, 1935
24. Prostatic and male pores combined (Argentine) . . . .
. . . . *Gatesia* Jamieson, 1962
- Prostatic and male pores separated (Argentine, Bolivia, Brazil) . . . .
. . . . *Belladrilus* Righi, 1984
25. Two gizzards, one in VI, one in VII 26
- Three gizzards, in VI–VIII (Brazil). . . . . *Lourdesia* Righi, 1994
26. Solid calciferous glands (without lumen) (Dominican Republic). . . . .
. . . . *Temanonegia* Gates, 1979
- Calciferous glands with large, central lumen (Central America, Africa, one
peregrine) . . . . *Nematogenia* Eisen, 1900
27. Proandric 28
- Holandric. 29
28. One pair of prostates in XVII (Brazil, Paraguay) . . . .
. . . . *Haplodrilus* Eisen, 1900
- Two pairs of prostates, one pair in XVII, one pair in XIX (Brazil) . . . .
. . . .*Kerriona* Michaelsen, 1924
29. One pair of spermathecal pores. 30
- Three pairs of spermathecal pores (in VII–IX) (Equatorial Guinea) . . . .
. . . . *Diaphorodrilus* Cognetti, 1910
30. Spermathecal pores in 6/7 or 7/8. 31
- Spermathecal pores in 8/9 or 9/10. 32
31. Spermathecal pores in 6/7 (Ghana). . . . . *Tazelaaria* Gates, 1962
- Spermathecal pores in 7/8 (Guyana, Brazil) . . . .*Liodrilus* Eisen, 1900
32. Trabeculated calciferous glands, complete or only in part (Africa, Mexico,
Central and South America, some peregrines) . . . .
. . . .*Phoenicodrilus* Eisen, 1895
- Calciferous glands with large central lumen. 33
33. Spermathecae with diverticula (Africa) . . . . *Pygmaeodrilus* Michaelsen, 1890
- Spermathecae without diverticulum (Central and South America, one
peregrine). . . . . *Ocnerodrilus* Eisen, 1878

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7.4 **CAPITULO 4:** Additions to *Andiorrhinus* (*Turedrilus*) (Rhinodrilidae, Clitellata) from Eastern Amazonia

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Abstract

Two new species of the earthworm family Rhinodrilidae (Clitellata), from Maranhão state, Brazil, were studied by dissection. *Andiorrhinus* (*Turedrilus*) *miricuri* n. sp. is the first record of a big earthworm for this region with three pairs of large spermathecae in segments 7–9 and one pair of tubercula pubertatis bands lateral to b line in XX–XXV. *Andiorrhinus* (*Turedrilus*) *barrosoi* n. sp. lacks spermathecae and has one pair of tubercula pubertatis bands lateral to b line in XX–(1/3) XXIV. The earthworms described are from the most threatened region of Amazonia where 75% of forest cover is already lost. More information is urgently necessary to assess their ecology and vulnerability status.

Key words: Earthworm, Brazil, Maranhão, Gurupi

Introduction

The Maranhão Amazon forest represents 2.3% of the total Brazilian Amazonia, with at least 65% of this area being part of the Belém Endemism Area, BEA (Braz et al. 2016). BEA is known for its high diversity of vertebrates like mammals and birds (Lopes & Ferrari 2008; Dornas et al. 2014; Lima et al. 2015) and of at least 29 major taxa of the soil macrofauna (Rousseau et al. 2014). More recently, Gutierrez et al. (2017) have found high ant diversity in this area. The principal limitations of distribution respond to clear physical and environmental limits, such as large rivers to the northwest, the Atlantic Ocean to the northeast and the transition to the drier Cerrado biome to the southeast. Almost 75% of original forest cover of BEA had been lost since 1960s, when the military government promoted the occupation of forest lands as a political strategy to solve social conflicts (Kohlhepp 2002, Silva et al. 2005). Removal of original vegetation causes fast degradation of the soil (Celentano et al. 2017), and is likely to disrupt soil macrofauna communities and therefore the ecological functions they perform (Rousseau et al. 2014). Within soil macrofauna communities, earthworms have important effects on soil function: they maintain soil porosity and promote nutrient cycling and carbon stabilization in many ecosystems (Bottinelli et al. 2010; Tikhonov et al. 2016). However, they are susceptible to environmental changes like land use changes or decrease of C input into the soil by the increase of atmospheric CO_2 (Pritchard 2011; Lapied & Lavelle 2003). Most of the Brazilian native earthworms belong to two families, Glossoscolecidae and Rhinodrilidae (Brown & James 2007; James & Davidson 2012; James 2012). Species of these genera were previously accomodated in one family, Glossoscolecidae, until molecular studies detected its polyphyly (James & Davidson 2012). Reports of earthworms from the BEA listed the Glossoscolecidae genera *Righiodrilus, Diaguita, Glossodrilus,* the Rhinodrilidae genera *Pontoscolex, Rhinodrilus, Urobenus, Atatina, Ocnerodrilus* and the Acanthodrilidae genus *Dichogaster* (Righi et al. 1978, Righi 1972, 1985, 1988, Zicsi & Csuzdi 1999, Rousseau et al. 2010, dos Santos et al. 2017). Although there are records of *Andiorrhinus* species for Pará state (Marichal et al. 2017), the genus has never been reported in the BEA. The Rhinodrilidae genus *Andiorrhinus* Cognetti, 1908 includes 39 species, distributed in Venezuela (19), Brazil (10), Bolivia (4), Colombia (3), Paraguay (1), Chile (1) and Guyana (1). Within the family, the main defining characters of the genus are three pairs of calciferous glands with lamellar structure in segments 7–9, holandric male sexual system and metagynous female sexual system (Cognetti 1908). *Andiorrhinus* can be divided into five subgenera based on number and positions of hearts (Righi 1993, Feijoo et al. 2017): *Amazonidrilus, Andiorrhinus, Meridrilus, Quibario* and *Turedrilus*, where *Turedrilus* subgenus has two pairs of lateral-esophageal hearts limited to segments 10 and 11.

Almost 80% of species in the subgenus *Turedrilus* are associated with minimally perturbed environments (Michaelsen 1892; Righi 1986, 1993; Righi & Nemeth 1983; Drachenberg 1991; Feijoo 2008; Feijoo & Celis 2012). Because *Andiorrhinus* earthworms are largely epi-endogeic or endo-anecic, their main ecological importance is to contribute to soil porosity (Feijoo et al. 2017), although they can also be used as food resources as it happens in Venezuelan indigenous tribes (Moreno & Paoletti 2004). There is a lack of information about the drilofauna of Maranhão state and the BEA, especially for the last remaining forests. In this manuscript we describe two new earthworm species of the subgenus *Andiorrhinus* (*Turedrilus*) from eastern Brazilian Amazonia, found in conserved forest, fallows and pasture.

Materials and methods

The earthworm specimens were collected by digging and hand-sorting in natural ecosystems, old forest remnants and agroforestry systems at the counties of Centro Novo and Itinga (Reserva Biológica do Gurupi), Alcântara, São Luis and Rosário, Maranhão State, Brazil (Fig. 1). Sampling was carried out with the quantitative modified (3 monolith by plot) Tropical Soil Biology and Fertility (TSBF) method (Anderson & Ingram 1993) complemented by an active search qualitative method (Decaëns et al. 2016). Earthworms caught were anesthetized in 50% (v/v) ethanol. Posterior body fragments of the specimens were preserved in 95% (v/v) ethanol and kept in a freezer for further molecular phylogenetic studies. The specimens were then fixed in 10% (v/v) formalin and preserved in 95% (v/v) ethanol. The holotypes and twenty-five paratypes were deposited at the Museu Paraense Emílio Goeldi, Belém - Pará, the accession identification codes begin with MPEG.

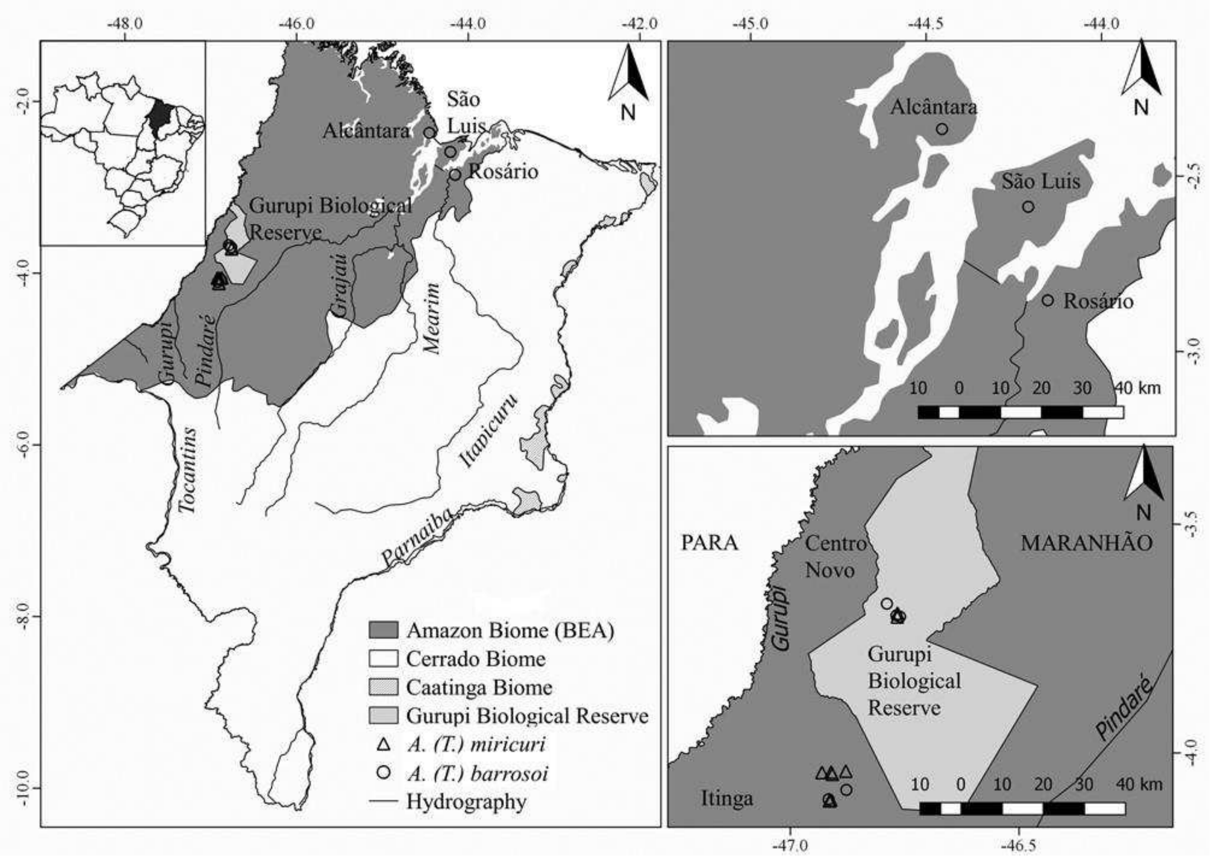

FIGURE 1. Occurrence of *Andiorrhinus* (*Turedrilus*) *miricuri* and *Andiorrhinus* (*Turedrilus*) *barrosoi* spp. nov. (black triangle and circle) at Gurupi Biological Reserve, Itinga, Alcântara, São Luis and Rosário, Maranhão State, Brazil. Dark-grey: Amazon biome (BEA). Light grey: Gurupi Biological Reserve within the Amazon biome.

Genus *Andiorrhinus* Cognetti, 1908

Andiorrhinus Cognetti, 1908; Michaelsen 1918; Cordero 1945; Righi 1971, 1986, 1993.

Type species. *Anteus brunneus* Michaelsen, 1892

Subgenus *Andiorrhinus* (*Turedrilus*) Righi, 1993

Type species. *Andiorrhinus samuelensis* Righi, 1986.

Diagnosis. Only two pairs of intestinal hearts in segments 10 and 11 (Righi 1986, 1993). Setae in 8 longitudinal lines, one gizzard in segment 6, one pair of intraclitellar male pores, spermathecae present or absent, three pairs of calciferous glands in segments 7–9 with lamellar structure. Sexual system holandric and metagynous with seminal vesicles short or long. Number of species: 12. Distribution: Venezuela (5), Brazil (5) and Colombia (2). Although *Andiorrhinus meansi* (James, 2009) from Guyana (1) has two pairs of intestinal hearts in segments 10 –11, it was recommended not to include this species in *Turedrilus* until a general revision (James 2009).

Andiorrhinus (*Turedrilus*) *miricuri* Hernández-García & James, sp. n.
(Figure 2 A–F)

Holotype. MPEG.ANL 000316 adult, incomplete (posterior end missing). Logged primary forest, PPBio Gurupi N, Centro Novo do Maranhão, Maranhão, Brazil, 3°41'10.76"S 46°45'6.91"W, 158 masl. 28 March 2015, Hernández-García, L.M. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000317, one adult, MPEG.ANL 000318, one adult, MPEG.ANL 000319, one adult. Same locality as holotype. MPEG.ANL 000320 one adult, amputee. Secondary forest, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'S 46°54'W, 228 masl. 27 June 2014, Muñoz J.A. & Rousseau G.X. colls.

Other material. MPEG.ANL 000367, one adult, complete. Secondary forest 15 years old, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°41'57.3"S 46°46'04.8"W, 131 masl. 22 April 2016, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000368, one adult, complete. Primary forest, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°41'27.7"S 46°46'02.7"W, 146 masl. 23 April 2016, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000369, one adult, complete. Primary forest, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°41'27.7"S 46°46'02.7"W, 146 masl. 23 April 2016, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000370, one adult, complete. Pasture, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'20.6"S 46°55'58"W, 196 masl. 18 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000371, one adult, complete. Pasture, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'18"S 46°54'45.5"W, 158 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000372, three adults, complete. Pasture, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'18"S 46°54'45.5"W, 158 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000373, one adult, complete. Degraded forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'34.5"S 46°54'36.1"W, 164 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000374, one adult, complete. Degraded forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'34.5"S 46°54'36.1"W, 164 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000375, two adults, complete. Conserved forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°5'57.6"S 46°54'48.8"W, 229 masl. 22 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000376, one adult, complete. Conserved forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°5'57.6"S 46°54'48.8"W, 229 masl. 22 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000377, one adult, complete. Conserved forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°5'57.6"S 46°54'48.8"W, 229 masl. 22 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000378, one adult, complete. Conserved forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°6'4"S 46°54'59"W, 185 masl. 22 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000379, one adult, complete. Secondary forest 15 years old, Galetti Farm, Itinga do Maranhão, Maranhão, Brazil, 4°2'9.9"S 46°52'50.2"W, 294 masl. 19 April 2017, HernándezGarcía, L.M. & Rousseau G.X. colls. MPEG.ANL 000380, three juveniles, complete. Pasture, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'18"S 46°54'45.5"W, 158 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000381, three juveniles, complete. Forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°2'34.5"S 46°54'36.1"W, 164 masl. 20 April 2017, Hernández-García, L.M. & Rousseau G.X. colls.

Etymology. The species name is the Guajajara indigenous language word for this earthworm species.

Description. Dimensions: holotype 216 mm (amputee) by 8.3 mm at X, 9.0 mm at clitellum, 6.5 mm at XXX, 203 segments; complete adult paratypes 210–350 mm by 9.0 mm at X, 10.0 mm at clitellum and 7.0 mm at XXX, 221–344 segments. In general the total segments and length are variable because the species presents a biological strategy of self-amputation to survive predator attack. Body cylindrical. Setae ab and cd commence on V and VII, respectively. Setae closely paired throughout; setal formula aa:ab:bc:cd:dd = 6.5:1.0:8.0:0.5:20.0 at XXX, dd > 1/2 circumference throughout. Prostomium tanylobous-tentacular, post-setal secondary annulations present in XXVIII–XXXV and CV–CLXXII behind clitellum. Body pigmented, brownish dorsally and greyish ventrally. Clitellum annular in XV–(1/2) XXVI (Fig. 2A) and beige pigmented. Protruding genital markings in XVII, XIX–XXIV with genital setae associated. Genital setae of XXII 3mm in length, ornamented with nine alternate semilunar excavations at the sub-apical concave part (Fig. 2B). Two pairs of bands forming the tubercula pubertatis present just near b line between segments XX–XXV. Nephropores intersegmental in cd line. Male and female pores not seen externally, but male sperm ducts enter body wall at 18/19.

Septa equally thick, conical and muscular in 6/7–8/9 and 11/12. Septa 9/10 and 10/11 absent, while septa 13/ 14–17/18 are thin. Alimentary canal with large cylindrical gizzard in VI; esophagus valvular in XVII, intestinal origin XIII; typhlosole origin XXV and sigmoid form in cross section. Three pairs of calciferous glands with ental appendices in VII–IX, dorsal esophageal connection, garlic clove shaped and lamellar structure (Fig. 2C,D). The external part of calciferous gland exposes to coelom is crusty and darker. Holonephric, vesiculate; ducts to body wall near level of cd. Post-clitellar nephridium with one loop connected directly with the bladder compartment (Fig. 2E). Vascular system with ventral trunk, single dorsal trunk, lateral vessels in VII–IX. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard, to segment 10. Paired hearts in X–XI, kidneyshaped, surrounded by seminal vesicles and testicular sacs. Supra-esophageal vessels in X–XIII. Ovaries in XIII. Three pairs of spermathecae in segments VII–IX opening in cd line (Fig. 2F). Ampulla bean shaped 3.4 x 9 mm in size and connected to the spermathecal pore by a thin tube. Spermathecal ducts as long as the maximum diameter of the ampulla. Male sexual system holandric, testes and funnels in single mid-ventral sub-esophageal sac in each of X and XI; lobulated seminal vesicles occupying segments XI–XIV, XVII. Deferent ducts opening in 18/19.

Remarks. *Andiorrhinus* (*Turedrilus*) *miricuri* n. sp. is similar to *Andiorrhinus* (*Turedrilus*) *baniwa* Righi & Nemeth, 1983, by the presence of three pairs of spermathecae in segments VII–IX and because both have an annular clitellum. The differences between *A.* (*T.*) *miricuri* n. sp. and *A.* (*Turedrilus*) *baniwa* are, basically, the length of the clitellum; 12 segments (15–1/2 26) in *A.* (*T.*) *miricuri* n. sp. and 11 segments (15–1/2 25) in *A.* (*T.*) *baniwa* (Table 1), the extension of the tubercula pubertatis, which is XX–XXV for *A.* (*T.*) *miricuri* n. sp. and XXI–XXIV for *A.* (*T.*) *baniwa*. On the other hand, the spermathecal duct-ampulla relation is shorter (1:1) in *A.* (*T.*) *miricuri* n. sp. than for *A.* (*T.*) *baniwa* (2:1); the calciferous gland in *A.* (*T.*) *baniwa* is pedunculated while for *A.* (*T.*) *miricuri* ducts are absent; intraclitellar genital papillae are absent or less protruding in *A.* (*T.*) *baniwa* than those of *A.* (*T.*) *miricuri.*

Andiorrhinus (*Turedrilus*) *barrosoi* Hernández-García & James, sp. n.
(Figure 2 G–K)

Holotype. MPEG.ANL 000321 adult, complete. Agroforestry System. Plano de Assentamento São João do Rosário, Rosário, MA, Brazil, 02º51'14.3"S, 44º09'11.4"W, 38 masl. 06 March 2014. Muñoz J.A. & Rousseau G.X. colls.

Paratypes. MPEG.ANL 000322 one adult, complete. Six years-old fallow, Espera, Alcântara, MA, Brazil, 2°22'13.0"S, 44º26'40.3"W, 38 masl. 19 May 2015. Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000323, adult, complete. Logged primary forest, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°41'10.76"S 46°45'56.54"W, 158 masl. 28 March 2015, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000324, one adult, complete. Forest 120 years old, Espera, Alcântara, MA, Brazil, 2º21'58.6"S, 44º27'16.5"W, 48 masl. 18 May 2015. Hernández-García, L.M. & Rousseau G.X. colls.

Other material. MPEG.ANL 000360 three adults, complete. Pasture, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°40'25.3"S 46°47'20.5"W, 139 masl. 20 April 2016. Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000361, one adult, complete. Secondary forest 15 years old, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°41'57.3"S 46°46'04.8"W, 131 masl. 22 April 2016, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000362, one adult, complete. Secondary forest 15 years old, Centro Novo do Maranhão, PPBio Gurupi N, Maranhão, Brazil, 3°42'07.3"S 46°45'37.1"W, 134 masl. 24 April 2016, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000363, one adult, complete. Conserved forest fragment, Plano de assentamento Gurupi, Vila Canãa, Itinga do Maranhão, Maranhão, Brazil, 4°6'4"S 46°54'59"W, 185 masl. 22 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000364, one adult, complete. Secondary forest 15 years old, Rio dos Bois, Itinga do Maranhão, Maranhão, Brazil, 4°4'50.3"S 46°52'39.4"W, 149 masl. 23 April 2017, Hernández-García, L.M. & Rousseau G.X. colls. MPEG.ANL 000365, one adult, complete. Secondary forest 16 years old, Fazenda Escola, Universidade Estadual do Maranhão, São Luis, Maranhão, Brazil, 2°35'14.53"S 44°12'31.57"W, 62 masl. 4 June 2017, Sousa, S. & Rousseau G.X. colls. MPEG.ANL 000366, one juvenil, complete. Secondary forest 16 years old, Fazenda Escola, Universidade Estadual do Maranhão, São Luis, Maranhão, Brazil, 2°35'14.53"S 44°12'31.57"W, 62 masl. 4 June 2017, Sousa, S. & Rousseau G.X. colls.

Etymology. The species is named for Mr. João Castro Barroso, owner and keeper of the remaining forests that cover the land where one of those paratypes was collected in Alcântara (Espera).

Description. Dimensions: holotype 165 mm by 5.8 mm at X, 5.5 mm at clitellum, 5 mm at XXX, 183 segments; paratype 64–128 mm by 4.7 mm at X, 5.6 mm at clitellum and 4.3 mm at XXX, 127–211 segments. Body cylindrical in anterior region and slightly flattened after clitellar area for Gurupi paratypes. Paratypes found in Alcântara, São Luis and Rosario are totally cylindrical. Setae ab commence on III, cd commences on IV. Setae closely paired throughout; setal formula aa:ab:bc:cd:dd = 7.0:1.0:6.3:0.7:32.0 at XXX, dd > 1/2 circumference throughout. For flattened earthworms dd ratio is 18.2. Prostomium prolobous, post-setal secondary annulations absent. Body pigmented brownish dorsally preserved, ventrally unpigmented. Annular clitellum in XV–XXV (Fig. 2G), dark brown preserved, tubercula pubertatis markings smooth straight band shape present between bc line on XX–XXIII, (1/3)XXIV. Genital setae of XX 0.7–1.1mm in length, eight alternate semilunar excavations at the subapical concave part (Fig. 2H). Microscopical ovipores are visible just behind ab line on white and small papilla of segment XIV. Male pores invisible. Preclitellar nephropores in d line, then in c line just after clitellum.

Conical septa equally thick and muscular in 6/7–8/9. Septa 9/10 and 10/11 absent, septum 11/12 is slightly muscular, those of 12/13–16/17 very thin. Alimentary canal with large cylindrical gizzard in VI; intestinal origin XVIII; typhlosole origin XXI, ventral margin of lamina curved upwards in a hook form. Calciferous glands paired VII–IX with dorsal esophageal connection, kidney shape and lamellar type (Fig. 2I,J). Holonephric, sphincters present; ducts to body wall near level of c. Post-clitellar nephridium with two loops, the first thicker than the second one, and the second connected with some loops that connect to bladder compartment (Fig. 2K). Vascular system with ventral trunk, supra-esophageal vessel in X, expanded in XII–XIII and then dorsally extended on intestine, latero-esophageal hearts in each of X and XI with latter pair enclosed in testis sacs. Extra-esophageal vessel from pharyngeal glands, along ventral-lateral face of gizzard. Metagynous, no spermathecae. Male sexual system holandric, testes and funnels in single mid-ventral sub-esophageal sac in each of X, XI; lobulated seminal vesicles are occupying the segments XI–XII. Deferent ducts running between bc line opening in 20/21.

Remarks. *Andiorrhinus* (*Turedrilus*) *barrosoi* n. sp. is similar to *Andiorrhinus* (*Turedrilus*) *bare* Righi & Nemeth, 1983 by the dimensions in length and a slightly annular shape of the clitellum. The main differences are the lack of spermathecae in *A.* (*T.*) *barrosoi* n. sp. while *A.* (*T.*) *bare* has two pairs of spermathecae in segments 9 and 10 (Table 1); *A.* (*T.*) *bare* has a clitellum between XVII–XXVI, while in *A.* (*T.*) *barrosoi* n. sp. it is in XV–XXV; the position and shape of the tubercula pubertatis in *A.* (*T.*) *barrosoi* n. sp. is XX–XXIII, (1/3)XXIV and has smooth straight band shape while in *A.* (*T.*) *bare* it is XXII–XXV and has transverse horseshoe shape protruding to body wall.

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TABLE 1. *Andiorrhinus* (*Turedrilus*) species comparison.

***Andiorrhinus* (*Turedrilus*) species**	**Pigmentation**	**Length (mm)**	**Diameter XXX (mm)**	**Segment number**	**Post-clitellar setal ratio aa:ab:bc:cd:dd**	**Prostomium**	**Clitellum**	**Tubercula pubertatis**	**Tub. pubert. shape**
A. (T.) brunneus Michaelsen, 1892	dark brown, dorsal	70–125	?	97–112	5.5:1.0:7.3:0.8:28.5	retracted	saddle XVI–XXIV	(1/2)XVIII–(1/2)XXIII	gutter
A. (T.) marcuzzi Omodeo, 1955	dark violet, segment I	66–77	3	105–124	4.0:1.1:5.5:1.0:20.0	tentacular	saddle, XVI–XXV	XXI–XXIII *B* line	smooth band
A. (T.) caudatus Righi *et al*., 1976	absent	90–105	2.0–3.0	227–242	1.47:1:1.05:0.82:2.58	?	annular, XVI–XXV	XXI–XXIV	parallelepiped
A. (T.) baniwa Righi & Nemeth, 1983	absent	225*	5	306*	2.5:1.0:2.7:1.4:3.1	tentacular	annular, XV(1/2)–XXV	XXI–XXIV, BC line	smooth band
A. (T.) bare Righi & Nemeth, 1983	absent	85	1.6	216	2.6:1.0:1.8:??:??	tentacular	annular, XVII–XX; saddle, XXI–XXVI	XXII–XXV, *B* line	protruding horseshoe
A. (T.) samuelensis Righi, 1986	absent	390	18	318	6.5:1:8.4:0.4:17.8	retracted	saddle, XV–XXV	(1/2)XX–(2/3)XXIV	smooth band
A. (T.) royeroi Drachenberg, 1991	dorsal, gray	230.7	6.2	267	9.9:1:9.8:0.9:22.3	tentacular	annular, XIII–XVI, saddle, XVII–XXIV	(1/2)XIX–(2/3)XXII, *BC* line	smooth band
A. (T.) acaciascensis Feijoo, 2008	dorsal	45–52	3	135–148	3:0.3:4:0.2:13.6	retracted	saddle, XV,XVI–XXIV	XIX–XX, *A* line	semicircle
A. (T.) yukuna Feijoo & Celis, 2012	dorsal, brown	74.5–78	2.9–3.1	131–134	3.0:0.25:3.2:0.25:9.6	tentacular	annular, XVI–XIX, saddle, XX–(1/2)XXIV, XXIV	absent	absent
A. (T.) duranti Feijoo *et al*., 2017	dorsal, yellow	170–218	6.2	129–164	10.5:1.0:11.5:0.8:43.0	retracted	saddle, XVI– XXV	XVIII–XXIII, *B* line?	smooth band?
A. (T.) miricuri **sp.nov.**	dorsal, brown	198–245	7.1–8.4	221–344	6.5:1.0:8.0:0.5:20.0	tanylobous-tentacular	annular, XV–(1/2) XXVI	XX–XXV, *B* line	smooth band
A. (T.) barrosoi **sp. nov.**	dorsal, brown	64-165	4.3	127–211	7.2:1.0:8.0:0.6:32.0	Prolobous	annular, XV–XXV	XX–XXIII,1/3 XXIV, *BC* line	smooth band

*Amputee

TABLE 1 (continued). *Andiorrhinus* (*Turedrilus*) species comparison.

***Andiorrhinus* (*Turedrilus*) species**	**Intraclitellar genital marks**	**Male pore**	**Spermathecae**	**Genital setae**	**Calciferous gland Shape**	**Typhlosole origin and shape**	**Locality**
A. (T.) brunneus Michaelsen, 1892	absent	microscopical	VII–IX, *CD* line	1000 µm, slightly curved 22 excavations	cylindrical, appendage	XXVI, bend as tube	Venezuela, Caracas
A. (T.) marcuzzi, Omodeo, 1955	XX–XXII	microscopical	VII–IX *AB* line, duct-ampulla ratio 2:1, chambers	610 µm, straight, 12 excavations	Sac	present, sigmoidal sheet	Venezuela, El Junquito
A. (T.) caudatus Righi *et al*., 1976	absent	21	VII, IX, *B* line	562–663 µm, 4–5 excavations	Sac	XXVI, sigmoidal sheet	Brazil, Amazonas (Sucunduri), Pará (Tapajos)
A. (T.) baniwa Righi & Nemeth, 1983	XVII	21/22	VIII–IX *CD* line, duct-ampulla ratio 2:1	692 µm, 5–7 excavations	flabellate, appendage	XXVI, double sigmoidal sheet	Venezuela, Amazonas (San Carlos de Rio Negro).
A. (T.) bare Righi & Nemeth, 1983	absent	20	XI–X *CD* line, small duct	600 µm, 6–8 excavations	triangle, appendage	XXV, wavy	Venezuela, Amazonas (San Carlos de Rio Negro).
A. (T.) samuelensis Righi, 1986	XII–XXV	microscopical	VIII–IX *CD* line, duct-ampulla ratio 1:2	1940–2500 µm, 12–15 excavations	flabellate, appendage	XXX, double sigmoidal sheet	Brazil, Rondonia (Porto velho)
A. (T.) royeroi Drachenberg, 1991	absent	19/20	VI–VIII, *C* line, duct-ampulla ratio 1:1, chamber	1640–1650 µm, 23–27 excavations	kidney	XXII, sigmoid	Venezuela, Amazonas (Rio Mavaca)
A. (T.) acaciascensis Feijoo, 2008	?	microscopical	VIII, IX	21 excavations	?	XVIII, simple sheet	Colombia, Sumapaz
A. (T.) yukuna Feijoo & Celis, 2012	XVII–XIX	19	IX, *B* line, duct-ampulla ratio 3:1	1000 µm,19 excavations	garlic clove	XXVII, bent tube	Colombia, Caquetá (Belém de los andaquíes, Florencia)
A. (T.) duranti Feijoo *et al*., 2017	XIX–XXIII	20	VII–VIII *B* line, duct-ampulla ratio 3:1	2140 µm, >15 excavations	Sac	XXVII, sigmoidal sheet	Brazil, Maranhão(Gurupi)
A. (T.) miricuri **sp.nov.**	XIX–XXIV	18/19 *BC line*	VII–IX *CD* line, duct-ampulla ratio 1:1	3000 µm, 9 excavations	garlic clove, appendage	XXV, sigmoidal sheet	Brazil, Maranhão(Gurupi)
A. (T.) barrosoi **sp. nov.**	absent	19/20 *BC line*	absent	700–1000 µm, 5–8 excavations	garlic clove, appendage	XXVI, hook	Brazil, Maranhão(Gurupi, Alcântara, São Luis, Rosário)

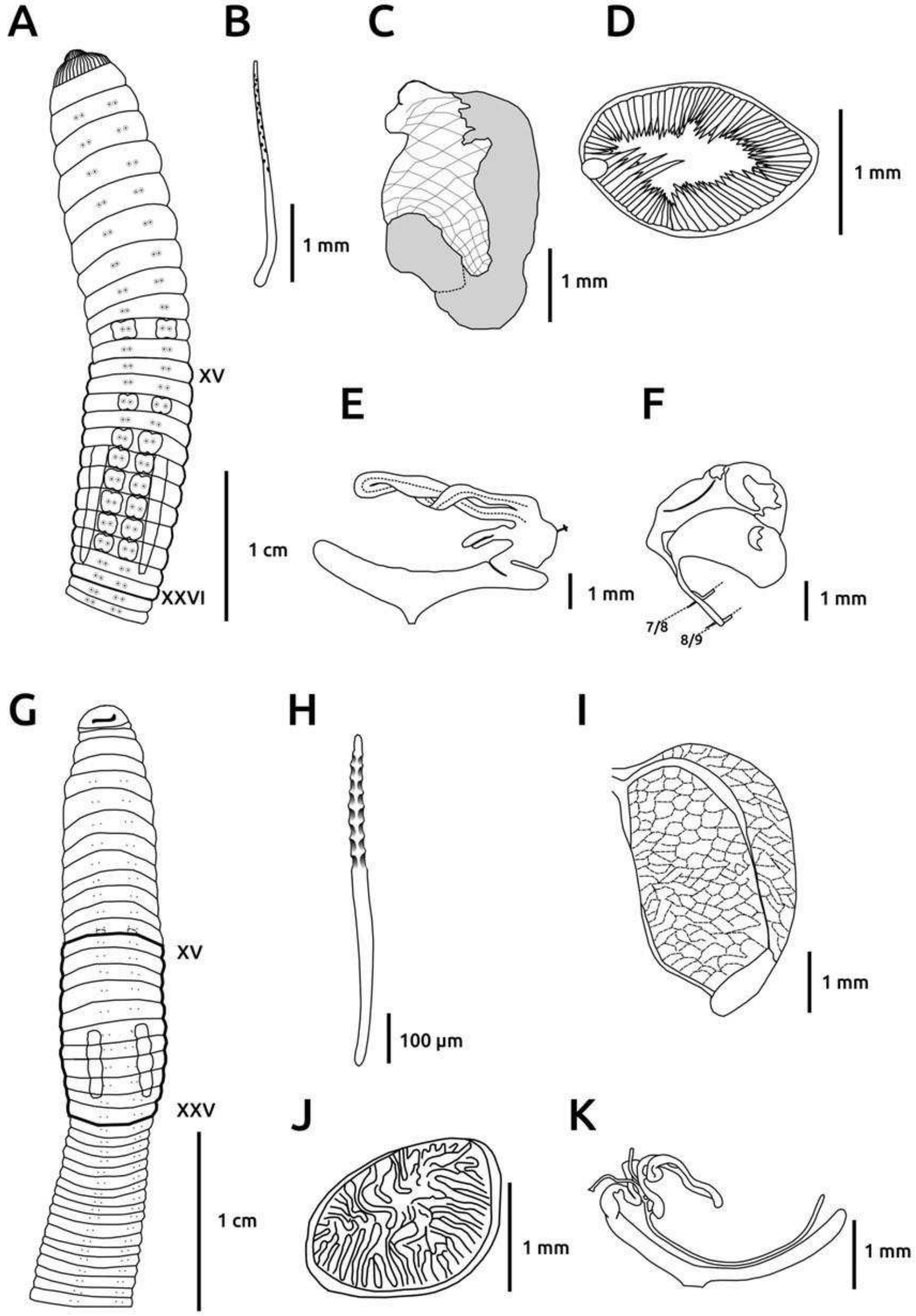

FIGURE 2. *Andiorrhinus* (*Turedrilus*) *miricuri* n. sp. (A–F) and *Andiorrhinus* (*Turedrilus*) *barrosoi* n. sp. (G–K). A. External ventral view of anterior body region, with clitellum, tubercula pubertatis and genital papilla. B. Genital setae of 22. C. View in cross section of right calciferous gland of 9. D. Dorsal view of transversal cut of right calciferous gland of 9, anterior body end to the top. E. View in cross section of left postclitellar nephridium. F. Dorsal view of spermathecae of 8 and 9. G. External ventral view of anterior body region, with clitellum, tubercula pubertatis and genital papilla. H. Genital setae of 20. I. View in cross section of left calciferous gland of 9. J. Dorsal view of transversal cut of right calciferous gland of 9, anterior body end to the top. K. View in cross section of right postclitellar nephridium.

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7.5 **CAPITULO 5:** A New species of *Pontoscolex* earthworm from the Gurupi Biological Reserve along with new records of *Pontoscolex corethrurus*, *Urobenus petrerei, Righiodrilus tocantinensis, Dichogaster affinis*, *Dichogaster bolaui, Liodrilus mendesi* and *Hyperiodrilus africanus* from Amazon region of Maranhão, Brazil.

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Abstract

We described a new species belonging to *Pontoscolex (Pontoscolex)* subgenus from the Amazon region of Maranhão State in Brazil. The region is the most deforested and degraded in the biome with only 25% of original forest cover. *Pontoscolex (Pontoscolex) awa* **sp. nov.** has regular setae, spermathecae absent, and with tubercula pubertatis band-shaped extending to *AB* line in XIX-XXII. The Gurupi Biological Reserve where the new species was found is the only integral protection area from the Belém Endemism Area and therefore of crucial importance for the conservation of earthworms and other endemic organisms.

Keywords: Belém Endemism Area, São Luís, Oligochaeta.

Introduction

Earthworms are ecosystem engineers and therefore important for soil function, such as aggregate formation, infiltration, nutrient cycling and, soil formation (Bartz *et al*. 2009). Currently, more than 6000 species are known, of which only 3200 are considered valid, being distributed in 38 families and over 811 genera (Csuzdi 2012). In Brazil, about 310 species/subspecies have been recorded, although this number does not approach the estimated 1.400 species (Brown; James 2007; Fragoso *et al*. 2003). The *Pontoscolex* genus displays high species richness in Roraima state (Righi 1984), as well as, in Venezuela, and the Guianas, therefore suggesting the Guyana plateau as its center of origin. In 1984 Righi subdivided the *Pontoscolex* genus into two subgenera *Pontoscolex* (*Pontoscolex*) and *Pontoscolex* (*Meroscolex*), taken from Cernosvitov

1934 based in the position and extension of the tubercula pubertatis. Borges (1992) added an additional subgenus, *Mesoscolex,* also based on tubercula pubertatis position. The subgenus *Pontoscolex* maintained the type species *Pontoscolex* (*Pontoscolex*) *corethrurus* (Müller, 1857), famous for its plasticity and wide distribution in 57 countries and four continents (Fragoso *et al.* 1999, Ortiz-Gamino *et al*. 2017). *P. corethrurus* occurs widely in the American continent with records from the United States, Mexico, and all countries of Central, and South America (Righi 1981; Brown & Fragoso 2007; Rodríguez 2007; Zicsi 2007). Other species of *Pontoscolex* are harder to find as one moves away from the center of origin, and particularly close to the border of Amazon biome in the Maranhão state. However, the soil fauna of Maranhão, and of the Amazonian biome, in general, have been poorly studied and further information is needed to fill the gaps in the earthworm species dispersion.

After the first earthworm studies led by Righi (1972, 1985), only eight new earthworms had been reported from Maranhão (Santos *et al*. 2017, Hernández-Garcia *et al.* 2018a, 2018b, 2018c). Many factors contribute to the low number of species cataloged, such as a large territorial extension, the small number of sites sampled, as well as the low number of effective taxonomists in Brazil (Brown; James 2007). To contribute to the taxonomic knowledge of earthworms in the Amazon region of Maranhão, we here describe a new species of *Pontoscolex (Pontoscolex)* subgenus and report the presence of seven other species.

Material and method.

Earthworms were collected in Centro Novo do Maranhão (3°41'56.7"S, 46°45'57.8"W), Itinga do Maranhão (4°4'20.8"S, 46°52'59.8"W), Alcântara (2°21'05.5"S, 44°27'51.4"W), São Luís (2°35'31.6"S, 44°12'35.9"W), São José de Ribamar (2°39'1.9"S, 44°08'49.4"W) and Rosário (2º51' 11"S, 44º09'30.1"W) counties in the state of Maranhão, Brazil. The method used was modified from the standard Tropical Soil Biology and Fertility Program (Anderson & Ingram 1993), complemented by an active survey using a qualitative sampling method in which a semi-qualitative sample of 1m^2 was collected inside the triangle area where cast activity was observed. Finally, a four hour/sampler search was performed in all the 1 ha plot in all areas susceptible to host earthworms: Soil mud, termite nest, decaying trunk, litter, epiphytic soil, the soil where earthworm cast was present (Decaëns *et al.* 2016). The specimens collected were fixed 99% alcohol and separated in flasks corresponding to each microhabitat in which they were found and labeled accordingly.

Genus *Pontoscolex* Schmarda, 1861

Diagnosis. Setae in eight longitudinal lines. Tubercula pubertatis extends in XIX-XXIII. Gizzard in VI. Tree pair of calciferous glands composite-tubular structure in segments VII-IX. Spermathecae present in VII-IX or absent. Sexual system metandric, a pair of seminal vesicles in XII usually long.

Type species. *Pontoscolex corethrurus* (Müller, 1857)

Subgenus *Pontoscolex (Pontoscolex)* (Righi, 1984)

Diagnosis. Tubercula pubertatis extend 3-5 segments in XIX-XXIII. Posterior setae arranged in regular series, or almost one serie regular, three pairs of calciferous glands composite-tubular structure in VII–IX. One pair of ovaries in XIII.

Type species: *Pontoscolex (Pontoscolex) cuasi* Righi, 1984

Pontoscolex (Pontoscolex) awa, Sousa & Hernández-García, n. sp.

Holotype: MPEG001587, adult, complete, Secondary Forest, Rio dos Bois, Bom Jesus do Maranhão, Maranhão, Brazil, 4°4'32"S, 46°52'49"W, 141 masl, 24 April 2017, Hernández-García, L.M, Sousa, S.C & Rousseau, G.X. colls.

Paratype: MPEG001588, MPEG001589, MPEG001590, MPEG001591, MPEG001592, five adults, complete, same data as for Holotype. One adult, complete, Secondary Forest, Rio dos Bois, Bom Jesus do Maranhão, Maranhão, Brazil, 4°4'50"S, 46°52'39"W, 149 masl, 24 April 2017, Hernández-García, L.M, Sousa, S.C & Rousseau, G.X. colls.

Etymology: The species name is in honor of the Awa indigenous group last hunter-gatherers of the continent that traditionally occupy the forest of the region and are now one of the most threatened indians of the world.

Description. Dimensions: holotype complete - 66 mm in length by 4.1 mm width at X, 4.2 mm at clitellum, 3.9 mm at XXX, 219 segments; paratypes entire 53-66 mm by 3.6-4.1 mm in X, 3.7-4.2 mm at clitellum and 3.5-3.9 mm at XXX, 218-219 segments. Body cylindrical, apigmented when fixed in alcohol. Prostomium prolobic. The first segments and the peristomium grooved. Setae *AB* and *CD* start on II, closely paired-type and arranged in eight parallel lines. Setal arrangement *aa:ab:bc:cd:dd* = 3.6:1.0:5.6:1.0:15.2 at XXX. Clitellum dark beige color after fixed, extended in XIII-XXIII, annular in XIII-XVIII and saddle-shaped in XIX-XXIII. A pair of tubercula pubertatis band-shaped are in XIX-(1/2) XXII limited to *BC* line, with darker pigmentation (Fig. 1A). Four pairs of genital markings are ventrally between the tubercula pubertatis field protruding along the segment. Male pores microscopical and not recognized externally. Smooth genital setae (Fig. 1B) present in XIX-XXI at *AB* line, 350 μm in length, straight at distal region, thickness near the apical region. Common setae smooth and slightly curve at the apical region, 250 μm in length (Fig. 1C). Microscopical ovipores between anterior and equatorial region of segment XIV, on oval light beige genital marks, just between *A* lines (0.25 mm apart) in segment XIV. Nephropores intersegmental, vesicled and aligned with *D* line, first nephropore visible in XIII-XIV.

Septa 6/7, 7/8, 8/9 with highly muscular structure, septa 9/10, 10/11, 11/12 slightly muscular structure, intraclitellar septa membranous. Gizzard in segment VI, 2.2 mm in wide and 1.9 mm in length, with strong musculature and thickness of 0.5 mm. In VII-IX are three pairs of pyriform calciferous glands with a small rounded appendage (Fig. 1D). The calciferous glands are tubular-composite structure (Fig. 1E) and open in the lateroesophagic region of each segment. Blood vessel passes over the glands to irrigate dorsally. Two pairs of lateroesophageal hearts in X-XI. A poor developed dorsal vessel running over the intestine.

Spermathecae absent. In XII is a pair of large seminal vesicles extends to segment XXX-XLV. A pair of testes in XI. One pair of deferent ducts go out from testes and enter into tissue body on XII near *B* line and then running to 20/21, opening into tubercula pubertatis. Holonephridial system, a long tube folded as a simple loop opening to the bladder with rough structure (Fig. 1F). The intestine begins in segment XVII. Typhlosole sigmoidal shaped in XXVII, occupying approximately 70% of the intestinal space. The typhlosole extents to CXLIX segment. One pair of ovary sacs in XIII. The ovary funnels are back side of the segment XIII just on membranous septa. Female pores open in segment XIV between *A* lines space.

Remarks. This new species is similar to *Pontoscolex (Pontoscolex) marcusi* Righi & Ayres 1976 by the presence of three pairs of pyriform calciferous glands with a composite-tubular structure in VII-IX, a pair of tubercula pubertatis in XIX-XXII. However, *P. (Pontoscolex) awa* lacks spermathecae and has all postclitellar setae with regular arrangement. Another difference is the clitellum extension; in *P. (Pontoscolex) marcusi* it is in XIV-XXIII and in *P. (Pontoscolex) awa* in XIII-XXIII.

Further records of species

***Pontoscolex (Pontoscolex) corethrurus* Müller, 1857**

Locality: Sitio Aguahi, Secondary Forest, São José de Ribamar, Maranhão, Brazil, 2°39'1.87"S 44°08'49.60"W, 55 masl (4 adults complete). 23 June 2017. Hernández-García, L.M., Sousa. S.C & Rousseau, G.X. colls. (MPEG 001573, MPEG 001574, MPEG 001575, MPEG 001576). Horizonte Azul settlement, Fragment Forest, Itinga do Maranhão, Maranhão, Brazil, 04º02'34.8"S 46º54'21.81"W, 175 masl. (5 adults complete). 21 April 2017. Rousseau, G.X, Hernández-García, L.M & Burgos, J.E. colls. (MPEG 001582, MPEG 001583, MPEG 001584, MPEG 001585, MPEG 001586). Rio Grande, Riparian Logged Forest, Alcântara, Maranhão, Brazil, 2°20'56.0" S 44°29'01.0" W, 35 masl. (3 adults complete). 15 June 2013. Rousseau, G.X, Hernández-García, L.M & Burgos, J.E. colls. (MPEG 001565, MPEG 001566, MPEG 001567). Marudá settlement, Secondary Forest, Alcântara, Maranhão, Brazil, 3°41'10.76"S 46°45'56.54"W, 38 masl. (5 adults complete). 19 June 2017. Rousseau, G.X, Hernández-García, L.M & Burgos, J.E. colls. (MPEG 001577, MPEG 001578, MPEG 001579, MPEG 001580, MPEG 001581). Maranhão State University School Farm, Secondary Forest, São Luís, Maranhão, Brazil, 4°35'14''S 44°12'31"W, 37 masl. (4 adults entire). 4 June 2017. Hernández-García, L.M., Sousa. S.C & Rousseau, G.X. colls. (MPEG 001558, MPEG 001559, MPEG 001560, MPEG 001561).

***Urobenus petrerei* Righi, 1985**

Locality: Maranhão State University School Farm, Secondary Forest near Paciencia River bank sediment, São Luís, Maranhão, Brazil 4°35'14"S 44°12'31"W, 37 masl. (5 adults complete). 8 February 2018. Sousa, S.C & Hernández-García, L.M. colls. (MPEG 001598, MPEG 001599, MPEG 001600, MPEG 001601, MPEG 001602).

***Hyperiodrilus africanus* Beddard, 1891**

Locality: Maranhão State University School Farm, Secondary Forest near Paciencia River bank sediment, São Luís, Maranhão, Brazil 4°35'14"S 44°12'31"W, 37 masl. (4 adults complete). 8 February 2018. Sousa, S.C & Hernández-García, L.M. colls. (MPEG 001608, MPEG 001609, MPEG 001610, MPEG 001611).

***Righiodrilus tocantinensis tocantinensis* Righi, 1972**

Locality: Maranhão State University School Farm, Secondary Forest near Paciencia River bank sediment, São Luís, Maranhão, Brazil 4°35'14"S 44°12'31"W, 37 masl. (5 adults complete). 8 February 2018. Sousa, S.C & Hernández-García, L.M. colls. (MPEG 001603, MPEG 001604, MPEG 001605, MPEG 001606, MPEG 001607).

***Dichogaster affinis* Michaelsen, 1890**

Locality: Gurupi Biological Reserve**,** Logged Forest, Itinga do Maranhão, Brazil, 03º55'11"S 46º45'42"W and 3°58’59”S 46°47’56”W, 224 and 181 masl. (5 adults complete). 25-27 April 2017. Hernández-García, L.M., Burgos, J.E & Rousseau, G.X. colls. (MPEG 001612, MPEG 001613, MPEG 001614, MPEG 001615, MPEG 001616).

***Dichogaster bolaui* Michaelsen, 1891**

Locality: Gurupi Biological Reserve, Logged Forest, Itinga do Maranhão, Maranhão, Brazil, 03º55'11"S 46º45'42"W, 224 masl. (5 adults complete). 25 April 2017. Hernández-García, L.M., Burgos, J.E & Rousseau, G.X. colls. (MPEG 001617, MPEG 001618, MPEG 001619, MPEG 001620, MPEG 001621).

***Liodrilus mendesi* Righi, 1994**

Locality: Maranhão State University School Farm, Secondary Forest, São Luís, Maranhão, Brazil 4°35'14"S 44°12'31"W, 37 masl. (5 adults complete). 8 February 2018. Sousa, S.C & Hernández-García, L.M. colls. (MPEG 001593, MPEG 001594, MPEG 001595, MPEG 001596, MPEG 001597).

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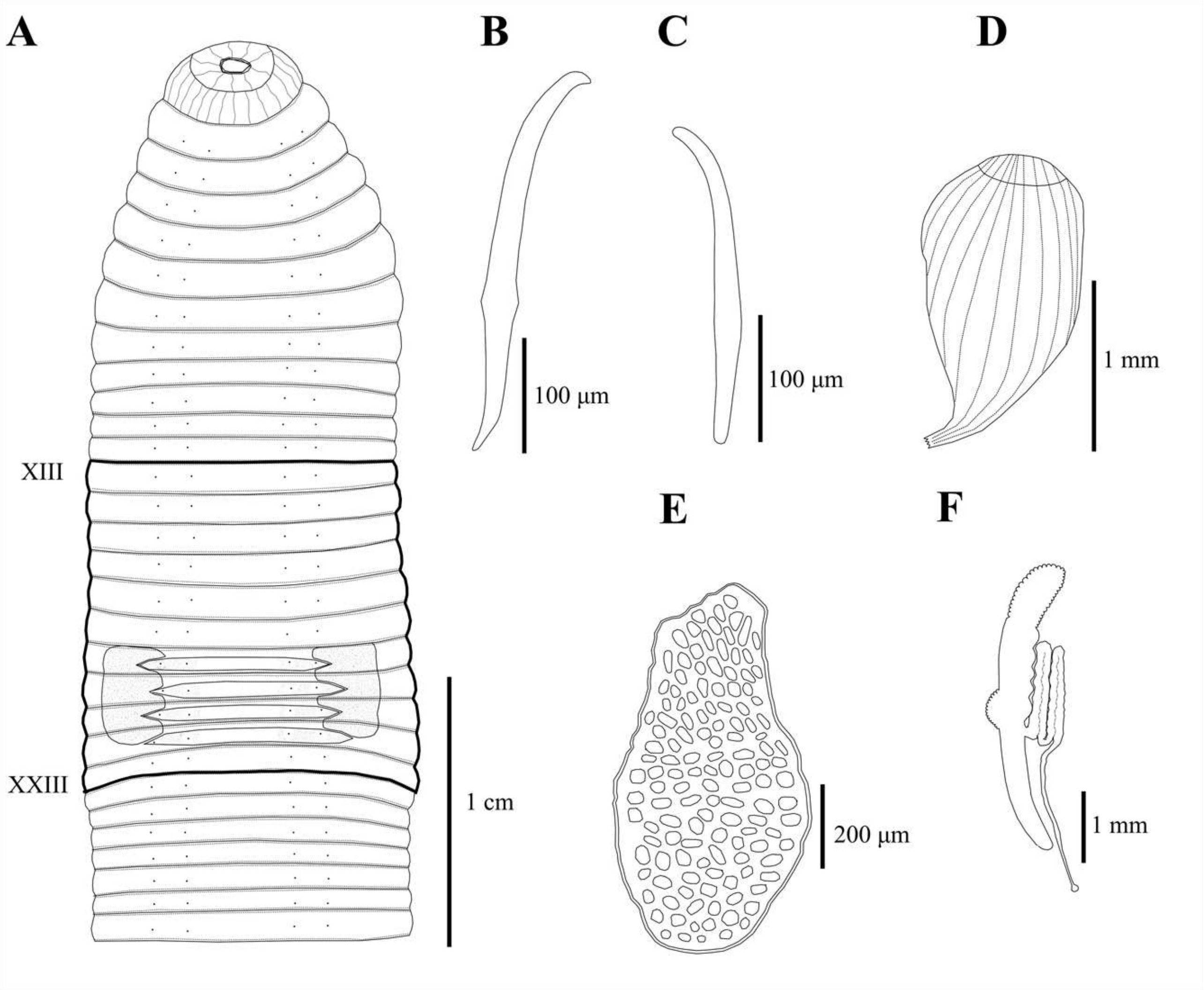

Figure 1. *Pontoscolex* (*Pontoscolex*) *awa* **n. sp.** **A**. Ventral view of anterior region. **B**. Right genital seta of XIX at *A* line. **C**. Right common setae of XXX at *B* line. **D**. View in cross section of left calciferous gland of VII. **E**. Dorsal view of transversal cut of left calciferous gland of VII. **F**. Dorsal view of post-clitellar nephridium.

7.6 **CAPITULO 6:** Earthworm community response to land use change in Eastern Amazonia, Brazil.

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Abstract:

The soil fauna in the Brazilian eastern Amazon is one of the most affected group because of the extensive land-use change. They are hidden in the soil and are generally poorly known and with little emotional reasons for conservation. When the forest is transformed to pasture the soil can be affected by the type of landscape and land morphology. In this study, we contrasted the assemblage of earthworm community and soil physical-chemical properties depends on different conditions of land uses, topography, landscape and climate. Our results suggest that only land use was an important determinant of difference in earthworm density and biomass. Logged forest had the lowest earthworm density while pasture had the lowest biomass. Calcium, Fine Sand, Temperature, Precipitation, the cubic product of geographical coordinates and Bulk density were sufficient to explain at least 44% of the total variance of data. When partitioned, the most important environmental variables explaining earthworm communities were climate (Precipitation and Temperature) and soil physical properties (Sand proportion and Bulk density). There was no effect of relief to explain the structure of earthworm communities but the conserved landscape was more efficient in maintaining native species than the degraded landscape. Earthworm communities in different land use in the degraded landscape had exotic or invasive species in all land-use types. Only big forest fragments in the degraded landscape were close to the continuous primary forest in the conserved landscape. These results show that the effect of transformation of forest to pasture is more dangerous when remnant fragments are constantly disturbed.

Key-Words: Gurupi Biological Reserve, oligochaetes, soil fauna.

Introduction

The remaining Amazonian forest fragments in the state of Maranhão are part of a biodiverse system supporting local vertebrates' diversity (Cracraft 1985, Maria et al. 1996, Ron 2000). Recently was also found than a remnant forest in eastern amazon can also guarantee soil biodiversity (Rousseau et al. 2014), especially for ant communities, as was reported a decrease of richness after deforestation (Muñoz et al. 2017).

With the advance of the deforestation arc in Eastern Amazonia, concerns about the loss of soil biodiversity are higher (Vieira et al. 2009, Rousseau et al. 2014). Earthworms are one of the less known soil group with a great importance as they participate actively in soil transformation and nutrient cycling (Chaoui et al. 2003, Coq et al. 2007, Sánchez-de León et al. 2014).

Authors had suggested than earthworm community assemblage was driven by physical-chemical (Fragoso and Lavelle 1992, Marichal et al. 2017), topography (Decaëns et al. 2016), and landscape factors (Paoletti 1999, Kim et al. 2015, Frazão et al. 2017). Despite Davidson et al. (2012) and Quesada et al. (2012) also recommended the climate as an important driver in amazon forest, earthworm studies did not take those variables in account.

Fragoso et al. (1992), and Marichal et al. (2017) found than in tropical areas the physical-chemical properties were the most important explaining earthworm assemblages. Decaëns et al. (2016) added topographical factor as important in explaining earthworm abundances as lowlands are more suitable to be inhabited by earthworms due to higher availability of food sources and water (Ayres and Guerra 1981).

Researchers also found that a landscape with more forest (i.e., conserved) maintain a higher earthworm diversity and even act as pool species maintaining the migration to agricultural areas (Paoleti 1999, Kim et al. 2015). Conserved continuous forest (Fragoso and Lavelle 1992, Feijoo et al. 2006) or even old-growth forest fragments (Paoleti 1999) contribute to maintain the native earthworm biodiversity. Even small disturbance in amazon forest (i.e., logging, selective extraction) can modify earthworm assemblage (Franco et al. 2018) but the transformation to pastures cause loss of native species and changes of soil physical-chemical properties (Fearnside and Barbosa 1998, Castilho et al. 2016, Marichal et al. 2017).

The main earthworm species colonizing disturbed areas in Brazilian eastern amazon are *D. bolaui* and *P. corethrurus* (Marichal et al. 2012, 2017, Castilho et al. 2016). Because the dispersion of earthworms is naturally limited, Marichal et al. (2012) suggested that the invasion of *P. corethrurus* in the landscape of the Amazon deforestation arc is mediated by humans.

On the other hand, there is necessary to understand the rank of importance than each effect has on assemblage earthworms in the eastern amazon because there are threatened ecosystems. Little is known about earthworm ecology in the Maranhão state because traditional studies focused on taxonomical description (Righi 1972, 1975, 1985, 1989, Righi et al. 1978, Santos et al. 2017, Hernández-García et al. 2018a, 2018b, 2018c). The objective of this work was to determine the effect of degradation of forest located in the Brazilian Eastern Amazon on earthworm community composition, by measuring the changes of soil variables explained by the climate, landscape, land-use, and topographical effects.

Material and methods

Sites

The study was conducted in the Amazon biome of southwestern Maranhão state, Brazil. Two contrasting landscapes were defined previously according to the history of disturbance intensity and quantity of remnant forest. The first with a forest matrix, thereafter named Conserved Landscape (CL) (, is located in the central area of the Gurupi Biological Reserve (GBR), inside the Centro Novo do Maranhão County, CN (3º46’21.2” S; 46º46’49.1” O). The second , with an agriculture matrix, thereafter named Degraded Landscape (DL), is located in the south area of the GBR, at the intersection of three counties : CN (4º1’28.4” S; 46º52’47.7” O), Bom Jardim, BJ (4º5’23.1” S; 46º53’32.3” O) and Itinga do Maranhão, IT (4º2’25.9” S; 46º55’34.8” O). The sampling took place in March 2015, and April 2016 in the more forested area and in April 2017 in the less forested area. The climate is tropical humid (Am), according to Köppen classification (Alvares et al. 2013), and the annual mean temperature is 25.4-25.9 ºC (WorldClim 2015). The average of the total annual precipitation measuring over 40 years is 1555-1725 mm (WorldClim 2015). The area is located on Itapecurú soil formation, with mainly yellow Latosols and Plintosols, and less frequently Vertisols and Oxisols (Santos et al. 2011). Topography is undulated with hilltops (Top) and lowlands (Low) ranging from 101 to 302 masl. The native common vegetation is typical of dense rainforest (Lima et al. 2014) with the Rutaceae, Leguminosae, and Euphorbiaceae as a dominant family (Muniz 2008). Seven main types of vegetation are present in the sampled area: continuous old-growth forest, fragmented old-growth forest, logged old-growth forest, secondary forest, grazed pasture, shifting cultivation and eucalyptus plantations (Rousseau et al., 2014), Fig. 1.

Experimental design

The design was a nested three-factorial model with three repetitions (2x4x2x3) and a total of 48 sampled plots (Table 1). The landscape was the main factor (Conserved Landscape, Degraded Landscape), followed by the land-use (Forest, Logged Forest, Secondary Forest, Pasture) and then topography (Top, Low). To guaranty the independence of each sample we established the criteria of minimal and maximum distance between plots of 200m and 5km, respectively. Five types of land use were selected: Old-growth forest (F) without intervention Logged old-growth forest (LF) with selective wood extraction; Secondary forest (SF) were fallow from pasture 2-15 years old, and Pasture (P) were grazed areas. Each type of land-use was sampled in triplicated in conserved and degraded landscape, as well as, on hilltop and lowland.

Landscape analysis

Satellite images (LandSat 8) for sampling area were obtained from the United States Geological Services (www.earthexplorer.usgs.gov) and uploaded to the Quantum Gis program (Quantum GIS Development Team 2015) to select the best three RGB bands (7:5:3) to improve visualization of the land-use type. The major distance between extremes sample points in each area was considered as a lower limit to create two circles of 16 km ratio that characterized the two types of landscape. Supervised classification (Congedo 2013) was carried out to create a raster with all land use type (Fig. 1). The raster image was vectorized and then all land-use type surfaces were measured, as well as, basic landscape metrics of fragmentation: area/perimeter ratio, dominance index (D), and the evenness Shannon index (Supplementary material, SM 1). These metrics along with forest cover were used as the criteria to define the landscape categories: Conserved Landscape (CL, 67% of forest cover) and Degraded Landscape (DL, 29% of forest cover). Around each sampling point was estimated the forest cover in a circle of 1km of ratio and used as a quantitative variable in the forward selection model (see Community structure section and SM 2).

Earthworm sampling

The earthworms were collected by digging and hand-sorting according to the modified Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram 1993), complemented by an active qualitative search method (Decaëns et al. 2016). Plots consisted of a 56 m-radius circle (1 Ha) centered on a geolocated point (Fig. 2). For quantitative sampling (modified TSBF), three blocks of soil 25x25 cm and 20 cm deep (divided into litter and two

10 cm layers) located at the extremities of a 20 m equilateral triangle, were dug out and hand-sorted on a white plastic tray. Semi-qualitative sampling was carried out initially by seeking earthworms related to casting activities in litter and soil in 1 m^2 of the soil surface, inside the triangle area delimited previously. Subsequently, all life stages of earthworms (i.e., adults, juveniles, and cocoons) were collected during a fixed period of four researcher-hours. Suitable microhabitats for oligochaetes included: soil layers with cast activity, mud sediments, tree base soil, termite nest, litter accumulations, decaying trunks on the soil surface (trunk and below-trunk soil), cattle dung and epiphytic soils.

Morphological analysis

All earthworms were preserved in 96% (v/v) ethanol and examined under a Coleman NSZ-606 stereoscopic light microscope. We used different keys for ordinary genera of South America earthworm (Righi 1985, Fragoso and Rojas 2009, Feijoo and Celis 2010, 2012),, as well as keys for exotic oligochaetes when necessary (Plisko and Nxele 2015). New species, when present, were described in detail and published in Santos et al. (2016) and Hernández-Garcia et al. (2018a, 2018b, 2018c).

Soil analysis

Soil samples adjacent to each TSBF monolith were collected in duplicate with rings (566 cm^3) at 0-10, and 10-20 cm in depth. For each replicate, a portion of 40g of soil was oven-dried at 105°C to obtain water content. The bulk density was calculated as the relation between soil dry mass and ring volume (m/v). Samples from the same depth were grouped to create composite samples and were used to estimate the physical-chemical parameters. Soil particle-size were estimated by the densitometry method after air drying and sieving of soil to 2 mm (Claessen et al. 1997). Each sample was analyzed to determine the pH (0.01 M CaCl2 suspension, 1:2.5 soil/solution, v/v), P-availability (extraction with Amberlite IRA 400 resin) and exchangeable K, Ca, Mg (resin), and H +Al (SMP method) following standards methods of the Agronomic Institute of Campinas (Van Raij et al. 2001). The cation exchange capacity (CEC) was calculated as the sum of K, Ca, Mg, and (H+Al); the sum of bases (BS) was calculated as the sum of K, Ca, and Mg; the base saturation percentage (V) was calculated as BS/CEC*100. The total soil organic matter (OM) was obtained by the Walkley and Black dichromate digestion (Nelson and Sommers, 1996), and carbon percentage (C) was calculated as the relation of OM/17.24.

Climate data.

Because there is not meteorological station near the plots, we used the WorldClim database (WorldClim 2015) to obtain climatic values of annual and monthly precipitation (P_A, P_M), and average of air Temperature (T_A and T_M). Average data over 40 years were downloaded separately by month as a raster of 30x30m in resolution for each variable used, and all information for plots were extracted as a matrix data. The monthly values corresponded to the months of sampling (i.e., March and April).

Earthworm total density and biomass

Data were previously checked for homogeneity of variance and normality distribution, and the logarithmic transformation was used when needed. All analyses were conducted in R

3.4.1 (R Core Team 2018) with auxiliary packages like Car (Fox and Weisberg 2011) and Agricolae (Mendiburu 2017).

Total density (individuals/m^{-2}) and biomass (g/m^{-2}) of earthworm were estimated for the plot from monolith samples (0.19 m^2) and then conducted a three-way ANOVA with Landscape, Land-use, and Topography as factors. A least significant difference test (LSD) was used as a post hoc test when variance explained by factor was significant.

Community structure

The total data set used for this analysis is available in the supplementary material (SM 2). To partition the variation explained by geography, soil physics, soil chemistry, climate, topography, and landscape in earthworm community, a canonical redundancy analysis (RDA) was conducted (Borcard et al. 2011). The geographical coordinates (UTMx, UTMy) were previously transformed to obtain a cubic trend by a surface regression model as shown in the expression:

$$z = b_1x+b_2y+b_3x^2+b_4y^2+b_5x^3+b_6y^3+b_7xy+b_8x^2y+b_9x^3y+b_{10}xy^2+b_{11}xy^3+b_{12}x^2y^2+ b_{13}x^2y^3+b_{14}x^3y^2$$

Each group of environmental data set was standardized and the species data was transformed to Hellinger using the package Vegan (Oksanen et al. 2018). A forward selection was conducted separately for each group of data set and called independent selection (Pinel-Alloul et al. 1995). The significant variables ($P \leq 0.05$) obtained by the forward selection, as well as, the variables altitude and cover forest were kept to build an additive model which was tested after for goodness of fit computing the adjusted r^2. The additive model was used to conduct a final forward selection and significant variables were used to build the final model. The canonical axes of the final model were tested for significance (ter Braak and Šmilauer 2002) and then computed the variance partitioning and tested the significance of the pure effect of each group of data (Borcard et al. 2011) remained in the final model

On the final model, we tested the importance of the interactions between the factors landscape, land use, and topography using a multivariate analysis of variance (MANOVA) on the Hellinger-transformed species matrix (Borcard et al. 2011). Balanced factors were transformed into an orthogonal matrix using the Hermert contrast within-group multivariate homogeneity was checked, then isolated effects and interactions were tested for significance (Borcard et al. 2011).

The species matrix, as well as, the factors landscape, land use and topography, along with environmental variables filtered by forwarding selection were used to conduct a co-inertia analysis using the package Ade4 (Dray and Dufour 2007). We estimated the total co-inertia between the principal component analysis (PCA) and between class analysis (BCA) of standardized environment matrix, Hellinger-transformed species matrix and all levels of significant factors selected by MANOVA. The total co-inertia was computed, and significance was tested by 999 Monte Carlo permutations (Borcard et al. 2011).

Results

Physical-Chemical

Degradation of the landscape was related to changes in physical variables as several land-use types located in the DL were less compacted and had lowest sandy content (Table 2, $p<0.05$). We found that pastures located on the hilltop of CL and DL were more compacted and sandier than other types of land use as SF and F ($p<0.05$). On the other hand, degradation of the landscape caused an increase of Ca concentration in soil, especially for the more disturbed land use types (i.e., P, SF) where Ca levels were the highest. Na in CL did not change between land use and topography but differed between landscape where pasture in CL had lower Na than those of DL. Degradation of the forest to pasture in CL is followed by an increase of Na content. Pasture in DL also had higher K and P content than more conserved vegetation and those located in lowlands had higher P content. There was a topographical effect related to DL where SF in lowlands was more compacted, sandier, and with lower Ca content than other vegetation types.

Earthworm total density and biomass

Landscape and topography conditions, as well as the land use type, had no significant effect ($p>0.05$) on earthworm total density (Table 3). However, we found that SF and LF on the hilltop of DL had less earthworm biomass ($p<0.01$ and $p<0.05$, respectively). We also found that LF located in lowland in DL had less total earthworm biomass than that located inside the CL ($p<0.05$). No interaction was significant (SM 3, 4). The average density earthworm for conserved and degraded landscape was 93.64 and 64.3 ind.m^2, while mean biomass was 13.60 and 4.76 g.m^2 (Table 4). The lowest average earthworm density was 41.67 ind.m^2 for LF, and the highest was 114.91 ind.m^2 for SF. The total earthworm biomass differed for land use ($p<0.05$) with SF having the highest earthworm biomass (16.27 g.m^2), and LF and P the lowest (7.22 and 3.53 g.m^2,). Table?

When separated by soil layer earthworm density and biomass was highest at 0-10 cm ($p<0.05$). Few individuals were found in the litter layer which was reflected in low values of density and biomass ($p<0.05$), Fig. 3.

Community structure

Physical-chemical, climate, landscape, and topography were important components of environmental variables explaining the earthworm community assemblage. Calcium, potassium, sodium, phosphorous, soil bulk density, fine sand, annual precipitation, altitude, and the cover forest were sufficient to explain 57% of the total variability of species data (Table 5, $p\leq0.05$). When partitioning the total variance, the physical-chemical variables explained 13% of pure effect followed by climate (6%), landscape (4%), and topography (3%) (Fig. 4). Na, soil bulk density, and the fine sand were the most important variables influencing the earthworm assemblage ($p<0.01$) (SM 5).

The distribution of some species of earthworm depends on the type of landscape, land use and topography as the interaction between factors were highly significant ($p<0.001$, Table 6). The landscape and land use were more significant factors ($p<0.001$) explaining the species assemblages than the topography ($p<0.006$, Supplementary 6). Species grouped by the landscape and land use ($p<0.001$Table 6) as is represented in the coinertia analysis showed the dominance of native species in the forest from the CL (Fig. 5).

The first two axes of the coinertia analysis explained 86 % of total inertia and the permutation test showed a high association between environment variables selected and species composition matrix ($r^2=0.69$, $p<0.001$). Degradation of landscape and transformation of forest to pasture caused the invasion of exotic *Dichogaster* spp and *P. corethrurus* while the presence of remnant F and LF in the CL showed to be the best habitat to the conservation of the native species *Holoscolex fernandoi*, and *Rigiodrilus gurupi*. Environmental variables were related to the species assemblage as DL was highest and dryer than CL and pastures located in the CL were the most compacted and sandiest, while those located in the DL were the richest in Ca, Na, K, and P

Discussion

Physical-Chemical

The landscape degradation is related to changes in the soil physical-chemical properties (Fearnside et al. 1998, Marichal et al. 2017). Nonetheless, soil physical properties are less impacted by degradation than chemical properties (Borcard et al. 2011). One of the most critical property affected by degradation is the porosity which is inversely related to bulk density. In the RBG where mechanical agriculture is absent the rise of bulk density is caused by cattle trampling (Villamil et al. 2001). Pastures in amazon are highly related to fire event as slash and burn is used for pasture installation and maintenance. After fire the nutrients stored in the forest biomass are volatilized or released in the soil (Hughes et al. 2000).

Earthworm total density and biomass

Means of earthworm density and biomass depend on sampling method, as well as, the depth of analysis. Values obtained in this study are compared with other studies using the same method in the Amazon biome (Supplementary 7). Earthworm density from conserved forest (i.e., LF and F) of the GBR was lower than reported for old-growth forest in eastern amazon (Rousseau et al. 2014, Castilho et al. 2016), central amazon (Harada and Bandeira 1994, Bandeira and Harada 1998, Barros et al. 2004, Viana 2012) and western amazon in Peru (Lavelle and Pashanasi 1989). However, our values exceeded those reported to Venezuela (Nemeth and Herrera 1982) and Brazilian south-western amazon (Guerra 1994, Barros et al. 2002).

Earthworm biomass for conserved forest of GBR was lower than those reported in central and western amazon (Lavelle and Pashanasi 1989, Barros et al. 2001, 2004) but was equal than those reported in northern amazon (Nemeth and Herrera 1982), and south-western amazon (Guerra et al. 1994, Barros et al. 2002).

Earthworm density and biomass increased from south to north as values in eastern (Maranhão state) and western amazon (Acre and Rondônia states) are the lowest (Guerra 1994, Harada and Bandeira 1994, Bandeira and Harada 1998, Barros et al. 2002, 2004, Viana 2012, Rousseau et al. 2014, Castilho et al. 2016). This pattern can depend on two most important factors driving soil biodiversity: soil origin and the climate, as soils in central (Amazonas state) and north-eastern amazon (Amapá and North of Pará state) are geologically more recently (Quesada et al. 2012) and with the highest levels of precipitation (Davidson et al. 2012).

After a disturbance of native forest, the soil fauna regeneration process will depend on additional conditions different of those previously cited and generally are related to anthropic effect and age of vegetation. Earthworm density in SF was lower than those reported to eastern amazon by Rousseau et al. (2010, 2014) but was similar (Lavelle and Pashanasi 1989, Alves 2010) or superior to those reports to others secondary forest (Barros et al. 2002, 2003)

On the other hand, earthworm biomass of SF was higher than those reported in another secondary forest from amazon biome (Lavelle and Pashanasi 1989, Barros et al. 2002, 2003). This higher values in biomass are due to the presence of the big worm *Andiorrhinus miricuri* (Hernández-García et al. 2018a) particularly in secondary forest.

Our result suggests that degradation of forest to pasture in eastern amazon of Maranhão affect drastically earthworm density, and biomass more than in other amazon areas as earthworm density was lower than in other pastures located inside the biome (Lavelle and Pashanasi 1989, Guerra 1994, Barros et al. 2002, 2004, Laossi et al. 2008, Pontes 2009, Alves 2010, Velasquez et al. 2012, Rousseau et al. 2014, Castilho et al. 2016).

Community structure

At least one variable of each environment component (i.e., topography, landscape, climate, and soil physical-chemical properties) was selected as important to explain earthworm community. but, soil physical-chemical properties were the most important. Few studies compared the topography effect on earthworm communities in the amazon (Decaëns et al. 2016). Recent information indicates that earthworm prefers to colonize lowlands more than upland (Decaëns et al. 2016). According to Ayres and Guerra (1981), 82% of earthworm species from central amazon occur in the vicinity of water which also depends on the interaction of landscape and land use type.

The second more important component of our study was the landscape, as earthworm assemblage differed significantly between CL and DL. There are two contrasting conclusions about the effect that degradation of landscape cause over earthworms. Kim et al. (2015) found that more conserved landscape (i.e., native forest or forest plantation) had less native, and exotic earthworm than anthropized ones (i.e., dairy farm without intensive management and fertilizer, sheep farm), and the colonization of oligochaetes was mediated by the ammonium content, which was lowest in conserved areas. Paoletti (1999) also suggest that mosaics landscape that includes hedgerows, field margins and riverbanks still maintain some diversity of species even if the major proportion of the area is under intense cultivation.

On the contrary, Frazão et al. (2017) concluded that management practices of arable fields overrule potential positive effects of the surrounding landscape and of soil properties on earthworm community diversity. Native earthworms also had been related to native plant diversity (Xu et al. 2013).

Landscape can be as important as soil variables (Frazão et al. 2017) when analyzed separately, and it is possible because changes in soil properties are a consequence of landscape change as higher soil pH, P and Ca^2 contents are characteristic of deforested soil (Fearnside et al. 1998).

In Brazilian Amazon, a small disturbance in the forest has no effect on earthworm assemblage (Franco et al. 2018) but the transformation to pastures cause a significant negative effect as exotic or invasive earthworms colonize new areas where pH, Ca^{+2}, and P rose (Fearnside et al. 1998, Marichal et al 2017).

Distribution of functional groups is determined by land-use practices (Sinha et al. 2003, Marichal et al. 2017). The most common species indicating disturbance is *P. corethrurus* which prefers clay soils (Marichal et al. 2012). Pastures located in CL were more susceptible to be colonized by *P. corethrurus* while those of DL were dominated by *D. bolaui*. Despite native large earthworms can be sensitive to disturbances (Marichal et al 2017) we found the big earthworm *A. miricuri* (Hernández-García et al. 2018a) inhabiting pasture in DL.

In tropical forest, where changes in temperature are minimal, soil properties are the main drivers of earthworm structure (Fragoso and Lavelle 1992, Marichal et al. 2017), however, we found than climatic component was also important, as precipitation explained the second major pure effect. Different rates of precipitation modified the main food source of earthworm as forest type and composition depend on water. Turnover rates of forest and wood production are related to physical-chemical and climate drivers (Quesada et al. 2012). Deforestation affects macrofauna abundance in amazon when precipitation and time of disturbances are over 2200 mm and 20 years, respectively (Franco et al. 2019).

In conclusion, our results suggest than earthworm community structure inhabiting pastures inside conserved landscape differed from the conserved forest, and even pasture located in the degraded landscape, and forest degradation (logging, fragmentation, and pasture) in degraded landscapes lead to drastic changes in earthworm composition

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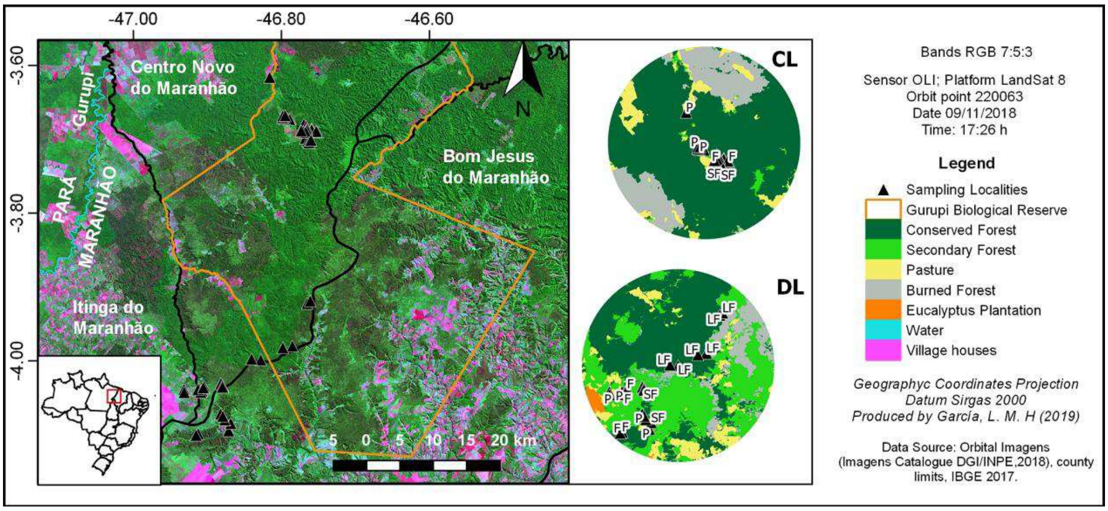


Fig. 1. Sampling locations in Centro Novo do Maranhão, Bom Jardim, and Itinga do Maranhão counties, Eastern Amazonia, Brazil.

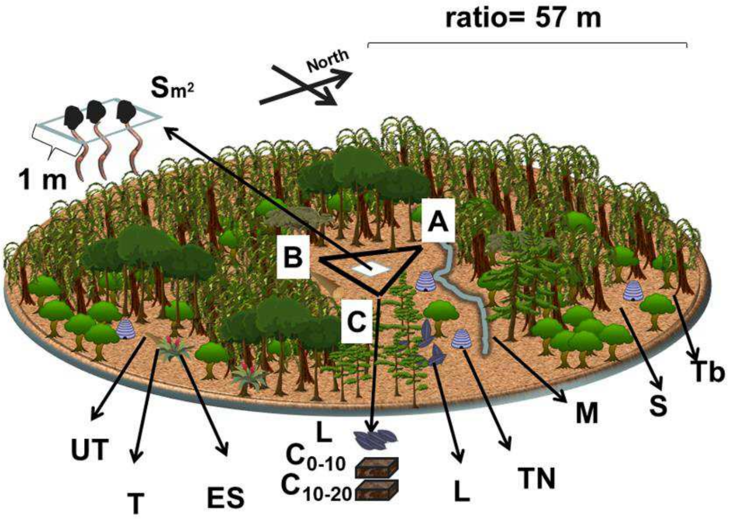


Fig. 2. Sampling method after Decaëns et al. (2016). A-C: monolith position, L: Litter, UT: Under Trunk, T: Trunk, ES: Ephyfitic soil, TN: Termite nest, M: Mud sediments, S: soil, Tb: Tree base..

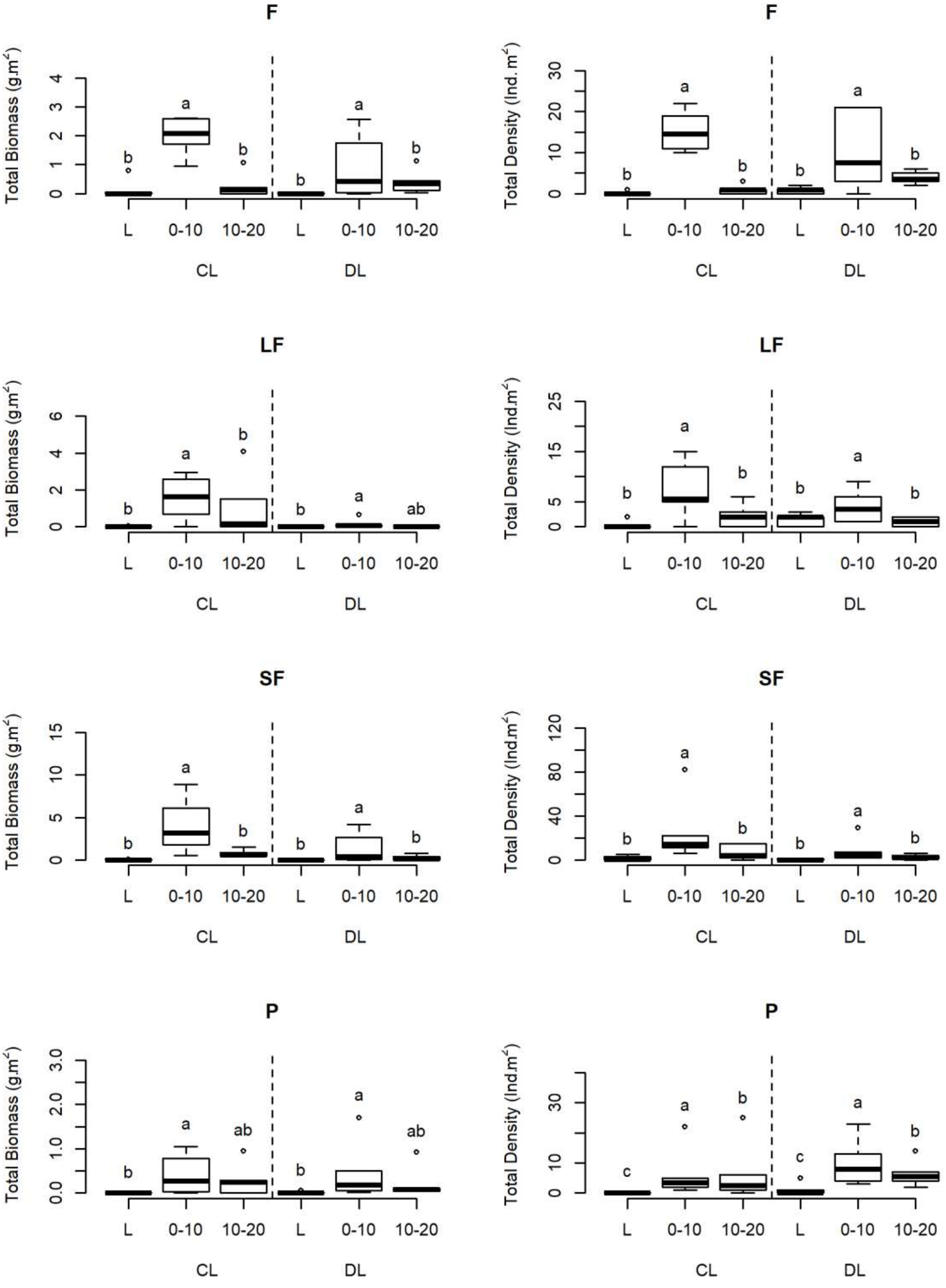


Fig. 3. Earthworm biomass (g/m^2) and density (ind/m^2) in different layers of soil. CL: Conserved Landscape, DL: Degraded Landscape, F: Old-growth forest, LF: Logged old-growth forest, SF: Secondary forest, P: Pasture. L: Litter. Different letters indicate difference between soil layers ($p<0.05$).

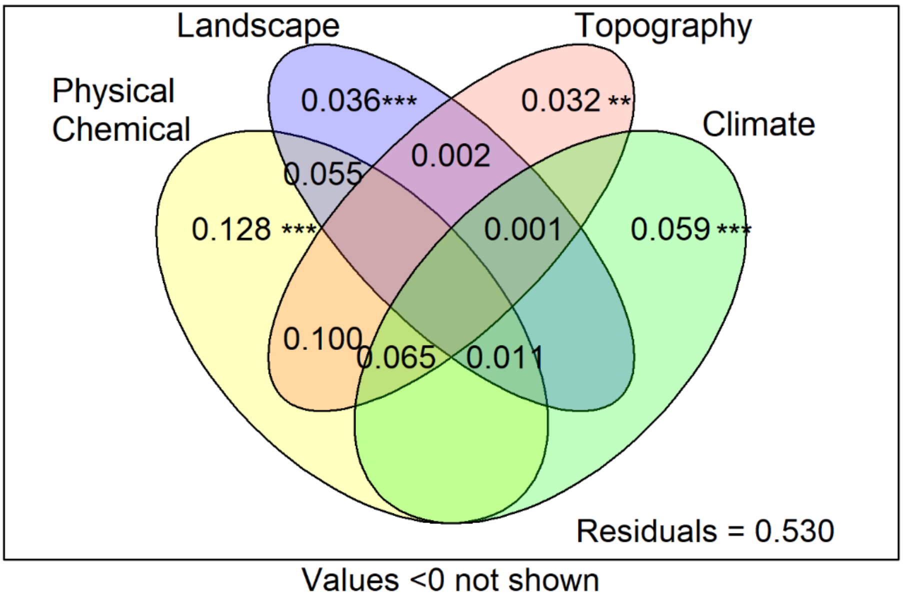


Fig. 4. Variance partition, and pure effects of Physical-Chemical, Landscape, Topography and Climate variables.

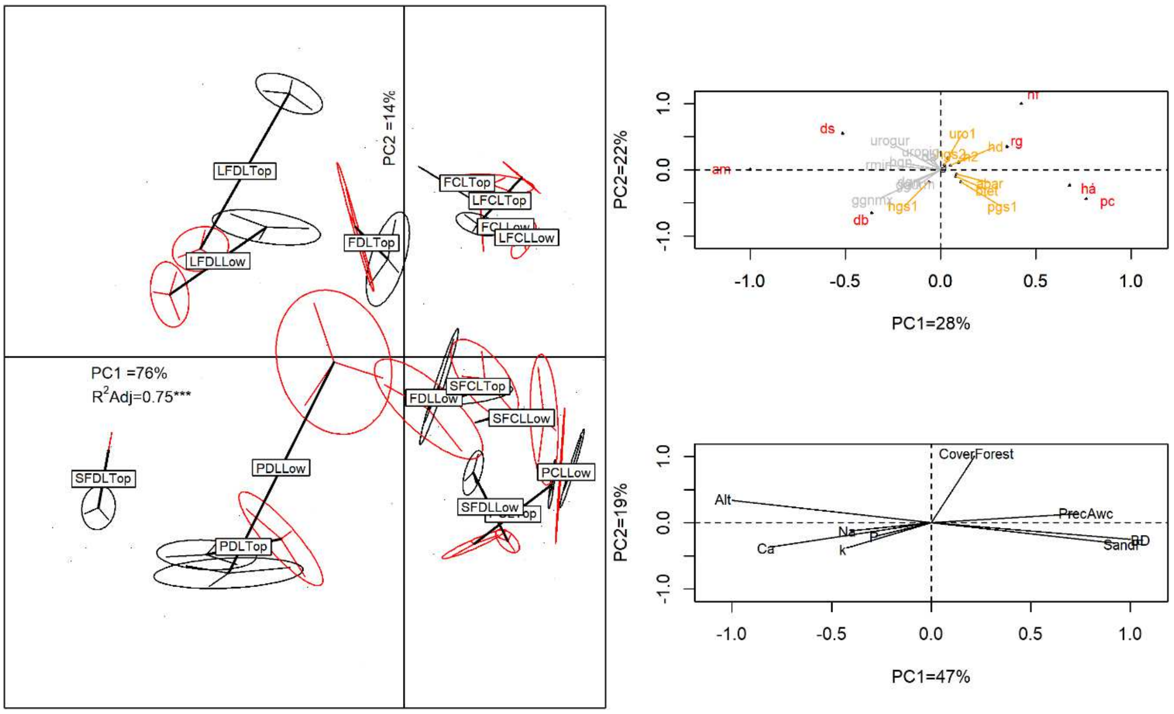


Fig. 5. Principal Component Analysis and Coinertia analysis showing the relationship between standardized environment matrix and species matrix Hellinger transformed. pc: *Pontoscolex corethrurus*, ha: *Holoscolex alatus*, pgs1: *Pontoscolex* gs1, abar: *Andiorrhinus barrosoi*, rg: *Righiodrilus grurupi*, hd: *Holoscolex dossantosi*, db: *Dichogaster bolaui*, btet: *Brasilisia* tet, ds: *Dichogaster saliens*, am: *Andiorrhinus* (*T.*) *miricuri*, uro1: *Urobenus* sp1, hgs2: *Holoscolex* gs2, hf: *Holoscolex fernandoi*. P_A: Annual Precipitation, P_M: Monthly Precipitation, T_M: Monthly Temperature,T_A: Annual Temperature.

Table 1. Earthworm sampling localities in the Centro Novo do Maranhão, Bom Jardim and Itinga do Maranhão counties, Eastern Amazonia, Brazil. GBR: Gurupi Biological Reserve; HAS: Horizonte Azul Settlement; ICMBio: Base for biological research “Chico Mendes Institute”; GF: Galetti Farm road; RB: Bois`River.

County	Site	Vegetation Type	Land Use	Landscape	Topography	Latitude (S)	Longitude (W)	Altitude (m)
CN	GBR	Old-growth forest	F	CL	Upland	3°41'11"	46°45'34"	189
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'16"	46°45'34"	175
CN	GBR	Old-growth forest	F	CL	Upland	3°41'27"	46°45'06"	210
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'24"	46°45'12"	188
CN	GBR	Old-growth forest	F	CL	Upland	3°41'18"	46°45'57"	155
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'27"	46°46'02"	146
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°40'50"	46°46'12"	154
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°40'59"	46°46'01"	182
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°41'10"	46°45'56"	158
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°41'20"	46°46'01"	172
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°41'26"	46°46'14"	134
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°41'21"	46°46'24"	146
CN	GBR	Secondary Forest	SF	CL	Upland	3°41'56"	46°45'58"	152
CN	GBR	Secondary Forest	SF	CL	Upland	3°41'57"	46°46'05"	131
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'09"	46°45'51"	109
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'07"	46°45'43"	127
CN	GBR	Secondary Forest	SF	CL	Upland	3°42'18"	46°45'47"	107
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'07"	46°45'37"	134
CN	GBR	Pasture	P	CL	Upland	3°40'25"	46°47'20"	139
CN	GBR	Pasture	P	CL	Lowland	3°40'22"	46°47'47"	105
CN	GBR	Pasture	P	CL	Upland	3°40'17"	46°47'55"	145
CN	GBR	Pasture	P	CL	Lowland	3°40'08"	46°47'54"	108
CN	GBR	Pasture	P	CL	Upland	3°40'07"	46°47'42"	128
CN	GBR	Pasture	P	CL	Lowland	3°37'00"	46°48'57"	101

Table 1. (**Continued**). Earthworm sampling localities in the Centro Novo do Maranhão (CN), Bom Jardim (BJ) and Itinga do Maranhão (IT) counties, Eastern Amazonia, Brazil. GBR: Gurupi Biological Reserve; HAS: Horizonte Azul Settlement; ICMBio: Base for biological research "Chico Mendes Institute"; GF: Galetti Farm road; RB: Bois`River.

County	Site	Vegetation Type	Land Use	Landscape	Topography	Latitude (S)	Longitude (W)	Altitude (m)
IT	HAS	Old-growth forest fragment	F	DL	Upland	4°02'29"	46°54'31"	206
IT	HAS	Old-growth forest fragment	F	DL	Lowland	4°02'34"	46°54'36"	164
IT	HAS	Old-growth forest fragment	F	DL	Upland	4°02'04"	46°54'21"	234
IT	HAS	Old-growth forest fragment	F	DL	Lowland	4°02'12"	46°54'24"	175
BJ	HAS	Old-growth forest fragment	F	DL	Upland	4°05'58"	46°54'49"	229
BJ	HAS	Old-growth forest fragment	F	DL	Lowland	4°06'04"	46°54'59"	185
CN	ICMBio	Logged old-growth forest	LF	DL	Lowland	3°59'58"	46°49'44"	262
CN	GBR	Logged old-growth forest	LF	DL	Lowland	3°55'23"	46°45'58"	212
CN	GBR	Logged old-growth forest	LF	DL	Upland	3°58'50"	46°47'10"	250
CN	GBR	Logged old-growth forest	LF	DL	Lowland	3°58'59"	46°47'56"	181
CN	GBR	Logged old-growth forest	LF	DL	Upland	3°55'11"	46°45'42"	224
CN	ICMBio	Logged old-growth forest	LF	DL	Upland	3°59'54"	46°50'28"	285
CN	GF	Secondary Forest	SF	DL	Upland	4°01'54"	46°52'58"	302
BJ	BR	Secondary Forest	SF	DL	Lowland	4°05'07"	46°52'10"	144
BJ	BR	Secondary Forest	SF	DL	Lowland	4°04'50"	46°52'39"	149
BJ	BR	Secondary Forest	SF	DL	Lowland	4°04'32"	46°52'49"	141
CN	GF	Secondary Forest	SF	DL	Upland	4°02'01"	46°52'50"	294
CN	GF	Secondary Forest	SF	DL	Upland	4°02'10"	46°52'50"	294
IT	HAS	Pasture	P	DL	Lowland	4°02'21"	46°55'58"	196
IT	HAS	Pasture	P	DL	Upland	4°02'35"	46°55'57"	242
IT	HAS	Pasture	P	DL	Lowland	4°02'18"	46°54'45"	158
IT	HAS	Pasture	P	DL	Upland	4°02'22"	46°54'50"	179
BJ	BR	Pasture	P	DL	Lowland	4°04'20"	46°53'00"	139
BJ	BR	Pasture	P	DL	Upland	4°05'43"	46°52'21"	160

Table 2. Soil physical-chemical parameters by the landscape, land use and topography in Eastern Amazonia, Brazil. Different letters indicate a significant difference ($\alpha \leq 0.05$). F: Old-growth forest, LF: Logged old-growth forest, SF: Secondary forest, P: Pasture. CL: Conserved Landscape, DL: Degraded Landscape. N= 3. Values represent the mean ± standard deviation.

Parameter	**Land-use**	**CL**		**DL**	
		Low	Top	Low	Top
Bulk Density	F	1.52±0.07aA^{a}	1.34±0.06bA^{a}	1.38±0.08abA^{a}	1.27±0.13aA^{a}
	LF	1.59±0.08aA^{a}	1.46±0.21abA^{a}	1.16±0.22cB^{a}	1.14±0.21abB^{a}
	SF	1.41±0.1aA^{a}	1.37±0.13bA^{a}	1.52±0.07aA^{a}	0.97±0.01bB^{b}
	P	1.6±0.03aA^{a}	1.63±0.06aA^{a}	1.19±0.28bcB^{a}	1.23±0.16aB^{a}
Fine Sand	F	52.03±1.89aA^{a}	31.19±15aA^{b}	54.5±6.54aA^{a}	37.17±12.09aA^{a}
	LF	36.94±2.8aA^{a}	32.27±10.06aA^{a}	17.67±25.84bA^{a}	9.5±9.18bB^{a}
	SF	48.97±9.44aA^{a}	41.17±11.73aA^{a}	58.33±3.62aA^{a}	12.33±16.6bB^{b}
	P	45.89±3.2aA^{a}	43.32±2.87aA^{a}	22.5±26.96bB^{a}	16.5±13.26bB^{a}
Ca	F	5±1.8bA^{a}	4±1.32aA^{a}	18.17±15.09bA^{a}	8.17±6.33bA^{a}
	LF	5.67±2.84bB^{a}	6.5±3.5aA^{a}	37±15.5abA^{a}	19.5±11.17bA^{a}
	SF	19.5±6.73aA^{a}	19.67±15.89aB^{a}	18.67±7.82bA^{b}	59.67±17.96aA^{a}
	P	14.5±7.94aB^{a}	11.17±4.54aB^{a}	48.5±24.78aA^{a}	48.5±32.6aA^{a}
Na	F	4.53±0.57aA^{a}	4.78±0.62aA^{a}	3.51±0.25cA^{a}	2.95±0.33bB^{a}
	LF	4.32±0.77aA^{a}	4.77±0.78aA^{a}	4.93±0.68abA^{a}	3.57±0.54bA^{b}
	SF	4.32±0.67aA^{a}	4.51±0.7aA^{a}	4.41±0.62bcA^{a}	5.43±1.17aA^{a}
	P	3.79±0.54aB^{a}	3.83±0.79aB^{a}	6.08±1.16aA^{a}	6.42±0.8aA^{a}
K	F	1.8±0.18a	1.91±0.13a	1.49±0.3b	1.18±0.17c
	LF	1.76±0.07a	1.85±0.19a	2.3±0.36ab	1.71±0.23bc
	SF	2.09±0.39a	2.29±0.62a	1.76±0.36b	2.38±0.6ab
	P	2.04±0.41a	2.12±0.41a	3.19±1.05a	3.31±0.49a
P	F	3.12±0.72cA^{a}	4.4±0.63aA^{a}	9.15±5.89bA^{a}	7.65±4.02aA^{a}
	LF	3.62±1.11bcA^{a}	3.6±0.66bA^{a}	5.71±5.31cA^{a}	2.73±1.24cA^{a}
	SF	4.52±2.53aA^{a}	3.17±1.29bcA^{a}	3.65±1.01dA^{a}	6.16±2.47bA^{a}
	P	3.87±1.02abB^{a}	2.6±0.3cA^{a}	19.77±21.63aA^{a}	7.64±4.12aA^{b}

Different lower-case letters indicate difference between land use (same level of landscape and topography).
Different uppercase letters indicate difference between landscape (same level of land use and topography).
Different superscript letters indicate difference between topography (same level of land use and landscape).

Table 3. Earthworm total density (individuals/m^2) and biomass (g/m^2) by the landscape, land use and topography in Eastern Amazonia, Brazil. Different letters indicate a significant difference (α≤0.05). F: Old-growth forest, LF: Logged old-growth forest, SF: Secondary forest, P: Pasture. CL: Conserved Landscape, DL: Degraded Landscape. N= 3. Values represent the mean ± standard deviation..

Earthworm parameter	Landscape	Topography	Land Use			
			F	LF	SF	P
Total Density (ind/m^2)	CL	Top	78.95±22.94	61.4±36.97	126.32±27.85	28.07±18.48
		Low	92.98±35.83	38.6±36.97	221.05±255.3	101.75±126.13
	DL	Top	68.42±50.21	36.84±10.53	31.58±18.23	119.3±71.84
		Low	84.21±58.61	29.82±33.84	80.7±80.4	61.4±15.19
Total Biomass (g/m^2)	CL	Top	9.46±3.03A	13.82±3.62A	23.53±17.65A	3.19±1.01A
		Low	15.82±4.79A	13.23±12.59A	25.7±22.46A	4.02±5.67A
	DL	Top	5.93±3.8A	1.49±1.77B	1.68±2.74B	1.95±1.51A
		Low	7.51±10.4A	0.33±0.32B	14.18±12.45A	4.98±7.63A

Different uppercase letters indicate difference between landscape (same level of land use and topography).

Table 4. Earthworm total density (individuals/m^2) and biomass (g/m^2) by the landscape and land use in Eastern Amazonia, Brazil. Different letters indicate a significant difference (α≤0.05). F: Old-growth forest, LF: Logged old-growth forest, SF: Secondary forest, P: Pasture. CL: Conserved Landscape, DL: Degraded Landscape. Values represent the mean ± standard deviation.

	Landscape	Land Use				
		F	LF	SF	P	Total (N=24)
Total Density (ind.m^{-2})	CL	85.96±27.98	50±35.35	173.68±170.51	64.91±90.16	93.64±104.53
	DL	76.32±49.57	33.33±22.74	56.14±58.67	90.35±56.24	64.03±50.64
	Total (N=12)	81.14±38.71	41.67±29.64	114.91±136.19	77.63±72.86	
Total Biomass (g.m^{-2})	CL	12.64±5abA	13.53±8.29abA	24.61±18.1aA	3.61±3.67bA	13.60±12.35A
	DL	6.72±7.06aA	0.91±1.3aB	7.93±10.57aB	3.46±5.19aA	4.76±7.02B
	Total (N=12)	9.68±6.60ab	7.22±8.68b	16.27±16.60a	3.53±4.29b	

Lower case letters: Different letters indicate difference between land use.

Upper case letters: Different letters indicate difference between landscape

Table 5. RDA sequence step to test environment variation font by group. Y is the species data after transformed as Hellinger distance; “x” and “y “ represents the geography coordinate (Longitude and Latitude), respectively. OM: Organic Mater, Bsum: Bases Sum, P_A: Annual Precipitation, P_M: Monthly Precipitation, T_M: Monthly Temperature,T_A: Annual Temperature.

Step	Variation Font	Model	p	R^2 adj.
Original independente model	Geographycal	$Y \sim xy+x^2y+xy^2+x^2y^2+x^3y+xy^3+x^2y^3+x^3y^2+x^3y^3$	0.001	0.48
	Chemical	Y~pH+HAL+K+Ca+Mg+P+OM+Na+H+Bsum+CTC+V	0.001	0.44
	Physical	Y~Moisture+Bulk Density+Thick Sand+Fine Sand+Silt+Clay	0.001	0.43
	Climate	$Y \sim P_A+P_M+T_A+T_M$	0.001	0.36
Independent model	Geographycal	$Y \sim x+xy^2+x^3y+x^3y^2+x^3y^3$	0.026	0.28
	Chemical	Y~Mg+V+Ca+k+Na+P	0.044	0.26
	Physical	Y~Moisture+Bulk Density+Fine Sand	0.002	0.31
	Climate	$Y \sim P_A+P_M+T_M$	0.014	0.29
	Topography	Y~Altitude	0.001	0.19
	Landscape	Y~Forest Cover	0.001	0.09
Additive model	All	Y~Geographycal+Chemical+Physical+Climate+Topography+Landscape	-	-
Final additive model	Environment	Y~Ca+K+Na+P+ Bulk Density+Fine Sand +P_A +Altitude+Forest Cover	0.042	0.57

Table 6. Table 6: MANOVA test permutation for different variation font in design.

Font	Df	Variance	F	P
Landscape	1	0.09346	14.873	0.001***
Land use	3	0.11286	5.9865	0.001***
Topography	1	0.02333	0.7129	0.006**
Landscape*Land use*Topography	10	0.14319	2.2786	0.001***

Landscape type: Conserved (CL) and Degraded (DL)
Land Use type: Old-growth forest/Old-growth forest fragment (F); Logged old-growth forest (LF); Secondary forest (SF); Pasture (P)
Topography type: Upland (Top); Lowland (Low)

SM 1. Percentage of forest cover by land use over a 16 km of ratio polygons on different Landscape condition.

Land Use Type	Forest cover (%)		Perimeter/Area	
	CL	DL	CL	DL
Old-growth forest	67	29	890	948
Secondary forest	7	34	1470	2158
Pasture	9	9	322	259
Recent burned forest	17	12	931	640
Eucalyptus forest	0	1	0	22
Old-growth forest fragment	0	14	0	1906
Urban area	0	1	0	640
Simpson dominance Index(D)	**0.46**	**0.33**		
Shannon Evenness Index (SHEI)	**0.54**	**0.67**		

SM 2: Experimental design data. Landscape and Topography are expressed as category factor and vector gradient (Cover Forest and altitude respectively). Land use factor classified as factor. F: Old growth forest , FF: Old growth forest fragment, LF: Logged old growth forest, SF: Secondary forest, P: Pasture. CL: Conserved landscape, DL: Degraded landscape. Top: Hilltop, Low: Lowland

Code	Landscape	Land use	Topography	Altitude	CoverForest (%)	Lat Dec	Long Dec
1	CL	F	Top	189	100	-3.68639	-46.7594
2	CL	F	Low	175	100	-3.68778	-46.7594
3	CL	LF	Low	154	100	-3.68056	-46.77
4	CL	LF	Top	182	100	-3.68306	-46.7669
5	CL	F	Top	210	100	-3.69083	-46.7517
6	CL	F	Low	188	100	-3.69	-46.7533
7	CL	LF	Low	158	100	-3.68611	-46.7656
8	CL	LF	Top	172	100	-3.68889	-46.7669
9	CL	P	Top	139	11.4	-3.67369	-46.789
10	CL	P	Low	105	23.7	-3.67278	-46.7963
11	CL	P	Top	145	37.3	-3.67144	-46.7986
12	CL	P	Low	108	37.8	-3.66894	-46.7984
13	CL	P	Top	128	24.3	-3.66867	-46.7951
14	CL	P	Low	101	58.9	-3.61664	-46.816
15	CL	SF	Top	152	62.8	-3.69908	-46.7661
16	CL	SF	Top	131	62.8	-3.69925	-46.768
17	CL	SF	Low	109	60.6	-3.70272	-46.7642
18	CL	SF	Low	127	57	-3.70219	-46.7618
19	CL	LF	Low	134	89	-3.69058	-46.7706
20	CL	F	Top	155	100	-3.68847	-46.7659
21	CL	F	Low	146	92.7	-3.69103	-46.7674
22	CL	LF	Top	146	78	-3.68917	-46.7734
23	CL	SF	Top	107	53.7	-3.70506	-46.7632
24	CL	SF	Low	134	57	-3.70203	-46.7603
25	DL	P	Low	196	46.4	-4.03906	-46.9328
26	DL	P	Top	242	35.4	-4.04314	-46.9324
27	DL	SF	Top	302	32.3	-4.03158	-46.8829
28	DL	P	Low	158	40.4	-4.03833	-46.9126
29	DL	FF	Top	206	39.29	-4.04133	-46.9086
30	DL	FF	Low	164	34	-4.04292	-46.91
31	DL	P	Top	179	36.8	-4.03939	-46.9138
32	DL	FF	Top	234	50.3	-4.03439	-46.9058
33	DL	FF	Low	175	43	-4.03672	-46.9066
34	DL	FF	Top	229	95	-4.09933	-46.9136
35	DL	FF	Low	185	100	-4.10111	-46.9164
36	DL	P	Low	139	33	-4.07244	-46.8833
37	DL	P	Top	160	31	-4.09528	-46.8725
38	DL	SF	Low	144	53	-4.08539	-46.8695
39	DL	SF	Low	149	42	-4.08064	-46.8776
40	DL	SF	Low	141	41	-4.07556	-46.8803
41	DL	LF	Low	262	93	-3.99936	-46.8288
42	DL	LF	Low	212	100	-3.92317	-46.766
43	DL	LF	Top	250	100	-3.98069	-46.7861
44	DL	LF	Low	181	100	-3.98306	-46.799
45	DL	LF	Top	224	100	-3.91972	-46.7617
46	DL	LF	Top	285	93	-3.99825	-46.8413
47	DL	SF	Top	294	17	-4.03372	-46.8806
48	DL	SF	Top	294	10.4	-4.03608	-46.8806

SM 2: **Continue.** Climate and Physical-chemical matrix.PA:Annual precipitation, PM:Montlhy precipitation, TA:Annual temperature, TM:Montlhy temperature, BD: Bulk density, Tsand: Thickness sand, Fsand: Fine sand, Bsum: Bases sum. K, Ca, Mg, Na, Al, H, HAl.(mmol/dm^3), P (mg/dm^3), OM (g/dm^3), C (%).

Code	PA	PM	TA	TM	Moisture	BD	Tsand	Fsand	Silt	Clay	pH	HAl	k	Ca	Mg	P	OM	C	Na	Al	H	Bsum	CTC	V
1	1676	343	25.6	25.2	9.66	1.37	7.38	37.03	23.75	32	3.63	71.08	1.91	4.5	6	3.8	25.4	1.47	4.89	18.5	52.58	12.41	83.49	15.01
2	1676	343	25.6	25.2	6.1	1.51	21.83	53.53	6.65	18	3.53	63.07	1.78	5.5	14	3.65	18.65	1.08	4.5	11.75	51.32	21.28	84.35	25.79
3	1679	344	25.6	25.29	5.84	1.67	30.13	39.88	9.43	21	3.74	47.85	1.79	8	8.5	4.4	17.25	1	4.54	10.25	37.6	18.29	66.14	27.26
4	1679	344	25.6	25.29	13.16	1.41	8.18	24.18	16.65	51	3.5	110.46	2.06	4	6	4.2	31.9	1.85	5.37	28	82.46	12.06	122.53	9.72
5	1676	343	25.4	25.1	13.89	1.28	5.58	14.15	17.5	63	3.51	105.86	2.04	2.5	10.5	4.35	30.05	1.74	5.33	25.75	80.11	15.04	120.9	12.37
6	1676	343	25.4	25.1	7.5	1.59	16.18	49.9	14.88	19	3.73	50.99	1.99	6.5	7.5	3.4	16.2	0.94	5.11	11.5	39.49	15.99	66.98	23.77
7	1674	344	25.6	25.2	11.12	1.59	19.03	36.63	19.5	24.5	3.82	44.74	1.81	2.5	7	4.1	15.35	0.89	4.96	10.5	34.24	11.31	56.05	19.63
8	1673	343	25.6	25.29	7.75	1.69	21.85	43.53	15.63	19	3.78	39.05	1.76	5	4	2.9	14.25	0.83	5.04	11.75	27.3	10.76	49.8	24.05
9	1690	271	25.8	25.7	13.55	1.7	23.88	40.25	14.13	21.5	4.65	24.8	1.82	16	12	2.25	19.6	1.14	3.17	0	24.8	29.82	54.62	54.51
10	1725	272	25.8	25.7	14.91	1.61	26.3	42.33	11.38	20	4.72	33.23	2.14	23.5	11	4.55	25.4	1.47	4.07	0	33.23	36.64	69.87	52.33
11	1725	272	25.8	25.7	15.76	1.59	11.98	43.78	18.58	26	4.1	36.64	1.95	7	11.5	2.8	18.2	1.06	3.61	4.25	32.39	20.45	57.09	35.1
12	1725	272	25.8	25.7	12.77	1.62	28.85	48.55	4.55	18	4.48	32.45	2.38	11.5	9	4.35	20.8	1.21	4.13	2.5	29.95	22.88	55.34	40.12
13	1725	272	25.8	25.7	11.68	1.61	28.88	45.93	8.53	17	4.11	37.98	2.58	10.5	10	2.75	20.75	1.2	4.7	5.25	32.73	23.08	61.06	35.13
14	1709	275	25.9	25.79	11.35	1.57	29.95	46.78	6	17	4.2	30.3	1.59	8.5	5	2.7	15.8	0.92	3.17	4	26.3	15.09	45.39	32.11
15	1674	269	25.6	25.5	21.47	1.27	6	31.28	27	35	4.88	27.5	2.96	37.5	11.5	2.8	27.65	1.6	5.3	0	27.5	51.96	79.46	62.33
16	1673	269	25.7	25.6	21.58	1.32	6.6	38.1	20.98	34	4.23	44.27	2.19	14.5	16	4.6	26	1.51	4.26	3	41.27	32.69	76.97	42.63
17	1673	268	25.7	25.6	20.41	1.37	10.1	52.93	17.25	20	4.15	35.11	1.91	12	5.5	7	21.7	1.26	3.72	4.25	30.86	19.41	54.52	32.15
18	1673	268	25.7	25.6	15.12	1.52	19.83	55.78	8.4	16	4.76	21.09	1.83	21.5	10	1.95	16.55	0.96	4.2	0	21.09	33.33	54.42	60.31
19	1677	271	25.6	25.5	13.98	1.51	9.43	34.3	29.27	27	3.75	53.11	1.68	6.5	2.5	2.35	17.6	1.02	3.46	10.5	42.61	10.68	63.79	16.13
20	1673	271	25.6	25.5	17.89	1.38	7.63	42.4	20.98	29	3.68	68.48	1.79	5	6.5	5.05	24	1.39	4.11	16	52.48	13.29	81.77	16.53
21	1677	271	25.6	25.5	14.8	1.45	17.9	52.65	15.45	14	3.65	46.95	1.64	3	6	2.3	18.8	1.09	3.98	10.5	36.45	10.64	57.59	18.5
22	1677	271	25.6	25.5	20.36	1.28	6.58	29.1	24.5	39	3.97	56.66	1.72	10.5	14.5	3.7	24.35	1.41	3.89	9	47.66	26.72	83.37	32.55
23	1673	268	25.7	25.6	15.42	1.51	12.6	54.13	11.28	22	3.83	53.02	1.73	7	8	2.1	20.45	1.19	3.96	10.5	42.52	16.73	69.75	23.12
24	1673	268	25.7	25.6	16.59	1.34	9.98	38.2	19.83	32	4.61	30.8	2.54	25	17	4.6	25.2	1.46	5.04	0.75	30.05	44.54	75.34	56.31
25	1582	251	25.3	25.2	27.15	0.95	2.5	4.5	50	43	5.97	16.9	3.88	74	11	7.01	36.15	2.097	6.85	0	16.9	88.88	105.79	83.6
26	1592	253	25.1	25	19.91	1.09	3	3.5	51.5	42	6.88	11.07	3.45	83.5	8.5	7.97	32.2	1.868	6.96	0	11.07	95.45	106.52	89.17
27	1578	253	25	24.89	27.6	0.98	2	3	51	44	6.26	17.92	1.91	55	14	7.08	34.5	2.001	4.2	0	17.92	70.91	88.83	78.55
28	1587	254	25.5	25.39	28.88	1.12	3.5	9.5	56	31	5.97	19.89	3.71	47	36	44.75	36.43	2.113	6.65	0	19.89	86.71	106.6	80.62
29	1587	254	25.5	25.39	22.17	1.12	7.5	26	26.5	40	4.66	45.48	1.37	15	23.5	12.03	25.11	1.457	3.33	11.5	33.98	39.87	85.35	42.44
30	1587	254	25.5	25.39	19.82	1.33	7	47	19	27	4.16	32.4	1.54	11	18.5	5.57	20.51	1.19	3.52	2	30.4	31.04	63.44	47.28
31	1587	254	25.5	25.39	22.75	1.2	4	16	38	42	5.35	22.89	2.76	43	11	11.59	23	1.334	5.5	0	22.89	56.76	79.65	68.62
32	1583	254	25.3	25.2	17.14	1.34	7.5	50	17.5	25	3.53	62.55	1.1	7	5.5	6.8	24.84	1.441	2.7	21.5	41.05	13.6	76.15	16.85
33	1583	254	25.3	25.2	18.6	1.34	9.5	57.5	13	20	5.02	19.51	1.77	35.5	7.5	15.95	24.29	1.409	3.76	0	19.51	44.77	64.28	68.24
34	1557	248	25.2	25.1	18.47	1.34	10	35.5	24.5	30	3.54	58.28	1.06	2.5	8	4.12	21.8	1.265	2.83	24.5	33.78	11.56	69.84	16.14
35	1555	247	25.5	25.39	14.93	1.48	12	59	12	17	3.91	28.89	1.17	8	8	5.94	17.02	0.987	3.26	5.75	23.14	17.17	46.06	36.49
36	1565	249	25.45	25.39	17.38	1.5	9.5	53.5	21	16	5.45	19.59	1.99	24.5	5	7.56	18.77	1.088	4.74	0	19.59	31.49	51.08	57.02
37	1555	247	25.65	25.6	18.82	1.4	5	30	25	40	4.63	36.26	3.71	19	10.5	3.36	26.12	1.515	6.8	4.5	31.76	33.21	69.46	44.58
38	1561	250	25.6	25.5	14.05	1.51	16	56	13	15	4.74	19.54	1.42	17.5	4	2.95	15.91	0.923	3.78	0	19.54	22.92	42.46	50.68
39	1562	249	25.6	25.5	15.8	1.59	5.5	62.5	13	19	4.3	17.77	2.14	11.5	7	3.19	15.55	0.902	5.02	0.5	17.27	20.64	38.41	52.77
40	1562	249	25.6	25.5	16.3	1.45	11.5	56.5	16	16	4.87	20.41	1.73	27	3.5	4.81	22.08	1.281	4.43	0	20.41	32.23	52.64	58.87
41	1587	252	25.05	24.89	28.15	0.91	3	3	48	46	4.73	29.98	2.51	49	13.5	2.26	39.83	2.31	5.63	0	29.98	65.01	95	67.45
42	1592	256	25.3	25.2	16.44	1.31	1.5	2.5	69	27	5.39	17.77	2.51	42.5	3.5	11.83	25.11	1.457	4.89	0	17.77	48.51	66.28	67.5
43	1577	252	25.34	25.1	22.27	1.14	1	3	43	53	3.7	59.69	1.69	7	8.5	2.09	24.75	1.435	3.43	13	46.69	17.19	76.88	21.47
44	1584	253	25.3	25.2	21.29	1.25	7.5	47.5	7	38	4.24	27.69	1.88	19.5	7.5	3.05	22.08	1.281	4.28	0.75	26.94	28.88	56.57	48.25
45	1592	256	25.3	25.2	14.43	1.35	8.5	20	42.5	29	4.5	24.06	1.49	23	5	4.16	20.42	1.185	3.11	0.5	23.56	29.49	53.55	53.76
46	1587	252	25	24.89	24.81	0.93	4.5	5.5	44	46	4.68	33.16	1.95	28.5	15.5	1.95	27.78	1.611	4.17	0	33.16	45.95	79.11	57.85
47	1576	253	25	24.89	28.17	0.96	7	31.5	22.5	39	5.53	20.47	3.06	44.5	17.5	3.36	29.25	1.697	6.54	0	20.47	65.06	85.54	70.83
48	1576	253	25	24.89	27.39	0.97	1	2.5	59.5	37	6.58	12.01	2.18	79.5	11.5	8.04	25.11	1.457	5.54	0.5	11.51	93.18	105.19	87.89

SM 2: **Continue.** Species matrix. TotB: Total Biomass, TotD: Total Density, pc: *Pontoscolex corethrurus*, pgs1: *Pontoscolex* gs1, ha: *Holoscolex alatus*, hf: *Holoscolex fernandoi*, hd: *Holoscolex dossantosi*, h2: *Holoscolex* sp2, hgn: *Holoscolex* gn, hgs1: *Holoscolex* gs1, hgs2: *Holoscolex* gs2, rg: *Righiodrilus grurupi*, rmin: *Righiodrilus* min, am: *Andiorrhinus* (*T.*) *miricuri*, abar: *Andiorrhinus barrosoi*, db: *Dichogaster bolaui*, ds: *Dichogaster saliens*, ds: *Dichogaster afinis*, dgn: *Dichogaster* gn, btet: *Brasilisia* tet, uro1: *Urobenus* sp1, uropig: *Urobenus* pig, urogur: *Urobenus* gur, ggurm: *Glossodrilus* gurm, ggnmx: *Glossodrilus* gnmx.

Code	TotB	TotD	pc	pgs1	ha	hf	hd	h2	hgn	hgs1	hgs2	rg	rmin	am	abar	db	ds	da	dgn	btet	uro1	uropig	urogur	ggurm	ggnmx
1	9.04	51	5	0	1	15	0	0	0	0	0	7	0	0	0	0	0	0	0	0	1	0	0	0	0
2	27.3	81	1	0	2	30	1	1	0	0	0	6	0	2	0	0	0	0	0	0	0	0	0	0	0
3	15.08	53	2	0	5	30	1	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
4	20.98	74	0	0	2	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	14.93	40	0	0	1	11	0	0	0	0	0	0	0	3	0	0	0	0	0	0	3	0	0	0	0
6	31.07	92	4	0	1	26	2	1	0	0	0	2	0	2	0	0	0	0	0	0	1	0	0	0	5
7	22.62	64	13	0	2	18	2	0	0	0	0	8	0	2	1	0	0	0	0	0	0	0	0	0	0
8	24.2	88	11	0	4	40	0	0	0	0	0	8	0	0	0	0	0	0	1	0	0	0	0	0	0
9	22.94	136	3	0	18	3	0	0	0	0	0	2	0	0	11	80	0	0	0	0	0	0	0	0	0
10	29.08	177	11	0	32	0	0	0	0	0	0	0	0	0	1	1	0	0	0	5	1	0	0	0	0
11	20.16	114	2	0	30	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0
12	23.59	108	16	0	13	1	0	0	0	0	0	2	0	0	0	7	0	0	0	0	0	0	0	0	0
13	12.63	54	0	0	17	0	0	0	0	0	0	1	0	0	0	4	0	0	0	2	2	0	1	0	0
14	9.12	22	1	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	33.88	110	3	0	4	6	0	0	0	0	0	0	0	5	0	1	0	0	0	0	2	0	0	0	0
16	45.86	92	4	0	6	11	0	0	0	0	0	2	0	5	1	0	0	0	0	0	1	0	0	0	0
17	70.87	272	223	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	33.18	112	75	1	2	8	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
19	30.96	94	6	0	4	29	1	0	0	0	0	5	0	2	0	3	0	0	0	0	2	0	0	0	0
20	22.12	88	1	0	4	31	0	1	0	0	0	10	0	0	0	0	0	0	0	0	1	0	0	1	0
21	22.83	89	6	0	7	15	3	0	0	0	0	14	0	4	0	2	0	0	0	0	0	0	0	0	0
22	35.7	150	10	0	1	103	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
23	55.79	211	122	0	5	11	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
24	16.12	67	2	0	4	28	0	0	0	0	0	1	0	0	3	1	0	2	0	0	0	0	0	0	0
25	16.03	45	0	0	0	8	0	0	0	4	0	0	0	2	0	0	5	0	0	0	0	0	0	0	0
26	9.68	12	0	0	0	1	0	0	0	0	0	0	0	4	0	3	0	0	0	0	0	0	0	0	0
27	17.12	14	0	0	0	0	0	0	0	0	0	0	0	6	0	1	0	0	0	0	0	0	0	0	0
28	42.6	96	4	0	0	4	0	0	0	0	0	0	0	10	0	29	1	0	0	0	0	0	0	0	0
29	27.1	47	0	0	0	8	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
30	31	48	0	3	1	1	0	0	0	0	1	0	0	3	0	0	1	0	0	0	0	1	0	0	0
31	4.63	71	1	0	1	3	0	0	0	0	0	0	0	0	0	36	3	0	0	0	0	0	0	0	0
32	47.08	102	10	1	1	4	0	0	0	0	0	0	0	8	0	0	0	0	0	0	1	0	0	0	0
33	57.66	221	175	0	2	4	0	0	0	0	1	0	0	0	0	5	0	0	0	0	0	0	0	0	0
34	71.83	116	0	1	4	20	0	0	0	1	1	0	0	7	0	0	4	0	0	0	1	0	0	0	0
35	54.89	59	8	4	2	11	0	0	0	0	2	0	0	4	1	0	0	0	0	0	0	0	0	0	0
36	63.24	215	11	10	8	3	0	0	0	2	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0
37	8.97	236	19	0	8	0	0	0	0	0	0	0	0	0	0	154	0	0	0	0	1	0	0	0	0
38	127.51	479	414	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
39	37.87	75	15	28	2	1	0	0	0	3	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
40	37.84	110	18	9	3	2	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	13.48	32	0	0	0	0	0	0	0	0	0	0	0	8	0	0	17	0	0	0	0	0	0	0	0
42	30.88	71	0	0	0	11	0	0	0	0	0	0	0	8	0	28	5	0	0	0	0	0	0	0	0
43	14.44	40	0	0	0	2	0	0	0	0	0	0	0	1	0	0	27	0	0	0	0	0	0	0	0
44	27.33	70	0	0	0	9	0	0	0	0	1	0	0	7	0	4	15	0	0	0	1	0	0	0	0
45	31.84	85	0	0	1	9	0	0	0	0	1	9	0	6	0	15	14	0	0	0	0	0	0	0	0
46	30.52	33	0	0	0	0	0	0	0	0	0	0	0	4	0	0	8	0	0	0	1	0	0	0	0
47	11.73	13	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
48	10.08	6	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0

SM 2. ANOVA summary result for earthworm total density.

Source	Df	SS	MS	F	P
Block	2	1.356	0.6181	0.693	0.508
Landscape	1	0.584	0.5841	0.597	0.446
Landscape:Landuse	6	13.156	2.1926	2.242	0.066
Landscape:Landuse:Topography	8	5.204	0.6505	0.665	0.717
Residuals	30	29.342	0.9781		

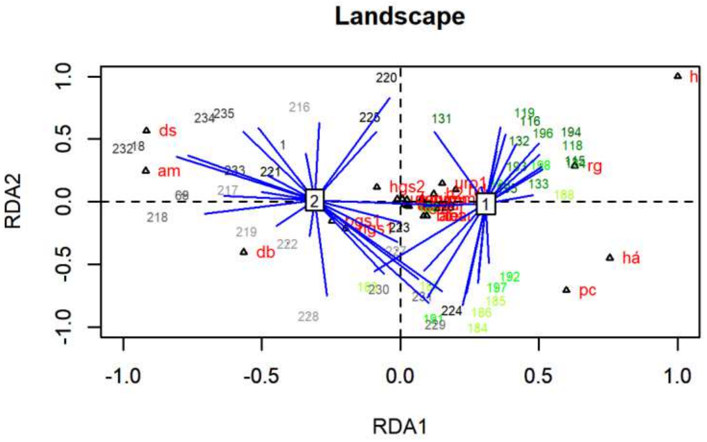
SM 3. ANOVA summary result for earthworm total biomass.

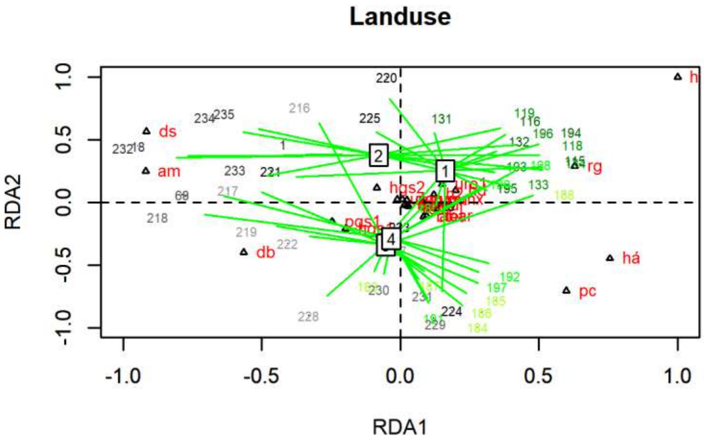
Source	Df	SS	MS	F	P
Block	2	0.271	0.135	0.137	0.872
Landscape	1	15.203	15.203	15.375	0.0004***
Landscape:Landuse	6	13.961	2.327	2.353	0.055
Landscape:Landuse:Topography	8	4.951	0.619	0.626	0.750
Residuals	30	29.664	0.989		

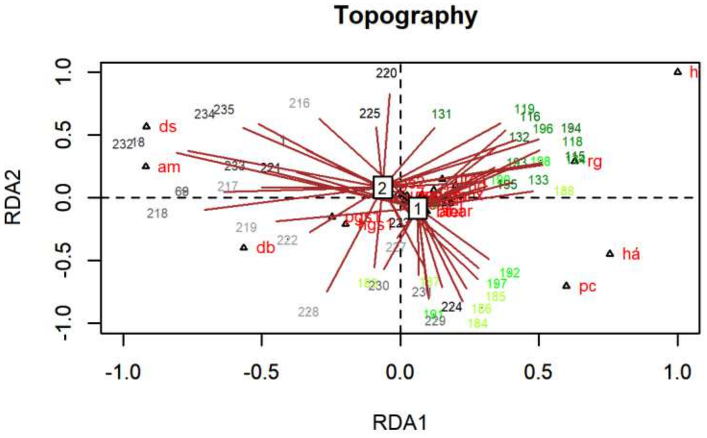
SM 4. List of importance values for environmental variables selected for the final additive model.

Group	Variable	P
Physical-Chemical	Ca	0.05 *
	K	0.018 *
	Na	0.002 **
	P	0.05 *
	Bulk Density	0.005 **
	Fine Sand	0.008 **
Landscape	Cover Forest	0.001 ***
Topography	Altitude	0.004 **
Climate	Precipitation	0.001 ***

SM 5. MANOVA result separated by factor. For levels of landscape: 1=CL, 2=DL. For levels of land use: 1-4 indicate F, LF, SF and P respectively. For levels of topography: 1=Top, 2=Low.







SM 6**.** Earthworm density (Individuals/m^2) and biomass (g/m^2) compare by country, state, and localities in the Amazonians ecosystems.

Country	State	County	Site	Vegetation type	Density (Individuals/m2)	Biomass (g/m2)	Monolith size (cm x cm)	Sample depth (cm)	Monoliths/ Plot	Author
Venezuela	Amazonas	RN	San Carlos	Old growth forest (clay)	54.8 ± 22.5	15.8 ± 8	50x50	30	10	Nemeth and Herrera 1982
				Old growth forest (sandy)	68.4 ± 25	16.6 ± 10.5	50x50	30	10	Nemeth and Herrera 1982
				Old growth forest (loam)	42.2 ± 20.2	10.3 ± 9	50x50	30	10	Nemeth and Herrera 1982
				Total	42.2-68.4	10.3-16.6	50x50	30	10	Nemeth and Herrera 1982
Perú	Loreto	AA	Yarimaguas	"moist" pasture	573 ± 59.2	116.4 ± 22	25x25	30	10	Lavelle and Pashanasi 1989
				"dry" pasture	474 ± 109.7	78 ± 17.1	25x25	30	10	Lavelle and Pashanasi 1989
				Secondary Forest (15 years)	85 ± 39	11.3 ± 4	25x25	30	10	Lavelle and Pashanasi 1989
				Old growth forest	120 ± 39.6	28.2 ± 8.9	25x25	30	10	Lavelle and Pashanasi 1989
Brazil	AC	RB	Federal University of Acre	Forest	3.9-26.8	2.4-12.6	50x50	30	80	Guerra et al. 1994
				Pasture	3.5-22.5	2.7-8.8	50x50	30	80	Guerra et al. 1994
	Amazonas	Manaus	INPA	Old growth forest	80±137.5		20x20	30	10	Harada and Bandeira 1994
				Old growth forest (Sandy soil)	120		20x20	30	10	Bandeira and Harada 1998
				Old growth forest (Clay soil)	102.5		20x20	30	10	Bandeira and Harada 1998
	Amazonas	RP	EMBRAPA	Old growth forest		44.4±11.25	25x25	30	10	Barros et al. 2001
				Pasture		45.4±7.24	25x25	30	10	Barros et al. 2001
	Rondônia	JP	-	Pasture 1	374 ± 141	146.01 ± 48.62	25x25	30	5	Barros et al. 2002
		Th	-	Pasture 2	80 ± 33	12.54 ± 5.68	25x25	30	5	Barros et al. 2002
			-	Secondary Forest 1	13 ± 5	1.35 ± 0.89	25x25	30	5	Barros et al. 2002
			-	Secondary Forest 2	3 ± 1	0.08 ± 0.03	25x25	30	5	Barros et al. 2002
			-	Old growth forest	26 ± 12	0.89 ± 0.57	25x25	30	5	Barros et al. 2002
	Acre	PP	-	Pasture	10 ± 2	0.02 ± 0	25x25	30	5	Barros et al. 2002
			-	Secondary Forest	106 ± 47	0.91 ± 0.23	25x25	30	5	Barros et al. 2002
		RECA	-	Old growth forest	109 ± 61	18.31 ± 10.56	25x25	30	5	Barros et al. 2002
		PP	-	Old growth forest	6 ± 2	0.12 ± 0.05	25x25	30	5	Barros et al. 2002
	Amazonas	RP	EMBRAPA	Secondary forest	43±24	4.88±4.71	25x25	25	15	Barros et al. 2003
				Old growth forest	136±70	44.32±26.24	25x25	30	10	Barros et al. 2004
				Pasture	284±114	48.91±29.21	25x25	30	10	Barros et al. 2004
	Pará	I	Benfica	Pasture (Brachiaria)	16.64±7.61		25x25	10	6	Laossi et al., 2008
				Pasture (Brachiaria ungrazed)	9±6		25x25	10	6	Laossi et al., 2008

SM 7. **Continue**. Earthworm density (Individuals/m^2) and biomass (g/m^2) compare by country, state, and localities in the Amazonians ecosystems.

Country	State	County	Site	Vegetation type	Density (Individuals/m2)	Biomass (g/m2)	Monolith size (cm x cm)	Sample depth (cm)	Monoliths/ plot	Author
Brazil	Amazonas	Manaus	Tarumã-Mirím	Pasture (after Agriculture)	14.0	1.84	50 x 50	30	9	Pontes 2009
			Tarumã Açu	Pasture (after cattle)	478.0	66.90	50 x 50	30	9	Pontes 2009
	Pará	IA	UFRPA University Campus	Secondary Forest	177-350	-	25x25	15	21	Rousseau et al 2010
		I	Benfica	Pasture	304 ± 33		25x25	10	6	Velasquez et al. 2012
	Amazonas	PF	Balbina hidroelectric plant	Old growth forest	416		25x25	20	8	Viana 2012
				Secondary forest	0		25x25	20	8	Viana 2012
		BC	-	Old growth forest	17.6	1.8	50x50	30	5	Alves 2012
			-	Old secondary forest	92	12.6	50x50	30	5	Alves 2012
			-	Young secondary forest	71.2	15.4	50x50	30	5	Alves 2012
			-	Pasture	221.6	58.6	50x50	30	5	Alves 2012
	Para-Maranhão	IA, TA, AL	Belem Endemism Area	Pasture	440	-	25x25	10	5	Rousseau et al 2014
		IA, TA. AL, CN, IT, R, Slz		Secondary Forest	227	-	25x25	10	5	Rousseau et al 2014
		TA, CN		Old growth forest	200	-	25x25	10	5	Rousseau et al 2014
	Pará	NI	Praialta Piranheira	Pasture	289	-	25x25	30	10	Castilho et al. 2016
				Old growth forest	165	-	25x25	30	10	Castilho et al. 2016
	Maranhão	CN, IT	Gurupi Biological Reserve	Pasture	65±90 - 90±56	3.5 ± 5.1 -3.6 ± 3.7	25x25	20cm	3	This study
				Secodary forest	56±59 - 174±171	7.9 ± 11 - 24.6 ± 18	25x25	20cm	3	This study
				Logged old growth forest	33±23 - 50±35	0.91 ± 1.3 - 13.5 ± 8.3	25x25	20cm	3	This study
				Old growth forest	76±50 - 86±28	6.7 ± 7- 12.6 ± 5	25x25	20cm	3	This study

7.7 **CAPITULO 7:** Environmental components explain earthworm beta diversity in Eastern Amazonia remnant, Maranhão State.

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Abstract:

Different environment components drive changes in earthworm communities, and land use may not necessarily show changes in local earthworm diversity patterns (alpha diversity), although species substitution may be occurring from one site to another. In this study, we dissected the causes of species turnover, by grouping geographical, climate and physical-chemical variables and measuring the pure effect on earthworm species substitution by land use. Sampling was carried out in the conserved remnant forest of the Gurupi Biological Reserve, as well as, three others class of land use (i.e., logged old-growth forest, secondary forest and pasture), all located in two different levels of topography and landscape. For all sites, earthworm α and β diversity was measured and compared to determine if only α diversity was sufficient as ecological indicator of community. β diversity was dissected to determine how much of each environmental component was responsible to explain the total turnover. Our results suggest than there are not change of α diversity with land use type (forest to pasture) but located in two relief types, and inside two landscape matrices (conserved and degraded). The earthworm's composition changed with the land use types, with the secondary forest and logged forest having high turnover values. Physical chemical variables or even climate, can explain better the turnover than geographical distance, and each land use was affected by this type of factor in different stages of vegetation development. Physical-chemical variables explained better the turnover on initial stages of regrowth forest, as pasture and secondary forest, while logged forest turnover was influenced more by climatic variables. Conversely, primary forest responded more to geographical conditions, because others effects were not significantly influencing the species substitution. Our results suggest that there are habitats filling when the forest is transformed to pasture and different natives' species could survive depending on physical-chemical conditions. Different gradients are activated depending on land use type and the heterogeneity is a crucial factor determining which type of species can survive to sum or decreasing the total diversity of the area.

Key-Words: Turnover, Gurupi Biological Reserve, soil fauna.

Introduction

The tropical forest of Maranhão state is located inside the eastern frontier of Amazon biome and had been considered as an essential component of the Belém Endemism Area (BEA). This biome borders the Atlantic Ocean to the north and the Cerrado biome to the east, and represents an important ecological transition area to study species turnover. In spite of that, recurrent anthropic impacts have transformed it into the most threatened area of Amazonia (Celentano et al. 2017).

Forest remnants are inside the Gurupi Biological Reserve (ReBio) and indigenous land, but economic problems of local people, in addition to politic failures, have allowed continuous degradation inside these areas, leaving only fragments surrounded by patches of secondary forest and areas of permanent pasture.

Forest disturbance affects its ecosystem services, and directly influences changes in species richness, promoting local extinction and changing ecological dynamics (Gorte and Sheikh 2010, Karp et al. 2012, Baan et al. 2013, Solar et al. 2016). Earthworms have been showed as susceptible to land use change, and richness estimates have been used as an important index of habitat conservation in Amazon (Smith et al. 2008, Castilho et al. 2016, Decaëns et al. 2018).

Nevertheless, this seems not to happen in poor soils from the Brazilian Eastern Amazon, where studies of earthworm (Rousseau et al. 2010) and ants communities (Solar et al. 2016) found same values of richness between land uses but difference in composition.

α and β-diversity depends of interaction of different factors as α-diversity is low in ecosystem controlled by physical factors (i.e., physical-chemical factors) and high in systems controlled by biological factors (i.e., competition, predation, mutualism, etc), (Begon et al. 2007). The area of isolated ecosystem is also important as was measured an increase of diversity of ants when islands were bigger (MacArthur and Wilson 1963). Likewise, land use area can be used as indicator perturbation as biggest areas of forest remnant can remain higher species diversity because α-diversity depend on habitat richness (Vanbergen et al. 2007).

According to Soininen et al. (2007) β-diversity depend of others conditions as communities of sessile species, or located in low latitudinal position, or having species with high body size, or in high tropic position, or belonging terrestrial has more probabilities to have higher β-diversity.

In bacteria communities the pH gradient is the main drivers of assemblage (Zheng et al. 2019). The turnover in secondary forest can be caused by recolonization (Amazonas et al. 2018) and habitat richness (Vanbergen et al. 2007). In plant communities factors explaining biodiversity depend of scale. According Wang et al. (2019) α and β-diversity can respond differently to environment factor as α-diversity of plant community was more related to climatic variable and β-diversity was relate to soil variables.

The last decade has been promoted specific index to estimate the turnover of species, like for example those of dissimilarities or beta diversity, one of the most famous is the Bray Curtis (Martin and Wilsey 2015, Fenton et al. 2016, Tomašových et al. 2016) and Sorensen (Baselga 2010).

Furthermore, the partition of beta diversity components has allowed determining the importance that a specific group of environment variables has in the turnover of species (Legendre et al. 2005, Baselga 2010). Following this method Decaëns et al. (2016) found an earthworm species turnover between 62.6 and 82.5% for different land uses in Amazon forest in French Guiana.

Unfortunately, there is not a detailed study indicating which environment factor is most important influencing the turnover in earthworm species and this is an opportunity to show how earthworm communities respond to natural gradients and land use in a transitional Amazonia area threatened by continuous deforestation and fire. The objective of this work is to determine which environmental factors are more important influencing earthworm β-diversity for different land use.

We hypothesized than: The spatial distribution of oligochaetes in the Belém Endemism Area has a high turnover of species (high β-diversity) and The deforestation of the Amazon in the BEA causes loss of native species.

Material and methods

The study site (Fig. 1), field history, experimental design (Table 1), earthworm sampling methods, and soil analysis method were detailed in the chapter 6.

Data analysis.

Richness and diversity estimation

We plotted the accumulation curve of species by landscape, land use, and topography. The richness and ecological index of Shannon-Weiner were used to estimate as indicator of community structure, as Shannon--Weiner index is insensible to rare species (Bandeira et al. 2013). Thereafter was checked the homogeneity of variance and normality and when necessary variables were transformed to logarithmic and then a three-way ANOVA was conducted to test possible interaction and difference on the mean variance of richness and diversity with the Landscape, Land Use, and Relief as a factor. A least significant difference test (LSD test) was used as a post hoc test when variance explained by factor was significant. All analyses were conducted in R 3.4.1 (R Core Team 2018): diversity indices were computed with the vegan package (Oksanen et al. 2018).

Beta-diversity

The total data set used to this analysis is available in the supplementary data (Supplementary 2, capítulo 6). The entire environment matrix was separated into four components; geographical, climate physical and chemical. Geographical variables were expressed as geographical coordinates (UTMx, UTMy). Climate components included the average of the forty years data of precipitation by month and the average of the total annual precipitation (WorldClim 2015). The average of monthly and annual temperature also included as climatic variable. Soil physical and soil chemical variables were also used separately as matrix as explainer of β-diversity components. The β-diversity index was computed according to Baselga (2010). The Sorensen index was applied into the Hellinger distance matrix of species to obtain three distance matrices by land use: the first one was for the total β-diversity, the second one for the turnover, and the third one for the nested component.

Each component of the environment matrix was transformed to Euclidean distance, and a correlation mantel test was run with each matrix total β-diversity component. Each Mantel test significance was tested by Pearson method after 1000 permutation. The matrix correlation was vectorized and plotted with the best fitted model. A null model constructed for each land use and by using a random logarithmic distribution was used as comparative data where environment component had no effect on β-diversity. Geographical distance was expressed in kilometers but other variables groups were expressed as distance class index in x-axes. We selected the most significant environmental variables explaining exclusively the turnover to create a correlation map indicating the significance by land use type.

Results

Richness and diversity estimation

A total of 725 individuals were collected in the TSBF samples and an additional 4010 individuals in the qualitative samples. The TSBF samples resulted in 17 species and the qualitative 23 species, of which 11 were unique to this method. The total earthworm list of species by the landscape, land use, and relief are showed in Tables 2 and 3. The qualitative method (Decaëns et al. 2016) was more efficient in recovery earthworm species than the monolith method. Approximately 71% of the total earthworm species collected in the monolith were native species. With the qualitative search, 18 species (78%) were native.

When considering the monoliths, species accumulation curves showed that the CL resulted in higher species richness (13 spp.) than the DL (10 spp.; Fig. 1A). Pastures had lower species richness (Table 2, Fig. 1B) but this is recovered in secondary forest. Moreover, low areas had a higher species richness than upper areas (Fig. 1C). When summed the qualitative data species obtained by the modified TSBF we found that pastures had richness approximately equal to the forest and secondary forest (Table 3, Supplementary 1) but rarefaction curves showed than old growth forest was higher in richness than pasture and there also was saturation of species only for top areas and pasture condition (Supplementary 2,3,4)..

The richness depends on landscape, land use and relief condition. In the CL the richness had no difference in land uses located in the top but in the low relief, where F had higher richness than SF and P, $P<0.05$ (Table 4). Otherwise, SF located in the top of DL had a lower value than P, LF, and F ($P<0.05$). Low areas of LF in CL was most rich in species than those of DL, while SF in the top of CL had higher richness than those of DL ($P<0.05$). Relief differences also were conditioned by the landscape and land use, in CL low areas located in LF was more richness than those of the top, while the same was found for SF located in DL ($P<0.05$).

Almost two types of vegetation presented differences in landscape, land use, and relief, and CL in the low area had a better condition to maintain highest values of richness both for LF and SF. We did not find a significative difference in diversity index (**H´**) by the landscape, land use, and relief.

Beta-diversity

The primary forest had higher total β-diversity than logged forest, pasture and secondary forest (Fig. 2). When partitioned, all land use showed high turnover, except by the secondary forest where the nested index was higher than turnover. Conserved forest and pasture were the highest in the turnover with values between 17 and 18%.

When dissecting the turnover in groups of environment components we found there are variations depending on land use. The geographical distance was most important in explaining the turnover for the LF ($r=0.84$, $p<0.001$) but not for the SF, where nestedness was more important independent of location ($r=0.13$, $p=0.116$). F and P also had a significant turnover ($p=0.005$ and0.012, respectively) but with low r values ($r=0.5$ and 0.3, respectively; Fig. 3). Climate variables, together, also explained turnover in LF ($r=0.79$, $p<0.001$). The LF and P also had a significant turnover due to climatic gradient ($p=0.005$ and $p=0.002$, respectively) but not F ($r=0.23$, $p=0.051$). Soil physical properties were more important in explaining turnover in SF ($r=0.57$, $p<0.001$) than in LF ($r=0.48$, $p=0.005$) and P ($r=0.41$, $p=0.006$) but was not significant in F ($r=0.02$, $p=0.6$). On the other hand, soil chemical attributes were more important in explaining turnover in P ($r=0.54$, $p<0.001$) than SF ($r=0.46$, $p<0.001$) and were not significant in explaining turnover of LF ($r=0.22$, $p=0.055$) and F ($r=0.21$, $p=0.055$), Fig 4.

The geographical distance was most important in explaining the turnover of F, while climate variables were more important in LF and physical and chemical factors in SF and P.

In conserved areas, climate, physical, and chemical variables were not important explaining the turnover, although there are differences in climate between the landscapes.

The correlation map of species and environmental variables showed the species responsible for creating turnover in a specific land use condition (Fig. 5). Univariate gradients explaining the turnover of species were found for each group of variables. *D. saliens* and *A. miricuri* in LF were negatively related to increase in precipitation and temperature, while *H. alatus* and *H. fernandoi* did positively, explaining the cause of turnover due to the climate gradient.

On the other hand, *A. miricuri* was related positively related to soil clay, moisture and silt contents in SF, while *P. corethrurus* was negatively related to clay and moisture. The opposite was found for bulk density where *A. miricuri* had a negative and highly significant correlation ($p<0.001$), contrary to *P. corethrurus* which had a positive correlation when increasing the soil compaction. In LF, *H. alatus* and *H. fernandoi* also preferred compacted soils different of *A miricuri*. This physical preference explained the gradient responsible for high turnover in SF and LF.

Finally, in pastures we found that Ca and pH were the most important chemical variables explaining the turnover with *H. alatus* preferring acid soils or low in Ca and *A. miricuri* inhabiting pastures with more alkaline soil.

Discussion

Richness and diversity estimation

This study represents the first formal report of earthworm diversity in the state of Maranhão, Brazil. More than 70% of the total species found in the different land use systems were native and several of them recently described as new species (Santos et al. 2017, Hernández-García et al. 2018a, 2018b), highlighting the importance of protected areas in maintaining soil biodiversity. Overall, twenty-three species were found in an area of X km^2, with 8-12 species per land use type. The accumulation species curves showed there was already saturating at the end of sampling. The knowledge of soil invertebrates species in this area is still poor and up to now only one study on the ant fauna was performed, which did not find species accumulation curve saturation in the land use system studied (Muñoz et al. 2017).

Qualitative sampling combined with quantitative is known to provide better species richness estimates in the Atlantic Forest biome (Bartz et al. 2014), and this was confirmed again in this study in Amazonia. Different species are known to occupy different niches, other than soil which are not contemplated (riverine habitats, seepages, termitaria, decomposing logs), or poorly contemplated (litter) in monolith sampling. Lower richness in upland, "terra firme" soils compared with lower lying riverine or wetland habitats had already been reported for the Manaus region (Ayres and Guerra 1981) and for French Guyana (Decaëns et al., 2016). This was also confirmed for our study site, and can be explained by factors, such as a lower soil moisture and nutrient efflux in upland soils. Low-lying areas also have higher chance to recruit different species coming from neighboring hilltops.

Using soil monoliths only, all forest systems always had higher species richness than pastures. However, with the combined monolith and qualitative data, these differences were maintained only in the CL, while in the DL, only FF had higher richness than P. On the other hand, differences in mean species richness between contrasting land uses such as forest and pasture were only found for low areas where F was richest. Richness in SF and LF also was conditioned to relief where top areas had low earthworm species richness.

Rare native species such as *Righiodrilus* or *Holoscolex* morphotypes increased species richness in Pasture when qualitative methods were used. On the other hand, species such as *A.*

miricuri and *P. awa* **n. sp**, inhabiting conserved forest or secondary forest survived in some pastures of DL, and this preference can explain why richness showed low changes in the vegetation gradient. The lack of significance of Shannon index suggests that alpha diversity is similar for all land uses, despite changes in local species composition. According to Wardle (2002) typical species richness values in earthworm communities is limited to 12 species. Richness values of forest found in this study were similar to that found by Nemeth and Herrera (1982) in Venezuelan primary forest, and higher than found by Rousseau et al. (2010) in secondary forests of the Para state, Brazil (Table 5). However, it was lower than those reported for primary forest (Lavelle and Pashanasi 1989, Barros et al. 2002, Castilho et al. 2016), secondary forest (Lavelle and Pashanasi 1989, Barros et al. 2002), and pasture of another Amazonian regions (Lavelle and Pashanasi 1989, Barros et al. 2002, Castilho et al. 2016). On the other hand, the Shannon diversity index was lower than those reported for similar land use type in the Brazilian Amazon biome and primary forest of Venezuela (Nemeth and Herrera 1982, Barros and Barbosa 2015, Castilho et al. 2016).

The Shannon index for the secondary forests was similar to that found by Rousseau et al. (2010) in a secondary forest of the Brazilian eastern Amazon, near to the Maranhão state. Rousseau et at. (2014) working with other groups of macrofauna in the Maranhão state suggested that there is an atypical low diversity of soil organism in the remnants forests of Maranhão and this can be critical when a disturbance occurs because of the high vulnerability in loss of native soil fauna. Traditionally, research had shown that primary forest or conserved forest have higher diversity than the most degraded areas, i.e. pastures (Lavelle and Pashanasi 1989, Fragoso and Lavelle 1992, Barros and Barbosa 2015, Castilho et al. 2016), but pastures near conserved areas of the Gurupi Biological Reserved are rich in native species remaining of conserved old growth forest. The species accumulation by land use is higher than the average of richness suggesting there is an important percentage of species substitution. Similar values found no changes in alpha diversity index, but in composition for ants communities inhabiting different land uses near to the Gurupi Biological Reserve (Solar et al. (2016).

We found that fragment forest between 0.17-21.6 km^2 in the degraded landscape can support equal earthworm diversity index that no interrupted forest of the conserved landscape, despite had been suggested that forest fragmentation affect other soil individuals as ants communities (Bierregaard et al. 2001). A degraded matrix as pasture can have a negative influence in soil eater-litter biodiversity when the fragment is reduced to less than 2 ha and could modify the soil moisture if the fragment is reduced to less than 100m in radius (Laurance et al. 2002).

Beta-diversity

According to (Legendre et al. 2009) there are different ways to understand the beta diversity patterns, por example Decaëns et al. (2016) studying the earthworm communities in different land use system from French Guiana dissected the total beta-diversity by region or station field and found a total value for Sorensen=0.69, which considered as a high value with a turnover representing 60.81 % of the total quantity. We decided to break the beta diversity by land use due to high heterogeneity when sum by the landscape, and we found maximum values less than 0.25 with the turnover representing more than 70% of the total beta diversity. In this situation, a great part of beta diversity is pure turnover, especially in the secondary forest.

The partition of different components (spatial, environmental, etc) is necessary to understand what is causing variation in the community as suggested initially for plant communities (Legendre et al. 2009) and then recommended to use by dissecting the total variability explained by each group of variables (Legendre and Cáceres 2013). This assumption was demonstrated recently in plants community where environmental variables were more important in explaining beta diversity patterns than spatial factors (Wang et al. 2019).

In this study, the spatial factor was strongly related to climate variables, and physical factors differed in pastures and secondary forest even if they were near each another. The turnover associated with the secondary forest was due to the physical and chemical gradients more than geographical.

The significance of each group of variables in determining land use indicates the main effect causing the change of species. For example, the geographical gradient is important in determining the turnover of logged forest, i.e. a great proportion of species found in the logged forest in conserved landscape change when we moved to the logged forest in the south. This proportion is less for pasture and forest. The same happens with chemical variables which indicate there is the chemical difference in pasture sampling that causes the turnover, i.e. pastures in the degraded landscape could have high turnover by differences in chemical properties of soil creating a gradient. Soil Physical and climate were the most significant group of variables in explains the turnover of earthworm species.

Inside each group of variables, there are one most important explaining the high variability, for example in pasture those variables relate to acidity-alkalinity (pH, Ca, Al) were determinant causing significative relationship with unique species as *Righiodrilus sp* MT1, *A. miricuri* (positive to acidity) and *P. corethrurus* (negative to acidity). pH had been found as the principal driver of different patterns in bacterial soil communities inhabiting anthropic areas (Zheng et al. 2019). On the other hand, others author suggested that pH was no important as a determinant of interspecific competition on earthworm but food font and native species can be advantaged in a poor environment (Winsome et al. 2006). The highest values of turnover in the secondary forest could be caused by two mechanisms: a high capacity of recolonization (Amazonas et al. 2018) and the highest habitat richness (Vanbergen et al. 2007) expressed as heterogeneity.

The dynamic of this process is complex and the beta diversity could be due to the fact that the species of soil fauna community also vary in the degree of sensitivity to various environmental factors (Zhukov et al. 2018). Our results suggest that there are habitats filling when the forest is transformed to pasture and different natives species could survive depending on physical-chemical conditions. Different gradients are activated depending on land use type and the heterogeneity is a crucial factor determining which type of species can survive to sum or decreasing the total diversity of the area.

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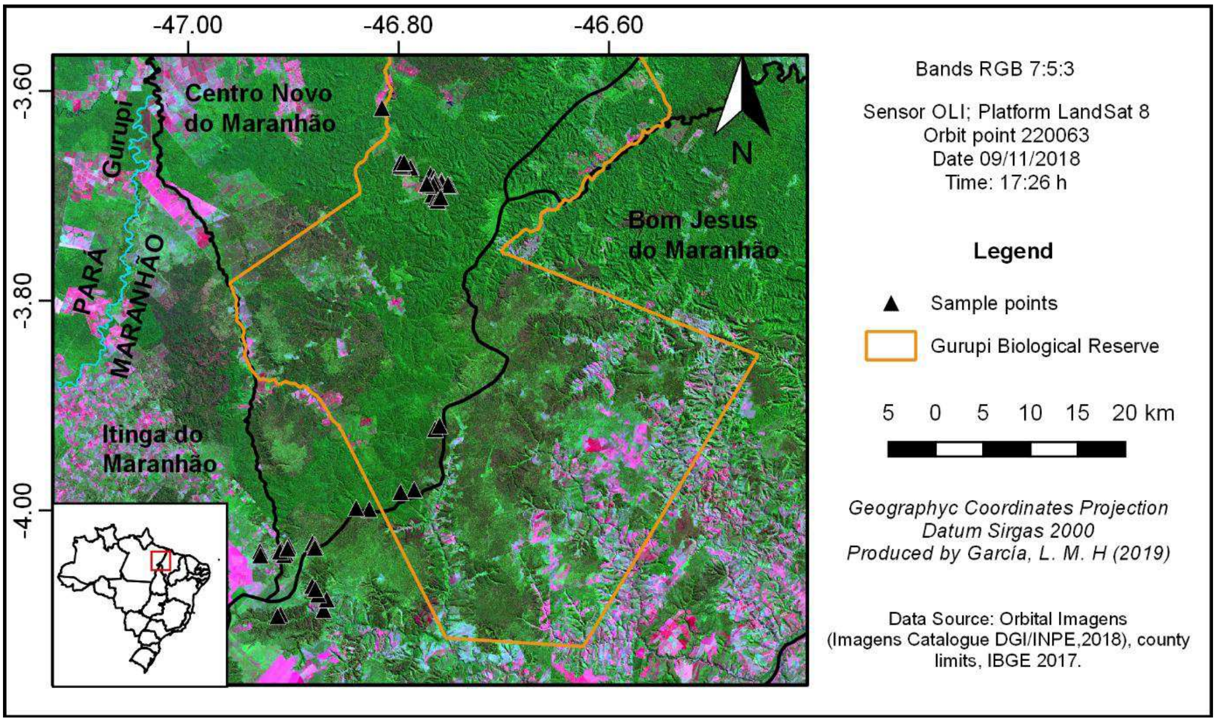


Fig 1. Sampling area in the Gurupi Biological Reserve and neighbor villages, Eastern Amazonia, Maranhão, Brazil.

Table. 1. Earthworm sampling localities in the Centro Novo do Maranhão, Bom Jardim and Itinga do Maranhão counties, Eastern Amazonia, Brazil. GBR: Gurupi Biological Reserve; HAS: Horizonte Azul Settlement; ICMBio: Base for biological research “Chico Mendes Institute”; GF: Galetti Farm road; RB: Bois`River.

County	Site	Vegetation Type	Land Use	Landscape	Topography	Latitude (S)	Longitude (W)	Altitude (m)
CN	GBR	Old-growth forest	F	CL	Upland	3°41'11"	46°45'34"	189
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'16"	46°45'34"	175
CN	GBR	Old-growth forest	F	CL	Upland	3°41'27"	46°45'06"	210
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'24"	46°45'12"	188
CN	GBR	Old-growth forest	F	CL	Upland	3°41'18"	46°45'57"	155
CN	GBR	Old-growth forest	F	CL	Lowland	3°41'27"	46°46'02"	146
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°40'50"	46°46'12"	154
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°40'59"	46°46'01"	182
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°41'10"	46°45'56"	158
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°41'20"	46°46'01"	172
CN	GBR	Logged old-growth forest	LF	CL	Lowland	3°41'26"	46°46'14"	134
CN	GBR	Logged old-growth forest	LF	CL	Upland	3°41'21"	46°46'24"	146
CN	GBR	Secondary Forest	SF	CL	Upland	3°41'56"	46°45'58"	152
CN	GBR	Secondary Forest	SF	CL	Upland	3°41'57"	46°46'05"	131
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'09"	46°45'51"	109
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'07"	46°45'43"	127
CN	GBR	Secondary Forest	SF	CL	Upland	3°42'18"	46°45'47"	107
CN	GBR	Secondary Forest	SF	CL	Lowland	3°42'07"	46°45'37"	134
CN	GBR	Pasture	P	CL	Upland	3°40'25"	46°47'20"	139
CN	GBR	Pasture	P	CL	Lowland	3°40'22"	46°47'47"	105
CN	GBR	Pasture	P	CL	Upland	3°40'17"	46°47'55"	145
CN	GBR	Pasture	P	CL	Lowland	3°40'08"	46°47'54"	108
CN	GBR	Pasture	P	CL	Upland	3°40'07"	46°47'42"	128
CN	GBR	Pasture	P	CL	Lowland	3°37'00"	46°48'57"	101

Table 1. (**Continued**). Earthworm sampling localities in the Centro Novo do Maranhão (CN), Bom Jardim (BJ) and Itinga do Maranhão (IT) counties, Eastern Amazonia, Brazil. GBR: Gurupi Biological Reserve; HAS: Horizonte Azul Settlement; ICMBio: Base for biological research "Chico Mendes Institute"; GF: Galetti Farm road; RB: Bois`River.

County	Site	Vegetation Type	Land Use	Landscape	Topography	Latitude (S)	Longitude (W)	Altitude (m)
IT	HAS	Old-growth forest fragment	F	DL	Upland	4°02'29"	46°54'31"	206
IT	HAS	Old-growth forest fragment	F	DL	Lowland	4°02'34"	46°54'36"	164
IT	HAS	Old-growth forest fragment	F	DL	Upland	4°02'04"	46°54'21"	234
IT	HAS	Old-growth forest fragment	F	DL	Lowland	4°02'12"	46°54'24"	175
BJ	HAS	Old-growth forest fragment	F	DL	Upland	4°05'58"	46°54'49"	229
BJ	HAS	Old-growth forest fragment	F	DL	Lowland	4°06'04"	46°54'59"	185
CN	ICMBio	Logged old-growth forest	LF	DL	Lowland	3°59'58"	46°49'44"	262
CN	GBR	Logged old-growth forest	LF	DL	Lowland	3°55'23"	46°45'58"	212
CN	GBR	Logged old-growth forest	LF	DL	Upland	3°58'50"	46°47'10"	250
CN	GBR	Logged old-growth forest	LF	DL	Lowland	3°58'59"	46°47'56"	181
CN	GBR	Logged old-growth forest	LF	DL	Upland	3°55'11"	46°45'42"	224
CN	ICMBio	Logged old-growth forest	LF	DL	Upland	3°59'54"	46°50'28"	285
CN	GF	Secondary Forest	SF	DL	Upland	4°01'54"	46°52'58"	302
BJ	BR	Secondary Forest	SF	DL	Lowland	4°05'07"	46°52'10"	144
BJ	BR	Secondary Forest	SF	DL	Lowland	4°04'50"	46°52'39"	149
BJ	BR	Secondary Forest	SF	DL	Lowland	4°04'32"	46°52'49"	141
CN	GF	Secondary Forest	SF	DL	Upland	4°02'01"	46°52'50"	294
CN	GF	Secondary Forest	SF	DL	Upland	4°02'10"	46°52'50"	294
IT	HAS	Pasture	P	DL	Lowland	4°02'21"	46°55'58"	196
IT	HAS	Pasture	P	DL	Upland	4°02'35"	46°55'57"	242
IT	HAS	Pasture	P	DL	Lowland	4°02'18"	46°54'45"	158
IT	HAS	Pasture	P	DL	Upland	4°02'22"	46°54'50"	179
BJ	BR	Pasture	P	DL	Lowland	4°04'20"	46°53'00"	139
BJ	BR	Pasture	P	DL	Upland	4°05'43"	46°52'21"	160

Table 2: Earthworm species abundance, families and origin collected by the TSBF method in different landscape and land uses in the state of Maranhão, Brazil. Families: G. Glossoscolecidae, R. Rhinodrilidae, A. Acanthodrilidae. Origin: NT. Native, EX. Exotic, PR. Peregrine. Landuse: F. Conserved Forest, FF. Conserved Forest remnants in fragment forest, LF. Logged Forest, SF. Secondary Forest, P. Pasture. Counties: CN. Centro Novo do Maranhão, It: Itinga do Maranhão, BJ. Bom Jesus do Maranhão. Numbers represent the sum of earthworms collected in six monoliths, each one measuring 25x25cm and 20cm in depth.

Genus/Species	Family	Origin	Conserved Landscape (CN)				Degraded Landscape (CN, It, BJ)				
			F	LF	SF	P	FF	LF	SF	P	Total
Holoscolex alatus	G	NT	5	1	-	2	2	-	-	-	10
Holoscolex fernandoi	G	NT	20	27	5	1	2	1	-	-	56
Holoscolex dossantosi	G	NT	1	-	-	-	-	-	-	-	1
Holoscolex sp (MT2)	G	NT	-	1	-	-	-	-	-	-	1
Holoscolex sp (MTgn)	G	NT	-	-	6	-	-	-	-	-	6
Holoscolex sp (MTgs1)	G	NT	-	-	-	-	-	-	1	-	1
Holoscolex sp (MTgs2)	G	NT	-	-	-	-	1	-	-	-	1
Righiodrilus gurupi	G	NT	2	1	-	-	-	-	-	-	3
Glossodrilus sp (MT1)	G	NT	2	-	-	-	-	-	-	-	2
Andiorrhinus (T) miricuri	R	NT	-	1	2	-	-	-	-	-	3
Andiorrhinus (T) barrosoi	R	NT	-	1	-	-	1	1	3	-	6
*Urobenu sp*1	R	NT	5	-	2	-	1	1	-	-	9
Pontoscolex awa. **sp.n**	R	NT	-	-	-	-	-	-	1	-	1
Pontoscolex corethrurus	R	PR	3	2	89	6	17	-	28	-	145
Dichogaster bolaui	A	EX	-	-	1	-	5	-	-	27	33
Dichogaster saliens	A	EX	-	-	-	-	3	14	-	5	22
Dichogaster afinis	A	EX	-	-	1	-	-	-	-	-	1
Juveniles (Not Identified)	-	-	173	284	245	304	252	34	104	319	1715
Richness	-	-	7	7	7	3	8	4	4	2	17
Total	-	-	211	318	351	313	284	51	137	351	2016

MT: Morphotype; G: Glossoscolecidae; R: Rhinodrilidae; A: Acanthodrilidae; NT: Native; EX: Exotic: PR: Peregrine

Table 3: Earthworm species abundance, families and origin collected in the plot (1ha) after the sum of the TSBF method and qualitative data obtained in different landscape and land uses in the state of Maranhão, Brazil. Families: G. Glossoscolecidae, R. Rhinodrilidae, A. Acanthodrilidae. Origin: NT. Native, EX. Exotic, PR. Peregrine. Landuse: F. Conserved Forest, FF. Conserved Forest remnants in fragment forest, LF. Logged Forest, SF. Secondary Forest, P. Pasture. Counties: CN. Centro Novo do Maranhão, It: Itinga do Maranhão, BJ. Bom Jesus do Maranhão.

Genus/Species	Family	Origin	CL (CN)				DL (CN, IT, BJ)				Total
			F	LF	SF	P	F	LF	SF	P	
Holoscolex alatus	G	NT	16	18	21	113	10	1	6	17	202
Holoscolex fernandoi	G	NT	128	261	64	9	48	31	3	19	563
Holoscolex dossantosi	G	NT	6	4	0	0	0	0	0	0	10
Holoscolex sp (MT1)	G	NT	3	2	0	0	0	0	0	0	5
Holoscolex sp (MT2)	G	NT	0	0	7	0	0	0	0	0	7
Holoscolex sp (MT3)	G	NT	0	0	0	0	1	0	20	6	27
Holoscolex sp (MT4)	G	NT	0	0	0	0	5	2	0	0	7
Righiodrilus gurupi	G	NT	39	24	6	6	0	9	0	0	84
Righidrilus sp (MT1)	G	NT	0	0	5	0	0	0	0	0	5
Glossodrilus sp (MT1)	G	NT	1	0	0	0	0	0	0	0	1
Glossodrilus sp (MT2)	G	NT	5	0	0	0	0	0	0	0	5
Andiorrhinus (T) miricuri	R	NT	11	4	10	0	23	34	10	16	108
Andiorrhinus (T) barrosoi	R	NT	0	1	4	12	1	0	1	0	19
Urobenu sp (MT1)	R	NT	6	2	3	3	2	2	0	1	19
Urobenu sp (MT2)	R	NT	0	0	0	0	1	0	0	0	1
Urobenu sp (MT3)	R	NT	0	0	0	1	0	0	0	0	1
Brasilisia sp (MT1)	O	NT	0	0	0	9	0	0	0	0	9
Pontoscolex awa. **sp.n**	R	NT	0	0	1	0	9	0	37	10	57
Pontoscolex corethrurus	R	PR	17	42	429	33	193	0	447	35	1196
Dichogaster bolaui	A	EX	2	3	2	92	5	47	2	222	375
Dichogaster saliens	A	EX	0	0	0	0	6	86	0	16	108
Dichogaster afinis	A	EX	0	0	2	0	0	0	0	0	2
Dichogaster sp (MT1)	A	EX	0	1	0	0	0	0	0	0	1
Juveniles (Not Identified)	-	-	161	130	242	298	258	118	114	323	1644
Richness	-	-	10	11	12	9	12	8	8	9	23
Total	-	-	395	492	796	576	562	330	640	665	4456

MT: Morphotype; G: Glossoscolecidae; R: Rhinodrilidae; O: Ocnerodrilidae; A: Acanthodrilidae; NT: Native; EX: Exotic: PR: Peregrine

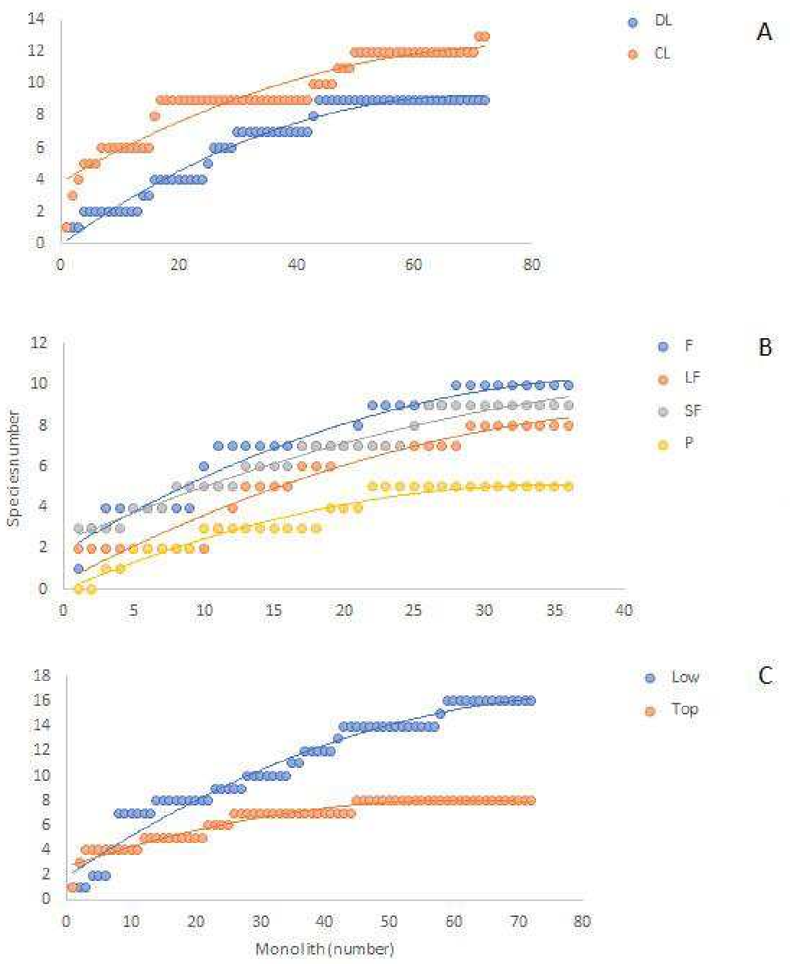


Fig 2. Accumulation curve for two different landscapes (A), and use (B), and relief (C) in the Grurupi Biological Reserve. CL: Conserved landscape, DL: Degraded landscape. F: Primary forest, LF: Logged forest, SF: Secondary forest, P: Pasture.

Table 4: Earthworm richness (S), and Shannon-Weiner diversity index (H´) by the landscape, land use, and relief. CL: Conserved landscape, DL: Degraded landscape. F: primary forest, LF: logged forest, SF: secondary forest, P: pasture.

Earthworm parameter	Landscape	Relief	Land Use			
			F	LF	SF	P
Richness	CL	Top	5.33±1.53aA^{a}	3.67±1.53aA^{b}	5.67±1.53aA^{a}	5.33±1.15aA^{a}
		Low	7.67±1.15aA^{a}	7±1abA^{a}	4.67±2.52 bA^{a}	4.67±1.53bA^{a}
	DL	Top	5.67±2.52aA^{a}	4.33±2.31aA^{a}	1.33±0.58bB^{b}	4±1aA^{a}
		Low	6.33±1.15aA^{a}	4±2aB^{a}	5±2aA^{a}	5±1aA^{a}
Shannon-Weiner	CL	Top	1.14±0.08$_{ns}$	0.56±0.48 $_{ns}$	1.3±0.63 $_{ns}$	0.93±0.33 $_{ns}$
		Low	1.42±0.33 $_{ns}$	1.34±0.28 $_{ns}$	0.63±0.51 $_{ns}$	1.1±0.18 $_{ns}$
	DL	Top	1.18±0.47 $_{ns}$	0.98±0.65 $_{ns}$	0.14±0.24 $_{ns}$	0.74±0.22 $_{ns}$
		Low	1.27±0.83 $_{ns}$	1.09±0.42 $_{ns}$	0.87±0.72 $_{ns}$	1.35±0.27 $_{ns}$

Small Letters: Difference between land use for a particular relief and landscape
Capital letters: Difference between landscape for a particular relief and land use
Superscripts: Difference between relief for particular land use and landscape
ns: no significative difference

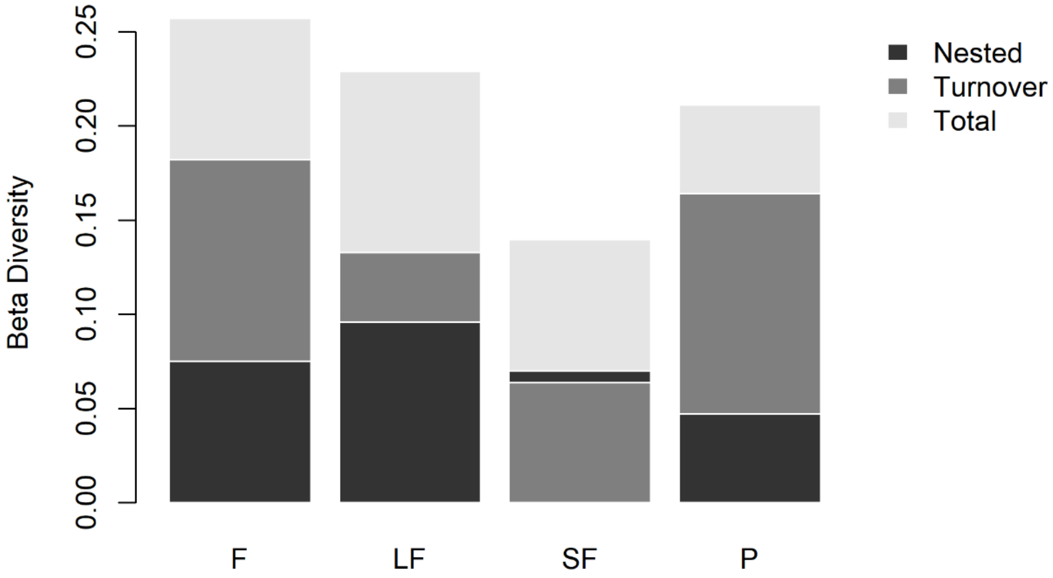


Fig 3. Beta diversity partitioning by different land use. F: primary forest, FF, fragment forest, LF: logged forest, SF: secondary forest, P: pasture.

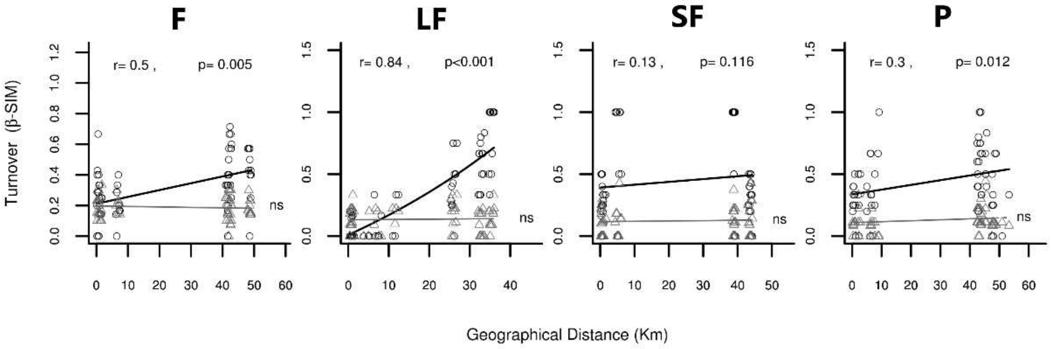


Fig 4. Beta diversity explained by the geographical distance in different land use. F: Old-growth forest, FF, Old-growth forest fragmented, LF: Logged old-growth forest, SF: secondary forest, P: pasture. black line: data model, gray line: null model.

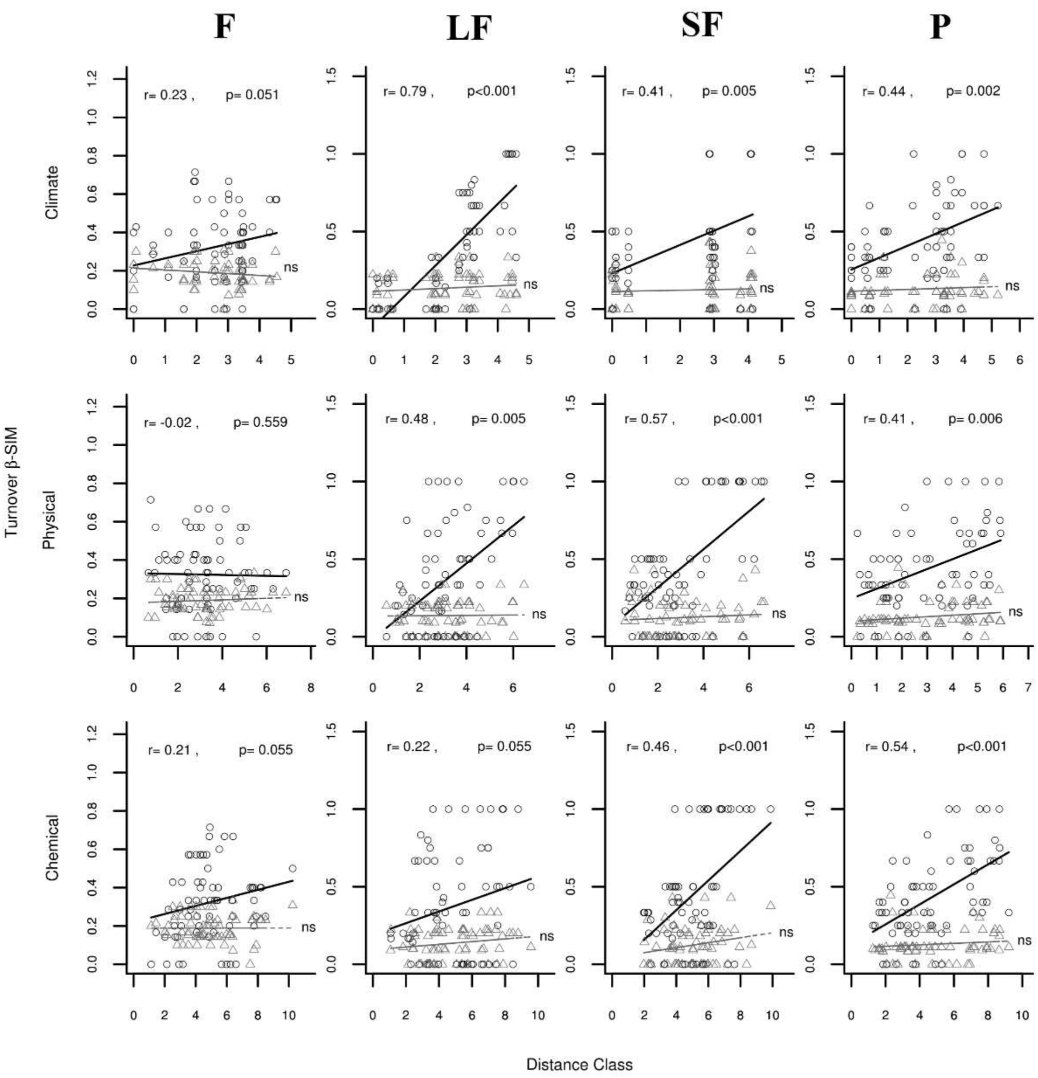


Fig 5. Beta diversity partitioning by different land use and according to environment components. F: Old-growth forest, FF, Old-growth forest fragmented, LF: Logged old-growth forest, SF: secondary forest, P: pasture. black line: data model, gray line: null model.

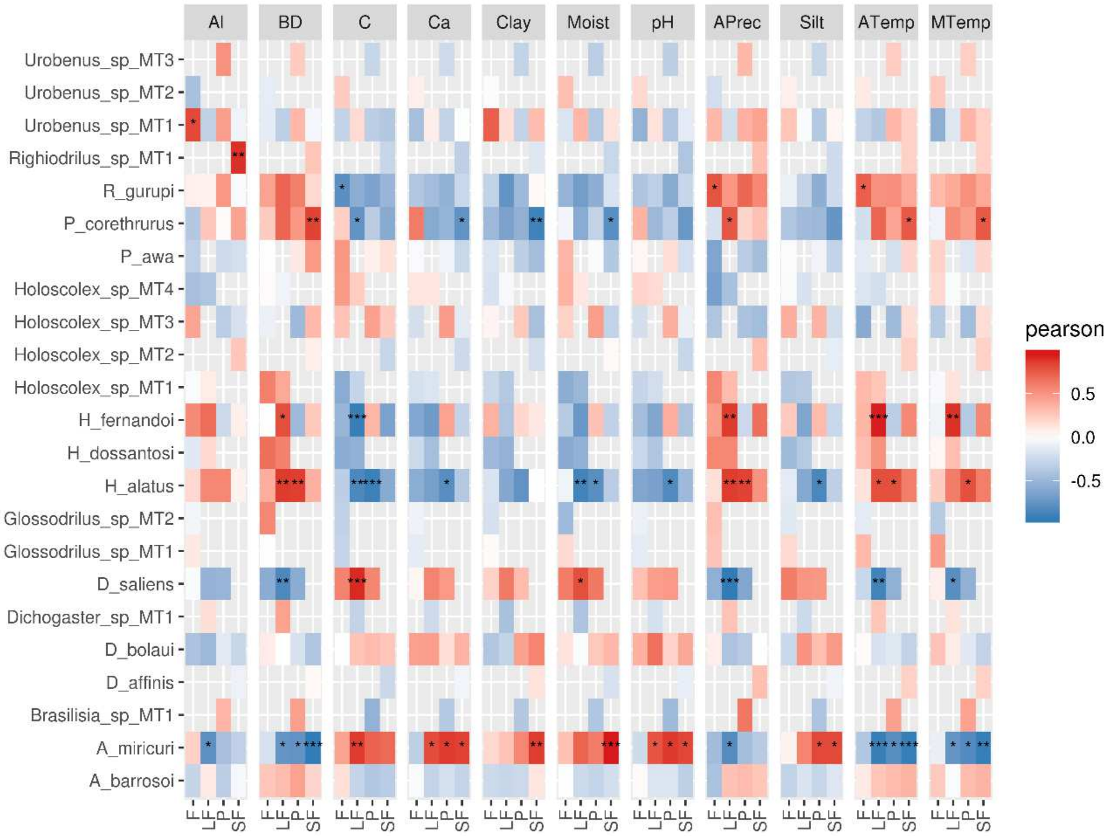
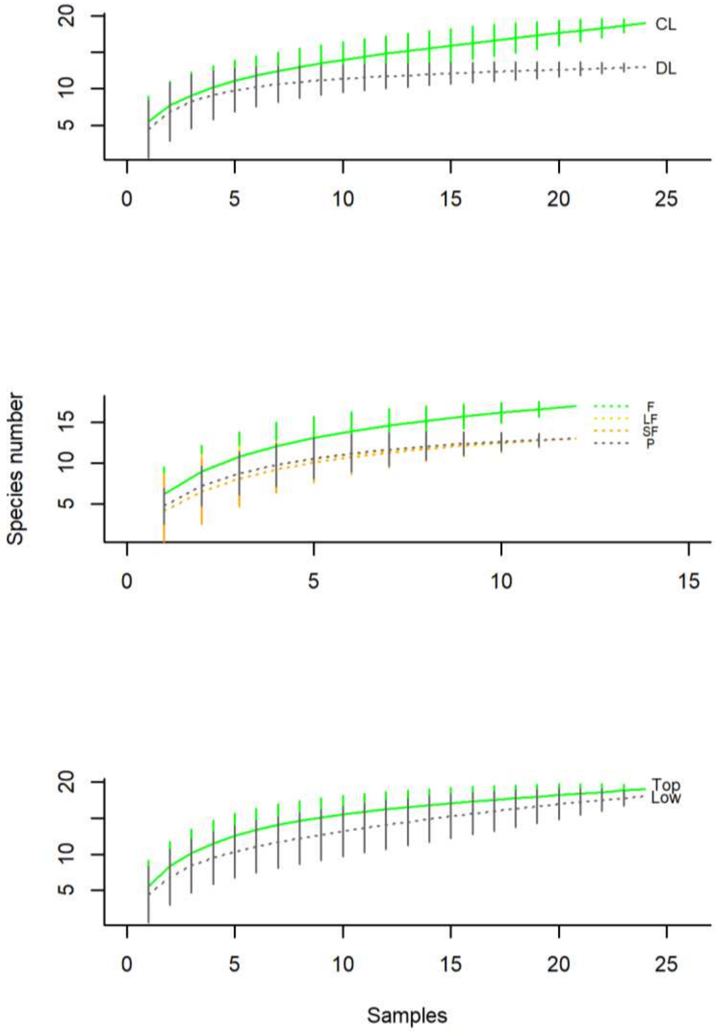


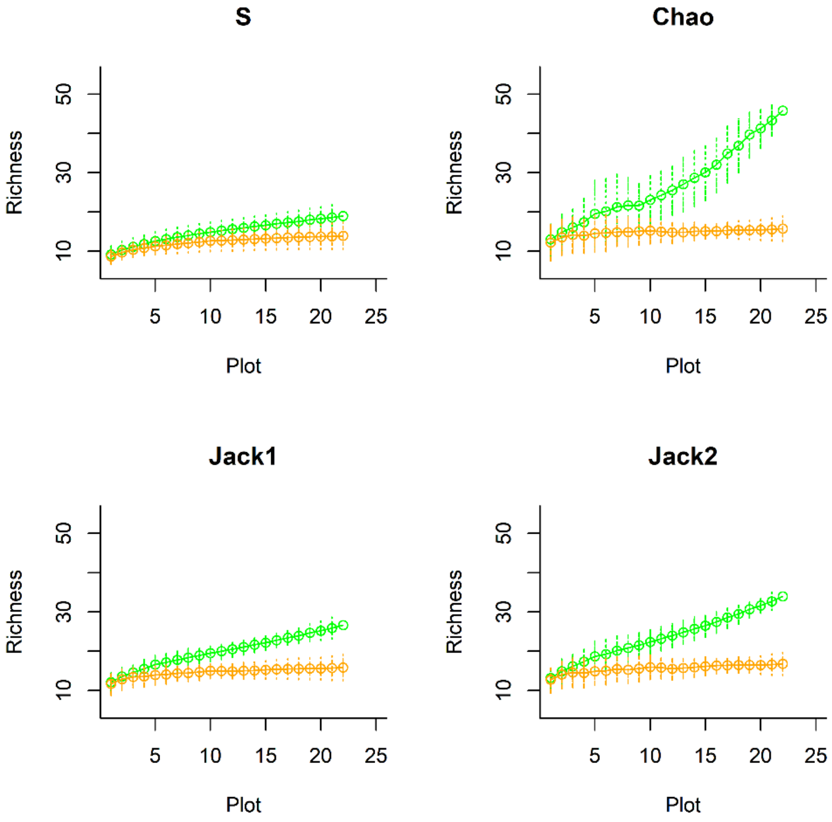
Fig 6. Correlations map for significant environmental variables and species according to land use. F: Old-growth forest, FF, Old-growth forest fragmented, LF: Logged old-growth forest, SF: secondary forest, P: pasture. pc: *Pontoscolex corethrurus*, ha: *Holoscolex alatus*, pgs1: *Pontoscolex* gs1, abar: *Andiorrhinus barrosoi*, rg: *Righiodrilus grurupi*, hd: *Holoscolex dossantosi*, db: *Dichogaster boloui*, btet: *Brasilisia* tet, ds: *Dichogaster saliens*, am: *Andiorrhinus* (*T.*) *miricuri*, uro1: *Urobenus* spn.1, hgs2: *Holoscolex* gs2, hf: *Holoscolex fernandoi*. rmin: *Righiodrilus* min, dgn: *Dichogaster* gn, uropig: *Urobenus* pig, urogur: *Urobenus* gur, ggurm: *Glossosdrilus* gurm, ggnmx: *Glossosdrilus* gnmx. Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 5: Comparative values for richness (S) and Shannon-Weiner (H`) parameters found in different land use system in the Amazon region.

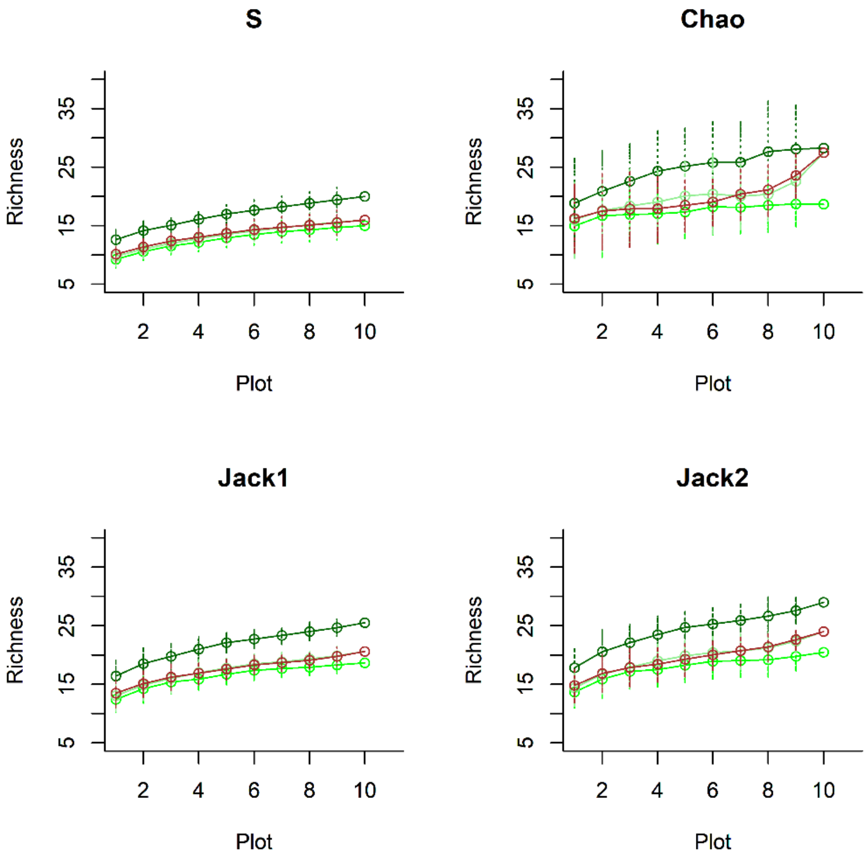
Country/State	Vegetation type	S	H`	Author
Venezuelan Amazon	Primary Forest	5-6	1.51-1.82	Nemeth and Herrera 1982
Peruvian Amazon	"moist" pasture	23	-	Lavelle and Pashanasi 1989
	"dry" pasture	20	-	
	Secondary Forest (15 years)	27	-	
	Primary Forest	41	-	
Brazilian Western Amazon, Rondônia	Pasture 1	9	1.34	Barros et al. 2002
	Pasture 2	10	2.57	
	Secondary Forest 1	15	1.96	
	Secondary Forest 2	17	1.7	
	Forest	16	2.26	
Brazilian Western Amazon, Acre	Pasture	10	1.27	
	Secondary Forest	14	2.75	
	Forest 1	13	2.37	
	Forest 2	9	2.02	
Brazilian Eastern Amazon, Pará	Secondary Forest	1.7 -2.3	0.25 - 0.72	Rousseau et al. 2010
Brazilian Eastern Amazon, Pará	Pasture	11	1.40	Castilho et al. 2016
	Forest	15	1.64	
French Guiana	Primary forest	7-12	-	Decaëns et al. 2016
	Secondary forest	10	-	
Brazilian Eastern Amazon, Maranhão	Pasture	4.50±1.05 - 5.00±1.26	1.02±0.26 - 1.05±0.40	This study
	Secondary Forest	3.17±2.40 - 5.17±1.94	0.50±0.63 - 0.97±0.63	
	Logged Forest	4.17±1.94 - 5.33±2.16	0.95±0.55 - 1.03±0.50	
	Forest	6.00±1.79 - 6.50±1.76	1.22±0.60 - 1.28±0.27	



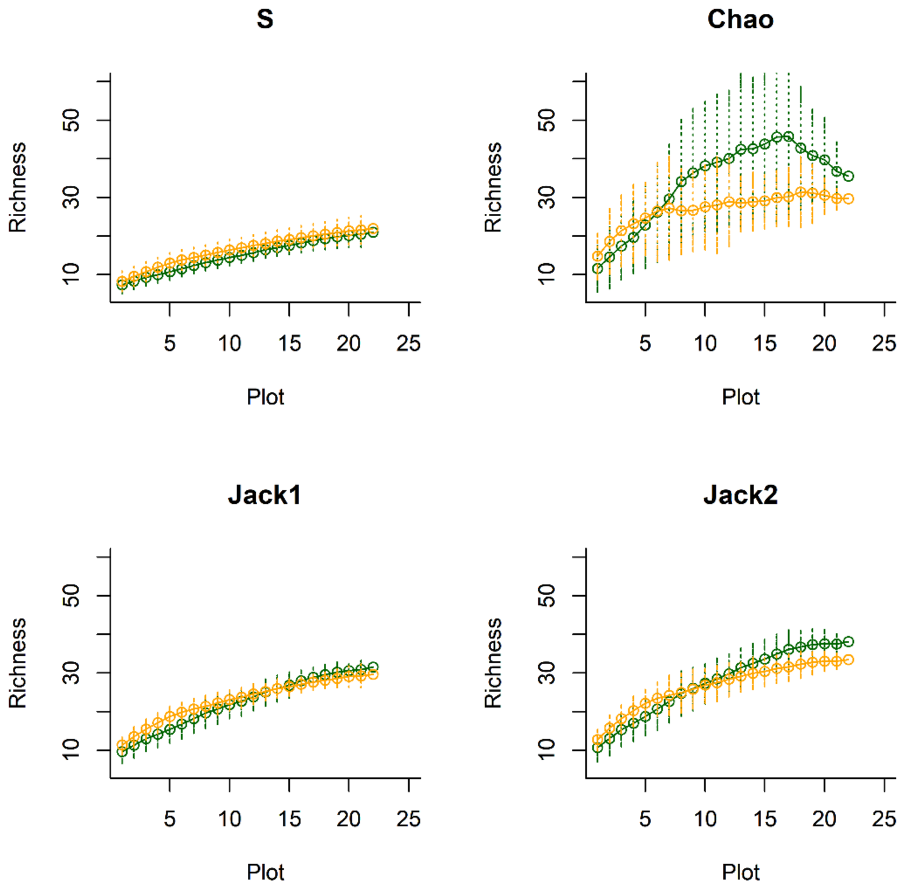
SM 1. Accumulation curve by sample plot (TSBF and qualitative) for two different landscapes (Upper), and use (Middle), and relief (bottom) in the Grurupi Biological Reserve. CL: Conserved landscape, DL: Degraded landscape. F: Primary forest, LF: Logged forest, SF: Secondary forest, P: Pasture.



SM 2. Earthworm richness rarefaction by topography. Green line: Lowland. Orange line: Upland.



SM 3. Earthworm richness rarefaction by land use. Wine line: Old-growth forest. Light blue: Logged old-growth forest. Dark green: Secondary forest. Green: Pasture.



SM 4. Earthworm richness rarefaction by landscape. Green line: Conserved. Orange line: Degraded.

8 DISCUSSÃO GERAL

Dentre os principais fatores limitantes para estimação adequada da diversidade das minhocas são a metodologia e o esforço amostral, portanto consideramos que o método proposto por Decaëns et al. (2016) valoriza alguns microhabitats que dificilmente poderiam ser amostrados com o método do TSBF clássico, e são esses microhabitats que permitiram obter espécies raras e inclusive espécies novas em estado adulto.

No total oito espécies novas foram divulgadas em revistas especializadas em taxonomia, das quais duas pertencem a dois gêneros novos (*Brasilisia* e *Arraia*) para a ciência e que são resistentes a ambientes halófitos. Duas espécies pertencentes ao gênero *Andiorrhinus* foram reportadas principalmente associadas com a floresta conservada na ReBio do Gurupi, no entanto a mesma espécie pode sobreviver em pastagens em paisagens degradadas. Geralmente o subgênero *Turedrilus* está associado à floresta amazônica (RIGHI, 1993; RIGHI; NEMETH, 1983), portanto este estudo é de grande importância porque relata como uma espécie de subgênero suporta o estresse causado pelo desmatamento.

Outros grupos representativos desta Floresta Amazônica oriental são as espécies nativas pertencentes aos gêneros *Holoscolex* e *Righiodrilus*, o que difere da região Sul do país onde os gêneros característicos são *Fimoscolex* e *Glossoscolex* (BARTZ et al., 2012, 2014). Segundo Fragoso et al. (1997, 1999) as florestas poderiam possuir maior riqueza que áreas degradadas, ou no pior caso transformados em culturas, contudo eles também enfatizam que se a perturbação não é forte nem prolongada as espécies nativas podem coexistir. Nossos resultados comprovam, diferentemente do encontrado por outros autores (CASTILHO et al., 2016a; FRAGOSO et al., 1999), que as florestas tropicais conservadas podem ter igual diversidade que pastagens.

Na Floresta da Amazônia Oriental brasileira, as minhocas exóticas com capacidade de colonizar o sistema perturbado são de origem africana, geralmente pertencentes ao gênero *Dichogaster*, o que difere das áreas temperadas da região Sul brasileira, onde minhocas de origem Européia ou Asiática também podem aparecer (GROSSO et al. 2006, NUNES et al. 2006; BARTZ et al. 2014). Assim como Bartz et al. (2014), em nosso estudo comprovamos que nas pastagens brasileiras onde não é introduzido pasto exótico podem coexistir certo grupo de minhocas nativas com as invasoras, mas dependendo da intensidade de perturbação as exóticas (mais adaptadas) podem acabar dominando certas pastagens com presença do gado.

Römbke et al. (2009) reportaram que *P. corethrurus* pode invadir as florestas e recentemente Marichal et al. (2012) mostraram que um pH alcalino e alto conteúdo de argila podem propiciar a colonização da espécie em áreas degradadas. Adicionalmente, Marichal et al. (2017) propuseram que o mecanismo de colonização nas pastagens está relacionado à presença de moela e tiflossol. Nossas amostragens revelaram que a maioria das espécies nativas que habitam as florestas apresenta moela desenvolvida, portanto poderia explicar a sua facilidade para sobreviver à perturbação.

Os valores médios de densidade de minhocas em florestas e pastagens foram menores que os reportados por outros autores na Amazônia Oriental brasileira (Rousseau et al. 2010, 2014, Castilho et al. 2016) e na Amazônia peruana (LAVELLE; PASHANASI, 1989), mas superou outros reportados para florestas secundárias em Rondônia e do Acre na Amazônia Ocidental brasileira (BARROS et al., 2002) e Amazônia venezuelana (NEMETH; HERRERA, 1982). Geralmente os trabalhos com foco na macrofauna do solo não reportam a biomassa das minhocas, portanto dificulta comparações. Os valores de biomassa neste estudo foram semelhantes aos encontrados por Nemeth e Herrera (1982) na Amazônia venezuelana e superaram os reportados por Barros et al (2002) para florestas primária e secundária nos estados de Rondônia e Acre. Contudo, foram inferiores aos reportados para a Amazônia peruana por Lavelle e Pashanassi (1989).

As variáveis mais importantes na determinação da estrutura comunitária das minhocas na localidade amostrada foram o clima, propriedades físicas do solo, propriedades químicas do solo e por último a localização geográfica. Fragoso et al. (1999) relataram que a temperatura parece não ter efeito como fator determinante no trópico para afetar significativamente a distribuição das minhocas, no entanto, nossos resultados mostram que pequenas variações poderiam ser significativas para determinar direta ou indiretamente a preferência de habitat.

No nosso modelo de β-diversidade as variáveis climáticas tiveram muita influência na explicação da distribuição das minhocas nos diferentes tipos de usos do solo. Igualmente podemos resumir como variáveis determinantes na física do solo a presença de areia grossa ou argila podendo criar gradientes físicos. Mas também este gradiente pode ser antrópico, por causa do efeito de pastoreio nas pastagens, que ocasiona um aumento considerável na densidade aparente. A variável química mais importante foi o teor de Ca, associado com muitas outras variáveis como pH, soma de bases, etc. Neste estudo consideramos um aumento de Ca na área degradada devido ao efeito do fogo recorrente, portanto também pode ser interpretado como um fator indireto de desmatamento.

Avaliando cada grupo de variável por separado constatamos que cada um tem efeito diferencial na substituição de espécies no sistema e cada tipo de uso de solo pode ser afetado por um grupo de variáveis distintas. É a primeira vez que se avalia separadamente o efeito de variáveis ambientais sobre a β-diversidade das comunidades de minhocas e sobre diferentes usos de solo. O último estudo abordando β-diversidade por uso de solo foi realizado por *Decaëns* et al. (2016), encontrando altos valores de substituição de espécies para duas áreas diferentes, mas considerando a soma dos usos de solo por localidade.

Neste estudo os valores globais são um pouco inferiores aos reportados por *Decaëns* et al. (2016), mas variáveis específicas permitem discernir a causa aparente dessas trocas de espécies e cada fator ambiental aporta uma porção. Nestas circunstâncias especiais onde os valores de abundância e biomassa de minhocas parecem não ter grandes efeitos, outros parâmetros populacionais podem mostrar mais claramente a dinâmica do ecossistema e estabelecer as bases de possíveis interações a serem estudadas futuramente. Para o caso de florestas isoladas consideramos que há grandes valores de substituição de espécies (alta β-diversidade), e esta substituição está refletida em parte pela resistência de algumas espécies nativas ao desmatamento (taxa de perda de espécies nativas é baixa).

Os resultados permitem comprovar que ambientes degradados dentro de paisagens conservadas hospedam tanta riqueza quanto ambientes conservados em paisagens degradadas e que grande parte da biodiversidade de oligoquetas endogéicos é recuperada nas florestas secundárias.

9 CONCLUSÕES

Os remanescentes florestais conservados da Amazônia oriental brasileira apresentam uma grande diversidade de espécies novas de oligoquetas para a ciência que deve ser considerada como uma realidade adicional para promover a conservação das áreas protegidas e incentivar políticas nas outras adjacentes. A abundância e biomassa de minhocas nesta área é muito menor que as estimadas para outras florestas próximas no bioma amazônico, mas a diversidade é muito maior. A transformação da floresta para pastagem ou capoeiras afeta a riqueza, mas não a diversidade local de espécies, no entanto apresenta alta substituição de espécies entre os usos do solo. A pesar dos fragmentos florestais apresentarem riqueza de espécies próximas a pastagens a sua estrutura comunitária é muito diferente sendo mais próxima de florestas conservadas.

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