

RESEARCH ARTICLE

Pontoscolex corethrurus: A homeless invasive tropical earthworm?

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Abstract

The presence of earthworm species in crop fields is as old as agriculture itself. The earthworms *Pontoscolex corethrurus* (invasive) and *Balanteodrilus pearsei* (native) are associated with the emergence of agriculture and sedentism in the region Amazon and Maya, respectively. Both species have shifted their preference from their natural habitat to the cropland niche. They contrast in terms of intensification of agricultural land use (anthropic impact to the symbiotic soil microbiome). *P. corethrurus* inhabits conventional agroecosystems, while *B. pearsei* thrives in traditional agroecosystems, i.e., *P. corethrurus* has not yet been recorded in soils where *B. pearsei* dwells. The demographic behavior of these two earthworm species was assessed in the laboratory over 100 days, according to their origin (OE; *P. corethrurus* and *B. pearsei*) food quality (FQ; soil only, maize stubble, *Mucuna pruriens*), and soil moisture (SM; 25, 33, 42%). The results showed that OE, FQ, SM, and the OE x FQ interaction were highly significant for the survival, growth, and reproduction of earthworms. *P. corethrurus* showed a lower survival rate (> mortality). *P. corethrurus* survivors fed a diet of low-to-intermediate nutritional quality (soil and stubble maize, respectively) showed a greater capacity to grow and reproduce; however, it was surpassed by the native earthworm when fed a high-quality diet (*M. pruriens*). Besides, *P. corethrurus* displayed a low cocoon hatching (emergence of juveniles). These results suggest that the presence of the invasive species was associated with a negative interaction with the soil microbiota where the native species dwells, and with the absence of natural mutualistic bacteria (gut, nephridia, and cocoons). These results are consistent with the absence of *P. corethrurus* in milpa and pasture-type agricultural niches managed by peasants (agroecologists) to grow food regularly through biological soil management. Results reported here suggest that *P. corethrurus* is an invasive species that is neither wild nor domesticated, that is, its eco-evolutionary phylogeny needs to be derived based on its symbionts.

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Citation: Ortíz-Ceballos AI, Ortiz-Gamino D, Andrade-Torres A, Pérez-Rodríguez P, López-Ortega M (2019) *Pontoscolex corethrurus*: A homeless invasive tropical earthworm? PLoS ONE 14(9): e0222337. <https://doi.org/10.1371/journal.pone.0222337>

Editor: Tunira Bhadauria, Feroze Gandhi Degree College, INDIA

Received: May 29, 2019

Accepted: August 27, 2019

Published: September 20, 2019

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0222337>

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Data Availability Statement: All relevant data are within the manuscript and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Although humans have produced novel niches prior to the advent of agriculture, the innovation of domestication led to changes in the life cycle of one or a few species, and the local microenvironments were manipulated, especially soil biota [1–4]. The artificial landscapes that resulted from these practices (anthropocentric ecology) were exported as agricultural packages from the centers of origin [1, 2, 4]. Thus, over a relatively short period in the history of mankind, the expansion of agriculture has brought about the remodeling of biodiversity as one of the most significant anthropogenic impacts on terrestrial ecosystems [1, 2, 5].

Agriculture has given rise to uniform and predictable disturbed ecological niches (invasible habitats), which have proven highly beneficial for non-domesticated species or weeds [1, 2, 6], and some earthworm species. Blakemore [7] has suggested that the origins of cosmopolitan (invasive) earthworms at family level are associated with domestication centers of plants and animals; that is, the presence of earthworms in crop fields is as old as agriculture itself [7–9]. The terms of the Millennium Ecosystem Assessment highlight the catalytic role of earthworms regarding two environmental services [10], namely the formation of soil and biogeochemical cycles, both of which are prerequisites for other environmental services [10–11].

Most of the studies focused on earthworms have used species adapted to crops, and most of them are currently considered as invasive [11]. It has been documented that 3% of the diversity of earthworms are invasive species [12]. As an example, European earthworms are frequently mentioned as the main cause of an irreversible change in the diversity and functioning of ecosystems in North America (Wisconsin glaciation areas) that were previously free from earthworms 12 thousand years ago [13–15]. However, there is a deeply rooted positive attitude toward earthworms in human populations in North America, acknowledging their beneficial effects on agricultural soils and urban gardens [10, 16].

Among the invasive tropical earthworms, the endogeic species *Pontoscolex corethrurus* was collected and described in crop fields in Blumenau, Brazil 160 years ago [17–18]; it has a broad distribution range and is the most studied tropical species [19–20]. Native species also move across a region in a similar way to invasive species, in addition to natural displacements [5, 7, 21]. The native endogeic earthworm *Balanteodrilus pearsei* was first collected and described from Gongora cave in Okcutzab, Yucatan 81 years ago [22]; it is distributed in the east and southeast of Mexico and Belize [19]; it dwells in natural and agricultural environments and is the most studied species native to Mexico. Most studies conducted with both species point to a positive influence of their biological activity on soil [20, 23], i.e., they do not meet the definition of pest [24]. For this reason, we use the term *invasive* with reference to the biogeographical status of the species, regardless of its impact on soil [24–25].

Similar to weeds [6, 8, 9, 26], it can be suggested that *P. corethrurus* and *B. pearsei* have shifted their preference from their natural habitat to agricultural environments, spreading geographically beyond their place of origin, and are currently key elements of agricultural environments. The presence of *P. corethrurus* and *B. pearsei* is associated with the development of pre-Columbian cultivation techniques in the Amazon [2, 27, 28, 29] and Maya [2, 30, 31] regions, respectively. For example, it is believed that *P. corethrurus* facilitated the formation of fertile soils in the Amazon area named "Terra Preta do Indo" [32, 33, 34, 35, 36, 37]. Both species have adapted to niches that emerged from agriculture [38], but contrast regarding the intensification of agricultural land use and/or the diversion of each from natural habitats (anthropic manipulation of soil). *P. corethrurus* is commonly found in conventional agroecosystems (use of fertilizers, herbicides, pesticides, and tillage), as well as in industrial (polluted with heavy metals, petroleum hydrocarbons, and others) and urban areas [20, 39, 40, 41]. *B. pearsei* inhabits soils managed under an agroecological approach (little human impact of the soil

microbiome), such as traditional agroecosystems (no use of industrial inputs) and in natural ecosystems [40, 42, 43]. *P. corethrurus* has been found coexisting with native species in some agroecosystems [41, 44, 45], but there are no records of its coexistence with *B. pearsei* so far [40, 42, 43].

A previous study of coexistence under controlled conditions showed no competitive interaction between *P. corethrurus* and *B. pearsei*, i.e., both can coexist [23]. However, the question to address is, why *P. corethrurus* has not invaded the agroecological niche of *B. pearsei*? Therefore, this work compared the demographic behavior of *P. corethrurus* vs. *B. pearsei* assuming that the survival rate of the invasive species decreases in soil populated by the native species.

Materials and methods

Ethics statement

No permits were required for the collection and laboratory trials. Soil and earthworms were provided by farmers with free of charge. The experimental procedure used in this study is detailed elsewhere [23].

Soil

Soil was collected from a maize field (MM) rotated with the tropical legume velvet bean (*Mucuna pruriens* var. *utilis*) located near the village Tamulté de las Sabanas (18°08'N, 92°47'W), 30 km east of Villahermosa, Tabasco, Mexico. The silty clay loam soil (41.5% sand; 26.8% clay; 31.6% silt) was air-dried in the shade at room temperature and sieved through a 2 mm mesh. The main chemical characteristics of this soil were: 2.7% organic matter; 0.14% total N; 11.4 C/N; pH (H₂O) of 6.3.

Earthworms

Two tropical endogeic earthworm species were used in this study: *B. pearsei* (native) and *P. corethrurus* (invasive). *B. pearsei* was collected from the MM field, whereas *P. corethrurus* was collected from pastures at Huimanguillo (79 km southwest Villahermosa, 17°48'N, 93°28'W), given its absence in the former site. All earthworms (120 for each species) were collected two weeks prior to the beginning of the experiment.

Food quality

The effects of food quality were assessed by using two different types of plant litter of contrasting nutritional quality: *M. pruriens* (52.4% C, 2.25% N, 23.3 C/N, and 9.67% ash) and maize stubble (52% C, 0.84% N, 61.9% C/N, and 10.3% ash). Both materials were obtained from the MM field, oven-dried at 60°C for 48 h, and sieved (1 mm).

Experiment

Growth, sexual maturity, reproduction (cocoons and juveniles), and mortality of *B. pearsei* and *P. corethrurus* were investigated during 100 days using a factorial design with three factors: origin of earthworms (OE), soil moisture (SM), and food quality (FQ). SM involved 3 levels, corresponding to the permanent wilt point (25%), field capacity (42%), and an intermediate level (33%). FQ included three levels: 300 g soil only (S), 294 g soil + 6 g maize stubble (MS), and 294 g soil + 6g *M. pruriens* (MP); the amounts added correspond to those commonly found in both maize monocultures and cultures rotating maize and *M. pruriens*. The earthworm species used belong to two different classes based on origin: Native (*B. pearsei*) and Invasive (*P. corethrurus*).

Table 1. F-values and significance levels (ANOVA) of the interaction of three factors on growth and reproduction of the tropical endogeic earthworm *Pontoscolex corethrurus* and *Balanteodrilus pearsei* at 100 days of culture in soil with low anthropic impact.

Independent variable	Biomass adult		Sexual maturity		Cocoon				Juveniles			
					Number		Biomass		Number		Biomass	
	F	P	F	P	F	P	F	P	F	P	F	P
Origen Earthworm (OE)	29.7	0.0000	22.3	0.0000	4.4	0.0390	2238.8	0.0000	25.1	0.0000	836.9	0.0000
Soil Moisture (SM)	20.4	0.0000	9.2	0.0002	22.2	0.0000	31.7	0.0000	14.8	0.0000	35.2	0.0000
Food quality (FQ)	191.7	0.0000	299.4	0.0000	109.0	0.0000	12.5	0.0004	67.0	0.0000	11.6	0.0006
OE×SM	0.03	0.9739	4.2	0.0164	4.4	0.0161	9.3	0.0000	5.0	0.0095	13.5	0.0000
OE×FQ	5.2	0.0068	3.2	0.0438	11.6	0.0000	3.5	0.0609	25.2	0.0000	10.8	0.0010
FQ×SM	7.3	0.0000	2.1	0.0897	13.4	0.0000	5.9	0.0029	11.4	0.0000	21.7	0.0000
OE×FQ×SM	1.1	0.3626	2.5	0.0442	6.4	0.0002	2.5	0.0859	6.2	0.0002	15.3	0.0000

<https://doi.org/10.1371/journal.pone.0222337.t001>

The combination of the three factors and three levels produced nine treatments with five replicates per treatment. Each replicate consisted of a plastic container (12×12×8 cm) containing 300 g dried soil of the corresponding food-soil mixture and soil moisture; two individuals of *B. pearsei* and two of *P. corethrurus* were transferred to each container (Table 1).

Earthworms were washed, dried on paper towels, weighed, and assigned randomly to each treatment. The baseline weight of the 45 replicates from the nine treatments was statistically similar in *B. pearsei* and *P. corethrurus* (76.06 ± 26.1 mg, n = 90 and 66.04 ± 31.1 mg, n = 90, respectively). Containers were incubated at 26 ± 1 °C. Body weight, mortality, clitellum appearance (sexual maturity), and number and biomass of cocoons and juveniles of *B. pearsei* and *P. corethrurus* were recorded at 10-day intervals, and soil was replaced. Before use, fresh soil (including the corresponding food-soil mixture and moisture) was preincubated for 8 days at 26 °C in order to trigger litter substrate decomposition. Each cocoon produced was incubated in a petri dish at 26 °C; incubation time as well as number and weight of all juveniles hatched were recorded.

Statistical analysis

Cocoon and juvenile weight, and growth were evaluated through Analysis of Variance (ANOVA). Mortality, sexual maturity, number of cocoons, and number of juveniles were analyzed using generalized linear models, specifically the Poisson distribution which is widely used for modelling count data. Differences between means were evaluated with Tukey’s HSD. All statistical analyses were performed using the Statistica software.

Results

At 100 days of culture, significant effects were observed between the origin of earthworms (OE), food quality (FQ) and soil moisture (SM), and the interaction between these three factors on sexual maturity, number of cocoons, and number and biomass of juveniles (Table 1).

Mortality

At the end of the culture, the invasive earthworm (*P. corethrurus*) had a 21.1% mortality rate in the soil treatment (33% and 25% SM), while that of the native earthworm (*B. pearsei*) had only a 1.1% mortality rate in the soil treatment (only 42% SM). In the *M. pruriens* and maize stubble treatments (25%, 33% and 42% SM) no mortality was observed in both earthworm species.

Growth

Growth of the endogeic earthworms clearly varied in response to EO, FQ, SM, and the EO \subseteq CF and SM \subseteq CF interactions (Table 1). At 100 days of culture, the growth of the invasive and native species (*P. corethrurus* and *B. pearsei*, respectively) was higher when food quality increased (Fig 1). In the three FQ levels (soil, maize stubble, and *M. pruriens*) the exotic species showed a faster growth (1.6, 9.4, and 12.3 mg/day, respectively) relative to the native species (0.34, 4.8, and 10.4 mg/day).

Reproduction

Sexual maturity (clitellum). When fed *M. pruriens*, the onset of sexual maturity in *P. corethrurus* and *B. pearsei* occurred at 30 days; when fed maize stubble, sexual maturity was observed at 30 and 70 days in *P. corethrurus* and *B. pearsei*, respectively.

At 100 days of culture, OE, FQ, SM, and the OE \subseteq CF \subseteq SM interaction significantly affected clitellum development (Table 1). The invasive and native earthworms reached sexual maturity in the treatments with *M. pruriens* (100% and 86.6%) and maize stubble (96.7% and 70.0%), respectively (Fig 2). No individuals reached sexual maturity after 100 days in the soil treatments; however, in the soil treatment with 33% SM, one earthworm of *P. corethrurus* (6.7%) reached sexual maturity at 80 days.

Cocoon production. *B. pearsei* and *P. corethrurus* displayed biparental and uniparental sexual reproduction, respectively. On *M. pruriens* treatments (25%, 33% and 42% SM), cocoon production started when *B. pearsei* and *P. corethrurus* reached a mean biomass of 773.5 ± 146.8 mg and 644.7 ± 71.1 mg (average of 25%, 33% and 42% SM), respectively. On maize stubble treatments, it started when *B. pearsei* and *P. corethrurus* reached a mean body weight of 593.0 ± 80.9 mg and 598.5 ± 95.2 mg (average of 25%, 33% and 42% SM), respectively. Cocoon production in *P. corethrurus* was observed in soil (6.7%), maize stubble (53.3%), and *M. pruriens* (86.7%) treatments, but in *B. pearsei* it was observed only in maize stubble (33.3%) and *M. pruriens* (86.7%) treatments.

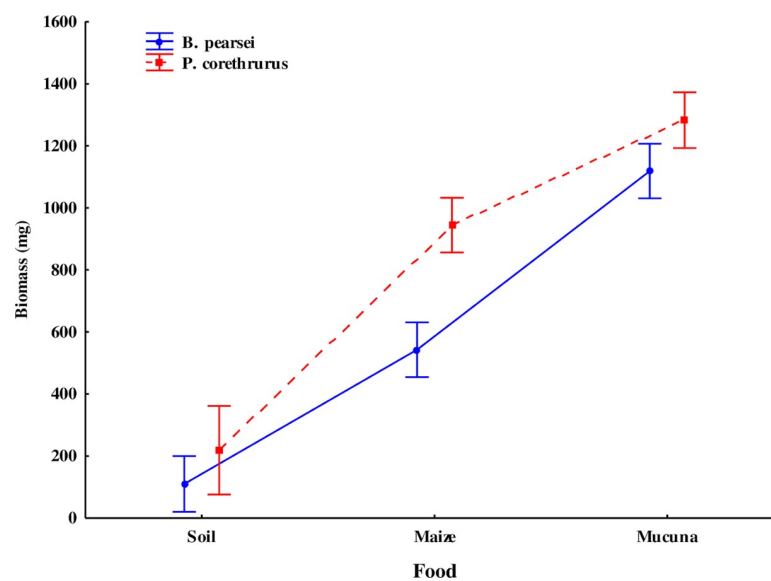


Fig 1. Biomass of the tropical endogeic earthworms *Pontoscolex corethrurus* (invasive) and *Balanteodrilus pearsei* (native) at 100 days of culture using three diets of different nutritional quality in soil with low anthropic impact. Vertical lines represent standard error.

<https://doi.org/10.1371/journal.pone.0222337.g001>

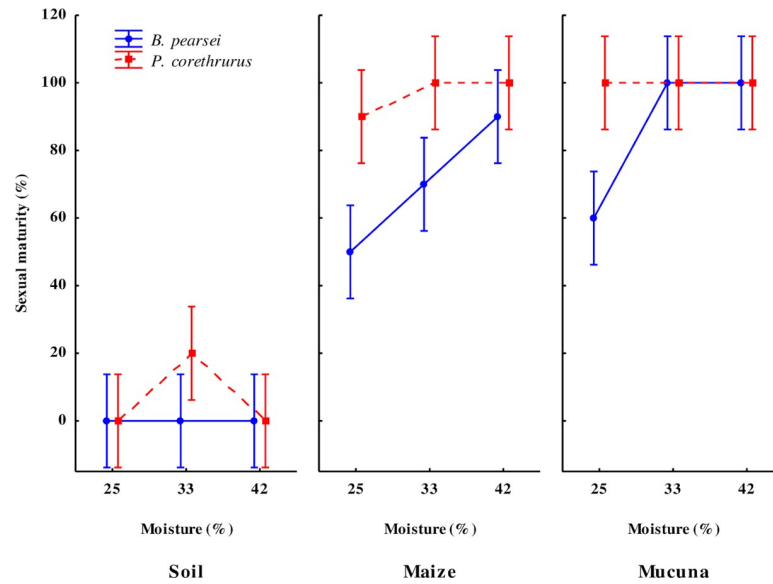


Fig 2. Sexual maturity (formation of the clitellum) in the tropical endogeic earthworms *Pontoscolex corethrurus* (invasive) and *Balanteodrilus pearsei* (native) at 100 days of culture under the interaction of three diets of different nutritional quality and three moisture content levels in soil with low anthropic impact. Vertical lines represent standard error.

<https://doi.org/10.1371/journal.pone.0222337.g002>

Mean cocoon production was significantly influenced by EO, CF, SM, and the interaction between these three factors (Table 1). After 100 days of culture, peak mean cocoon production in *B. pearsei* and *P. corethrurus* was observed in *M. pruriens* treatments, with 59.7 ± 40.8 and 35.5 ± 21.5 cocoons (average of 25%, 33% and 42% SM treatments), respectively (Fig 3). When fed maize stubble, *B. pearsei* and *P. corethrurus* produced 7.9 ± 3.2 and 14.4 ± 9.2 cocoons

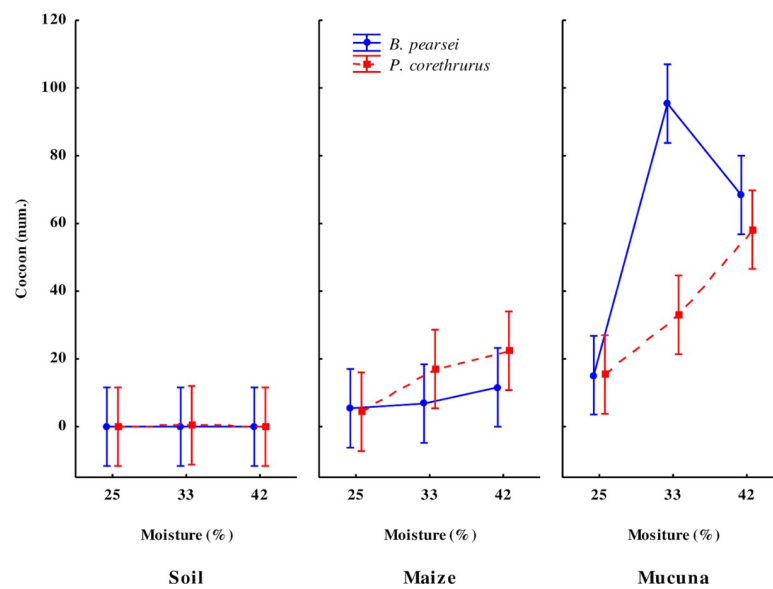


Fig 3. Number of cocoons produced by the tropical endogeic earthworms *Pontoscolex corethrurus* (invasive) and *Balanteodrilus pearsei* (native) at 100 days of culture under the interaction of three diets of different nutritional quality and three moisture content levels in soil with low anthropic impact. Vertical lines represent standard error.

<https://doi.org/10.1371/journal.pone.0222337.g003>

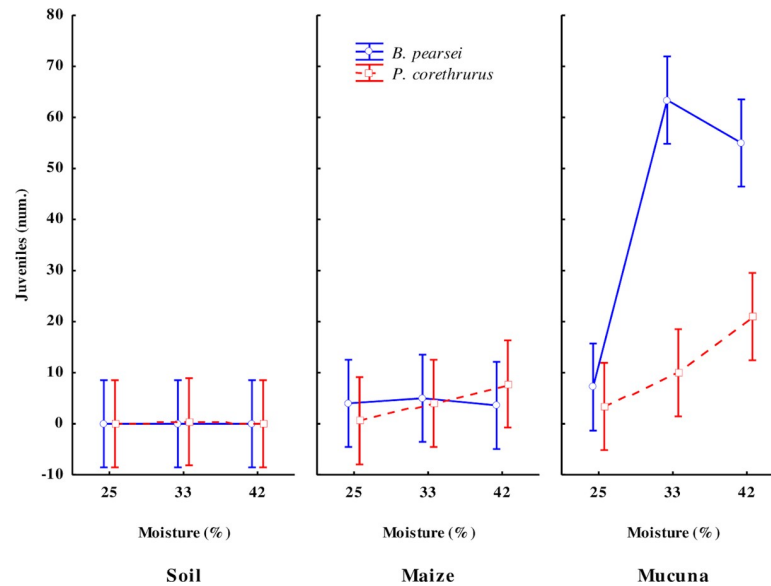


Fig 4. Number of juveniles hatched from cocoons produced by the tropical endogeic earthworms *Pontoscolex corethrurus* (invasive) and *Balanteodrilus pearsei* (native) at 100 days of culture under the interaction of three diets of different nutritional quality and three moisture content levels in soil with low anthropic impact. Vertical lines represent standard error.

<https://doi.org/10.1371/journal.pone.0222337.g004>

(average of 25%, 33% and 42% SM treatments), respectively. Finally, when fed soil only (33% SM), *P. corethrurus* (448 mg body weight) produced only two cocoons.

Cocoon biomass varied significantly in response to EO, FQ, SM and the OE x SM and FQ x SM interactions (Table 1). Average cocoon biomass produced by *B. pearsei* and *P. corethrurus* with SM treatments (25%, 33% and 42%) was 10.2 ± 1.4 mg and 27.7 ± 3.7 mg, respectively.

Juvenile production. The mean cocoon incubation time was similar among treatments ($P > 0.05$). In general, mean cocoon incubation time was 20.4 ± 5.2 days (*B. pearsei*) and 30.3 ± 2.2 days (*P. corethrurus*), with one individual hatching per cocoon in all cases. Of the total number of cocoons produced by *B. pearsei* and *P. corethrurus* in *M. pruriens* and corn stubble treatments, the average number of hatched juveniles was $64.7 \pm 16.6\%$ and $29.5 \pm 7.0\%$ (average of 25%, 33% and 42% SM treatments) and 59.5 ± 24.7 and 24.0 ± 10.6 (average of 25%, 33% and 42% SM treatments), respectively.

The number of hatched juveniles of *B. pearsei* and *P. corethrurus* varied significantly with OE, CF, SM, and the interaction between these three factors (Table 1; Fig 4). The mean number of hatched juveniles of *B. pearsei* and *P. corethrurus* increased in adults fed *M. pruriens*, as well as with increasing soil moisture (mean 59.7 ± 40.8 and 35.5 ± 21.5 individuals, respectively), and corn stubble (mean 7.9 ± 3.3 and 14.6 ± 9.2 individuals).

At hatching, in the *M. pruriens* and corn stubble treatments, mean biomass of *P. corethrurus* juveniles (21.2 ± 1.0 and 18.6 ± 7.4 mg, respectively) was higher vs. *B. pearsei* juveniles (8.5 ± 0.7 and 8.5 ± 1.3 mg, respectively).

Discussion

Domesticated, wild populations respond to changing selective pressures, which are reflected in their adaptation to agricultural niches [2, 46]. From an ecological perspective, the endogeic earthworm *P. corethrurus* resembles non-domesticated species or weeds given its strong profile (invading species) regarding growth rate, fertility, plasticity, interspecific competition, and

environmental tolerance [7, 8, 9, 26,]. This suggests that the four *P. corethrurus* ecotypes described by Taheri et al. [47] are likely the result of the selective forces imposed by cultivation, agricultural practices, and industrial and urban activities [20]. In the present study, soil in the habitat for *B. pearsei* was observed to restrain the presence of *P. corethrurus*.

The conversion of the Amazon forest to pastures led to the homogenization of soil biota [3, 48]. The potential resistance of soil (i.e., predators, low species richness, etc.) to earthworms has been documented [15, 49, 50]. For instance, the endogeic tropical earthworm *Millsonia anomala* from the savannah was unable to prosper in forest soil [49], similar findings have been reported with *P. corethrurus* from fallow (slash-and-burn) to mature forest [35]. Also, the shift in vegetation from grass to woody plants decreased in the density and biomass of *P. corethrurus* [51]. Our results showed that the survival of *P. corethrurus* was lower in the environment where *B. pearsei* thrives, maybe due to a negative interaction with a more diverse edaphic microbiome [49, 50, 52], because it has been suggested that *P. corethrurus* has a high ability to utilize soil organic resources as an energy source [39].

Earthworms harbor symbiotic microbiomes that are essential for their life history in the nephridia (excretory organs), and cocoons in tropical species such as *P. corethrurus* is poorly studied [53–58]. The microbiome is known to improve the nutritional status of low-quality diets [57–58]. For example, Topoliantz and Ponge [35] observed that the behaviour of two populations of *P. corethrurus* separated along the Maroni river (French Guiana, South America) differed significantly: fallow populations produced more cast on charcoal in the presence of forest soil, while the casting activity of the forest population was higher on soil regardless of the soil origin. Our findings show that *P. corethrurus* and *B. pearsei* differ in their diet preference (*M. pruriens*, corn stubble, and control), i.e., the invasive species displayed faster growth than the native species when nutritional quality improved. This suggests that *P. corethrurus* consumes and degrades a greater variety of organic materials given its greater ability (efficiency), evidenced by: a) producing endogenous cellulases [59–62]; b) its association with the gut microbiota [63–66]; c) gene expression (transcriptome) that contribute to the adaptation of its digestive system [65]; d) improving its digestion efficiency according to the type of cecum [59, 67]; and e) its association with nephridial bacteria [50, 68, 69].

It is known that in diets of low nutritional quality, mutualistic bacteria residing in earthworm nephridia (in 19 of 23 species studied) provide vitamins to its host, stimulate earlier sexual maturity, and contribute to pesticide detoxification [56, 57, 58, 60, 70, 71]. The results reported here showed that the invasive species of smaller size (biomass) fed on a lower nutritional diet (*M. pruriens* > corn stubble > soil) reached sexual maturity earlier than the native earthworm. This suggests that the nephridial symbionts of *P. corethrurus* are generalists, while those of *B. pearsei* are specialists.

Earthworms produce external cocoons that are colonized by bacteria from parents and soil [vertical and horizontal transmission, respectively 53, 58] and could be used as biovectors for the introduction of beneficial bacteria [55]. In a new habitat, cocoons of invasive earthworms may be affected by the native microbiota, but they can survive if they carry a parental microbial inoculum. Our results show that *P. corethrurus* produced cocoons when fed either of the three diets, while *B. pearsei* fed the control diet (only soil) failed to produce cocoons. In contrast, cocoons of *P. corethrurus* had a low hatching rate (births), which was lower (diet with *M. pruriens*) compared to *B. pearsei*. These results suggest the absence and/or loss of parental symbionts bacteria, i.e., the loss of a parental care strategy to control predators, detoxify nitrogenous wastes, conserve nitrogen, and supply vitamins and essential cofactors to the offspring [55, 56, 57, 68, 69, 70, 72]. Thus, the likely symbiotic evolution of *P. corethrurus* with the microbiome (gut, nephridia and cocoons) should be explored as a source of biogeography and phylogenetic

information [11, 57, 68, 70, 71, 73, 74]. That is, we could “. . .explain why *P. corethrurus* is rare or absent in undisturbed lands” [39].

The human-mediated translocation of species dates back to the Late Pleistocene [2, 5, 75]. Invasive plant species are usually divided in two groups according their residence time: archaeophytes were found from 1500 AD, and neophytes are found after this date [76]. This approach can contribute to elucidate the history of the invasion of *P. corethrurus* in Mexico. Until now, only two ecotypes have been recorded [47] and the cryptic lineage used in this study corresponds to L1 (the most widespread). The origin of *P. corethrurus* may be related to anthropogenic soil formation (“terras mulatas” and “terras pretas”). The domestication of manioc (bitter and sweet) and peach palm staple food that facilitated sedentary lifestyles in the Amazon region [5, 27, 28, 29, 32] has evolved to the point that we cannot recognize the predecessors of *P. corethrurus*, as evidenced by the recent designation of the *P. corethrurus* neotype from an anthropogenic environment [18] and temperate climate [77], and by the ambiguity used for assigning its place of origin [12, 78].

Based on the results reported here, we conclude that the invasive tropical earthworm *P. corethrurus* had lower survival and cocoons hatching rates (offspring) in the agro-ecological niche of the native endogeic earthworm, i.e., a finding consistent with the absence of *P. corethrurus* in parcels where maize- and *M. pruriens* crop rotation is practiced, as well as in pastures and other traditional tropical agroecosystems [40, 41, 42, 43, 44, 45]. This suggests that *P. corethrurus* is an invasive species that thrives far from its natural status, i.e., has no wild ancestry in the study area. Therefore, it is important to determine the preference of the four *P. corethrurus* ecotypes [47] in terms of soil type, cultivation, response to stressors and climate change.

Supporting information

S1 Table. Results fitting linear model of the earthworm biomass.
(PDF)

S2 Table. Results fitting logistic model of sexual maturity of the earthworms.
(PDF)

S3 Table. Results fitting zero inflated poisson of the earthworms cocoons.
(PDF)

Acknowledgments

The authors thank Mario M. Osorio-Arce, Angel Ramos-Sánchez and Efraín Hernández-Xolocotzi, promoters of agroecology in Mexico. In addition, we are grateful to anonymous reviewers and Diana Pérez-Staples for valuable comments and careful revision of the manuscript.

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References

1. Bender SF, Wagg C, van der Heijden MGA. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. *Trends in Ecology and Evolution* 2016; 31(6):440–452. <https://doi.org/10.1016/j.tree.2016.02.016> PMID: 26993667
2. Boivin NL, Zeder MA, Fuller DQ, Crowther A, Larson G, Erlandson JM, et al. Ecological consequences of human niche construction: Examining long-term anthropogenic shaping of global species distributions. *Proceedings of the National Academy of Sciences*. 2016; 113(23):6388–6396. <https://doi.org/10.1073/pnas.1525200113> PMID: 27274046
3. Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology*. 2016; 90(6):635–644. <https://doi.org/10.1007/s11103-015-0337-7> PMID: 26085172
4. Fuller DQ, Lucas L. Adapting crops, landscapes, and food choices: Patterns in the dispersal of domesticated plants across Eurasia. In: Boivin N, Crassard R, Petraglia M, editors. *Human Dispersal and Species Movement: From Prehistory to the Present*. Cambridge: Cambridge University Press; 2017. pp. 304–331.
5. Lodge DM. Biological invasions: lessons for ecology. *Trends in Ecology & Evolution*. 1993; 8(4):133–137
6. Fuller DQ, Stevens CJ. Open for Competition: Domesticates, Parasitic Domesticoids and the Agricultural Niche. *Archaeology International*. 2017; 20:110–121. <https://doi.org/10.5334/ai.359>
7. Blakemore RJ. Cosmopolitan Earthworms—A Global and Historical Perspective. In Shain DH, editor. *Annelids in Modern Biology*. New York: John Wiley & Sons, Inc.; 2009. pp. 257–283.
8. Vigueira CC, Olsen KM, Caicedo AL. The red queen in the corn: Agricultural weeds as models of rapid adaptive evolution. *Heredity*. 2013; 119:303–311. <https://doi.org/10.1038/hdy.2012.104> PMID: 23188175
9. Mercuri AM, Fornaciari R, Gallinaro M, Vanin S, Di Lernia S. Plant behaviour from human imprints and the cultivation of wild cereals in Holocene Sahara. *Nature Plants*. 2018; 4(2):71–81. <https://doi.org/10.1038/s41477-017-0098-1> PMID: 29379157
10. Plaas E, Meyer-Wolfarth F, Banse M, Bengtsson J, Bergmann H, Faber J, et al. Towards valuation of biodiversity in agricultural soils: A case for earthworms. *Ecological Economics*. 2019; 159:291–300. <https://doi.org/10.1016/j.ecolecon.2019.02.003>
11. Brussaard L, Aanen DK, Briones MJ, Decaëns T, Deyn GBD, Fayle TM, et al. Biogeography and Phylogenetic Community Structure of Soil Invertebrate Ecosystem Engineers: Global to Local Patterns, Implications for Ecosystem Functioning and Services and Global Environmental Change Impacts. In: *Soil Ecology and Ecosystem Services*. Wall DH, Bardgett RD, Behan-Pelletier V, Henrick J, Jones H, Ritz K, et al. editors. Oxford: University Press; 2013. pp. 201–232.
12. Dupont L, Decaëns T, Lapied E, Chassany V, Marichal R, Dubs F, et al. Genetic signature of accidental transfer of the peregrine earthworm *Pontoscolex corethrurus* (Clitellata, Glossoscolecidae) in French Guiana. *European Journal of Soil Biology*. 2012; 53:70–75. <https://doi.org/10.1016/j.ejsobi.2012.09.001>
13. Uvarov AV. (Inter- and intraspecific interactions in lumbricid earthworms: Their role for earthworm performance and ecosystem functioning. *Pedobiologia* 2009; 53(1):1–27. <https://doi.org/10.1016/j.pedobi.2009.05.001>
14. Lobe JW, Callahan MA, Hendrix PF, Hanula JL. Removal of an invasive shrub (Chinese privet: *Ligustrum sinense* Lour) reduces exotic earthworm abundance and promotes recovery of native North American earthworms. *Applied Soil Ecology*. 2014; 83:133–139. <https://doi.org/10.1016/j.apsoil.2014.03.020>
15. Vestergård M, Rønn R, Ekelund F. Above-belowground interactions govern the course and impact of biological invasions. *AoB PLANTS*. 2015; 7:plv025. <https://doi.org/10.1093/aobpla/plv025> PMID: 25854693
16. Simmons W, Dávalos A, Blossey B. Forest successional history and earthworm legacy affect earthworm survival and performance. *Pedobiologia*. 2015; 58(4):153–164. <https://doi.org/10.1016/j.pedobi.2015.05.001>
17. Müller F. II.—Description of a new species of Earthworm (*Lumbricus corethrurus*). *Annals and Magazine of Natural History*. 1857; 20(115):13–15. <https://doi.org/10.1080/00222935709487865>

18. James SW, Bartz MLC, Stanton DWG, Conrado AC, Dupont L, Taheri S., et al. A neotype for *Pontoscolex corethrurus* (Müller, 1857) (Clitellata). *Zootaxa*. 2019; 4545(1):124–132. <https://doi.org/10.11646/zootaxa.4545.1.7> PMID: 30647239
19. Fragoso GC. Importancia de las lombrices de tierra (Oligochaeta) en el monitoreo de áreas prioritarias de conservación del centro, este y sureste de México. CONABIO. 2018. Available from: <https://doi.org/10.15468/omvnpi> accessed via [GBIF.org](https://www.gbif.org) on 2019-05-01
20. Taheri S, Pelosi C, Dupont L. Harmful or useful? A case study of the exotic peregrine earthworm morphospecies *Pontoscolex corethrurus*. *Soil Biology and Biochemistry*. 2018b; 116:277–289. <https://doi.org/10.1016/j.soilbio.2017.10.030>
21. Nackley LL, West AG, Skowno AL, Bond WJ. The Nebulous Ecology of Native Invasions. *Trends in Ecology and Evolution*. 2017; 32(11):814–824. <https://doi.org/10.1016/j.tree.2017.08.003> PMID: 28890126
22. Orell T. NMNH Extant Specimen Records. Version 1.2. National Museum of Natural History, Smithsonian Institution. 2019. Available from: 10.15468/hnhr3 accessed via [GBI.org](https://www.gbif.org) on 2019-05-01. <https://www.gbif.org/occurrence/1318951655>
23. Ortiz-Ceballos AI, Fragoso C, Equihua M, Brown GG. Influence of food quality, soil moisture and the earthworm *Pontoscolex corethrurus* on growth and reproduction of the tropical earthworm *Balanteodrilus pearsei*. *Pedobiologia*. 2005; 49(1):89–98. <https://doi.org/10.1016/j.pedobi.2004.08.006>
24. Richardson DM, Pyšek P, Rejmánek M, Barbour MG, Panetta FD, West CJ. Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*. 2000; 6(2):93–107. <https://doi.org/10.1046/j.1472-4642.2000.00083.x>
25. Ricciardi A, Cohen J. The invasiveness of an introduced species does not predict its impact. *Biological Invasions*. 2007; 9(3):309–315. <https://doi.org/10.1007/s10530-006-9034-4>
26. Willcox G. The Beginnings of Cereal Cultivation and Domestication in Southwest Asia. In: Potts DT, editor. *A Companion to the Archaeology of the Ancient Near East*. London: Blackwell Publishing Ltd. 2012; pp. 161–180. <https://doi.org/10.1002/9781444360790.ch9>
27. Clement CR, Denevan WM, Heckenberger MJ, Junqueira AB, Neves EG, Teixeira WG, et al. The domestication of amazonia before european conquest. *Proceedings of the Royal Society B: Biological Sciences* 2015; 282:20150813. <https://doi.org/10.1098/rspb.2015.0813> PMID: 26202998
28. Levis C, Flores BM, Moreira PA, Luize BG, Alves RP, Franco-Moraes J, et al. How People Domesticated Amazonian Forests. *Frontiers in Ecology and Evolution*. 2018 Jan 17. Available from: <https://doi.org/10.3389/fevo.2017.00171>
29. Watling J, Shock MP, Mongeló GZ, Almeida FO, Kater T, De Oliveira PE, et al. Direct archaeological evidence for Southwestern Amazonia as an early plant domestication and food production centre. *Plos One*. 2018; 13(7):e0199868. <https://doi.org/10.1371/journal.pone.0199868> PMID: 30044799
30. Ford A, Nigh R. Origins of the Maya Forest Garden: Maya Resource Management. *Journal of Ethnobiology*. 2009; 29(2):213–236. <https://doi.org/10.2993/0278-0771-29.2.213>
31. McNeil CL. Deforestation, agroforestry, and sustainable land management practices among the Classic period Maya. *Quaternary International*. 2012; 249:19–30. <https://doi.org/10.1016/j.quaint.2011.06.055>
32. Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W. Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Geochemistry*. 2000; 31:669–678. [https://doi.org/10.1016/S0146-6380\(00\)00044-9](https://doi.org/10.1016/S0146-6380(00)00044-9)
33. Lima HN, Schaefer ER, Mello JWV, Gilkes RJ, Ker JC. Pedogenesis and pre-Colombian land use of “Terra Preta Anthrosols” (“Indian black earth”) of Western Amazonia. *Geoderma*. 2002; 110:1–17.
34. Schaefer CEGR Lima HN, Gilkes RJ Mello JWV. Micromorphology and electron microprobe analysis of phosphorus and potassium forms of an Indian Black Earth (IBE) Anthrosol form Western Amazonia. *Australian Journal of Soil Research*. 2004; 24(4):401–409.
35. Topoliantz S, Ponge JF. Charcoal consumption and casting activity by *Pontoscolex corethrurus* (Glossoscolecidae). *Applied Soil Ecology*. 2005; 28:217–224.
36. Ponge JF, Topoliantz S, Ballof S, Rossi JP, Lavelle P, Betsch JM, et al. Ingestion of charcoal by the Amazonian earthworm *Pontoscolex corethrurus*: A potential for tropical soil fertility. *Soil Biology and Biochemistry*. 2006; 38(7):2008–2009. <https://doi.org/10.1016/j.soilbio.2005.12.024>
37. Kim JS, Sparovek G, Longo RM, de Melo WJ, Crowley D. Bacterial diversity of terra and pristine forest soil from Western Amazon. *Soil Biology & Biochemistry*. 2007; 39:684–690.
38. Laland KN, Odling-Smee FJ, Feldman MW. Evolutionary consequences of niche construction and their implications for ecology. *Proceedings of the National Academy of Sciences*. 1999; 96(18):10242–10247.
39. Lavelle P, Barois I, Cruz I, Fragoso C, Hernandez A, Pineda A, Rangel P. Adaptive strategies of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta), a peregrine geophagous earthworm of the humid tropics. *Biology and Fertility of Soils*. 1987; 5(3):188–194. <https://doi.org/10.1007/BF00256899>

40. Fragoso C, Leyequién E, García-Robles M, Montero-Muñoz J, Rojas P. Dominance of native earthworms in secondary tropical forests derived from slash-and-burn Mayan agricultural practices (Yucatán, Mexico). *Applied Soil Ecology*. 2016; 104:116–124. <https://doi.org/10.1016/j.apsoil.2015.12.005>
41. Marichal R, Martinez AF, Praxedes C, Ruiz D, Carvajal AF, Oszwald J, et al. Invasion of *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta) in landscapes of the Amazonian deforestation arc. *Applied Soil Ecology*. 2010; 64(3):443–449. <https://doi.org/10.1016/j.apsoil.2010.09.001>
42. Ortiz-Ceballos AI, Fragoso C. Earthworm populations under tropical maize cultivation: the effect of mulching with Velvetbean. *Biol. Fert. Soils*. 2004; 39:438–445
43. Huerta E, Fragoso C, Rodríguez-Olan J, Evia-Castillo I, Montejo-Meneses E, Cruz-Mondragon M, García-Hernández R. Presence of exotic and native earthworms in principal agro- and natural systems in Central and Southeastern Tabasco, Mexico. *Caribbean Journal of Science*. 2006; 42(3):359–365.
44. Lavelle P, Maury ME, Serrano V. Estudio cuantitativo de la fauna del suelo en la región de Laguna Verde, Veracruz. *Publicaciones Instituto de Ecología (México)*. 1981; 6:75–105.
45. Ortiz-Gamino D, Pérez-Rodríguez P, Ortiz-Ceballos AI. Invasion of the tropical earthworm *Pontoscolex corethrurus* (Rhiodrilidae, Oligochaeta) in temperate grasslands. *PeerJ*. 2016; 4:e2572. <https://doi.org/10.7717/peerj.2572> PMID: 27761348
46. Stitzer MC, Ross-Ibarra J. Maize domestication and gene interaction. *New Phytologist*. 2018; 220:395–408. <https://doi.org/10.1111/nph.15350> PMID: 30035321
47. Taheri S, James S, Roy V, Decaëns T, Williams BW, Anderson F, et al. Complex taxonomy of the ‘brush tail’ peregrine earthworm *Pontoscolex corethrurus*. *Molecular Phylogenetics and Evolution*. 2018a; 124:60–70. <https://doi.org/10.1016/j.ympev.2018.02.021> PMID: 29501375
48. Rodrigues JLM, Pellizari VH, Mueller R, Baek K, da C. Jesus E, Paula FS, et al. Conversion of the Amazon rainforest to agriculture results in biotic homogenization of soil bacterial communities. *PNAS*. 2013; 110(3):988–993. <https://doi.org/10.1073/pnas.1220608110> PMID: 23271810
49. Gilot-Villeneuve C. Determination of the origin of the different growing abilities of two populations of *Milnesonia anomala* (Omodeo and Vaillaud), a tropical geophagous earthworm. *European Journal of Soil Biology*. 1994; 39(3):125–131.
50. De Menezes AB, Prendergast-Miller MT, Macdonald LM, Toscas P, Baker G, Farrell M, et al. Earthworm-induced shifts in microbial diversity in soils with rare versus established invasive earthworm populations. *FEMS Microbiology Ecology*. 2018; 94(5):fiy051. <https://doi.org/10.1093/femsec/fiy051> PMID: 29579181
51. Sánchez-De León Y, Zou X. Plant influences on native and exotic earthworms during secondary succession in old tropical pastures. *Pedobiologia*. 2004; 48(3):215–226. <https://doi.org/10.1016/j.pedobi.2003.12.006>
52. Philippot L, Raaijmakers JM, Lemanceau P & Van Der Putten WH. Going back to the roots: The microbial ecology of the rhizosphere. *Nature Reviews Microbiology*. 2013; 11:789–799. <https://doi.org/10.1038/nrmicro3109> PMID: 24056930
53. Zachmann JE, Molina JAE. Presence of culturable bacteria in cocoons of the earthworm *Eisenia fetida*. *Applied and Environmental Microbiology*. 1993; 59(6):1904–1910. PMID: 16348968
54. Schramm A, Davidson SK, Dodsworth JA, Drake HL, Stahl DA, Dubilier N. Acidovorax-like symbionts in the nephridia of earthworms. *Environmental Microbiology*. 2003; 5(9):804–809. PMID: 12919416
55. Daane LL, Häggblom MM. Earthworm egg capsules as vectors for the environmental introduction of biodegradative bacteria. *Applied and Environmental Microbiology*. 1999; 65:2376–2381. PMID: 10347016
56. Lund MB, Davidson SK, Holmstrup M, James S, Kjeldsen KU, Stahl DA, et al. Diversity and host specificity of the *Verminephrobacter*-earthworm symbiosis. *Environmental Microbiology*. 2010a; 12(8):2142–2151. <https://doi.org/10.1111/j.1462-2920.2009.02084.x> PMID: 21966909
57. Lund MB, Holmstrup M, Lomstein BA, Damgaard C, Schramm A. Beneficial effect of *Verminephrobacter* nephridial symbionts on the fitness of the earthworm *Aporrectodea tuberculata*. *Applied and Environmental Microbiology*. 2010b; 76(14):4738–4743. <https://doi.org/10.1128/AEM.00108-10> PMID: 20511426
58. Aira M, Pérez-Losada M, Domínguez J. Diversity, structure and sources of bacterial communities in earthworm cocoons. *Scientific Reports*. 2018; 8:6632. <https://doi.org/10.1038/s41598-018-25081-9> PMID: 29700426
59. Nozaki M, Miura C, Tozawa Y, Miura T. The contribution of endogenous cellulase to the cellulose digestion in the gut of earthworm (*Pheretima hilgendorfi*: Megascolecidae). *Soil Biology and Biochemistry*. 2009; 41(4):762–769. <https://doi.org/10.1016/j.soilbio.2009.01.016>
60. Shweta M. Cellulolysis. A transient property of earthworm or symbiotic/ingested microorganisms? *International Journal of Scientific and Research Publications*. 2012; 2(11):1–8.

61. Ueda M, Ito A, Nakazawa M, Miyatake K, Sakaguchi M, Inouye K. Cloning and expression of the cold-adapted endo-1,4- β -glucanase gene from *Eisenia fetida*. *Carbohydrate Polymers*. 2014; 101:511–516. <https://doi.org/10.1016/j.carbpol.2013.09.057> PMID: 24299806
62. Park IY, Cha JR, Ok SM, Shin C, Kim JS, Kwak HJ, et al. A new earthworm cellulase and its possible role in the innate immunity. *Developmental and Comparative Immunology*. 2017; 67:476–480. <https://doi.org/10.1016/j.dci.2016.09.003> PMID: 27614272
63. Thakuria D, Schmidt O, Finan D., Egan D., & Doohan F.M. (2010). Gut wall bacteria of earthworms: A natural selection process. *ISME Journal* 4, 357–366. <https://doi.org/10.1038/ismej.2009.124> PMID: 19924156
64. Liu D, Lian B, Wu C, Guo P. Earthworms' Transcriptome and Gut Microbiota Response to Mineral Weathering. *Acta Geologica Sinica—English Edition*. 2017; 91(1):1–2. <https://doi.org/10.1111/1755-6724.13232>
65. Liu D, Lian B, Wu C, Guo P. A comparative study of gut microbiota profiles of earthworms fed in three different substrates. *Symbiosis* 2018; 74(1):21–29. <https://doi.org/10.1007/s13199-017-0491-6>
66. Gong X, Jiang Y, Zheng Y, Chen X, Li H, Hu F, Liu M, Scheu S. Earthworms differentially modify the microbiome of arable soils varying in residue management. *Soil Biology and Biochemistry*. 2018; 121:120–129. <https://doi.org/10.1016/j.soilbio.2018.03.011>
67. Ikeda H, Fukumori K, Shoda-Kagaya E, Takahashi M, Ito MT, Sakai Y, Matsumoto K. Evolution of a key trait greatly affects underground community assembly process through habitat adaptation in earthworms. *Ecology and Evolution*. 2018; 8(3):1726–1735. <https://doi.org/10.1002/ece3.3777> PMID: 29435247
68. Davidson SK, Powell R, James S. (A global survey of the bacteria within earthworm nephridia. *Molecular Phylogenetics and Evolution* 2013; 67:188–200. <https://doi.org/10.1016/j.ympev.2012.12.005> PMID: 23268186
69. Davidson S, Stahl DA. Transmission of nephridial bacteria of the earthworm *Eisenia fetida*. *Applied and Environmental Microbiology*. 2006; 72(1):769–775. <https://doi.org/10.1128/AEM.72.1.769-775.2006> PMID: 16391117
70. Møller P, Lund MB, Schramm A. Evolution of the tripartite symbiosis between earthworms, *Verminephrobacter* and *Flexibacter-like* bacteria. *Frontiers in Microbiology*. 2015; 6:529. <https://doi.org/10.3389/fmicb.2015.00529> PMID: 26074907
71. Ponesakki V, Paul S, Mani DK S, Rajendiran V, Kanniah P, Sivasubramaniam S. Annotation of nerve cord transcriptome in earthworm *Eisenia fetida*. *Genomics Data*. 2017; 14:91–105. <https://doi.org/10.1016/j.gdata.2017.10.002> PMID: 29204349
72. Ortiz-Ceballos AI, Pérez-Staples D, Pérez-Rodríguez P. Nest site selection and nutritional provision through excreta: a form of parental care in a tropical endogeic earthworm. *PeerJ*. 2016; 4:e2032. <https://doi.org/10.7717/peerj.2032> PMID: 27231655
73. Schult N, Pittenger K, Davalos S, McHugh D. Phylogeographic analysis of invasive Asian earthworms (*Amyntas*) in the northeast United States. *Invertebrate Biology*. 2016; 135(4):314–327. <https://doi.org/10.1111/ivb.12145>
74. Fernández-Marchán DF, Díaz-Cosín DJ, Novo M. Why are we blind to cryptic species? Lessons from the eyeless. *European Journal of Soil Biology*. 2018; 86:49–51. <https://doi.org/10.1016/j.ejsobi.2018.03.004>
75. Denevan WM. The Pristine Myth: The Landscape of the Americas in 1492. *Annals of the Association of American Geographers*. 1992; 82(3):369–385. <https://doi.org/10.1111/j.1467-8306.1992.tb01965.x>
76. Kowarik I. On the role of alien species in urban flora and vegetation. In: Marzluff JM, Shulenberger E, Endlicher w, Alberti M, Bradley G, Ryan C, et al. editors. *Urban Ecology: An International Perspective on the Interaction Between Humans and Nature*. USA: Springer Us; 1995. pp. 321–338. https://doi.org/10.1007/978-0-387-73412-5_20
77. Peel MC, Finlayson BL, McMahon TA. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences*. 2007; 11(5):1633–1644. <https://doi.org/10.5194/hess-11-1633-2007>
78. Righi G. *Pontoscolex* (Oligochaeta, Glossoscolecidae), a New Evaluation. *Studies on Neotropical Fauna and Environment*. 1984; 19(3):159–177. <https://doi.org/10.1080/01650528409360653>