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Invasion of the tropical earthworm Pontoscolex corethrurus (Rhinodrilidae, Oligochaeta) in temperate grasslands

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The tropical earthworm Pontoscolex corethrurus presents a broad distribution (56 countries from four continents) with climates that resemble the one in its native area of distribution. In invasive earthworms, it is generally assumed that temperature appears to limit the success of tropical exotic species in temperate climates. With the global climate change, the edge of the distribution range of this species could advance towards higher elevations (with lower temperatures) where no tropical species currently occur. The aim of this study was to evaluate the soil and climatic variables that could be closely associated with the distribution of P. corethrurus in four sites along an altitudinal gradient in central Veracruz, Mexico. We tested the hypothesis that the global migration of P. corethrurus appears to be limited only by temperature. Five sampling points (monoliths) were established at each of four sites along an altitudinal gradient: Laguna Verde (LV), Ingenio La Concepción (IC), Naolinco (NA) and Acatlán (AC) at 20, 982, 1542 y 1751 masl, respectively. Our results showed that the climate along the altitudinal gradient ranged from tropical to temperate. Ten earthworm species were found along the gradient, belonging to three families (Rhinodrilidae, Megascolecide and Lumbricidae). Soil properties are associated with the abundance of the earthworm community along the altitudinal gradient. P. corethrurus was recorded at three sites (LV, IC and NA) along the altitudinal gradient. Our results reveal that the premise that low temperature limits the distribution of P. corethrurus in not supported; that is, this species may survive and reproduce at the site NA with an average annual temperature of 17 ºC. These results suggested that P. corethrurus might be colonizing temperate environments.

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Invasion of the tropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Oligochaeta) in temperate grasslands

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Abstract

The tropical earthworm *Pontoscolex corethrurus* presents a broad distribution (56 countries from four continents) with climates that resemble the one in its native area of distribution. In invasive earthworms, it is generally assumed that temperature appears to limit the success of tropical exotic species in temperate climates. With the global climate change, the edge of the distribution range of this species could advance towards higher elevations (with lower temperatures) where no tropical species currently occur. The aim of this study was to evaluate the soil and climatic variables that could be closely associated with the distribution of *P. corethrurus* in four sites along an altitudinal gradient in central Veracruz, Mexico. We tested the hypothesis that the global migration of *P. corethrurus* appears to be limited only by temperature. Five sampling points (monoliths) were established at each of four sites along an altitudinal gradient: Laguna Verde (LV), Ingenio La Concepción (IC), Naolinco (NA) and Acatlán (AC) at 20, 982, 1542 y 1751 masl, respectively. Our results showed that the climate along the altitudinal gradient ranged from tropical to temperate. Ten earthworm species were found along the gradient, belonging to three families (Rhinodrilidae, Megascolecide and Lumbricidae). Soil properties are associated with the abundance of the earthworm community along the altitudinal gradient. *P. corethrurus* was recorded at three sites (LV, IC and NA) along the altitudinal gradient. Our results reveal that the premise that low temperature limits the distribution of *P. corethrurus* in not supported; that is, this species may survive and reproduce at the site NA with an average annual temperature of 17 ºC. These results suggested that *P. corethrurus* might be colonizing temperate environments.

Introduction

Within soil biodiversity, earthworms stand out as part of the guild of ecosystem engineers [1, 2, 3]. They provide a considerable level of ecosystem services, such as contributing to biogeochemical cycling and crop productivity [4, 5, 6]. Depending on their ecological classification (epigeic, endogeic or anecic), they can also modify the distribution and abundance of soil biodiversity, mainly by constructing structures and galleries within the soil profile and by producing casts, mucus and urine [7, 8, 9].

Most earthworm species display an aggregated spatial distribution in response to soil variables such as texture and the quality and amount of organic matter [10, 11]. However, most studies of the ecological distribution of earthworms consider soil type and characteristics as the primary determinant [8, 10, 11, 12, 13], largely because of the limited capabilities of earthworms for horizontal displacement (between 4 and 10 m per year) [12, 14, 15]. Nevertheless, climate has a substantial influence on earthworms (physiology, development or activity) that is reflected in the seasonal dynamics of their life history [16, 7, 8, 4, 17].

At the global level, little recognition has been given to invasions of soil organisms [7, 10]. However, studies of earthworm invasions have focused on *Pontoscolex corethrurus* (Rhinodrilidae, [18]), *Eudrilus eugeniae* (Eudrilidae), various species of the genus *Amynthas*, *Microscolex*, *Dichogaster*, and *Perionyx* (all Megascolecidae), and at least 10 species of Lumbricidae [7]. These species have reached a broad distribution in many tropical agroecosystems and natural ecosystems. For example, grasslands are more commonly invaded due to disturbance that allows exotic species to reduce or avoid the intensity of biological resistance, which is usually expressed through interspecific competition, predation or parasitism

[7]; however, this has been facilitated by the road networks and vehicle transport to surmount important biogeographic barriers [7, 8, 19, 20], horticulture [21], waste management industries and fishing bait [20].

P. corethrurus (Müller, 1857) is native to the Guiana Shield area of the Amazon [7,15, 22, 23]. Due to its parthenogenetic clonal populations [7, 22, 23, 24, 25], its adaptive strategies include a high tolerance to soil conditions and climatic variables (precipitation) [7, 12, 26]. For this reason, *P. corethrurus* has become established throughout the tropical regions of over 56 countries [7, 12, 15, 19, 26]. Moreover, given the wide distribution range of *P. corethrurus*, the species can be used as a model organism to investigate and compare the effects of native and introduced earthworm species on ecosystem processes in the tropics [19]. Besides, it has a potential for use in biotechnological packages to improve plant growth and productivity [9, 27, 28], as a biomarker for sites contaminated with hydrocarbons [29, 22, 30] and as a bioindicator of regional climate variability. Although *P. corethrurus* may have a negative impact on soil ecosystems under certain circumstances; for example, their activity (associated with the reduction in soil biodiversity) may cause negative effects on soil [31, 32, 21] and plant growth [27], and tends to eliminate native species [33]. For this reason, it has been suggested that *P. corethrurus* can be considered a non-obligatory ecosystem engineer [2, 3], i.e., its activity does not affect its fitness characteristics.

In Mexico, since the early twentieth century, *P. corethrurus* has been the endogeic earthworm species most commonly found in human-altered tropical ecosystems [12, 26, 34]. The altitudinal distribution of this species in Mexico and other tropical countries ranges from 5 to 2000 masl [13, 27, 34, 35, 36, 37]. However, with a warming climate, the edge of the earthworm's distribution range could advance towards higher elevations (with temperate climates) where few or no tropical species currently occur [7, 40, 24]. Therefore, it is important to understand the climatic and geographical factors which may be responsible for facilitating or preventing the invasion of *P. corethrurus.*

The aim of this study was to evaluate the soil and climatic variables that could be closely associated with the distribution of *P. corethrurus*. In addition, we compared the occurrence of four possible situations in the altitudinal gradient: a) presence of *P. corethrurus* only, b) coexistence of *P. corethrurus* and other species (native and invasive), c) absence of *P. corethrurus* but presence of other species (native and exotic), and d) absence of earthworms. We assumed that this species is tolerant of a wide range of soil conditions (eurytopic) and that its global migration appears to be limited only by temperature [7, 37, 23, 24]. We tested this hypothesis through a study of the earthworm community along an altitudinal gradient in central Veracruz, Mexico.

Methods

Study area

An altitudinal transect, ranging from 20 to 1751 masl, was established in the central region of the State of Veracruz, Mexico. Five sampling points (monoliths) were established at each of four sites along this altitudinal gradient (Fig. 1 and Table 1): Laguna Verde (LV), Ingenio La Concepción (IC), Naolinco (NA) and Acatlán (AC) at 20, 982, 1542 y 1751 masl, respectively. The sampling points were chosen based on the following criteria: a) each site constitutes an environment (agroecosystem) that has been disturbed by human activity; b) the agroecosystem most commonly invaded is the grassland; c) the grassland is a suitable agroecosystem that fosters the growth of earthworms; and d) this agroecosystem can be found all along the gradient adjacent to rural populations. For this reason, we chose the grassland as the agroecosystem for determining the composition of earthworm communities and the distribution of *P. corethrurus* along the altitudinal gradient.

Climate information from Mexican National Water Commission weather stations (http://www.conagua.gob.mx) was compiled for each site along the altitudinal transect. The monthly and annual 30-year averages were obtained for the following climate variables: average temperature (AT), average maximum temperature (AMT), average minimum temperature (AmT), total annual precipitation (TAP) and total evaporation (TE). In addition, climate types along the altitudinal gradient were determined using the Clima2 software (http://www.pablo-leautaud.com/home/proyectos/python/clima) and classified into one climate type according to the Köppen-Geiger system [41].

Earthworm sampling

The quantitative sampling of earthworms was conducted along the altitudinal gradient [42]. One monolith (25 x 25 x 30 cm deep) was sampled at each of the five earthworm sampling points established at least 200 m apart [37], located at each of the four sites on the altitudinal transect, for a total of 20 monoliths along the altitudinal transect. Each monolith was separated into four strata: above-ground plant biomass, 0-10, 10-20 and 20-30 cm. Earthworms were then manually removed from each layer and preserved in ethanol. In the laboratory, all specimens were fixed in 4% formaldehyde and then identified (to species or morphospecies), quantified and weighed. The sampling was conducted at the end of the rainy season, i.e., when the earthworms peaked in abundance and biomass [42]. Abundance and biomass data of the earthworms were converted into densities per square metre (ind. $\cdot m^{-2}$ and g $\cdot m^{-2}$, respectively) for each site [42].

Soil and foliage sampling

Prior to removing the earthworms, the above-ground plant biomass was harvested from each monolith. In the laboratory, this plant material was dried (60 °C for 72 h) and weighed, and its total nitrogen content (Ngrass) was determined using the methods described in the Mexican Official Standard NOM-02-RECNAT-2000 [43].

Following the removal of earthworms, a 1-kg soil sample was taken from each stratum of each monolith. Soil samples were air-dried to constant weight and sieved (5 mm), and a 200-g subsample was taken to determine the texture (clay, silt and sand), water-holding capacity, Permanent Wilting Point (PWP), pH, organic matter, Total C, Total N, P and K, using the methods described in the Mexican Official Standard NOM-02-RECNAT-2000 [43].

Statistical analysis

A one-way ANOVA was used to test for significant differences ($P < 0.05$) in soil properties between sites, using the Statistica ver. 7 software.

We used a Linear Model (LM) to study earthworm abundance (ind.$\cdot$m^{-2}) using soil properties and climatic elements as covariates (factors). The effect of the climatic elements was included in the model through the variable “site” because it was possible to clearly distinguish the sites in a scatter plot (figure not shown) of the scores for the first two principal components obtained from a PCA of the climatic variables. The dependent variable (earthworm abundance) was transformed using natural logarithms because the empirical distribution of earthworms was highly asymmetric. All the analyses were performed using the R version 3.2.3 software [44]. We also fitted Linear Mixed Models to take into account the sampling design consisting in clustered samples and the response variable measured at two different scales, i.e., soil properties at the sample scale and climatic conditions at the site scale. The results (data not shown) showed that the variance component associated with the site effect was misleading. Apart from the linear models and before log-transforming the data, we also fitted generalized linear models with different families (e.g., Poisson, Negative Binomial); however, some convergence issues arose when fitting the models. Consequently, only the results for the fitted LM are reported here.

Results

Site climate

The climate along the altitudinal gradient, from the lowest to the highest elevation sites, ranged from warm to humid tropical (Aw) to temperate (Cfb) [41]. There is a difference of approximately 11 °C in average, minimum (15 °C) and maximum (26 °C) temperatures between the lowest (LV, 20 masl) and the highest elevation sites (AC, 1751 masl) along the gradient (Table 2). Rainfall was higher in site IC (1676.8 mm) than in site LV (1143 mm), whereas sites NA (1542 masl) and AC (1751 masl) had intermediate values (1461 mm).

Soil properties

The physical and chemical variables of soil along the altitudinal gradient displayed significant variations. Soils from sites LV and IC have a heavy textured (25.6-27.6% clay, 21-8-34.4% silt and 40.0-50.6% sand); were mildly acidic (pH 6.6); and displayed low values for water-holding capacity (32.9-36.6%), permanent wilting point (20.0-20.2%), organic matter (8.0-8.4%), total N (0.31%), P (13.2-54.7 mg kg^{-1}), and K (1.1-1.7 cmol$_c$ kg^{-1}). In contrast, soils from sites NA and AC had a light textured (12.0-13.6% clay, 28.8-35.8% silt and 50.6-59.2% sand); greater water-holding capacity (70.5-83.5%) and permanent wilting point (40.4-53.0%); were slightly acidic (pH 5.5-5.6); and were rich in organic matter (34.2-43.7%), total N (0.83-1.13%), P (14.2-49.8 mg kg^{-1}) and K (1.4-2.8 $cmol_c$ kg^{-1}).

Earthworm communities

Ten earthworm species (Annelida: Oligochaeta: Crassiclitellata) were found in 19 of the 20 samples along the altitudinal gradient (Table 3). Seven of these were well-known, ubiquitous species, some of which are considered invasive, belonging to three different families (Rhinodrilidae, Megascolecide and Lumbricidae). The remainder of the earthworms were native morphospecies (differentiated from others only by morphological features). The highest diversity was found at site AC, with five species. The total abundance of the earthworm community ranged from 0 to 864 ind.·m^{-2}, with an average of 332 ind.·m^{-2}.

The LM analysis showed that the total abundance of the 10 earthworm species was significantly influenced by clay, water-holding capacity, pH, P and Ngrass, while the climatic factors (sites) had no such effect; that is, positive coefficients are associated with an increase in the number of earthworms, and negative coefficients are associated with a decrease in the number of earthworms (Table 4). Furthermore, abundance was positively correlated with certain soil properties and negatively correlated with others (Table 4).

Pontoscolex corethrurus

Populations of *P. corethrurus* were found in 10 of the 20 samples from the gradient (Table 3, Fig. 1a): LV (1/5), IC (5/5) and NA (4/5), but the species was absent in all samples of site AC (situated at 1751 masl).

On average, the abundance of *P. corethrurus* accounted for 72.6% of the total earthworm density throughout the samples where the species was present. This percentage varied between sites LV, IC and NA at 91.7, 79.3 and 46.9%, respectively (Fig. 2b). In the sites where the species occurred, its average density was 273.5 ind.·m^{-2}$_{,}$ ranging from 16 to 704 ind.·m^{-2} (Fig. 2c).

Pontoscolex corethrurus coexisted with exotic (2 and 4 of the 5 samples in IC and NA, respectively) and native (1/5 in LV) species (Table 5). In contrast, *P. corethrurus* was found alone in 3 of the 5 monoliths of site IC, while only native species were found alone in site LV.

Discussion

To date, 102 earthworm species have been reported in Mexico [34]. Along the altitudinal gradient studied here, 10 species (seven exotic and three native morphospecies) were recorded in the grassland. The exotic species are among the 51 exotic species recorded in Mexico, and the three morphospecies can be added to the 40 native species that are already known but still undescribed [34]. Among the exotic species commonly known as invasive [7, 26], two are Neotropical (*P. corethrurus* and *O. windlei),* five are Western Paleartic (*L. rubellus*, *A. trapezoids*, *O. tyrtaeum* and *B. parvus*), and one is Eastern Paleartic (*A. gracilis*). A number of factors may contribute to the invasiveness of these species. Endogenous factors include reproductive strategies (high fecundity, short incubation time and high hatching success), ability to survive while traveling under dormancy or as cocoons, and wide environmental tolerance, [7, 12, 25]. Several exogenous factors including phoresy, gravity, anemochory and hydrochory are important in facilitating the displacement of earthworms across areas that are not unlikely to be traversed by earthworms on their own [7, 20, 21, 25]. However, anthropogenic vectors now dominate the movements of earthworms and their propagules at a global scale [7, 20, 21, 25].

The total abundance of earthworms, irrespective of the species is explained by the properties of soil rather than site (climate). In addition, the regression coefficients showed that Ngrass, PWP, pH, organic matter and P are all associated with an increase in earthworm abundance, while water-holding capacity, K and total N apparently reduce it. This has also been documented in other field and laboratory studies [7, 12, 45, 46, 47]. The diversity and density along the altitudinal gradient (ranging from 1-10 and 80-864 ind.·m^{-2}, respectively) measured in this study fall within the range reported in the literature (1-35 and 12-850 ind.·m^{-2}, respectively) for grasslands, croplands and forests of different tropical regions (from 10 to 2000 masl) of Latin America [13, 26, 35, 36, 37].

Among the 3700 earthworm species described, approximately 3% (100-120) have been identified as invasive [7, 15, 23, 24]. The current state of knowledge allows little generalization about the distribution patterns of invasive earthworm, as is the case of *P. corethrurus*. The observations of Beddard (1912) are still maintained worldwide [7], i.e., tropical earthworms only tend to invade tropical regions and low temperatures restrain them from colonizing temperate areas [7, 23, 24]. Temperate species, however, tend to invade temperate regions and montane areas in tropical regions [7]. Our results show that site (i.e., climatic variables) had a significant influence on the presence (LV, IC and NA) and absence (AC) of *P. corethrurus* throughout the altitudinal gradient, while temperate earthworms were only found in sites NA and AC. In this regard, several field studies [13, 35, 36, 37, 32, 40, 48, 49, 50, 51, 52] in different tropical regions of Mexico (e.g., State of Veracruz, with 145 records) and other countries have documented that *P. corethrurus* populations (from 0-804 ind.·m^{-2}) are only found in environments with an average annual temperature of 24.1 ± 3.9 °C (range: 16 to 33 °C), similar to sites LV and IC that show average earthworm densities of 704 and 329 ind.·m^{-2}, respectively.

In contrast, we observed populations of *P. corethrurus* (with an average of 133 ind.·m^{-2}) at site NA, where the average annual temperature is 17 °C. In the Antsirabe region of Madagascar [41, 53, 54], the Azores Archipelago (São Miguel island) [24, 41], Curitiba (Paraná State, Brazil) [22, 41], and Chatham (New Jersey, USA) [21, 41], *P. corethrurus* has become established under similar temperatures. This suggests that the growth and reproduction of *P. corethrurus* may no longer be limited by temperature, as indicated by [12], with ranges of 20-30 °C and 23-27 °C, respectively, at these locations and similarly in several microcosm [22, 46, 47, 53, 54, 55, 56, 58, 59, 60, 61, 62] and greenhouse [45, 63, 64] studies conducted in areas with an average of 24.8 ± 4.5 °C and a ranging from 15 to 39.5 °C. Rapid adaptations or mutations in known invasive species should be considered as likely mechanisms that could facilitate their spread into new habitats [7, 25]. Our findings also suggest that *P. corethrurus* might be colonizing environments characterized by a temperate climate, and that the population in site NA could be a clone adapted to the local environment as a strategy that may facilitate the colonization of temperate enviroments as was the case in their place of origin and in the Azores Archipelago.

There is also the possibility that *P. corethrurus* has developed adaptations to survive in the temperate climate of site NA, as is the case with *Amynthas hawayanus* [7, 65], *Lumbricus terrestris* [65], *Eisenia nordenskioldi* [66], *Dendrobaena octaedra* [39, 73] and with the local linage of *P. corethrurus* found in Chatham, USA since 1937 [21], namely: a) migrating to deeper soil layers during winter, b) accumulating high glucose concentrations, c) lowering body temperature with low enzymatic capacities and inverse compensation of mitochondrial membrane, d) mass-producing cocoons that survive the cold season, and e) losing water to avoid the freezing of cocoons in winter. However, this hypothesis requires further investigation under controlled laboratory conditions.

Finally, it is interesting to speculate on the potential changes in invasive earthworms as a result of climate change [7, 8, 67]. Since *P. corethrurus* is a species that is native to tropical latitudes, it is likely to be limited by temperature (e.g., in site AC > 1751 masl, with a mean annual temperature of 15 °C) rather than precipitation or some soil property. Under a scenario of regional climate change involving rising temperatures (0.2 °C per decade over the next two decades) [8], it is reasonable to hypothesize that the distribution range of *P. corethrurus* will eventually reach elevations above the limit observed today (1542 masl or site NA). It is also possible that, under the same scenario, the distribution ranges of *L. rubellus*, *B. parvus* and *O. tyrtaeum* will likely become restricted to the mountainous areas of Mexico and other countries around the tropics and elsewhere [67].

Conclusions

Our results showed that soil properties are associated with the abundance of the earthworm community along an altitudinal gradient. The presence of the endogeic tropical earthworm *P. corethrurus* in a temperate zone showed that this species is capable to survive and reproduce at a temperature below its normal range. This suggests that it may be able to invade new habitats characterized by a temperate climate. Also, these findings suggest the need to elucidate the impacts of this earthworm species in both soil biota and plant community.

Competing interests

The authors declare that they have no conflict of interest. This article does not involve studies on human or higher-order animal subjects.

Authors' contributions

DOG and AIOC conceived and designed the experiments. DOG and AIOC performed the experiments. DOG, AIOC, PPR, analyzed the data. DOG, AIOC and PPR wrote the manuscript.

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Figure 1(on next page)

Figure 1. Sampling sites of earthworms along an altitudinal gradient in central Veracruz, Mexico.

For each site, the geographical coordinates (14N zone, Datum WGS84) are shown. LV = Laguna Verde (20 masl), IC = Ingenio La Concha (982 masl), NA = Naolinco (1542 masl), AC = Acatlán (1751 masl). Digital elevation model created using the geographical data provided by Instituto Nacional de Estadística y Geografía.

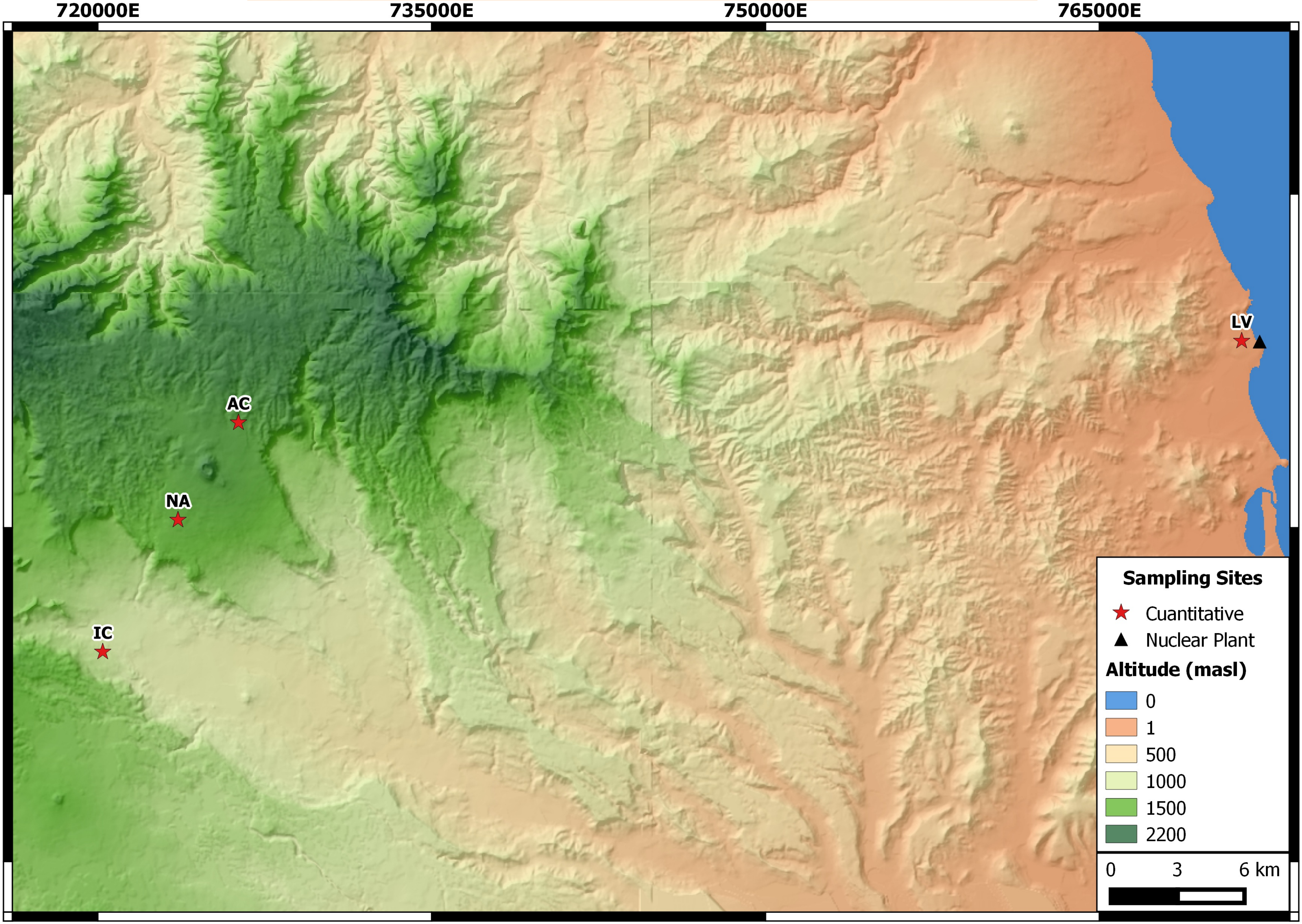

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Table 1(on next page)

Table 1. Location of five earthworm sampling points by site along an altitudinal gradient in central Veracruz, Mexico.

For each sampling point per site, the geographical coordinates (14N zone, Datum WGS 84) are shown. LV = Laguna Verde (20 masl), IC = Ingenio La Concha (982 masl), NA = Naolinco (1542 masl), AC = Acatlán (1751 masl).

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Site	Locally	GPS coordinates	
		Latitude	Longitud
LV	1. Laguna Verde, Alto Lucero	19.685234	-96.40943
	2. Laguna Verde, Alto Lucero	19.768824	-96.42992
	3. Laguna Verde, Alto Lucero	19.768691	-96.429868
	4. Laguna Verde, Alto Lucero	19.726981	-96.421836
	5. Laguna Verde, Alto Lucero	19.597558	-96.388257
IC	1. Ingenio La Concepción	19.60877	-96.895282
	2. Ingenio La Concepción	19.60713	-96.892446
	3. Ingenio La Concepción	19.619955	-96.878518
	4. Ingenio La Concepción	19.603434	-96.889606
	5. Ingenio La Concepción	19.615873	-96.883049
NA	1. Naolinco	19.654457	-96.868536
	2. Naolinco	19.654457	-96.867994
	3. Naolinco	19.661429	-96.85499
	4. Naolinco	19.661417	-96.854993
	5. Naolinco	19.652782	-96.866057
AC	1. Acatlán	19.696656	-96.84695
	2. Acatlán	19.693611	-96.851334
	3. Acatlán	19.693839	-96.857626
	4. Acatlán	19.693838	-96.857626
	5. Acatlán	19.694813	-96.862185

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Table 2(on next page)

Table 2. Climate variables at the four sampling site along an altitudinal gradient in central Veracruz, Mexico.

LV = Laguna Verde (20 masl), IC = Ingenio La Concepción (982 masl), N = Naolinco (1542 masl), AC = Acatlán (1751 masl). AT = Average temperature, AMT = Average maximum temperature, AmT = Average minimum temperature, TAP = total annual precipitation, TE = total evapotranspiration.

Site	Temperature (˚C)			TAP	TE	Month most	
	AT	AMT	AmT	(mm)		Warmer	Cooler
LV	26.0 ± 2.5	30.0 ± 2.5	21.0 ± 2.0	1143.0	1618.1	June	January
IC	20.0 ± 2.0	26.0 ± 2.2	14.0 ± 1.8	1676.8	1322.0	May	January
N	17.0 ± 2.0	22.2 ± 2.0	12.0 ± 1.9	1462.0	1554.8	May	January
AC	15.0 ± 1.8	20.0 ± 1.9	10.0 ± 1.8	1461.0	1190.8	May	January

Table 3(on next page)

Table 3. Earthworm species recorded in four sampling sites along an altitudinal gradient in central Veracruz, Mexico.

LV = Laguna Verde, IC = Ingenio La Concepción, N = Naolinco, AC = Acatlán.

Species	**Family**	**Origin**		**Ecological category**	**Sites and Altitude (masl)**			
		Exoctic	**Native**		**LV 20**	**IC 982**	**NA 1542**	**AC 1751**
Pontoscolex corethrurus	Rhinodrilidae	x		Endogeic	x	x	x	
Onychochaeta windlei		x		Endogeic		x		
Amynthas gracilis	Megascolecidae	x		Epi-endogeic		x	x	x
Octolasion tyrtaeum	Lumbricidae	x		Endogeic			x	x
Aporrectodea trapezoides		x		Endogeic				x
Bimastos parvus		x		Endogeic				x
Lumbricus rubellus		x		Epi-endogeic				x
Morfo 1 (LV)	Morfospecies		x	Endogeic	x			
Morfo 2 (LV)			x	Endogeic	x			
Morfo 3 (LV)			x	Endogeic	x			

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Table 4(on next page)

Table 4. Estimated regression coefficients that predict total earthworm abundance along an altitudinal gradient in central Veracruz, Mexico.

Sites: LV = Laguna Verde (20 masl), IC = Ingenio La Concepción (982 masl), N = Naolinco (1542 masl), AC = Acatlán (1751 masl).

Coefficients	Estimate	Std. Error	t	*P*
Site AC*	-0.394	0.792	-0.497	0.645
Site IC*	0.626	0.554	1.130	0.322
Site NA*	-0.324	1.095	-0.296	0.782
Clay, %	-0.133	0.067	-1.98	0.118
Sand, %	0.001	0.034	0.06	0.957
Water-holding capacity, %	-0.208	0.061	-3.43	0.026
Permanent wilting point, %	0.484	0.128	3.77	0.019
pH, (H_2O)	0.920	0.322	2.86	0.045
Organic matter, %	0.156	0.044	3.32	0.029
Total N, %	-18.090	4.420	-4.01	0.015
P, mg kg^{-1}	0.018	0.006	3.26	0.031
K, cmol$_c$ kg^{-1}	-1.053	0.263	-4.01	0.016
Ngrass, %	3.559	0.770	4.62	0.009

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Table 5(on next page)

Table 5. Composition of earthworm communities in each of the five monoliths of the four sites along an altitudinal gradient in central Veracruz, Mexico:

no earthworms, Pontoscolex corethrurus only, coexistence (exotics and natives), other species (but no P. corethrurus). LV = Laguna Verde (20 masl), IC = Ingenio la Concepcion (982 masl), NA = Naolinco (1542 masl), AC = Acatlán (1751 masl).

Sites	**No Earthworm**	***P. corethrurus* only**	**Coexistence**		**Others species only**		**Total**
			Exotic	Native	Exotic	Native	
LV	0	0	0	1	0	4	5
IC	0	3	2	0	0	0	5
NA	1	0	4	0	0	0	5
AC	0	0	0	0	5	0	5
Total	1	3	6	1	5	4	20

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