



Invasive lumbricid earthworms in North America—Different life histories but common dispersal?

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Abstract

Aim: Lumbricid earthworms are invasive across northern North America, causing notable changes in forest ecosystems. During their range expansion, they encountered harsher climatic conditions compared to their native ranges in short time (~400 years). This study investigated if (a) dispersal barriers, (b) climatic selection or (c) anthropogenic activities, that is fishing bait disposal, structure the dispersal of free-living earthworm populations.

Location: North America, forest habitats along former Wisconsinan glaciation line.

Taxon: *Lumbricus terrestris*, *L. rubellus*.

Methods: *Lumbricus terrestris* and *L. rubellus* co-occur in the same habitats but differ in ecology and use as fishing bait. Both species were sampled in five transects ranging from the east to the west coast of northern North America, including major dispersal barriers, three different climate zones, and bait shops near sampling locations. Genetic diversity and structure were compared between the two species, and the presence of free-living bait shop genotypes was assessed using four markers (COI, 16S rDNA, 12S rDNA, and H3).

Results: Populations of both species were genetically diverse with some geographic structure, which was more pronounced in *L. terrestris* than in *L. rubellus*. Common haplotypes were present in all regions, but locally restricted haplotypes also occurred. Furthermore, two distinct genetic clades of *L. terrestris* co-occurred only in the two most distant transects (Alberta and Minnesota). Genotypes identical to bait individuals were omnipresent in field populations of *L. terrestris*.

Main Conclusions: Genetic diversity was high in both species, and invasive populations represented a genetic subset of European earthworms. Geographic and climatic dispersal barriers affected the less mobile species, *L. terrestris*, resulting in differences in genetic structure between the two species. Our results indicate common long-distance dispersal vectors and vectors affecting only *L. terrestris*. The roles of climate and anthropogenic activities are discussed, providing additional explanations of dispersal and new insights into establishment of invasive earthworm populations.

KEYWORDS

agriculture, biological invasion, climate, colonization, dispersal barriers, genetic clades

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1 | INTRODUCTION

European lumbricid earthworms are among the most successful invasive species in North America (James & Hendrix, 2004). European settlers introduced them at the east coast about 400 years ago, both accidentally and intentionally (Gates, 1976). Similar to many invasive species living above the ground, earthworms substantially alter the functioning of invaded ecosystems (Bohlen et al., 2004; Eisenhauer, Partsch, Parkinson, & Scheu, 2007; Hendrix et al., 2008; Mooney & Hobbs, 2000; Scheu & Parkinson, 1994). They change physical and biotic properties of the soil, which affects the density of other soil invertebrates, plant community composition, and aboveground food webs (Lee, 1985; Edwards & Bohlen, 1996; Eisenhauer et al., 2007; Eisenhauer 2010; Craven et al., 2017; Ferlian et al., 2018). In general, where earthworms are native they beneficially affect plant growth (van Groenigen et al., 2014) and plant competition (Eisenhauer & Scheu, 2008), but earthworms can exert contrasting effects on ecosystems that developed without their presence (Bohlen et al., 2004; Craven et al., 2017; Hale, Herms, Hansen, Clausen, & Arnold, 2005).

As successful invaders, earthworms possess high tolerance for a wide range of environmental conditions, though they prefer clay soils with near neutral pH that restricts their distribution (Curry, 2004; Fisichelli, Frelich, Reich, & Eisenhauer, 2013; Laverack, 1961). Due to their ability to tolerate disturbances, they also occur in agricultural fields and meadows, with varying frequencies and abundances (Hendrix et al., 1992). In general, earthworms are susceptible to prolonged freezing periods, drought and geographic barriers like mountain ranges and large water bodies, which usually restrict their natural dispersal pattern (Eggleton, Inward, Smith, Jones, & Sherlock, 2009; Reynolds, 1994). However, they recently were recorded from interior Alaska and Fennoscandia suggesting that they can also withstand very low temperatures (Booyesen, Sikes, Bowser, & Andrews, 2018; Wackett, Yoo, Olofsson, & Klaminder, 2018). Active dispersal of earthworms is slow, but they were able to spread across northern North America within a few hundred years by passive dispersal or repeated introductions, and today they are present in large areas from the east coast to the Midwest, east of the Rocky Mountains in Canada, and the Pacific coast (Hale et al., 2005; Holdsworth, Frelich, & Reich, 2007; Reynolds, 1977, 1994, 2016; Reynolds, Linden, & Hale, 2002; Scheu & Parkinson, 1994). The pronounced ecological consequences of earthworm invasions in North America are well documented, making earthworms one of the best-studied invasive animal species living below the ground (Wardle, Bardgett, Callaway, & Putten, 2011) and thus, a unique model system for biological invasion and accompanying effects (Hendrix et al., 2008).

During their expansion across northern North America, European earthworms established in distinct climate zones that differ in the amount and distribution of precipitation across the year, as well as frost intensity and duration, two abiotic factors that are known to drive earthworm distribution (Curry, 2004; Fisichelli et al., 2013; Holmstrup, 2003; Uvarov, Tiunov, & Scheu, 2011). At the west coast, precipitation is high (1,200 mm/year), mild frost occurs sporadically and lasts for only few weeks between December and January. By

contrast, in the central plains of North America, precipitation is low (400–600 mm/year), and strong frost conditions typically persist between November and March, with occasional night frost already starting in late August and extending into early June. In the east, precipitation is intermediate (800–1,000 mm/year), and frost conditions typically last from December to February. Given this wide range of climatic conditions, knowledge on genetic diversity and relationships among populations across North America is needed for a better understanding of dispersal mechanisms and population establishment.

We investigated the genetic structure of *Lumbricus rubellus* and *L. terrestris*, two exotic earthworm species that are widespread and common across northern North America. Both feed on litter but have distinct ecological preferences and life histories (Sims & Gerard, 1999). *Lumbricus rubellus* is an epi-endogeic species, living in horizontal burrows up to 30-cm deep and moves freely within the litter layer for foraging. It prefers neutral to slightly acidic soils and generally has a higher pH and frost tolerance than *L. terrestris* (Addison, 2009; Tiunov, Hale, Holdsworth, & Vsevolodova-Perel, 2006). In contrast, *L. terrestris* prefers neutral to slightly alkaline soils, lives in permanent, vertical burrows of up to 2-m depth, and collects litter in the vicinity of its burrow entrance (Addison, 2009; Sims & Gerard, 1999; Tiunov et al., 2006). Active dispersal rates of the two earthworm species range between 2–4 m/year for *L. terrestris* and 10–14 m/year for *L. rubellus* (Marinissen & van den Bosch, 1992). As epi-endogeic species, passive dispersal of *L. rubellus* by human activities may be more likely by moving animals and cocoons in surface soils, such as through activities related to forestry or tourism (i.e., by hiking or wheels of vehicles). In contrast, *L. terrestris* lives in permanent, vertical burrows that are only left for foraging and mating (usually at night), making it less likely to be passively transported by human activities above the ground. *Lumbricus terrestris* is commonly used as fishing bait and sold in bait shops, which likely facilitates its dispersal. By contrast, *L. rubellus* is rarely sold in bait shops (A. Klein, pers. obs.). Disposal of fishing baits contributes substantially to the introduction and establishment of earthworm populations in recreational and fishing areas (Holdsworth et al., 2007; Keller et al., 2007), but the long-term establishment of these populations and further dispersal in the field remain unclear.

We sampled earthworms from five transects of ~150- to 300-km length (north-south orientation) in three climatic regions in two provinces in Canada and three states in the USA: the warm and moist region of British Columbia, Canada (BC), the cold and dry regions of Alberta, Canada (AL) and Minnesota, USA (MN), and the cold and moderately moist regions of Michigan, USA (MI) and New York State, USA (NY), respectively. This is the first study investigating the invasion of detritivorous soil animals on continental scale, including two different dispersal barriers and distinct climate zones in its sampling design.

We tested three hypotheses to understand if climate (H1), dispersal barriers (H2), and/or human migrations and transport (H3) predominantly structured the distribution and establishment of European earthworm species in northern North America: We tested, if (H1) distinct genetic clades from genetically diverse

TABLE 1 Overview of sampling area, abbreviations of sampling locations and climatic characteristics of each transect

Sampling transect	Climate zone	Climate characteristics	Sampling location
British Columbia (BC)	Mixed Mediterranean, oceanic and continental	Warm and moist AMP: ~1,200 mm/year AMT: 6–16°C	Cypress Provincial Park BC_I
			Golden Ears Provincial Park BC_II
			Cultus Lake BC_III
Alberta (AL)	Cold continental	Cold and dry AMP: 400–750 mm/year AMT: –2 to 9°C	Crandell Lake AL_I
			Waterton Springs AL_II
			Maycroft AL_III
			Eden Valley AL_IV
			Fish Creek Park, Calgary AL_V
			Nose Hill Park, Calgary AL_VI
Minnesota (MN)	Cold continental	Cold and dry AMP: 400–750 mm/year AMT: –2 to 9°C	Nerstrand MN_I
			Wood-Rill SNA MN_II
			Wolsfeld Wood SNA MN_III
			Warner Nature Center MN_IV
			Pine Needles Preserve MN_V
			Rush City MN_VI
Michigan (MI)	Moderate continental	Cold and moderately moist AMP: 800–1000 mm/year AMT: 0–9°C	Turner MI_I
			Tawas City MI_II
			Alpena MI_III
			Gaylord MI_IV
New York (NY)	Moderate continental	Cold and moderately moist AMP: 800–1000 mm/year AMT: 0–9°C	Hamilton NY_I
			Norwich NY_II
			Newcomb NY_III
			Lower Saranac Lake NY_IV
			Lake Placid NY_V
			Portland Waterfront NY_VI

Note: See Appendix S1 Table S1 for GPS coordinates.

Abbreviations: AMP, annual mean precipitation; AMT, annual mean temperature.

source populations established in the different climate zones. Due to environmental filtering we expected to find monophyletic clades in the different regions, if only individuals survived that were better adapted to regional drought or cold conditions. Furthermore, the Rocky Mountains and the Great Plains may mark potential dispersal barriers for earthworms and we tested if (H2) earthworms were introduced independently in areas that are separated by potential dispersal barriers resulting in distinct genetic clades in the west (BC, AL), but less distinct clades or no genetic structure in the east (MN, MI, NY) where geographic dispersal barriers are of minor importance. Finally, we tested if human-mediated dispersal of earthworms counteracts local genetic structure and disregards dispersal barriers (H3), resulting in diverse earthworm populations and genotypes that are represented in all regions without any local clades occurring. To account for human-mediated dispersal by dumping of fishing baits, which is a severe problem in northern North America (Hale, 2008; Holdsworth et al., 2007; Seidl & Klepeis, 2011), we purchased earthworms from bait shops near sampling locations in all transects to test if bait

genotypes contribute to free-living populations, thereby increasing local diversity.

2 | MATERIALS AND METHODS

2.1 | Sampling design—dispersal barriers and climate

Between May and July 2014 and in June 2015, we collected *L. terrestris* and *L. rubellus* along five transects (regions) spanning from east to west of the northern North American continent, ranging in the USA from New York State (Adirondack Mountains, transect NY), to the Midwest, that is Michigan (upper peninsula, transect MI) and Minnesota (near Minneapolis/St. Paul, transect MN; Table 1). In Canada, we collected earthworms east and west of the Rocky Mountains in Alberta (south of Calgary, transect AL) and British Columbia (near Vancouver, transect BC). Distances among transects ranged between 700 and 1,600 km, and within transects earthworms were collected at five sampling locations with north–south



orientation that were 20–80-km apart. The two major dispersal barriers for plants and animals are the extensive dry grassland areas of the Great Plains extending between the transects of Minnesota (USA) and Alberta (Canada), and the Rocky Mountains, which separate the two Canadian transects Alberta and British Columbia. Climate in east and central northern North America is similar to continental climate in Europe, but seasonality in North America is harsher with hotter and drier summers, and longer and colder winters, which is most extreme in Alberta and Minnesota. Climate in British Columbia differs from that in Europe, as three different climate zones (Mediterranean, Continental and Oceanic) co-occur in the Greater Vancouver area.

Earthworms were collected in forests by turning over logs, hand sorting of litter, digging or applying mustard solution to extract earthworms from soil. Additionally, we purchased earthworms sold as fishing baits in bait shops close to sampling locations; all bait shops exclusively sold *L. terrestris*, restricting the bait shop dataset to a single species. Earthworms were washed, stored in 75% ethanol in the field and later transferred in the laboratory into 95% ethanol and stored at 16°C. One centimetre of tail tissue of each individual was cut and shipped to the University of Göttingen (Germany) for molecular analyses; remaining body parts are stored as voucher specimens at the University of Minnesota (Minneapolis-St. Paul, MN) and the University of British Columbia (Vancouver, BC).

2.2 | Genetic analyses

Genomic DNA was extracted with the Genaxxon DNA Tissue Mini Prep Kit (Genaxxon) following the manufacturer's protocol. Four molecular markers were amplified: the mitochondrial genes COI (~600 bp; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), 16S rDNA (~750 bp; Pérez-Losada et al., 2009), and 12S rDNA (~400 bp; Simon et al., 1994), and the nuclear gene Histone 3 (~350 bp; Colgan et al., 1998). The PCR cycling conditions had an initial activation step at 95°C for 3 min, 40 amplification cycles (denaturation at 95°C for 30 s, annealing at 53°C for 60 s, elongation at 72°C for 60 s), and a final elongation step at 72°C for 10 min and were sequenced at the Göttingen Genome Sequencing Laboratory (Georg August University Göttingen) and SeqLab Göttingen (Microsynth). Sequences were submitted to the GenBank databases under the accession numbers (MK730610–MK731484) (GenBank www.ncbi.nlm.nih.gov/genbank). Sequences were checked with Sequencher 4.9 (Gene Codes Corporation), and ambiguous positions were coded as wobble bases. Consensus sequences of the individual genes were assembled in BioEdit 7.0.1 (Hall, 1999) and aligned with ClustalW. Genes were analysed individually and in a combined matrix of 2,150 bp; all positions with wobble bases were deleted for further analyses. Sequence alignments (single genes and combined) were collapsed into haplotype alignments using FaBox 1.41 (Villesen, 2007). The best-fit models of sequence evolution were estimated with TOPALi v2.5 (Milne et al., 2004) using the Akaike information criterion (AIC; Akaike, 1973). Trees were constructed using MrBayes 3.2. (Ronquist et al., 2012), partitioning the combined alignment to

the following Iset parameters for *L. rubellus* (COI: nst = 2, rates = invgamma; 16S rDNA: nst = 2, rates = invgamma; 12S rDNA: nst = 6, rates = invgamma; H3: nst = 1, rates = invgamma) and *L. terrestris* (COI: nst = 6, rates = gamma; 16S rDNA: nst = 6, rates = invgamma; 12S rDNA: nst = 2, rates = invgamma; H3: nst = 1, rates = equal). A mcmc run of 4 million generations with default settings was performed. We analysed the North American haplotype identities with European earthworms using Bayesian phylogenetic trees of the COI and H3 datasets and included sequences available from NCBI. A list of the data sources is found in Appendix S1 Table S4. Parameter settings were nst = 6, rates = invgamma and default settings for the mcmc run.

2.3 | Phylogeography and genetic differentiation across putative dispersal barriers

Spatial distribution of genetic clades was analysed with haplotype networks and constructed for 16S rDNA, which provided the most informative resolution. Median-joining (MJ) networks (Bandelt, Forster, & Röhl, 1999) were constructed with PopART (University of Otago, Dunedin, New Zealand) and edited using Inkscape (Software Freedom Conservancy). Parameters were set to equal weights for all mutations and the epsilon parameter to zero to restrict the choice of possible links in the final network.

To test hypotheses about climatic and geographic dispersal barriers, we used analyses of molecular variance (AMOVA) and analysed genetic differentiation among populations using the distance method of Tajima & Nei, pairwise differences without Gamma correction, and pairwise genetic distances using Arlequin 3.5.2.2 (Excoffier et al., 2005). AMOVAs were calculated with COI, the most variable gene regarding nucleotide diversity (Appendix S2 Table S5), and earthworm populations were assigned a priori according to our first hypotheses (H1) into climate zones separating populations from British Columbia (mixed climate), Alberta and Minnesota (cold continental climate), Michigan and New York (moderate continental climate). To test for the relevance of geographic barriers (H2), populations were analysed in three different combinations: Great Plains as main dispersal barrier (BC, AL vs. MN, MI, NY), Rocky Mountains as main dispersal barrier (BC vs. AL, MN, MI, NY), and Rocky Mountains and Great Plains as main dispersal barriers (BC vs. AL vs. MN, MI, NY). Human influence on reducing the effect of dispersal barriers was tested by comparing genetic variance among transects (BC vs. AL vs. MN vs. MI vs. NY). If human transport plays a significant role for earthworms across large geographic distances (H3), genetic variance should be similar among regions.

2.4 | Climate data

The responses of genetically diverse earthworms to ecological factors were inspected using a multiple regression matrix (MRM). Bioclimatic data were retrieved from WorldClim v2 bioclimatic variables database (Fick & Hijmans, 2017) and had a spatial resolution of ~5 km². The response matrix compared genetic pairwise differences

of the COI sequence data and was calculated with the Analysis of Phylogenetics and Evolution (APE) package (Paradis, Claude, & Strimmer, 2004) in R (<http://www.R-project.org>). Tested factors were (a) environmental abiotic parameters, that is, annual mean temperature (BIO01), maximum temperature of the warmest month (BIO05), minimum temperature of the coldest month (BIO06), mean temperature of the wettest quarter (BIO08), mean temperature of the driest quarter (BIO09), annual precipitation (BIO12), precipitation of the driest month (BIO14), precipitation seasonality (BIO15), and (b) the geographical parameter spatial distance and elevation. Data were first transformed into scaled explanatory distance matrices using Euclidean distances for standardization and then normalized. Information on the correlation of the environmental variables is provided in Appendix S2 (Table S6). With the present dataset from the sampling locations provided in Table 1, we have limited power to clearly separate the different climatic drivers that are also likely to interactively drive earthworm communities. As a consequence, our results included several correlated climate variables and should be interpreted with caution, but inspire future work on the clear identification of different environmental drivers and their interactions in affecting earthworm distribution patterns. The spatial distance between each pair of samples was calculated using the Geographic Distance Generator v1.2.3 (Ersts, 2014) with the World Geodetic

System (1984) setting for the reference spheroid and then normalized by dividing the values by the maximum distance value, thus measuring the absolute but normalized distances. The MRM function was executed with the R package *ecodist* (Goslee & Urban, 2007).

We also examined if additional environmental factors (i.e. sampling location, soil pH and human population density) correlated with earthworm abundance and genetic diversity (see Appendix S2 Figures S2–S3). Results indicated a trend that human densities affect genetic diversity and abundances of *L. terrestris* but not of *L. rubellus*.

3 | RESULTS

3.1 | Sampling and genetic diversity

In total, 120 *L. rubellus* (LR) and 122 *L. terrestris* (LT) individuals were sampled from the 25 locations. The number of individuals per transect varied from 12 to 48 for *L. terrestris* and from 12 to 37 for *L. rubellus* (Appendix S1 Figure S1). Nucleotide (NUD) and haplotype diversity (HTD) was greater in *L. rubellus* and decreased in both species from COI to 16S rDNA to 12S rDNA to H3. Overall, nucleotide diversity of COI was two or three times higher in *L. rubellus* than in *L. terrestris* and varied among transects (Appendix S1 Tables S2, S3).

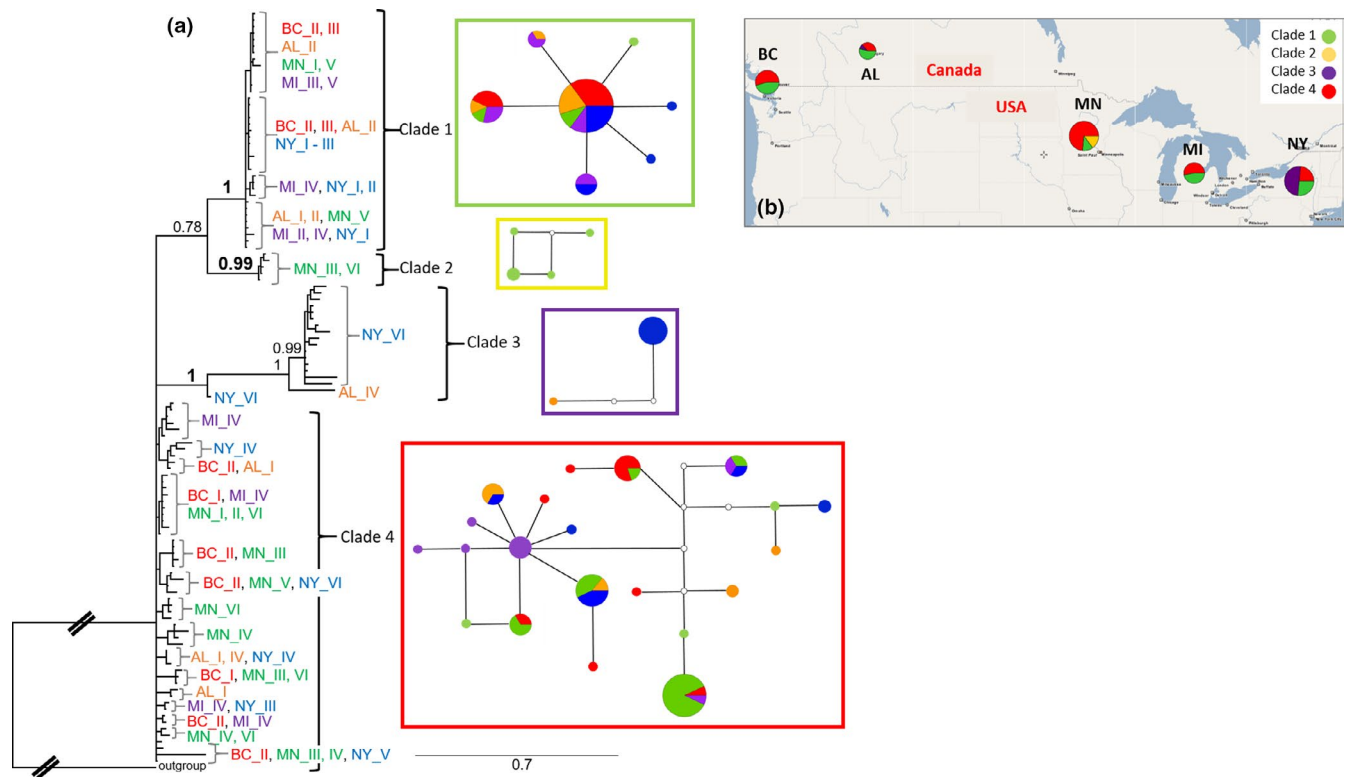


FIGURE 1 Bayesian phylogenetic tree based on a supermatrix of four genes (COI, 16S, 12S and H3) of 120 individuals of *Lumbricus rubellus* (a), and distribution and abundance of the four genetic clades in the five transects across northern North America (b). The corresponding clades of the haplotype network analysis based on 16S are provided next to each clade, the area of each circle is proportional to the numbers of individuals for each haplotype, the colour code refers to the five transects British Columbia (BC, red), Alberta (AL, orange), Minnesota (MN, green), Michigan (MI, violet) and New York (NY, blue). For abbreviations of sampling locations see Table 1, posterior probabilities of well-supported clades are highlighted in bold

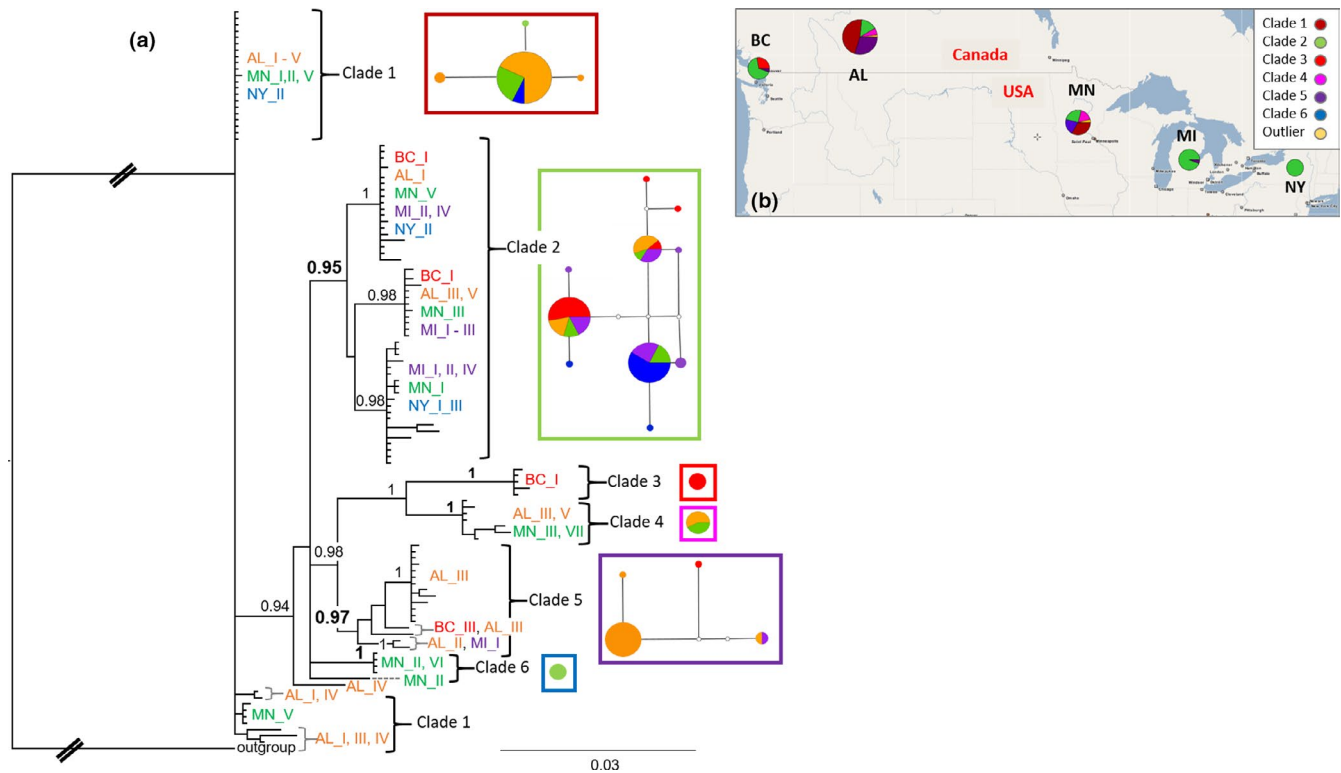


FIGURE 2 Bayesian phylogenetic tree based on a supermatrix of four genes (COI, 16S, 12S and H3) of 122 individuals of *Lumbricus terrestris* (a), and distribution and abundance of the seven genetic clades in the five transects across northern North America (b). The corresponding clades of the haplotype network analysis based on 16S are provided next to each clade, the area of each circle is proportional to the numbers of individuals for each haplotype, the colour code refers to the five transects as in Figure 1. For abbreviations of sampling locations see Table 1, posterior probabilities of well-supported clades are highlighted in bold

3.2 | Relatedness and spatial distribution

In both species, earthworms were closely related resulting in phylogenetic trees with a weakly supported backbone and clades with mixed geographic origin. Accordingly, phylogenetic and geographic structure was generally weak, in particular in *L. rubellus*. However, in both species, some populations formed well-supported clades (posterior probabilities: 0.95–1; Figure 1a) that were also recovered by haplotype network analyses. In *L. rubellus*, two clades comprised closely related individuals from all transects (mixed clades 1 and 4 with 37 and 60 individuals, respectively). However, five individuals from Minnesota (clade 2, green) and 18 individuals from New York (clade 3, blue) were distinct and did not occur in other transects (Figure 1b). All North American COI haplotypes of *L. rubellus* could be assigned to lineages from Europe (Giska, Sechi, & Babik, 2015; Sechi, 2013). Haplotypes of clade 4 corresponded to the widespread European lineages A1–A3. Haplotypes in clade 1 and 2 clustered with European lineages C and D from Eastern Europe (Poland, Hungary, Balkans), and haplotypes in clade 3 clustered with lineage H, which is restricted to Germany and Austria. We compared COI lineages with the H3 dataset to check if mitochondrial and nuclear markers corresponded. The North American haplotypes of the COI clades 1, 2 and 4 carried the same H3 lineage that is also common in Europe (Martinsson & Erséus, 2017). Three individuals from Michigan (clade 4) carried a different H3

lineage, which is undescribed in Europe. Clade 3 comprised several H3 haplotypes, one known from Europe (Martinsson & Erséus, 2017) and one also present as widespread H3 lineage in the common COI clade 4.

Genetic distances among populations of *L. terrestris* were less distinct but had more haplotypes separating into more clades than *L. rubellus* (Figure 2a,b). The largest clade of *L. terrestris* (clade 2, 52 individuals) included haplotypes from all transects. The second largest clade (clade 1, 32 individuals) consisted of a haplotype predominantly found in Alberta (orange) and Minnesota (green) and in one individual from New York (blue). Furthermore, haplotypes from Alberta also occurred in separate clades together with Minnesota (clade 4, 7 individuals), British Columbia (red, single individual) and Michigan (violet, clade 5, 16 individuals). Notably, Minnesota and British Columbia also had distinct haplotypes that formed isolated monophyletic clades (clades 6, 5 individuals and 3, 5 individuals).

Most haplotypes of *L. terrestris* from bait shops were identical to common and widespread haplotypes from field populations (Figure 3). Only few haplotypes formed separate clades (mainly AL and BC) or were related to rare field haplotypes (BC) from the same sampling region. The North American COI and H3 haplotypes of *L. terrestris* were closely related or identical to haplotypes described from Europe or North America in previous studies (Appendix S1 Table S4, Appendix S3 Figures S4–S5).

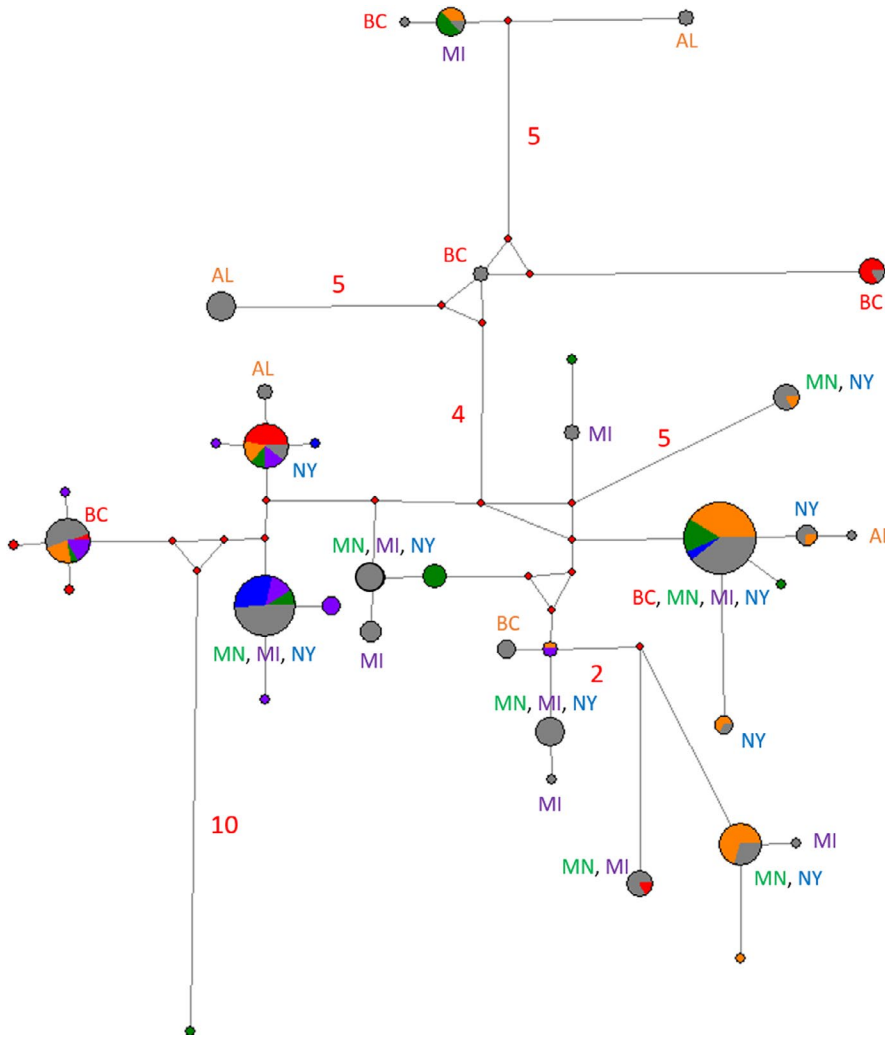


FIGURE 3 Haplotype network based on 16S of 122 individuals of *Lumbricus terrestris* sampled in 25 field locations and of 104 individuals purchased in nearby bait shops. Bait shop individuals are grey and labelled with transects of their origin. Colour codes of transects and field individuals correspond to transects in Figures 1 and 2. Inferred mutation steps between haplotypes that are >1 are indicated by numbers on lines

3.3 | Genetic differentiation across putative barriers

Analysis of molecular variance (AMOVA) across all four genes showed that most of the molecular variance was at local scale (within sampling points = populations, Appendix S2 Table S5), with ~92%–94% of variance in *L. rubellus* and ~70% to 73% in *L. terrestris* in the most variable gene (COI, Table 2). In both species, molecular variance predominantly resided at population level but was much clearer in *L. rubellus* with only 3.75% of variance among populations compared to *L. terrestris* with 17.92% (Table 2). Analyses based on a priori assigned populations to test for effects of climate (H1: transects BC vs. AL, MN vs. MI, NY), geographic barriers (H2: Great Plains = transects BC, AL vs. MN, MI, NY; Rocky Mountains = transects BC vs. AL, MN, MI, NY; Great Plains and Rocky Mountains = transects BC vs. AL vs. MN, MI, NY), and distance (H3: transects BC vs. AL vs. MN vs. MI vs. NY) on population structure also showed very little variance for *L. rubellus* within (3.75%–5.81%) and among geographic populations (0.71%–2.86%), thereby rejecting all hypotheses for this species. However, *L. terrestris* generally showed a higher genetic structure with 11.22% variance among climate regions (H1) followed by distance among regions (9.46%).

3.4 | Importance of bioclimatic factors

The MRM showed contrasting results for the two earthworm species; the permutation test indicated that 22% and 4% of the variance were explained by climatic variables for the complete datasets of *L. rubellus* and *L. terrestris*, respectively (Appendix S2 Table S7). *Lumbricus rubellus* correlated significantly ($p < 0.002$) with all tested bioclimatic factors except for the minimum temperature in the coldest month (BIO06; $p = 0.755$) and seasonality of precipitation (BIO15; $p = 0.084$). For the complete dataset of *L. terrestris*, correlations generally were not significant ($p > 0.130$), except for the minimum temperature of the coldest month (BIO06; $p = 0.022$). We repeated the analysis with reduced datasets containing only the local clades (clades 2 and 3 of *L. rubellus*, all clades except the widespread clade 2 of *L. terrestris*) and the widespread clades (clades 1 and 4 of *L. rubellus*, clade 2 of *L. terrestris*). In the reduced datasets, the variance explained by climatic factors decreased strongly for *L. rubellus*, but increased for the local clades of *L. terrestris* (Table 3). Temperature and seasonal precipitation explained 22% ($r^2 = 0.22$, $p = 0.001$) of the variance of the local clades of *L. terrestris*, correlations for the widespread clade of *L. terrestris* were not significant.

TABLE 2 Analyses of molecular variance (AMOVA) of *Lumbricus rubellus* and *L. terrestris* assigned a priori into populations separated by climate regions (Climate), geographic barriers (Great Plains and Rocky Mountains) or by geographic distance between transects (Transect)

Tested potential barriers	<i>L. rubellus</i>					<i>L. terrestris</i>										
	Within sp	df	Within gg	df	Among gg	df	FCT	p-value	Within sp	df	Within gg	df	Among gg	df	FCT	p-value
Climate																
(BC vs. AL, MN vs. MI, NY)	93.32	104	4.92	17	1.76	2	0.02	0.022	70.58	102	18.20	16	11.22	2	0.11	0.001
Great Plains & Rocky Mountains																
(BC vs. AL vs. MN, MI, NY)	92.47	104	4.81	17	2.71	2	0.03	0.011	71.96	102	20.00	16	8.04	2	0.08	0.015
Great Plains																
(BC, AL vs. MN, MI, NY)	93.48	104	5.81	18	0.71	1	0.01	0.157	72.04	102	22.44	17	5.51	1	0.06	0.036
Rocky Mountains																
(BC vs. AL, MN, MI, NY)	92.52	104	5.43	18	2.06	1	0.02	0.057	72.47	102	24.84	17	2.69	1	0.03	0.224
Transect																
(BC vs. AL vs. MN vs. MI vs. NY)	93.39	104	3.75	15	2.86	4	0.03	0.001	72.62	102	17.92	14	9.46	4	0.09	0.006

Note: Molecular variance is given in percent within sampling points (sp), and within and among geographic groups (gg), *df* = degrees of freedom, FCT = variance among a priori assigned populations.

4 | DISCUSSION

4.1 | Genetic diversity

This study shows that northern North American populations of the two earthworm species *L. rubellus* and *L. terrestris* share the same genetic lineages with populations of their native range in Europe. However, genetic diversity is lower in North America than in Europe, which is typical for invasive species (Sakai et al., 2001; Allendorf & Lundquist, 2003; King, Tibble, & Symondson, 2008; Gailing et al., 2012; Donnelly et al., 2013; Donnelly, Harper, Morgan, Pinto-Juma, & Bruford, 2014; Giska et al., 2015). Consistent with studies in Europe, genetic diversity in *L. rubellus* was higher than in *L. terrestris* (King et al., 2008; Martinsson & Erséus, 2017), and intraspecific genetic distances of COI were comparable with those reported from Europe (King et al., 2008; James et al., 2010; Klarica, Kloss-Brandstätter, Traugott, & Juen, 2011).

In North America, common and widespread haplotypes dominated in both species, but genetic and geographic structure differed. Among populations of *L. rubellus*, haplotypes divided into two genetic lineages that predominantly occurred in all sampling regions (except in New York), which belonged to common and widespread lineages from Europe (Sechi, 2013). Future studies with higher spatial resolution sampling should explore if different nationalities of European settlers are mirrored by the genetic structure of earthworm populations.

The co-occurring pattern of omnipresent lineages of *L. rubellus* and *L. terrestris* across northern North America suggests a common origin and mode of dispersal for both species. In particular road constructions, traffic, logging, fishing and agriculture have been identified as main drivers of earthworm range expansion in North America (Marinissen & van den Bosh, 1992; Dymond, Scheu, & Parkinson, 1997; Casson et al., 2002; Holdsworth et al., 2007; Gundale, Jolly, & Deluca, 2005; Cameron, Bayne, & Coltman, 2008; Cameron & Bayne, 2009) and certainly also apply here. Human-mediated long-range dispersal by passive transport is more likely for *L. rubellus*, which frequently moves in leaf litter near or on the soil surface than for soil-dwelling anecic species (Terhivuo & Saura, 1997). However, the presence of locally occurring lineages in *L. rubellus* and the distinct genetic assembly of *L. terrestris* in Alberta and Minnesota indicate that additional factors affected the dispersal and introduction of these two earthworm species.

The relevance of bait abandonment for the distribution of *L. rubellus* is difficult to assess. This species has been commonly used as fishing bait (Reynolds, 1977), but was not sold in any bait shops we purchased earthworms from. However, dispersal via bait abandonment in the past cannot be excluded. In contrast, *L. terrestris* is the most commonly sold live fishing bait in northern North America today, and a large fraction of individuals from bait shops and the field shared identical or closely related haplotypes, indicating that bait abandonment contributes significantly to the



Bioclimatic factor	Local		Widespread	
	<i>L. rubellus</i>	<i>L. terrestris</i>	<i>L. rubellus</i>	<i>L. terrestris</i>
Annual mean temp.	n/a	/	/	ns
Max. temp. warmest month	n/a	/	0.005**	ns
Min. temp. coldest month	n/a	0.006***	0.019***	ns
Mean temp. wettest month	n/a	0.005**	-0.007*	ns
Mean temp. driest month	n/a	/	-0.016***	ns
Annual precipitation	n/a	/	/	ns
Precipitation driest month	n/a	-0.006***	/	ns
Precipitation seasonality	n/a	0.003**	0.002*	ns
	$r^2 = \text{n/a}$ $p = \text{n/a}$	$r^2 = 0.22$ $p = 0.001$	$r^2 = 0.04$ $p = 0.001$	$r^2 = \text{ns}$ $p = \text{ns}$

Note: n/a, not available due to small sample size; ns, not significant; r^2 , standardized coefficient of a regression analysis indicating the influence of the bioclimatic factors (independent variable) on genetic variance (dependent variable).

* $p > 0.05$.

** $0.001 < p < 0.01$.

*** $p < 0.001$.

spread of *L. terrestris*. Historically, earthworms sold as fishing baits were collected from fields and sold locally, but establishment of refrigerated warehouses by large distributors selling pre-packed baits nationwide might additionally contribute to long-distance spread of genetic diversity. This assumption is supported by a study at local scale in Calgary, Alberta, that demonstrated the genetic relatedness of bait and field populations with fine resolution markers (Klein et al., 2017). Here, at large scale, bait haplotypes from Alberta and British Columbia in part did not match the haplotypes of nearby field populations, but rather field populations of far distant transects. However, bait cannot be the only source and vector for dispersal of *L. terrestris*, since the two most distant transects of Alberta and Minnesota contained three genetic clades that occurred nowhere else, indicating the existence of a distinct dispersal vector that connects these two transects.

4.2 | Climate and dispersal barriers

Genetic variation among regions was very low for *L. rubellus*, and bioclimatic factors or dispersal barriers did not explain the distribution of common lineages, which agrees with its higher tolerance to frost (Fisichelli et al., 2013; Sims & Gerard, 1999; Tiunov et al., 2006). The ability of epi-endogeic earthworms to quickly adapt to cold and fluctuating temperatures through behavioural and physiological changes (Holmstrup, 2003), and their persistence to perturbations, such as heavy metal pollution by fertilizers and intoxication by pesticides, are well known (Edwards & Bohlen,

TABLE 3 Genetic variance of *Lumbricus rubellus* and *L. terrestris* explained by bioclimatic factors, for local and widespread genetic clades

1996; Kruse & Barrett, 1985; Levine, Hall, Barrett, & Taylor, 1989). Although consecutive summer droughts can have strong effects on epi-endogeic earthworms, drought resistance of cocoons allows persistence through dry periods (Eggleton et al., 2009; Holmstrup & Loeschcke, 2003).

In contrast to *L. rubellus*, genetic variance in the common lineages of *L. terrestris* in part was related to climate factors, in particular frost, drought and seasonality. These results corresponded to findings that anecic earthworm species are negatively affected by prolonged drought periods, high frequency of freeze-thaw cycles and low soil moisture during their prime reproductive periods in spring and autumn (Addison, 2009; Curry, 2004; Sims & Gerard, 1999). Conform to these findings, the distinct genetic composition of populations in Alberta and Minnesota correlated with the continental climate in both transects. However, both species were recorded from areas with harsh frost conditions (Booyesen et al., 2018; Wackett et al., 2018). These areas were associated with recent human introductions and human land use indicating potential new stepping stones of earthworm invasions. If the more severe frost and drought periods in these regions facilitated genetic diversity by continuous extinctions and reintroductions, or if only climatically pre-adapted lineages were able to establish viable populations in these areas needs to be investigated under controlled experimental conditions (Holmstrup, 2003). Additionally, joined analyses of more molecular datasets from North America and Europe would be an important step to explore the historical and recent invasion routes of exotic earthworms. Moreover, the application of more variable markers, for example, SNPs or microsatellites, would provide a more detailed picture on gene flow



from which we can infer dispersal barriers and migration routes or the extent of genetic bottlenecks earthworms experienced during their invasion.

5 | CONCLUSIONS

Genetic diversity and structure of the two invasive earthworm species *L. rubellus* and *L. terrestris* was homogenous across all regions indicating a dominant common dispersal vector and the ability to adjust to most environmental conditions in northern North America. However, *L. terrestris* was genetically more structured, and here its genetic variance positively correlated with harsh climatic conditions in central North America. In contrast to *L. rubellus*, this species is common in arable fields with frequent disturbances, and distinctness of genetic lineages occurring predominantly in transects of Alberta and Minnesota could be explained by their position at the edges of the North American corn belt. Genetic patterns indicate that both species have common long-distance distribution vector(s). For *L. terrestris*, nation-wide bait distributors potentially play a major role as dispersal agent of field populations. In the past two decades, the globalization of economy has changed infrastructure, intensity and range of traffic including commercial distribution of soil-related goods, and potentially will increase dispersal of *L. rubellus* and *L. terrestris*.

Our present study exemplifies how earthworms as belowground invaders with substantial differences in life history traits can be used to test broad questions in invasion ecology, such as the genetic underpinnings of successful invasion events, geographic and climatic dispersal barriers, as well as the human role in ecologically relevant invasions. Thus, the present results may inspire future work on the role of different hypothesized main drivers of invasive species that can be explored with comparative genetic analyses (Sovic, Carstens, & Gibbs, 2016).

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BIOSKETCH

Andreas Klein is interested in phylogeography, invasion biology and population genetics of lumbricid earthworms, with particular interest in dispersal vectors and climate adaptation. This study is part of his PhD work at the universities of Göttingen and Leipzig on the spread of European earthworms in North America.

Author contributions: A.K., N.E. and I.S. conceived the original idea. A.K. conducted the field work, collected and analysed the data, and wrote the manuscript. All authors contributed to this study and the manuscript in form of discussions, suggestions and revisions, and approved the final manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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