



A comparison of latitudinal species diversity patterns between riverine and terrestrial earthworms from the North American temperate zone

Hiroshi Ikeda¹ | Mac A. Callaham Jr.² | Richard P. Shefferson³ | Evelyn S. Wenk² | Carlos Fragoso⁴

¹Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Aomori, Japan

²Center for Forest Disturbance Science, Southern Research Station, USDA Forest Service, Athens, GA, USA

³Organization for Programs on Environmental Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan

⁴Biodiversity and Systematics Network, Instituto de Ecología A.C., Xalapa, Veracruz, Mexico

Correspondence

Hiroshi Ikeda, Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan.
Email: hikeda@hirosaki-u.ac.jp

Funding information

Japan Society for the Promotion of Science, Grant/Award Number: 22-6882 and 25891002

Handling Editor: Evan Economo

Abstract

Aim: Latitudinal clines of species diversity are widely documented in terrestrial and marine ecosystems. However, the processes governing species diversity gradients in riverine ecosystems have not been well-studied. We addressed this issue by comparing species diversity between riverine aquatic and terrestrial earthworm groups (genus *Sparganophilus* and *Diplocardia*, respectively).

Location: North American temperate zone.

Taxon: *Sparganophilus* and *Diplocardia*.

Methods: We collected 556 *Sparganophilus* earthworms from 64 sites spanning 27 degrees of latitude (18.77°–45.90°N), and 165 *Diplocardia* earthworms from 23 sites (21 degrees, 19.77°–41.20°N). We split potential species from the phylogenetic trees based on four genes and compared the latitudinal pattern of species diversity between these two groups.

Results: We estimated the number of potential species to be 10 for *Sparganophilus* and 8 for *Diplocardia*, respectively, from 526 haplotypes (403 in *Sparganophilus* and 123 in *Diplocardia*). *Sparganophilus* species diversity was higher at mid-latitudes (32° to 40°) due to a preponderance of species with limited geographical distributions, whereas all specimens collected north of 40° belonged to broadly distributed species. Species with limited geographical distributions were more often collected at higher elevations than broadly distributed species in *Sparganophilus*. For *Diplocardia*, species diversity was higher at lower latitudes (28° to 32°).

Main Conclusions: These results suggest that, in *Sparganophilus*, species composition at higher latitudes above 40° is derived from range expansion by broadly distributed species from lower latitudes. The high elevation area in the native distribution range of *Sparganophilus* is limited to the Appalachian Mountains, which ranges above 33° in latitude. The high species diversity of *Sparganophilus* with limited distributions at mid-latitude (32°–40°) suggests that the headwater regions at the Appalachian Mountains are sites for more frequent speciation.

KEYWORDS

Diplocardia, latitudinal cline, range size, riverine ecosystem, *Sparganophilus*, speciation

1 | INTRODUCTION

Latitudinal clines of species diversity are one of a few generalized patterns in biogeography. Although the pattern has been widely characterized, the processes driving the latitudinal pattern are still a matter of debate (e.g. Allen, Gillooly, Savage, & Brown, 2006; Jablonski, Roy, & Valentine, 2006; Weir & Schluter, 2007). This latitudinal pattern is mainly thought to be due to more speciation events in a lower latitudinal area and due to a distribution range expansion of each species from lower to higher latitudes (Jablonski et al., 2006). Allen et al. (2006) indicate that a higher individual metabolic rate in a warmer area is a primary determinant of a higher speciation rate at lower latitudes. On the other hand, Weir and Schluter (2007) suggest that a faster turnover (higher speciation and extinction rates) at higher latitudes contributes to the latitudinal diversity gradient. In addition, the major factors affecting the latitudinal pattern can vary at different latitudes, and the evolutionary time hypothesis holds for temperate areas affected by extremely cold climate during past glacial periods (Gaston & Blackburn, 2000; Mittelbach et al., 2007; Wallace, 1878; Wiens, Pyron, & Moen, 2011). The evolutionary time hypothesis suggests that the species richness in an area is related to the length of time available for speciation to occur.

Riverine ecosystems are characterized globally by a range of diverse and unique species. Although there have been many studies concerning the latitudinal pattern of species diversity in terrestrial and marine ecosystems (e.g. Allen et al., 2006; Jablonski et al., 2006; Weir & Schluter, 2007), the findings from these studies might not be appropriate for interpreting species diversity patterns in riverine systems. The processes governing species diversity in fish and invertebrates in riverine ecosystems have not been well-studied. One meta-analysis reported that latitudinal gradients of species diversity are much weaker in freshwater than in terrestrial and marine habitats (Hillebrand, 2004), while another showed that species diversity of rheophilic insects was higher in tropical streams compared to mid-latitude streams (Stout & Vandermeer, 1975). However, these studies only compared species diversity, and thus did not reveal the processes causing a species diversity gradient such as speciation and genetic differentiation patterns. Although several researchers have examined the genetic differentiation of fish along rivers in North America (e.g. Avise, 2000; Soltis, Morris, McLachlan, Manos, & Soltis, 2006), speciation cannot be detected because of the low genetic differentiation among populations due to their high dispersal ability. There are some studies concerning the genetic structure in riverine aquatic insects that have a lower dispersal ability (e.g. Galacatos, Cognato, & Sperling, 2002; Zickovich & Bohonak, 2007), but these studies are localized samples and hence are not useful for understanding latitudinal patterns.

As with freshwater ecosystems, there have been relatively few studies examining the general relationship between latitude and diversity in soil ecosystems. Among these studies, very few indeed have dealt with latitude/soil animal diversity explicitly (i.e. not focusing on soil microbial community diversity/structure/function). The general

lack of studies in this area was pointed out by Decaëns (2010); but recently, a meta-analysis study for the global pattern of earthworm diversity has been published (Phillips et al., 2019). Interestingly, they found that the regional diversity of earthworms was higher at mid-latitudes, but that of unique earthworms was higher at lower latitudes. They also found that the effects of previous glaciations had a strong impact on earthworm diversity. In North America, glacial history has long been hypothesized to have structured earthworm communities (Gates, 1977; James, 2004), and this glacial history has been used to explain a latitudinal cline in species diversity for other organisms in the continent as well.

In this study, we used a molecular phylogenetic approach to address the speciation and dispersal patterns in riverine ecosystems along a latitudinal gradient in the North American temperate zone by comparing patterns between two genera within the native earthworm fauna. The first of these genera, *Sparganophilus* (Sparganophilidae) (Figure 1a), is composed of riverine sediment-dwelling earthworms, and the second genus, *Diplocardia* (Acanthodrilidae) (Figure 1b), is composed of terrestrial, soil-dwelling earthworms. *Sparganophilus* earthworms are obligately aquatic inhabitants of humus and sediments along rivers and ponds, as they are never encountered in litter or soils of truly terrestrial habitats such as forests or grasslands. Although several studies for freshwater fish have revealed the intraspecific and interspecific phylogenetic differentiation along the length of rivers (Avise, 2000; Bernatchez & Osinov, 1995; Haponski & Stepien, 2008), earthworms are expected to have a poorer dispersal ability than fish, and therefore to have experienced more allopatric speciation than fishes. *Sparganophilus* species are mainly distributed in the North American continent from the southern part of Canada to Mexico throughout the temperate zone (Reynolds, 2008; Reynolds & Wetzel, 2008). The genus *Sparganophilus* is native to North America, but has been introduced in other parts of the world, notably in the River Thames, as well as in continental Europe (Rota, Bartoli, & Laini, 2014). Therefore, *Sparganophilus* earthworms are an excellent candidate group of organisms for examining latitudinal patterns of speciation in riverine ecosystems.

Thirteen *Sparganophilus* species are currently known based on their morphological differences in North America (Reynolds, 2008; Reynolds & Wetzel, 2008). However, it is difficult to identify earthworm species using their morphological characters because these external morphological characters are relatively limited in number and depend on the full development of sexual characters in individual specimens (e.g. Ikeda et al., 2018; Ikeda, Tsuchiya, Nagata, Ito, & Sota, 2012). Species-level classification of the genus *Sparganophilus* has not been seriously pursued since the 1970s, but more work in this area is expected to reveal many more species than currently described (Carrera-Martínez, 2018). Fortunately, in the light of the difficulty in identifying specimens, it has become an increasingly accepted practice in recent years to use molecular designations of species identity to allow previously unidentifiable specimens to be used in ecological studies (Ikeda et al., 2018; Richard et al., 2010).

We also used a native North American terrestrial earthworm group, *Diplocardia*, to allow comparisons between aquatic and

FIGURE 1 (a) An adult and (c) the castings of *Sparganophilus* species, and (b) an adult of *Diplocardia* species. Examples of the sampling sites for (d) *Sparganophilus* species and (e) *Diplocardia* species. Photo credits: R. Carerra-Martinez (a–c) and H. Ikeda (d and e)



terrestrial species. *Diplocardia* inhabits forest and grassland soils, with 50 species known in North America based on their morphological differences (Damoff, 2018; Damoff & Reynolds, 2017; Fragoso & Rojas, 2016; Reynolds, & Wetzel, 2008, 2012). The native distribution range of *Diplocardia* is limited to North America (Gates, 1977), and is similar to that of *Sparganophilus*. Therefore, terrestrial *Diplocardia* is an appropriate earthworm group for comparing speciation and dispersal patterns with riverine *Sparganophilus*. Generally, terrestrial earthworms are mainly classified into three ecological types (epigeic, endogeic and anecic; Bouché, 1977), and most *Diplocardia* species are endogeic (Callahan, personal observation). Species living in unstable environments tend to have a high dispersal ability, and move among habitats frequently, causing low rates of allopatric speciation, whereas species living in stable environments tend to have a low dispersal ability, causing high rates of allopatric speciation (Papadopolou, Anastasiou, Keskin, & Vogler, 2009; Ribera, Barraclough, & Vogler, 2001). Several disturbances can occur in riverine ecosystems (e.g. floods and droughts), whereas the soil environment is relatively stable. In addition, the river flow can cause the migration of earthworm individuals and cocoons. Therefore, we hypothesized that the riverine *Sparganophilus* species would be genetically less differentiated among populations than the terrestrial *Diplocardia* species.

2 | MATERIALS AND METHODS

2.1 | Sampling

We collected earthworms over a wide geographical area in North America from 2011 to 2013. *Sparganophilus* samples were collected from humus and sediments along rivers and small streams (Figure 1c,d), and *Diplocardia* samples were collected from nearby forest or grassland soils (Figure 1e; see Table S1.1

in Appendix S1). We collected earthworms for about half an hour at each site.

2.2 | DNA sequencing

The total genomic DNA of earthworms was extracted using DNeasy Blood & Tissue Kit (Qiagen). The following primers were used for PCR amplification and direct sequencing: mitochondrial COI gene: LCO1490 (forward), 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' (Folmer, Black, Hoeh, Lutz, & Virjenhoek, 1994), COI2198E (reverse), 5'-TAW ACT TCW GGG TGW CCR AAR AAT CA-3' (Ikeda et al., 2018); mitochondrial 16S gene: 16SF2 (forward), 5'-CGA CTG TTT AAC AAA AAC ATT GC-3' (Pérez-Losada, Ricoy, Marshall, & Domínguez, 2009), 16SR2 (reverse), 5'-GTT TAA ACC TGT GGC ACT ATT C-3' (Pérez-Losada et al., 2009); nuclear 28S gene: 28s-RD3.3f (forward), 5'-GAA GAG AGA GTT CAA GAG TAC G-3' (Pérez-Losada et al., 2009), 28SD1 (forward), 5'-CCT AGG AGT CGG GTT GTT TG-3' (this study), 28SD3 (forward), 5'-GAG TCG GGT TGT TTG AGA TTG-3' (this study), 28s-rD5b (reverse), 5'-CCA CAG CGC CAG TTC TGC TTA C-3' (Whiting, 2002); and nuclear H3 gene: H3F (forward), 5'-ATG GCT CGT ACC AAG CAG ACV GC-3' (Colgan et al., 1998), H3R (reverse), 5'-ATA TCC TTR GGC ATR ATR GTG AC-3' (Colgan et al., 1998), H3R4 (reverse), 5'-TGG GCA TGA TGG TGA CGC GCT-3' (Ikeda et al., 2018). Sequencing of the purified PCR products was performed using the services of Macrogen. Sequencing of the COI region of some specimens was performed at the Canadian Centre for DNA Barcoding (CCDB), Canada. Samples were sequenced from forward first, and if the results were ambiguous or unclear, we also obtained the reverse sequence data for these samples. We obtained at least one gene region from all specimens. Sequence data were deposited in GenBank (Table S1.2 in Appendix S1).

We used MAFFT v.7.304 software (qinsi method) to develop the 16S and 28S alignments (Katoh & Standley, 2013). The alignments of all genes were inspected by eye for obvious misalignment. Haplotypes

were detected using the 'pgglimduseq' command in Phylogears2 v. 2.0 software (Tanabe, 2008), and collapsed for phylogenetic analyses.

2.3 | Phylogenetic analysis

We used 1,035 bp of the 16S gene, 637 bp of the COI gene, 898 bp of the 28S gene and 282 bp of the H3 gene for phylogenetic analysis. The optimum substitution models of each data set for phylogenetic analysis were estimated using Kakusan4 software (Tanabe, 2011). Bayesian analysis was performed using BEAST v. 1.10.4 software (Drummond, Suchard, Xie, & Rambaut, 2012) with substitution models selected according to the BIC4 goodness-of-fit measure (COI: GTR+G; 16S: GTR+G; 28S: GTR+G; H3: HKY+G). The COI sequences were partitioned by codon positions without unlinking parameters among codon positions. Enchytraeidae, Tubificidae and Moniligastridae samples were used as outgroups (GenBank accession numbers: LC199985, LC199992, LC200017, LC200019, LC200023, LC200054, LC200078, LC200082, LC200152, LC200156, LC200175, LC200185, LC200240, LC200250, LC200254, LC200325, LC200329, LC200349, LC200363, LC475699, LC475700, LC476004, LC476187–LC476189, LC476547–LC476550, MK971718). We also included some other families (Lumbricidae, Megascolecidae and Glossoscolecidae) to construct the phylogenetic tree (GenBank accession numbers: LC475687–LC475698, LC475984–LC476003, LC476525–LC476546, MK971710, MK971712, MK971723, MK971736, MK971739, MK971740, MK971743, MK971756).

First, we constructed a haplotype tree with all haplotypes to include divergence within species. We used the lognormal relaxed clock model for the analysis. We performed three Markov chain Monte Carlo runs for 500 million generations, with trees sampled every 10,000 generations; the first 400 million generations were discarded as burn-in. We combined the remaining trees from the three runs and used them for constructing a tree. Maximum likelihood analyses were performed using RAxML ver. 8.2.9 (Stamatakis, 2014) based on the models selected by AIC (separate model among regions and non-partitioned model among codons, GTR+G for all regions) with 1,000 bootstrap replications.

We split the potential species (molecular operational taxonomic units: MOTUs) from the phylogenetic trees that include divergence within species (haplotype tree) because morphological identification of earthworms is difficult and relies on a relatively small number of characters, including sexual characters that may or may not be fully developed at the time of collection (e.g. Ikeda et al., 2018; Ikeda et al., 2012). Species-level classification of *Sparganophilus* has also not been well-studied and is currently in development (Carerra-Martínez, 2018). Thus, we used MOTUs as potential species in this study. We used a multilocus species delimitation method implemented in the program 'tr2' (Fujisawa, Aswad, & Barraclough, 2016). This program resolves species limits with multilocus sequence data using a guide tree constructed from multiple genes and each gene tree. Species delimitation programs have been developed for a single gene and/or a single tree (e.g. Monaghan et al., 2009), and

these programs can overestimate species number. However, the tr2 program uses multiple gene trees and prevents such an overestimation (Fujisawa et al., 2016). The haplotype tree constructed from all four genes was used as a guide tree for analysis. We also constructed each gene tree for analysis using BEAST v. 1.10.4 software (Drummond et al., 2012) for multilocus species delimitation. We used the same substitution models and settings for calibration that were used for the haplotype tree to construct each gene tree. We performed a Markov chain Monte Carlo run for 400 million generations, with sampling every 10,000 generations for each gene. The first 200 million generations were discarded as burn-in. We used the remaining trees for constructing each gene tree. The separated species (MOTUs) were considered as potential species in the analysis to construct the species tree. Potential species with more than five latitudinal degrees of distribution range were considered as broadly distributed (broad species), and those with less than five latitudinal degrees of distribution range were considered as locally distributed (local species).

We constructed the species tree, in which the divergence among haplotypes within species was considered as the variation within species, in the program *BEAST (Heled & Drummond, 2010) in BEAST v. 1.8.4 software (Drummond et al., 2012). We used the same substitution models and settings for calibration that were used for the haplotype tree except for the random local

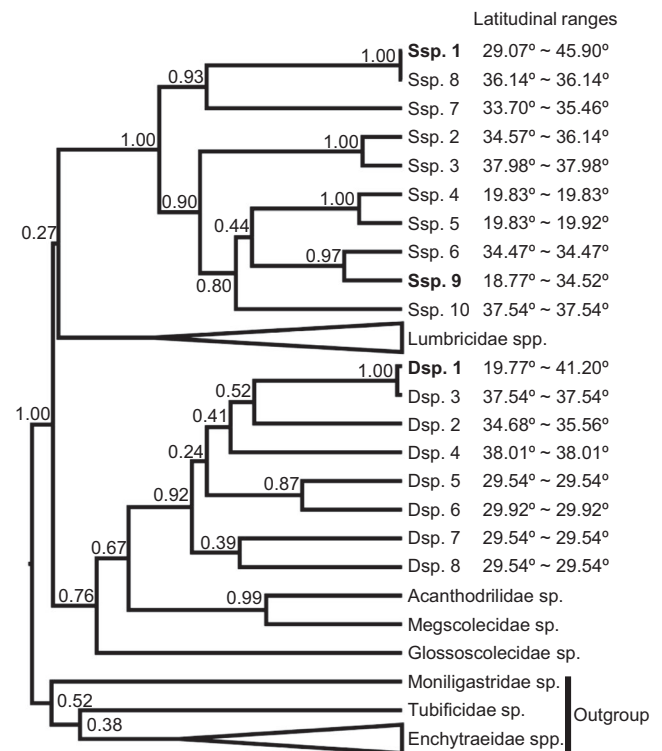


FIGURE 2 Bayesian inference tree of potential species of *Sparganophilus* (Ssp. 1 ~ Ssp. 10) and *Diplocardia* (Dsp. 1 ~ Dsp. 8) collected from North America with their latitudinal distribution ranges constructed from all genes (COI, 16S, 28S, H3). Posterior probabilities are shown for nodes. The potential species with broad distribution (broad species) are shown in bold

clock model. We performed a Markov chain Monte Carlo run for 300 million generations starting from a UPGMA tree, with trees sampled every 10,000 generations, and the first 200 million generations were discarded as burn-in. We used the remaining trees to construct gene trees.

2.4 | Relationship between latitude, elevation and species structure

We divided the sampling sites at intervals of four latitudinal degrees and calculated the number of species at each latitudinal interval to examine the pattern of species diversity along latitude. To compare species diversity among latitudinal intervals while considering the different sampling efforts used, we calculated the rarefaction curves of potential species in each latitudinal interval where more than two species were collected using EstimateS v. 9 software (Colwell, 2013). To examine the differences in the

elevational distributions between the locally and broadly distributed species, we compared the elevations of the collected sites between them. We used the R statistical software version 3.5.0 (R Core Team, 2018) to perform a generalized linear model analysis with a Gamma distribution and log link.

2.5 | Genetic differentiation among populations

We conducted an analysis of molecular variance using ARLEQUIN version 3.5.2.2 (Excoffier, Laval, & Schneider, 2005) to examine a genetic differentiation in the COI, 16S, 28S and H3 gene regions among sites in broadly distributed species (broad species) collected from more than five sites. Then we examined the effect of isolation by geographical distance on uncorrected pairwise genetic distances. We conducted a Mantel test with Pearson's correlation coefficient using the 'mantel' command with one million permutations in the 'vegan' package in R ver. 3.5.0 to assess the relationship between geographical distance and

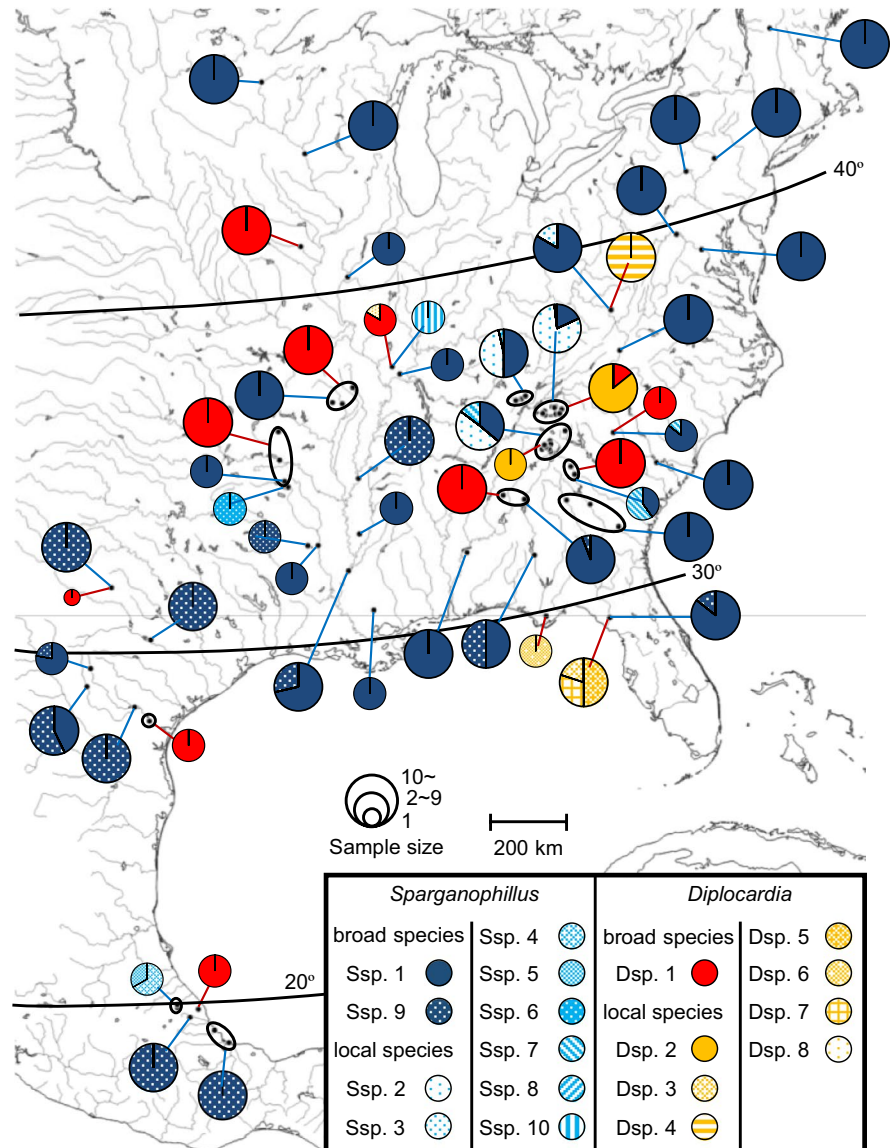


FIGURE 3 Geographical distribution pattern of each potential species of *Sparganophilus* and *Diplocardia* in North America

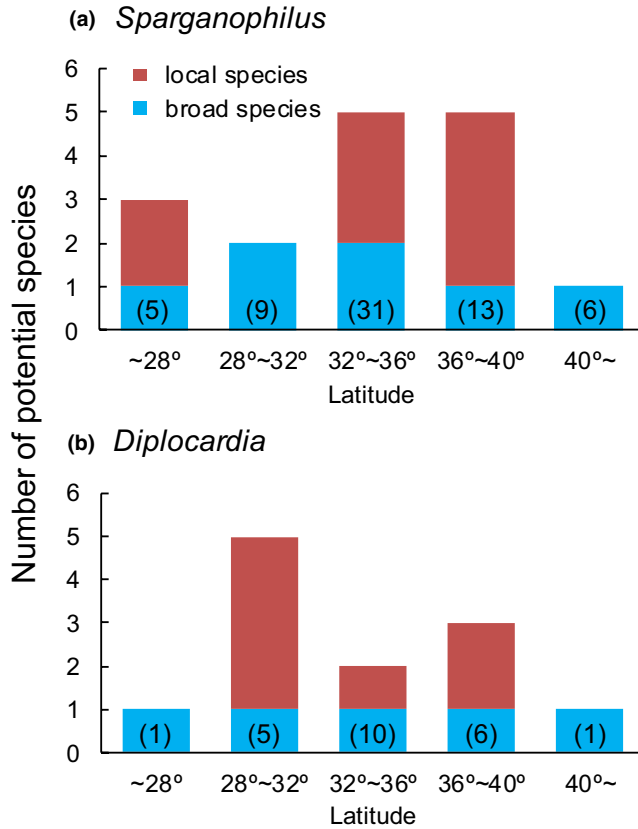


FIGURE 4 Latitudinal pattern of the diversity of potential species in (a) *Sparganophilus* and (b) *Diplocardia*. Values in parentheses indicate the number of sampling sites in each latitudinal range

genetic differentiation (R Core Team, 2018). Poor PCR amplification for the 28S gene in one *Diplocardia* species (Dsp. 1) limited the sample number so no analyses were possible. Geographical distance was $\log_{10}$ -transformed in all analyses.

3 | RESULTS

We collected 556 *Sparganophilus* earthworms from 64 sites spanning 27 degrees of latitude (18.77°–45.90°N), and 165 *Diplocardia* earthworms from 23 sites (21 degrees, 19.77°–41.20°N) (Table S1.1 and S1.2 in Appendix S1). We estimated the number of potential species to be 10 for *Sparganophilus* and 8 for *Diplocardia*, respectively, from 526 haplotypes (403 in *Sparganophilus* and 123 in *Diplocardia*; Figure 2, Table S1.3 in Appendix S1) using the haplotype tree (Figure S1.1 in Appendix S1). There were two potential species with broad distribution (broad species) in *Sparganophilus*, and one in *Diplocardia*. One broad species in *Sparganophilus* was collected at higher latitudes (Ssp. 1), while one species was collected at lower latitudes (Ssp. 9).

Species richness was high at latitudes ranging from 32° to 40° (Figures 3 and 4) in *Sparganophilus*. All individuals collected above 40° were broadly distributed (Ssp. 1). Conversely, locally distributed *Sparganophilus* species were more often collected at latitudes below

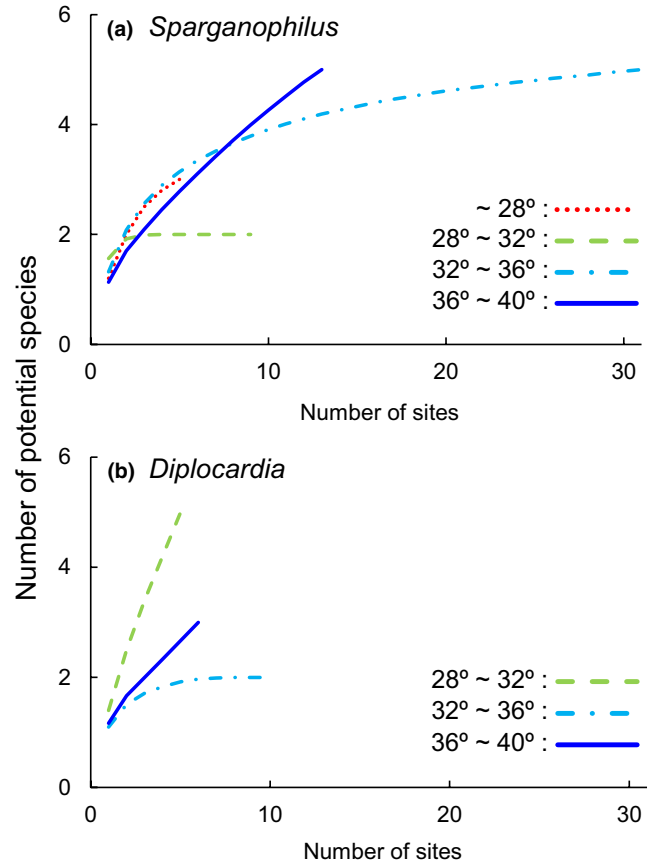


FIGURE 5 Rarefaction curves for potential species of (a) *Sparganophilus* and (b) *Diplocardia*

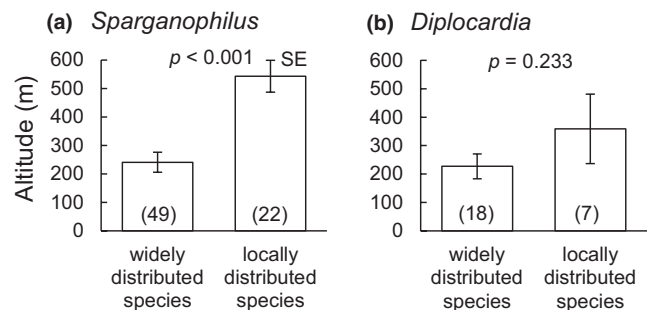


FIGURE 6 Elevational distributions of widely and locally distributed species in (a) *Sparganophilus* and (b) *Diplocardia*. Values in parentheses indicate the number of sampling sites

40°, specifically at latitudes ranging from 32° to 40°. For *Diplocardia*, a broad species was collected from all latitudinal ranges examined in this study. We did not collect locally distributed species at latitudes below 28° or above 40°, although the number of sampling sites was low at these latitudinal ranges. We collected some locally distributed *Diplocardia* species at the latitudinal range from 28° to 40°, specifically from 28° to 32°. Species structure in the *Diplocardia* was markedly different among latitudinal ranges because there were more local species than broad species. Rarefaction curves showed increased species richness in *Diplocardia* (mainly composed of local species) at



TABLE 1 Statistical results for genetic differences among populations. Significant *p* values are in **bold**

COI	16S				28S				H3			
	<i>F_{st}</i> value	<i>p</i> for <i>F_{st}</i>	Mantel <i>r</i>	<i>p</i> for Mantel test	<i>F_{st}</i> value	<i>p</i> for <i>F_{st}</i>	Mantel <i>r</i>	<i>p</i> for Mantel test	<i>F_{st}</i> value	<i>p</i> for <i>F_{st}</i>	Mantel <i>r</i>	<i>p</i> for Mantel test
<i>Sparganophilus</i>												
Ssp. 1	0.714	<.001	0.190	<.001	0.615	<.001	0.180	.004	0.537	<.001	0.184	<.001
Ssp. 9	0.776	<.001	0.206	.042	0.837	<.001	0.172	.063	0.638	<.001	0.332	.002
<i>Diplocardia</i>												
Dsp. 1	0.571	<.001	0.403	<.001	0.627	<.001	0.591	<.001	0.629	<.001	0.366	<.001

lower latitudes ranging from 28° to 32°, whereas species richness in *Sparganophilus* (also mainly composed of local species) was lower at latitudes ranging from 28° to 32° (Figure 5). More local species were collected at higher elevations than broad species in *Sparganophilus* (Figure 6a; *t*-value: 3.54, *p* < .001), whereas such a difference was not detected in *Diplocardia* (Figure 6b; *t*-value: 1.23, *p* = .233).

Genetic differentiation among populations (*F_{st}*) was significantly supported for all gene regions of all examined broad species (Table 1). Positive correlations between geographical distances and pairwise genetic differences were also significantly supported in at least two of the four examined gene regions for all broad species. These results indicate the incidence of geographical genetic differentiation within broad species for both *Sparganophilus* and *Diplocardia*, suggesting potential for future allopatric speciation. For the *Diplocardia* species, genetic differences were significantly positively correlated with geographical distances for all examined gene regions, indicating a stronger geographical differentiation than that observed for the *Sparganophilus* species.

4 | DISCUSSION

For *Sparganophilus*, locally distributed species were collected at latitudes below 40° but not above 40°. These results suggest that most of the speciation in *Sparganophilus* has occurred at lower and middle latitudes below 40°, whereas the *Sparganophilus* species composition at higher latitudes above 40° is mainly derived from range expansion by the broadly distributed species from lower latitudes. These worms could not migrate to higher latitudes during the glacial periods in the Quaternary because of the low temperatures (i.e. most waterways and suitable habitat would have been frozen or too cold for earthworm survival), and thus broadly distributed species living at higher latitudes above 40° in the present day must have migrated there relatively recently within the 12,000 years since the last glacial period. This type of species diversity relationship with latitude is well-explained by the evolutionary time hypothesis (Gaston & Blackburn, 2000; Mittelbach et al., 2007; Wallace, 1878; Wiens et al., 2011). There has not been enough time for speciation since the species have migrated to habitats newly available due to the recession of glaciers. However, we did observe a genetic differentiation with geographical distance within the broadly distributed species, suggesting that future speciation is possible if the populations at higher latitudes persist for a longer period.

Rarefaction curves showed a higher species diversity at lower latitudes (28° to 32°) than at higher latitudes (32° to 40°) in *Diplocardia*; however, we could not obtain the rarefaction curve below 28° because we did not have enough samples for analysis. Phillips et al. (2019) showed that the number of unique earthworm species was higher at lower latitudes. *Diplocardia* is the native earthworm group in North America, and our result may correspond to this latitudinal pattern of native species diversity. In contrast, species diversity was higher at mid-latitudes (32° to 40°)



in *Sparganophilus* due to the higher diversity of the local species. Rapoport's rule, where mean range size is narrower at lower latitudes, is widely observed in various organisms such as animals and plants (Stevens, 1989), marine bacteria (Sul, Oliver, Ducklow, Amaral-Zettler, & Sogin, 2013) and soil fungi (Tederloo et al., 2014). This latitudinal gradient of range size leads to the latitudinal gradient of species diversity (Stevens, 1989). However, local *Sparganophilus* species were often collected at mid-latitudes (32° to 40°) as well as at lower latitudes (below 28°) in our study, causing a high species diversity at the mid-latitudes. In addition, more local species were collected at higher elevations where headwaters exist. Most sampling records for *Sparganophilus* are from the central and eastern USA (Reynolds & Wetzel, 2008); the Appalachian Mountains, which ranges above 33° in latitude, is the only high elevation area. For these reasons, more potential species, specifically more local species, were obtained at mid-latitudes (32° to 40°) with high elevation areas. These headwater regions might have been sites for frequent speciation because *Sparganophilus* populations in these sites have likely experienced very little gene flow due to the geographical isolation among rivers. Therefore, species diversity of *Sparganophilus* may be less explained by latitude and related to the specific factors of the river habitat: the regional pattern of geographical discontinuity among headwater watersheds and the connectedness among habitats related to drainage networks.

Our results showed that a similar number of potential species was in *Diplocardia* as in *Sparganophilus*. However, only one *Diplocardia* species was broadly distributed, whereas two *Sparganophilus* species were classified as such. The latitudinal distribution ranges of the species were relatively higher in *Sparganophilus* than in *Diplocardia* (*Sparganophilus*: 3.60 ± 2.13 [SE], *Diplocardia*: 2.79 ± 2.67). Species living in unstable environments tend to have a high dispersal ability, causing low rates of allopatric speciation, whereas species living in stable environments tend to have a low dispersal ability, causing high rates of allopatric speciation (Papadopoulou et al., 2009; Ribera et al., 2001). Different disturbances can occur in riverine ecosystems relative to the soil environment, and the latter may even be more stable. Horizontal movement is also less likely to occur in soils than in river sediments because most *Diplocardia* species are endogeic living in the soil, and during our field sampling, surface layer collection was never done. In addition, the river flow can cause the migration of earthworm individuals and cocoons in *Sparganophilus*. Therefore, it is likely that animals in riverine habitats are less genetically differentiated among populations or have fewer new species with local distributions through allopatric speciation relative to those inhabiting the soil.

We can infer from the rarefaction curves that even more locally restricted species are likely to be discovered at latitudes ranging from 28° to 32° for *Diplocardia*, as well as at latitudes below 28°, and from 32° to 40° for *Sparganophilus*, because these curves were steep and did not reach saturation with the sampling effort used for this study. Indeed, for the *Sparganophilus*, 5 of 10 species were collected from a single location, and for the *Diplocardia*, 6 of 8 species were collected from a single location (Figure 2, Table S1.3 in Appendix S1). This implies that sampling in more locations will very likely yield more species in each group.

Our study revealed that for the *Sparganophilus* species living in aquatic habitats, those that were broadly distributed were dominant at high latitudes above 40°. The absence of *Sparganophilus* species at high latitudes during the last glacial periods suggests that the development of species diversity along latitude in riverine ecosystems is characterized by a rapid range expansion of broadly distributed, extant species. Our study also showed that locally distributed *Sparganophilus* species were often collected at mid-latitudes (32° to 40°) where high elevation areas with headwaters are distributed, suggesting that the species diversity of the riverine ecosystem is affected by the regional pattern of discontinuity and connectedness among habitats regardless of the latitude. We will be able to confirm the generality of our results by examining riverine animals with a low dispersal ability, such as aquatic invertebrates, in future studies.

ACKNOWLEDGEMENTS

We thank Antonio A. Varela, Kayoko Fukumori, Saaya Higuchi, Anita Juen, Shuhei Kosuda, Rina Kudo, Joshua W. Lobe, Junco Nagata, Saori Ogasawara-Ohashi, Teiji Sota and Taichi Suzuki for assistance with sampling and giving us advice on our study. We also thank Samuel W. James and John W. Reynolds for providing important information, Rima Lucardi for assistance with our DNA analysis, and the CCDB team, especially David Porco, for assistance with sequence acquisition and registration. Materials from the Great Smoky Mountains National Park were collected under permit number GRSM-2011-SCI-0029. This work was supported by Grant-in-Aid for scientific research from JSPS (25891002), and Grant-in-Aid for JSPS Fellows (22-6882) and JSPS Overseas Research Fellows.

DATA AVAILABILITY STATEMENT

All data used in this manuscript are presented in the manuscript and its Supporting Information or deposited in GenBank (accession numbers: LC475528–LC476550, MK971688–MK971759).

ORCID

Hiroshi Ikeda  <https://orcid.org/0000-0002-3610-697X>

REFERENCES

- Allen, A. P., Gillooly, J. F., Savage, V. M., & Brown, J. H. (2006). Kinetic effects of temperature on rates of genetic divergence and speciation. *Proceedings of the National Academy of Sciences*, 103(24), 9130–9135. <https://doi.org/10.1073/pnas.0603587103>
- Avice, J. C. (2000). *Phylogeography: The history and formation of species*. Cambridge, MA: Harvard University Press.
- Bernatchez, L., & Osinov, A. (1995). Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. *Molecular Ecology*, 4, 285–297. <https://doi.org/10.1111/j.1365-294X.1995.tb00222.x>
- Bouché, M. B. (1977). Strategies lombriciennes. In U. Lohm, & T. Persson (Eds.), *Soil organisms as components of ecosystems*, (*Ecological Bulletins No 25*) (pp. 122–132). Stockholm, Sweden: Swedish Natural Science Research Council.
- Carerra-Martínez, R. (2018). *Distribution and diversity of semi-aquatic Sparganophilidae earthworms in the Southern Appalachian Piedmont, USA*. Master's thesis. Athens, GA: University of Georgia.

- Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., ... Gray, M. R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, 46, 419–437. <https://doi.org/10.1071/ZO98048>
- Colwell, R. K. (2013). EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application. Retrieved from <http://purl.oclc.org/estimates>.
- Damoff, G. A., & Reynolds, J. W. (2017). *Diplocardia deborahae*, a new earthworm species (Annelida: Oligochaeta: Acanthodrilidae) from eastern Tennessee, USA. *Megadrilogica*, 22, 229–237.
- Damoff, G. A. (2018). Earthworms of the Canyonlands of the East Frio River Watershed in Real County Texas, USA, including *Diplocardia hebi*, a new earthworm species (Annelida: Oligochaeta: Acanthodrilidae). *Megadrilogica*, 23, 109–120.
- Decaëns, T. (2010). Macroecological patterns in soil communities. *Global Ecology and Biogeography*, 19, 287–302. <https://doi.org/10.1111/j.1466-8238.2009.00517.x>
- Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Excoffier, L. G., Laval, L. G., & Schneider, S. (2005). Arlequin ver.3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 1, 47–50.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Virjenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Fragoso, C., & Rojas, P. (2016). *Lavellodrilus notosetosus* sp. nov. (Annelida, Crassicitellata): A new Mexican earthworm with uncommon characters, revealed by a preliminary revision of subfamily Acanthodrilinae. *Zootaxa*, 4154(2), 101–138. <https://doi.org/10.11646/zootaxa.4154.2.1>
- Fujisawa, T., Aswad, A., & Barraclough, T. G. (2016). A rapid and scalable method for multilocus species delimitation using Bayesian model comparison and rooted triplets. *Systematic Biology*, 65, 759–771. <https://doi.org/10.1093/sysbio/syw028>
- Galacatos, K., Cognato, A. I., & Sperling, F. A. H. (2002). Population genetic structure of two water strider species in the Ecuadorian Amazon. *Freshwater Biology*, 47, 391–399. <https://doi.org/10.1046/j.1365-2427.2002.00811.x>
- Gaston, K. J., & Blackburn, T. M. (2000). *Pattern and process in macroecology*. Victoria, Australia: Blackwell.
- Gates, G. E. (1977). More on the earthworm genus *Diplocardia*. *Megadrilogica*, 3(1–48), 13.
- Haponski, A. E., & Stepien, C. A. (2008). Molecular, morphological, and biogeographic resolution of cryptic taxa in the Greenside Darter *Etheostoma blennioides* complex. *Molecular Phylogenetics and Evolution*, 49, 69–83. <https://doi.org/10.1016/j.ympev.2008.07.013>
- Heled, J., & Drummond, A. J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, 27, 570–580. <https://doi.org/10.1093/molbev/msp274>
- Hillebrand, H. (2004). On the generality of the latitudinal diversity gradient. *The American Naturalist*, 163, 192–211. <https://doi.org/10.1086/381004>
- Ikeda, H., Fukumori, K., Shoda-Kagaya, E., Takahashi, M., Ito, M. T., Sakai, Y., & Matsumoto, K. (2018). Evolution of a key trait greatly affects underground community assembly process through habitat adaptation in earthworms. *Ecology and Evolution*, 8, 1726–1735. <https://doi.org/10.1002/ece3.3777>
- Ikeda, H., Tsuchiya, Y., Nagata, N., Ito, M. T., & Sota, T. (2012). Altitudinal life-cycle and body-size variation in ground beetles of the genus *Carabus* (subgenus *Ohomopterus*) in relation to the temperature conditions and prey earthworms. *Pedobiologia*, 55, 67–73. <https://doi.org/10.1016/j.pedobi.2011.10.008>
- Jablonski, D., Roy, K., & Valentine, J. W. (2006). Out of the tropics: Evolutionary dynamics of the latitudinal diversity gradient. *Science*, 314, 102–106. <https://doi.org/10.1126/science.1130880>
- James, S. W. (2004). Planetary processes and their interactions with earthworm distributions and ecology. In C. A. Edwards (Ed.), *Earthworm ecology* (pp. 53–62). Boca Raton, FL: CRC Press.
- Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772–780. <https://doi.org/10.1093/molbev/mst010>
- Mittelbach, G. G., Schemske, D. W., Cornell, H. V., Allen, A. P., Brown, J. M., Bush, M. B., ... Turelli, M. (2007). Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. *Ecology Letters*, 10, 315–331. <https://doi.org/10.1111/j.1461-0248.2007.01020.x>
- Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J. G., ... Vogler, A. P. (2009). Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Systematic Biology*, 58, 298–311. <https://doi.org/10.1093/sysbio/syp027>
- Papadopoulou, A., Anastasiou, I., Keskin, B., & Vogler, A. P. (2009). Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: The effect of dispersal ability and habitat preference. *Molecular Ecology*, 18, 2503–2517. <https://doi.org/10.1111/j.1365-294X.2009.04207.x>
- Pérez-Losada, M., Ricoy, M., Marshall, J. C., & Domínguez, J. (2009). Phylogenetic assessment of the earthworm *Aporrectodea caliginosa* species complex (Oligochaeta: Lumbricidae) based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, 52, 293–302. <https://doi.org/10.1016/j.ympev.2009.04.003>
- Phillips, H. R. P., Guerra, C. A., Bartz, M. L. C., Briones, M. J. I., Brown, G., Crowther, T. W., ... Eisenhauer, N. (2019). Global distribution of earthworm diversity. *Science*, 366, 480–485. <https://doi.org/10.1126/science.aax4851>
- R Core Team. (2018). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>.
- Reynolds, J. W. (2008). Sparganophilidae (Annelida, Oligochaeta) distribution in North America and elsewhere, revisited. *Megadrilogica*, 12, 125–143.
- Reynolds, J. W., & Wetzel, M. J. (2008). Terrestrial Oligochaeta (Annelida: Clitellata) in North America, including Mexico, Puerto Rico, Hawaii, and Bermuda. *Megadrilogica*, 12, 157–210.
- Reynolds, J. W., & Wetzel, M. J. (2012). Terrestrial Oligochaeta (Annelida: Clitellata) in North America, including Mexico, Puerto Rico, Hawaii, and Bermuda. III. *Megadrilogica*, 15, 191–211.
- Ribera, I., Barraclough, T. G., & Vogler, A. P. (2001). The effect of habitat type on speciation rates and range movements in aquatic beetles: Inferences from species-level phylogenies. *Molecular Ecology*, 10, 721–735. <https://doi.org/10.1046/j.1365-294x.2001.01218.x>
- Richard, B., Decaëns, T., Rougerie, T., James, S. W., Porco, D., & Hebert, P. D. N. (2010). Re-integrating earthworm juveniles into soil biodiversity studies: Species identification through DNA barcoding. *Molecular Ecology Resources*, 10, 606–614. <https://doi.org/10.1111/j.1755-0998.2009.02822.x>
- Rota, E., Bartoli, M., & Laini, A. (2014). First time in Italy. Is the elusive aquatic megadrile *Sparganophilus Benham* 1892 (Annelida, Clitellata) accelerating its dispersal in Europe? *Journal of Limnology*, 73, 482–489. <https://doi.org/10.4081/jlimnol.2014.939>
- Soltis, D. E., Morris, A. B., McLachlan, J. S., Manos, P. S., & Soltis, P. S. (2006). Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, 15, 4261–4293. <https://doi.org/10.1111/j.1365-294X.2006.03061.x>



- Stevens, G. C. (1989). The latitudinal gradient in geographical range: How so many species coexist in the tropics. *The American Naturalist*, 133, 240–256. <https://doi.org/10.1086/284913>
- Stout, J., & Vandermeer, J. (1975). Comparison of species richness for stream-inhabiting insects in tropical and mid-latitude streams. *The American Naturalist*, 109, 263–280. <https://doi.org/10.1086/282996>
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Sul, W. J., Oliver, T. A., Ducklow, H. W., Amaral-Zettler, L. A., & Sogin, M. L. (2013). Marine bacteria exhibit a bipolar distribution. *Proceedings of the National Academy of Sciences*, 110(6), 2342–2347. <https://doi.org/10.1073/pnas.1212424110>
- Tanabe, A. S. (2008). Phylogears2 ver. 2.0. Retrieved from <http://www.fifthdimension.jp/>.
- Tanabe, A. S. (2011). Kakusan4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, 11, 914–921. <https://doi.org/10.1111/j.1755-0998.2011.03021.x>
- Tedersoo, L., Bahram, M., Pölme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., ... Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, 346, 1256688. <https://doi.org/10.1126/science.1256688>
- Wallace, A. R. (1878). *Tropical nature and other essays*. New York, NY: Macmillan.
- Weir, J. T., & Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science*, 315, 1574–1576. <https://doi.org/10.1126/science.1135590>
- Wiens, J. J., Pyron, R. A., & Moen, D. S. (2011). Phylogenetic origins of local-scale diversity patterns and the causes of Amazonian megadiversity. *Ecology Letters*, 14, 643–652. <https://doi.org/10.1111/j.1461-0248.2011.01625.x>
- Whiting, M. F. (2002). Mecoptera is paraphyletic: Multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, 31, 93–104. <https://doi.org/10.1046/j.0300-3256.2001.00095.x>
- Zickovich, J. M., & Bohonak, A. J. (2007). Dispersal ability and genetic structure in aquatic invertebrates: A comparative study in southern California streams and reservoirs. *Freshwater Biology*, 52, 1982–1996. <https://doi.org/10.1111/j.1365-2427.2007.01822.x>

BIOSKETCH

Hiroshi Ikeda is interested in evolutionary ecology and community ecology of invertebrates. He is also interested in their diversification process over evolutionary time scales.

SUPPORTING INFORMATION

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

How to cite this article: Ikeda H, Callaham MA Jr., Shefferson RP, Wenk ES, Fragoso C. A comparison of latitudinal species diversity patterns between riverine and terrestrial earthworms from the North American temperate zone. *J Biogeogr.* 2020;00:1–10. <https://doi.org/10.1111/jbi.13826>