

DNA BARCODING REVEALS A SINGLE GENETIC LINEAGE OF THE PEREGRINE EARTHWORM *LUMBRICUS RUBELLUS* (ANNELIDA, LUMBRICIDAE) IN THE NORTHEASTERN PALEARCTIC

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Lumbricus rubellus, a peregrine earthworm, is believed to trace its origins to Western Europe, from where it has dispersed across the temperate regions of the Northern Hemisphere. It exhibits significant genetic variation, with at least eight recognized cryptic genetic lineages in Europe. This study aimed to assess whether populations from the Northeastern Palearctic possess genetic diversity comparable to that of Western Europe. Our findings revealed that only a single genetic lineage (2/A) colonized the vast territories from Belarus to Kamchatka. All studied populations contained closely related haplotypes, with no evident geographic subdivisions. Consequently, only a small fraction of the *L. rubellus* genetic pool was found outside Western Europe. This limited distribution may be attributed to the 2/A lineage's adaptation to local conditions or its enhanced dispersal capabilities. Alternatively, phylogeographic factors might play a role, suggesting that the ancestral region of this lineage was situated along suitable dispersal corridors.

Keywords: peregrine, *Lumbricus rubellus*, phylogeography, mitochondrial DNA, *coxI*, COI
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REFERENCES

- Admassu B., Juen A., Traugott M. Earthworm primers for DNA-based gut content analysis and their cross-reactivity in a multi-species system. *Soil Biol. Biochem.* 2006, vol. 38, pp 1308–1315. <https://doi.org/10.1016/j.soilbio.2005.08.019>
- Anderson C., Cunha L., Sechi P., Kille P., Spurgeon D. Genetic variation in populations of the earthworm, *Lumbricus rubellus*, across contaminated mine sites. *BMC Genet.* 2017, vol. 18, no. 1, p. 97. <https://doi.org/10.1186/s12863-017-0557-8>
- Andre J., King R.A., Stürzenbaum S.R., Kille P., Hodson M.E., Morgan A.J. Molecular genetic differentiation in earthworms inhabiting a heterogeneous Pb-polluted landscape. *Environ. Pollut.* 2010, vol. 158, pp. 883–890. <https://doi.org/10.1016/j.envpol.2009.09.021>
- Audusseau H., Vandenbulcke F., Dume C., Deschins V., Pauwels M., Gigon A., Bagard M., Dupont L. Impacts of metallic trace elements on an earthworm community in an urban wasteland: Emphasis on the bioaccumulation and genetic characteristics in *Lumbricus castaneus*. *Sci. Total Environ.* 2020, vol. 718, p. 137259. <https://doi.org/10.1016/j.scitotenv.2020.137259>
- Berman D.I., Bulakhova N.A., Meshcheryakova E.N., Shekhovtsov S.V. Cold Resistance and the Distribution of Genetic Lineages of the Earthworm *Eisenia nordenskioldi* (Oligochaeta, Lumbricidae). *Biol. Bull.* 2019, vol. 46, no. 5, pp. 430–437. <https://doi.org/10.1134/S1062359019050042>
- Bienert F., De Danieli S., Miquel C., Coissac E., Poillot C., Brun J., Taberlet P. Tracking earthworm communities from soil DNA. *Mol. Ecol.* 2012, vol. 21, no. 8, pp. 2017–2030. <https://doi.org/10.1111/j.1365-294X.2011.05407.x>
- Blakemore R.J. Cosmopolitan earthworms—a global and historical perspective, in *Annelids Mod. Biol.* 2009, pp. 257–283.

- deWaard J.R., Ratnasingham S., Zakharov E.V., Borisenko A.V., Steinke D., Telfer A.C., Perez K.H.J., Sones J.E., Young M.R., Levesque-Beaudin V., Sobel C.N., Abrahamyan A., Bessonov K., Blagoev G., DeWaard S.L., Ho C., Ivanova N.V., Layton K.K.S., Lu L., Manjunath R., McKeown J.T.A., Milton M.A., Miskie R., Monkhouse N., Naik S., Nikolova N., Pentinsaari M., Prosser S.W.J., Radulovici A.E., Steinke C., Warne C.P., Hebert P.D.N. A reference library for Canadian invertebrates with 1.5 million barcodes, voucher specimens, and DNA samples. *Sci. Data*. 2019, vol. 6, no. 1, p. 308. <https://doi.org/10.1038/s41597-019-0320-2>
- Donnelly R.K., Harper G.L., Morgan A.J., Orozco-Terwengel P., Pinto-Juma G.A., Bruford M.W. Nuclear DNA recapitulates the cryptic mitochondrial lineages of *Lumbricus rubellus* and suggests the existence of cryptic species in an ecotoxicological soil sentinel. *Biol. J. Linn. Soc.* 2013, vol. 110, no. 4, pp. 780–795. <https://doi.org/10.1111/bij.12171>
- Ermolov S.A., Shekhovtsov S.V., Geraskina A.P., Derzhinsky E.A., Kotsur V.M., Poluboyarova T.V., Peltek S.E. Morphological and genetic analysis of *Dendrodrilus rubidus* (*Bimastos rubidus*) (Oligochaeta, Lumbricidae) in Russia and Belarus. *Russ. J. Ecosyst. Ecol.* 2023, vol. 8, no. 1, pp. 15–27. <https://doi.org/10.21685/2500-0578-2023-1-2>
- Fernández R., Novo M., Marchán D.F., Díaz Cosín D.J. Diversification patterns in cosmopolitan earthworms, pp. similar mode but different tempo. *Mol. Phylogenet. Evol.* 2016, vol. 94, pp. 701–708. <https://doi.org/10.1016/j.ympev.2015.07.017>
- Folmer O., Hoeh W.R., Black M.B., Vrijenhoek R.C. Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Mol. Mar. Biol. Biotechnol.* 1994, vol. 3, pp. 294–299.
- Giska I., Sechi P., Babik W. Deeply divergent sympatric mitochondrial lineages of the earthworm *Lumbricus rubellus* are not reproductively isolated. *BMC Evol. Biol.* 2015, vol. 15, no. 1, pp. 217. <https://doi.org/10.1186/s12862-015-0488-9>
- Hale C.M., Frelich L.E., Reich P.B. Exotic European earthworm invasion dynamics in northern hardwood forests of Minnesota, USA. *Ecol. Appl.* 2005, vol. 15, no. 3, pp. 848–860. <https://doi.org/10.1890/03-5345>
- Hendrix P.F., Baker G.H., Callahan M.A., Damoff G.A., Fragoso C., González G., James S.W., Lachnicht S.L., Winsome T., Zou X. Invasion of exotic earthworms into ecosystems inhabited by native earthworms, in *Biological Invasions Belowground: Earthworms as Invasive Species* Springer Netherlands: Dordrecht, 2006, pp. 87–100. https://doi.org/10.1007/978-1-4020-5429-7_9
- Hendrix P.F., Callahan M.A., Drake J.M., Huang C.-Y., James S.W., Snyder B.A., Zhang W. Pandora's Box Contained Bait: The Global Problem of Introduced Earthworms. *Annu. Rev. Ecol. Evol. Syst.* 2008, vol. 39, no. 1, pp. 593–613. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173426>
- Ikeda H., Callahan M.A., Shefferson R.P., Wenk E.S., Fragoso C. A comparison of latitudinal species diversity patterns between riverine and terrestrial earthworms from the North American temperate zone. *J. Biogeogr.* 2020, vol. 47, no. 6, pp. 1373–1382. <https://doi.org/10.1111/jbi.13826>
- James S.W., Hendrix P.F. Invasion of exotic earthworms into North America and other regions, in *Earthworm Ecology* CRC press, 2004, pp. 75–88.
- Jones G.L., Wills A., Morgan A.J., Thomas R.J., Kille P., Novo M. The worm has turned: Behavioural drivers of reproductive isolation between cryptic lineages. *Soil Biol. Biochem.* 2016, vol. 98, pp. 11–17. <https://doi.org/10.1016/j.soilbio.2016.03.015>
- Kille P., Andre J., Anderson C., Ang H.N., Bruford M.W., Bundy J.G., Donnelly R., Hodson M.E., Juma G., Lahive E. DNA sequence variation and methylation in an arsenic tolerant earthworm population. *Soil Biol. Biochem.* 2013, vol. 57, pp. 524–532. <https://doi.org/10.1016/j.soilbio.2012.10.014>
- King R.A., Tibble A.L., Symondson W.O.C. Opening a can of worms: Unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Mol. Ecol.* 2008, vol. 17, no. 21, pp. 4684–4698. <https://doi.org/10.1111/j.1365-294X.2008.03931.x>
- Klarica J., Kloss-Brandstätter A., Traugott M., Juen A. Comparing four mitochondrial genes in earthworms—implications for identification, phylogenetics, and discovery of cryptic species. *Soil Biol. Biochem.* 2012, vol. 45, pp. 23–30. <https://doi.org/10.1016/j.soilbio.2011.09.018>
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K. MEGA X – molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 2018, vol. 35, no. 6, pp. 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Leigh J.W., Bryant D. POPART: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 2015, vol. 6, no. 9, pp. 1110–1116. <https://doi.org/10.1111/2041-210X.12410>
- Lund M.B., Davidson S.K., Holmstrup M., James S., Kjeldsen K.U., Stahl D.A., Schramm A. Diversity and host specificity of the Verminephrobacter – earthworm symbiosis. *Environ. Microbiol.* 2010, vol. 12, no. 8, pp. 2142–2151. <https://doi.org/10.1111/j.1462-2920.2009.02084.x>
- Marchán D.F., Hedde M., Laped E., Maggia M.-E., Novo M., Domínguez J., Decaëns T. Contrasting phylogeographic patterns of earthworms (Crassicitellata, Lumbricidae) on near-shore mediterranean islands. *Eur. J. Soil Biol.* 2020, vol. 101, p. 103242. <https://doi.org/10.1016/j.ejsobi.2020.103242>
- Marinissen J.C.Y., van den Bosch F. Colonization of new habitats by earthworms. *Oecologia*. 1992, vol. 91, no. 3, pp. 371–376. <https://doi.org/10.1007/BF00317626>
- Martinsson S., Erséus C. Cryptic speciation and limited hybridization within *Lumbricus* earthworms (Clitellata, Lumbricidae). *Mol. Phylogenet. Evol.* 2017, vol. 106, pp. 18–27. <https://doi.org/10.1016/j.ympev.2016.09.011>
- Michaelsen W. Die Lumbriciden-Fauna Eurasiens. *Annu. du Museum St.-Petersbg.* 1900, pp. 213–225.
- Minamiya Y., Yokoyama J., Fukuda T. A phylogeographic study of the Japanese earthworm, *Metaphire sieboldi* (Horst, 1883) (Oligochaeta, Megascolecidae), Inferences from mitochondrial DNA sequences. *Eur. J. Soil Biol.* 2009,

- vol. 45, no. 5–6, pp. 423–430. <https://doi.org/10.1016/j.ejsobi.2009.06.004>
- Pinadero S.J., Marchán D.F., Navarro A.M., Tilikj N., Novo M., Domínguez J., Díaz Cosín D.J., Trigo D. Comparative phylogeography and integrative systematic revision of Iberian endemic earthworms (Crassiclitellata, Lumbricidae). *Zool. Scr.* 2023, vol. 52, no. 4, pp. 345–357. <https://doi.org/10.1111/zsc.12586>
- Porco D., Decaëns T., Deharveng L., James S.W., Skarżyński D., Erséus C., Butt K.R., Richard B., Hebert P.D.N. Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America. *Biol. Invasions.* 2013, vol. 15, no. 4, pp. 899–910. <https://doi.org/10.1007/s10530-012-0338-2>
- Richard B., Decaëns T., Rougerie R., James S.W., Porco D., Hebert P.D.N. Re-integrating earthworm juveniles into soil biodiversity studies: species identification through DNA barcoding. *Mol. Ecol. Resour.* 2010, vol. 10, no. 4, p. 606–614. <https://doi.org/10.1111/j.1755-0998.2009.02822.x>
- Ronquist F., Teslenko M., Van Der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A., Huelsenbeck J.P. MrBayes 3.2, efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 2012, vol. 61, no. 3, pp. 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Rybak A.V., Belykh E.S., Maystrenko T.A., Shadrin D.M., Pylina Y.I., Chadin I.F., Velegzhaninov I.O. Genetic analysis in earthworm population from area contaminated with radionuclides and heavy metals. *Sci. Total Environ.* 2020, vol. 723, p. 137920. <https://doi.org/10.1016/j.scitotenv.2020.137920>
- Shekhovtsov S.V., Ermolov S.A., Poluboyarova T.V., Kim-Kashmenskaya M.N., Derzhinsky Y.A., Peltek S.E. Morphological differences between genetic lineages of the peregrine earthworm *Aporrectodea caliginosa* (Savigny, 1826). *Acta Zool. Acad. Sci. Hungaricae.* 2021, vol. 67, no. 3, pp. 235–246. <https://doi.org/10.17109/AZH.67.3.235.2021>
- Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Invasive lumbricid earthworms of Kamchatka (Oligochaeta). *Zool. Stud.* 2014a, vol. 53, no. 1, p. 52. <https://doi.org/10.1186/s40555-014-0052-0>
- Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Genetic diversity of the earthworm *Octolasion tyrtaeum* (Lumbricidae, Annelida). *Pedobiologia*, 2014b, vol. 57, no. 4–6, pp. 245–250. <https://doi.org/10.1016/j.pedobi.2014.09.002>
- Shekhovtsov S.V., Sundukov Y.N., Blakemore R.J., Gongalsky K.B., Peltek S.E. Identifying earthworms (Oligochaeta, Megadrili) of the Southern Kuril Islands using DNA barcodes. *Anim. Biodivers. Conserv.* 2018b, vol. 41, no. 1, pp. 9–17. <https://doi.org/10.32800/abc.2018.41.0009>
- Shekhovtsov S.V., Berman D.I., Bulakhova N.A., Makarova O.L., Peltek S.E. Phylogeography of earthworms from high latitudes of Eurasia. *Acta Zool. Acad. Sci. Hungaricae.* 2018a, vol. 64, no. 4, pp. 369–382. <https://doi.org/10.17109/AZH.64.4.369.2018>
- Shekhovtsov S.V., Derzhinsky Y.A., Poluboyarova T.V., Golovanova E.V., Peltek S.E. Phylogeography and genetic lineages of *Aporrectodea rosea* (Lumbricidae, Annelida). *Eur. J. Soil Biol.* 2020, vol. 99, p. 103191. <https://doi.org/10.1016/j.ejsobi.2020.103191>
- Shekhovtsov S.V., Golovanova E.V., Peltek S.E. Different dispersal histories of lineages of the earthworm *Aporrectodea caliginosa* (Lumbricidae, Annelida) in the Palearctic. *Biol. Invasions.* 2016, vol. 18, no. 3, pp. 751–761. <https://doi.org/10.1007/s10530-015-1045-6>
- Stamatakis A. RAxML version 8, a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 2014, vol. 30, no. 9, pp. 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Vsevolodova-Perel T.S. The earthworms of the fauna of Russia, Nauka: Moscow, 1997.