

Genetic diversity of earthworms (*Oligochaeta*, *Annelida*) of the genera *Octodrilus* Omodeo, 1956 and *Octodriloides* Zicsi, 1986 in Croatia

Dino de Bona¹, Mišel Jelić², Goran Klobučar¹, Branimir K. Hackenberger³, Roman Ozimec⁴, Davorka K. Hackenberger³

¹ Faculty of Science, Department of Biology, Rooseveltov trg 6, HR-10000 Zagreb, Croatia

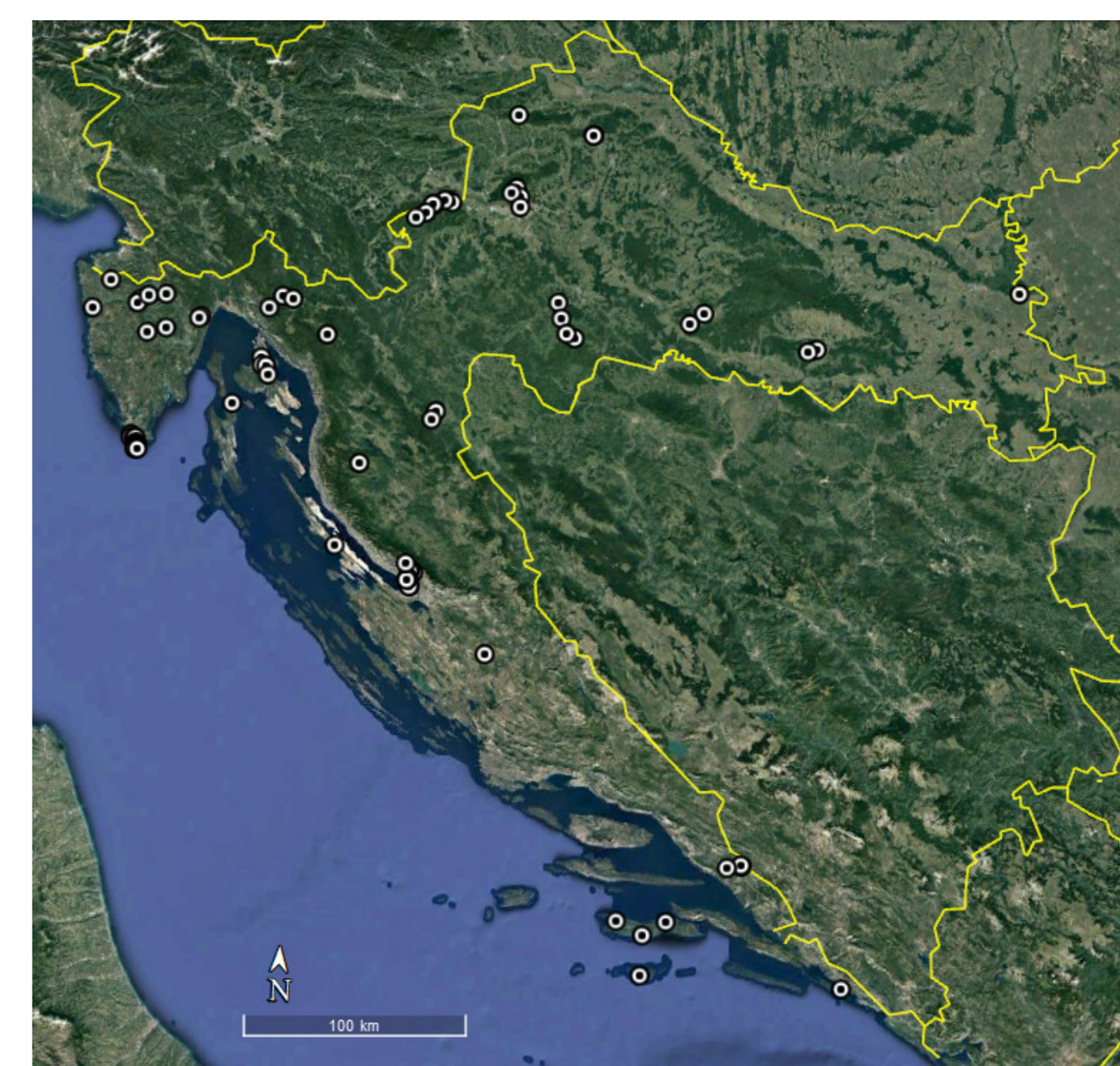
² Natural History Department of the Varaždin City Museum, Šetaliste Josipa Jurja Strossmayera 1, HR-42000 Varaždin, Croatia

³ J. J. Strossmayer University, Department of Biology, Cara Hadrijana 8A, HR-31000 Osijek, Croatia

⁴ ADIPA Society for Research and Conservation of Croatian Natural Diversity, Orehovečki ogranak 37, HR-10040 Zagreb, Croatia

INTRODUCTION

Octodrilus and *Octodriloides* genera represent a diverse group of lumbricid earthworms. The species are widespread throughout Europe, but the Trans-Aegean subdomain, which includes Alpine-Balkan-Carpathian region, is considered as an evolution origin. Croatian territory is one of the centers of divergence of these genera. *Octodrilus* includes at least 53, and *Octodriloides* at least 22 described species, with numerous subspecies within both genera. So far, 15 *Octodrilus* species (seven endemic), and five *Octodriloides* species (four endemic) have been recorded in Croatia (Table 1). In databases (e.g. BOLD and GenBank) publicly available sequences of these genera from Croatia are almost non-existent, making this molecular research a first study of its kind in Croatia.



Octodrilus species

- Oc. bretscheri*
- Oc. kvarnerus*
- Oc. croaticus*
- Oc. lissaensis*
- Oc. pseudolissaensis*
- Oc. complanatus*
- Oc. istrianus*
- Oc. velebiticus*
- Oc. mimus*
- Oc. rucneri*
- Oc. slovenicus*
- Oc. tergestinus*
- Oc. transpadanus*
- Oc. pseudotranspadanus*
- Oc. pseudozirianus*

Octodriloides species

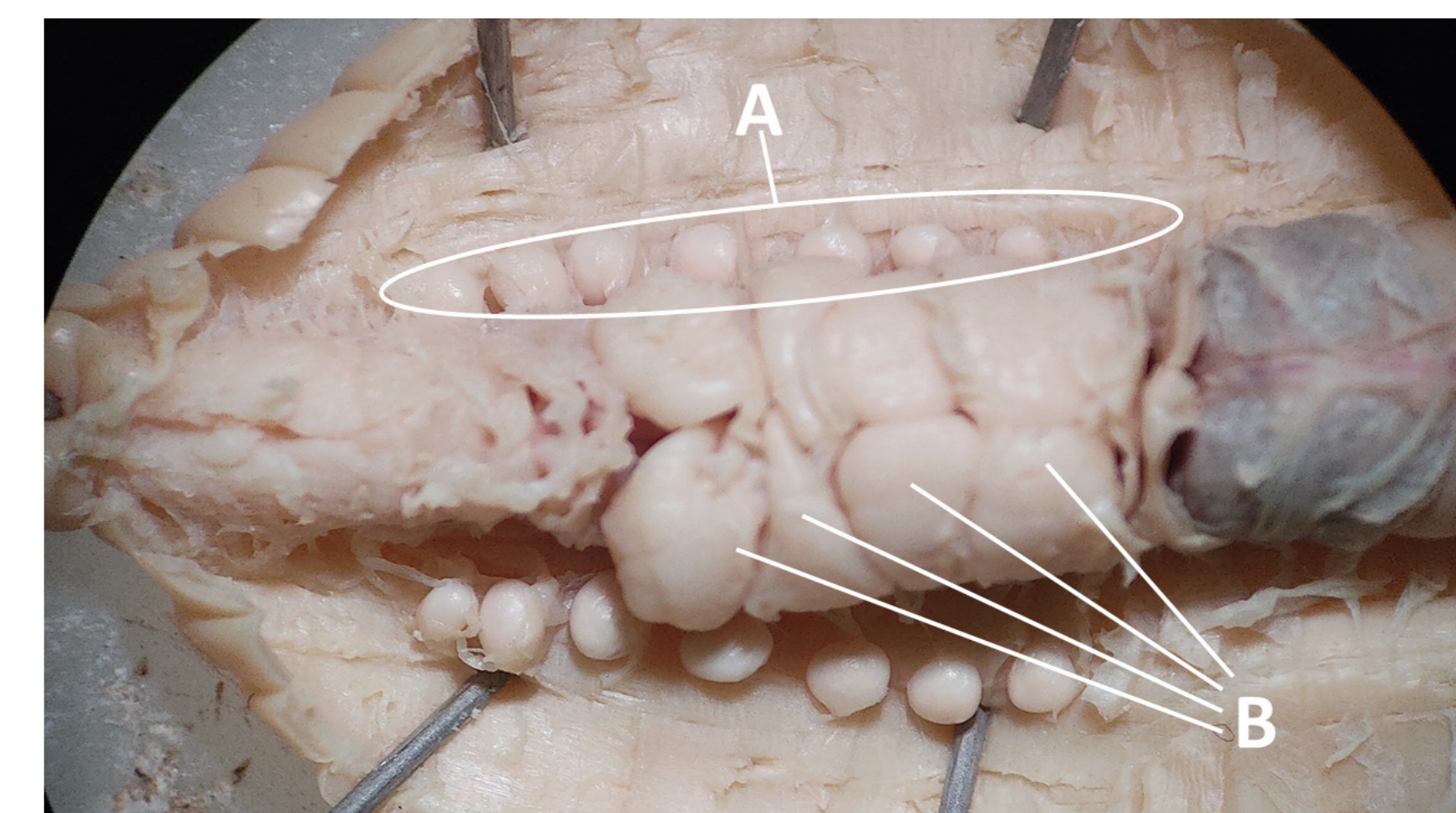
- Oi. bolei*
- Oi. poklonensis*
- Oi. kammensis* (syn. *karawankensis*)
- Oi. kovacevici*
- Oi. janetscheki*

MATERIALS & METHODS

180 specimens were isolated from several collections, sampled at 76 sites throughout Croatia. The specimens were identified to species level according to identification keys. Specimens were barcoded by isolating, amplifying and sequencing the COI region of mtDNA from the epithelial tissue of each specimen. Using the SEQUENCHER (4.1.4) and MEGA-X computer programs, the obtained sequences of each specimen were processed and compared, and one phylogenetic tree of both genera was constructed by the method of the highest probability.

RESULTS

By tree analysis, according to the degree of support and genetic distance, 28 lines representing potential species and/or subspecies were separated. 25 lines were included in 13 described species. Four species form complexes of at least two lines, and three lines are unique in their dislocation from others and do not lead to the described species using identification keys due to specific morphological features or due to specimen's juvenile stage. Consensus among published research indicates that two specimens of earthworms can be considered separate species if their genetic distance calculated by Kimura 2-parameter is greater than 15%, and if there are morphological differences between sister lines when the genetic distance value is 9-15%. The largest genetic distance between lines in this study is 25.3%, while the lowest is 11.8%.



Species from *Octodrilus* and *Octodriloides* species have a high number of spermathecae (A)



A) *Octodrilus istrianus*; B) *Octodrilus tergestinus*

CONCLUSION

The existence of several lines with significant genetic divergences within the species show the advantage of using molecular methods and molecular-phylogenetic analyzes for species identification, especially on complexes of cryptic species that cannot be classified into separate species based on morphological and anatomical characters. However, only one molecular marker (COI) was used in this study and it is not possible to reliably determine the hierarchical relationships of *Octodrilus* and *Octodriloides* genera species, as well as the monophyletic origin of each genus.

Contact: davorka@biologija.unios.hr



Maximum likelihood (ML) phylogenetic tree of the investigated genera *Octodrilus* and *Octodriloides*. The first number on the branches corresponds to the self-loading value (*p*), and the second to the posterior values (*pp*) of the Bayesian method.