Acta Veterinaria-Beograd 2025, 75 (4), 513-520
UDK: 595.161:575.22(497.6)
639.111.1.09:616.995.132(497.6)
DOI: 10.2478/acve-2025-0039

Short communication

NEW INSIGHTS ON THE DISTRIBUTION OF *SETARIA TUNDRA*: A CASE REPORT FROM BOSNIA AND HERZEGOVINA AND GENETIC VARIATION OF THE *COX1* GENE

Srđan GLIGORIĆ[1*], Andrea RADALJ[2], Dragan KNEŽEVIĆ[1],
Drago NEDIĆ[1], Željko SLADOJEVIĆ[1], Ana VASIĆ[3],
Darko MARINKOVIĆ[4], Sanja ALEKSIĆ-KOVAČEVIĆ[4],
Oliver STEVANOVIĆ[1]

[1]PI Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka, Banja Luka, Bosnia and Herzegovina; [2]University of Belgrade, Faculty of Veterinary Medicine, Department of Microbiology and Immunology, Belgrade Serbia; [3]Scientific Institute for Veterinary Medicine of Serbia, Belgrade, Serbia; [4]University of Belgrade, Faculty of Veterinary Medicine, Department of Pathology, Belgrade, Serbia.

(Received 28 August, Accepted 13 October 2025)

Filaroid nematode *Setaria tundra* is a parasite well adapted to cervids, especially to roe deer (*Capreolus capreolus*). Adult forms are located in the abdominal cavity, and infections are usually asymptomatic. During 2024, a total od 31 carcasses of roe deer were examined in the Republic of Srpska (Bosnia and Herzegovina). During gross pathologic examination nematodes were found in two cases and were identified as *S. tundra* by morphological and molecular analysis. In the Balkan region, this nematode has previously been described in Croatia and Serbia. This study represents the first report of *S. tundra* in Bosnia and Herzegovina. Genetic diversity parameters and neutrality tests of 50 *cox1* sequences belonging to *S. tundra* from Europe indicate a genetic signature consistent with recent population expansion.

Keywords: Filaroid nematode, *Setaria tundra*, Roe deer, molecular analysis;

INTRODUCTION

Setaria tundra is a significant filarial nematode that has expanded across Europe in recent decades. This filaria was first described in Russia by Raevskaya (1928) [1]. Mosquitoes from the genera *Aedes* and *Culex* are the main vectors for transmitting infectious L3 larvae of this filaria, where the larvae develop from L1 to L3 in the abdomen and salivary glands [2]. The definitive hosts for this parasite are various species of cervids, with roe deer (*Capreolus capreolus*) being the most frequently reported, as well as reindeer

---

*Corresponding author: e-mail: s.gligoric996@gmail.com

(*Rangifer tarandus*), moose (*Alces alces*), and red deer (*Cervus elaphus*). Adults or L5s of *S. tundra* are typically found in the abdominal cavity and usually do not cause significant pathological changes, indicating the asymptomatic nature of the disease in definitive hosts [3]. According to the literature, the primary morphological difference among the most relevant species in Europe is their size [4-6]. The species *Setaria cervi*, *Setaria labiatopapillosa*, and *Setaria equina* are significantly longer than *S. tundra*. In fact, the females of *S. tundra* can reach a maximum length of 5 cm, while the males can be up to 3.7 cm long [4,6]. Additionally, certain species within the genus *Setaria* have well-defined definitive hosts. However, there are some morphological similarities between *S. tundra* and other species from the genus *Setaria* Viborg. *Setaria tundra* and *Setaria yehi* are almost morphologically identical species, leading some authors to suggest that they may be the same species [6]. Therefore, it is necessary to confirm the initial parasitological findings of *S. tundra* in new geographical areas using molecular diagnostic methods. The recent discovery of an unknown sister taxon of *S. tundra* highlights the need for additional phylogenetic studies to more effectively distinguish differences within the genus [2] . In Southeastern Europe, *S. tundra* has been identified in roe deer in Croatia, while its infectious larvae (L3s) have been detected in mosquitoes in Serbia [7,8]. Most reports regarding the distribution of this species are primarily from Central Europe [2,3] . With the presence of *S. tundra* in the Balkans, it can be inferred that infected cervid species may be found in Bosnia and Herzegovina. A preliminary study was conducted to investigate the presence and distribution of *S. tundra* species in roe deer (*Capreolus capreolus*) to better understand its epidemiological distribution in Southeast Europe. We also conducted a study on the genetic diversity of the *cox1* gene in *S. tundra* using the obtained sequence and other available sequences from European isolates in the GenBank database (NCBI).

MATERIAL AND METHODS

In 2024, a total of 31 carcasses of roe deer (*Capreolus capreolus*) were examined at the Laboratory for Pathology at the Public Veterinary Institute “Dr. Vaso Butozan” in Banja Luka, Bosnia and Herzegovina. The carcasses underwent a gross pathological examination. During the examination of the abdominal cavity, nematodes were observed in two animales, which were then subjected to detailed morphological and molecular analysis. Species identification was initially based on morphological characteristics, following the criteria described by Nikander et al. (2007) [6]. Genomic DNA was extracted from an adult worm using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). In the PCR study, we amplified the *cox1* (cytochrome oxidase 1) gene using primers designed by Casirhagi et al. (2001) [9]. After obtaining the desired PCR products, sequencing was performed in both directions using the Sanger method. Confirmation of the species was conducted via BLAST analysis. For the phylogenetic analysis, we utilized 50 *cox1* gene sequences from Europe available in the NCBI gene database (originating from Austria, Bosnia and Herzegovina, Croatia, Poland, Portugal, Denmark, Serbia, Slovenia, Slovakia, Germany, France, and Italy). These sequences were downloaded in FASTA format and trimmed to a length of 401 base pairs in MEGA XII. A maximum likelihood (ML) tree was constructed in MEGA XII using the Tamura-Nei model with adaptive bootstrap analysis [10]. We evaluated the genetic diversity of *cox1* gene, including the number of haplotypes, haplotype diversity, nucleotide diversity, average number of pairwise differences, number of segregating sites, and number of singleton segregating sites. Neutrality tests (Tajima's D, Fu's Fs, and R2) and mismach distribution were performed using DnaSP version 6 [11, 12, 13, 14]. A haplotype network was generated using PopART software [15]. A partial *cox1* sequence of *S. tundra* from Bosnia and Herzegovina was deposited in the NCBI GenBank under accession number PV696749.

RESULT AND DISCUSSION

Two roe deer (*Capreolus capreolus*) from the Laktaši region (44°54'N 17°18'E) of Bosnia and Herzegovina were subjected to necropsy, and their organs examined. During the *post mortem* examination of the abdominal cavity, one filarial nematode was found in each animal. No gross changes were observed. Based on their morphological characteristics and length (up to 7 cm), these nematodes were identified as belonging to the genus *Setaria* (Figure 1).

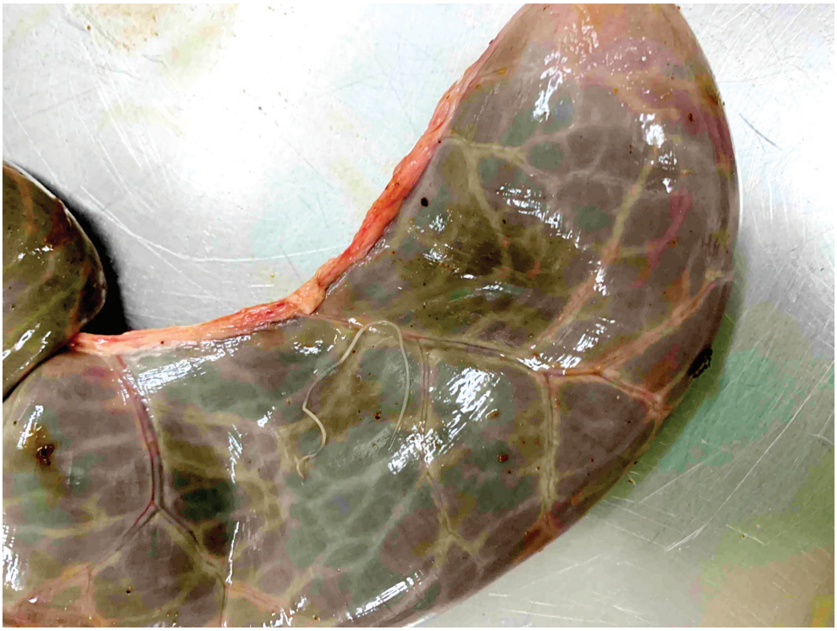

Figure 1. Female *Setaria spp.* – in situ from abdominal cavity of roe deer (*Capreolus capreolus*)

One specimen was subjected to morphological analysis, while the other, a female, was used for DNA extraction, PCR, and sequencing. After being processed in Amman's lactophenol, a typical peribuccal crown and filarid eggs in the uterus were observed, while the posterior end displayed a knob-like tail tip without spines.

It was not possible to distinguish *S. tundra* from *S. yehi* and *S. labiatopapillosa* with certainty based on a single female specimen. After successful amplification and Sanger sequencing, BLAST analysis revealed that the obtained sequence was 99.70% similar to the *cox1* sequence of *S. tundra* from Austria (MF695092). The phylogenetic tree generated using MEGA XII software showed that the European *cox1* sequences of *S. tundra* are clearly grouped in a well-defined clade, distinct from the related species *S. yehi* (PQ282508), *S. equina* (MK541847), *S. digitata* (EF174427), *S. cervi* (PP270178), and *S. labiatopapillosa* (KX570601) (Figure 2). The intraspecific p-distance of *cox1* sequences among European isolates of *S. tundra* ranged from 0% to 1.7%.

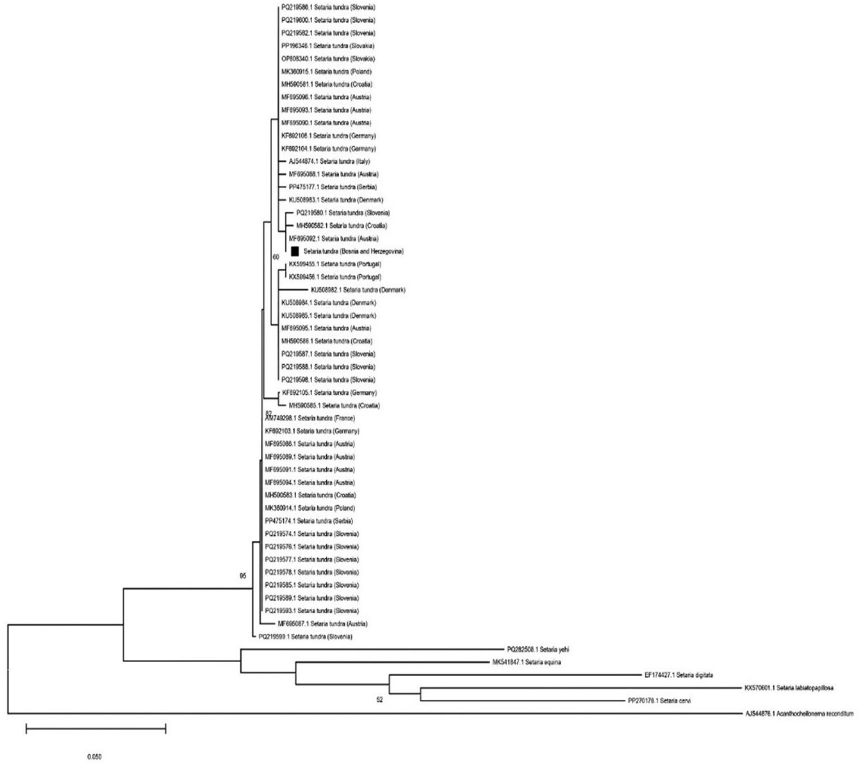

Figure 2. The phylogenic tree of 50 *cox1* partial sequences of *Setaria tundra* was generated using the Maximum Likelihood method and Tamura-Nei (1993) model of nucleotide substitutions. The tree with the highest log likelihood (-1,452.46) is shown. The percentage of replicate trees in which the associated taxa clustered together, with the number of replicates (102) determined adaptively (Kumar et al. 2024). Partial cox sequences of congeneric *Setaria yehi* (PQ282508), *Setaria equina* (MK541847), *Setaria digitata* (EF174427), *Setaria cervi* (PP270178), and *Setaria labiatopapilossa* (KX570601) were also included in tree. Partial cox sequence of *Acanthocheilonema reconditum* (AJ544876) was included as outgroup. Bootstrap values greater than 50% are shown. Evolutionary analyses were conducted in MEGA XII (Kumar et al. 2024).

Parameters of genetic variation and values of neutral tests calculated in DnaSP are shown in Table 1. The haplotype network indicates that the *cox1* sequence from *S. tundra* from Bosnia and Herzegovina belongs to haplotype 11 (Figure 3).

Table 1. Genetic diversity parameters and neutrality test results based on *cox1* sequences of *Setaria tundra* from Europe

Parametar	Value
Number analyzed cox sequences of *Setaria tundra* (n)	50
Number of haplotypes (Hn)	16
Haplotype diversity (Hd)	0.829
Nucleotide diversity (π)	0.004
Number of segregation sites (S)	18
Number of singleton segregation sites (η)	12
Average number of pairwise nucleotide differences (k)	1.917
Tajimas D test	-1.64
Fu's F test	-8.867
R2 test	0.05
Raggedness index (r) (mismatch distribution)	0.04

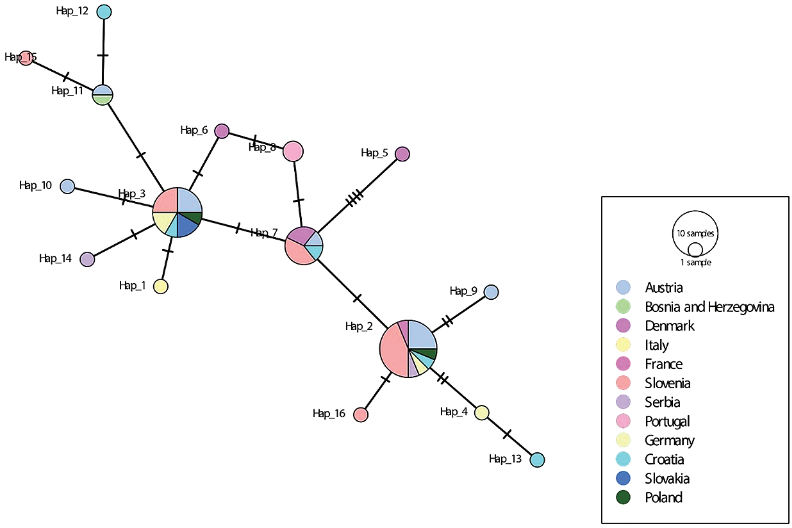

Figure 3. TSC haplotype network of partial *cox1* sequence from European *Setaria tundra* isolates

For the first time, the filaroid nematode *S. tundra* has been confirmed in Bosnia and Herzegovina, representing a significant epidemiological contribution to the Balkans. This confirmation completes the current understanding of the species' distribution in the region, following previously reported cases in Croatia and Serbia [7,8]. The NCBI gene database contains numerous *cox1* gene sequences from Slovenia, however, to the best of the authors' knowledge, the results of these studies have not yet been publicly published.

Currently, there is limited data on the presence of filaroid nematode from the genus *Setaria* in domestic and wild animals in Bosnia and Herzegovina, making this study a pioneering investigation in the country. Alongside *S. tundra,* the species *S. labiatopapillosa* has been identified in Croatia, while *S. digitata* was diagnosed in the neighboring Republic of Serbia [16,17] . However, these findings were based solely on morphological diagnoses, which can be challenging and heavily reliant on the quality of available samples, particularly when only females and larvae are present for analysis [6].

The genetic diversity parameters, characterized by high haplotype diversity, low nucleotide diversity, negative Tajima's D and Fu's F values, and extremely low R2 and raggedness index (r), indicate a clear genetic signal consistent with a recent population expansion of this species in Europe. These findings align with the review article by Olos et al. (2021) [2], which identified *S. tundra* as an expansive species across the continent. A limitation of this study is the small number of processed samples, the absence of nuclear gene analysis, and the lack of vector surveillance. Further research is necessary to investigate the distribution of *S. tundra* in Bosnia and Herzegovina and to explore its presence in other geographical areas [18].

CONCLUSION

This study confirms the presence of filaroid nematode *Setaria tundra* for the first time in Bosnia and Herzegovina, establishing that this filarid species is widespread in the Balkans. An analysis of the genetic diversity of the mitochondrial *cox1* gene reveals clear genetic signals indicative of recent population expansion in Europe. These findings suggest that the prevalence and geographical distribution of this parasite may continue to increase in the future.

Authors' contributions

SG collected samples in the field, performed autopsies on corpses, performed macroscopic examinations, interpreted macroscopic findings, interpretation of molecular and genetic tests. AR performed molecular genetic tests. DK and DN supervised all stages during the study, performed the overall review of the short communication, and provided funding for all research in the short communication. ŽS organized the collection of samples. SAK and DM interpreted the results of the pathological part of the study, reviewed the relevant literature, approved the final version of the short communication. AV performed interpretation of molecular genetic tests. OS carried out the identification of parasites, carried out molecular genetic analyses, interpreted the results of molecular genetic analyses, worked on the preparation of tables and graphic displays.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs

Srđan Gligorić https://orcid.org/0009-0004-8542-0596
Andrea Radalj https://orcid.org/0000-0003-2287-7818
Drago Nedić https://orcid.org/0000-0002-1494-3435
Ana Vasić https://orcid.org/0000-0002-0653-2935
Darko Marinković https://orcid.org/0000-0002-5456-1343
Sanja Aleksić-Kovačević https://orcid.org/0000-0001-6508-8400

REFERENCES

1. Raevskaya ZA: Setaria and their pathogenic significance. Trudy Gosudarstvennogo Instituta Eksperimental'noi Veterinarii (1928) 5: 1-58
2. Grzegorz O, Julita N, Renata WF: *Setaria tundra*, what do we know, what is still to be discovered? Ann Parasitol 2021, 67(1):1-10
3. Lazár J, Šmigová J, Šmiga Ľ, Berrilli F, Lazár P, Čurlík J, Papajová I: Molecular detection of *Setaria tundra* (Issaitshikoff & Rajewskaya, 1928) and Setaria cervi (Rudolphi, 1819) in red deer in Slovakia. Vet Res Commun 2024, 48(4):2835-2840
4. Yeh LS: A revision of the genus Setaria Viborg, 1795, its host-parasite relationship, speciation and evolution. J Helminthol 1959, 33:1-98.
5. Favia G, Cancrini G, Ferroglio E, Casiraghi M, Ricci I, Rossi L: Molecular assay for theidentification of *Setaria tundra*. Vet Parasitol 2003, 177:139-145
6. Nikander S, Laaksonen S, Saari S, Oksanen A: The morphology of the filaroid nematode *Setaria tundra*, the cause of peritonitis in reindeer Rangifer tarandus. J Helminthol 2007, 81(1):49-55.
7. Čurlík J, Konjević D, Bujanić M, Sabol Ž, Martinković F, Sindičić M: The first description of *Setaria tundra* (Issaitshikoff & Rajewskaya, 1928) in Roe Deer from Croatia. Helminthologia 2019, 56(3):252-255.
8. Šiljegović S, Mouillaud T, Jiolle D, Petrić D, Ignjatović-Ćupina A, Vasić A, Paupy C, Kavran M: Dirofilaria sp. and blood meal analysis in mosquitoes collected in Vojvodina and Mačva, and the first report of *Setaria tundra* (Issaitshikoff & Rajewskaya, 1928) in Serbia. Animals (Basel) 2024, 14(9):1255.
9. Casiraghi M, Anderson TJC, Bandi C, Bazzochi C, Genchi C: A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of Wolbachia endosymbionts. Parasitology 2001, 122:93-103.

10. 10. Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K: Molecular Evolutionary Genetics Analysis Version 12 for adaptive and green computing. Mol Biol Evol 2024, 41:1-9
11. Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A: DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evol 2017, 34(12):3299–3302
12. Tajima F: Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 1989, 123(3):585-595.
13. Fu YX: Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 1997, 147(2):915-925.
14. Ramos-Onsins SE, Rozas J: Statistical properties of new neutrality tests against population growth. Mol Biol Evol 2002, 19(12):2092-2100.
15. Leigh JW, Bryant D: POPART: full-feature software for haplotype network construction. Methods Ecol Evol 2015, 6(9):1110–1116.
16. Mikačić D: Parasitic fauna of our cattle. Vet Arhiv 1941, 11:28-50.
17. Jezdimirović N, Kureljušić B, Pavlović I, Cvetojević Đ, Kureljušić J, Radanović O, Stanojević S: The occurence of setaria digitata in dairy cows in Serbia. Second International Symposium of Veterinary Medicine (ISVM2016) 2016:150.
18. Cipriano IA, Favare GM, Carmo TA, Mena MO, Vicari DVF, Pagnozzi FC, Pacheco TM, Rodrigues MGF, Pavan BE, Soutello RVG: Epigenetic investigation related to gastrointestinal helimnth resistance and performance in cattle. Acta Vet-Beograd 2024, 74 (1):91 – 105.

NOVI ASPEKTI O RASPROSTRANJENOSTI *SETARIA TUNDRA*: PRIKAZ SLUČAJA IZ BOSNE I HERCEGOVINE SA PRIKAZOM GENETSKE VARIJACIJE GENA *COX1*

Srđan GLIGORIĆ, Andrea RADALJ, Dragan KNEŽEVIĆ, Drago NEDIĆ, Željko SLADOJEVIĆ, Ana VASIĆ, Darko MARINKOVIĆ, Sanja ALEKSIĆ – KOVAČEVIĆ, Oliver STEVANOVIĆ

Filaroidna nematoda *Setaria tundra* je dobro adaptiran parazit na jelensku divljač, a posebno na srneću divljač (*Capreolus capreolus*). Odrasli oblici se nalaze u abdominalnoj šupljini, a infekcije su obično asimptomatske. Tokom 2024. godine u Republici Srpskoj (Bosna i Hercegovina) pregledan je ukupno 31 leš srna. Makroskopskim pregledom, nematode su pronađene u dva slučaja i identifikovane kao *S. tundra*, morfološkom i molekularnom analizom. U regionu Balkana, ova nematoda je ranije opisana u Hrvatskoj i Srbiji. Parametri genetske raznolikosti i testovi neutralnosti 50 *cox1* sekvenci koje pripadaju *S. tundra* iz Evrope, ukazuju na genetski kod koji je u skladu sa skorašnjim širenjem populacije.