Annals of Parasitology 2021, 67(1), 1–10
doi: 10.17420/ap6701.306

Review article

Setaria tundra, what do we know, what is still to be discovered?

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ABSTRACT. Over the past years, the nematode species from the Onchocercidae family, *Setaria tundra*, has expanded its range by thousands of kilometres from subpolar to subtropical climate zone. Its presence has been confirmed in seven new countries. The appearance of this parasite in many other European countries, especially central and southern ones, is highly probable. In recent years, its mass appearance has led to the numerous fall of reindeer in Fennoscandia, thus causing significant economic damage. There is also a growing risk of its gradation in Central Europe, which threatens overpopulated wild deer species and possibly farm animals, however there is no information if other than mosquitoes blood-sucking arthropods act as its vector. This paper covers the range of occurrence of *S. tundra* in Europe, all of the reported intermediate and definitive hosts, phylogeny, biology, morphology, health effects, and treatment methods.

Keywords: *Setaria tundra*, roe deer, reindeer, mosquito, filariosis, setariosis

Introduction

Nematodes of the *Setaria* Viborg genus are common parasites of different ungulates including wild ruminants. In recent years, one of the most expansive species of *Setaria* genus in Europe is *S. tundra*. Adult, L5 forms of *S. tundra* live in the definitive hosts (wild cervids) peritoneal cavity, while L1 larvae (microfilariae) are released into host's bloodstream. From there, microfilariae infect blood-sucking arthropods during blood meal. So far, the only known vectors of *S. tundra* are mosquitoes [1–3]. After two moulds microfilariae of *S. tundra* develop to L3 invasive larvae and may infect definitive hosts during another mosquito blood meal. *S. tundra* infection may be life threatening during its gradation or development of L4 and/or L5 in accidental hosts (mainly due to neurological disorders) [4–10]. In the past there have been significant economical and veterinary gradations of this species, which took place in Fennoscandia and the last of which was in 2003 [9,11]. This gradation has led to the numerous fall of semi-domesticated reindeer. In recent years *S. tundra* has been found for the first time in many European countries. This expansion is probably propagated by climate change and warm, wet summers, during which intermediate hosts appear in vast numbers [12], however numerously occurring wild deer species probably favour this process. In most western and middle European countries, the red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) populations has increased in recent years [13,14] above a level not previously reported. This makes a gradation of *S. tundra* possible due to high population density of its definitive hosts (as an effect of overpopulation) [15]. Due to the wide range of definitive hosts of closely related *Setaria* species, domestic species such as sheep, goats, cattle, and horses are also potentially at risk [5,16–18]. Because another outbreak of *S. tundra* is very possible, the purpose of this work was to collect vital information on that

species in one place with particular emphasis on when and where the presence of microfilariae or adult forms of *S. tundra* were recorded in across Europe, including both host type species. Any available data on biology, taxonomy and medical treatment of *S. tundra* was given, but important knowledge gaps were also identified.

Morphology of L5 of *S. tundra*

Adult forms of the *Setaria* genus are light-pale, narrow and longitudinal helminths with invisible details by the naked eye. When opening the host's body they wriggle intensively, which makes them easier to spot. Females of *S. tundra* are longer than males, which are about 4.6–7.7 cm to 2.6–3.7cm, respectively [1,19–21]. Adult forms of other *Setaria* species present in Europe are much longer; *Setaria cervii*: (♀) 11.6–14.2 cm, (♂) 4.7–7.6 cm, *Setaria equina*: (♀) 10.0–15.0 cm, (♂) 8.0–10.0 cm, *Setaria labiatopapillosa*: (♀) 12–15 cm, (♂) 4–6 cm [1,17, 22–24]. The width of the adult form of *S. tundra* ranges from 605–792 µm (♀) and 320–347 µm (♂) [1,20]. From the available descriptions in the literature, the characteristic traits of the cephalic end of their body can be determined under the scanning electron microscope (Fig. 1): (♀) have four protruding cephalic papillae (cp) and four smaller externolabial papillae (elp) and a pair or one lateral amphid. These features surround the oval mouth, where the dorsal and ventral bifid projections (bp) are present at the edge of the peribuccal crown [20,21,26]. A caudolateral appendage (ca) is visible on the tip of the caudal end (posterior end). Close to this appendage, there are two, small, lateral, subterminal caudal (lsc) appendixes. Numerous small papillae (sp) are visible all over the posterior end [1,20,21,26]. The posterior end of (♂) is spirally twisted and has a number of papillae pairs. In the direction from the center to the posterior end of the body there are nine pairs of asymmetrical papillae, three precloacal, one medial precloacal, one adcloacal, and three pairs of ventral postcloacal papillae [20]. There are no tail wings. The tail is up to 625 µm long.

Reproductive cycle and hosts of *S. tundra*

Cervids species are the definitive hosts for *S. tundra*. In northern Europe, reindeer (*Rangifer tarandus*) is the main definitive host, while roe deer is the main definitive host in central and southern Europe [11,26]. In addition moose (*Alces alces*) is also a definitive host [21,26] and according to recent reports red deer (*Cervus elaphus*) as well [27]. Adult females in definitive host may carry up to 200,000 eggs (microfilariae) that are ready to be released into the host's bloodstream [28]. Only different species of mosquitoes are known act as a vector to *S. tundra*. These were: *Aedes vexans*, *Aedes geminus*, *Culex pippens complex*, *Culex torretium*, *Coquillettidia richiardii*, *Ochlerotatus sticticus*, *Ochlerotatus cantans*, *Ochlerotatus caspius*, *Anopheles daciae*, *Anopheles rossicus* [29–35]. Nothing is known if other blood-sucking parasites (ticks, horse flies, keds) are vector for *S. tundra*. When blood containing microfilariae is taken from hosts by mosquitoes, L2 larvae develop (the so called sausage-like state) which later migrate from the insect's abdomen to salivary glands where

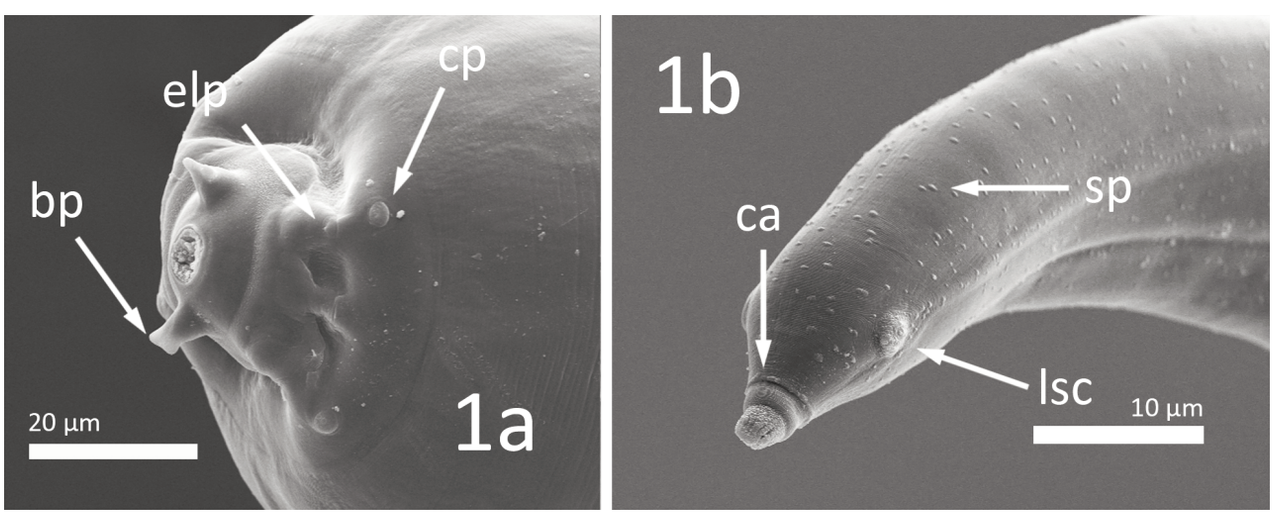

Figure 1. Morphology of female *Setaria tundra*: 1a – anterior end, 1b – posterior end

they reach L3 stage and about 1500 μm in length. This process takes probably about two weeks [36] (data for *Setaria digitata* as there is no specific data for *S. tundra* so far). The L3 larvae can be transmitted to a definitive host's bloodstream during another mosquito blood feeding. In the definitive host, larvae migrate and develop to stage L4 (prepatent period lasts for about 4 months [11]) and finally to L5. Almost certainly adult form of *S. tundra* can live in definitive host for a year, probably longer [11,37] but there are no certain data on that subject as well.

Phylogeny

There are 147 sequences of *Setaria* genus with 54 of *S. tundra* deposited in GenBank. Molecular tests of nematodes from the Onchocercidae family are generally based on sequencing of the mitochondrial cytochrome c oxidase (COI) gene, which is available in GenBank, and the longest of has 689 bp (excluding the one of COI+NADH). Analysis based on this gene prove that the following species: *S. cervii*, *S. digitata* and *S. labiatopapillosa* form a sister clade to one which consists of *S. tundra* and *S. equine*. All these species form a monophyletic group [33,38,39]. Close affinity of these species has been confirmed by analysis of amplification products of the ribosomal 5S region of which two shorter sections (long for 400 and 800 bp) where much the same for *S. tundra*, S. *labiato-papillosa*, *S. cervi* and *S. digitata* with only one section, 1200 bp, different [19]. In other studies, 94 and 400 bp long sections of the 12S rRNA gene from the mitochondrial genome where analyzed and gave similar results [29,32]. Moreover, an unknown, sister species of *S. tundra* was identified, as well as some local differences among the discussed species. The most, up-to-date phylogeny show, that the Setariinae group most probably derived from an independent speciation to Onchocercidae family [40].

The occurrence of *S. tundra* in Europe

S. tundra has been found in 15 European countries so far. It was most likely (as this report goes far before the systematic of *Setaria* nematodes was established by Yeh's (1959) and Desset's (1966) publications) first described in Russia in 1928 [41]. The first numerous reports of its presence took place in the 1970s. Since 1969 this parasite has been found in

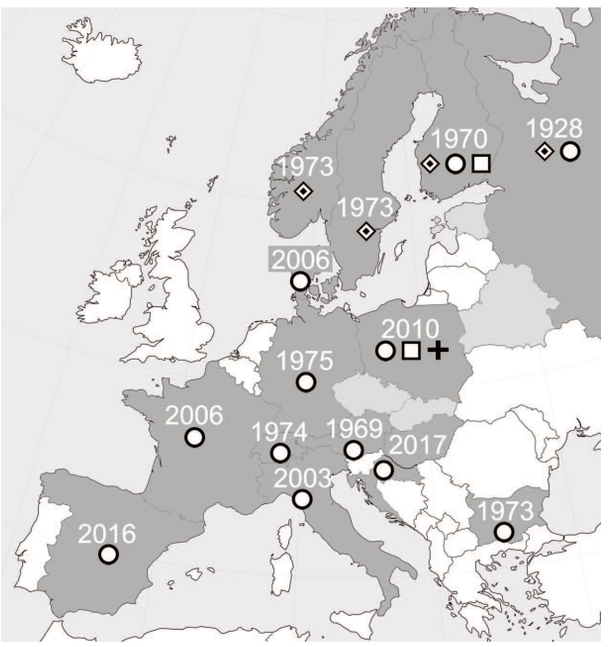

Figure 2. The date of first occurrence and the definitive hosts of *S. tundra* in Europe. ◈ is for reindeer, ❒ for moose, O for roe deer and + for red deer, no symbol refers to mosquito only. Dark grey is where *S. tundra* is present in cervids or mosquitos. Bright grey indicates countries which have a high probability of *S. tundra*. White indicates countries and islands where *S. tundra* was not found or that have no data available.
Picture of Europe was provided by freeworldmaps.net

Austria [42], Bulgaria [43], Switzerland [43], Sweden [45], Norway [46], Finland [47], and Germany [48]. First reports of *S. tundra* began again in the early 2000s: Italy [19], France [49], Poland [50], Hungary [32,33], Spain [51], Croatia [52], and Denmark [25]. Across Europe the main definitive hosts are reindeer and roe deer (Fig. 2), while intermediate hosts are different mosquitoes species.

Northern Europe and Russia

S. tundra is present in all of Fennoscandia. Russia (Archangelsk region) was the first country in which *S. tundra* has been described in reindeer [41]. It is very likely that this species has later been found in roe deer in the region of Vladivostok [53]. However, *S. tundra* has not been detected in Siberian roe deer (*Capreolus pygargus*) in far Eastern Russia, Primorsky Krai [54].

Finland is one of the most northern countries where *S. tundra* is present, where it was described in 1970s [47]. Several times its gradations has led to significant economic losses in semi-domesticated reindeer (*R. tarandus*) [9]. Thanks to numerous studies in Finland [11,12,20,28] much knowledge

has been gathered regarding of *S. tundra* life cycle.

In 1973 a mass fall of reindeer took place in Sweden, Norway, and Finland [55,11]. Intensity of *S. tundra* infection in reindeer was very high then, and over the years it has declined. The only confirmed definitive host species in Norway and Sweden is reindeer (*R. tarandus*) [44,56,57].

In Denmark this parasite was found in 2006 [25]. From all Northern European countries, it is the only one in which the only definitive host species is a roe deer. All examined specimens of roe deer were found with adult *Setaria*, and in one case nematodes were encapsulated under the liver.

Data from Latvia and Estonia was inconclusive, however *S. labiatopapillosa* was detected in roe deer in Lithuania [58]. In Estonia, *Setaria transcaucasica* was detected [59], but as current taxonomic status of this species is very unclear it could have been, due to many similarities, *S. tundra*. *S. tundra* has not been reported so far in Great Britain or in Ireland, and Iceland.

Central Europe

Among all countries of this region, *S. tundra* has been found in Poland, Bulgaria, and Hungary. Poland, just after Finland, has the most comprehensive literature about this species and eight years have passed since the first report of its presence [50]. Main definitive host in Poland is roe deer [50,60–62], but L5 of *S. tundra* where also found in moose and red deer [21,27]. Intensity of infection by this parasite reached 9% among roe deer, 23% among moose and 9% among red deer. In examined specimens anywhere from 1 to 14 adult worms were found. Microfilariae has been detected in central and western Poland among different mosquitoes species, mostly in *Aedes vexans*, but also in *Ochlerotatus caspius*, *Culex pipiens* and *Cx. torretium* [34,63–65]. *S. tundra* has been reported throughout Poland, including places located close to the borders, which creates a risk of transferring this parasite abroad.

DNA from *S. tundra* has been found in intermediate hosts, precisely, *Ochlerotatus annulipes*, *O. sticticus*, *Aedes vexans*, *A. rossicus*, *Culex richiardii* [32,33] in two regions located in southern Hungary: Segedyn and Baranya. Microfilariae were reported in all samples across several years [32], which can lead to the assumption that occurrence of this parasite in southern Hungary is common. Due to closeness of the Serbian and Romanian border high risk of transferring it abroad is possible. It's occurrence in Bulgaria, which was reported almost 50 years ago, is surprising [43]. This is the farthest place from Finland or northern Russia (more than 2.000 km in a straight line) where *S. tundra* has been found in those years, and it's presence was not reported later.

There has been no report of *S. tundra* from the Czech Republic so far [66]. However, other species of *Setaria* has been found here (*S. transcaucasica*, *S. cervii*, *S. labiatopapillosa*), with an undefined *Setaria* spp. microfilariae in *Aedes vexans* [31,67].

There is a high possibility that *S. tundra* is also present in Slovakia [35]. In Romania, only *S. labiatopapillosa* [68] and an undefined *Setaria* spp. with a 57% prevalence has been reported so far [69]. Unfortunately the current literature regarding helminthofauna focuses on lung and gastral nematodes [70]. Studies in the Balkans regarding helminths have not been focused on *Setaria* nematodes, as only faecal samples were analyzed [71].

Western Europe

S. tundra has been reported in Germany, Austria, Switzerland, and France. The first report from Germany was in 1975, when up to 12.3% of examined roe deer were infected [48]. Later, prevalence of *S. tundra* declined to 1.6% [72]. In recent years, microfilariae DNA has been found among various mosquitoes: *Aedes vexans*, *Culex* spp., *Ochlerotatus sticticus*, *Oc. cantans* [29,30], and *Anopheles daciae*. Intensity of infection among mosquitoes reached as high as 39% [29]. *S. tundra* has been found in Bawaria [29,30,48], Westfalia [72], Baden-Württemberg, Niedersachsen [30] and in Rhineland-Palatinate [29].

S. tundra was reported in Austria in 1969, where up to 11% of the examined roe deer were infected [42]. Among infected intermediate hosts *Aedes vexans*, *A. geminus*, *Culex pippens complex*, and *Coquillettidia richiardii* were found [35].

S. tundra is also present in Switzerland and France. In both countries, roe deer is the only definitive host for this parasite [44,49,73].

There is no current data on *S. tundra* from the Netherlands, Lichtenstein or Belgium.

Southern Europe

In recent years *S. tundra* has been found in Spain, Croatia, and Italy, with Spain being the farthest country in relation to the original occurrence of this parasite. According to the

literature [51], domesticated roe deer in Spain have had a great (75% of the population) population drop in recent years. In one of these deer, two specimens of *Setaria* were found. Analysis by mitochondrial cytochrome c oxidase subunit 1 (COI) gene indicated that these specimens were *S. tundra*. This was the first report of *S. tundra* from Iberian Peninsula.

In Croatia up to 22.2% of wild roe deer were infected. Species affiliation of the found specimens were also indicated to be *S. tundra* by analysis of the cox1 gene [52].

The report of *S. tundra* form Italy took place in 2003, from Piedmont in northern Italy [19].

There is no data currently from Albania, Bosnia and Herzegovina, North Macedonia, Monaco, Monte Negro, Greece, and Serbia. In Turkey *S. tundra* has not yet been found [74], as well as in Slovenia [75].

Eastern Europe

In Belarus and Ukraine, only *S. cervii* has been found so far [76–78]. Information from Moldavia is inconclusive.

Health effects and treatments of setariosis

The presence of L5 worms (setariosis) and/or microfilariae (microfilariosis) in accidental hosts, or their mass appearance cause negative health effects. Classic symptoms include: fever, weight loss, and weakness. *S. tundra* may cause severe fibrinous perihepatitis and severe peritonitis [20], in which calves are particularly vulnerable. Closely related species, *S. yehi*, can cause intestine inflammation, secondary and bacterial peritonitis in calves and adult reindeer [4,8]. Most *Setaria* species can cause neurological disorders usually in accidental hosts (lumbar paralisys, "Kumri" and cerebrospinal nematodis) [36,79], which can be lethal to the host. According to recent findings this can take place also in definitive hosts like buffalo [80]. *S. equina* and *S. labiatopapillosa* can cause intestine or hind inflammation and pain [80,81]. Together with *S. digitata* and *S. cervii* these species can also cause ocular filariosis or even blindness [10]. As microfilariae are not capable to penetrate through bearing, thus newborns can be infected only by mosquito bites [11]. The risk of being infected by milk is an open question as adult forms of *S. labiatopapillosa* have been found in milk of infected buffalo cows [82].

The main drugs against *S. tundra* in Fennish reindeer is ivermectin. It was administered preventively through injections at doses of 200 µg/kg subcutaneously [83]. Injections seem to be the most effective method of administering this drug [84]. At 300 µg/kg, this drug can also cure ocular filariosis caused by *S. equina* [85]. In the destruction of microfilaria of *S. equina*, *S. digitata* and *S. cervii*, diethylcarbamazine citrate (DEC) can also be used at doses of 25 to 100 mg/kg [86–88]. The preventive use of this drug is associated with the necessity of its use throughout the period of activity of mosquitoes [89]. Unfortunately, literature data on the efficacy of pharmacological treatments (DEC, avermectins, fenbendazole) of ocular filariosis are contradictory [85,86]. In neurological setariosis, combined therapy with ivermectin and fenbendazole (at a dose of 50mg/kg) is recommended [90]. Fenbendazole in dosage of 50 mg/kg together with 30 mg/kg trimethroprim sulphonamides, 0.1 mg/kg dexamethasone, and with vitamin B and E helped to cure parasitic encephalitis in horse [91]. Much is yet to be discovered with potential antifilarial medicines of traditional, Indian medicine based on plants and herbs, with *Streblus asper* being the most promising [92].

Discussion

In the post-war years systematics of *Setaria* genus underwent significant changes [1,93,94]. Technological advancements in genetics and electron microscopy allowed for the correct determination and classification of these species in later years. Recent reports of *S. tundra,* mainly from southern Europe, indicate that this nematode has expanded its range of occurrence in a few decades by thousands of kilometers. A report from Bulgaria made almost 50 years ago is in contradiction with these new findings [43]. Either the species could have been wrongly identified, or the original range of *S. tundra* was not only limited to Fennoscandia. In the light of recent studies high diversity of the COI gene sequences isolated from various hosts across Europe suggests that *S. tundra* has been established in most of Europe for a very long time [63]. Despite the process, *S. tundra* is now present across Europe, from Finland to Italy, and one can expect its further expansion [11,95].

It is very likely that the presence of all *Setaria* species would be reported more often if studies of helminth fauna were not limited to gastral/lung worms only. For this reason one can suspect, that

although no data is available from several countries in Europe, *S. tundra* is probably present there. *S. tundra* has been found in Poland, very close to the Belarus border in more than one definitive host species [21], making it very possible that this nematode is present also on the other side of border (so far, no geographical barrier for definitive hosts occurs). A similar situation for the Czech Republic also occurs. Despite the fact that this nematode was not indicated in research of the Czech Republic's Cervidae, it is worth to notice that *S. tundra* is present in 3 out of 4 neighboring countries, and is very much likely in the fourth one. *S. tundra* probably is also present in Slovakia by the fact it has been reported from Austria, very close to the Slovakian border [35]. By the same reasoning, we can also expect the presence of *S. tundra* in Slovenia where it has not been reported so far [75], but is present in all surrounding countries.

According to some authors, it is possible that *S. tundra* and *S. yehi* (parasite of various ungulates in North America) are the same species [20,96]. This assumption is based on smaller anatomic intra-species differences vs. interspecies ones, similar types of hosts, and the original range of its presence in the subpolar zone. Due to the wide range of definitive hosts of *S. yehi*: caribou (*R. tarandus*), bison (*Bison bison*) [97], fallow deer (*D. dama*) [98], sambar (*Rusa unicolor*) [99], mule deer (*Odocoileus hemionus*) [100], white-tailed deer (*Odocoileus virginianus*) [101], and moose [102] we can expect reports of *S. tundra* in other host species such as fallow deer or sika deer (*Cervus nippon*) in the near future. Like *S. tundra*, *S. yehi* has also widened its range, reaching Florida. The question of the degree of relationship between these two taxa could be resolved by molecular studies. If sika and/or fallow deer a definitive host for *S. tundra* (like in case of *S. yehi*) is an open question.

Conclusions

S. tundra has been reported in 15 European countries so far. In recent years it has widened its range from the subpolar to subtropical zone. Its presence in Belarus, Czech Republic, Slovakia, Slovenia, and Estonia is very possible. Four species of cervids have been found among the following definitive hosts: reindeer, roe deer, red deer and moose. Ten species of mosquitoes have been found as intermediate hosts, mostly from: *Aedes*, *Culex*, *Ochlerotatus,* and the *Anopheles* genus. Besides Fennoscandia, it has not yet caused significant losses among livestock and wild species. Further geographic and specie-host expansion of this nematode can be expected. There are important gaps in knowledge of its life cycle, intermediate hosts and affinity with other setaria nematodes.

Acknowledgements

I would like to express my gratitude to Natasha Ng from Arizona University for help in writing this article.

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Received 09 April 2020
Accepted 04 December 2020