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Onchocerca (Nematoda: Filarioidea) infection in reindeer (*Rangifer tarandus*) in Russia: two case reports and the first molecularly confirmed record of *O. flexuosa* in this host species

Olga A. Loginova

ABSTRACT. The family Onchocercidae comprises tissue-dwelling nematodes transmitted by hematophagous arthropods, including taxa of medical, veterinary, and ecological importance, with known zoonotic potential and symbioses such as *Onchocerca–Wolbachia*. Five genera infect cervids; three are reported in reindeer (*Rangifer tarandus*). *Onchocerca* spp. can induce granulomatous lesions and secondary infections with economic and ecological impact. Molecular data on *Onchocerca* spp. in Palearctic reindeer remain lacking. This study describes two cases of *Onchocerca* infection in semiwild male reindeer. Both males died in 2021 in different private menageries. One animal was kept in the Republic of Karelia (66.3015° N, 33.0777° E), whereas the other was kept in Samara Oblast (53.3449° N, 50.2224° E). In the first case, necropsy revealed multiple dorsal subcutaneous nodules (1.5 × 2 cm) with nematodes. Morphological, histological, and molecular analyses (12S and 16S rRNA genes) identified the parasites as *O. flexuosa*. In the second case, a 4 × 3 mm nodule near the hock joint contained filarioid nematodes assigned to *Onchocerca* sp. Delayed fixation (~24 h post-mortem) and suboptimal preservation impaired morphology and DNA quality, precluding species identification despite sequencing attempts. Co-infections with *Elaphostrongylus rangiferi* and rumen flukes (Paramphistomatoidea), identified by the author during life-time coproscopic examination of this reindeer one month before its death, together with mixed-species housing, indicate a complex epidemiological setting. This work provides the first molecular data on *Onchocerca* in Palearctic reindeer and confirms *O. flexuosa* in this host.

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Инвазия *Onchocerca* (Nematoda: Filarioidea) у северного оленя (*Rangifer tarandus*) в России: два случая и первое молекулярно подтверждённое обнаружение *O. flexuosa* у этого вида-хозяина

О.А. Логинова

РЕЗЮМЕ. Семейство Onchocercidae включает тканевых нематод, передающихся кровососущими членистоногими и имеющих медицинское, ветеринарное и экологическое значение, включая зоонозный потенциал и симбиозы, такие как *Onchocerca–Wolbachia*. У оленевых отмечено пять родов; три из них зарегистрированы у северного оленя (*Rangifer tarandus*). Виды *Onchocerca* могут вызывать гранулематозные поражения и вторичные инфекции с экономическими и экологическими последствиями. Молекулярные данные по *Onchocerca* для палеарктических северных оленей отсутствуют. В работе описаны два случая инвазии *Onchocerca* у полудиких самцов северных оленей. Оба самца умерли в 2021 году в разных частных зверинцах. Одно животное содержалось в Республике Карелия (66.3015° N, 33.0777° E), а другое — в Самарской области (53.3449° N, 50.2224° E). В первом случае при вскрытии в дорсальной области выявлены множественные подкожные узлы (1,5 × 2 см) с нематодами. Морфологический, гистологический и молекулярный анализы (гены 12S и 16S рРНК) идентифицировали паразитов как *O. flexuosa*. Во втором случае узел размером 4 × 3 мм в области скакательного сустава содержал филяриевых нематод, отнесённых к роду *Onchocerca*. Поздняя фиксации (~24 ч после смерти) и несоответствующие условия хранения ухудшили сохранность морфологических признаков и качество ДНК, что не позволило установить вид, несмотря на попытки секвенирования. Сопутствующие инвазии *Elaphostrongylus rangiferi* и рубцовыми трематодами (Paramphistomatoidea), обнаруженные автором у этого оленя посредством прижизненной копроскопии за месяц до падежа, а также содержание с животными разных видов указывают на сложную

эпизоотическую обстановку. Работа предоставляет первые молекулярные данные по *Onchocerca* у палеарктических северных оленей и подтверждает наличие *O. flexuosa* у данного хозяина.

КЛЮЧЕВЫЕ СЛОВА: филяроидные нематоды, Cervidae, Onchocercidae.

Introduction

The family Onchocercidae (Chromadorea, Rhabditida) comprises highly specialized tissue-dwelling nematodes. These parasites are transmitted to their vertebrate hosts by blood-feeding arthropod vectors (Kulpa & Verocai, 2026). Medical interest in onchocercids is driven by human diseases, such as river blindness (caused by *Onchocerca volvulus* Leuckart, 1893), filariasis or elephantiasis (caused by *Wuchereria bancrofti* Cobbold, 1877 and *Brugia* Buckley, 1960 species), as well as occasional zoonotic infections associated with *Dirofilaria immitis* Leidy, 1856 and species of *Onchocerca* Diesing, 1841 (including *O. jakutensis* Gubanov, 1964, a parasite of cervids) (Cambra-Pellejà *et al*., 2020). Biological interest in onchocercids has focused on the model system of *Onchocerca* and its symbiotic *Wolbachia* Hertig, 1936 (likely acquired from arthropod hosts) within the framework of nematode-bacteria interactions (Latrofa *et al*., 2026). Onchocercids also draw attention of biologists and veterinarians who study vertebrates hosting these parasites. Five genera of Onchocercidae are known to parasitize cervids: *Alcefilaria* Oshmarin et Belous, 1951 (venous vessels of the liver and mesentery), *Cercopithifilaria* Eberhard, 1980 (subcutaneous tissues), *Mansonella* Faust, 1929 (dermis), *Onchocerca* (subcutaneous tissues and ligaments), and *Rumenfilaria* Lankester et Snider, 1982 (lymphatic vessels of the rumen) (Appendix 1 and 2). To date, three of these genera have been reported in reindeer (*Rangifer tarandus* Linnaeus, 1758): *Alcefilaria* (restricted to the Palearctic), *Onchocerca*, and *Rumenfilaria* (both distributed in the Nearctic and Palearctic regions) (Mitskewitch, 1967; Tryland & Kutz, 2019).

Species of *Onchocerca* were previously considered to have low pathogenicity for reindeer (Laaksonen *et al*., 2017). However, they can facilitate secondary bacterial infections, including those caused by corynebacteria and fusobacteria (the agent of necrobacillosis). In cases of heavy infection, *O. skrjabini* Ruklyadev, 1961 (syn. *O. tarsicola* Bain et Schulz-Key, 1974) may induce the formation of granulomatous nodules in the abdominal wall, diaphragm, rumen, and especially the liver (Rehbinder *et al*., 1976). This results in the condemnation of affected carcass parts during meat inspection, raising food safety concerns (Rehbinder & Nikander, 1999). In addition, infection may lead to hemorrhagic tarsitis, causing pain and lameness and thereby compromising reindeer survival (Rehbinder & Nordkvist, 1983). Consequently, *Onchocerca* infections are of economic importance in semi-domesticated reindeer husbandry and are also relevant for the conservation of wild reindeer populations.

Species *O. cervipedis* Wehr et Dikmans, 1935 has been reported in reindeer from both the Nearctic: Canada and USA (Verocai *et al*., 2012) and the Palearctic: USSR, present Russia (Gubanov, 1964). Species *O. flexuosa* Wedl, 1856 has been recorded in the Palearctic: USSR (Kadenatsii, 1963). Species *O. skrjabini* has been reported from the Palearctic: Sweden (Rehbinder, 1973; Rehbinder *et al*., 1976; Bain *et al*., 1979) and Finland (Bylund *et al*., 1981). It is possible that additional species of *Onchocerca* remain undetected in reindeer populations worldwide, both within and outside their native range. Recent molecular studies have revealed considerable cryptic diversity within *Onchocerca* (Kulpa *et al*., 2024). At the same time, historical diagnoses of reindeer onchocercids should be interpreted with caution. In the pre-molecular era, identifications relied largely on morphology, histopathology, and gross pathological findings, and other insect-borne, tissue-dwelling nematodes parasitizing reindeer could have confounded the diagnosis of onchocercids, especially when only larval or otherwise poorly preserved material was available. These include *Lappnema auris* Bain et Nikander, 1983 (Enoplea, Robertdollfusidae) and several species of *Setaria* Viborg, 1795 (Chromadorea, Setariidae) (Lisitzin, 1964, 1976; Bain & Nikander, 1983; Tryland & Kutz, 2019).

The earliest reports by Russian researchers on *Onchocerca* infections in reindeer were, in fact, the first for the entire Palearctic region. Nikolay M. Gubanov, summarizing the results of a nine-year survey of helminth fauna in game animals of the Yakut ASSR (1954–1962), presented data based on lethal sampling — helminthological dissections following Skrjabin's method (Skrjabin, 1928). A total of 11 wild reindeer were examined. Seventy specimens of *O. flexuosa* were recovered from the subcutaneous tissue of one reindeer from the Gorniy District (62.0637° N, 127.2773° E), while 6–80 specimens of *O. cervipedis* were found in two reindeer from the Olyokminskiy District (60.7271° N, 120.301° E) (Gubanov, 1964).

From June to September 1960, Anton N. Kadenatsii participated in the 314th (Amur) All-Union Helminthological Expedition (Skrjabin *et al*., 1963, pp. 392–402). During this survey, he performed complete helminthological dissections on five domestic reindeer of the forest "Karagas" type, aged one to five years. These animals originated from a herd of 500 individuals at the "Severnaya Zarya" collective farm in the Verkhnebureinskiy District of Khabarovsk Krai, USSR, approximately 30 km from the now abandoned settlement of Umalta (51.9347° N, 133.5694° E). *Onchocerca* nematodes were detected in three of the examined reindeer (Ryzhikov *et al*., 1962). The parasites were located beneath the skin in the dorsal region, forming dense nod-

ules ranging in size "from a large bean to a hazelnut," and were identified as *O. flexuosa* (Kadenatsii, 1963).

In a monograph on helminths of reindeer, Vera Yu. Mitskewitch (who never detected *Onchocerca* herself) referred to an oral communication by Leonid D. Nikolaevskiy reporting the presence of *Onchocerca* in the subcutaneous tissue and tendons of reindeer (Mitskewitch, 1967, p. 118). She noted that these helminths had never been studied, formally described, or published in the scientific literature. Importantly, this statement appears in the section on the history of *Onchocerca* research in reindeer, where Mitskewitch wrote that Nikolaevskiy was the first to report their presence. Although this observation was undated, it is followed by references to targeted searches conducted by Efim P. Pushmenkov in 1940, Maria V. Polyanskaya in 1941, and her own surveys (all yielded negative results), and subsequently by records from the 1960s (when the parasites were finally obtained by Gubanov and Kadenatsii). This sequence suggests that Nikolaevskiy's report predates 1940. In a chapter on reindeer diseases in a handbook on reindeer husbandry, Nikolaevskiy mentioned *Onchocerca* as having been found (apparently in domestic reindeer) in "the Asian part of the North" (Nikolaevskiy, 1961, p. 399). Earlier, Konstantin I. Skrjabin and Nadezhda P. Shikhobalova, in their monograph on filariae, noted preliminary indications of *Onchocerca* in reindeer, but added that these observations required confirmation (Skrjabin & Shikhobalova, 1948, p. 83). These references may plausibly derive from Nikolaevskiy's unpublished observations.

However, an even earlier publication may contain the first report of *Onchocerca* in reindeer. In his account of a journey to the Okhotsk–Kamchatka region in 1898, Nikolay V. Slyunin described a parasitic disease of reindeer caused by a "long and thin (like a thread) worm" that the "Tungusy" (Evenki) reportedly found beneath the skin of animals (Slyunin, 1900, p. 329). Current evidence suggests only two plausible candidates for this subcutaneous helminth: *Elaphostrongylus rangiferi* Mitskewitch, 1958 (Mitskewitch, 1967) or species of *Onchocerca*, such as *O. skrjabini* or *O. garmsi* Schulz-Key et Bain, 1976, which are large enough to be visible to the naked eye and are known to occur freely beneath the skin in cervids (Schulz-Key *et al*., 1976; Manzanell *et al*., 2022).

Semyon I. Isakov conducted complete helminthological dissections in three reindeer and incomplete ones in 12776 reindeer during 1981–1985 in Yakut ASSR. He reported *O. cervipedis* and *O. flexuosa* (Isakov, 1992).

Despite the availability of a growing body of data, contemporary monographs still provide an incomplete account of *Onchocerca* in reindeer. Thus, in the monograph *Diseases of Reindeer*, onchocerciasis is not mentioned at all in the section on helminth infections (Zabrodin *et al*., 2019). The book «Ecology of Ungulates: A Handbook of Species in Eastern Europe and Northern and Central Asia» lists only the genus *Onchocerca* for reindeer (Baskin & Danell, 2003), as has the book «Know the Reindeer: Reindeer Diseases and Health Care» (Laaksonen, 2016), while the 2019 monograph «Reindeer and Caribou: Health and Disease» mentions only two out of three species, *O. cervipedis* and *O. skrjabini* (Tryland & Kutz, 2019).

No molecular studies of *Onchocerca* from reindeer in Palearctic have been conducted to date. Therefore, the aim of this study is to report two cases of *Onchocerca* infection in Russian reindeer and to provide the first molecular confirmation of *O. flexuosa* in reindeer.

Material and methods

Sample collection

Case 1. A male semiwild reindeer died in a private menagerie in the Republic of Karelia (66.3015° N, 33.0777° E) on 11 February 2021. No information on clinical history or prior condition was available. During the mandatory necropsy, subcutaneous nodules were detected on the dorsal region. These were excised, and one nodule was transported frozen to the Center for Parasitology, IEE RAS (Moscow, Russia) for further examination. Due to prior freezing of the specimen, two intact male worms as well as fragments of females were successfully recovered after thawing. Nematodes intended for scanning electron microscopy (SEM) were fixed and preserved in 10% buffered HistoSafe formalin (ErgoProduction LLC, Russia). Nematode specimens intended for DNA analysis were preserved in 70% ethanol. Female nematode fragments were preserved in 80% ethanol as a voucher. The other intact subcutaneous nodule tissues dissected from infected reindeer were fixed in 10% buffered HistoSafe formalin (ErgoProduction LLC, Russia) for histopathological examination.

Case 2. A male semiwild reindeer died in a private menagerie in Samara Oblast (53.3449° N, 50.2224° E) on 27 August 2021 and was also subjected to mandatory necropsy. A small nodule was observed in the region of the muscle fascia near the hock joint of the pelvic limb and was excised. The material was submitted in ethanol of inadequate concentration; furthermore, fixation was delayed, with ethanol being introduced only approximately 24 hours post mortem. The animal had previously been examined, and coproscopic analysis performed by the author one month before death revealed additional helminth infections, including rumen flukes (superfamily Paramphistomatoidea) and the meningeal nematode *E. rangiferi*. It originated from the Khanty-Mansi Autonomous District and, at the time of death in Samara Region, was housed in the same enclosure with other reindeer, two sika deer (*Cervus nippon* Temminck, 1838), and a Bactrian camel (*Camelus bactrianus* Linnaeus, 1758).

Histopathological examination

Samples underwent standard histological processing, including graded ethanol dehydration, xylene clearing, and paraffin embedding. Histological slides were produced using a Digital Pathology system at the commercial Unim Pathomorphological Laboratory (Moscow, Russia; https://unim.su/), in collaboration

with VetUnion Company (Moscow, Russia; https://vetunion.ru/). Paraffin sections of 5 μm thickness were prepared following conventional histological protocols on a HistoCore BIOCUT Rotary Microtome (Leica Biosystems Nussloch GmbH, Germany) and subsequently stained with hematoxylin and eosin (H&E).

Light microscopy

Nematodes were examined under a stereomicroscope (MBS-10) in a flat-bottomed dish filled with tap water, and under a light microscope (Micmed-6) in tap water on glass slides with or without coverslips (both microscopes manufactured by LOMO-MA, Russia). Light microscopy was performed using bright-field and dark-field techniques. Temporary mounts of nematodes were studied at magnifications of 20×, 100×, 200×, and 400×. Images were captured using a Canon 5D Mark II digital camera (Canon, Japan) connected via an optical-mechanical C-mount adapter (LOMO-MA, Russia).

Scanning electron microscopy

Prior to examination, samples were rinsed in distilled water, passed through a graded ethanol series followed by acetone dehydration, and dried at the critical point using a Leica EM CPD300 (Leica Microsystems Vertrieb GmbH, Austria). The dried specimens were mounted on stubs, sputter-coated with gold using an S150A Sputter Coater (Quorum Technologies Ltd., UK), and examined with a MIRA 3 LMH scanning electron microscope (TESCAN, Czech Republic) in the Joint Usage Center "Instrumental Methods in Ecology" of the IEE RAS (https://sev-in.ru/kabinet-elektronnoy-mikroskopii/).

DNA analysis

Genomic DNA extraction from a fragment of nematode female body was performed by digestion in the presence of proteinase K (Holterman *et al*., 2006). For species identification, fragments of the mitochondrial 12S rRNA gene were amplified using the forward primer 12SOvC (TCGGCTATGCGTTTTAATTTT) and the reverse primer 12SOvB (CAACTTACGCCCCTTTAGGC). PCR conditions included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. The 16S rRNA gene fragment was amplified using primers 16SOvC (AGCCTTAGCGTGATGGCATA) and 16SOvB (ACCCACATTGCATTCCTTTC), (Morales-Hojas *et al*., 2006), under the same cycling conditions. Each PCR run concluded with a cooling step to 12 °C. Negative controls without DNA template were included to monitor contamination. Positive controls consisted of previously obtained *O. flexuosa* samples from red deer *Cervus elaphus* Linnaeus, 1758 (Karelia; GenBank accession numbers: OR744845 (12S), OR741778 (16S)). PCR products were visualized on a 1% agarose gel. Bands of the expected size were excised and purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA). Following additional purification by ethanol precipitation with ammonium acetate, the products were directly sequenced by Genotech (Moscow, Russia). Chromatograms were processed in Chromas 2.6.6 (Technelysium Pty Ltd, Australia; RRID:SCR_000598). Sequence similarity searches were conducted using BLASTN 2.13.0+ against the GenBank database (Zhang *et al*., 2000; Morgulis *et al*., 2008).

Statistical analysis

Only descriptive statistics were applied due to the small sample size. Morphometric data were compared with literature reports without inferential statistical testing. Linear measurements were obtained via LM and SEM images using Fiji/ImageJ Version 1.2.4 software, RRID:SCR_003070 (National Institutes of Health, USA). The following parameters were recorded where applicable: total body length, maximum body width, and dimensions of diagnostically relevant structures. All measurements are provided in micrometers (μm) and presented as median values with corresponding ranges due to the limited sample size. Median values were calculated using R Version 4.3 software, RRID:SCR_001905 (R Foundation for Statistical Computing, Austria). The obtained morphometric data were qualitatively compared with previously published descriptions to assess consistency with known species characteristics.

Results

Case 1

In a connective tissue nodule measuring 1.5 × 2 cm, numerous nematodes densely embedded in the tissue were found (Fig. 1A, B, Fig. 2A). The median body length of a male (n=2) was 61150 μm (51470–70830). The total body length of a female is unknown. The body surface displayed transverse striation formed by cuticular ridges (Fig. 1C), with an interruption of these ridges in the lateral field (Fig. 3B). The cuticle was two-layered, with fine striation present in the medullary layer (Fig. 2D). An anterior end was rounded, without thickenings or swellings. There, the nematodes exhibited Y-shaped mouth opening, two pairs of labial papillae, two pairs of cervical papillae, and two amphids (Fig. 3A). Males possessed two unequal and dissimilar spicules. The longer spicule was tubular, with a widened proximal end and a tapered distal end. The shorter spicule was sickle-shaped and equipped with a terminal hook (Fig. 2E, F). The median lengths (*n*=2) were 589 μm (583–595) for the large spicule and 185 μm (163–207) for the small spicule. No pairs of caudal papillae were observed anterior to the cloaca. A group of four pairs of lateral postcloacal papillae was present; between them, at the level of the first to second pairs, a pair of median postcloacal papillae was located. This was followed by four pairs of terminal caudal papillae: the first two pairs were spaced apart from each other and from other papillae, whereas the last two pairs were located close to the caudal end of the male (Fig. 2B, E, Fig. 3C, D).

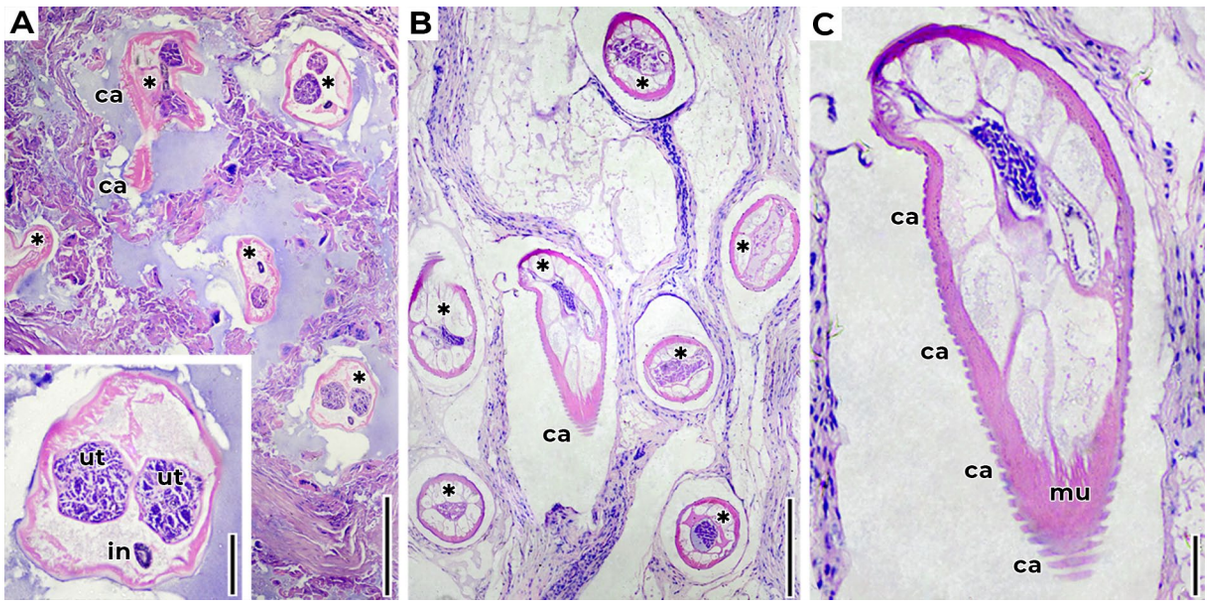


Fig. 1. Histological sections of subcutaneous nodules from the reindeer (Case 1): A — overview of a lesion with multiple specimens of *Onchocerca flexuosa* (asterisks) and inflammatory infiltrate surrounding them; evenly spaced cuticular annulations are visible; 100×; scale bar 25 µm; inset shows higher magnification cross-section of a female with a relatively small intestine and paired uteri filled with microfilariae; 400×; scale bar 50 µm; B — section of another nodule with helminths embedded in subcutaneous tissue, including an obliquely sectioned specimen with visible cuticular annulations; 100×; scale bar 25 µm; C — higher magnification of an obliquely sectioned specimen highlighting cuticular annulations and coelomyarian muscles; 200×; scale bar 50 µm. Abbreviations: ca — cuticular annulations; in — intestine; mu — muscles; ut — uterus. Bright-field microscopy, H&E staining.

The number and location of the caudal papillae varied. In male 1, the lateral postcloacal papillae of pairs 1, 2 and 4 were aligned, whereas the papilla of pair 3 was positioned more medially; on the other side, the papillae of pairs 2 and 3 were fused (Fig. 3C). In male 2, the papillae of all four lateral postcloacal pairs were aligned, whereas on the other side the papilla of pair 3 was positioned more medially than the others (Fig. 3D). The vulva of the female (n=1) was located 430 µm from the anterior end. The body width at this level was 182 µm. In females, two uteri were observed, initially filled with round eggs, followed by lemon-shaped eggs (Fig. 2C), and subsequently containing microfilariae. The body width of another fragment of the female in the region of the uteri filled with embryonated eggs was 319 µm. The median uterine thickness (*n*=2) was 106 µm (97–115). The ratio of intestinal thickness to the median uterine thickness at this level was therefore 37:106 (approximately 1:2.86).

Partial 12S (485 bp) and 16S (388 bp) sequences were obtained from the material and compared with sequences available in GenBank, confirming its identity as *O. flexuosa*. The 12S sequence showed 100% identity to an *O. flexuosa* sequence from an adult female nematode in Karelian red deer (GenBank: OR712112; deposited by Loginova O.A. and Spiridonov S.E. on 19 October 2023) and 99.20% identity to a sequence from Spanish red deer (GenBank: JQ733522; Hidalgo et al., 2015). The 16S sequence was 100% identical to an *O. flexuosa* sequence from an adult helminth in Karelian red deer (GenBank: OR741781; deposited by Loginova O.A. and Spiridonov S.E. on 29 October 2023) and 99.48% identical to a sequence from red deer in Sweden (GenBank: LT732687; submitted by Boijsen B., U H., Ågren E., and Höglund J. on 03 February 2017). Following molecular confirmation, a voucher sample consisting of female fragments preserved in 80% ethanol was deposited in the Museum of Helminthological Collections of the Center for Parasitology, IEE RAS (Moscow, Russia), under accession number 14473. The newly generated sequences were deposited in GenBank under accession numbers PP461396 (12S) and PP461397 (16S).

Case 2

From a connective tissue nodule measuring 4×3 mm (Fig. 4A), only nematode fragments were recovered, including a single anterior end (Fig. 4B, C); no intact specimens or posterior ends were obtained, which markedly hindered identification. The total body length could not be determined. An anterior end was also rounded having no thickenings or swellings (Fig. 4C, D). The body width of one nematode fragment (presumably a male) measured 56 µm at 50 µm from the anterior end and 84 µm at 2382 µm from the anterior end (which was the maximum length). The maximum

body width of another fragment (presumably a female) was 93 µm. Sex could not be established due to insufficient visualization of the reproductive system and poor preservation of the material. The cuticle, as in case 1, was transversely striated throughout, two-layered with fine striae in the medulla (Fig. 4E). Attempts to extract and analyze DNA failed.

Discussion

The first case represents the first well-substantiated record of *O. flexuosa* in *R. tarandus* from Russia, supported by concordant histological, morphological, and molecular evidence. This record is of particular interest in a broader regional context, given that a different species, *O. skrjabini*, has previously been reported from reindeer in neighbouring areas, including Finland (Bylund *et al*., 1981) and Sweden (Rehbinder, 1973; Rehbinder *et al*., 1976; Bain *et al*., 1979). At the same time, the unpublished data by Sergei V. Bugmyrin, Olga A. Loginova, and Sergei S. Spiridinov indicate the occurrence of *O. flexuosa* in red deer in Karelia, i.e. within the same general region as the reindeer examined here. Taken together, these observations suggest that the distribution of *O. flexuosa* in northern Europe may be broader than currently documented and that its circulation in different cervid hosts in northwestern Russia warrants further investigation.

The convergence of multiple independent methods strengthens diagnostic confidence and minimizes the likelihood of misidentification in the first case report. The lesions and parasite burden observed in this case are comparable to those described in cervids (including reindeer), indicating a typical manifestation of infection (Kadenatsii, 1963; Barbušinová *et al*., 2020; Martinuš *et al*., 2026). All observed parameters fall within the range reported in previous studies of *O. flexuosa* infections in ungulates. Morphological measurements, parasite localization, and genetic profiles are consistent with published data, suggesting no substantial deviation from known species characteristics (Skrjabin & Shikhobalova, 1948; Sonin, 1975; Bain, 1981; Hidalgo *et al*., 2015).

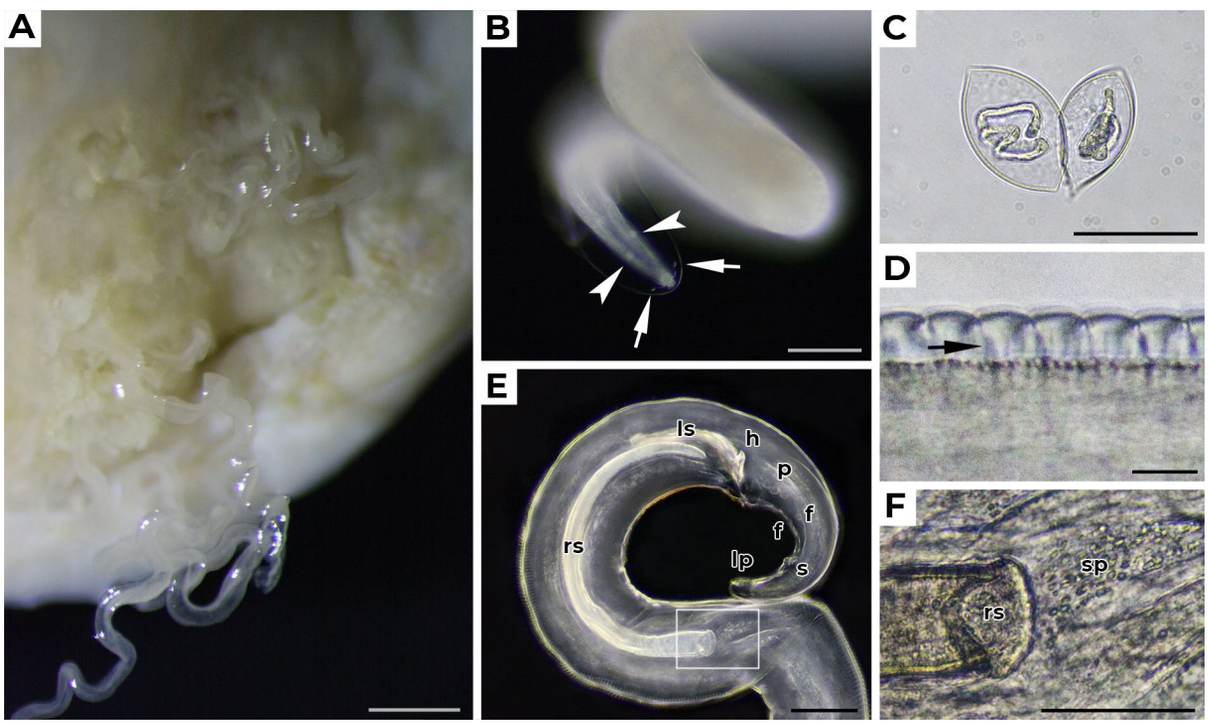


Fig. 2. Light microscopy of *Onchocerca flexuosa* recovered from subcutaneous nodules of the reindeer (Case 1): A — curved females in situ, no coverslip; 20×; scale bar 1000 µm; B — posterior end of the male coiled in two turns showing the second pare (arrowheads) and two last pairs (arrows) of terminal caudal papillae, no coverslip; 200×; scale bar 100 µm; C — lemon-shaped embryonated eggs derived from the uterus, coverslip; 400×; scale bar 50 µm; D — details of two-layered cuticle with fine striae in medulla (arrow), coverslip; 400×; scale bar 10 µm; E — posterior end of the male showing spicules (the left spicule bearing a hook) and caudal papillae (four postcloacal and four terminal pairs), coverslip; 200×; scale bar 100 µm; F — higher magnification of the posterior region of the male, indicated by a rectangular in panel E, showing distal part of the right spicule and sperm, coverslip; 400×; scale bar 50 µm. Abbreviations: f — first pair of terminal caudal papillae; h — hook; lp — last two (third and fourth) pairs of terminal caudal papillae; ls — left spicule; p — four pairs of postcloacal caudal papillae; rs — right spicule; s — second pair of terminal caudal papillae; sp — sperm. A — stereoscopic microscope, B–F — light microscope; B, E — dark-field, C, D, F — bright-field; all the specimens depicted in tap water without staining.

This agreement reinforces the validity of the case and supports its inclusion within the current understanding of host-parasite associations in northern ungulates.

In the second case, several findings support a diagnosis of onchocerciasis in reindeer, including the anatomical localization of the parasite (nematodes embedded within a connective tissue nodule in the tarsal region), as well as overall body morphology and cuticular features consistent with *Onchocerca* spp. A major diagnostic limitation, however, is the

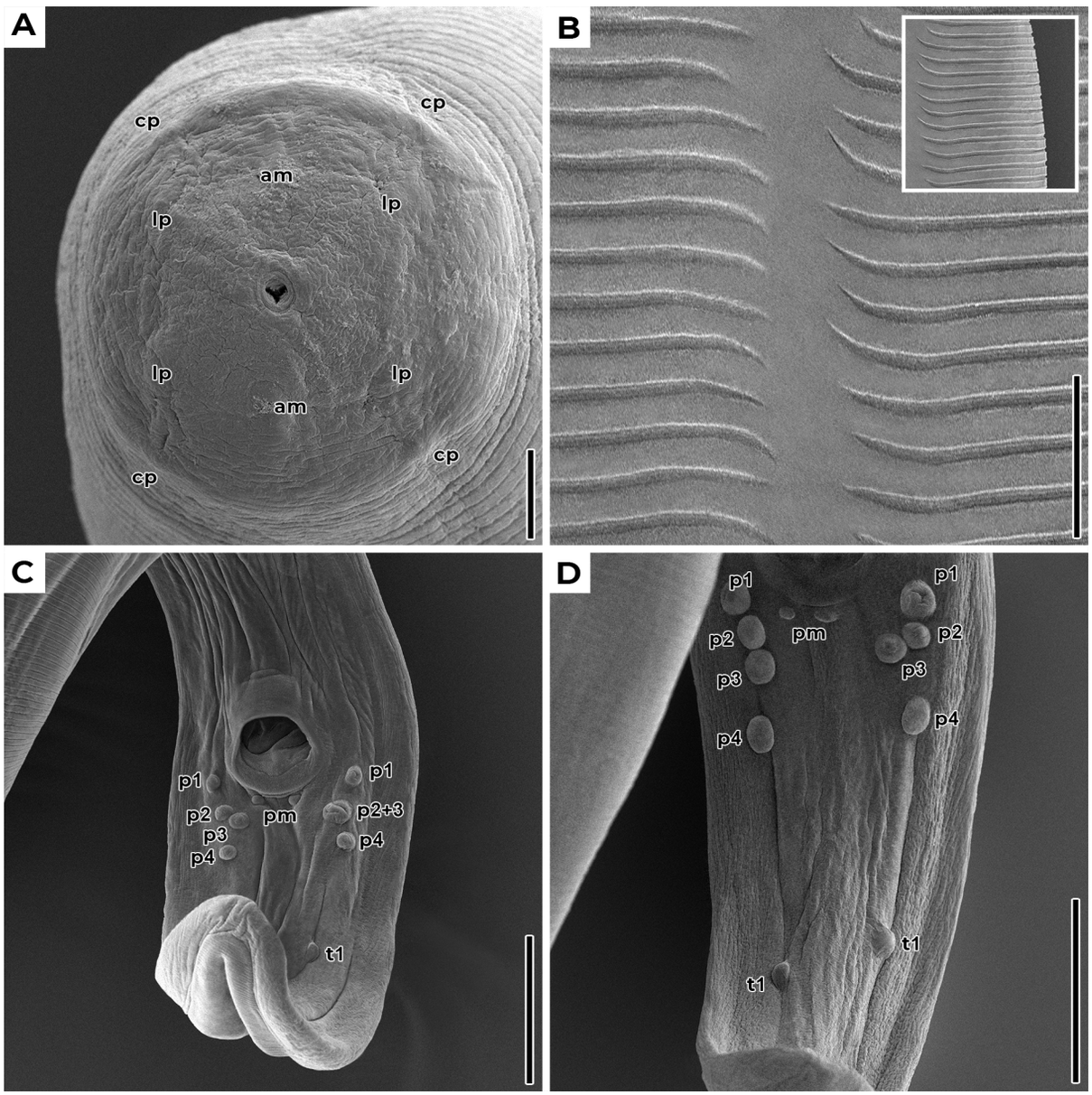


Fig. 3. Scanning electron microscopy of *Onchocerca flexuosa* recovered from subcutaneous nodules of the reindeer (Case 1): A — anterior end showing Y-shaped mouth opening, two amphids, two pairs of labial papillae, and two pairs of cephalic papillae; scale bar 10 µm; B — detail of cuticular ornamentation with the interruption of cuticular ridges in the lateral field, inset shows a broader view of the same cuticle region, including the lateral field and the body margin of the nematode; scale bar 25 µm; C — posterior end of the male nematode showing four pairs of the lateral postcloacal caudal papillae, one pair of the median postcloacal caudal papillae, and one terminal caudal papilla of the first pair; on the left side of the image, the lateral postcloacal papillae of pairs 1, 2 and 4 are aligned, whereas the papilla of pair 3 is positioned more medially; on the right side, the papillae of pairs 2 and 3 are fused; scale bar 50 µm; D — posterior end of the different male nematode also showing postcloacal and terminal caudal papillae, but differently arranged; on the left side of the image, the papillae of all four lateral postcloacal pairs are aligned, whereas on the right side the papilla of pair 3 is positioned more medially than the others; scale bar 50 µm. Abbreviations: am — amphid; cp — cervical papilla; lp — labial papilla; p1–4 — papillae of the first to fourth lateral postcloacal caudal pairs; pm — median postcloacal caudal papillae; t1 — papilla of the first terminal caudal pare.

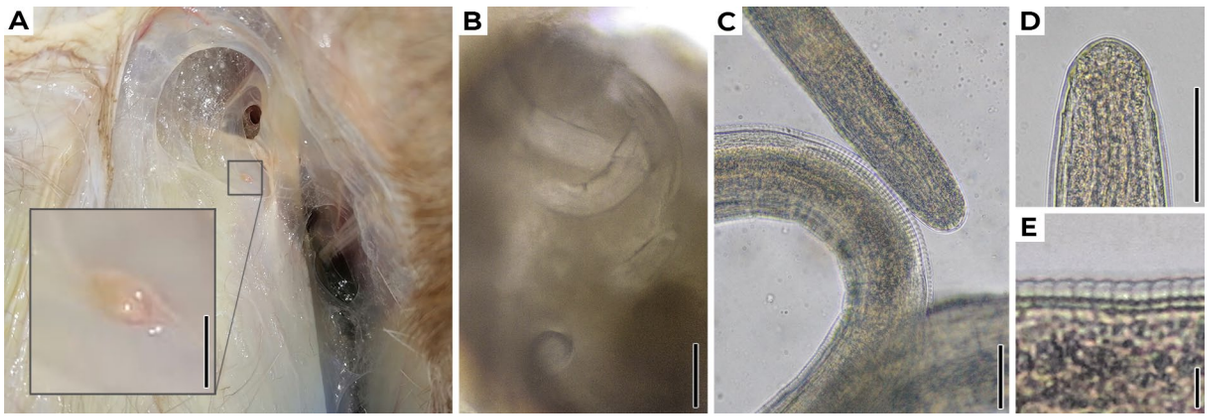


Fig. 4. *Onchocerca* nematodes in the reindeer subcutaneous nodule, macro and microscopy (Case 2): A — necropsy photograph of a reindeer showing a connective tissue nodule on the muscle fascia in the region of the hock joint of the pelvic limb, inset shows a magnified view of the nodule; scale bar 5000 µm; B — nematodes in situ embedded in subcutaneous tissue, no coverslip; 100×; scale bar 200 µm; C — fragments of nematodes obtained from the nodule, coverslip; 400×; scale bar 50 µm; D — anterior end of the nematode, coverslip; 400×; scale bar 50 µm; E — cuticle details of the nematode, coverslip; 100×; scale bar 10 µm. A — smartphone camera photo; B–E — bright-field microscopy, all the specimens depicted in tap water without staining.

absence of molecular data and the lack of morphological characteristics indicative of at least sexual maturity of the nematodes. At the same time, their firm embedding within host tissue suggests a prolonged presence in the host, sufficient to induce an inflammatory response and subsequent connective tissue encapsulation. Nevertheless, certain observations complicate interpretation. The size of the nodule (3 × 4 mm) is notably smaller than those typically described for *O. flexuosa* or *O. cervipedis* infections, where nodules usually reach approximately 2 cm (Wehr & Dikmans, 1935; Martinuš *et al*., 2026). In addition, the size of the recovered nematodes is inconsistent with this species. While their measurements are more compatible with *O. skrjabini*, some authors report that this species does not form well-defined nodules but rather occurs relatively freely within tissues (Bosch *et al*., 2016; Manzanell *et al*., 2022). Thus, the observed specimens do not fully correspond to any *Onchocerca* species described in reindeer. Co-housing with other ruminant species (sika deer and Bactrian camel) may suggest that the search for the detected *Onchocerca* species should also consider taxa known to parasitize these ungulates. Despite this uncertainty, given the new geographic context of the finding, we consider it important to report this case, even in the absence of definitive species-level identification.

Conclusion

This study describes two cases of *Onchocerca* infection in semiwild reindeer from Russia, originating from geographically distinct regions and differing management conditions. Importantly, this work represents the first molecular study of *Onchocerca* in reindeer in the Palearctic and provides the first molecular confirmation of *O. flexuosa* in reindeer.

The first case, involving a male reindeer from Karelia, provides robust evidence of *O. flexuosa* infection. Subcutaneous nodules located in the dorsal region were detected during necropsy and examined using an integrative approach. Morphological, histological, and molecular data were fully concordant and consistent with previous descriptions of *O. flexuosa* in cervids. These findings confirm a typical presentation of the species and expand its molecularly verified distribution in *R. tarandus*.

More broadly, this finding may also be viewed in the context of ongoing climate change, which is increasingly recognized as a driver of the emergence, northward expansion, and altered transmission dynamics of filarioid nematodes in Arctic and sub-Arctic regions. In this respect, the molecularly confirmed detection of *O. flexuosa* in reindeer from Karelia may reflect a wider and still insufficiently documented circulation of filarioids in northern cervids under changing environmental conditions (Laaksonen *et al*., 2010). Given the importance of reindeer as a key subsistence and food resource in the North, improved surveillance of these parasites is also relevant from the perspectives of animal health, food security, and, potentially, food safety.

The second case, recorded in a male reindeer originating from the Khanty-Mansi Autonomous Okrug and later maintained in a private menagerie in Samara Oblast, remains unresolved at the species level. A small nodule located near the hock joint contained nematodes attributable to *Onchocerca* spp.; however, delayed fixation approximately 24 hours post-mortem and suboptimal preservation limited both morphological resolution and molecular

analysis. Additionally, the small size of the nodule and discrepancies in parasite measurements complicate species identification. The presence of co-infections with rumen flukes and *E. rangiferi*, as well as housing in a mixed-species enclosure with other ungulates, further highlights the epidemiological complexity of this case.

Overall, these findings demonstrate both the value of integrative diagnostics and the limitations imposed by suboptimal sample preservation. While the first case provides a significant molecular benchmark for *O. flexuosa*, the second underscores the need for improved sampling protocols. Reporting such cases contributes to a better understanding of the diversity, distribution, and host associations of *Onchocerca* species in reindeer across the Palearctic.

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Appendix 1. A list of Onchocercidae records attributed to the eight cervid host species from the Palearctic and Nearctic regions. Information given includes initial or singular report for every host species in the region. Site and type of localization are cited verbatim from the source. The list is arranged alphabetically according to scientific name. Host species are given according to Wilson & Reeder (2005).

Onchocercidae species	Cervid host species	Site and type of localization	Biogeographic realm of helminth collection	Reference
Alcefilaria abramovi Oschmarin et Belous, 1951	moose *Alces alces* (Linnaeus, 1758) red deer *Cervus elaphus* Linnaeus, 1758 caribou *Rangifer tarandus* (Linnaeus, 1758)	venous vessels of the liver, mesentery	PALEARCTIC	Gubanov, 1964
Cercopithifilaria rugosicauda (Böhm et Supperer, 1953) Lefoulon et al., 2014	roe deer *Capreolus capreolus* (Linnaeus, 1758)	subcutaneous tissues	PALEARCTIC	Lefoulon *et al*., 2014
Mansonella wenki Uni, Bain et Takaoka, 2004	sika deer *Cervus nippon* Temminck, 1838	in the skin	PALEARCTIC	Uni *et al*., 2004
Mansonella wenki (Bain et Schulz-Key, 1974) Uni, Bain et Takaoka, 2004	red deer *Cervus elaphus* Linnaeus, 1758	intradermally	PALEARCTIC	Bain & Schulz-Key, 1974a
Mansonella wenki (Bain et Schulz-Key, 1974) Uni, Bain et Takaoka, 2004	fallow deer *Dama dama* (Linnaeus, 1758)	in the skin	PALEARCTIC	Uni *et al*., 2004
Onchocerca alcis Bain et Rehbinder, 1986	moose *Alces alces* (Linnaeus, 1758)	in the tendon insertions around the knee, primarily the distal insertions of the *M. artorius* and *M. semitendinosus* on the tibia	PALEARCTIC	Bain & Rehbinder, 1986

Onchocercidae species	Cervid host species	Site and type of localization	Biogeographic realm of helminth collection	Reference
Onchocerca cervipedis Wehr et Dikmans, 1935	black-tailed deer *Odocoileus hemionus* (Rafinesque, 1817) white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780)	foot (subcutaneous); hindquarters under skin, ankle joints to toe	NEARCTIC	Wehr & Dikmans, 1935
	moose *Alces alces* (Linnaeus, 1758) red deer *Cervus elaphus* Linnaeus, 1758 reindeer *Rangifer tarandus* (Linnaeus, 1758)	the knee and fetlock [probably, metatarsophalangeal] joints of legs	PALEARCTIC	Gubanov, 1964
	moose *Alces alces* (Linnaeus, 1758) caribou *Rangifer tarandus* (Linnaeus, 1758)	subcutaneous nodular lesions	NEARCTIC	Verocai *et al*., 2012
	sika deer *Cervus nippon* Temminck, 1838 fallow deer *Dama dama* (Linnaeus, 1758)	subcutaneous tissue in the area of the joints of the extremities	PALEARCTIC	reviewed in Pryadko, 1976
Onchocerca eberhardi Uni et Bain, 2007	sika deer *Cervus nippon* Temminck, 1838	deep inside the carpal ligaments or tarsal joints	PALEARCTIC	Uni *et. al.*, 2007
Onchocerca flexuosa Wedl, 1856	reindeer *Rangifer tarandus* (Linnaeus, 1758)	beneath the skin in the dorsal region, occurring within dense nodules ranging in size from a large bean to a hazelnut	PALEARCTIC	Kadenatsii, 1963
	red deer *Cervus elaphus* Linnaeus, 1758 sika deer *Cervus nippon* Temminck, 1838 fallow deer *Dama dama* (Linnaeus, 1758)	in subcutaneous tissue	PALEARCTIC	reviewed in Sonin,1975
Onchocerca garmsi Schulz-Key et Bain, 1976	red deer *Cervus elaphus* Linnaeus, 1758	freely in the subcutaneous tissues on the chest	PALEARCTIC	Schulz-Key *et al.*, 1976
Onchocerca jakutensis Gubanov, 1964 (syn. *O. tubingensis* Bain et Schulz-Key, 1974)	red deer *Cervus elaphus* Linnaeus, 1758	in neoplasms (balls) of subcutaneous tissue	PALEARCTIC	Gubanov, 1964

Onchocercidae species	Cervid host species	Site and type of localization	Biogeographic realm of helminth collection	Reference
Onchocerca skrjabini Ruklyadev, 1961 (syn. *O. tarsicola* Bain et Schulz-Key, 1974)	red deer *Cervus elaphus* Linnaeus, 1758	in the subcutaneous tissue and ligaments of the hock and carpal joints	PALEARCTIC	Rukhlyadev, 1964
	reindeer *Rangifer tarandus* (Linnaeus, 1758)	in the ligaments and tendons of the legs, in small ventral subcutaneous nodules, and under the peritoneum (subperitoneal nodules)	PALEARCTIC	Bain *et al*., 1979
Rumenfilaria andersoni Murray, Lankester et Snider, 1982	moose *Alces alces* (Linnaeus, 1758)	in vessels in subserosal connective tissue of the rumen	NEARCTIC	Murray, Lankester & Snider, 1982
Rumenfilaria andersoni Murray, Lankester et Snider, 1982	roe deer *Capreolus capreolus* (Linnaeus, 1758) white-tailed deer *Odocoileus virginianus* (Zimmermann, 1780) reindeer *Rangifer tarandus* (Linnaeus, 1758)	lymphatic vessels adjacent to the rumen	PALEARCTIC	Laaksonen, Oksanen & Hoberg, 2015

Appendix 2. A list of obsolete but still encountered synonyms for Onchocercidae in cervids.

Actual scientific name	Synonym	Reference
Cercopithifilaria rugosicauda	*Dipetalonema rugosicauda*	Mészáros & Sugár, 1976
	Wehrdikmansia rugosicauda	Böhm & Supperer, 1953 Sonin, 1975
Mansonella wenki	*Cutifilaria wenki*	Bain & Schulz-Key, 1974a Laaksonen *et al*., 2017
Onchocerca cervipedis	*Acanthospiculum cervipedis*	Skrjabin & Shikhobalova, 1948 Gubanov, 1964 Mitskewitch, 1967
Onchocerca flexuosa	*Acanthospiculum flexuosa*	Skrjabin & Shikhobalova, 1948 Gubanov, 1964 Mitskewitch, 1967
	Wehrdikmansia flexuosa	Sonin,1975
Onchocerca jakutensis	*Acanthospiculum jakutensis*	Gubanov, 1964
	Wehrdikmansia jakutensis	Sonin, 1975