

First record of *Diplotrriaena obtusa* (Rudolphi, 1802) Henry & Ozoux, 1909 from the Eurasian nuthatch (*Sitta europaea* Linnaeus, 1758)

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Summary

The nematode *Diplotrriaena obtusa* (Rudolphi, 1802) Henry & Ozoux, 1909 was found in a nuthatch (*Sitta europaea* Linnaeus, 1758) from Wangen im Allgäu, Baden-Württemberg, Germany, and was examined using light microscopy and DNA analysis, and compared with GenBank sequences. The sequence obtained herein showed 99.75 % identity with other isolates of *D. obtusa*. This is the first detection of this nematode species in this bird species, expanding the known host range in Europe. An overview of the known host records for *D. obtusa* is given.

Keywords: Eurasian nuthatch; *Diplotrriaena obtusa*; molecular analysis

Introduction

The nematode phylum is a highly diverse group of animals. Estimates range from 100.000 to 100 million species (Bouamer *et al.*, 2006; Hammond, 1992; Hugot *et al.*, 2001; Lambhead, 1993). Many species are free-living; others live as parasites in plants, arthropods and vertebrates, including humans (Anderson, 2000; Yamaguti, 1961; Yeates *et al.*, 2009). Among parasitic nematodes, a wide range of species are found in birds as final hosts (Cram, 1927; Rebelo *et al.*, 2024; Leung & Koprivnikar, 2016; Malik *et al.*, 2022; Movsesyan *et al.*, 2020; Okulewicz, 2013; Saparov *et al.*, 2013; Santoro *et al.*, 2016; Zhang *et al.*, 2012). There, they can cause air sac inflammation, pneumonia, bronchopneumonia, and other diseases. Symptoms include deterioration in general condition, lethargy, and reduced flight performance (Abdu *et al.*, 2023; Freeman *et al.*, 2025; Leung & Koprivnikar, 2016; Sterner & Cole, 2008).

Here we report the first record of the nematode *Diplotrriaena obtusa* in a Eurasian nuthatch (*Sitta europaea*) from southwestern Germany.

Material and Methods

Sampling and necropsy of the bird

The nuthatch was caught in a net on 5 September 2024 in Wangen im Allgäu, Baden-Württemberg, Germany (47°41'N, 9°48'E; Fig. 1) and ringed with the ring number Hiddensee C4E9091 (Fig. 1).

The bird was recognized as a juvenile male and weighed 23.2 g. It was noticed that the bird was rattling. Two days later, the nuthatch was found dead nearby and then dissected to determine the cause of death and confirm its age and sex. There were no signs of injury, for example, from being hit by a windscreen or a predator, but two nematodes were found in the area of the esophagus.

Altogether, three nematodes were fixed in ethanol (70 %) and sent to the first author as an incidental finding as part of a research program funded by members of the Saxon State Parliament to record louse flies (Freick *et al.*, 2025). The morphological and molecular examinations and species determination were conducted at the Institute of Parasitology, University of Leipzig.

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Fig. 1. Collection locality (Wangen im Allgäu, Baden-Württemberg, Germany, 47°41' N, 9°48' E, red dot).

Morphological identification of parasites

Two fixed specimens were examined via light microscopy using a Leica DMLB clinical microscope. For clearing, one nematode was placed in lactophenol. Unfortunately, specific morphologic features of the helminth were unrecognizable. Therefore, a single female nematode was dissected, and eggs were obtained for morphological characterization.

DNA extraction, amplification and sequencing

One nematode was used for molecular analysis, and DNA was extracted using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Afterward, a PCR was conducted to amplify an approximately 900 bp fragment of the 18S small subunit rRNA gene using the primer pair Nem_18SF (5'-cgcgaaatrgctcattacaacagc-3') and Nem_18SR (5'-gggcggatctgatcgcc-3') according to Floyd *et al.* (2005). Each reaction mixture contained: 2.5 µl 10X DreamTaq Buffer (Thermo Scientific™), 0.8 µl dNTP-Mix (0.2 µM of each deoxynucleoside triphosphate; Thermo Scientific™), 0.5 µl of Primer each (25 µM), 0.1 µl DreamTaq Green DNA Polymerase (Thermo Scientific™), 3 µl of the DNA sample and DEPC water to a total volume of 25 µl. The initial denaturation at 94°C for 5 min was followed by

35 cycles at 94°C (30s), 54°C (30s), and 72°C (1 min). A positive and a negative control were included to confirm the PCR result. Final extension was performed at 72°C for 7 min. After gel electrophoresis, the PCR products were visualized on a 1.5 % agarose gel. The purified PCR product was bidirectionally sequenced by a commercial service (Microsynth Seqlab, Göttingen, Germany).

Data analysis

The consensus sequence was compared with sequences in GenBank using the Basic Local Alignment Search Tool (Altschul *et al.*, 1990), and exemplary sequences (E-value < 0.0; percent identity > 96 %) were used for phylogenetic comparison. All sequences (inclusive references) used for this analysis are given as supplementary data (Supplementary data 1). A *Toxocara cati* sequence (EF180059) was used as the outgroup. Evolutionary analysis was performed twice on the Phylogeny. fr platform (Dereeper *et al.*, 2010; Dereeper *et al.*, 2008), with MUSCLE (v3.8.31) for alignment and Gblocks (v0.91b) for refinement (Edgar, 2004; Castresana, 2000). Subsequently, the maximum-likelihood method (100 bootstrap replicates) implemented in PhyML (v3.1/3.0 aLRT) was used, and the default substitution model was selected to construct the phylogenetic tree, which was edited in TreeDyn (v198.3) (Anisimov & Gascuel, 2006; Chevenet *et al.*, 2006; Guindon & Gascuel, 2003). Two analyses were conducted, one with a final length of 791 bp (Fig. 3A) and another with a length of 492 bp (Fig. 3B), including exemplary sequences of *D. bargusina* and *D. anthrepitis*.

Ethical Approval and/or Informed Consent

For this study, formal consent was not required.



Fig. 2. Spirurid eggs removed from a female specimen. Shells were thick and smooth with a larval stage inside.

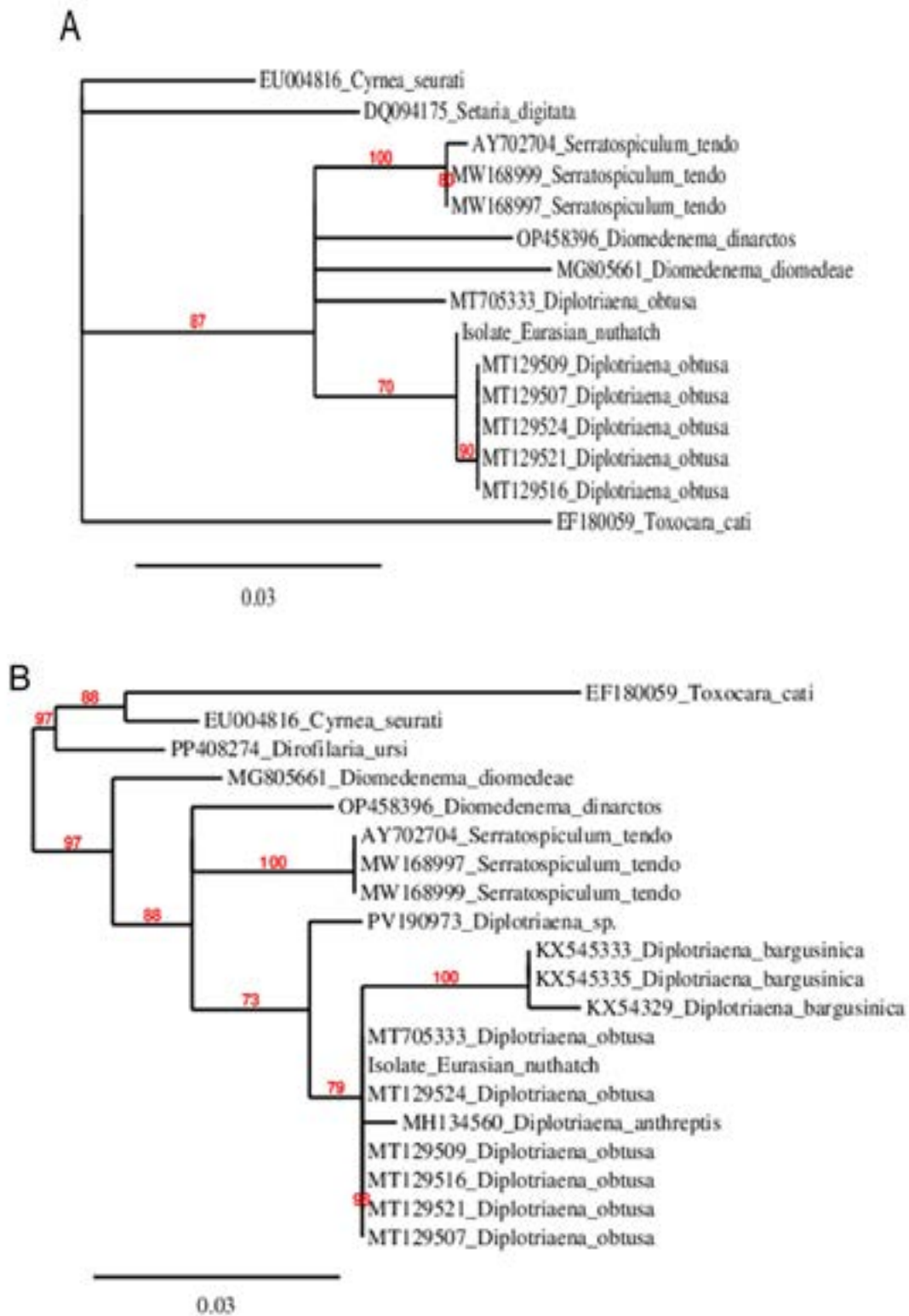


Fig. 3. Phylogenetic analysis of herein obtained sequence for 791 bp (A) and 492bp (B) with those available from GenBank (e.g., MT129516, MT129509) using the maximum likelihood method. The sequence of *T. cati* (EF180059) was used as an outgroup. Only branches with support values greater than 70% are shown (bootstrapping method; 100 bootstrap replicates).

Results

Since no specific morphological features were recognizable via light microscopy in the adult nematodes, as might be expected in the case of *Diplotrriaena* spp. Eggs, such as those with size, specific trident structures at the anterior end, and lateral pores at the oral opening (Freeman *et al.*, 2025; Rentería-Solís *et al.*, 2021,

Anderson *et al.*, 2009), were removed from a female specimen for morphological characterization. These nematodes' eggs were identified as Spirurid eggs according to Schmäscke (2025) and Beck & Pantchev (2013), with a thick, smooth wall and a larval stage inside (Fig. 2). Measurements averaged 46 µm x 30 µm. Phylogenetic analysis of the herein obtained sequence (Isolate_Eurasian_nuthatch) for 791 bp (Fig. 3A) and 492bp (Fig. 3B)

Table 1. Bird species with records of *Diplotrriaena obtusa* (names of subspecies only if given in the cited references).

Family	Species	Location	References
Apodidae Hartert, 1897	<i>Apus apus</i> (Linnaeus, 1758)	Czech Republic	Sitko & Heneberg (2023),
Hirundinidae Rafinesque, 1815	<i>Delichon urbicum</i> (Linnaeus, 1758)	No information	Schreibers (1811)
		Poland	Jaroń (1969)
		Czech Republic	Sitko & Heneberg (2023), Sitko & Okulewicz (2010)
		No information	Leidy (1890)
	<i>Hirundo rustica</i> Linnaeus, 1758	Poland	Jaroń (1969)
		Slovenia	Brglez (1982)
		Slovakia	Birová (1990)
		Czech Republic	Sitko & Heneberg (2023), Sitko & Okulewicz (2010)
		Upper Midwest, United States	Michalski <i>et al.</i> (2021)
		No information	Rudolphi (1802)
	<i>Hirundo rustica gutturalis</i> Scopoli, 1786	Yunnan, China	Wu (1973)
	<i>Hirundo rustica savignii</i> Stephens, 1817	Egypt	Ahmed (1997)
	<i>Petrochelidon pyrrhonota</i> (Vieillot, 1817)	Upper Midwest, United States	Michalski <i>et al.</i> (2021)
	<i>Progne chalybea</i> (Gmelin, JF, 1789)	No information	Diesing (1851)
	<i>Progne subis</i> (Linnaeus, 1758)	No information	Diesing (1851)
		Ontario, Canada	Webster (1974)
	<i>Riparia riparia</i> (Linnaeus, 1758)	No information	Schreibers (1811)
		Czech Republic	Sitko & Heneberg (2023)
	<i>Tachycineta bicolor</i> (Vieillot, 1808)	Colorado, United States	Sherwin & Schmidt (1988)
Muscicapidae Fleming, 1822	<i>Ficedula albicollis</i> (Temminck, 1815)	Czech Republic	Sitko & Heneberg (2023),
Paridae Vigors, 1825	<i>Cyanistes caeruleus</i> (Linnaeus, 1758)	Germany	Rentería-Solís <i>et al.</i> (2021)
	<i>Parus major</i> Linnaeus, 1758	Russia	Sonin (1958)
		Poland	Rząd <i>et al.</i> (2023)
Parulidae Wetmore <i>et al.</i> , 1947	<i>Setophaga occidentalis</i> (Townsend, 1837)	Wyoming, United States	Orrell T, Informatics and Data Science Center - Digital Stewardship (2025)
Ploceidae Sundevall, 1836	<i>Foudia madagascariensis</i> (Linnaeus, 1766)	Madagascar	Henry & Ozoux (1909)
Sittidae Lesson, 1828	<i>Sitta europaea</i> Linnaeus, 1758	Germany	This paper
Sturnidae Rafinesque, 1815	<i>Sturnus vulgaris</i> Linnaeus, 1758	Armenia	Akhumian (1966), Movsesyan <i>et al.</i> (1985)
Sylviidae Leach, 1820	<i>Sylvia atricapilla</i> (Linnaeus, 1758)	Poland	Stanicka <i>et al.</i> (2021)

with those available from GenBank (e.g., MT129516, MT129509) employed the maximum likelihood method. The *T. cati* sequence (EF180059) was used as the outgroup. Only branches with support values greater than 70 % are shown (bootstrapping method; 100 bootstrap replicates). The sequence obtained was submitted to GenBank (accession number PV942266, 861 bp) and showed the highest percent identity (99.75 %) with isolates of *Diplotriae-na obtusa* (e.g., accession number MT129516, query cover 95 %; accession number MT129509, query cover 94 %). Evolutionary analysis showed that the herein obtained sequence clustered together with other *D. obtusa* sequences (Fig. 3A). The specimen branched between a specimen of *D. obtusa* found in a blue tit from Germany (accession number: MT705333) (Fig. 3A) and the other specimens of *D. obtusa* (accession numbers: MT129516, MT129509, MT129524, MT129521, MT129507) which showed very high percent identities (99.75 %) and were detected in barn swallows and cliff swallows from the USA hinting on some potential genetic variations due to geographical conditions, which should be confirmed with higher-resolution markers and broader sampling. Including sequences from other *Diplotriae-na* species in the phylogenetic analysis further supported the consensus between the isolate herein found and other isolates of *D. obtusa* (Fig. 3B).

Discussion

Among the nematodes affecting birds, particularly passerines, there were approximately 77 described species of the genus *Diplotriae-na* sp. (Hong *et al.*, 2019), to which *D. obtusa* found in the nuthatch was attributed. *D. obtusa* was recorded in different passerines of the northern hemisphere and from Madagascar, whereby, according to the frequency of publication, swallows (Hirundinidae Rafinesque, 1815) were particularly frequently observed as hosts (Tab. 1). The assignment of *D. obtusa* to the hosts *Junco hyemalis* (Linnaeus, 1758) and *Quiscalus quiscula* (Linnaeus, 1758) by Leidy (1885, 1886) and Walton (1927) was incorrect and based on a misidentification of the parasites (Anderson, 1959).

Until now, there has been no scientific evidence that *D. obtusa* has been found in the European nuthatch, of which only the species *Acuaria subula* (Dujardin, 1845) and *D. henryi* (Blanc, 1919) have been known as helminth parasites (Sitko & Okulewicz, 2010; Okulewicz, 2013).

The only available study of *D. obtusa* from Germany identified the blue tit as the host (Rentería-Solís *et al.*, 2021). A report of *D. obtusa* parasitizing the great tit was from Poland (Rząd *et al.*, 2023). European nuthatches live in loose associations with tits during autumn and winter, and also in similar breeding habitats (Blotzheim, 1993). It is therefore possible that the birds were infected with nematodes in habitats similar to those in which they were found.

Parasitic helminths of the genus *Diplotriae-na* sp. can cause significant pathology in their hosts (Abdu *et al.*, 2023; Michalski *et al.*, 2021; Stanicka *et al.*, 2021). Many are (Leung & Koprivnikar,

2016). Sitko & Heneberg (2023) showed that the prevalence, and thus the infection intensity, of this nematode in Europe has increased in recent years.

The trivial name “air sac worm” refers to the leading site of infection, which can also affect the body cavity (Sonin, 1975). In our case, the nematodes were found in the tracheal region, but they likely originated from the neighboring air sacs. The dissected nuthatch showed no external injuries. However, a rattle was throughout its life, likely due to the nematode infection. This symptom may be associated with the dead bird, given the nuthatch’s ringing. Although *Diplotriae-na* infections are usually subclinical, a high parasite load can lead to morbidity and mortality (Freeman *et al.*, 2025; Stanicka *et al.*, 2021). Whether the nematode infection itself was the cause of death of the bird cannot be said with certainty, as no histological or pathological evidence was available. However, it is at least possible that it contributed to the bird’s death.

A limitation of this study is the small number of specimens, or, rather, the limited potential for morphometric analysis. However, this matter was mitigated by the clear identification of the parasites’ eggs and subsequent molecular analysis to identify the species.

D. obtusa was found for the first time in a European nuthatch. This finding broadens the spectrum of potential hosts for this nematode species and, consequently, its distribution, as this bird species is (Blotzheim, 1993). The larvae are transmitted when they are ingested by their definitive hosts (Anderson, 2000). This occurs via invertebrates as intermediate hosts (Abdu *et al.*, 2023; Rentería-Solís *et al.*, 2021; Sitko & Heneberg, 2023), which are part of the European nuthatch’s and many other passerines’ diet (Brehm, 1867) as part of the natural chain of infection.

Conflict of Interest

Authors state no conflict of interest.

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