



The first case of genetically confirmed sparganosis (*Spirometra erinaceieuropaei*) in European reptiles

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Abstract

Sparganosis is a zoonosis caused by the spargana (larvae) of *Spirometra* sp. (Diphyllbothriidae). Reptiles are particularly important vectors for the transmission of this parasite in Asia; however, their role in sparganosis spread in European wildlife is unrecognized. We investigated the infection of reptiles with *Spirometra* sp. in NE Poland, where several mammalian hosts have been identified recently and in the past. Of the 59 dead reptiles, plerocercoids were found in two grass snakes (*Natrix natrix*) from the Białowieża Primeval Forest (BPF). The *Spirometra erinaceieuropaei* species was genetically confirmed using the evolutionary conserved nuclear 18S rRNA gene, and then compared to GenBank deposits. The sequences were identical to previously investigated *Spirometra* sp. found in Eurasian badger and wild boar from BPF. Our finding is the first genetically confirmed record of *Spirometra* sp. in reptiles in Europe. Since reptiles are often a component of mammalian diet, they can be a source of *Spirometra* tapeworm infection in European wildlife; however, further studies are needed to investigate the prevalence of infection in reptiles and other non-mammalian hosts.

Keywords *Natrix natrix* · Sparganum · Plerocercoid · Non-mammalian host · Zoonosis · DNA analyses

Introduction

Sparganosis is a food- and water-borne disease still little-known in Europe. Most research regarding it has been conducted in Asia, where sparganosis is a serious danger for public health (Wang et al. 2011, 2014; Hong et al. 2016). It is caused by spargana (plerocercoids)—the second larval stadium of the tapeworm *Spirometra* sp. (Diphyllbothriidae); however, knowledge of the *Spirometra* sp. life cycle is still limited. Adult *Spirometra* sp. reproduces mainly in the intestines of felids and canids such as the Eurasian lynx (*Lynx lynx*)

and wolf (*Canis lupus*) (Furmaga 1953; Kołodziej-Sobocińska et al. 2018). Eggs are shed with animal feces. The parasite's first intermediate hosts are copepods (*Cyclops* sp.)—planktonic crustaceans in which proceroids (the first larval stadium) develop. The second intermediate or paratenic hosts can be vertebrates, such as amphibians, reptiles, birds, or mammals (including human) (Wongkulab et al. 2011; Hong et al. 2016). Proceroids develop into plerocercoids which settle in organs and tissues of intermediate hosts (Bearup 1953). The life cycle of *Spirometra* sp. may also include paratenic hosts, in which spargana once more settle in the tissues after passing through the intestinal wall (Wongkulab et al. 2011). These hosts, however, are not necessary for the cycle's completion.

Two species of *Spirometra* were found in Europe: *S. erinaceieuropaei*, found in several studies in Europe and Asia (Wang et al. 2011; Kołodziej-Sobocińska and Miniuk 2018), and *S. janickii*, described for the first time in the Białowieża Primeval Forest (BPF) (north-eastern Poland) in mammalian hosts such as the Eurasian lynx, wolf, red fox (*Vulpes vulpes*), and common shrew (*Sorex*

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Fig. 1 Plerocercoid larvae of *Spirometra* sp. isolated from grass snake (*Natrix natrix*) subcutaneous tissue (photo: M. Kołodziej-Sobocińska)

araneus) in the 1950s (Furmaga 1953). The second species has never been confirmed in other studies; thus, its taxonomic status remains problematic. In recent years, tapeworms have been found in other hosts in BPF, including the wild boar (*Sus scrofa*) and Eurasian badger (*Meles meles*) (Kołodziej-Sobocińska et al. 2014, 2016). Genetic analysis and comparison to Gene Bank sequences indicate systematic status of the species in BPF as *S. erinacei* (Liu et al. 1997).

Intermediate hosts for *Spirometra* sp., such as amphibians and reptiles, are already considered important vectors for parasite transmission in Asia (Wang et al. 2011, 2014; Nelli et al. 2014; Hong et al. 2016). In Europe, the only three cases of infected reptiles, in a common European viper (*Vipera berus*) and a grass snake (*Natrix natrix*), have been recorded in Italy, Germany, and Belarus (Joyeux and Baer 1927; Odening et al. 1980; Shimalov et al. 2000).

The aim of our study was the survey of reptiles as hosts of *Spirometra* sp. and genetic confirmation of the species identification. Current knowledge indicates that reptiles in particular have never been reported as a host of *Spirometra* sp. in Poland.

Materials and methods

A total of 59 dead reptiles from Białowieża Primeval Forest (23 ind.) and Biebrza National Park (BNP) (36 ind.) were found and necropsied. These included 53 grass snakes, two sand lizards (*Lacerta agilis*), two common European vipers, one viviparous lizard (*Zootoca vivipara*), and one slowworm (*Anguis fragilis*). Reptiles were sexed, measured, and checked for *Spirometra* sp.

presence. The skin of each individual was slit from the neck to the end of the tail along the spine. Visceral mass was checked for the presence of *Spirometra* sp. plerocercoids.

To identify a species of isolated larvae we used primers described by Liu et al. (1997). An over 240 bp length sequence of evolutionary conserved nuclear 18S rRNA gene was used for this test. We compared obtained sequences with the ones acquired from three wild boars and three badgers from BPF (Kołodziej-Sobocińska et al. 2014, 2016), as well as from GenBank deposits, using the ClustalW Multiple alignment test. All molecular analyses were performed online using Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results and discussion

During the necropsies, we found spargana of *Spirometra* sp. in two grass snakes from BPF (Fig. 1). Both snakes were adults—one a 32-cm-long female found in the forest and the other a 64-cm-long male found in Białowieża village—and were infected by one and three larvae, respectively. In both individuals, spargana were located subcutaneously near the cloaca. The average length of a sparganum was 50 mm (range 5–120 mm).

Due to the lack of specific plerocercoid morphological features, parasite identification was performed using molecular methods. The analyzed 18S rRNA gene fragment of larvae isolated from snake tissue showed 99% identity with the GenBank-deposited *Spirometra erinacei* (KX528100.1) and 100% identity with *Spirometra erinacei* (D64072.1) from both GenBank and previously investigated sequences of plerocercoids in badgers and wild boars from BPF (Kołodziej-Sobocińska et al. 2014, 2016) (Fig. 2a, b).

Our finding is the first genetically confirmed record of *Spirometra* sp. in reptiles in Europe, and the first infected reptiles in Poland. In Asia, where this tapeworm occurs frequently, many species of snakes act as second intermediate hosts (Wang et al. 2011, 2014; Nelli et al. 2014). Reptiles infected with spargana were also recorded in South America (Oda et al. 2016), Australia (Zhu et al. 2002), and Africa (Pantchev and Tappe 2011). Morphological identification of *Spirometra* sp. plerocercoids is very unreliable. Thus, molecular methods are the most recommended confirmation of their systematic classification (Wongkulab et al. 2011; Kołodziej-Sobocińska et al. 2014, 2016).

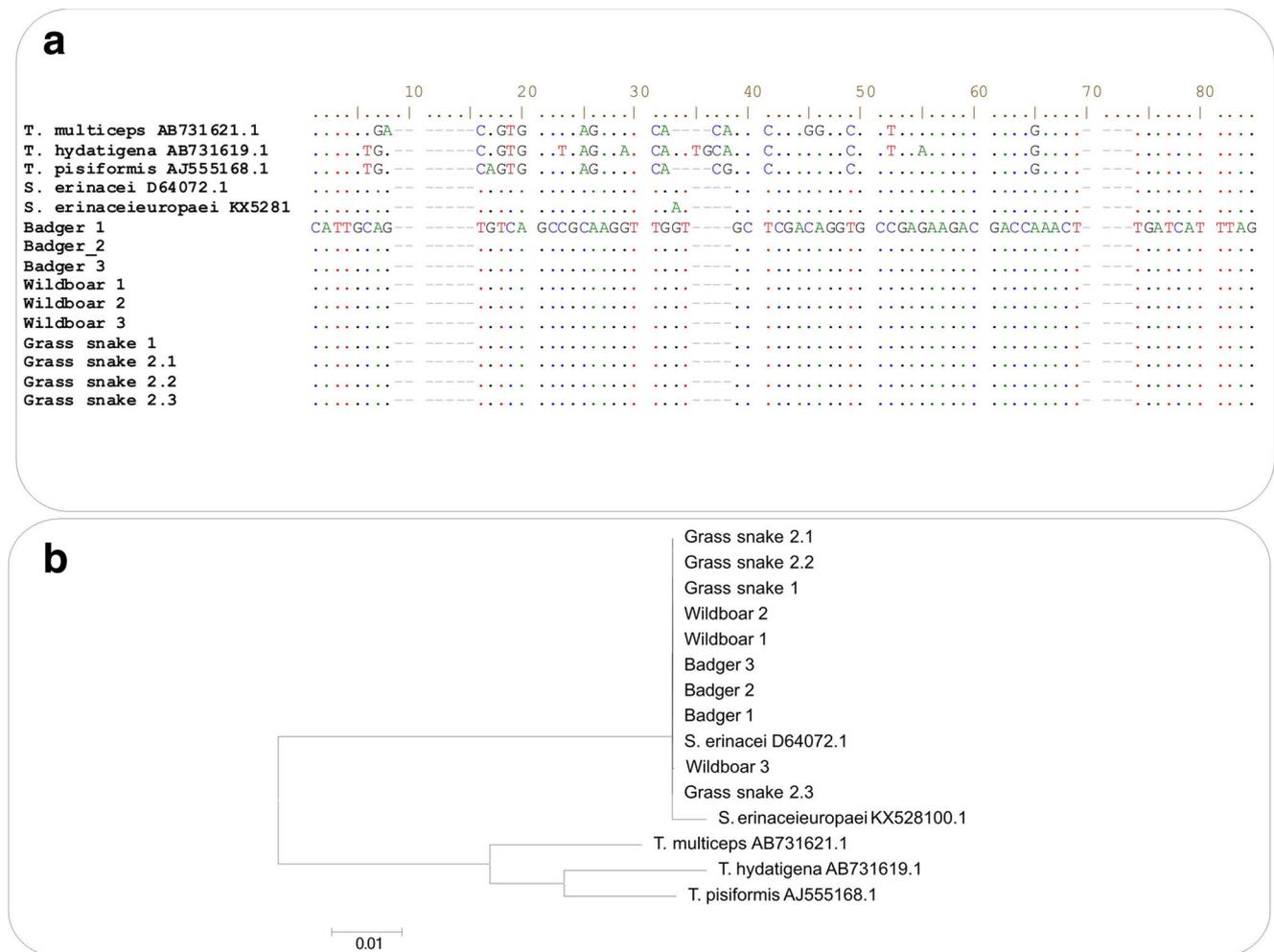


Fig. 2 Results of molecular analysis. **a** Partial alignment of 240 bp fragments of 18S rRNA gene fragments extracted from *Spirometra* sp. plerocercoids found in grass snakes, badgers, and wild boars, as well as other Cestoda species from NCBI deposits, along with their access IDHere. The reference sequence is *Spirometra* sp. described by Kołodziej-Sobocińska et al. (2014). Dotted nucleotides are identical to

those of a reference. **b** Molecular phylogenetic analysis of the studied sequences from Fig. 2a, by the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013)

Grass snakes are strongly associated with wetlands, where they can most likely become infected with procerocoids by swallowing water copepods or consuming amphibians (Luiselli et al. 1997). Moreover, they can be preyed upon by several species of mammals and birds that are known to occasionally feed on reptiles (Jędrzejewska and Jędrzejewski 1998), transferring spargana to subsequent trophic levels. This report also confirms the role of reptiles as parasite transmitters outside Asia and reveals additional routes of sparganosis transmission in European wildlife. Further studies are recommended to provide a deeper explanation of the role

of non-mammalian hosts in the spread of *Spirometra* sp. in the natural environment.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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