Journal of Helminthology

cambridge.org/jhl

Prevalence and molecular characterization of *Spirometra erinaceieuropaei* spargana in snakes in Hunan Province, China

W. Liu[1,2,3], L. Tan[1,2], Y. Huang[3], W.C. Li[1], Y.S. Liu[1,2] and L.C. Yang[1,2]

[1]Hunan Provincial Key Laboratory of Protein Engineering in Animal Vaccines, College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan 410128, China; [2]Hunan Co-Innovation Center of Animal Production Safety, Changsha, China and [3]College of Veterinary Medicine, South China Agricultural University, Guangzhou, China

Short Communication

Cite this article: Liu W, Tan L, Huang Y, Li WC, Liu YS, Yang LC (2020). Prevalence and molecular characterization of *Spirometra erinaceieuropaei* spargana in snakes in Hunan Province, China. *Journal of Helminthology* **94**, e131, 1–7. https://doi.org/10.1017/S0022149X20000139

Received: 1 January 2020
Accepted: 4 February 2020

Key words:
Hunan province; pleroceroid; *Spirometra einaceieuropaei*; mitochondrial gene; phylogenetic analysis

Author for correspondence:
L. Yang,
E-mail: lcyang@hunau.edu.cn

Abstract

Sparganosis is an important foodborne parasitic zoonosis; however, few reports on the prevalence of snake-infecting plerocercoids from Hunan province in China are available. Therefore, we investigated the prevalence of spargana infection in wild snakes from this region in 2018, and identified an astonishing prevalence rate of 91.7% (344/375). Spargana parasites were found in 99.1% of *Zaocys dhumnades*, 94.1% of *Elaphe carinata* and 86.7% of *Elaphe taeniura*. Parasites exhibited various distributions: 50% were located in muscular tissue, 32.1% in subcutaneous tissue and 17.9% in the coelomic cavity. To identify the specific status of spargana collected from wild snakes, partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene sequences were amplified, sequenced and analysed. Sequence variations for *cox*1 among all the examined plerocercoids ranged between 0.0 and 2.9%, with 21 variable sites identified (4.71%, 21/446). Phylogenetic analyses identified that all plerocercoids isolated from Hunan province were *Spirometra erinaceieuropaei*. This is the first report of *S. erinaceieuropaei* infection in snakes in Hunan province. The risks and harms of sparganosis should be publicized, and illegal wildlife trade should be controlled.

Introduction

Sparganosis, an important foodborne parasitic zoonosis, is caused by the plerocercoid larvae of genus *Spirometra*. Humans become infected with spargana mainly by eating raw or inadequately cooked frog and snake meat, or by using raw frog and snake meat as poultice on open wounds (Li *et al.*, 2011). Human sparganosis has been reported in many countries, but most of the cases occur in eastern and south-eastern Asian countries (Kim *et al.*, 2018). Infection with spargana can cause serious illnesses, including blindness, paralysis and even death (Liu *et al.*, 2015).

Thus far, more than 1350 cases of human sparganosis have been reported in China, and most of them were documented in Guangdong and Hunan provinces (Lu *et al.*, 2014). Before 2010, only approximately 40 cases have been reported in Hunan province. However, more than 100 cases have emerged since 2010 in Hunan province, which may be correlated with the local customs. In recent years, eating frog and snake meat has become increasingly popular as they are both delicious and nutritious. Furthermore, swallowing raw snake gall bladder was very common as some people believe they possess therapeutic effects for many diseases (Su & Li, 2004). Such customs in this province may facilitate human infection with spargana (Wang *et al.*, 2011; Tan *et al.*, 2015; Yang *et al.*, 2015). The second intermediate hosts of *Spirometra* spp. are mainly frogs and snakes, which play an important epidemiological role (Mo *et al.*, 2013; Liu *et al.*, 2015). In our previous work, we showed a high prevalence (20.2%) of spargana in wild frogs from Hunan province (Liu *et al.*, 2010). To evaluate the risks of human infected with sparganosis in Hunan province and to strengthen public safety awareness, we investigated the prevalence of spargana infection in wild snakes in Hunan province.

For all these reasons, the objective of the target work was to determine the risk of plerocercoid infection in snakes from Hunan province. Sequence analysis of a portion of the cytochrome *c* oxidase subunit 1 (*cox*1) gene was performed to identify the specific identity of collected plerocercoids. Based on *cox*1 sequences, phylogenetic relationships among *Diphyllobothrium* tapeworms were also reconstructed.

Materials and methods

Sample collection

Wild snakes were obtained from food markets in 14 administrative regions covering the whole Hunan province between April and September 2018 (table 1). Snakes were sacrificed after

CAMBRIDGE UNIVERSITY PRESS

Table 1. Prevalence of spargana in snakes in Hunan province, China.

Variable	No. positive/no. tested	Prevalence, % (95% CI)	Intensity of infection	*P*-value	Odds ratio (95% CI)
Region					
Changde	18/20	90 (76.852–100.000)	5–25	0.1274	4.846 (0.863–27.220)
Changsha	50/60	83.3 (73.903–92.763)	3–60	0.1143	2.692 (0.859–8.439)
Chenzhou	18/20	90 (76.852–100.000)	7–20	0.1274	4.846 (0.863–27.220)
Huaihua	18/20	90 (76.852–100.000)	6–35	0.1274	4.846 (0.863–27.220)
Hengyang	13/20	65 (44.096–85.904)	4–15	reference	Reference
Loudi	20/20	100 (100.000–100.000)	8–49	0.0083	N
Shaoyang	20/20	100 (100.000–100.000)	10–60	0.0083	N
Xiangtan	58/60	96.7 (92.125–100.000)	5–70	$6.25e^{-4}$	15.620 (2.903–84.010)
Xiangxi	19/20	95 (85.448–100.000)	10–43	0.0436	10.230 (1.121–93.350)
Yiyang	35/35	100 (100.000–100.000)	5–40	$3.82e^{-4}$	N
Yongzhou	18/20	90 (76.852–100.000)	1–18	0.1274	4.846 (0.863–27.220)
Yueyang	17/20	85 (69.351–100.000)	2–10	0.2733	3.051 (0.659–14.140)
Zhangjiajie	20/20	100 (100.000–100.000)	10–38	0.0083	N
Zhuzhou	20/20	100 (100.000–100.000)	2–32	0.0083	N
Species					
Bungarus multicinctus	0/10	– (0.000–0.000)	–	$1.181e^{-6}$	N
Elaphe carinata	80/85	94.1 (89.115–99.120)	2–43	0.2369	2.462 (0.615–9.856)
Elaphe taeniura	26/30	86.7 (74.502–98.831)	1–25	Reference	Reference
Naja atra	0/10	– (0.000–0.000)	–	$1.181e^{-6}$	N
Zaocys dhumnades	238/240	99.2 (98.017–100.000)	5–70	0.0016	21.770 (3.805–124.600)
Total	344/375	91.7 (88.946–94.521)	1–70		

N = No significant

Fig. 1. Morphological photographs of spargana in snakes: (a) spargana in subcutaneous tissue of the snake; (b) spargana in muscular tissue of the snake; (c) spargana isolated from snakes.

anesthetizing with ethyl ether and skinned. The muscular, subcutaneous tissue and coelomic cavity were carefully observed for the presence of spargana parasites (fig. 1). The number of spargana plerocercoids was recorded to estimate the intensity and locations of infection. All obtained spargana parasites were washed in physiological saline, and identified preliminarily based on morphological characters and predilection sites (Daly, 1982).

DNA extraction and polymerase chain reaction (PCR) amplification

Samples preserved with 70% ethanol were repeatedly washed with double-distilled water. Genomic DNA of individual spargana plerocercoids was extracted using the Wizard® SV Genomic DNA Purification System (Promega, Madison, Wisconsin, USA) according to the manufacturer's recommendations.

A region of the *cox*1 gene was amplified with universal primers JB3 and JB4.5 (Bowles *et al.*, 1992; Zhu *et al.*, 2002; Li *et al.*, 2008). Each reaction mix contained 12.5 µl of Ex-Taq polymerase (TaKaRa, Beijing, China), 0.5 µl of each primer (50 µmol/l) and 2 µ of DNA template. Deionized H_2O was added to a final volume of 25 µl. The PCR reaction was performed in a thermocycler (Biometra, Jena, Germany) under the following conditions: initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 7 min. Each PCR series included no-DNA controls and host-DNA controls,

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press

Fig. 2. Phylogenetic relationships among members of the family *Diphyllobothriidae* inferred by neighbour-joining analysis and minimum-evolution analysis using the partial sequence of the *cox*1 gene, with *Taenia solium* as outgroup. The scale bar (0.02) indicates the genetic distance.

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press

where no amplicons were detected (not shown). PCR products were identified after agarose gel electrophoresis, and target amplicons were recovered, purified and sequenced.

Sequence analysis and reconstruction of phylogenetic relationships

The partial *cox*1 sequences were submitted to GenBank™ databases with accession numbers (Appendix 1) that were aligned with reference sequences using Clustal X 2.0 (Thompson *et al.*, 1997). Neighbour-joining (NJ) and minimum-evolution methods (ME) were used for phylogenetic reconstructions. The consensus tree was set up after bootstrap analysis, with 1000 replications. To reveal the genetic relationships in *Diphyllobothrium* tapeworms, other parasites of the Diphyllobothriidae family (Appendix 1) were taken into consideration in the experiment, with *Taenia solium* (GenBank™ accession number AB271234) as outgroup. Phylograms were completed with the Tree View program version 1.65 (Page, 1996).

Statistical analyses

All statistical analyses were performed using Statistical Analysis System Version 9.1 (SAS, North Carolina, USA); 95% confidence intervals (CI) are given; and $P < 0.05$ was considered statistically significant.

Results and discussion

The overall prevalence of spargana parasites in the examined wild snakes was 91.7% (344/375) (table 1) in Hunan province, which was almost twofold higher than the average prevalence observed in its neighbouring province (Guangdong: 29.8%, 37/124 and 55%, 251/456) (Wang *et al.*, 2011, 2014). The detection rate of spargana in wild snakes ranged from 65% to 100% (table 1). The difference of positive rates in wild snakes from different geographical locations was not significant, except for Loudi, Shaoyang, Xiangxi, Xiangtan, Yiyang, Zhangjiajie and Zhuzhou ($P > 0.05$). Logistic regression analysis showed that Xiangtan and Xiangxi had 15 (odds ratio (OR) = 15. 620, 95% CI = 2.903–84.010, $P = 6.25e^{-4}$) and ten (OR = 10.230, 95% CI = 1.121–93.350, $P = 0.0436$) times higher risks of being positive compared to Hengyang. In the present study, *Zaocys dhumnades* (99.1%) had the highest spargana infection prevalence, followed by *Elaphe carinata* (94.1%) and *Elaphe taeniura* (86.7%) (table 1), consistent with previous results (Wang *et al.*, 2011, 2014). Logistic regression analysis showed that *Z. dhumnades* had 21 (OR = 21.770, 95% CI = 3.805–124.600, $P = 0.0016$) times higher risk of being positive compared to *E. taeniura*. Spargana plerocercoids were identified in the muscular tissue (50.0%), subcutaneous tissue (32.1%) and coelomic cavity (17.9%); these differences in the distribution density were significant ($P < 0.05$). Interestingly, spargana infection in some snakes, such as *Bungarus multicinctus* and *Naja atra*, in which spargana infection had been reported previously in Guangdong province (Wang *et al.*, 2014) was not found in the present investigation.

Sequence variations of the *cox*1 gene fragment from 48 spargana isolates ranged between 0 and 2.9%, and 21 variable sites were detected (4.71%, 21/446). These sequences were deposited in GenBank (accession numbers MG762037–MG762084). The sequence of *cox*1 was 446 bp, and the A + T contents of the sequence was 62.56–63.45%. While the interspecific sequence differences among members of *Diphyllobothrium* were significantly higher than the intraspecific sequence variations among different populations of spargana isolates, being 15.0–27.4% and were 0–12.8%. In addition, the sequence variations of spargana isolates obtained here and others in China were 0.0–3.8%; that of spargana isolates obtained here and others from different species in other Asian countries (including in Laos, Japan and Thailand) and Australia ranged from 0.0 to 4.5%, but the sequence differences among spargana isolates obtained here and others in Poland were more obvious, being 11.7–12.8%. In addition, based on this molecular marker, 26 representative spargana isolates gained in the present study and others available in the GenBank database were employed to reconstruct the phylogenetic tree using the NJ method. Results showed that all isolates obtained from Hunan Province in the present study grouped with *Spirometra erinaceieuropaei* isolates available in the GenBank database, and they formed two separate branches. The upper branch was composed of several sister clades, and the isolates obtained here and gained from other hosts/regions in Asian countries and Australia were randomly distributed in this branch, and the lower branch consisted of *S. erinaceieuropaei* isolates from different species in Poland (fig. 2), which was in line with that of recent research (Kołodziej-Sobocińska *et al.*, 2019). Moreover, the phylogenetic tree based on sequences of the *cox*1 region successfully discriminates *Diphyllobothrium* and *Spirometra* species, and allowed identification of spargana isolates as belonging to *S. erinaceieuropaei*, confirming that *cox*1 is an appropriate marker for molecular epidemiology (Yamasaki & Kuramochi, 2009; Wicht *et al.*, 2010; Zhang *et al.*, 2015; Jeon *et al.*, 2018; Kołodziej-Sobocińska *et al.*, 2019).

Wild snakes have been extensively traded at food markets in some regions of Hunan province; eating the rare-cooked meat of wild snakes is still popular in some remote or poor areas in this province, contributing to the high risk of those people contracting sparganosis. The present results showed that risks of wild snake infections with spargana reached 91.8% in Hunan province, posing significant public health threats. Another study (Wu *et al.*, 2007) has indicated that high sparganosis cases rates (53.9%) closely correlated with patients that had consumed uncooked frogs and snakes. To ensure public safety, the major risk behaviour and harms of sparganosis should be publicized and popularized; in addition, the illegal trade of wild snakes should be effectively controlled and punished.

Conclusions

The present study indicated that the infection of snakes with *S. erinaceieuropaei* was severe in Hunan Province, China, which might contribute to huge threats to public health. The results obtained from the present study provide baseline data for the implementation of effective measures and strategies to control and prevent snake and human infection with spargana.

Financial support. This work was supported, in part, by the Department of Education, Hunan Province (17B126); the Bureau of Animal Husbandry and Fisheries, Hunan Province (2130199); the Department of Science and Technology, Hunan Province (2016NK2014); and the Youth Department of Science and Technology, Orient Science and Technology College of Hunan Agricultural University (17QNZ04).

Conflicts of interest. None.

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press

Ethical standards. The authors assert that all procedures contributing to this work comply with the Guidelines and Recommendations for the Care and Use of Animals of the Ministry of Health of the People' s Republic of China. The protocols of the animal experiments reported herein were approved by The Life Science Ethics Committee of Hunan Agricultural University. All efforts were made to minimize animal suffering during the course of these studies.

Author contributions. W. Liu and L. Tan contributed equally to this article.

References

Bowles J, Blair D and McManus DP (1992) Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–173.

Daly JJ (1982) *Sparganosis*. Vol **l**. Boca Raton, CRC Press.

Jeon HK, Park H, Lee D, *et al.* (2018) Genetic and morphologic identification of *Spirometra ranarum* in Myanmar. *The Korean Journal of Parasitology* **56**, 275–280.

Kim JG, Ahn CS, Sohn WM, Nawa Y and Kong Y (2018) Human sparganosis in Korea. *Journal of Korean Medical Science* **33**, e273.

Kołodziej-Sobocińska M, Stojak J, Kondzior E, Ruczyńska I and Wójcik J (2019) Genetic diversity of two mitochondrial DNA genes in *Spirometra erinaceieuropaei* (Cestoda: Diphyllobothridae) from Poland. *Journal of Zoological Systematics and Evolutionary Research* **57**, 764–777.

Li L, Yu LY, Zhu XQ, Wang CR, Zhai YQ and Zhao JP (2008) *Orientobilharzia turkestanicum* is grouped within African schistosomes based on phylogenetic analyses using sequences of mitochondrial genes. *Parasitology Research* **102**, 939–943.

Li MW, Song HQ, Li C, Lin HY, Xie WT, Lin RQ and Zhu XQ (2011) Sparganosis in mainland China. *International Journal of Infectious Diseases* **15**, e154–e156.

Liu W, Zhao GH, Tan MY, Zeng DL, Wang KZ, Yuan ZG, Lin RQ, Zhu XQ and Liu Y (2010) Survey of *Spirometra erinaceieuropaei* spargana infection in the frog *Rana nigromaculata* of the Hunan Province of China. *Veterinary Parasitology* **173**, 152–156.

Liu Q, Li MW, Wang ZD, Zhao GH and Zhu XQ (2015) Human sparganosis, a neglected food borne zoonosis. *Lancet Infectious Diseases* **15**, 1226–1235.

Lu G, Shi DZ, Lu YJ, Wu LX, Li LH, Rao LY and Yin FF (2014) Retrospective epidemiological analysis of sparganosis in mainland China from 1959 to 2012. *Epidemiology and Infection* **142**, 2654–2714.

Mo ZS, Li XH, Lei ZY and Xie DY (2013) Clinical analysis of 25 sparganosis cases. *Chinese Journal of Parasitology & Parasitic Diseases* **31**, 218–220.

Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**, 357–358.

Su HL and Li CQ (2004) Research on pharmacological action and application of snake gall. *Northwest Pharmacological Journal* **19**, 285–287.

Tan ZR, Chen CG and Ding HF (2015) A Case of visceral sparganosis misdiagnosed as tuberculous pericarditis. *Journal of Critical Care in Internal Medicine* **21**, 154–158.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882.

Wang F, Zhou L, Gong S, Deng Y, Zou J, Wu J, Liu W and Hou F (2011) Severe infection of wild-caught snakes with *Spirometra erinaceieuropaei* from food markets in Guangzhou, China involves a risk for zoonotic sparganosis. *Journal of Parasitology* **97**, 170–171.

Wang F, Li W, Hua L, Gong S, Xiao J, Hou F, Ge Y and Yang G (2014) *Spirometra* (Pseudophyllidea, Diphyllobothriidae) severely infecting wild-caught snakes from food markets in Guangzhou and Shenzhen, Guangdong, China: implications for public health. *Scientific World Journal* **2014**, 874014.

Wicht B, Ruggeri-Bernardi N, Yanagida T, Nakao M, Peduzzi R and Ito A (2010) Inter- and intra-specific characterization of tapeworms of the genus *Diphyllobothrium* (Cestoda: Diphyllobothriidea) from Switzerland, using nuclear and mitochondrial DNA targets. *Parasitology International* **59**, 35–39.

Wu ZJ, Chen Y, Qiu XL and Jiang HT (2007) An investigation of plerocercoid infection of frogs in Guiyang city and an analysis on clinical characteristics of 104 cases. *Journal of Guiyang Medical College* **32**, 140–141.

Yamasaki H and Kuramochi T (2009) A case of *Diphyllobothrium nihonkaiense* infection possibly linked to salmon consumption in New Zealand. *Parasitology Research* **105**, 583–586.

Yang GD, Wang FM, Gong SP, Li WY, Wei YF, Ge Y and Xiao JJ (2015) Investigation of *Spirometra mansoni* infection in snakes from Guangdong Province. *Guangdong Forestry Science and Technology* **31**, 80–83.

Zhang X, Wang H, Cui J, Jiang P, Fu GM, Zhong K, Zhang ZF and Wang ZQ (2015) Characterisation of the relationship between *Spirometra erinaceieuropaei* and *Diphyllobothrium* species using complete cytb and cox1 genes. *Infection, Genetics and Evolution* **35**, 1–8.

Zhu XQ, Beveridge I, Berger L, Barton D and Gasser RB (2002) Single-strand conformation polymorphism-based analysis reveals genetic variation within *Spirometra erinacei* (Cestoda: Pseudophyllidea) from Australia. *Molecular and Cellular Probes* **16**, 159–165.

Appendix 1. Sequence information for diphyllobothroid tapeworms used in the present study.

Species	Location	Sample codes	Host	GenBank™ accession number
Spirometra erinaceieuropaei	Hunan, China	SeECD1	Snake	MG762037
Spirometra erinaceieuropaei	Hunan, China	SeECD2	Snake	MG762038
Spirometra erinaceieuropaei	Hunan, China	SeECD3	Snake	MG762039
Spirometra erinaceieuropaei	Hunan, China	SeECS1	Snake	MG762040
Spirometra erinaceieuropaei	Hunan, China	SeECS2	Snake	MG762041
Spirometra erinaceieuropaei	Hunan, China	SeECS3	Snake	MG762042
Spirometra erinaceieuropaei	Hunan, China	SeECZ1	Snake	MG762043
Spirometra erinaceieuropaei	Hunan, China	SeECZ2	Snake	MG762044
Spirometra erinaceieuropaei	Hunan, China	SeECZ3	Snake	MG762045
Spirometra erinaceieuropaei	Hunan, China	SeELY1	Snake	MG762046
Spirometra erinaceieuropaei	Hunan, China	SeELY2	Snake	MG762047
Spirometra erinaceieuropaei	Hunan, China	SeELY3	Snake	MG762048

(*Continued*)

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press

Appendix 1. (*Continued.*)

Species	Location	Sample codes	Host	GenBank™ accession number
Spirometra erinaceieuropaei	Hunan, China	SeENX1	Snake	MG762049
Spirometra erinaceieuropaei	Hunan, China	SeENX2	Snake	MG762050
Spirometra erinaceieuropaei	Hunan, China	SeENX3	Snake	MG762051
Spirometra erinaceieuropaei	Hunan, China	SeEXTX1	Snake	MG762052
Spirometra erinaceieuropaei	Hunan, China	SeEXTX2	Snake	MG762053
Spirometra erinaceieuropaei	Hunan, China	SeEXTX3	Snake	MG762054
Spirometra erinaceieuropaei	Hunan, China	SeEYY1	Snake	MG762055
Spirometra erinaceieuropaei	Hunan, China	SeEYY2	Snake	MG762056
Spirometra erinaceieuropaei	Hunan, China	SeEYY3	Snake	MG762057
Spirometra erinaceieuropaei	Hunan, China	SeEZHJ1	Snake	MG762058
Spirometra erinaceieuropaei	Hunan, China	SeEZHJ2	Snake	MG762059
Spirometra erinaceieuropaei	Hunan, China	SeEZHJ3	Snake	MG762060
Spirometra erinaceieuropaei	Hunan, China	SeEFH1	Snake	MG762061
Spirometra erinaceieuropaei	Hunan, China	SeEFH2	Snake	MG762062
Spirometra erinaceieuropaei	Hunan, China	SeEFH3	Snake	MG762063
Spirometra erinaceieuropaei	Hunan, China	SeZAH1	Snake	MG762064
Spirometra erinaceieuropaei	Hunan, China	SeZAH2	Snake	MG762065
Spirometra erinaceieuropaei	Hunan, China	SeZAH3	Snake	MG762066
Spirometra erinaceieuropaei	Hunan, China	SeZHS1	Snake	MG762067
Spirometra erinaceieuropaei	Hunan, China	SeZHS2	Snake	MG762068
Spirometra erinaceieuropaei	Hunan, China	SeZHS3	Snake	MG762069
Spirometra erinaceieuropaei	Hunan, China	SeZLD1	Snake	MG762070
Spirometra erinaceieuropaei	Hunan, China	SeZLD2	Snake	MG762071
Spirometra erinaceieuropaei	Hunan, China	SeZLD3	Snake	MG762072
Spirometra erinaceieuropaei	Hunan, China	SeZSY1	Snake	MG762073
Spirometra erinaceieuropaei	Hunan, China	SeZSY2	Snake	MG762074
Spirometra erinaceieuropaei	Hunan, China	SeZSY3	Snake	MG762075
Spirometra erinaceieuropaei	Hunan, China	SeZXX1	Snake	MG762076
Spirometra erinaceieuropaei	Hunan, China	SeZXX2	Snake	MG762077
Spirometra erinaceieuropaei	Hunan, China	SeZXX3	Snake	MG762078
Spirometra erinaceieuropaei	Hunan, China	SeZYX1	Snake	MG762079
Spirometra erinaceieuropaei	Hunan, China	SeZYX2	Snake	MG762080
Spirometra erinaceieuropaei	Hunan, China	SeZYX3	Snake	MG762081
Spirometra erinaceieuropaei	Hunan, China	SeZYZ1	Snake	MG762082
Spirometra erinaceieuropaei	Hunan, China	SeZYZ2	Snake	MG762083
Spirometra erinaceieuropaei	Hunan, China	SeZYZ3	Snake	MG762084
Spirometra erinaceieuropaei	Guangxi China	–	Snake	KF745147.1
Spirometra erinaceieuropaei	Guangxi China	–	Snake	KF745148.1
Spirometra erinaceieuropaei	Guangxi China	–	Snake	KF745146.1
Spirometra erinaceieuropaei	Vietnam	–	Snake	KY552887.1
Spirometra erinaceieuropaei	Laos	–	Snake	KM099136.2
Spirometra erinaceieuropaei	Laos	–	Snake	KM099124.2
Spirometra erinaceieuropaei	Australia	–	Snake	AJ308262.1

(*Continued*)

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press

Appendix 1. (*Continued.*)

Species	Location	Sample codes	Host	GenBank™ accession number
Spirometra erinaceieuropaei	Thailand	–	Snake	KM099139.2
Spirometra erinaceieuropaei	Hunan, China	–	Frog	KF745145.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999950.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999952.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999948.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999946.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999954.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999951.1
Spirometra erinaceieuropaei	Hunan, China	–	Frog	GQ999956.1
Spirometra erinaceieuropaei	Zhejiang, China	–	Frog	KP738287.1
Spirometra erinaceieuropaei	Zhejiang, China	–	Frog	KP738288.1
Spirometra erinaceieuropaei	Zhejiang, China	–	Frog	KP738274.1
Spirometra erinaceieuropaei	Zhejiang, China	–	Frog	KP738269.1
Spirometra erinaceieuropaei	Zhejiang, China	–	Frog	KP738262.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656739.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656740.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656736.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656735.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656741.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656742.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656743.1
Spirometra erinaceieuropaei	Henan, China	–	Frog	KF656744.1
Spirometra erinaceieuropaei	Australia	–	Fox	AJ308259.1-
Spirometra erinaceieuropaei	Australia	–	Fox	AJ308260.1
Spirometra erinaceieuropaei	Japan	–	Human	AB480297.1
Spirometra erinaceieuropaei	Thailand	–	Human	KF539840.1
Spirometra erinaceieuropaei	Thailand	–	Human	KF539836.1
Spirometra erinaceieuropaei	Australia	–	Cat	AJ308261.1
Spirometra erinaceieuropaei	Iran	–	Cat	KY009916.1
Spirometra erinaceieuropaei	Poland	–	*Nyctereutes procyonoides*	MK523402.1
Spirometra erinaceieuropaei	Poland	–	*Nyctereutes procyonoides*	MK523408.1
Spirometra erinaceieuropaei	Poland	–	*Nyctereutes procyonoides*	MK523394.1
Spirometra erinaceieuropaei	Poland	–	*Meles meles*	MK523404.1
Spirometra erinaceieuropaei	Poland	–	*Meles meles*	MK523411.1
Spirometra erinaceieuropaei	Poland	–	*Mustela putorius*	MK523396.1
Diphyllobothrium ditremum	UK	–	*Salvelinus alpinus*	FM209182.1
Diphyllobothrium nihonkaiense	Japan	–	Human	AB015755.1
Echinococcus granulosus	Tunisia	–	Dog	KT001403.1
Echinococcus multilocularis	Poland	–	Fox	KY205685.1
Ligula sp.	UK	–	*Phoxinus phoxinus*	EU241247.1
Hymenolepis microstoma	USA	–	Rice	AB494473.1

https://doi.org/10.1017/S0022149X20000139 Published online by Cambridge University Press