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Molecular characterization of *Spirometra* isolates across the United States of America

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Abstract

Spirometra is a genus of zoonotic cestodes with an ambiguous species–level taxonomic history. Previously, Spirometra mansonoides was considered the only species present in North America. However, recent molecular data revealed the presence of at least three distinct species in the United States of America (USA): *Spirometra* sp. 2 and 3, and *Spirometra mansoni*. This study aimed to elucidate the diversity and potential host associations of *Spirometra* species among companion animals in the USA. Samples (N=302) were examined from at least 13 host species, including mammals, amphibians, and reptiles. Sample types included eggs isolated from faeces (n=222), adult specimens (n=71), and plerocercoids (n=9) from 18 different states and 2

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territories across the USA. Extracted genomic DNA was subjected to PCR targeting a fragment of the mitochondrial cytochrome c oxidase subunit 1 (cox1) gene. Generated sequences (n=136) were included in a phylogenetic analysis. *Spirometra mansoni* was detected in domestic cats (n=76), dogs (n=12), a White's tree frog (n=1), a Cuban knight anole (n=1), a green iguana (n=1), and a serval (n=1) across 15 states and Puerto Rico. *Spirometra* sp. 2 was found only in dogs (n=3) from Florida, and *Spirometra* sp. 3 was found only in cats (n=41) from 17 states. All plerocercoid samples were consistent with S. mansoni. The results confirm that at least three distinct Spirometra species are present and established in companion animals, such as dogs and cats, and likely are using various native and exotic species as paratenic hosts within the USA.

Keywords: Genetic diversity, Diphyllobothriidea, North America, sparganosis, species complex, *Spirometra mansoni, Spirometra mansonoides,* Zoonotic diseases

Introduction

Broad tapeworms within the genus *Spirometra* (Cestoda: Diphyllobothriidea) are known to cause sparganosis (i.e., larval infection), a potentially life–threatening zoonotic disease, and spirometrosis (i.e., adult infection), with reports from every continent except Antarctica (Scholz *et al*., 2019; Kuchta *et al*., 2024). *Spirometra* species are known to parasitize mammals, amphibians, reptiles, birds, and rarely fish (Kuchta *et al*., 2024; Vettorazzi *et al*., 2023). The lifecycle begins when an infected definitive host defecates faeces containing eggs. The egg must reach freshwater where it hatches and releases a coracidium. The lifecycle requires two intermediate hosts, the first intermediate of which is a freshwater cyclopoid copepod which becomes infected upon the ingestion of a coracidium. The coracidium develops into the first metacestode larval stage, known as the procercoid, within the copepod. The second intermediate

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host, typically an amphibian or reptile but occasionally mammals such as humans, become infected upon ingestion of the copepod-containing procercoids; however, experimental studies have shown that infection through direct penetration by the procercoid is also possible (Li *et al*., 1929; Mueller *et al*., 1938). The procercoid develops into the final metacestode larval stage, the plerocercoid, within the second intermediate host; and this plerocercoid can establish itself in various organs or tissues for several years (Mueller, 1974). Plerocercoids, also termed spargana, can elicit a serious clinical manifestation known as sparganosis within the second intermediate host and a wide range of paratenic hosts. Typically, plerocercoids are located within the subcutaneous tissue, but they can migrate to the viscera, muscles, eyes, and central nervous system (Kuchta *et al*., 2021). The definitive host, typically a carnivoran mammal, becomes infected by ingesting tissues of a paratenic or second intermediate host containing the plerocercoid. The plerocercoid may also infect an individual if exposed to an open wound, for instance, in cases where amphibians such as frogs are used as poultices and placed on the wound (Liu *et al*., 2015). The plerocercoid develops into an adult cestode within the host's small intestine and releases eggs into the faeces. In the environment, these eggs embryonate and hatch in freshwater, releasing a coracidium that then completes the life cycle through infection of and development in a freshwater cyclopoid copepod (Mueller, 1974). The prepatent period of this cestode is relatively short with eggs being passed in faeces as early as two weeks in cats (Kuchta *et al*., 2024).

Adult *Spirometra* is generally nonpathogenic within the carnivoran definitive host; however, in some cases, it can cause gastrointestinal disease resulting in vomiting, diarrhoea, and weight loss (Conboy, 2009). Infection with the plerocercoid may result in more serious disease and even death depending on the degree of pathology and the tissues involved (Conboy, 2009). In humans,

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there have been approximately 70 reported cases of sparganosis in North America with the most recent case of a 12-year-old child from Florida in 2024 who presented with a painful subcutaneous mass from which a plerocercoid was surgically excised (Griffin *et al*., 1996; Hawkins *et al*., 2024; Kuchta *et al*., 2015; Mueller al., 1963; Taylor *et al*., 1976). In extreme and rare cases, the plerocercoid asexually reproduces in multiple organs causing a fatal condition known as proliferative sparganosis (Buergelt *et al*., 1984; Conboy, 2009; Tokiwa *et al*., 2024; Woldemeskel, 2014).

Advancements in molecular diagnostic methods have contributed to the recent reclassification of *Spirometra* species; and, while still controversial, species or lineages are generally confined to specific geographic regions. Kuchta *et al*. (2024) proposed a new classification scheme based on molecular data and geographic location in which there are seven distinct lineages: *Spirometra erinaceieuropaei* in Europe, *Spirometra theileri* in Africa, *Spirometra asiana* in Korea and Japan, *Spirometra decipiens* in South America, *Spirometra* sp. 2 in both North and South America, *Spirometra* sp. 3 in North America, and *Spirometra mansoni* found worldwide (Kuchta *et al*., 2024; Yamasaki *et al*., 2024). Prior to this new classification scheme, *Spirometra* sp. 2 was denoted as *S. decipiens* complex 1 and *Spirometra* sp. 3 was within the *S. decipiens* complex 2. Both species complexes have been molecularly confirmed in North and South America. Kuchta *et al*. (2024) proposed the *S. decipiens* complex 2 be divided into North and South American lineages with what is now the free-standing *S. decipiens* in South America and *Spirometra* sp. 3 in North America. Additionally, specimens within *S. decipiens* complex 1 are referred to as *Spirometra* sp. 2. This newly proposed naming schematic by Kuchta *et al*. (2024) is used henceforth.

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In North America, the first species to be formally described was named *Diphyllobothrium* (*Spirometra*) *mansonoides*, based on specimens from a domestic cat in the northeastern USA (Mueller, 1935). At the time, *Spirometra* was classified as a subgenus of *Diphyllobothrium*, but it was later elevated to genus (Mueller, 1938). In fact, the first reports of *Spirometra* in the USA pre-date the 1935 description of *S. mansonoides*; in 1927, a cestode was collected from a cat in Louisiana and was morphologically identified as belonging to the genus *Dibothriocephalus,* previously *Diphyllobothrium*, subgenus *Spirometra.* Additionally, tapeworms collected from a cat in Puerto Rico, also in 1927, were classified as *Diphyllobothrium mansoni* (Dikmans, 1931). *Spirometra mansonoides* was originally described based on the morphology of the terminal loops of the uterus. However, this morphological feature is also found within the *S. decipiens* complex and with no supporting molecular data of *S. mansonoides*, it is currently not recognized as a valid species (Kuchta *et al*., 2024). Nevertheless, *Spirometra* eggs have been routinely observed through faecal examinations of dogs and cats mainly from the eastern USA across decades, and often diagnosed either to genus level or as *S. mansonoides* (Conboy, 2009, Wyrosdick *et al*. 2017, Hoggard *et al*., 2019, Nagamori *et al*., 2020; Sobotyk *et al*. 2021). Currently, molecular evidence suggests that the *Spirometra* species present in the USA include: *S. mansoni* from a captive Samar cobra (*Naja samarensis*) in Texas, *S. mansoni* from an unknown host in Nebraska, *Spirometra* sp. 2 from a bobcat (*Lynx rufus*) in Illinois, and *Spirometra* sp. 3 from captive meerkats (*Suricata suricatta*) in South Carolina, a black rat snake (*Pantherophis obsoletus*) in Louisiana, an eastern racer snake (*Coluber constrictor*), and a western ribbon snake (*Thamnophis proximus*) both from Mississippi (Jeon *et al*., 2016; Kuchta *et al*., 2024; McHale *et al*., 2020; Verocai *et al*., 2023; Waeschenbach *et al*., 2017; Yamasaki *et al*., 2021).

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This study aimed to elucidate the genetic diversity and potential host associations of *Spirometra* cestodes in the USA and its territories through molecular analysis of specimens collected from naturally infected animals and submitted to veterinary diagnostic laboratories nationwide.

Materials and Methods

Sample acquisition

Samples were requested utilizing the VetPDx (Veterinary Parasitology Diagnostic Network), a listserv founded in 2017 to connect parasitology diagnostic laboratories across North America. A total of 302 samples were received from various locations across the USA, including 25 states and 2 territories (Puerto Rico and the US Virgin Islands). The specimens represented adults (n=71), plerocercoids (n=9), and faecal samples from which *Spirometra* eggs were isolated (n=222). Of the adult specimens, 16 were collected from domestic dogs (*Canis lupus familiaris*), 52 from domestic cats (*Felis silvestris catus*), one from an ocelot (*Leopardus pardalis*), one from a serval (*Leptailurus serval*), and one from an unspecified host. The faecal samples consisted of 29 from domestic dogs, 188 from domestic cats, and five from unspecified hosts. Finally, of the plerocercoids, one was obtained from a green iguana (*Iguana iguana*), one from a rattlesnake of unknown species, one from a wild boar (*Sus scrofa*), one from an opossum (*Didelphis virginiana*), one from a White's tree frog (*Litoria caerulea*), one from a Cuban knight anole (*Anolis equestris*), and three from New England cottontail rabbits (*Sylvilagus transitionalis*). Overall, samples included both fresh and archival samples from teaching and diagnostic laboratory collections in which samples were collected from 1993–2024 in various preservatives such as formalin and ethanol.

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Sample preparation

The different sample types were prepared as follows for DNA extraction: 1) Faecal samples (n=222): The faecal samples were processed by double centrifugation sugar flotation using 2 grams of faeces (Hoggard *et al*. 2019, Zajac *et al*. 2021). After centrifugation, the top 2 mL of the faecal mixture was siphoned from the 15 mL centrifuge tube and added to a 50 mL conical centrifuge tube. Next, 40 mL of water was added to the tube, vortexed, centrifuged at 400 rcf for 10 minutes, and the supernatant was discarded. This step was repeated. Most of the supernatant was removed, leaving the pellet in approximately 2 mL of water. The pellet and water were then vortexed and strained through a 100 µM ÜberStrainer® (PluriSelect, Leipzig, Germany), followed by straining through a 30 µM ÜberStrainer®. The eggs were then collected from the mesh of the 30 µM ÜberStrainer® and stored in 50 mL conical centrifuge tubes. 2) Adults (n=71) and plerocercoids (n=9): Approximately 1–2 cm of each specimen was placed in a 1.5 mL Eppendorf microtube. The preservative in which each specimen had been kept was then evaporated using a Vacufuge® plus centrifuge concentrator (Eppendorf, Hamburg, Germany) for 6 minutes.

Genomic DNA extraction

Genomic DNA was extracted using different methods and kits for the sample types listed. 1) Eggs: For 68 of the 222 samples from which eggs were isolated, an Omni International Bead Ruptor Elite Bead Mill Homogenizer® (Thermo Fisher Scientific, Waltham, MA, USA) was used to break apart the eggs before DNA extraction. To do this, the faecal sediment was added to a 2 mL tube with 1.4 mm ceramic beads and processed with the following settings: 2-minute cycle, 10-second dwell, 5.00 m/s, and run for 2 cycles. DNA was then extracted from the

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samples using the Maxwell® RSC Tissue DNA kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The remaining 154 samples containing egg samples were extracted using the Maxwell® RSC Faecal Microbiome DNA kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. 2) Adult worms and plerocercoids: These were initially beaten with beads in a 2 mL tube with 2.8 mm ceramic beads and processed with the following settings: 2-minute cycle, 10-second dwell, 5.65 m/s, and run for 2 cycles. DNA was then extracted using the Maxwell® RSC Tissue DNA kit (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Molecular analysis

All samples were subjected to polymerase chain reaction (PCR) using primers (forward primer PlatCOI F (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and reverse primer PlatCOI R (5'-TAA AGA AAG AAC ATA ATG AAA ATG-3')) that targeted a portion of the cytochrome *c* oxidase subunit 1 (*cox1*) gene in the mitochondrial DNA (mtDNA). A modified protocol from Bowles *et al*. (1995) was utilized to amplify a fragment of the *cox1* gene region in a 25 µL reaction that included Go™ TaqGreen Master Mix (Promega, Madison, WI, USA), 2.5 µL of DNA template, and 10 µM of each of primer. Cycling conditions were as follows: denaturation at 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 52°C for 1 minute, and 72°C for 1 minute, followed by a final extension of 72°C for 5 minutes. DNA extracted from a fragment of an adult *Spirometra* sp. was used as a positive control, and nuclease-free water was used as the negative control. The PCR products were run on a 1.5% agarose gel and visualized using an ultraviolet transilluminator. The expected size of the amplified products was approximately 400 bp in size. Samples that amplified were purified using the E.Z.N.A.® Cycle

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Pure Kit (Omega Bio–tek, Norcross, GA, USA) and were sequenced in both directions in a 3730xl DNA Analyser at Eurofins Genomics (Louisville, KY, USA).

Phylogenetic and haplotype analysis

For phylogenetic analysis, sequences were aligned and trimmed using MEGA X version 10.0.5 (Kumar *et al*., 2018) and were compared to related sequences from NCBI. The phylogenetic tree was constructed from the generated *cox1* gene sequences using the maximum–likelihood method with 1000 bootstrap support and the Tamura-Nei with gamma-distribution (TN93+G) best–fit substitution model (Tamura and Nei, 1993). *Schistocephalus solidus* (AP017669) was used as an outgroup.

For the haplotype network analysis, sequences of *S. mansoni* were analysed. This included 83 sequences (330bp) and 41 reference sequences available in GenBank from Vietnam (n=1), India (n=1), Australia (n=4), Korea (n=2), China (n=3), Iran (n=2), Japan (n=7), Romania (n=1), Indonesia (n=3), New Zealand (n=1), Colombia (n=1), Thailand (n=3), Myanmar (n=2), Tanzania (n=1), Cambodia (n=4), USA (n=1), and Laos (n=4). Nine of the *S. mansoni* sequences generated in our study were removed due to their shorter length. The sequences were prepared in Mega X version 10.0.05 (Kumar *et al*., 2018) and DnaSP (v6) (Rozas *et al*., 2017). The prepared sequences were imported into PopART (v1.7) and the median-joining network method was utilized to construct the haplotype network (Bandelt *et al*., 1999).

Results

Of the 302 samples received, quality sequences were obtained from 45% (n=136) of them and were included in the phylogenetic analysis (Table 1) and submitted to GenBank (Supplementary

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Table 1). Of these 136 samples, 83.1% (n=113) came from isolated eggs, 14.7% (n=20) from adult worms, and 2.2% (n=3) from plerocercoids. The phylogenetic analysis indicated three distinct clades among the samples that grouped with previously submitted sequences of *Spirometra mansoni* and *Spirometra* spp. 2 and 3 (Figure 1). Of the 136 sequences, 67.6% (n=92) were most similar to *S. mansoni*, 2.2% (n=3) were similar to *Spirometra* sp. 2, and 30.1% (n=41) were similar to *Spirometra* sp. 3 (Table 1). Within each sample type, the species breakdown is as follows: 1) Of the 113 egg samples, 67.3% (n=76) were identified as *S. mansoni*, *Spirometra* sp. 3 comprised 32.7% (n=37), and *Spirometra* sp. 2 was not identified in any of the egg samples. 2) Of the 20 adult specimens, 65% (n=13) were identified as *S. mansoni*, 15% (n=3) were identified as *Spirometra* sp. 2, and 20% (n=4) were identified as *Spirometra* sp. 3. 3) Of the plerocercoids, 100% (n=3) were identified as *S. mansoni*. Regarding host association, samples that grouped with *Spirometra* sp. 3 were found only in domestic cats, whereas samples grouping with *Spirometra* sp. 2 were only found in domestic dogs (Table 1). No host association was observed for *S. mansoni*. Geographically, *S. mansoni* was identified in 15 different states and Puerto Rico. Whereas, *Spirometra* sp. 3 was found in 17 different states, and *Spirometra* sp. 2 was restricted to Florida (Figure 2). *Spirometra mansoni* and *Spirometra* sp. 3 had a geographic overlap and were found in 14 of the same states. Additionally, all three lineages were found in Florida.

Regarding the haplotype network of *S. mansoni*, overall, there were 26 different haplotypes identified from the 124 *cox1* gene sequences (Figure 3). The network analysis indicated two predominant haplotypes to which most of the samples aligned Haplotype 2 (Hap_2) and Haplotype 4 (Hap_4). Hap_2 had a haplotype frequency of 33.9% (n=42) among samples, including samples from Connecticut, Florida, Georgia, Indiana, Louisiana, Maryland,

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New Hampshire, New Jersey, Pennsylvania, and South Carolina and sequences from Oceania (e.g., Australia) and Asia (e.g., China, Japan, Iran, and Thailand). Hap_4 had a frequency of 30.6% (n=38) among samples, including samples from Connecticut, Florida, Illinois, Massachusetts, New Hampshire, Pennsylvania, and Texas, and sequences from Oceania (e.g., Australia and New Zealand), Asia (e.g., Japan, and Indonesia) and South America (e.g., Colombia). A summary of the geographic origins and haplotype frequencies can be found in Supplementary Table 2.

Discussion

The genus *Spirometra* is likely the oldest lineage of diphyllobothriidean cestodes; and, since its original description over 200 years ago, the genus has undergone multiple reclassifications (Kuchta *et al*., 2024). Before this study, the molecular characterization of *Spirometra* isolates from the USA was limited with few studies having been done leading to a significant knowledge gap in species diversity and distribution (Kuchta *et al*., 2021; McHale *et al*., 2020; Verocai *et al*., 2023; Waeschenbach *et al*., 2017). Despite *S. mansoni* being found worldwide, reports of molecular characterization from cases occurring in the Americas have only been recently described (Brabec *et al*., 2022; Verocai *et al*., 2023; Alvarado–Hidalgo *et al*., 2024; Wu *et al*., 2024); however, it has not been determined if *S. mansoni* is established and circulating in the Americas, specifically the USA. The first molecular report of *S. mansoni* in the Americas was from a crab-eating fox (*Cerdocyon thous*) in 2022 from Colombia (Brabec *et al*., 2022), followed by a Samar cobra in 2023 from the USA (Verocai *et al*., 2023). In 2024, there were 3 published reports that included four dogs, three cats, and one coyote (*Canis latrans*) from Costa Rica (Alvarado–Hidalgo *et al*., 2024), and two Puerto Rican crested anoles (*Anolis cristatellus*) from

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Puerto Rico (Wu *et al*., 2024). These cases confirm the presence of *S. mansoni* in the Americas. In the case of the Samar cobra from the USA, which was imported from the Philippines, it was suspected that the snake was infected prior to importation to the USA (Verocai *et al*., 2023). Additionally, another specimen from an unknown host in Nebraska, USA that was originally labelled as *Spirometra mansonoides* based on morphology was molecularly misidentified as *S. decipiens* and later reclassified as *S. mansoni* (Jeon *et al*., 2016; Yamasaki *et al*., 2021; Yamasaki *et al*., 2024). The results now confirm that *S. mansoni* is likely well established, across the USA, using dogs and cats as definitive hosts, and has had multiple introductions over time, followed by some events of within-country expansion through animal movement. The latter claim is supported by the results of the haplotype network analysis, which indicates intraspecific genetic diversity within *S. mansoni* (Figure3). While various haplotypes comprised sequences from the USA and other continents, their specific origin cannot be determined.

Regarding *Spirometra* sp. 2, previously named *S. decipiens* complex 1, there has been only a single molecular report from the USA in a bobcat (*L. rufus*), whereas all other reports have originated primarily from both wild and domestic canid and felid hosts in South America (Waeschenbach *et al*., 2017; Fredes *et al*., 2022; Petrigh *et al*., 2015; Almeida *et al*., 2016; Arrabal *et al*., 2020; Kuchta *et al*., 2024). In contrast, samples from this study included only three cases that grouped with *Spirometra* sp. 2, all originating from domestic dogs from Florida (Figure 1). It is plausible that the importation of domestic dogs and cats from South America has led to the establishment of *Spirometra* sp. 2 in North America as well (Jeon *et al*., 2016). However, *Spirometra* has been reported from a grey fox (*Urocyon cinereoargenteus*) and Florida panthers (*Puma concolor coryi*) (Conti, 1984; Foster *et al*., 2006), bobcats from Arkansas (Heidt *et al*., 1988), and coyotes and raccoons (*Procyon lotor*) from various states (Harkema and Miller,

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1964; Schaffer *et al*., 1981; Gompper *et al*., 2003) without molecular characterization therefore, it is plausible that *Spirometra* sp. 2 was already established and circulating within the USA. Considering the variety of both wildlife and domestic animal host species that *Spirometra* sp. 2 infects across North and South America, determining any host association within this complex remains challenging. This highlights the need for broader sampling and molecular characterization of specimens from the USA, as well as Central and South America, to better understand the species composition and host associations among different genetic lineages.

Molecular confirmation of *Spirometra* sp. 3, previously within *S. decipiens* complex 2, infections have been reported prior to this study in the USA in reptiles and exotic mammals (Waeschenbach *et al*., 2017; McHale *et al*., 2020; Kuchta *et al*., 2024). In South America, *S. decipiens*, previously within *S. decipiens* complex 2, has been found in a maned wolf (*Chrysocyon brachyurus*) in Bolivia, a Patagonian green racer (*Philodryas patagoniensis*) in Uruguay, and a Geoffroy's cat (*Leopardus geoffroyi*) and an ocelot (*Leopardus pardalis*) in Brazil (Kuchta *et al*., 2024). The samples that grouped with *Spirometra* sp. 3 were exclusively detected in domestic cats, suggesting a potential host association within the USA. The sequences from this study represent the first molecular characterization of *Spirometra* isolates from the geographic region in which *S. mansonoides* was first described by Mueller (1935), supporting the idea that *S. mansonoides* belongs in the *Spirometra* sp. 3. Complex. According to Kuchta *et al*. (2024), isolates within *S. decipiens* complex 2, should be separated into a North and a South American lineage, with those from South America being referred to as *S. decipiens* and those from North America as *Spirometra* sp. 3. The results of this study further support the hypothesis of *S. mansonoides* to be a valid species, possibly represented by *Spirometra* sp. 3 (i.e., Kuchta *et al*. 2024). Nevertheless, integrated molecular and morphological evidence would be required for

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resurrecting the species. This could be accomplished by reassessing the type-material of the original *S. mansonoides* description by Mueller (1935), in addition to an attempt of molecular characterization of the material, which may not be fruitful given preservation methods, DNA quality, and destructive use. Alternatively, adult specimens could be collected from the type-host and the type-locality (i.e., Syracuse, New York) to provide a robust molecular characterization followed by phylogenetic analysis for comparison with the data generated by our study.

There are many challenges associated with determining a species based solely on morphology. Historically, the reproductive system of adults was used for identification, specifically the number of uterine coils; however, this can vary as the worm develops and is considered unreliable (Yamasaki *et al*., 2021; Iwata, 1972). Additionally, the morphology of museum or archival specimens may be distorted, depending on the fixation technique used, and result in misidentification. Furthermore, the opportunistic nature of our sampling limited the ability for morphology to be assessed as the amount of specimen provided was enough only for molecular analysis. Another challenge when working with archival samples, such as the ones obtained in this study, that had an unknown preservation history, is that preservatives such as formalin can greatly inhibit molecular analysis (Zimmermann *et al*., 2008). Furthermore, most samples included in our study were eggs isolated from faeces, a biological sample known to contain PCR inhibitors such as complex polysaccharides, bilirubin, and metabolites from digestion that may have contributed to the reduced number of high–quality sequences suitable for inclusion in the phylogenetic analysis (Roncancio–Duque *et al*., 2024). To have a complete taxonomic description, future studies should focus on both molecular and morphologic

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characterization of larval and adult stages using standardized fixation techniques that are compatible with molecular techniques, (Chávez-González *et al*., 2022; Chervy, 2024).

This study identified patent infections in both dogs and cats, which may increase the risk of transmission in companion animals and pose an additional threat to public health due to the zoonotic potential of this parasite. Infections with *Spirometra* adults in the small intestine of cats and dogs are typically subclinical, but there are rare instances in which a fatal proliferative sparganosis can occur in companion animals and humans. Proliferative sparganosis develops when the plerocercoids asexually reproduce and migrate into multiple tissues and organs as opposed to non–proliferative infections in which only a few plerocercoids migrate in a confined area within connective tissue (Kikuchi *et al*., 2020). Historically, reported cases of symptomatic or fatal proliferative sparganosis in humans or animals, thought to be caused almost exclusively by an isolate phylogenetically closely related to *Spirometra* isolates from South America belonging to *Spirometra* species 2, therefore within the *S. decipiens* complex 1, incorrectly referred to as *Sparganum proliferum* (Fredes *et al*., 2022; Kikuchi *et al*., 2020; Miyadera *et al*., 2001); however, a recent case of proliferative sparganosis in a cat in Japan was due to *S. mansoni* (Tokiwa *et al*., 2024). The Japanese case highlights the importance of determining the taxonomic status and distribution of *Spirometra*, as different species may be associated with a higher degree of pathogenicity. Furthermore, feral cats and other wild carnivoran mammals such as raccoons, may act as reservoirs for *Spirometra* species and potentiate their spread to vulnerable or endangered species in zoological facilities, as was demonstrated by McHale *et al*. (2020) in which three cases of sparganosis in captive meerkats at a zoological facility was reported.

Although *Spirometra* infections have been reported in Oklahoma, Hawaii, North Carolina, and West Virginia, no samples from these areas were available for inclusion in the

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analysis (Nagamori *et al*., 2020; Little *et al*., 2000). Due to the opportunistic and voluntary nature of the sampling strategy employed in the present study, we were unable to confirm the absence of *Spirometra* spp. in states without sample representation. Notwithstanding the limitation of sampling bias, the results still demonstrated an extensive geographic overlap of *S. mansoni* and *Spirometra* sp. 3 with both in 14 of the same states (Figure 2). It is plausible that cats in these states are at an increased risk for co–infection with both lineages. These findings raise the question of how both lineages became established in the same geographic area in the USA. To answer this, we must consider the origins of both the parasite and its host. *Spirometra* isolates within *Spirometra* sp. 3 may have already been present in North America with the bobcat (*L. rufus*), a felid native to North America, serving as the definitive host, and native amphibian and reptile species acting as the second intermediate host (Mueller, 1974). With the colonization of North America by European settlers in the early 16th century, various domestic animals were introduced, including domestic cats. It can be speculated that these animals preyed upon the second intermediate hosts, which led to their infection and integration into the *Spirometra* life cycle (Serpell, 2014).

In contrast, the results indicate that *S. mansoni* is currently well established throughout the USA, and while it is speculated that *S. mansoni* currently has a cosmopolitan distribution with potential origins in Asia, prior to this study there was little molecular evidence to confirm its presence and establishment in North America. Our haplotype analysis further supports the hypothesis of multiple introduction events throughout modern times resulted in the establishment and expansion in the USA. Another explanation, especially pertinent in the last few decades, is the increased travel and rehoming efforts of companion animals such as dogs and cats both domestically and internationally may have further contributed to the introduction of *S. mansoni*

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into new geographic areas within the USA (Wright *et al*. 2020, Giannelli *et al*., 2024). Additionally, the legal and illegal importation of exotic animals to the United States may play an extensive role in modern times in the introduction and spread of zoonotic pathogens such as species of *Spirometra* (Rush *et al*., 2021, Verocai *et al*., 2023).

Regarding treatment and prevention, there are currently no FDA–approved products labelled for the treatment of *Spirometra* in companion animals. However, treatment recommendations consist of oral or subcutaneous administration of praziquantel at 25 mg/kg once a day for two consecutive days for both cats and dogs (Conboy, 2009). In cases of proliferative sparganosis, one of the only known successful treatments was of a dog, administered 3 weeks of mebendazole at 20 mg/kg orally once a day followed by 3 weeks of praziquantel at 5 mg/kg orally or subcutaneously once a day, and alternating this regimen for three months (Beveridge *et al*., 1998). Effective prevention strategies must consider the various transmission routes the parasite utilizes to complete the life cycle. A vertebrate host may become infected by i) ingestion of an infected copepod first intermediate host with a procercoid through contaminated water or food; ii) ingestion of a second intermediate or paratenic host infected with a plerocercoid; or iii) migration of a plerocercoid into an open wound of a potential host (Mueller, 1974; Li *et al*., 2011). Infections in humans primarily occur due to poor food safety and hygiene practices, such as the ingestion of raw or undercooked second intermediate or paratenic hosts, or the use of amphibian poultices on open wounds to facilitate healing in certain cultures (Li *et al*., 2011; Liu *et al*., 2015). Education is likely the most vital tool in prevention of human sparganosis and should focus on educating people about how infections occur, emphasizing food safety, (i.e. thoroughly cooking meat, especially wildlife), and filtering potentially contaminated drinking water. In regions where the use of frog and snake poultices is

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common, discouraging this practice and educating about the risk of infection is warranted (Li *et al*., 2011; Liu *et al*., 2015). In companion animals, prevention could consist of restricting pets' access to areas which wildlife inhabit and may contaminate the environment, and monitoring pets to prevent ingestion of potential second intermediate or paratenic hosts. Zoological facilities should consider similar mitigation strategies by implementing surveillance of potential reservoir hosts such as feral cats and raccoons that may contribute to contaminating the environment (McHale *et al*., 2020).

Conclusion

Spirometra mansoni is well established in the USA and likely has had multiple introductions throughout history. There are two other distinct species of *Spirometra* present within the United States that correspond to *Spirometra* species 2 and 3. Two lineages within *Spirometra* sp. 3 should be considered, one which represents the North American lineage and the other the South American lineage of *Spirometra*. Pending additional integrated classical and molecular assessment, *S. mansonoides* may be resurrected as the taxon shown to infect domestic cats in North America. Overall, this study reinforces the need for further molecular characterization of different *Spirometra* life stages in domestic animals, animals housed in zoological facilities, and wildlife.

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Ethical standards. Not applicable.

Data availability. Data supporting the conclusions of this study are included in the article. Generated sequences were submitted to GenBank database under accession numbers: PQ673870 – PQ674005.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

References

Almeida, GG, Coscarelli, D, Melo, MN, Melo AL and Pinto, HA (2016) Molecular identification of *Spirometra* spp. (Cestoda: Diphyllobothriidae) in some wild animals from Brazil. *Parasitology International* **65,** 428–431. doi: 10.1016/j.parint.2016.05.014.

Alvarado-Hidalgo, I, Campos-Camacho, J, Arguedas-Morales, Y, Romero-Vega, LM, Alfaro-Alarcón, A, Anchia-Ureña, G, Bass, LG, Berrocal-Ávila, I, Hagnauer, I, Olivares, RWI, Solano-Barquero A, Traube-Rivera, R, Montenegro-Hidalgo, V and Rojas, A (2024) Molecular, morphological, and histopathological evidence of *Spirometra mansoni* in wild and domestic animals from Costa Rica. *Veterinary Parasitology: Regional Studies and Reports* **51**. doi: 10.1016/j.vprsr.2024.101030.

Arrabal, JP, Pérez, MG, Arce LF and Kamenetsky, L (2020) First identification and molecular phylogeny of *Sparganum proliferum* from endangered felid (*Panthera onca*) and other wild definitive hosts in one of the regions with highest worldwide biodiversity *International Journal of Parasitology: Parasites and Wildlife* **13**, 142–149. doi: 10.1016/j.ijppaw.2020.09.002.

Bandelt, H, Forster, P and Röhl, A (1999). Median–joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**(1), 37–48.

Beveridge, I, Friend, SE and Jeganathan, N (1998) Proliferative sparganosis in Australian dogs *Australian Veterinary Journal* **76**, 757–759. doi: 10.1111/j.1751–0813.1998.tb12309.x

Bowles, J, Blair, D and McManus, DP (1995) A molecular phylogeny of the genus *Echinococcus. Parasitology* **110**(3):317–328. doi: 10.1017/S0031182000080902.

Brabec, J, Uribe, M, Chaparro-Gutíerrez, JJ and Hermosilla, C (2022) Presence of

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Spirometra mansoni, causative agent of sparganosis, in South America. *Emerging infectious diseases* **28**, 2347–2350. doi: 10.3201/eid2811.220529.

Buergelt, CD, Ellis, CG, Senior, DF (1984) Proliferative sparganosis in a cat. *The journal of parasitology.* 70 (1). https://www.jstor.org/stable/3281933.

Chávez-González, LE, Morales-Calvo, F, Mora, J, Solano-Barquero, A, Verocai, GG and Rojas, A (2022) What lies behind the curtain: Cryptic diversity in helminth parasites of human and veterinary importance. *Current Research in Parasitology & Vector-Borne Diseases* **2.** 1000094. https://doi.org/10.1016/j.crpvbd.2022.100094

Chervy L (2024) Manual for the study of tapeworm (Cestoda) parasitic in ray–finned fish, amphibians and reptiles. *Folia Parasitologica* **71,** 1–24. doi:10.14411/fp.2024.001.

Cobbold TS (1883) Description of *Ligula mansoni*, a new human cestode. *Zoological journal of the Linnean Society* **17**(98). 78–83. doi: 10.1111/j.1096–3642.1883.tb00237.x.

Conboy G (2009) Cestodes of dogs and cats in North America. *Veterinary Clinics: Small Animal Practice* **39** (6), 1075–1090. doi: 10.1016/j.cvsm.2009.06.005.

Conti JA (1984) Helminths of Foxes and Coyotes in Florida. *Proceedings of the Helminthological Society of Washington* **51**, 365–367.

Dikmans G (1931) Society Proceedings: Helminthological Society of Washington. *The Journal of Parasitology* **18**(1) 44–56.

Fredes, F, Mercado, R, Salas, IP, Sugiyama, H, Kobayashi H and Yamasaki, H (2022) Morphological observation and molecular phylogeny of *Spirometra decipiens* complex 1 (Cestoda: Diphyllobothriidae) found in cat from Chile. *Parasitology International* **87.** doi: 10.1016/j.parint.2021.102493.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Foster, GW, Cunningham, MW, Kinsella, JM, McLaughlin, G and Forrester DJ (2006) Gastrointestinal helminths of free–ranging Florida Panthers (*Puma concolor coryi*) and the efficacy of the current anthelmintic treatment protocol. *Journal of Wildlife Diseases* **42**(2), 402–406. doi: 10.7589/0090–3558–42.2.402

Giannelli, A, Schnyder, M, Wright, I and Charlier, J (2024) Control of companion animal parasites and impact on One Health. *One Health* **18.** 100679. doi: 10.1016/j.onehlt.2024.100679

Gompper, ME, Goodman, RM, Kays, RW, Ray, JC and Fiorello, CV (2003) A survey of the parasites of coyotes (*Canis latrans*) in New York based on faecal analysis. *Journal of Wildlife Diseases* **39**(3) 712–717. doi: 10.7589/0090–3558–39.3.712

Griffin, MP, Tompkins, KJ and Ryan, MT (1996) Cutaneous Sparganosis. *The American Journal of Dermatopathology* **18**(1), 70–72.

Harkema R and Miller GC (1964) Helminth parasites of the raccoon, *Procyon lotor* in the southeastern United States. *The Journal of Parasitology* **50**(1), 60–66.

Hawkins, RB, Feely, M, Saulino, D, and Raymond, SL (2024) Symptomatic cutaneous sparaganosis (tapeworm) in a child: A case report. *Journal of Pediatric Surgery Case Reports* **110.** doi: 10.1016/j.epsc.2024.102864.

Heidt, GA, Rucker RA, Kennedy, ML and Baeyena ME (1988) Hematology, intestinal parasites, and selected disease antibodies from a population of bobcats (*Lynx rufus*) in central Arkansas. *Journal of Wildlife Diseases* **24**(1), 180–183. doi: 10.7589/0090–3558–24.1.180

Hoggard, KR, Jarriel, DM, Bevelock, TJ, Verocai, GG (2019) Prevalence survey of gastrointestinal and respiratory parasites of shelter cats in northeastern Georgia, USA.

Veterinary Parasitology Regional Studies and Reports. **16.** 100270. doi: 10.1016/j.vprsr.2019.100270

Iwata S (1972) Experimental and morphological studies of Manson's tapeworm, *Diphyllobothrium erinacei*, Rudolphi. Special reference to its scientific name and relationship with *Sparganum proliferum*, Ijima. *Progress Medicine Parasitology Japan* **4**, 533–590.

Jeon, HK, Park, H, Lee, D, Choe, S, Sohn, WM and Eom, KS (2016) Molecular Detection of *Spirometra decipiens* in the United States. The *Korean Journal of Parasitology* **54**(4), 503–507. doi: 10.3347/kjp.2016.54.4.503.

Kikuchi T and Maruyama H (2020) Human proliferative sparganosis update. *Parasitology International* **7.** doi: 10.1016/j.parint.2019.102036.

Kikuchi, T, Dayi, M, Hunt, VL, Ishiwata, K, Toyoda, A, Kounosu, A, Sun, S, Maeda, Y, Kondo, Y, de Noya, BA, Noya, O, Kojima, S, Kuramochi, T and Maruyama, H (2021) Genome of the fatal tapeworm *Sparganum proliferum* uncovers mechanisms for cryptic life cycle and aberrant larval proliferation. *Communications Biology* **4**(1) doi: 10.1038/s42003–021–02160–8.

Kuchta, R, Scholz, T, Brabec, J and Narduzzi–Wicht, B (2015) *Diphyllobothrium*, *Diplogonoporus* and *Spirometra*. In: Xiao, L, Ryan, U, Feng, Y (Eds.). *Biology of Foodborne Parasites. Section III Important Foodborne Helminths.* CRC Press, Boca Raton, Florida, USA pp. 299–326.

Kuchta, R, Kołodziej–Sobocńska, M, Brabec, J, Młocicki, D, Sałamatin, R and Scholz, T (2021) Sparganosis (*Spirometra*) in Europe in the molecular era. *Clinical infectious diseases* **72,** 882–890. doi: 10.1093/cid/ciaa1036.

Kuchta, R, Phillips, AJ and Scholz, T (2024) Diversity and biology of *Spirometra* tapeworms (Cestoda: Diphyllobothriidea), zoonotic parasites of wildlife: A review. *International Journal for Parasitology: Parasites and Wildlife* **24** doi: 10.1016/j.ijppaw.2024.100947.

Kumar, S, Stecher, G, Li, M, Knyaz, C and Tamura, K (2018) Mega X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35** doi: 10.1093/molbev/msy096.

Li HC (1929) Life histories of *Diphyllobothrium decipiens* and *D. erinacei*. *American Journal of Hygiene* **10**, 527–550.

Li, MW, Song, HQ, Li, C, Lin, HY, Xie, WT, Lin, RQ, Zhu, XQ (2011) Sparganosis in mainland China. *International Journal of Infectious Diseases* **15**, 154–156. doi: 10.1016/j.ijid.2010.10.001

Liu, Q, Li, MW, Wang, ZD, Zhao, GH and Zhu, XQ (2015) Human sparganosis, a neglected food borne zoonosis. *The Lancet Infectious Diseases* **15**(10) 1226–1235. doi: 10.1016/S1473–3099(15)00133–4.

McHale, B, Callahan, RT, Paras, KL, Weber, M, Kimbrell, L, Velázquez–Jiménez, Y, McManamon, R, Howerth, EW and Verocai, GG (2020) Sparganosis due to *Spirometra* sp. (Cestoda; Diphyllobothriidae) in captive meerkats (*Suricata suricatta*). *International Journal for Parasitology: Parasites and Wildlife* **13**, 186–190. doi: 10.1016/j.ijppaw.2023.02.001.

Miyadera, H, Kokaze, A, Kuramochi, T, Kita, K, Machinami, R, Noya, O, B Alarcón de Noya, B, Okamoto, M, and Kojima, S (2001) Phylogenetic identification of *Sparganum proliferum* as a pseudophyllidean cestode by the sequence analyses on mitochondrial COI and nuclear *sdhB* genes. *Parasitology International* **50**, 93–104. doi: 10.1016/s1383–5769(01)00071–x.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Mueller JF (1935) A Diphyllobothrium from cats and dogs in the Syracuse region. *The Journal of Parasitology* **21**, 114–121.

Mueller JF (1937) A repartition of the genus *Diphyllobothrium*. *The Journal of Parasitology* **23**, 308–310.

Mueller JF (1938) The life history of *Diphyllobothrium mansonoides* Mueller, 1935, and some considerations with regard to sparganosis in the United States. *The American Journal of Tropical Medicine* **18**, 41–58.

Mueller, JF, Hart, EP and Walsh, WP (1963) Human sparganosis in the United States. *The Journal of Parasitology* **49**, 294–296.

Mueller JF (1974) The biology of *Spirometra*. *The Journal of Parasitology* **60**(1), 3–14.

Nagamori, Y, Payton, ME, Looper, E, Apple, H and Johnson, EM (2020) Retrospective survey of endoparasitism identified in faeces of client–owned dogs in North America from 2007 through 2018. *Veterinary Parasitology* **282**. doi: 10.1016/j.vetpar.2020.109137.

Petrigh, RS, Scioscia, NP, Denegri, GM and Fugassa, MH (2015) *Cox-1* gene sequences of *Spirometra* in Pampas foxes from Argentina. *Helminthologia* **52**(4), 355–359. DOI 10.1515/helmin–2015–0056.

Roncancio-Duque, N, García–Ariza, JE, Rivera-Franco N, Gonzalez-Ríos, AM and López-Alvarez (2024) Comparison of DNA quantity and quality from faecal samples of mammals transported in ethanol or lysis buffer. *One Health* **18**. doi: 10.1016/j.onehlt.2024.100731.

Rozas, J, Ferrer-Mata, A, Sánchez-Del Barrio, JC, Guirao-Rico, S, Librado, P, Ramos-Onsins, SE and Sánchez–Gracia, A (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Molecular Biology and Evolution* **34**(12) 3299–3302 https://doi.org/10.1093/molbev/msx248.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Rush, ER, Dale, E, and Aguirre, AA (2022) Illegal Wildlife Trade and Emerging Infectious Diseases: Pervasive Impacts to Species, Ecosystems, and Human Health. *Animals* **11**(6) https://doi.org/10.3390/ani11061821.

Scholz, T Kuchta, R and Brabec, J (2019) Broad tapeworms (Diphyllobothriidae), parasites of wildlife and humans: Recent progress and future challenges. *International Journal for Parasitology: Parasites and Wildlife* **9**, 359–369. https://doi.org/10.1016/j.ijppaw.2019.02.001.

Schaffer, GD, Davidson, WR, Nettles, VF and Rollor III, EA (1981) Helminth parasites of translocated raccoons (*Procyon lotor*) in the southeastern United States. *Journal of Wildlife Diseases* **17**(2). https://doi.org/10.7589/0090–3558–17.2.217

Serpell JA (2014) Domestication and history of the cat. In Turner, DC and Bateson, P (eds). *The Domestic Cat: The Biology of its Behavior 3rd edition.* Cambridge, UK: Cambridge University Press, pp. 83–99.

Sobotyk, C, Upton KE, Lejeune, M, Nolan, TJ, Marsh, AE, Herrin, BH, Borst, MM, Piccione, J, Zajac, AM, Camp, LE, Pulaski, CN, Starkey, LA, von Simson, C, Verocai, GG (2021) Retrospective study of canine endoparasites diagnosed by faecal flotation methods analysed across veterinary parasitology diagnostic laboratories, United States, 2018. *Parasites and Vectors* **14**, 1-10. https://doi.org/10.1186/s13071-021-04960-7

Tamura, K and Nei, M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10,** 512-526.

Taylor RL (1976) Sparganosis in the United States. *The American Journal of Clinical Pathology* **66**, 560–564.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Tokiwa, T, Fusimi, M, Chou, S, Yoshida, A, Kinoshita, K, Hikima, A, Kikuchi, T and Ozaki, K (2024) Aberrant sparganosis in cat caused by *Spirometra mansoni* (Cestoda: Diphyllothriidae): a case report. *BMC Veterinary Research* **20**(148). https://doi.org/10.1186/s12917–024–03995–z**.**

Tran, QR, Tran, MC and Mehanna, D (2019) Sparganosis: an under–recognized zoonosis in Australia*? BMJ Case Reports* **12.** doi:10.1136/bcr–2018228396

Uribe M, Brabec, J, Chaparro–Gutiérrez, JJ and Hermosilla, C (2023) Neglected zoonotic helminthiases in wild canids: new insights from South America. *Frontiers in Veterinary Science* **10**. doi 10.3389/fvets.2023.1235182.

Verocai, GG, Harvey, TV, Sobotyk, C, Siu, RE, Kulpa, M and Connolly, M (2023) *Spirometra* infection in a captive Samar cobra (*Naja samarensis*) in the United States: An imported case? *International Journal for Parasitology: Parasites and Wildlife* **20** 133–137. https://doi.org/10.1016/j.ijppaw.2023.02.001.

Vettorazzi, R, Norbis, W, Martorelli, SR, García, G, Rios, N (2023) First report of *Spirometra* (Eucestoda; Diphyllobothriidae) naturally occurring in a fish host. *Folia Parasitologica* **70,** doi**:** 10.14411/fp.2023.008.

Waeschenbach, A, Brabec, J, Scholz, T, Littlewood, DTJ and Kuchta, R (2017) The catholic taste of broad tapeworms–multiple routes to human infection. *International Journal for Parasitology*. **47**(13), 831–843. http://dx.doi.org/10.1016/j.ijpara.2017.06.004

Woldemskel M (2014) Subcutaneous sparganosis, a zoonotic cestodiasis, in two cats. *Journal of Veterinary Diagnostic Investigation.* 26(2). https://doi.org/10.1177/10406387135176.

Wright I, Jongejan F, Marcondes M, Peregrine A, Baneth G, Bourdeau P, Bowman DD, Breitschwerdt EB, Capelli G, Cardoso L, Dantas-Torres F, Day MJ, Dobler G, Ferrer

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

L, Gradoni L, Irwin P, Kempf VAJ, Kohn B, Krämer F, Lappin M, Madder M, Maggi RG, Maia C, Miró G, Naucke T, Oliva G, Otranto D, Pennisi MG, Penzhorn BL, Pfeffer M, Roura X, Sainz A, Shin S, Solano-Gallego L, Straubinger RK, Tasker S, Traub R, Little S. Parasites and vector-borne diseases disseminated by rehomed dogs. *Parasites and Vectors* **13**:546. doi: 10.1186/s13071-020-04407-5.

Wu, TK, Kaneko, S, Lucio-Forster, A, Spagnoli, S, Schultz-Powell, L, Liotta, J and Bowman, D (2024) Cestodiasis in 2 Puerto Rican crested anoles. *Journal of Veterinary Diagnostic Investigation* **36**(2). 258–261. doi: 10.1177/10406387241229072.

Wyrosdick, HM, Chapman, A, Martinez, J, Schaefer, JJ, (2017) Parasite prevalence survey in shelter cats in Citrus County, Florida. *Veterinary Parasitology Regional Reports and Studies* **10**, 20–24. https://doi.org/10.1016/j.vprsr.2017.07.002

Yamasaki, H, Sanpool, O, Rodpai, R, Sadaow, L, Laummaunwai, P, Un, M, Thanchomnang, T, Laymanivong, S, Aung, WPP and Intapan, PM (2021) *Spirometra* species from Asia: genetic diversity and taxonomic challenges. *Parasitology International* **80**. https://doi.org/10.1016/j.parint.2020.102181.

Yamasaki, H, Sugiyama, H, Morishima, Y and Kobayashi, H (2023) Description of *Spirometra asiana* sp. nov. (Cestoda: Diphyllobothriidae) found in wild boars and hound dogs in Japan. *Parasitology International* **98**. https://doi.org/10.1016/j.parint.2023.102798.

Zajac, AM, Conboy, GA, Little, SE, Reichard, MW (2021) Veterinary Clinical Parasitology, 9th Edn. New Jersey, USA: John Wiley & Sons, Inc.

Zimmermann, J, Hajibabaei, M, Blackburn, DC, Hanken, J, Cantin, EL, Posfai, J and Evans Jr, TC (2008) DNA damage in preserved specimens and tissue samples: a molecular assessment. *Frontiers in Zoology* **5**(18). https://doi.org/10.1186/1742–9994–5–18.

https://doi.org/10.1017/S003118202500054X Published online by Cambridge University Press

Figure 1. Maximum likelihood tree inferred from partial *cox1* gene sequences of *Spirometra* samples from this study and related taxa. Sequences from this study are denoted by a solid black circle (•). The best substitution model used was Tamura-Nei + Gamma distribution. *Schistocephalus solidus* was used as outgroup. (AUS – Australia; ARG – Argentina; BO – Bolivia; BRA – Brazil; CHL – Chile; CHI – China; COL – Colombia; CR – Costa Rica; ETH – Ethiopia; FIN – Finland; INDO – Indonesia; IND – India; IRA – Iran; KOR – Korea; JPN – Japan; NZE – New Zealand; PR – Puerto Rico; POL – Poland; RUS – Russia; SSU – South Sudan; TZN – Tanzania; UKR – Ukraine; USA – United States of America; VEN – Venezuela; VNM – Vietnam; CT – Connecticut; FL – Florida; GA – Georgia; IL – Illinois; ID – Idaho; IN – Indiana; KS – Kansas; LA – Louisiana; MA – Massachusetts; MD – Maryland; MN – Minnesota; NJ – New Jersey; NH – New Hampshire; NY – New York; PA – Pennsylvania; SC – South Carolina; TN – Tennessee; TX – Texas; WI – Wisconsin).

Figure 2. Distribution of *Spirometra* species in the United States of America. **A.** Distribution of *Spirometra* sp. 2. **B.** Distribution of *Spirometra* sp. 3. **C.** Distribution of *Spirometra mansoni*. **D.** Historical distribution of molecularly confirmed cases in the USA with pictograms of host species.

Figure 3. Median-joining haplotype network of *Spirometra mansoni* isolates. A total of 124 sequences, 83 from this study and 41 from GenBank, were included for analysis. The size of the circle corresponds to the number of sequences belonging to each haplotype. The network is color-coded to represent the geographical origins of samples within each haplotype.

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Table 1. Geographic distribution and species identity of samples (n=136) included in the phylogenetic analysis. Percentages are defined as the proportion of samples from a particular state belonging to dog, cat, and other* hosts and the *Spirometra* spp. identified. Other* includes samples from a serval (*Leptailurus serval*), a green iguana (*Iguana iguana*), a White's tree frog (*Litoria caerulea*), and a Cuban knight anole (*Anolis equestris*).

Origin	Total (n of samples)	*S. mansoni*			*Spirometra* sp. 2	*Spirometra* sp. 3
		Dog	Cat	Other*	Dog	Cat
Connecticut	5	80% (n=4)	NA	NA	NA	20% (n=1)
Florida	59	12% (n=7)	64% (n=38)	5% (n=3)	5% (n=3)	13% (n=8)
Georgia	2	NA	NA	50% (n=1)	NA	50% (n=1)
Idaho	3	NA	33% (n=1)	NA	NA	67% (n=2)
Illinois	3	NA	33% (n=1)	NA	NA	67% (n=2)
Indiana	3	NA	67% (n=2)	NA	NA	33% (n=1)
Louisiana	2	NA	50% (n=1)	NA	NA	50% (n=1)
Massachusetts	8	NA	25% (n=2)	NA	NA	75% (n=6)
Maryland	4	NA	100% (n=4)	NA	NA	NA
Minnesota	4	NA	50% (n=2)	NA	NA	50% (n=2)
New Hampshire	6	17% (n=1)	67% (n=4)	NA	NA	17% (n=1)
New Jersey	4	NA	50% (n=2)	NA	NA	50% (n=2)
New York	1	NA	NA	NA	NA	100% (n=1)

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Pennsylvania	5	40% (n=2)	40% (n=2)	NA	NA	20% (n=1)
South Carolina	7	NA	43% (n=3)	NA	NA	57% (n=4)
Tennessee	3	NA	NA	NA	NA	100% (n=3)
Texas	12	17% (n=2)	75% (n=9)	NA	NA	8% (n=1)
Wisconsin	4	NA	NA	NA	NA	100% (n=4)
Puerto Rico	1	NA	100% (n=1)	NA	NA	NA

Figure 1:

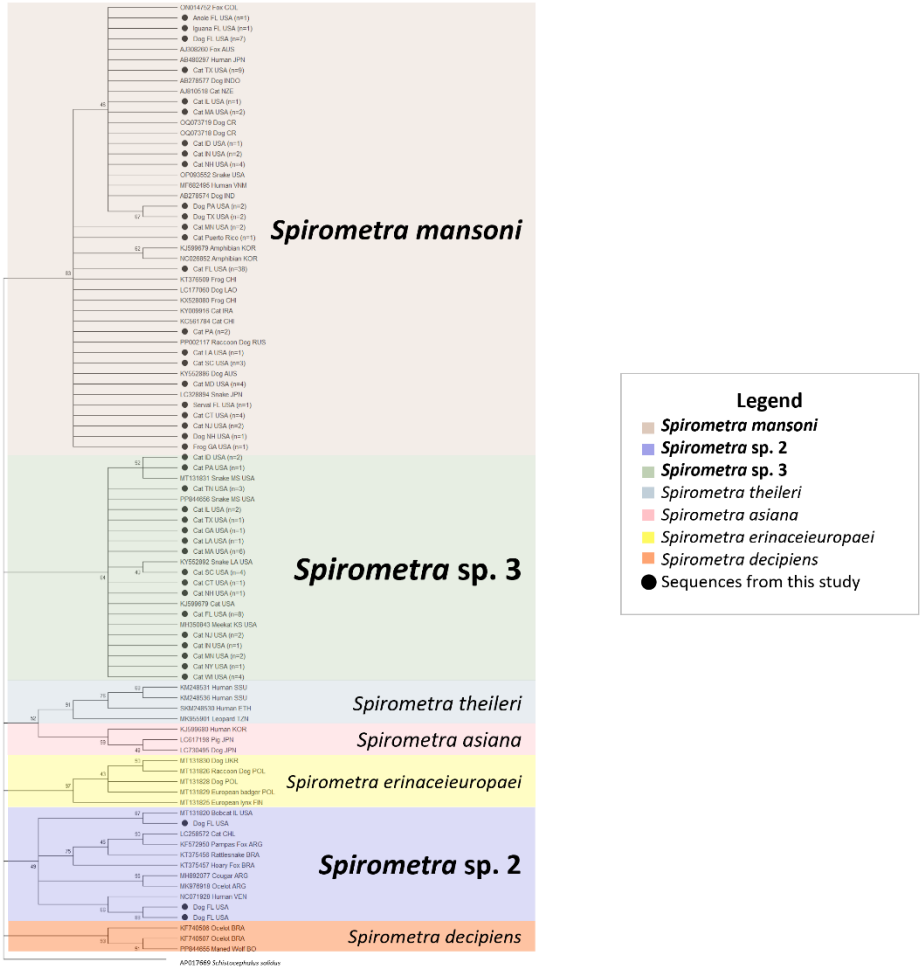

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Figure 2:

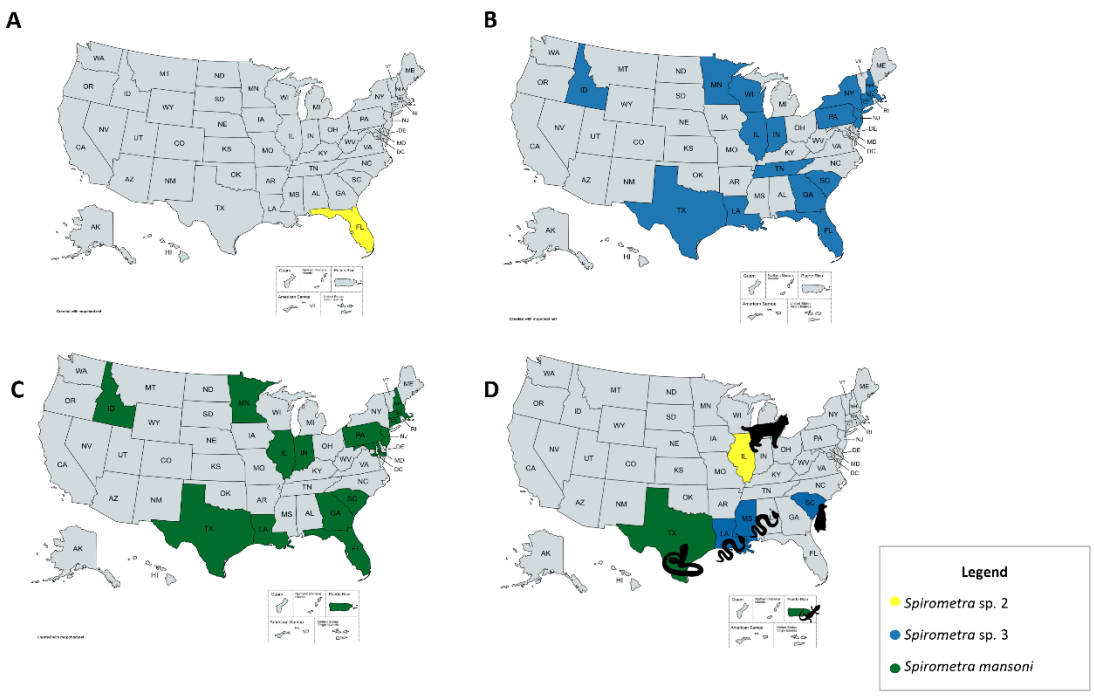
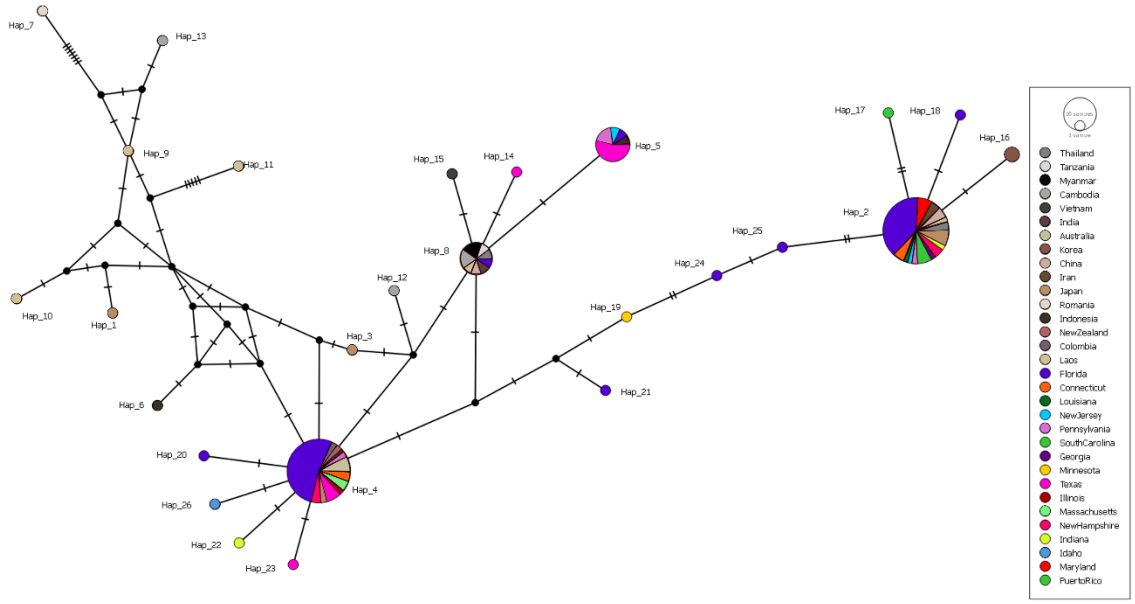


Figure 3:



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