Original Article

Molecular, morphological and histopathological evidence of *Spirometra mansoni* in wild and domestic animals from Costa Rica

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ABSTRACT

Spirometra mansoni is a diphyllobothroid cestode and one of the causing agents of sparganosis, a zoonotic foodborne and waterborne infection in humans. This parasite has an indirect life cycle with domestic and wild canids or felids as definitive hosts. The last report of *S. mansoni* in Costa Rica was done in 2004 by morphological assessment of worms, whereas molecular evidence of this species was obtained recently in the Americas. Herein, we present seven cases of spirometrosis in four dogs, three cats and a coyote from different regions of Costa Rica occurring in a time span of a year. Dog cases presented vomiting, hyporexia, lethargy and diarrhea, whereas cats were mostly asymptomatic. Moreover, the coyote was found with *Spirometra* sp. proglottids incidentally. Cytochrome oxidase subunit 1 (*cox*1) sequences of eggs or proglottids derived from all cases were analyzed with a Bayesian Inference phylogenetic tree and a haplotype network. These analyses showed the clustering of *S. mansoni* from Costa Rica with other sequences derived from Asia and America. Moreover, *cox*1 sequences clustered in two separate haplotypes, suggesting the high genetic diversity of the species. The present cases represent the first molecular evidence of the parasite in Central America; thus, extending its known range in the American continent.

1. Introduction

Spirometrosis is a parasitosis of canids and felids caused by adult stages of different *Spirometra* spp. (Cestoda: Diphyllobothriidea). The development into the infecting stage requires two hosts: freshwater copepod crustaceans as first intermediate hosts and vertebrates, like frogs and snakes, as second intermediate hosts. In addition, paratenic hosts like wild boars, raccoons and other wild animals increase the dispersion of the parasite in the environment (Kolodziej-Sobocinska et al., 2016; Schaffer et al., 1981). Domestic and wild canids and felids act as definitive hosts and become infected by predation of secondary intermediate or paratenic hosts with plerocercoids (Liu et al., 2015).

Spirometrosis in dogs or cats remain subclinical in most cases, although diarrhea, enteritis, weight loss and vomiting have been reported during infection (Bowman, 2014).

Humans act as accidental, dead-end hosts in the life cycle of *Spirometra* spp., where the plerocercoid stage causes sparganosis, a neglected foodborne and waterborne zoonotic parasitic disease. Infection occurs by the ingestion of raw or undercooked flesh of second intermediate or paratenic host species or by drinking water contaminated with infected first intermediate hosts, i.e. copepods containing procercoids. Placing raw infected frog meat to wounds and sore eyes as a traditional poultice has also been described as an infection route for humans (Bowman, 2014; Kuchta et al., 2021; Liu et al., 2015).

Clinical signs of human sparganosis vary widely and depend on the anatomical region where larvae migrate. The most common manifestations include subcutaneous nodules, lesions in the brain, spinal cord, eyes, muscles, and other internal organs, and a proliferative form of the parasite in humans has also been described (Kim et al., 2018; Kuchta et al., 2021).Sparganosis has been described worldwide and is recognized as a zoonotic infection in Asia, Europe and, in less extent, in the Americas (Alvarez et al., 2022; Beltran Fabian et al., 2015; Jones et al., 2013). However, the classification of *Spirometra* spp. and their geographical distribution have been controversial. Moreover, diagnosis to species based on parasite morphology is currently considered inaccurate due to the presence of conserved morphological characters, high intra-specific variability, and lack of consensus regarding species delimitation (Kuchta et al., 2021). Thus, molecular diagnosis using PCR is currently considered the only method for reliable species assignation (Jeon et al., 2018; Scholz et al., 2019).

Six well-defined species have been proposed based on phylogenetic analysis, including *Spirometra mansoni, Spirometra folium, Spirometra erinaceieuropaei, Spirometra decipiens* complex 1 and 2, and an additional uncharacterized *Spirometra* species (Kuchta et al., 2021). Isolates circulating in the Americas have been phylogenetically grouped into three genetic lineages: *S. decipiens* complex 1 and 2, and more recently, *S. mansoni*. The latter was detected in a crab-eating fox from Colombia (Brabec et al., 2022), which confirmed the presence of *S. mansoni* in South America. A case of *S. mansoni* was also molecularly detected in a Samar cobra imported to the United States from the Phillipines (Verocai et al., 2023). However, circulation of the parasite in North America has not yet been determined yet, due to the high likelihood the latter case was imported. Moreover, *Spirometra* sp. eggs have been found in cats from Brazil (Silva et al., 2023), thus highlighting active circulation in domestic animals.

In Costa Rica, *S. mansoni* was reported for the first time in two cats from Guanacaste and Alajuela in 2004 (Valerio et al., 2004) using morphological methods. Since then, no other cases have been detected in the country. Herein, we report seven cases from 2022 to 2023, in domestic dogs and cats as well as a coyote from several geographical locations of the country, diagnosed to the species level by using molecular techniques.

2. Case presentation

2.1. Cases 1 and 2

In May 2022, a 3-month-old female Dachshund dog from Santa Ana, San Jose (Fig. 1), was referred to a veterinary clinic due to acute onset of vomiting and diarrhea. The patient lived with three more dogs and a cat that did not present any clinical manifestations at that time. They lived indoors and outdoors, with access to a fenced yard consisting of a small area of standing water. Owners reported their animals hunting frogs frequently. In addition, other animals, such as toads, snakes, and opossums, were frequently observed in the property.

The results of the physical examination were unremarkable, besides mild dehydration. An antigen test for parvovirus and canine coronavirus (Anigen Rapid CPV/CCV Ag®; Bionote, Gyeonggi, South Korea) tested negative. Initial treatment with Metoclopramide (Pharmotil ®; Pet's Pharma), meloxicam (Meloxivet®; Pet's Pharma, Mexico City, Mexico), and long-acting amoxicillin (Amoxicilina LA®; Bayer, Norbrook Laboratories, Northern Ireland, United Kingdom) were prescribed, with no improvement.

Three fecal samples collected on consecutive days were sent for coproscopic examination to Diagnóstico Albeitar laboratories and *Spirometra* spp. eggs (Fig. 2a-d) and *Giardia* sp. cysts were observed in a direct smear and zinc sulfate centrifugal flotation technique, respectively. Samples were later submitted for DNA extraction and PCR (Isolate K, Table 1).

Anthelmintic treatment with a combination oral product (One®; Bio Zoo, Jalisco, Mexico) containing fenbendazole (50 mg/kg), toltrazuril (15 mg/kg), and praziquantel (5 mg/kg) once a day for four days, and metronidazole (25 mg/kg), p.o., twice daily (Metronidazol ®; Lisan, San José, Costa Rica) for seven days was established.

In September 2022, the dog was re-evaluated. No abnormalities were detected at physical examination, but the owner reported intermittent loose stool deposition. The patient and the other dogs in the same household were examined for *Spirometra* sp. eggs by concentration with sedimentation at the University of Costa Rica. The dog did not present any parasites; however, hookworm eggs were observed in all three other dogs, and one of them presented *Spirometra* eggs (Case 2, Table 1). No samples from the cat could be obtained, despite its owner reporting intermittent vomiting of adult worms.

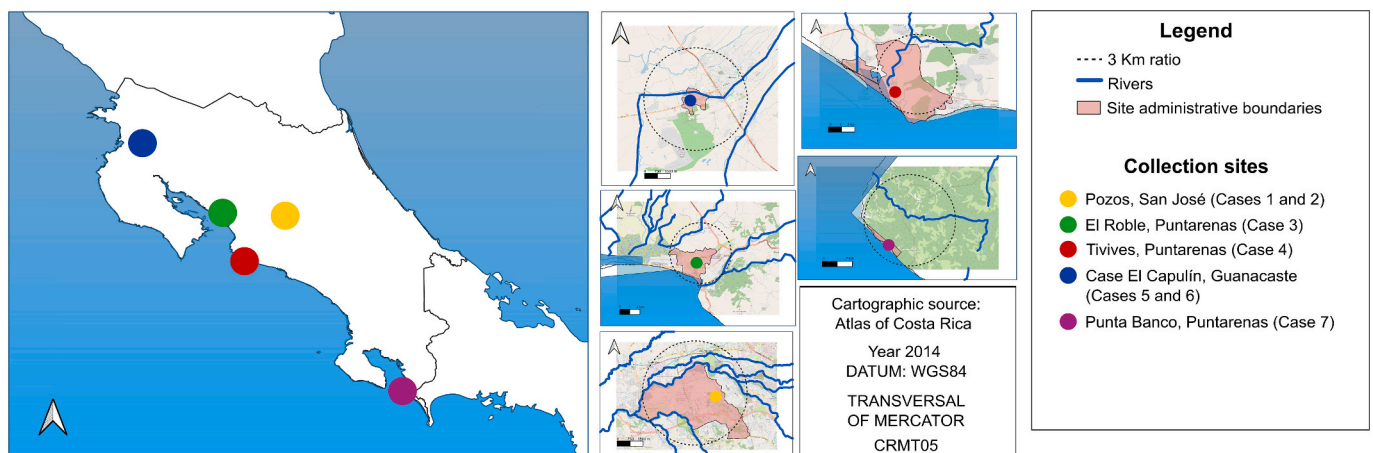All dogs from the household were dewormed with a combination oral

Fig. 1. Distribution of *Spirometra* cases in Costa Rica overlaid with the main river branches of the country. This map was generated with QGIS.

Legend

- - - - 3 Km ratio
——— Rivers
▨ Site administrative boundaries

Collection sites

🟡 Pozos, San José (Cases 1 and 2)
🟢 El Roble, Puntarenas (Case 3)
🔴 Tivives, Puntarenas (Case 4)
🔵 Case El Capulín, Guanacaste (Cases 5 and 6)
🟣 Punta Banco, Puntarenas (Case 7)

Cartographic source:
Atlas of Costa Rica
Year 2014
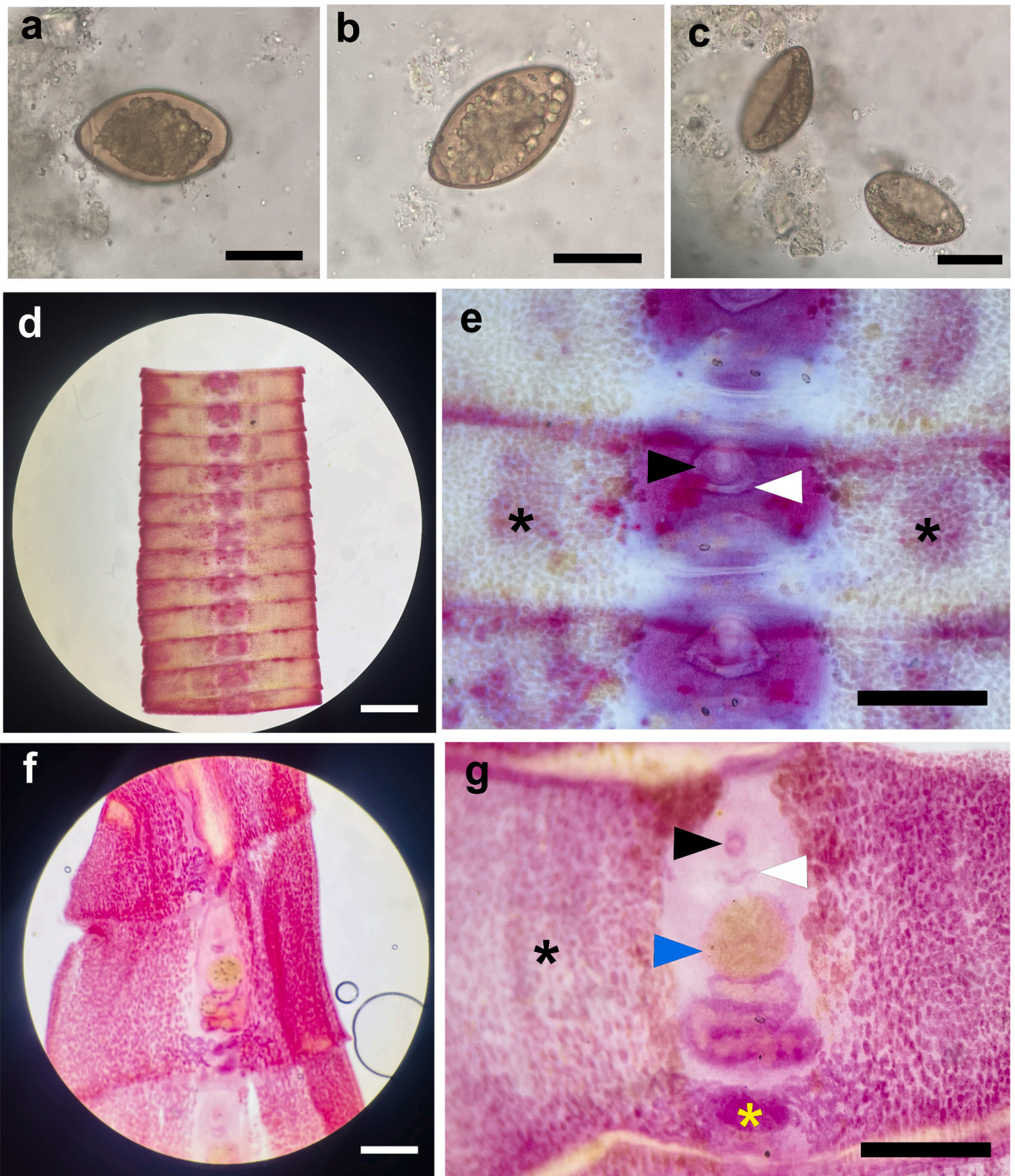DATUM: WGS84
TRANSVERSAL OF MERCATOR
CRMT05

Fig. 2. *Spirometra mansoni* parasitic stages collected from dogs, cats and a coyote. **A-B**. Eggs with typical morphology collected from fecal samples and observed in a light microscope with 400× magnifications (bar = 30 μm). **C.** Eggs with atypical morphology or observed from a different angle collected from fecal samples and observed in a light microscope with 400× magnifications (bar = 30 μm). **D.** Immature proglottids stained with hydrochloric Carmin collected from Case 7 (bar = 3 mm). **E.** Close-up of immature proglottid showing the genital pore (black arrowhead), uterine pore (white arrowhead) and vitelline glands (asterisks) (bar = 0.75 mm). **F.** Mature proglottids stained with hydrochloric Carmin collected from Case 7 (bar = 3 mm). **G.** Close-up of mature proglottid showing the genital pore (black arrowhead), uterine pore (white arrowhead), coiled uterus filled with eggs (blue arrowhead), ovary (yellow asterisk) and vitelline glands (black asterisk) (bar = 0.75 mm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

*cox*1 sequences obtained from *S. mansoni* of domestic dogs and cats from Costa Rica.

Case	Isolate ID	Sample type	Accession number	Host	Locality
1	K	Feces	OQ073718	*C. l. familiaris*	San José, Costa Rica
2	R	Feces	OQ073719	*C. l. familiaris*	San José, Costa Rica
2	R	Proglottid	OQ073720	*C. l. familiaris*	San José, Costa Rica
3	Tv	Proglottid	OQ695655	*F. catus*	Puntarenas, Costa Rica
4	Pn	Proglottid	OQ695656	*F. catus*	Puntarenas, Costa Rica
5	1118	Feces	OQ990499	*F. catus*	Guanacaste, Costa Rica
6	1124	Feces	OQ990500	*C. l. familiaris*	Guanacaste, Costa Rica
7	CY	Proglottid	OR814168	*C. latrans*	Osa, Costa Rica

product (One®; Bio Zoo, Jalisco, Mexico) containing fenbendazole (50 mg/kg), toltrazuril (15 mg/kg), and praziquantel (5 mg/kg) once a day for four days. The dog of Case 2 vomited an adult worm a few days after the antiparasitic treatment was initiated. The worm was preserved in 70% ethanol for molecular identification and a fecal sample was analyzed at the University of Costa Rica (Isolate R, Table 1). No follow-up on any of the animals was reported.

2.2. Case 3

In September 2022, fecal samples were collected from six free-ranging domestic shorthaired cats of unknown clinical history, captured near the El Roble prison in Puntarenas (Fig. 1). Direct fecal smear, Sheather's sugar flotation, and sedimentation were performed in fecal samples for five consecutive days. *Spirometra* spp. eggs were identified in five of the six (5/6) cats by using the three techniques. Coinfections with hookworms (4/5) and *Platynosomum* sp. (5/5) eggs were observed.

Four of the cats were euthanized due to concomitant pathology with

leukemia virus (3/5) and microfilariae (1/5). Cadavers were submitted to the Pathology Laboratory of the San Francisco de Asís School for necropsy and histopathologic examination. Histological sections from the small intestine stained with hematoxylin and eosin showed slight hyperplasia of the mucosa's goblet cells with a moderate mixed inflammatory infiltrate on the lamina propria (Fig. 3a). Several proglottids were identified in the intestinal lumen, described as irregular parenchymal-like structures surrounded by an eosinophilic stained tegumentary layer. Below this layer, longitudinally and circularly aligned muscle fibers were observed, along with calcareous corpuscles, vitellin glands, and a genital pore, continued with a gravid uterus containing numerous *Spirometra* spp. eggs (Fig. 3b). Collected parasites were fixed in 70% ethanol and submitted to the Laboratory of Helminthology at the University of Costa Rica, where DNA extraction and PCR were performed (Isolate Pn, Table 1).

The not-euthanized *Spirometra*-positive cat was dewormed with a combination oral product (Ovistop ®; Lisan, San José, Costa Rica) containing praziquantel (37.5 mg/kg), mebendazole (100 mg/kg), and pyrantel pamoate (100 mg/kg), with repeated doses 15 days later. A fecal exam was run after the second dose which resulted in no *Spirometra* sp. or hookworm eggs, but remained positive for *Platynosomum* sp., and was euthanized two months later due to severe cholangiohepatitis. At necropsy, *Platynosomum illiciens* and *Dipylidium caninum* adults were recovered in the liver and intestine, respectively. No evidence of remaining *Spirometra* parasites was found.

2.3. Case 4

In January 2023, a 6-month-old domestic short-haired cat from Tivives, Puntarenas, was taken to a veterinary clinic to remove stitches from a prior surgery. No signs of disease were reported by owners, and no findings were revealed during physical examination. The cat lived on a large, fenced property near a stream and a shoreline. Hunting activity was reported in the cat, with evidence of rodent consumption. Sedation with xylazine (0.5 mg/kg, i.m; Xilazina 2%®; Alfasan, Utrecht, The Netherlands) and ketamine (1.25 mg/kg, i.m; Ketamina 50®; Holliday Scott, Buenos Aires, Argentina) was performed to facilitate handling, which led to immediate retching and expelling of an adult worm.
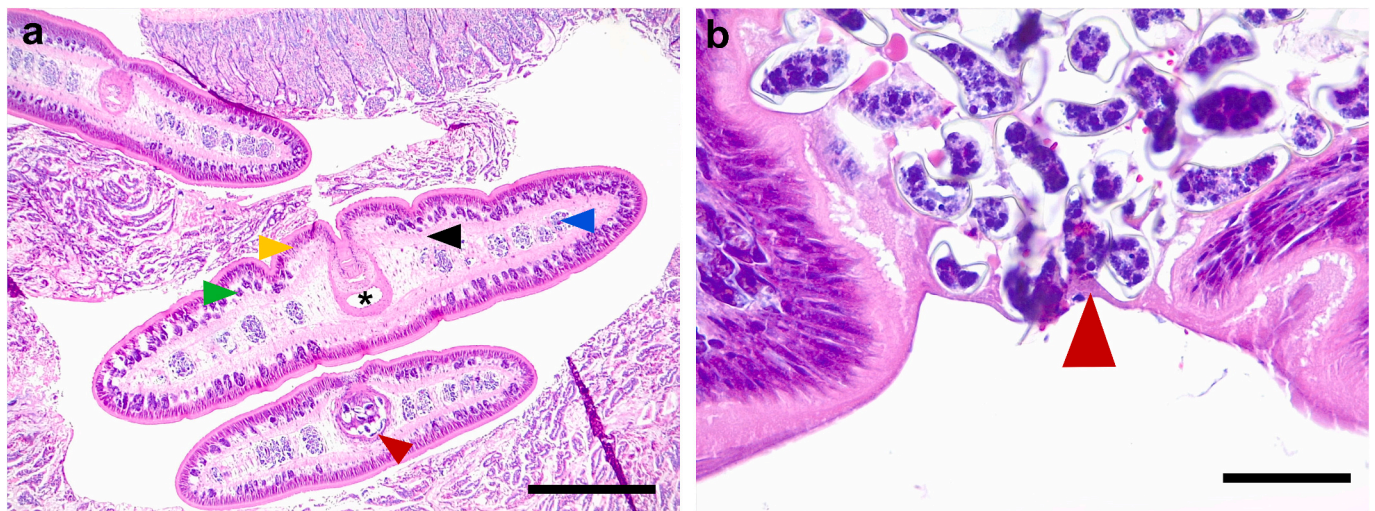

Fig. 3. Histopathology sections stained with hematoxylin and eosin of the small intestine of Case 3. **A.** Mild hyplerplasia of the mucosal goblet cells is observed, with presence of a mild to moderate inflammatory infiltrate composed of macrophages, lymphocytes, plasma cells and eosinophils located in the lamina propria. In the lumen there are multiple parasitic structures. Some of them are compatible with proglottids that correspond to irregular structures of parenchymal aspect surrounded by an acidophilic tegument. Under the tegument there are longitudinal (yellow arrowhead) and circular (black arrowhead) muscle fibers, among which there are basophilic staining concretions compatible with calcareous corpuscles, vitelline glands (green arrowhead), testis (blue arrowhead), and a centrally located genital pore (black asterisk) that continues with a uterus (red arrowhead) containing numerous morulated eggs that exhibit an operculum on one of its poles compatible with *Spirometra* spp. (Magnification at 40×, bar = 500 μm). **B.** Close up of the uterus of a mature proglottid containing numerous eggs (red arrowhead) (Magnification at 400×, bar = 50 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The parasite was collected in 70% ethanol and submitted to the Parasitology Laboratory of the School of Veterinary Medicine, National University of Costa Rica, where eggs were released from a proglottid and identified as *Spirometra* sp. (Fig. 2a-d). The rest of the parasite was sent to the Laboratory of Helminthology at the University of Costa Rica for molecular characterization (Isolate Tv adult worm).

The cat was dewormed with an oral combination product (Ovistop ®; Lisan, San José, Costa Rica) containing praziquantel (7.5 mg/kg), mebendazole (20 mg/kg), and pyrantel pamoate (20 mg/kg) with a repeated dose after 15 days. No record of the patient's return to consultation, coproscopy analyses, or new incidents of the expulsion of adult worms were reported.

2.4. Cases 5 and 6

In February 2023, a 4-month-old male Dachshund dog from Liberia, Guanacaste, received veterinary attention due to the acute onset of hyporexia, lethargy and one episode of vomiting and deposition of liquid stools, with the expulsion of an adult worm. Vaccination status was up to date, and the last anthelmintic treatment was administered four weeks prior to the onset of signs, with a combination product (Endogard®, Ipanema Indústria de Produtos Veterinários, São Paulo, Brazil) containing praziquantel (18.5 mg/kg), pyrantel pamoate (53.28 mg/kg), ivermectin (0.02 mg/kg) and febantel (55.5 mg/kg). On physical examination, the patient presented abdominal pain, enlarged submandibular lymph nodes, and mild hyperthermia of 39.4 °C. A single dose of maropitant (1 mg/kg, s.c, Cerenia ®; Zoetis Manufacturing & Research, Girona, Spain) was injected, and an oral prebiotics formula (Reinforces®; Viyo, Antwerp, Belgium) was prescribed.

The dog lived with another dog having mostly indoor habits, and a cat with reported hunting and unsupervised outdoor activity. These two other animals did not show any clinical signs of infection. Fecal samples from the patient and the household cat were sent to the Diagnóstico Albéitar laboratories for coproscopic examination, including a direct smear and zinc sulfate centrifugal flotation. Fecal samples could not be obtained from the other dog in the household. *Spirometra* sp. eggs were identified in a direct smear in both animals; and were submitted to the Laboratory of Helminthology of the University of Costa Rica for DNA extraction and molecular description.

Both animals were initially dewormed with a combination oral product (Albendazole + Prazicuantel®; Calox, San José, Costa Rica) at a dose of praziquantel (15 mg/kg) and albendazole (43.4 mg/kg). Anthelmintic therapy was repeated 15 days later at a dose of praziquantel (7.5 mg/kg) and albendazole (21 mg/kg) once daily for two days in the dog, and praziquantel (30 mg/kg) and albendazole (86.8 mg/kg) once daily in the cat.

No other gastrointestinal signs or excretion of adults were reported after treatment. Both animals were negative for *Spirometra* sp. eggs in three serial samples evaluated by direct smea three weeks after the second anthelmintic dose.

2.5. Case 7

In May 2023, a juvenile female coyote (*Canis latrans*) was received from Punta Banco, Osa, Puntarenas, the animal was rescued by people who claimed to have found it alone in a farm. They kept it for five days in their house, in contact with domestic dogs, until it was transferred to Rescate Wildlife Rescue Center, Alajuela, by government officials. The animal had a poor body condition, seborrhea, seborrheic alopecia, generalized lymphadenomegaly and was non-ambulatory. Because of clinical conditions suspicious of scabies, the animal was given oral Fluralaner at a dose of 33.3 mg/kg (Bravecto® Intervet GmbH, Vienna, Austria). The animal also received 25 mg/kg of metronidazole (Metronidazole®; Lisan, San José, Costa Rica) and amoxicillin at 12.5 mg/kg with clavulanic acid at 12.5 mg/kg (Clavaseptin®; Vetoquinol, Lure, France) because of severe leukocytosis and diarrhea. Screening tests for

Canine Distemper Virus were negative (Bionote®; Gyeonggi, South Korea).

One month later, a fecal sample was analyzed by flotation using Sheater's sugar solution, which was positive for *Capillaria* sp. and hookworms. Therefore, 0.15 mg/kg of ivermectin and 2.5 mg/kg of praziquantel (Sarnil CES®; Unimedical, Montevideo, Uruguay) were administered. Since there was no clinical improvement, the patient was humanely euthanized. Minutes prior to sedation, the animal defecated an almost complete *Spirometra* sp. adult. The parasite strobila was collected from the animal's large intestine in 70% ethanol for morphological and molecular assessment in the Laboratory of Helminthology of the University of Costa Rica. Immature (Fig. 2e and f) and mature (Fig. 2g and h) proglottids were stained with hydrochloric Carmin according to previous protocols (Castro and Guerrero, 2006) and DNA was extracted from one proglottid.

Necropsy of the animal was done, and lymphoid hyperplasia was found in mesenteric, iliac, submandibular, prescapular and popliteal lymph nodes as found in histopathological analysis. In addition, focal suppurative pneumonia and generalized cutaneous hyperkeratosis presumptively caused by *Malassezia pachydermatis* were found in those observations.

3. Materials and methods

3.1. Morphological analysis

Coprological methods were performed as previously described (Castro and Guerrero, 2006) for all cases except for case 4. Moreover, eggs were morphologically identified as *Spirometra* sp. according to published descriptions (Bowman, 2014). In case 4, eggs released from a proglottid were microscopically assessed. In addition, employed techniques for each case differed according to the primary laboratory that identified cases: direct fecal smears were done in cases 1, 3, 5, and 6. Flotations were run in cases 1, 3, 5, 6, and 7, with zinc sulfate as a flotation agent was used in cases 1, 5, and 6 and Sheather's sugar solution in cases 3 and 7. Concentration by sedimentation was performed for cases 1, 2 and 3. Immature proglottids obtained in case 7 were stained with hydrochloric Carmin as described by Castro and Guerrero (2006) and the taxonomically relevant structures were reviewed according to dichotomous keys for cestodes (Khalil et al., 1994).

3.2. DNA analysis

DNA was extracted from fecal samples or proglottids (Table 1) using the QIAamp Fast DNA stool kit and DNeasy Blood & Tissue kit (Qiagen®; Hilden, Germany), respectively, following the instructions of the manufacturer with some modifications previously described (Rojas et al., 2017). Then, PCRs targeting a 395 bp fragment of the cytochrome oxidase subunit 1 (*cox*1) were run using primers JB3 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′) and JB4.5 (5′- TAAAGAAAGAACATAATGAAAATG-3′) (Bowles et al., 1995) using modifications described before (Rojas et al., 2018). Amplicons were verified in 1.5% agarose gels stained with SYBR Safe® (Invitrogen, Massachusetts, Unites States) and positive reactions were Sanger sequenced (Macrogen®; Seoul, South Korea) using primers in both directions with the Big-Dye Terminator cycle sequencing chemistry (Applied Biosystems ABI3700 DNA analyzer) and ABI's Data Collection and Sequence Analysis software (Applied Biosystems, Thermo Fisher Scientific Inc., Waltham, USA).

Obtained sequences were deposited in Genbank (Table 1) and aligned together with other *Spirometra* spp. sequences publicly available. The best nucleotide substitution model was determined using MEGA 11.0. Then, a Bayesian Inference (BI) phylogenetic tree was built with the BEAST package (Drummond and Rambaut, 2007) using *Dibothriocephalus latus* (accession number: AB269325) as an outgroup. Accordingly, 10^8 Markov-Monte Carlo chains were run with 10% burn-

in length. Effective sample sizes were verified in Tracer (https://beast.community/tracer) and all priors were confirmed to be greater than 300. The generated phylogenetic trees were summarized in TreeAnotator (https://beast.community/treeannotator) and the consensus tree was visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) annotating country and host origin for each sequence. In addition, a Templeton-Crandall-Sing haplotype network was drawn using PopART software (http://popart.otago.ac.nz) with 95% connection limit.

4. Results

4.1. Morphological analysis

Collected eggs were operculated, brown in coloration, 60 to 70 μm long and 30–40 μm wide. Eggs were wider in their equatorial portion. The operculum was located in one distal end of the egg and was flat-shaped (Figs 2a and 2b). In addition, depending on the position of the egg, some could be observed as elliptical, whereas others were more rounded or convex (Fig. 2c). Morulated structures were observed in the interior of eggs.

Morphology of immature and mature proglottids were consistent with *S. mansoni* according to classification keys (Khalil et al., 1994). Immature proglottids were rectangular, the genital and uterine pores were observed, as well as vitelline glands (Fig. 2d and e). However, testis, ovary or uterus could not be distinguished. Moreover, mature proglottids were trapezoidal (Fig. 2f) and showed a coiled uterus filled with eggs located at the medial portion, an ovary below the uterus and abundant vitelline glands in both sides of the proglottid (Fig. 2g).

4.2. Molecular analysis

The seven sequences obtained from all cases were confirmed as *S. mansoni* based on phylogenetic and haplotype network analyses (Fig. 4). BI tree showed that sequences from different geographical locations were intermixed among them without any sign of clustering according to geographical region or host. For instance, three *S. mansoni* sequences were retrieved from cases 1 and 2 which belonged to the same household. These three sequences were separated in the BI tree by sequences from Cambodia, China, and Japan. In addition, sequences of cases 5 and 6 of the same household were separated between them by *S. mansoni* of South Korea and China. Additionally, higher level branches, i.e. among *S. mansoni* sequences, had posterior probabilities lower than 0.638, whereas separation between *Spirometra* spp. were larger than 0.542. Finally, the haplotype network clearly separated *S. mansoni* from other *Spirometra* spp. Moreover, *S. mansoni* was clustered into seven haplotypes, and Costa Rican specimens were placed into two different haplotypes with sequences from other geographical locations. Genbank accession numbers derived from specimens from Costa Rica are available in Table 1.

5. Discussion

Herein, we report seven cases of *S. mansoni* in domestic dogs (*C.* l. *familiaris*), domestic cats (*F. catus*) and a coyote (*C. latrans*), that appeared during a 1-year period from different geographical locations of Costa Rica. This represents the first molecular evidence of the parasite in Central America; thus, extending its known range in the American continent.

Molecular data of *S. mansoni* circulating in the Americas first became available in 2022 in a crab-eating fox from Colombia (Brabec et al., 2022) and in 2023 in a Samar cobra imported to the USA (Verocai et al., 2023). A few reports of *S. mansoni* based on the morphological assessment of specimens have been available since the 1960s in the continent, including a report from Costa Rica (Valerio et al., 2004). Nevertheless, the use of fixation techniques may modify some morphologic characters and the absence of molecular evidence cannot support robust species

identification (Brabec et al., 2022).

Both morphological and molecular methods were performed in our cases. The former provided an initial diagnosis of spirometrosis and DNA sequencing of eggs or proglottids allowed the characterization to the species level. Importantly, differential diagnosis with other sympatric *Spirometra* spp. was done since other species have been reported in North and South America, namely *Spirometra decipiens* complex 1 and 2 (Kuchta et al., 2021). Herein, only the specimen collected from the coyote could be properly stained and analyzed. Specimens from dog and cat cases could not be morphologically assessed due to fixation without prior relaxation of the worm's muscles, leading to a stiff parenchyma resistant to differential staining (Castro and Guerrero, 2006). Importantly, proglottid inner organs may not be always evident, and eggs in fecal samples are undistinguishable between *Spirometra* spp. (Bowman, 2014). Nevertheless, correct identification to the species level may be accomplished with the uterus shape and position of the ovary when specimens are freshly collected and all steps during staining are correctly performed (Khalil et al., 1994). This highlights the need for proper training in classical taxonomy tools, so accurate morphological identification can be accomplished as suggested before (Bradbury et al., 2022). Therefore, molecular confirmation based on DNA sequencing of partial or complete *cox*1 is considered the only method for differentiating *Spirometra* spp. (Jeon et al., 2018). Even so, careful interpretation of DNA sequences should be taken during species assignation (Kuchta et al., 2021), since data in Genbank may be incorrect for some *Spirometra* spp.

The current dataset highlights the possible underdiagnosis of spirometrosis during the last two decades in Costa Rica, since the last cases were detected in 2004 (Valerio et al., 2004). The apparent absence or epidemiological silence of the infection may be explained by the use of flotation assays for coprological analysis in routine diagnostics as well as in prevalence studies (Conrad et al., 2021; Scorza et al., 2011). This technique may be unsuitable for the detection of pseudophyllidean cestode or trematode species which generally have operculated eggs (Bartlett et al., 1978) Therefore, other methods like sedimentation should complement coprological analysis in definitive hosts to obtain real estimates of *S. mansoni* prevalence (Dib et al., 2019). However, a re-emergence of the parasite due to a change of land use for urbanization and the proximity of companion animals to wild hosts (Baker et al., 2022) is a possibility that requires exploration.

In the present study, *S. mansoni* adult stages were identified in carnivore definitive hosts, thus, with signs of spirometrosis. As explained before, the prevalence of spirometrosis in Costa Rica has been unknown in the last decades, but the frequency of this parasite has been studied in other countries. For instance, the frequency of *Spirometra* spp. infection in dogs ranges from 0.3% in Northern Portugal (Cardoso et al., 2014) to 21.34% in Cambodia (Liu et al., 2015), whereas in cats varies from 0.4% in some areas of the US (Lucio-Forster and Bowman, 2011) to 17.1% in Guizhou, China (Han et al., 1995) and 17.3% in cats from Northeast Brazil (Silva et al., 2023). This shows the varying incidence of *Spirometra* in different geographical locations due to the presence of infected intermediate or paratenic hosts and other environmental risk factors (Brabec et al., 2022). The sources of infection for the cases presented herein could not be identified and the intermediate or paratenic host species used by *S. mansoni* in Costa Rica remain unknown.

Diagnosis of intermediate and paratenic hosts may be challenging since these are normally detected as infected with plerocercoids after necropsy of animals (Armúa-Fernández et al., 2021; Verocai et al., 2023). For instance, intermediate or paratenic hosts of *Spirometra decipiens* complex 1 (Morales et al., 2022; Armúa-Fernández et al., 2021) and complex 2 (McHale et al., 2020); were detected in the Americas using *cox*1 analysis. *S. mansonoides* was morphologically identified in raccoons from the USA (Schaffer et al., 1981) and a *Spirometra* sp. plerocercoid was found in a captive meerkat of the USA (McHale et al., 2020). Furthermore, wild boars serve as paratenic hosts of *S. erinacei* in Poland (Kolodziej-Sobocinska et al., 2016), and thus their role in the life
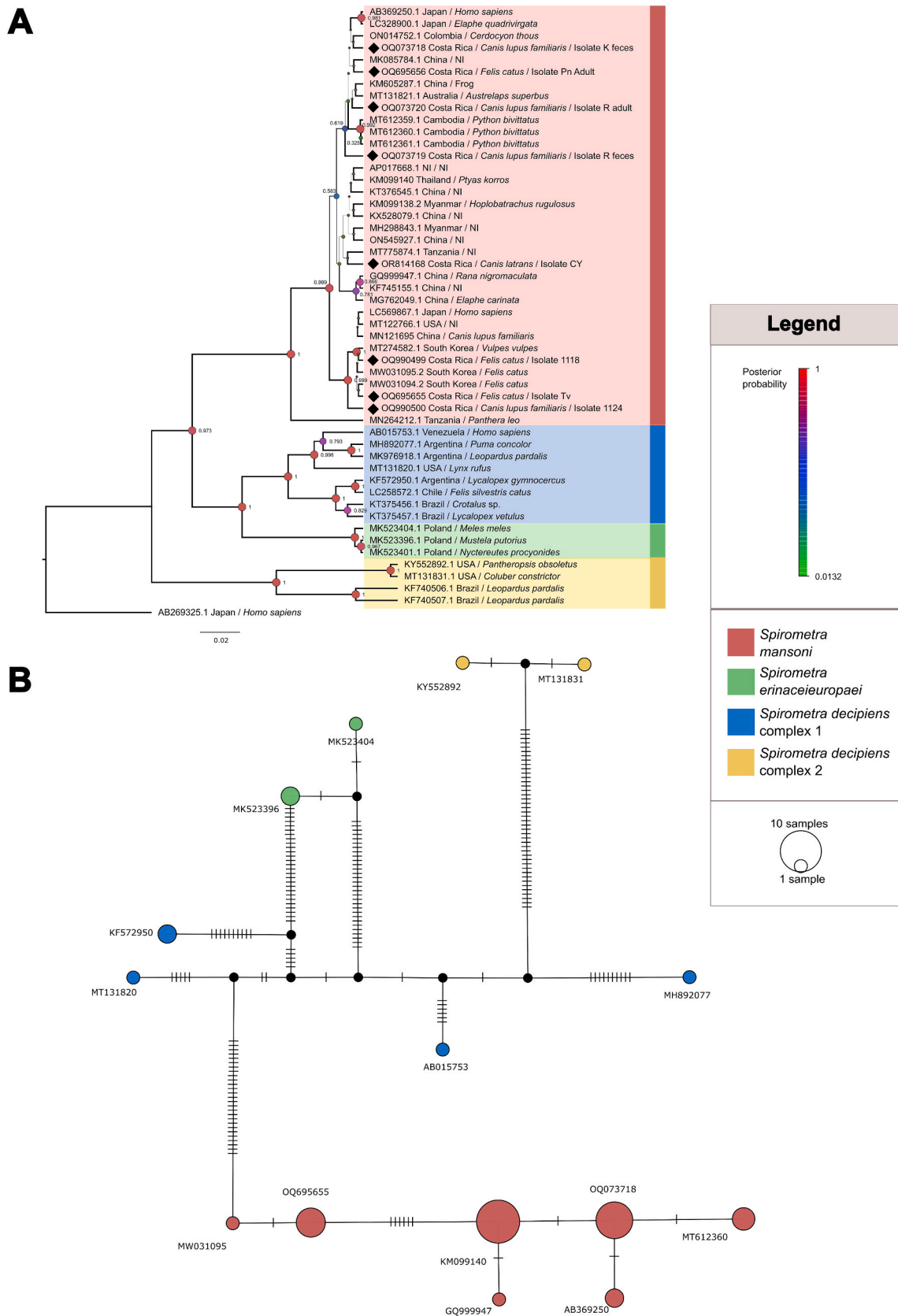
Fig. 4. Phylogenetic analysis of *S. mansoni cox*1 sequences obtained from dogs, cats and a coyote from Costa Rica. **A.** Bayesian Inference phylogenetic tree of *Spirometra* spp. Size and colour of node circles are proportional to posterior probabilities. Sequences obtained in the present study are denoted with black diamonds. **B.** Templeton-Crandall-Sing haplotype network of *Spirometra* spp. Circle size is proportional to the number of sequences belonging to the haplotype, black circles represent hypothetical haplotypes separating two groups and hatch marks mutational steps between two different haplotypes.

cycle of *S. mansoni* in the Americas should be explored due to their abundance in the continent. Reports of intermediate or paratenic hosts for *S. mansoni* in the Americas are currently lacking. Therefore, our research should lead to the study of the infection status of amphibian, reptile, and other vertebrate species in locations where cases were found.

Most cases from the current study reported outdoor access or hunting behavior, which has been described as a factor for higher prevalence for spirometrosis in cats in Japan (Itoh et al., 2012). Additionally, cases were reported from geographical locations with proximity to natural water sources, a factor that may enable potential reproduction of amphibian intermediate hosts. This has been formerly hypothesized as a contributing factor for *Spirometra decipiens* transmission to a dog in Cuba (Morales et al., 2022). Also, wet habitats have been determined as a factor for a higher *Spirometra erinaceieuropei* infection prevalence in wild carnivores of Poland (Kolodziej-Sobocinska et al., 2023). Even though these studies have been conducted in other geographical locations different to Central America, the information derived from them helps us understand the epidemiology of the infection.

More than 1600 cases of human sparganosis have been reported in the world, but it seems to be an uncommon zoonosis in the Americas (Liu et al., 2015) and, to the authors' knowledge, there is no published evidence of this neglected food-borne infection in Central America. However, awareness of sparganosis should be raised among human clinicians given the circulation of *S. mansoni* in urban areas of Costa Rica. Gaining knowledge of the implicated second intermediate and paratenic hosts in the region will help to understand the parasite's distribution, epidemiology, and risk factors, and to design prevention and control strategies for spirometrosis in companion animals and human sparganosis.

6. Conclusions

This study analyzed seven autochthonous cases of *S. mansoni* in dogs, cats, and a coyote from different regions of Costa Rica. Importantly, parasites were molecularly characterized to the species level, thus confirming the circulation of this zoonotic pathogen in the region in domestic animals and wild reservoirs. Future studies should be aimed at the description of second intermediate and paratenic hosts used by *S. mansoni*. This knowledge will promote the design of prevention and control campaigns of spirometrosis in animals and sparganosis in humans.

Ethical statement

This study did not require any ethical statement. Data from patient was completely anonymized.

CRediT authorship contribution statement

Irene Alvarado-Hidalgo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Josué Campos-Camacho:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Yuliana Arguedas-Morales:** Writing – review & editing, Data curation, Conceptualization. **Luis M. Romero-Vega:** Writing – review & editing, Visualization, Data curation, Conceptualization. **Alejandro Alfaro-Alarcón:** Investigation, Data curation, Conceptualization. **Gabriela Anchia-Ureña:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Laura G. Bass:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Ivan Berrocal-Ávila:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Isabel Hagnauer:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Roberto W.I. Olivares:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Alberto**

Solano-Barquero: Writing – review & editing, Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rodolfo Traube-Rivera:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Víctor Montenegro-Hidalgo:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Alicia Rojas:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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