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Case Report

The first *Linguatula serrata* case in an imported dog in Finland

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A R T I C L E I N F O

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A B S T R A C T

Linguatula serrata is a pentastomid parasite infecting carnivores as definitive hosts and herbivores as intermediate hosts. In carnivores, including dogs, it usually parasitises the nasal cavity and sinuses, causing upper respiratory signs. This case report presents the first canine *Linguatula* case in Finland in an imported dog originating from Spain. In addition to the unremarkable clinical history of the dog, the treatment, parasite's morphology and molecular analysis are described, and the zoonotic potential is discussed.

1. Introduction

L. serrata is a zoonotic pentastomid crustacean that resides as an adult in the nasal cavities and sinuses of carnivores, mainly canids (Acha and Szyfres, 2003). Eggs are shed to the environment in the faeces and nasal discharge by the infected animal. Intermediate hosts, mainly herbivores, are infected by ingesting the eggs, which hatch in the intestine and larvae penetrate into the tissues. Nymphs developed from larvae, are found encapsulated in the lymph nodes, liver and lungs. If a dog is fed with infected offals, the nymphs are released in its intestinal tract. They then migrate to the nasopharyngeal area and develop into adults (Acha and Szyfres, 2003).

L. serrata has been reported worldwide (Oluwasina et al., 2014; Hajipour et al., 2018; Nagamori et al., 2019; Shamsi et al., 2020), but its prevalence varies greatly. While the parasite is common and locally abundant in the Middle East and Africa (Oluwasina et al., 2014; Hajipour et al., 2018), it might be overlooked in Europe; there are several recent reports of cases from dogs from Romania (Ionita and Mitrea, 2016; Springer et al., 2018; Thomas, 2018; Villedieu et al., 2017). This is the first time *L. serrata* is reported in a dog in Finland.

2. Case presentation

In December 2020, a 2-year-old Spanish galgo, imported one year earlier to Finland from Malaga province, Spain, expectorated a suspected parasite of an unusual shape. The dog's owner brought the parasite and a faecal sample from three consecutive days to a local veterinarian for analysis. The dog had no respiratory or other clinical signs, according to the owner.

The parasite, preserved in ethanol, was sent to the Veterinary parasitology laboratory, University of Helsinki, Finland. Macroscopically, the parasite measured 3 cm in length, and was identified as *Linguatula* sp. The stereomicroscope examination revealed typical structures (Fig. 1, A–C), which strengthened the macroscopic identification. In addition to the earlier negative result from the faecal examination in a commercial laboratory, the faecal sample analysed with magnesium sulphate flotation in the University laboratory did not show any *Linguatula*-type eggs with paired hooks either.

A part of the parasite was embedded in paraffin and routinely processed for histopathological examination. Sections were stained with haematoxylin-eosin. Parasite's body was pseudosegmented, and cuticular ridges often possessed sclerotised openings. A distinct layer of striated muscle fibres, arranged in circular and longitudinal pattern, was present in the subcuticular tissue. The coelom was occupied by the uterus filled with large double-shelled eggs (Fig. 1, D). The outer shell-layer stained eosinophilic and the inner yellowish.

The tip of the tail was genetically analysed at the University of Eastern Finland. The specimen was DNA barcoded by sequencing the standard 5′ *Co1* sequence using LCO 1490 and HCO 2198 primers (Folmer et al., 1994). Briefly, DNA was isolated using NucleoSpin™ Tissue kit (Macherey-Nagel™, Düren, Germany) following the manufacturers' instructions. The PCR reaction was carried out essentially as in

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Folmer et al. (1994). The PCR product was purified using the Illustra™ GFX PCR DNA and Gel Band Purification Kit (Merck, Kenilworth, NJ, USA) and sequenced to both directions using the same LCO 1490 and HCO 2198 primers (Mix2Seq service, Eurofins Genomics, Cologne, Germany). After removing the primer sequences, the obtained sequence was then aligned with other pentastomid *Co1* sequences available in the GenBank (Table 1, Fig. 2), and a maximum likelihood tree of *Co1* genealogies was constructed using MrBayes v3.2 (Ronquist et al., 2012) with GTR-Γ model, assessing node support through 1,000,000 MCMC generations with 500 sample and 5000 generation diagnostic frequencies. The resulting tree was illustrated and edited using FigTree v1.4.4. (http://tree.bio.ed.ac.uk/software/figtree/). Molecular analysis confirmed *L. serrata* infection in this imported dog.

The dog was treated with fluralaner according to weight (Bravecto®, MSD Animal Health, Walton, UK). The treatment was selected from the literature (Nagamori et al., 2019), although, fluralaner's efficacy against pentastomids is poorly documented. Monitoring the efficacy of the treatment was considered impossible since there were no clinical signs, and eggs were not found in the faecal samples. No rhinoscopy was made before or after medication.

3. Discussion

We describe the first diagnosed *L. serrata* case in a dog in Finland. While *L. serrata* is not endemic in any of the Nordic countries, *Linguatula arctica* is prevalent in reindeer (*Rangifer tarandus*) (Nikander and Saari, 2006). However, that species possesses a direct life cycle that does not involve dogs.

As imported canine linguatulosis cases have been reported in EU countries, we have expected to see them also in Finland. The country of origin of these cases has been, almost invariably, Romania (Ionita and Mitrea, 2016; Springer et al., 2018; Thomas, 2018; Villedieu et al., 2017). The dog of the present report was imported from Spain, where *L. serrata* is considered rare the last autochthonous case being diagnosed in 2013 (Bello Gavete et al., 2013). The plausible explanations for the low abundance of the parasite in Spain might be a control of stray dog populations and broken reproductive cycle of the parasite, if the grazing herbivores are not exposed to canine faeces. It is also possible that the routine treatment of livestock and dogs with macrocyclic lactones is deleterious for *Linguatula*.

Linguatulosis may be subclinical, like in the present case, or manifest in the upper respiratory tract causing nasopharyngitis, rhinitis, or sinusitis. The parasite is relatively large, and it may cause irritation, nasal discharge and obstruction of the upper airways resulting in sniffling and pronounced respiratory sounds.

Eggs were not found in the faecal samples collected 1–3 days after the parasite was expectorated. This may suggest, that only one female was infecting the dog. Inside the expectorated female, developing and embryonated eggs were observed in the histological sample, but the female was small, only 3 cm long. The adult *Linguatula* females are 7.0–8.5 cm (Shamsi et al., 2020) or up to 8 cm in length (Oryan et al., 2008). The prepatent period of *Linguatula* is about six months, and the dog was imported about a year ago, so the infection should have been in the patent phase. Linguatula eggs have intermittent shedding to the faeces (Hajipour and Tavassoli, 2019), so it is possible that faecal samples are negative for the eggs during patent infection. An autochthonous infection taken place in Finland is unlikely, as Finnish fauna does not consist *L. serrata*, and the infected galgo has been kept as a pet in Finland, with limited outdoor access to the fenced yard and fed with processed feed only after import.

Although *L. serrata* can be morphologically differentiated from *L. arctica* by the number of abdominal annuli (~65 vs. ~90; Gjerde, 2013) and from *L. recurvata* as well as *Neolinguatula nutallii* from the lack of terminal cleft (Shamsi et al., 2020), DNA barcoding provides an easy and relatively rapid means for reliable species identification for non-experts (Ondrejicka et al., 2014). In the ML-tree (Fig. 2), our *Linguatula* specimen clustered tightly with the *L. serrata* samples obtained from GenBank and were distinct from the congeneric *L. arctica* samples. While *Co1* is not always sufficient in differentiating between animal species (e.g. van Velzen et al., 2012), the markable sequence differences to the other known pentastomatids, geographical origin and a dog as host species, rule out any other possibilities than *L. serrata*.

The zoonotic potential of *L. serrata* should be addressed upon the diagnosis. As canine linguatulosis is often subclinical, the infection goes readily unnoticed, and the dog owner may be exposed to the zoonotic parasitic disease without being aware of the risk.

The human can serve as *L. serrata*'s intermediate host, when eggs from an infected dog's nasal discharge or faeces reach the human's

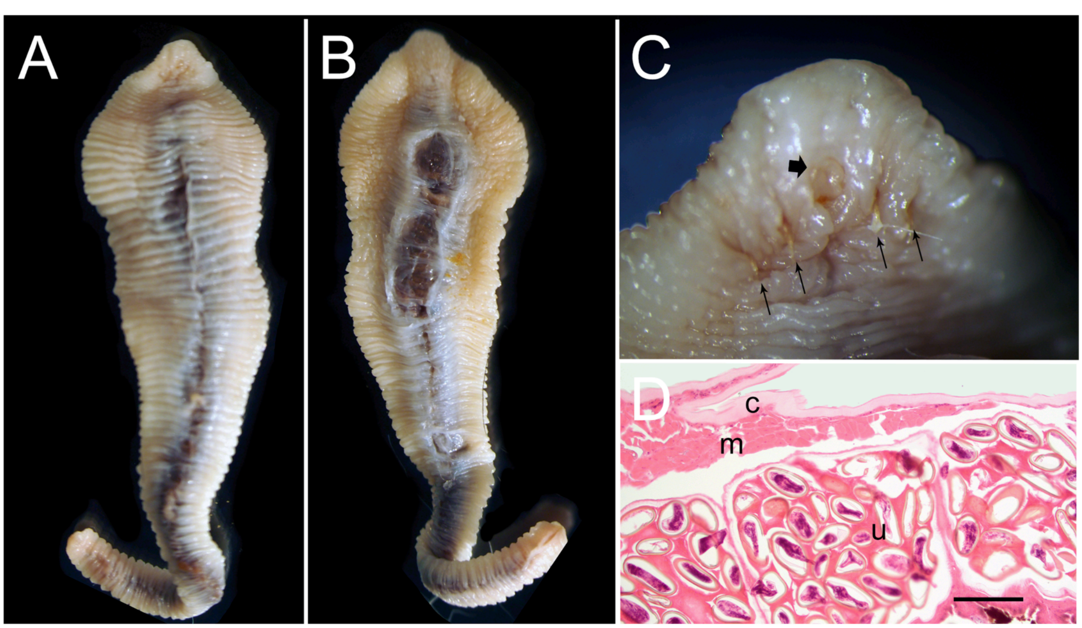

Fig. 1. Morphology of the Linguatula female sent as a sample. A) ventral view, B) dorsal view, C) head-like anterio-ventral protuberance with a mouth (thick arrow) and four grasping hooks (thin arrows), D) HE-stained histological transversal section, c = cuticle, m = striated muscle, u = uterus with thick celled eggs. Scale bar = 100 μm. The eggs appearing smaller than 100 μm in length, is probably due to the shrinkage during the tissue procession.

Table 1
GenBank reference sequences and specimen details for Maxillopodan parasites included in the sequence analysis.

GenBank #	Species	Country	Host	Developmental stage	Reference
KF723419	*Argulus foliaceus*	India	Goldfish (*Carassius auratus*)	Adult	Unpublished
JF975594	*Raillietiella hebitihamata*	Australia	Asian house gecko (*Hemidactylus frenatus*)	Adult	Kelehear et al. (2011)
MK695636	*Linguatula recurvata*	Mexico	Bairds Tapir (*Tapirus bairdii*)	Adult	Pérez Flores et al. (2019)
MN905338	*Neolinguatula nuttalli*	South Africa	Lion (*Panthera leo*)	Adult	Shamsi et al. (2020b)
KF029444	*Linguatula arctica*	Norway	Reindeer	Adult	Gjerde (2013)
KF029445	*Linguatula arctica*	Norway	Reindeer	Adult	Gjerde (2013)
KF029446	*Linguatula arctica*	Norway	Reindeer	Adult	Gjerde (2013)
MG589379	*Linguatula serrata*	India	Unknown	Unknown	Unpublished
MN481629	*L. serrata*	Iran	Cow (*Bos taurus*)	Nymph	Unpublished
KY829108	*L. serrata*	Peru	Vicuña (*Vicugna vicugna*)	Nymph	Gomez-Puerta et al. (2017)
MN893769	*L. serrata*	Australia	Dog (*Canis familiaris*)	Adult	Shamsi et al. (2020a)
MT371890	*L. serrata*	Australia	Red-necked wallaby (*Notamacropus rufogriseus*)	Nymph	Barton et al. (2019)
MZ052082	*L. serrata*	Italy	Dog (*C. familiaris*)	Adult	Unpublished
MK248491	*Sebekia missisippensis*	USA	Spotted gar (*Lepistosteus oculatus*)	Adult	Woodyard et al. (2019a)
MN062096	*Levisunguis subequalis*	USA	Mosquito fish (*Gambusia affinis*)	Nymph	Woodyard et al. (2019b)
KU975385	*Alofia merki*	Australia	Fish	Nymph	Barton and Morgan (2016)
MT271602	*Armillifer armillatus*	Republic of Congo	Potto (*Perodicticus potto*)	Nymph	Lemarcis et al. (2020)
FJ607340	*Armillifer agkistrodontis*	China	Unknown	Unknown	Unpublished
MG559658	*Kiricephalus coarctatus*	USA	Brown water snake (*Nerodia taxispilota*)	Adult	Miller et al. (2017)
MG559655	*Porocephalus crotali*	USA	Cottonmouth (*Agkistrodon piscivorus*)	Adult	Miller et al. (2017)

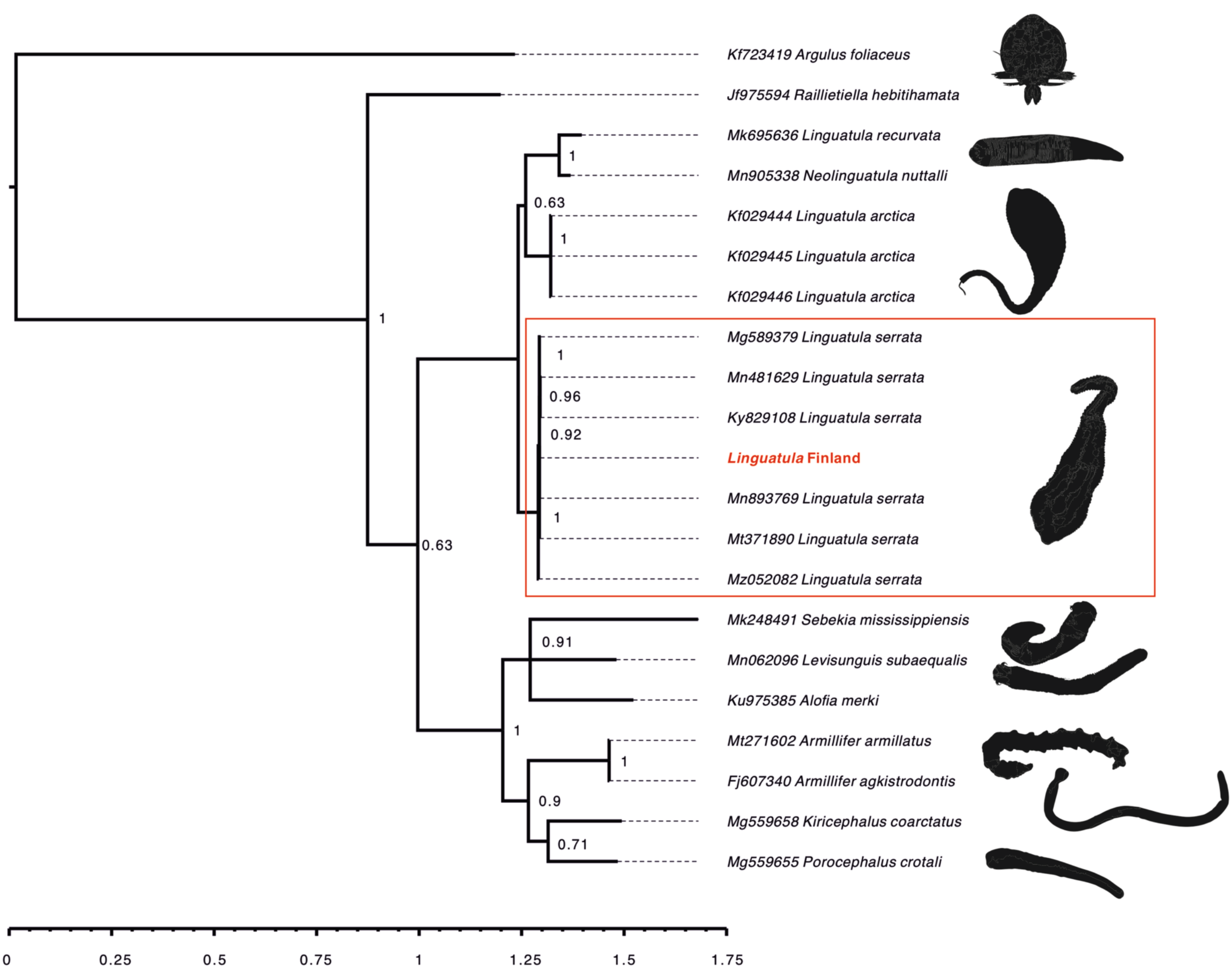

Fig. 2. Maximum-likelihood phylogenetic tree of *Linguatula* based on *Co1* gene sequence alignments. The *Linguatula* sample from Finland, marked in red, is almost identical with the L. *serrata* samples from elsewhere in the world. X-axis depicts the sequence difference and values at branch nodes the Bayesian posterior probabilities for their support.

intestine. The hatched larvae may cause tissue damage by migrating through lymph nodes to the liver and lungs and forming a cyst (Pampiglione et al., 2001; Machado et al., 2006), which grows larger and becomes more space-occupying

Humans may also become definitive hosts by eating raw and undercooked visceral organs of an infected intermediate host, usually a sheep or a goat. Nasopharyngeal inflammation will result. There are also rare reports about human ocular linguatulosis caused by larval or nymphal stages of *L. serrata*, which might have resulted from ingestion or direct eye contact with *L. serrata* eggs (Koehsler et al., 2011; Lazo et al., 1999). The zoonotic potential should not be overlooked in dog-import, especially considering the close contact between the dog and its owner.

4. Conclusion

Our case proves that dogs may carry zoonotic *L. serrata* without any clinical signs and with negative results in the faecal examination. Dog-import brings the risk to non-endemic areas too.

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Ethical statement

This article does not contain any experimental studies with human or animal subjects performed by any of the authors.

Declaration of Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Acha, P.N., Szyfres, B., 2003. Pentastomiasis. In: Zoonoses and Communicable Diseases Common to Man and Animals, , 3rd edVol. III. Pan American Health Organization, Washington D.C., U.S.A, pp. 345–350.

Barton, D.P., Morgan, J.A., 2016. A morphological and genetic description of pentastomid infective nymphs belonging to the family Sebekidae Sambon, 1922 in fish in Australian waters. Folia Parasitol. 63, 026.

Barton, D.P., Porter, M., Baker, A., Zhu, X., Jenkins, D.J., Shamsi, S., 2019. First report of nymphs of the introduced pentastomid, *Linguatula serrata*, in red-necked wallabies (*Notamacropus rufogriseus*) in Australia. Aust. J. Zool. 67, 106.

Bello Gavete, A.M., Layos, C., Meana, A., Rupérez, C., Miró, G., 2013. Linguatulosis canina. In: Parasitología clínica a través de cinco casos. Canis et Felis 125, 62–72 (in Spanish).

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Gjerde, B., 2013. Phylogenetic position of *Linguatula arctica* and *Linguatula serrata* (Pentastomida) as inferred from the nuclear 18S rRNA gene and the mitochondrial cytochrome c oxidase subunit I gene. Parasitol. Res. 112, 3517–3525.

Gomez-Puerta, L.A., Pacheco, J.I., Angulo-Tisoc, J.M., Lopez-Urbina, M.T., Gonzalez, A. E., 2017. First finding of nymphal stages of Linguatula serrata in a South American camelid, a vicuna from Peru. Vet. Parasitol. 244, 21–24.

Hajipour, N., Tavassoli, M., 2019. Prevalence and associated risk factors of *Linguatula serrata* infection in definitive and intermediate hosts in Iran and other countries: a systematic review. Vet. Parasitol. Reg. Stud. Rep. 16, 100288.

Hajipour, N., Soltani, M., Mirshekar, F., 2018. Effect of age, sex, and season on the prevalence of *Linguatula serrata* infestation in mesenteric lymph nodes of goats slaughtered in Tabriz, Iran. Trop. Anim. Health Prod. 51, 879–885.

Ionita, M., Mitrea, I., 2016. *Linguatula serrata* (Pentastomida: Linguatulidae) infection in dog, Romania: a case report. AgroLife Sci. J. 5, 85–89.

Kelehear, C., Spratt, D.M., Dubey, S., Brown, G.P., Shine, R., 2011. Using combined morphological, allometric and molecular approaches to identify species of the genus *Raillietiella* (Pentastomida). PLoS One 6, E24936.

Koehsler, M., Walochnik, J., Georgopoulos, M., Pruente, C., Boeckeler, W., Auer, H., Barisani-Asenbauer, T., 2011. *Linguatula serrata* tongue worm in human eye. Austria. Emerg. Infect. Dis. 17, 870–872.

Lazo, R.F., Hidalgo, E., Lazo, J.E., Bermeo, A., Llaguno, M., Murillo, J., Teixeira, V.P., 1999. Ocular linguatuliasis in Ecuador: case report and morphometric study of the larva of *Linguatula serrata*. Am. J. Trop. Med. Hyg. 60, 405–409.

Lemarcis, T., Chesnais, C.B., Pion, S.D.S., Boussinesq, M., Locatelli, S., 2020. Detection of a Larva of *Armillifer armillatus* in a Potto (*Perodicticus potto*) from the Republic of the Congo. J. Parasitol. 106, 654–662.

Machado, M.A.C., Makdissi, F.F., Canedo, L.F., Martino, R.B., Crescentini, F., Chieffi, P. P., Bacchella, T., Machado, M.C.C., 2006. Unusual case of pentastomiasis mimicking liver tumor. J. Gastroenterol. Hepatol. 21, 1218–1220.

Miller, M.A., Kinsella, J.M., Snow, R.W., Hayes, M.M., Falk, B.G., Reed, R.N., Mazzotti, F. J., Guyer, C., Romagosa, C.M., 2017. Parasite spillover: indirect effects of invasive Burmese pythons. Ecol. Evol. 8, 830–840.

Nagamori, Y., Ramachandran, A., Kuzma, C., Nafe, L., Johnson, E.M., 2019. A zoonotic parasite, *Linguatula serrata*, infection in a dog imported from Ethiopia to the United States. Vet. Parasitol. Reg. Stud. Rep. 16, 100273.

Nikander, S., Saari, S., 2006. A SEM study of the reindeer sinus worm (*Linguatula arctica*). Rangifer 26, 15–24.

Oluwasina, O.S., ThankGod, O.E., Augustine, O.O., Gimba, F.I., 2014. *Linguatula serrata* (Porocephalida: Linguatulidae) infection among client-owned dogs in Jalingo, North Eastern Nigeria: prevalence and public health implications. J. Parasitol. Res. 2014, 916120. https://doi.org/10.1155/2014/916120.

Ondrejicka, D.A., Locke, S.A., Morey, K., Borisenko, A.V., Hanner, R.H., 2014. Status and prospects of DNA barcoding in medically important parasites and vectors. Trends Parasitol. 30, 582–591.

Oryan, A., Sadjjadi, S.M., Mehrabani, D., Rezaei, M., 2008. The status of *Linguatula serrata* infection of stray dogs in Shiraz, Iran. Comp. Clin. Pathol. 17, 55–60.

Pampiglione, S., Gentile, A., Maggi, P., Scattone, A., Sollitto, F., 2001. A nodular pulmonary lesion due to *Linguatula serrata* in an HIV-positive man. Parassitologia. 43, 105–108.

Pérez Flores, J., Lagunas-Calvo, O., Gonzáles-Solis, D., Oceguera-Figueroa, A., 2019. First molecular characterization of *Linguatula recurvata* (Pentastomida) and First Record in Baird's Tapir (*Tapirus bairdii*) from Calakmul, Mexico. Comp. Parasitol. 86, 135.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542.

Shamsi, S., Barton, D.P., Zhu, X., Jenkins, D.J., 2020a. Characterisation of the tongue worm, *Linguatula serrata* (Pentastomida: Linguatulidae), in Australia. Int. J. Parasitol. Parasites Wildl. 11, 149–157.

Shamsi, S., Halajian, A., Barton, D.P., Zhu, X., Smit, W.J., Roux, F., Luus-Powell, W.J., 2020b. Occurrence and characterisation of tongue worms, *Linguatula* spp., in South Africa. Int. J. Parasitol.: Parasites Wildl. 11, 268–281.

Springer, A., Fiedler, H., Raue, K., Strube, C., 2018. *Linguatula serrata*-Befall bei einem Importhund aus Rumänien [*Linguatula serrata*-infection in a dog imported from Romania to Germany] [article in German]. Tierarztl. Prax. Ausg. K. Kleintiere. Heimtiere. 46, 260–264.

Thomas, M., 2018. *Linguatula serrata* in an imported Romanian street dog. Vet. Rec. 182, 112–113.

van Velzen, R., Weitschek, E., Felici, G., Bakker, F.T., 2012. DNA barcoding of recently diverged species: relative performance of matching methods. PLoS One 7, e30490.

Villedieu, E., Sanchez, R.F., Jepson, R.E., Ter Haar, G., 2017. Nasal infestation by *Linguatula serrata* in a dog in the UK: a case report. J. Small Anim. Pract. 58, 183–186.

Woodyard, E.T., Baumgartner, W.A., Rosser, T.G., Bodin, E.N., Ferrara, A.M., Noto, T.W., Ford, L.M., Scott, A., Rush, S.A., 2019a. Morphological, molecular, and histopathological data for *Sebekia mississippiensis* Overstreet, Self, and Vliet, 1985 (Pentastomida: Sebekidae) in the American Alligator, *Alligator mississippiensis* Daudin, and the Spotted Gar, *Lepisosteus oculatus* Winchell. J. Parasitol. 105, 283–298.

Woodyard, E.T., Stilwell, J.M., Camus, A.C., Rosser, T.G., 2019b. Molecular and histopathological data on *Levisunguis subaequalis* Curran, Overstreet, Collins & Benz, 2014 (Pentastomida: Eupentastomida: Porocephalida: Porocephaloidea: Sebekidae: Sebekinae) from *Gambusia affinis* in Alabama, USA. J. Parasitol. 105, 827–839.