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Molecular phylogenetic relationships of *Metastrongylus* nematodes with emphasis on specimens from domestic pigs in Vietnam

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Short Communication

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Abstract

Lungworms of the genus *Metastrongylus* are parasitic nematodes in the respiratory tract of swine. Although they infect both wild boars and domestic pigs, studies on *Metastrongylus* infections in wild boars in Europe, the Americas and Africa are numerous, while those in domestic pigs are few. There are several studies analysing the molecular phylogenetic relationships of few individual *Metastrongylus* species with other nematode taxa, but there are no studies on the phylogenetic relationships of species within the genus *Metastrongylus*. In Southeast Asia, reports on swine lungworms are extremely scarce and do not include any nucleotide sequence data. Therefore, the aim of the present study is to survey *Metastrongylus* infection in domestic pigs raised in Dien Bien Province, Northern Vietnam, and to analyse the molecular phylogenetic relationships of *Metastrongylus* species. Based on morphological and molecular data, we identified two species: *Metastrongylus apri* and *Metastrongylus* pudendotectus. The prevalence of the former species was found to be significantly higher than the latter one (24.1% vs. 2.3%). We observed pigs exhibiting a coinfection with the two lungworm species or a single infection with only *M. apri*. However, we did not observe any pigs being infected with just *M. pudendotectus*. Vietnamese *Metastrongylus* specimens showed slight morphological and molecular differences compared to those from other countries. The molecular analyses revealed a close genetic relationship between *M. apri* and *Metastrongylus* salmi, while both these species were far distant from *M. pudendotectus*. The present study highlights the needs for further studies to clarify the morphological features and ecological and phylogenetic relationships of *Metastrongylus* species at the global scale.

Introduction

Lungworms of the genus *Metastrongylus* Molin, 1861 are parasitic nematodes in the respiratory tract of swine. The infection with these nematodes leads to verminous pneumonia and secondary disorders of the hosts resulting in weight loss and abortions (Alcaide *et al.*, 2005). The infection may also cause immunodepression of the hosts, making them more susceptible to negative health impacts (Gassó *et al.*, 2014). Importantly, although rarely seen, *Metastrongylus* infection in humans has been reported (Miloshev, 1956; Calvopina *et al.*, 2016). Thus, *Metastrongylus* nematodes can be potential health risks to both animals and humans. There are numerous studies on *Metastrongylus* infections in wild boars in Europe, the Americas and Africa, reporting high prevalence, while surveys in domestic pigs are few (Nssien & Adesehinwa, 1999; Adedokun *et al.*, 2001; Carstensen *et al.*, 2002; Li *et al.*, 2016, 2018). At present, six *Metastrongylus* species are considered to be valid, including *Metastrongylus apri* (Gmelin, 1790) Vostokov, 1905 (Synonym *Metastrongylus elongatus* (Dujardin, 1845) Railliet & Henry, 1911, *Metastrongylus salmi* (Gedoelst, 1823), *Metastrongylus pudendotectus* (Wostokow, 1905), *Metastrongylus confusus* (Jansen, 1964), *Metastrongylus asymetricus* (Noda, 1973) and *Metastrongylus madagascariensis* Chabaud & Gretillat, 1956. Three species – *M. apri*, *M. salmi* and *M. pudendotectus* – are reported worldwide and are usually present in mixed infections with variable frequencies, while the distribution of three other species – *M. confusus*, *M. asymetricus* and *M. madagascariensis* – is restricted to specific areas (Epe *et al.*, 1997; Adedokun *et al.*, 2001; Carstensen *et al.*, 2002; García–González *et al.*, 2013; Gassó *et al.*, 2014; Poglayen *et al.*, 2015).

Metastrongylus species are distinguished by the following key morphological features: copulatory bursa and spicules in males; and prevulvar cuticular valve (swelling), dilatation, position of the vulva and anus in females. Gassó *et al.* (2014) provided a key to the identification of five species, which excludes *M. madagascariensis*. However, mis-identifications when observing morphological features under microscope are possible, especially in the case of similar species, such as *M. confusus*, *M. apri* and *M. salmi* (Gassó *et al.*, 2014). Molecular data have been used to

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distinguish species and to study the genetic variation of *Metastrongylus* species based on Random Amplified Polymorphic DNA (RAPD) analysis or Polymerase chain reaction - Restriction Fragment Length Polymorphism (PCR–RFLP) (Leignel *et al.*, 1997; Conole *et al.*, 1999). Nucleotide sequence data, with the majority from European countries, have been used to analyse the phylogenetic relationships of few individual *Metastrongylus* species with other nematode taxa (Conole *et al.*, 2001; Carreno & Nadler, 2003; Jex *et al.*, 2010; Li *et al.*, 2016, 2018; Yong *et al.*, 2016). In Vietnam, as well as in other Southeast Asian countries, studies on swine lungworms are extremely scarce and do not include any sequence data (Arambulo *et al.*, 1967), even though domestic pigs are commonly raised in outdoor systems in many locations throughout the region. Therefore, the aim of this study is to survey *Metastrongylus* infection in domestic pigs in Dien Bien Province, Northern Vietnam, where pigs are raised freely, and to analyse the phylogenetic relationships of *Metastrongylus* species.

Materials and methods

Sample collection

The lungs of 739 domestic pigs from slaughterhouses in Dien Bien Province, Northern Vietnam, were examined for lungworms. The lungs and trachea were removed from each animal and placed in a washbasin containing water. The respiratory pathways were opened from the trachea into the bronchi and into the bronchioles using sharp scissors. The lungs were washed with water to remove contents in the respiratory pathways. The contents in the washbasin were filtered through a 100-mesh (149 µm) sieve. Lungworms in the sieve were collected and washed in 0.9% physiological saline. They were checked under a stereomicroscope and separated based on morphology. Depending on the number of specimens of each morphological type of lungworm, one to five worms were preserved in 96% ethanol for molecular analyses, and the other specimens were treated with hot 4% formaldehyde solution, then preserved in 4% formaldehyde solution for further morphological examination.

Morphological studies

Metastrongylus nematodes were immersed in lactophenol solution (phenol, lactic acid, glycerol and distilled water in a volume of 1:1:2:1) until the specimens became transparent for observation of taxonomic characteristics under a light microscope. Morphological examination and photographs were taken using a light microscope (ECLIPSE N*i*, Nikon, Tokyo, Japan). The main taxonomic morphometrics of 30 adult nematodes (15 males and 15 females) of each species were measured. A paired-samples *t*-test was performed to examine the significance of differences.

Molecular analyses

Total genomic DNA was extracted from individual worms from different hosts using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The internal transcribed spacer 2 (ITS2) region of the nuclear ribosomal DNA and a partial region of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) were chosen for analysis. They were amplified via the polymerase chain reaction (PCR) method with primer set 3S and A28 (Bowles *et al.*, 1995), and JB3 and JB4.5 (Bowles *et al.*, 1993), respectively. The amplification reactions were carried out in a total volume of 25 µl, containing: 13 µl of Dream Taq Green PCR master mix (2X) (Thermo Fisher Scientific, Foster City, California, USA), 2 µl of Template DNA of 10 ng/µl, 1 µl of each forward and reverse primer (working concentration: 10 pmol/L), 8 µl of nuclease-free water. The PCR was performed with the following cycling protocol: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 1 min and elongation at 72°C for 1 min. After cycling, a final elongation step at 72°C for 10 min was performed. The PCR products were visualized by gel electrophoresis and purified using a Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany), and were subsequently sequenced by an Ab3730 sequencer using a Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany). Nucleotide sequence data obtained in this study were deposited in the GenBank™, EMBL and DDBJ databases under the accession numbers: LC625785–LC625790 (*cox*1 sequences) and LC625791–LC625793 (ITS2 sequences).

The ITS2 and *cox*1 sequence data obtained in the present study were aligned with sequences downloaded from GenBank, using ClustalW with default options in MEGA7 (Kumar *et al.*, 2016). Short or abnormal sequences from GenBank were excluded. The final data set included 20 ITS2 and 18 *cox*1 sequences of three species (*M. apri*, *M. salmi* and *M. pudendotectus*) in the analyses. The sequence (KF316481) of *Aelurostrongylus abstrusus* (Metastrongyloidea) was used as an outgroup for the *cox*1 tree, whereas no outgroup was used for the ITS2 tree due to the *Metastrongylus* sequences being too phylogenetically distant from those of the other genera of nematodes. Phylogenetic trees were constructed using the Maximum Likelihood method based on the best model: Tamura 3-parameter model for the ITS2 tree, and Tamura-Nei model combined with the proportion of invariable sites (+*I*) for the *cox*1 tree. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach and then selecting the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated. We evaluated the statistical confidence of branching patterns using 1000 bootstrap replicates.

Results

Morphology

The nematode specimens collected from the lungs of domestic pigs in Dien Bien Province, Vietnam, were identified as *M. apri* and *M. pudendotectus* based on the morphological key of Gassó *et al.* (2014). The two species varied in size and in morphology with the following main features.

Metastrongylus apri: the body length of females (39.4 ± 5.0 mm) is greater than that of males (19.8 ± 1.7 mm). In males, two long spicules (4.2 ± 0.5 mm in length) are usually protruded out the body (fig. 1a). In females, prevulva cuticular dilatation is absent. Prevulva swelling is 108.2 ± 8.0 × 84.3 ± 6.7 µm in size. The distance from the anus and from the vulva to the posterior end of the body is 89.5 ± 2.8 µm and 109.4 ± 7.0 µm, respectively (fig. 1b).

Metastrongylus pudendotectus: the body length of females (20.8 ± 0.9 mm) is greater than that of males (14.2 ± 0.8 mm). In females, the prevulva swelling is 92.0 ± 1.1 × 52.3 ± 1.9 µm in size, surrounded by well-developed spherical cuticular dilatation of 259.9 ± 2.9 × 238.6 ± 5.0 µm. The vulva is posterodorsal to

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Fig. 1. The caudal ends of *Metastrongylus apri* and *Metastrongylus pudendotectus* collected from domestic pigs in Vietnam: (a) *M. apri* male showing two long spicules protruded out the body; (b) *M. apri* female showing prevulvar cuticular swelling, but no dilatation; (c) *M. pudendotectus* female showing prevulvar cuticular swelling and well-developed dilatation; (d) *M. pudendotectus* male showing two medium spicules indented into the body..

prevulvar dilatation. The distance from the anus and from the vulva to the posterior end of the body is 221.7 ± 6.0 µm and 328.0 ± 4.5 µm, respectively (fig. 1c). In males, two medium spicules, 1.6 ± 0.05 mm in length, were usually indented in the body, distant to the posterior end of the body 1.5–2.0 mm (fig. 1d)

The statistical analyses revealed that the body length, spicule length, prevulva swelling, distance from the vulva and from the anus to the posterior end of the body of the two species were all significantly different ($P < 0.001$).

Molecular analyses

We obtained three *cox*1 sequences of each species, which were morphologically identified as *M. apri* and *M. pudendotectus*, and three ITS2 sequences of *M. apri*, but we did not obtain any ITS2 sequence of *M. pudendotectus* from the 17 pigs infected with this species.

For ITS2, three sequences of *M. apri* collected from Vietnam were completely identical to each other but differed from the Danish and Estonian *M. apri* sequences (Estonian sequences registered as *M. elongatus*) at a distance of 2.0–2.3%. Intraspecific nucleotide variation within *M. apri* (including *M. elongatus* as a synonym) ranged from 0.0 to 2.3%. Interspecific nucleotide distances between *M. apri* and *M. salmi* ranged from 1.3 to 3.6%, while the distances between *M. pudendotectus* and *M. apri* were much greater (from 13.1 to 15.0%), almost similar to that between *M. pudendotectus* and *M. salmi* (from 13.0 to 13.4%). In the phylogenetic tree (fig. 2a), three species belonged to distinct clades: *M. apri* was close to *M. salmi*, and separated from *M. pudendotectus*.

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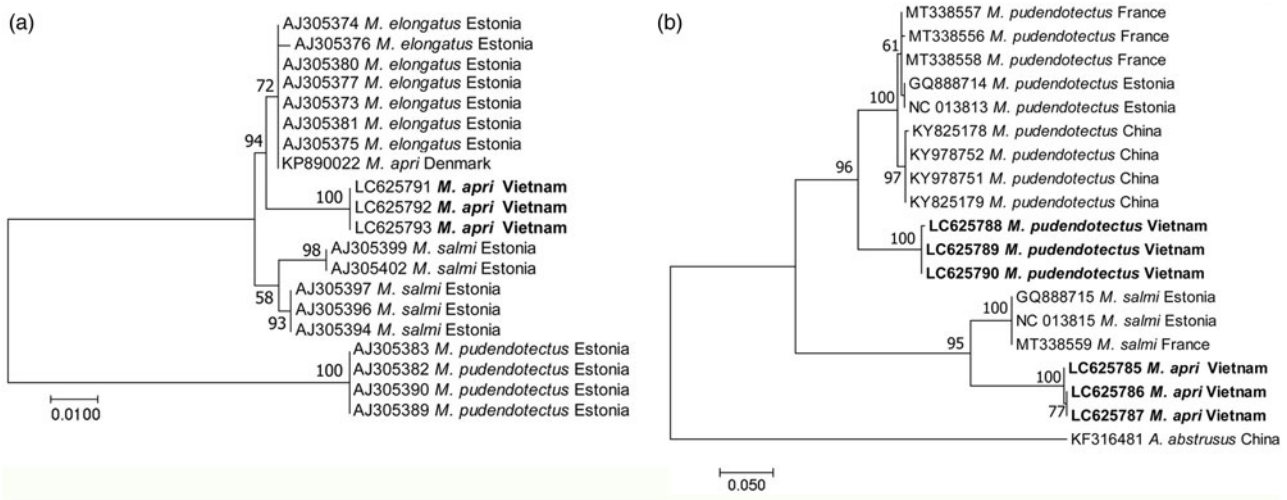

Fig. 2. Phylogenetic relationships of *Metastrongylus* species reconstructed from ITS2 sequences (a) and from *cox*1 sequences (b). Nucleotide sequences downloaded from GenBank are shown with accession number, species name and country name of their geographical origin. The sequences obtained in the present study are printed in bold. Bootstrap values are shown at the nodes. Scale bar shows the number of substitutions per site.

In the *cox*1 region of Vietnamese *Metastrongylus*, there was a single nucleotide difference among three *M. apri* specimens. Similarly, there was one single nucleotide difference among three *M. pudendotectus* sequences. At present, there are no *cox*1 sequences of *M. apri* available in GenBank for comparison. For *M. pudendotectus*, the specimens from Vietnam differed from the Chinese, French and Estonian sequences from 7.2 to 8.8%. Intraspecific nucleotide distance within *M. pudendotectus* specimens ranged from 0.0 to 8.8%. In comparison between species, nucleotide distances between *M. apri* and *M. salmi* ranged from 7.7 to 9.0%, smaller than that between *M. pudendotectus* and *M. salmi* (from 12.6 to 13.3%), and between *M. pudendotectus* and *M. apri* (from 13.8 to 14.9%). In the phylogenetic tree (fig. 2b), similar to the ITS2 tree, each species belonged to distinct clades, with *M. apri* and *M. salmi* close to each other, and separated from *M. pudendotectus*.

Prevalence of infection

Of the 739 domestic pigs examined, *M. apri* was found in 178 (24.1%) pigs with an intensity ranging from 7 to 113 (average: 30.1 ± 20.6) nematodes per infected pigs, and *M. pudendotectus* was found in 17 (2.3%) pigs with an intensity ranging from 2 to 10 (average: 5.3 ± 2.4) nematodes per infected pigs. There was a statistically significant difference ($P < 0.0001$) between the two species in terms of the infection rate and the intensity. We found pigs being infected with only *M. apri* or co-infected with the two species (*M. apri* and *M. pudendotectus*), but there were no pigs found to be infected with only *M. pudendotectus*.

Discussion

The morphological taxonomic features of the two lungworm species, *M. apri* and *M. pudendotectus*, found in domestic pigs in Vietnam were compatible with that described by Gassó *et al.* (2014). The two species are distinguishable as follows: in females, *M. pudendotectus* bears a well-developed prevulvar cuticular dilatation, whereas *M. apri* does not; and in males, *M. apri* bears two spicules much longer than those of *M. pudendotectus* (4.2 ± 0.2 mm vs. 1.38 ± 0.07 mm) (Gassó *et al.*, 2014). In addition to these diagnostic features, we found that the body size of *M. apri* was greater than *M. pudendotectus*, and the spicules of *M. apri* usually protruded out the body, whereas those of *M. pudendotectus* usually indented into the body, distant to the posterior end of the body 1.5–2.0 mm (about the length of the spicules). The protruded or indented status of the spicules should be a typical feature of each species because the specimens of the two species were all preserved in 4% formaldehyde solution by the same method. To our knowledge, this particular feature has not been mentioned previously. The spicules of *Metastrongylus* males should be examined in more detail to see if indentation of the spicules is a typical feature of *M. pudendotectus* or just a unique feature of Vietnamese specimens.

In the present study, we did not obtain ITS2 sequences of *M. pudendotectus*. However, as expected, the *cox*1 sequences of *M. pudendotectus* and the ITS2 sequences of *M. apri* from the specimens collected in Vietnam supported the morphological identification of the two species. They were clustered with reference sequences of these two species from other countries with some differences. It should be noted that almost all ITS2 sequences of *M. elongatus* from Estonia were completely identical to the *M. apri* sequence (KP890022) from Denmark and were placed in the same clade in the ITS2 tree. It is not surprising, because the two species names are considered synonyms based on morphology. The issue is that the two names are used inconsistently; some scientists use *M. apri* as the validated name and consider *M. elongatus* a synonym (Gassó *et al.*, 2014; Poglayen *et al.*, 2015), whereas others accept *M. elongatus* and place *M. apri* as a synonym (Nssien & Adesehinwa, 1999; Nosal *et al.*, 2010). Furthermore, the nucleotide sequences under accession numbers AJ305373–AJ305381, registered in GenBank with the name *M. elongatus*, were originated from the source organism of *M. apri* (Conole *et al.*, 2001). Taxonomically, however, the validated name of this species should be used correctly and consistently. Historically, Railliet & Henry (1911) used the name *M. elongatus* in place of *M. apri* without explanation (Dougherty, 1944). Since then, this proposal was not always but usually adopted. Importantly, based on the basis of priority of the International Code of Zoological Nomenclature, *M. elongatus* (Dujardin, 1845) Railliet & Henry, 1911 should be

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a synonym of *M. apri* (Gmelin, 1790) Vostokov, 1905. The molecular analysis supported this synonymization, as ITS2 sequences of *M. elongatus* were completely identical to that of *M. arpi*. Genetically, *M. apri* was found to be closely related to *M. salmi* but far distant from *M. pudendotectus* in both ITS2 and *cox*1 regions. Currently, there are relatively few nucleotide sequences of few *Metastrongylus* species in comparison to their abundance and wide distribution. Therefore, more sequence data of *Metastrongylus* species from various geographical locations should be obtained and analysed for a full understanding of their molecular phylogenetic relationships.

Regarding prevalence, most investigations focused on *Metastrongylus* spp. in wild boars in Europe, the Americas and Africa, reporting high infection rates up to 100% (Forrester *et al.*, 1982; Epe *et al.*, 1997; Poglayen *et al.*, 2015; Amayour *et al.*, 2016). There are few reports on lungworms in domestic pigs (Nssien & Adesehinwa, 1999; Adedokun *et al.*, 2001; Carstensen *et al.*, 2002; Li *et al.*, 2016, 2018). In the present study in Vietnam, we found the infection rate of *M. apri* in the pigs studied was much higher than that of *M. pudendotectus*. Our data agrees with previous studies that *M. apri* was the dominant species and usually co-infected with *M. pudendotectus* (Nssien & Adesehinwa, 1999; Nosal *et al.*, 2010), although the composition of *Metastrongylus* spp. and the prevalence varies from place to place (Nosal *et al.*, 2010). Moreover, we did not find *M. pudendotectus* infecting any pig individuals alone. Similarly in Florida, USA, a single infection with *M. apri* and concurrent infections with *M. apri* and *M. pudendotectus* or with *M. apri* and *M. salmi* were found, but a single infection with only *M. pudendotectus* or *M. salmi* was not observed (Forrester *et al.*, 1982). This may suggest that infection of *M. pudendotectus* is dependent on or requires prior infection with *M. apri*, as previously speculated on the mutualistic association between *M. apri* and *M. pudendotectus* (Ewing *et al.*, 1982). On the other hand, Poglayen *et al.* (2015) found random infections of different *Metastrongylus* species within the same host, they did not observe dependence or competition among them. Thus, more surveys for *Metastrongylus* nematodes should be conducted to confirm this ecological point.

In conclusion, the present study provided the prevalence, morphological characteristics and molecular data of *M. apri* and *M. pudendotectus* from domestic pigs in Northern Vietnam, with a slight morphological and genetic variation compared to those from other countries. Our analyses also reveal a close relationship between *M. apri* and *M. salmi*, but far distant from *M. pudendotectus*, and highlight the needs for further studies to clarify the morphological characteristics, ecological and molecular phylogenetic relationships of *Metastrongylus* species at the global scale.

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Conflicts of interest. None.

Ethical standards. The present study was approved by the rector of Thai Nguyen University of Agriculture and Forestry (decision no. 225/QĐ-ĐHNL-ĐT). The lungs of domestic pigs were purchased from the local owners in Dien Bien province where pork meat and visceral parts of pigs are sold in local markets. The use of the lungs of domestic pigs for research purposes was informed to the owners with their approval.

References

Adedokun OA, Adejinmi JO and Ukoikpoko GB (2001) Effect of husbandry system on the incidence of lungworm (*Metastrongylus* spp.) in pigs in Ibadan, Nigeria. *Tropical Veterinarian* **19**, 175–177.

Alcaide M, Frontera E, Rodríguez MJ, Sáez IE, Domínguez–Alpízar JL, Reina D and Navarrete I (2005) Parasitosis pulmonares del cerdo ibérico: situación actual de la metastrongilosis en España. *Mundo Ganadero* **176**, 40–44.

Amayour A, Alaoui Z, Alkhali A, Hassouni T, Kharrim K and Belghyti D (2016) Lung parasites of the genus *Metastrongylus* Molin, 1861 (Nematoda: Metastrongilidae) in wild boar (*Suc scrofa barbarus*) in middle atlas region of Morocco: an epidemiological study. *Journal of Biological Research* **6**, 2020–2025.

Arambulo PV, Hernandez VC and Soria-Abaga A (1967) On the prevalent species of swine lungworm (genus *Metastrongylus* Molin, 1861) in the Philippines. *Journal of Parasitology* **53**, 988.

Bowles J, Hope M, Tiu WU, Liu SX and McManus DP (1993) Nuclear and mitochondrial genetic markers highly conserved between Chinese and Philippine *Schistosoma japonicum*. *Acta Tropica* **55**, 217–229.

Bowles J, Blair D and McManus DP (1995) A molecular phylogeny of the human schistosomes. *Molecular Phylogenetics and Evolution* **4**, 103–109.

Calvopina M, Caballero H, Morita T and Korenaga M (2016) Human pulmonary infection by the zoonotic *Metastrongylus salmi* nematode. The first reported case in the Americas. *American Journal of Tropical Medicine and Hygiene* **95**, 871–873.

Carreno RA and Nadler SA (2003) Phylogenetic analysis of the metastrongyloidea (Nematoda: Strongylida) inferred from ribosomal RNA gene sequences. *Journal of Parasitology* **89**, 965–973.

Carstensen L, Vaarst M and Roepstorff A (2002) Helminth infections in Danish organic swine herds. *Veterinary Parasitology* **106**, 253–264.

Conole JC, Chilton NB, Järvis T and Gasser RB (1999) Intraspecific and interspecific variation in the second internal transcribed spacer (ITS-2) sequence for *Metastrongylus* (Nematoda: Metastrongyloidea) detected by high resolution PCR-RFLP. *International Journal for Parasitology* **29**, 1935–1940.

Conole JC, Chilton NB, Jarvis T and Gasser RB (2001) Mutation scanning analysis of microsatellite variability in the second internal transcribed spacer (precursor ribosomal RNA) for three species of *Metastrongylus* (Strongylida: Metastrongyloidea). *Parasitology* **122**, 195–206.

Dougherty EC (1944) The genus *Metastrongylus* Molin, 1861 (Nematoda: Metatorngylidae). *Proceedings of the Helminthological Society of Washington* **11**, 68–73.

Epe C, Spellmeyer O and Stoye M (1997) Investigations on the occurrence of endoparasites in wild boars. *Zeitschrift für Jagdwissenschaft* **43**, 99–104.

Ewing MS, Ewing SA, Keener MS and Mulholland RJ (1982) Mutualism among parasitic nematodes: a population model. *Ecological Modelling* **15**, 353–366.

Forrester DJ, Porter JH, Belden RC and Frankenberger WB (1982) Lungworms of feral swine in Florida. *Journal of the American Veterinary Medical Association* **181**, 1278–1280.

García–González ÁM, Pérez–Martín JE, Gamito–Santos JA, Calero–Bernal R, Alcaide–Alonso M and Frontera–Carrión EM (2013) Epidemiologic study of lung parasites (*Metastrongylus* spp.) in wild boar (*Sus scrofa*) in southwestern Spain. *Journal of Wildlife Diseases* **49**, 157–162.

Gassó D, Rossi L, Mentaberre G, *et al.* (2014) An identification key for the five most common species of *Metastrongylus*. *Parasitology Research* **113**, 3495–3500.

Jex AR, Hall RS, Littlewood DT and Gasser RB (2010) An integrated pipeline for next-generation sequencing and annotation of mitochondrial genomes. *Nucleic Acids Research* **38**, 522–533.

Kumar S, Stecher G and Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870–1874.

Leignel V, Humbert JF and Elard L (1997) Study by ribosomal DNA ITS-2 sequencing and RAPD analysis on the systematics of four *Metastrongylus* species (Nematoda: Metastrongyloidea). *Journal of Parasitology* **83**, 606–611.

Li K, Shahzad M, Zhang H, Mehmood K, Jiang X, Luo H, Zhang L, Dong X and Li J (2018) Characterization of the complete mitochondrial genome of *Metastrongylus salmi* (*M. Salmi*) derived from Tibetan pigs in Tibet, China. *Acta Parasitologica* **63**, 280–286.

Li K, Luo H, Zhang H, *et al.* (2016) First report of *Metastrongylus pudendotectus* by the genetic characterization of mitochondria genome of cox1 in pigs from Tibet, China. *Veterinary Parasitology* **223**, 91–95.

https://doi.org/10.1017/S0022149X21000353 Published online by Cambridge University Press

Miloshev B (1956) Case of triple infection with *Metastrongylus elongatus*, *Thaeniarhynchus saginatus* and *Enterobius vermicularis*. *Suvremenna Meditsina* **7**, 94–97 (in Bulgarian).

Nosal P, Kowal J and Nowosad B (2010) Structure of Metastrongylidae in wild boars from southern Poland. *Helminthologia* **47**, 212–218.

Nssien MAS and Adesehinwa AOK (1999) Seasonal variation and prevalence of lungworm infection in the three different breeds of pigs slaughtered at Bodija municipal government abattoir, Ibadan, Nigeria. *Tropical Animal Science Journal* **2**, 209–214.

Poglayen G, Marchesi B, Dall'Oglio G, Barlozzari G, Galuppi R and Morandi B (2015) Lung parasites of the genus *Metastrongylus* Molin, 1861 (Nematoda: Metastrongilidae) in wild boar (Sus scrofa L., 1758) in central-Italy: an eco-epidemiological study. *Veterinary Parasitology* **217**, 45–52.

Railliet A and Henry A (1911) Helminthes du pore recueillis par *M. Bauche* en Annam. *Bulletin de la Societe de Pathologie Exotique* **4**, 693–699.

Yong HS, Song SL, Eamsobhana P and Lim PE (2016) Complete mitochondrial genome of *Angiostrongylus malaysiensis* lungworm and molecular phylogeny of metastrongyloid nematodes. *Acta Tropica* **161**, 33–40.

https://doi.org/10.1017/S0022149X21000353 Published online by Cambridge University Press