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Genetic characteristics and phylogenetic relationship of *Parascaris* spp. from *Equus zebra, E. caballus*, and *E. asinus*

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ABSTRACT

The equine *Parascaris* spp. is large, parasitic nematodes, and predominantly focuses on the intestine of foals and young weanlings. There are two roundworms, *Parascaris equorum* and *Parascaris univalens*, recognized among equine hosts. In this study, all fifty-nine *Parascaris* worms were harvested from three different equine hosts (twenty specimens from *Equus zebra*, twenty specimens from *E. caballus,* and nineteen specimens from *E. asinus*). The ribosomal gene (ITS) and mitochondrial genes (*cox*1 and *nadh*1) were amplified to identify and genetically characterize these worms. Analysis of ITS sequences revealed five genotypes among the fifty-nine worms, and the sequence similarity among the worms from *E. zebra* and *E. caballus* was at a high level (99.87%), while the one of *E. asinus* worms showed an apparent difference from the worms either from the *E. zebra* or from the *E. caballus* (sequence similarity ranging from 93.04 to 93.42%). Analysis of mitochondrial genes revealed that twenty-one (*cox*1 gene) and thirteen (*nadh*1 gene) unique haplotypes were defined among the fifty-nine worms. The shared haplotypes (four *cox*1 haplotypes and one *nadh*1 haplotype) only occurred between the worm populations from *E. zebra* and *E. caballus*. The *cox*1 and *nadh*1 haplotype sequences were respectively applied to construct phylogenetic trees. Although the topologies showed that *E. asinus* worm population had an obvious boundary with the worm populations of the *E. zebra* and the *E. caballus*, however, no noticeable boundary was found within the two later worm populations. Meanwhile, the *E. asinus* worm population showed an obvious genetic differentiation and an extremely low gene flow (close to zero) with the worm populations from *E. zebra* and *E. caballus*, indicating that the genetic characteristics of the worms from the *E. asinus* have an obvious difference with the one from *E. zebra* and *E. caballus*.

1. Introduction

Equine roundworms are large parasitic nematodes that predominantly infects foals and weanlings. *Parascaris* spp. have a direct lifecycle where infective eggs ingested from the environment hatch in the horse's stomach, larvae then penetrate the intestinal mucosa and migrate through the liver and lungs before returning to the small intestine to mature to adult worms and reproduce. The *equus* hosts can show signs of coughing, anorexia, rough coat, and unthrifty appearance. Lungs had focal areas of necrosis with hemorrhage and interstitial pneumonia with hyalinization of the alveolar walls (Srihakim and Swerczek, 1978; Martin et al., 2018).

There are two species of roundworms, *Parascaris equorum* (*P. equorum*) and *Parascaris univalens* (*P. univalens*), that infect equine hosts. These two species are morphologically identical and it is necessary to perform karyotyping techniques of chromosomes to determine the specie. *P. univalens* has one pair of chromosomes while *P. equorum* have two (Goday and Pimpinelli, 1984; Martin et al., 2018). Typically, the *P. equorum* was found to be more frequency than the *P. univalens* as that was recognized as a “forgotten” and overlook species (Jabbar et al., 2014). However, two studies using the karyotyping techniques demonstrated that the *P. univalens* was the only roundworm occurring on farms in Kentucky (USA), and in on farms from different regions of Sweden (Nielsen et al., 2014; Martin et al., 2018). The probable reason

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for it is that the *P. univalens* was misrecognized as *P. equorum* to identify the *Parascaris* spp. in previously researches.

Some scholars reported that *P. equorum* and *P. univalens* are synonyms (Gao et al., 2018), while the majority of parasitologists believed that they are two different species (Nielsen et al., 2014; Martin et al., 2018). Additionally, there is a question worth considering, if they are two different parasites and if they are specific or nonspecific host parasites.

Comprehending the taxonomic position and genetic characteristics has important implications for controlling *Parascaris* worms (Tydén et al., 2013). Studies on population genetic structure of *Parascaris* spp. have been conducted in North America, Australasia, and Europe continents (Tydén et al., 2013; Jabbar et al., 2014; Nielsen et al., 2014). However, no adequate research on this topic has been carried out yet in China. The *Equus* species, such as *E. zebra*, *E. caballus,* and *E. asinus*, are the main reservoir hosts for *Parascaris* worms. In the present study, we harvested *Parascaris* spp. worms from three common *equus* hosts (*E. zebra*, *E. caballus,* and *E. asinus*). Consequently, according to ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) sequences, our objectives were to explore the genetic characteristics and phylogenetic relationships of *Parascaris* worms collected from the three different hosts.

2. Materials and methods

2.1. Parasite materials and isolation of genomic DNA

Here, six *E. asinus* used for Colla Corii Asini, a traditional Chinese medicine, and four *E. caballus* used were captive in two nearby farms in Chifeng, China (N42° 14′, E119° 19′). In addition, eight *E. zebra* were always captive in the Harbin Northern Forest Zoo, China (N45° 23′, E127° 06′). Twenty adult worms from *E. caballus* and nineteen adult worms from *E. asinus* were collected deworming with albendazole (10 mg/kg; Zentel®), and twenty adult worms from *E. zebra* were collected deworming with fenbendazole (5 mg/kg; Panacur®). All the 59 worms were rinsed in saline and then fixed in 70% ethanol until DNA was extracted. Genomic DNA of all the specimens was extracted using a QIAamp DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and then stored at −70 °C until use.

2.2. Polymerase chain reaction (PCR) and sequencing of all the specimens

For identification of species, partial sequence (˜770-bp) of the internal transcribed spacer (ITS, including partial ITS1, 5.8 s, and ITS2) was amplified by PCR with a pair of universal primers: NC5 (5´-GTA GGTGAACCTGCGGAAGGATCATT-3´) and NC2 (5´-TTAGTTTCTTTTC CTCCGCT-3´) (Zhu et al., 2002). Two fragment genes of mtDNA, cytochrome c oxidase subunit 1 (*cox*1) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nadh*1), were amplified to explore the genetic characteristics and phylogenetic relationships. Primer F5 (5´-TCATAAGGATATTGGGACC-3´) and primer F6 (5´-GCAAAATGTA AAGGGAAAA-3´) (Franssen et al., 2013) were applied to amply *cox*1 gene (996-bp) of each specimen. Primer F7 (5´-GCTTTTRTTACYTTR-TATGAGCGTCA-3´) and primer F8 (5´-ATYAGRTCATAACGAAA-ACG-3´) (Franssen et al., 2013) were applied to amply *nadh*1 gene (639-bp) of each specimen. Regarding the three PCR reactions, all the volumes were 25 μl, included 10 × Taq buffer, 2.5 mM of dNTPs, 2 mM $MgCl_2$, 220 ng/μl DNA sample, 100 pmol of each primer, 12 μl ddH_2O water, and 5 U Thermo Scientific *Taq* DNA polymerase under the following conditions: initial denaturation at 94 °C for 5 min, then 30 cycles at 94 °C for 30 s (denaturation); at 55 (ITS)/50 (*cox*1)/48 °C (*nadh*1) for 30 s for annealing, at 72 °C for 60 (ITS)/90 (*cox*1)/60 s (*nadh*1) for extension, followed by a final extension at 72 °C for 7 min. The PCR product was examined on a 1.0% agarose gel to verify that the product contained a single band of the appropriate size. Column-purified PCR products were sent to Beijing Genomics Institute (Beijing, China) for sequencing in forward and reverse directions.

2.3. Data and phylogenetic analyses

The raw ITS sequences were aligned using ClustalX v2.0 software (Thompson et al., 1997). The raw *cox*1 and *nadh*1 sequences were trimmed and edited aligned by a codon-based alignment that was performed using MUltiple Sequence Comparison by Log-Expectation (MUSCLE) algorithm (Robert, 2004) within MEGA 5 (Tamura et al., 2011). The DnaSP 5.10 software (Librado and Rozas, 2009) was applied to establish sequence haplotypes and calculate haplotype diversity, average number of nucleotide differences, and nucleotide diversity.

For phylogenetic analysis, all the *cox*1 and *nadh*1 haplotypes defined in the present study were used to hypothesize phylogenetic relationships, respectively. The complete mitochondrial genome of *Ascaris suum* (*A. suum;* GenBank Accession No. NC001327) acted as the outgroup. Phylogenetic analysis was hypothesized using Bayesian inference (BI) and maximum likelihood (ML). The best-fitting nucleotide substitution model was selected by Modeltest 3.7 software with Akaike information criterion (AIC) (Posada and Crandall, 1998). For ML, the best models of *cox*1 and *nadh*1 sequences were HKY + I and HKY + G, respectively. Besides, the phylogenetic analysis was conducted by using PhyML 3.0 software (Guindon et al., 2009). Bootstrap branch support values (MLBS) were obtained with 1000 rapid bootstrap inferences, and thereafter searched thorough ML search on the dataset. For BI, the best models of *cox*1 and *nadh*1 sequences were HKY + I and HKY + G, respectively. In addition, the phylogenetic analysis was performed by using MrBayes 3.2 software (Fredrik and Huelsenbeck, 2003). The parameters were set to nst = 2 (both *cox*1 and *nadh*1sequneces), rates = propinv (*cox*1 sequences)/gamma (*nadh*1 sequences), with four Markov chain Monte Carlo (MCMC) runs for 2 runs from random starting trees for 3 million generations, and the trees were sampled every 100 generations. Moreover, 25% of generations were discarded as "burn-in", and the remaining samples were used to calculate Bayesian posterior probabilities (BPP). Phylograms were plotted using FigTree 1.4.2 software (http://tree.bio.ed.ac.uk/software/figtree).

3. Results and discussion

A portion (~770-bp) of the ITS gene was successfully amplified and sequenced from all the 59 *Parascaris* worms. After editing and alignment of sequences, 5 genotypes (GenBank Accession Nos. MK209646-MK209650) were found among the 59 ITS sequences. Genotype 1 represented 6 *Parascaris* worms, which all worms were from *E. zebra.* Genotype 2 had 34 *Parascaris* worms, including 14 *Parascaris* worms from *E. zebra* and 20 *Parascaris* worms from *E. caballus*. The *Parascaris* worms from *E. asinus* were dispersed in Genotypes 3, 4, and 5 (Table 1). We artificially divided the five genotypes into two groups (Group A and Group B) according to the sequence similarity. The Genotypes 1 and 2, which formed Group A, were all from *E. zebra* and *E. caballus*, and the sequence similarity between the two genotypes 1 and 2 was at a high-level (99.87%). The Group B was formed by genotypes 3, 4, and 5, and the sequence similarities within the three genotypes were more than 99.30% (Table 1). Otherwise, each genotype form Group A had low similarity compared with any genotype of Group B (not more than 93.42%, see Table 1), in which those values were remarkably lower than normal values in intra-species, and were likely general values in inter-species. For instance, a study on the *Toxascaris leonina* and *Toxocara cati* of the Asiatic lion indicted that the similarities of ITS2 sequences in the intra-species were more than 97% and 99%, respectively (Pawar et al., 2012). The similarity of ITS1 between *Asaris lumbricoides* and *A. suum* was 100%, in which similarities among *Baylisascaris schroederi*, *B. procyonis,* and *B. transfuga* were 91.8%, 96.9%, and 93.5%, respectively (Peng et al., 2016). ITS analysis indicated that the two worm groups have apparently different genetic characteristics.

The *cox*1 and *nadh*1 genes of all 59 *Parascaris* worms from the three different hosts were successfully amplified. After editing and alignment of sequences, 996-bp gene fragment of *cox*1 and 639-bp gene fragment

Table 1
Sequence similarity (%) of 5 ITS genotypes from 59 *Parascaris* spp. worms from three different equid hosts.

Group	Genotype	GT1	GT2	GT3	GT4	GT5	Number of parasites			Total
							PEz[a]	PEc[b]	PEa[c]	
Group A										
	GT1						6	0	0	6
	GT2	99.87					14	20	0	34
Group B										
	GT3	93.29	93.42				0	0	13	13
	GT4	93.04	93.16	99.37			0	0	3	3
	GT5	93.04	93.16	99.49	99.87		0	0	3	3
	Total	–	–	–	–	–	20	20	19	59

[a] PEz, *Parascaris* spp. worms from *Equus zebra*.
[b] PEc, *Parascaris* spp. worms from *Equus caballus*.
[c] PEa, *Parascaris* spp. worms from *Equus asinus*.

Table 2
Details of nucleotide and haplotype diversity of *cox*1 and *nadh*1 sequences of the 59. *Parascaris* spp. worms from three different equid hosts.

Parasite	*N*	*h*	hd	Ps	Pi	Si	π
Cytochrome oxidase c subunit 1 (996 bp length)							
PEz[a]	20	10	0.8632	12	7	5	0.00377
PEc[b]	20	10	0.8632	15	10	5	0.00377
PEa[c]	19	5	0.7368	14	10	4	0.00319
Nicotine amide dehydrogenase subunit 1 (639 bp length)							
PEz[a]	20	2	0.5263	5	5	0	0.00412
PEc[b]	20	8	0.8316	8	6	2	0.00272
PEa[c]	19	4	0.5848	5	5	0	0.00205

Abbreviations: NParascaris spp. population size; hNumber of haplotypes; hdhaplotype diversity; Pspolymorphic sites; Piparsimony informative sites; Sisingleton sites; πnucleotide diversity.
[a] PEz, *Parascaris* spp.worms from *Equus zebra*.
[b] PEc, *Parascaris* spp. worms from *Equus caballus*.
[c] PEa, *Parascaris* spp. worms from *Equus asinus*.

of *nadh*1 were obtained. For *cox*1 gene, 10, 10, and 5 unique haplotypes (GenBank Accession Nos. MK209651-MK209671) were defined respectively within the *Parascaris* populations from *E. zebra*, *E. caballus,* and *E. asinus*. The haplotype diversity of the three worm populations was 0.8632, 0.8632, and 0.7368, respectively (Table 2). Those haplotypes showed four shared haplotypes between the *E. zebra* and *E. caballus* worm populations, while no shared haplotypes were found with *E. asinus*. Analysis of the fifty-nine individual roundworms for *nadh*1 gene revealed thirteen unique haplotypes (GenBank Accession Nos. MK209672-MK209684), and one shared haplotype was found between the *E. zebra* and *E. caballus* worm populations, whereas no shared haplotypes were detected with *E. asinus*, that is in agreement with the results of analysis of the *cox*1. The haplotype diversity on *nadh*1 gene for the three populations was 0.5263, 0.8316, and 0.5848, respectively. Other sequence information of *cox*1 and *nadh*1 genes is presented in Table 2.

To explore the genetic relationships of *Parascaris* worms from the three different hosts, the *cox*1 and *nadh*1 haplotype sequences were respectively applied to constructed phylogenetic trees using the two methods. The *cox*1 gene trees were based on 21 haplotype sequences of 59 *Parascaris* worms from the three different hosts, in which the topology of the BI tree (Fig. 1) showed that all the five haplotype sequences representing 19 *Parascaris* worms from *E. asinus* formed a distinct Clade (Clade 2, Fig. 1). Sequences of the *Parascaris* worms isolated from the *E. zebra* and *E. caballus* were randomly dispersed within the Clade 1 (Fig. 1). The topology of *cox*1 gene tree resulting from ML method (not shown) was corresponded with the BI inference. Meanwhile, the BI tree (Supplementary Fig. S1) created by the 13 *nadh*1 haplotype sequences of fifty-nine *Parascaris* worms from the three different hosts showed the same results that *Parascaris* individuals isolated

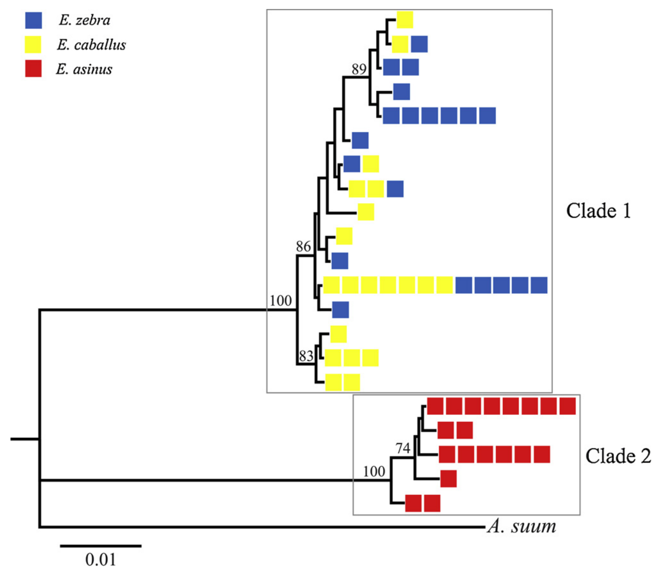

Fig. 1. Phylogenetic tree resulting from Bayesian analysis (3 million generations) constructed with 21 *cox*1 sequences haplotypes representing 59 *Parascaris* worms from three different equid hosts. Different colored squares represent haplotypes from different sampling hosts. Posterior probabilities lower than 60 were not displayed in the tree.

from *E. asinus* had an obvious boundary with the worms from the *E. zebra* and *E. caballus*, while no noticeable boundaries were present within the two later worm populations. Furthermore, the *nadh*1 gene tree (not shown) resulting from ML also revealed the same phenomenon. As a result, being consistent with analysis of the sequence of ITS, the two groups had apparently different genetic characteristics, which found to be helpful in analysis of the sequences of *cox*1 and *nadh*1.

To our knowledge, the current study for the first time presented molecular comparison (rDNA and mtDNA) of *Parascaris* worm populations from *equus* hosts. Additionally, we further explored the genetic structure of the three worm populations based on *cox*1 and *nadh*1 genes to test the taxonomic position of the equine roundworm. Firstly, we hypothesized that all the roundworms from *equus* hosts were the same species. The pairwise fixation index (*Fst*) values, an index of genetic differentiation among the three different *equus* hosts (*E. zebra*, *E. caballus* and *E. asinus*), were calculated using Arlequin 3.5 software (Excoffier and Lischer, 2010). Moreover, the migration number of each generation (*Nm*) value was calculated according to the following formula: $Fst = 1 / (4Nm + 1)$ (Weir and Cockerham, 1984) to directly estimate gene flow between the equine roundworm populations. *Fst* values based on *cox*1 and *nadh*1 genes between the *Parascaris* worm populations isolated from *E. zebra* and *E. caballus* were 0.20775 and

0.29542, respectively (Supplementary Table. S1), and the *Nm* values were closed to one (Supplementary Table. S2). However, the *E. asinus* worm population and the worm populations from *E. zebra* and *E. caballus* showed high levels in *Fst* values (Supplementary Table. S1) and the *Nm* values were closed to zero (Supplementary Table. S2). The above mentioned results suggested that no obvious genetic differentiation occurred between the *E. zebra* and *E. caballus* worm populations, while the *E. asinus* worm population showed extremely low gene flow with the worm populations from *E. zebra* and *E. caballus*.

For the parasitic nematodes, the gene flow was found to be influenced by the host movement, parasite life histories, geographical barriers, and effective population sizes (Hussain et al., 2014). Tydén et al (2013) studied the *P. equorum* populations from Europe and North America continents based on DNA fingerprinting, and indicated that there was no genetic differentiation among the worm populations from the two different continents. For the *Parascaris* worms, the geographical barriers between the continents played an insignificant role in blocking the gene flow. Host movement caused by the trade of livestock throughout China is currently frequent (Yin et al., 2013); consequently, there are no geographical barriers among the *Parascaris* worms harvested from the *E. asinus* and *E. caballus*. However, it should be noted that an obvious genetic differentiation occurred between the *E. asinus* and *E. caballus* worm populations.

Due to the fact that the *E. zebra* was always captive in the zoo, the *E. zebra* worm population had geographical isolation with the two worm populations from the *E. asinus* and *E. caballus*, however, it was surprising that the obvious genetic differentiation only appeared between the *E. zebra* and *E. asinus*, while there was no a notable genetic differentiation between the *E. zebra* and *E. caballus* worm populations. Nevertheless, the obvious genetic differentiation only occurred between the *E. zebra* and *E. asinus* worm populations was worthy of consideration. Therefore, using genetic analysis of ITS, *cox*1 and *nadh*1, and gene flow of the *Parascaris* worms from the three different hosts, it can be concluded that the ascarid worms from *equus* hosts are two *Parascaris* species, however, this speculation needs to be further confirmed by the method of karyotyping in the future.

If the *Parascaris* spp. in three equine hosts were two independent species, then one *Parascaris* species might infect *E. zebra* and *E. caballus*, and another one would infect *E. asinus*, which can answer the question of why the roundworm populations from *E. caballus* and *E. asinus* have no gene flow between them even if there are no geographical barrier.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.06.013.

References

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567.
Fredrik, R., Huelsenbeck, J.P., 2003. MrBayes 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Franssen, F., Xie, K., Sprong, H., Van Der Giessen, J., 2013. Molecular analysis of *Baylisascaris columnaris* revealed mitochondrial and nuclear polymorphisms. Parasit. Vectors 6, 124.
Gao, J.F., Zhang, X.X., Wang, X.X., Li, Q., Li, Y., Xu, W.W., Gao, Y., Wang, C.R., 2018. According to mitochondrial DNA evidence, *Parascaris equorum* and *Parascaris univalens* may represent the same species. J. Helminthol. 1–6.
Goday, C., Pimpinelli, S., 1984. Chromosome organization and heterochromatin elimination in Parascaris. Science 224, 411–413.
Guindon, S., Delsuc, F., Dufayard, J.F., Gascuel, O., 2009. Estimating maximum likelihood phylogenies with PhyML. Methods Mol. Biol. 537, 113–137.
Hussain, T., Periasamy, K., Nadeem, A., Ellahi Babar, M., Pichler, R., Diallo, A., 2014. Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates from domestic ruminants in Pakistan. Vet. Parasitol. 206, 188–199.
Jabbar, A., Littlewood, D.T.J., Mohandas, N., Briscoe, A.G., Foster, P.G., Müller, F., Von Samson-Himmelstjerna, G., Jex, A.R., Gasser, R.B., 2014. The mitochondrial genome of *Parascaris univalens* - implications for a "forgotten" parasite. Parasit. Vectors 7, 1–8.
Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Martin, F., Höglund, J., Bergström, T.F., Karlsson Lindsjö, O., Tydén, E., 2018. Resistance to pyrantel embonate and efficacy of fenbendazole in *Parascaris univalens* on Swedish stud farms. Vet. Parasitol. 264, 69–73.
Nielsen, M.K., Wang, J., Davis, R., Bellaw, J.L., Lyons, E.T., Lear, T.L., Goday, C., 2014. *Parascaris univalens* - a victim of large-scale misidentification? Parasitol. Res. 113, 4485–4490.
Pawar, R.M., Lakshmikantan, U., Hasan, S., Poornachandar, A., Shivaji, S., 2012. Detection and molecular characterization of ascarid nematode infection (*Toxascaris leonina* and *Toxocara cati*) in captive Asiatic lions (*Panthera leo persica*). Acta Parasitol. 57, 67–73.
Peng, Z., Liu, S., Hou, Z., Xing, M., 2016. Ascarid infestation in captive Siberian tigers in China. Vet. Parasitol. 226, 74–77.
Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Robert, C.E., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.
Srihakim, S., Swerczek, T.W., 1978. Pathologic changes and pathogenesis of *Parascaris equorum* infection in parasite-free pony foals. Am. J. Vet. Res. 39, 1155–1160.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.
Tydén, E., Morrison, D.A., Engström, A., Nielsen, M.K., Eydal, M., Höglund, J., 2013. Population genetics of *Parascaris equorum* based on DNA fingerprinting. Infect. Genet. Evol. 13, 236–241.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution. 38, 1358–1370.
Yin, F., Gasser, R.B., Li, F., Bao, M., Huang, W., Zou, F., Zhao, G., Wang, C., Yang, X., Zhou, Y., Zhao, J., Fang, R., Hu, M., 2013. Genetic variability within and among *Haemonchus contortus* isolates from goats and sheep in China. Parasit. Vectors 6, 279.
Zhu, X.Q., D'Amelio, S., Palm, H.W., Paggi, L., George, N.M., Gasser, R.B., 2002. SSCP-based identification of members within the *Pseudoterranova decipiens*complex (Nematoda: Ascaridoidea: Anisakidae) using genetic markers in the internal transcribed spacers of ribosomal DNA. Parasitology 124, 615–623.