

Genetic and morphological characterization of *Trichuris myocastoris* found in *Myocastor coypus* in the Czech Republic

K. Rylková¹ · E. Tůmová² · A. Brožová¹ · I. Jankovská¹ · J. Vadlejch¹ · Z. Čadková¹ · J. Frýdlová¹ · P. Peřínková¹ · I. Langrová¹ · D. Chodová² · S. Nechybová¹ · Š. Scháňková¹

Received: 29 June 2015 / Accepted: 3 July 2015 / Published online: 15 July 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract *Trichuris* sp. individuals were collected from *Myocastor coypus* from fancy breeder farms in the Czech Republic. Using morphological and biometrical methods, 30 female and 30 male nematodes were identified as *Trichuris myocastoris*. This paper presents the first molecular description of this species. The ribosomal DNA (rDNA) region, consisting of internal transcribed spacer (ITS)-1, 5.8 gene and ITS-2, was sequenced. Based on an analysis of 651 bp, *T. myocastoris* was found to be different from any other *Trichuris* species for which published sequencing of the ITS region is available. The phylogenetic relationships were estimated using the maximum parsimony methods and Bayesian analyses. *T. myocastoris* was found to be significantly closely related to *Trichuris* of rodents than those of ruminants.

Keywords *Myocastor coypus* · *Trichuris myocastoris* · Parasites · ITS1-5.8S-ITS2 · rDNA

Introduction

Myocastor coypus (coypu or nutria) is a South American semi-aquatic rodent with a wide geographical distribution,

from central Bolivia and southern Brazil to Tierra del Fuego (Argentina), and as a result of escapes and release from fur farms, feral populations now occur in Europe, Asia and North America (Woods et al. 1992).

In the Czech Republic, nutrias were first bred in 1925, when several animals were imported from Argentina. Within a short time, nutria breeding greatly expanded. Currently, nutrias are increasingly used not only for their fur but also for their high-quality meat.

Nutrias, like other animals, suffer from many diseases as well as many parasitic infections. However, studies on nutria parasites are sporadic and focus mainly on wild nutria from the Americas (Babero and Lee 1961; Rossin et al. 2009; Gayo et al. 2011; Martino et al. 2012). The most common endoparasites of wild nutria are *Eimeria* spp., *Longistriata maldonadoi*, *Strongyloides myopotami* and *Trichuris myocastoris* (Babero and Lee 1961; Pastuszko 1991; Rossin et al. 2009; Martino et al. 2012); for European nutria, they are mainly coccidia, *S. myopotami* and *T. myocastoris* (Zajíček 1955; Hohner 1961; Pastuszko 1991). Pellérdy (1963, 1974) reported the following coccidia in nutria: *Eimeria myopotami*, *Eimeria pellucida*, *Eimeria coypi*, *Eimeria seideli* and *Eimeria nutriae*.

T. myocastoris was first described in 1933 by Enigk; Baruš et al. (1975) redescribed this species. On the basis of a detailed study of the morphology and metrical characters of their specimens, which were compared to the original description of *T. myocastoris* and *Trichuris nutria*, Baruš et al. (1975) demonstrated that *T. myocastoris* and *T. nutria* (Petrov, 1941) are synonyms.

In the Czech Republic, nutrias are widespread and they are often bred on farms for their meat and fur. On these farms, they live in close proximity to various domestic animals (especially small ruminants). It is thus questionable whether *Trichuris* sp., which are very common in nutria in the

✉ I. Langrová
langrova@af.czu.cz

¹ Department of Zoology and Fisheries, Faculty of Agrobiolgy, Food and Natural Resources, Czech University of Life Sciences in Prague, Kamýcka 129, 165 21 Prague, 6-Suchdol, Czech Republic

² Department of Animal Husbandry, Faculty of Agrobiolgy, Food and Natural Resources, Czech University of Life Sciences in Prague, Kamýcka 129, 165 21 Prague, 6-Suchdol, Czech Republic

Czech Republic, are the same species described by Enigk in 1933 (England) and redescribed by Baruš in 1975 (Azerbaijan). It is also possible that *Trichuris* sp. originated from another host; Dobrovol'skii (1952) reported that *Trichuris ovis* had been found in the muskrat. A genetic analysis of *Trichuris* sp. has yet to be made from a nutria.

The aim of this study was to identify *T. myocastoris* from different fancy breeder farms in the Czech Republic using morphometrical as well as genetic molecular methods.

Materials and methods

Morphological study

Trichuris sp. individuals were collected from *M. coypus* (young and adult animals) that originated from three nutria farms in the Czech Republic. A total of 60 worms (30 males and 30 females) were separated and rinsed in a physiological saline solution (0.9 w/v NaCl) in order to remove contaminants. These specimens were used for morphometrical determination.

In order to identify *Trichuris* species found in the caecum and colon of the hosts, morphological and biometric studies were performed with an Olympus microscope (BX51).

A total of 60 *Trichuris* individual specimens were identified at the species level according to Enigk (1933) and Baruš et al. (1975).

The following morphometrical features were measured in each specimen: total body length, length of oesophagus, posterior body length, body width at oesophagus end, maximum posterior body width and spicule length.

Molecular study

We analyzed a total of six *Trichuris* sp. individuals. Genomic DNA from individual worm was extracted using a QIAamp Tissue Kit (Qiagen) according to the manufacturer's protocol. Genomic DNA was detected using 0.8 % agarose gel electrophoresis and GelRed (Biotium). The ribosomal DNA (rDNA) region, consisting of internal transcribed spacer (ITS)-1, 5.8 gene and ITS-2, was amplified by the polymerase chain reaction (PCR), using a universal pair of primers: forward NC5 5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3' and reverse NC2 5'-TTA GTT TCT TTT CCT CCG CT-3' (Zhu et al. 2000). The PCR reaction consisted of 15.5 µl of Combi PPP Master Mix (Top-Bio), 5 µl of each primer, 5 µl of template DNA and PRC water (Top-Bio), for a total volume of 50 µl. The PCR profile carried out on MJ Mini™ thermocycler (Bio-Rad) started with a 10-min period of initial denaturation at 94 °C, followed by 35 cycles. Each cycle consisted of a denaturation step at 94 °C for 1 min, a primer annealing step at 55 °C for 1 min and an elongation step at

72 °C for 1 min. The PCR was terminated at a final elongation period of 72 °C for 10 min. Products of the PCR were detected using 0.8 % agarose gel electrophoresis with GelRed (Biotium). The PCR products were purified and sequenced by Macrogen Inc., Korea. Each sample was sequenced from both (3' and 5') ends of both fragments using the same primers that are used for double-strand PCR amplification.

The raw chromatograms were manually assembled and checked by eye for potential errors using computer software BioEdit 5.0.9 (Hall 1999); the same program was used to align the sequences using the ClustalW algorithm. Sequences were compared with the online GenBank (NCBI) nucleotide database using the BLASTn program.

In addition to our samples, we used sequences of several *Trichuris* species downloaded from the GenBank database to set the phylogenetic position of our individuals within the genus. Detailed information regarding material used in our molecular study is listed in Table 1.

Our new six sequences of rDNA region consisting of ITS-1, 5.8 gene and ITS-2 were deposited into the GenBank database under the following accession numbers: KM877518–KM877523.

The phylogenetic relationships were estimated using the maximum parsimony (MP) method and Bayesian analysis (BAY). Equal weight was employed for all sites for the MP analysis, which was conducted using PAUP* version 4.0b10 (Swofford 2000). Tree searches were performed using heuristic searches with a stepwise addition of taxa and TBR branch swapping algorithm. Bootstrap analyses (1000 replicates) were used to assess the relative robustness of tree nodes. The Bayesian tree was constructed using MrBayes version 3.0 (Huelsenbeck and Ronquist 2001). Six Monte Carlo Markov chains ran simultaneously for 1,000,000 generations, with a sampling frequency of 100. Likelihood scores reached stability after c. 30,000 generations. The corresponding trees were discarded as a burn-in, and the remaining trees were used to construct a 50 % majority-rule consensus tree. The posterior probabilities were used to indicate branch supports in the final tree.

Results

Morphological study

The following is a brief description of the whipworm body found in nutria in the Czech Republic. This coincides with a description from Azerbaijan (Baruš et al. 1975); however, nematodes in our study were slightly longer (see Table 2).

The body of whipworms was white, the anterior part of the body gradually tapers towards the anterior end, and this part of body is distinctly narrower than the oesophageal region. The total male body length ranged from 21.38 to 49.44 mm, with

Table 1 List of samples used in molecular study

| GenBank Acc. No. | Species | Host | Reference |
|------------------|----------------------------|-----------------------------|----------------------|
| FN543201 | <i>T. muris</i> | <i>Mus domesticus</i> | Callejón et al. 2008 |
| FN543197 | <i>T. muris</i> | <i>Mus domesticus</i> | Callejón et al. 2008 |
| JX683525 | <i>Trichuris mastomysi</i> | <i>Mastomys natalensis</i> | Ribas et al. 2013 |
| JX683517 | <i>T. mastomysi</i> | <i>Mastomys natalensis</i> | Ribas et al. 2013 |
| JX683524 | <i>Trichuris carlieri</i> | <i>Gerbilliscus vicinus</i> | Ribas et al. 2013 |
| JX683522 | <i>T. carlieri</i> | <i>Gerbilliscus vicinus</i> | Ribas et al. 2013 |
| FR849687 | <i>T. arvicolae</i> | Unknown | Unpublished |
| FN543185 | <i>T. arvicolae</i> | <i>Myodes glareolus</i> | Unpublished |
| KM877518 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| KM877519 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| KM877520 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| KM877521 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| KM877522 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| KM877523 | <i>T. myocastoris</i> | <i>Myocastor coypus</i> | This study |
| JX218223 | <i>T. discolor</i> | <i>Capreolus capreolus</i> | Salaba et al. 2013 |
| JX218222 | <i>T. discolor</i> | <i>Capreolus capreolus</i> | Salaba et al. 2013 |
| JX218218 | <i>T. ovis</i> | <i>Capreolus capreolus</i> | Salaba et al. 2013 |
| JF680987 | <i>T. ovis</i> | Domestic sheep | Unpublished |
| AJ489248 | <i>T. skrjabini</i> | <i>Capra hircus</i> | Cutillas et al. 2004 |

Table 2 Biometrical data (mm) ± standard deviation (SD) of adults *Trichuris myocastoris*

| | Locality | | | |
|--|----------------------------|---------------------|---------------|----------------------|
| | Czech Republic Study | Azerbaijan | Azerbaijan | Germany |
| | Present study | Baruš et al. (1975) | Petrov (1941) | Enigk (1933) |
| Number of individuals | 60 | 43 | | |
| Males | | | | |
| Total length | 37.86 (21.38–49.44)±7.02 | 22.94–35.25 | 38–46 | 30–38 |
| Length of oesophagus | 23.03 (13.01–32.25)±4.99 | 13.26–19.65 | 23.7–25.9 | 18–24 |
| Length of posterior part | 14.69 (8.31–17.72)±2.33 | 9.69–15.6 | – | – |
| Width of body at level of oesophagus end | 0.23 (0.14–0.30)±0.04 | 0.22–0.37 | 0.28–0.36 | – |
| Maximum width of body | 0.31 (0.21–0.47)±0.06 | 0.39–0.51 | 0.43–0.49 | 0.38–0.42 |
| Length of spicule | 3.89 (2.55–4.9)±0.69 | 3.47–3.74 | 3.7–3.9 | 2.7–3.2 |
| Width of the spicule at the middle | 0.031 (0.024–0.038)±0.0043 | 0.037–0.042 | – | 0.038–0.044 |
| Females | | | | |
| Total length | 47.79 (33.89–61.56)±6.04 | 38.36–43.05 | 46–55 | 43–50 |
| Length of oesophagus | 30.21 (20.85–38.43)±3.76 | 24.64–28.08 | 27–34 | 26–32 |
| Length of posterior part | 17.61 (12.03–22.69)±2.35 | 13.14–14.97 | – | 16–18 |
| Width of body at level of oesophagus end | 0.27 (0.16–0.37)±0.05 | 0.29–0.33 | 0.24–0.28 | 0.17–0.22 |
| Maximum width of body | 0.37 (0.29–0.40)±0.04 | 0.48–0.66 | 0.35–0.39 | 0.48–0.54 |
| Distance vulva to sphincter of uterus | 1.39 (1.17–1.62)±1.5 | 1.12–1.85 | – | 0.7–0.9 ^a |
| Length of eggs | 0.058 (0.043–0.066)±0.04 | 0.052–0.06 | 0.060–0.063 | 0.06 |
| Width of eggs | 0.027 (0.023–0.033)±0.02 | 0.03–0.034 | 0.030–0.033 | – |

^a Straight line of the vagina

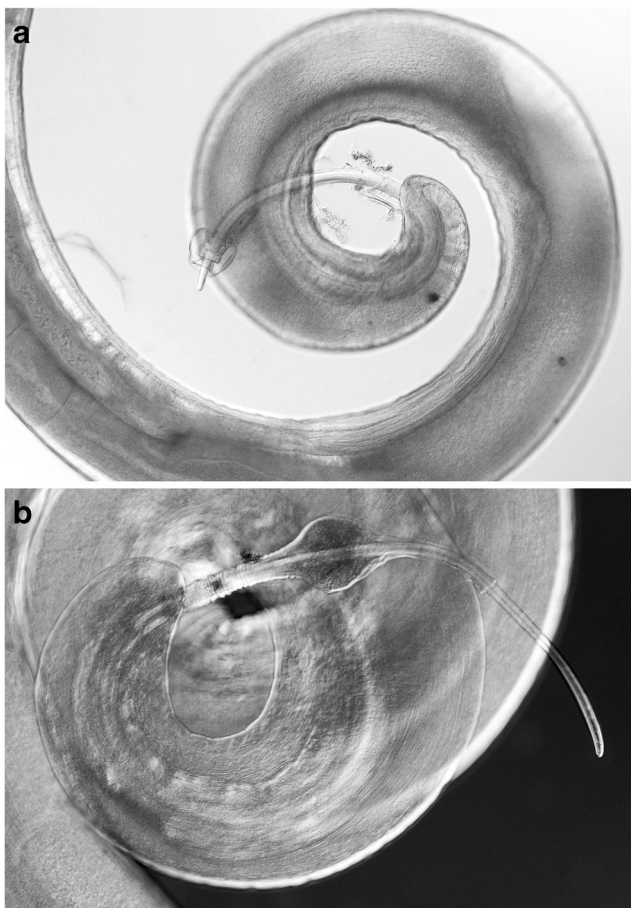


Fig. 1 a, b Males of *Trichuris myocastoris* isolated from nutria

the anterior part measuring 13.01–32.25 mm in length. Small rounded papillae are located on each side of the cloaca. The posterior end of the body is rounded. The spicule is markedly sclerotized, measuring 2.55–4.90 mm in length and 0.024–0.038 mm in width in the middle section and 0.012–0.017 mm in the distal section. Spicule sheath is tubular and covered with spines; however, the distal part of the bulb is covered with spines and the distal tubular part is without spines. The shape of the spicule sheath varies according to the evagination of the spicule, from tubular shape to the bulb (Fig. 1a, b).

The female body is 33.89–61.56 mm in length, and the anterior portion is 20.85–38.43 mm in length. The posterior part of the body measures 12.03–22.69 mm in length and 0.29–0.40 mm in maximum width. The vulva is located at the oesophagus end, and it is in the shape of a transverse slit with slightly frilled margins. It is situated in the centre of a circular cuticular disc, which has a distinctly smooth surface. The vagina extends in a straight line, loops three times and finally connects to the uterus (Fig. 2a, b). The distance from the vulva to the uterus ranged from 1.17 to 1.62 mm. The biometrical parameters of individuals are shown in Table 2.

Molecular study

The final matrix of 19 sequences consisted of 166 bp containing 20 variable characters with 14 parsimony informative sites. Altogether, the analyzed specimens of *Trichuris* revealed 14 haplotypes. Our six specimens from *M. coypus* represent six unique haplotypes. Employed methods have recovered trees of very similar topologies with high statistical supports and sorted sequences into well-supported clusters (Fig. 3).

Discussion

The nutria is indigenous to the southern part of South America, and both wild and captive-raised animals are used for fur and meat. Nutria breeding was mainly used to produce fur during the 1990s. Due to recent changes in the world market situation, meat has changed and become the main product of nutria breeding. In the Czech Republic, nutria meat is

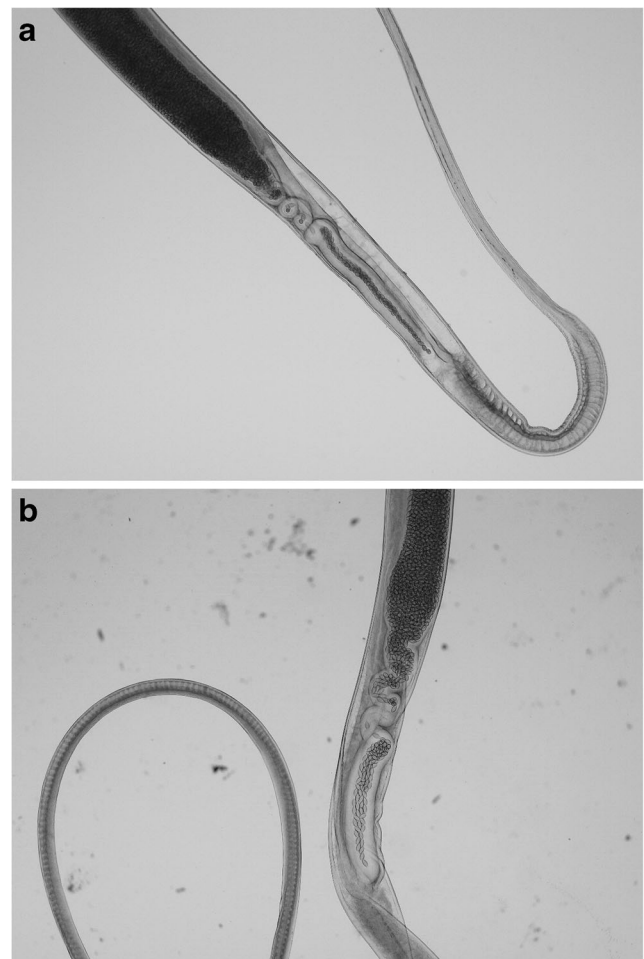
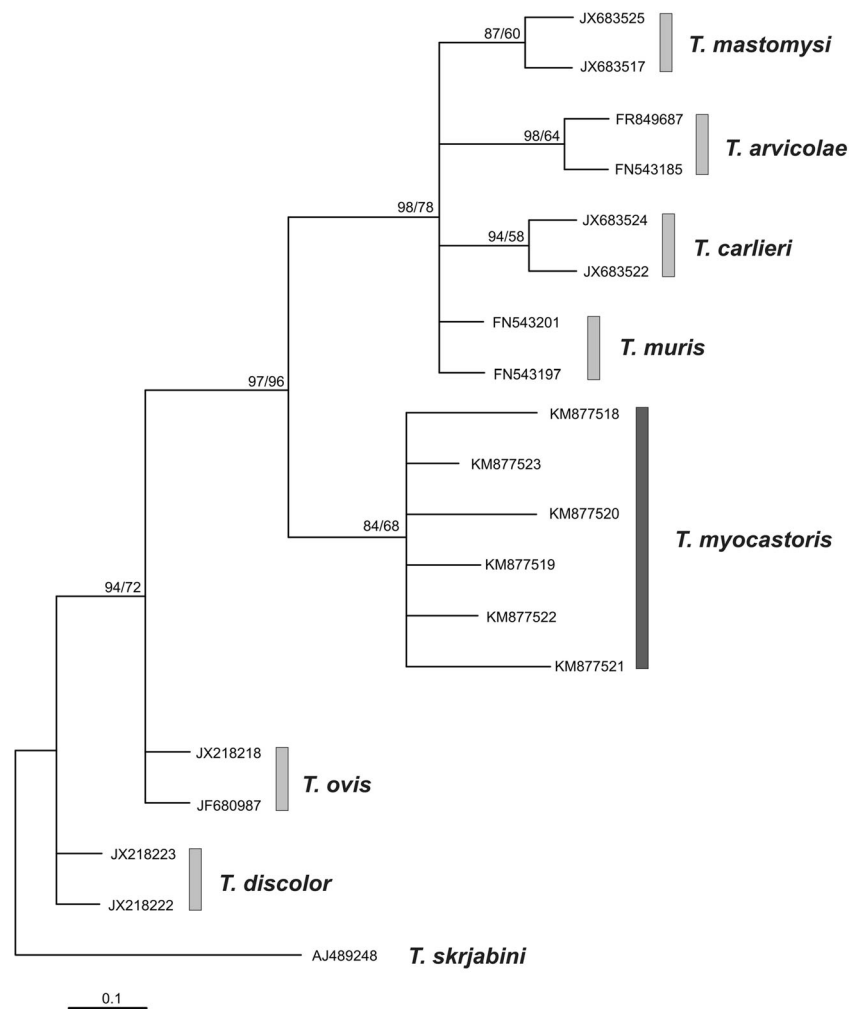


Fig. 2 a, b The vulval regions of *Trichuris myocastoris* isolated from nutria

Fig. 3 Reconstructed phylogeny of the *Trichuris* sequences. The numbers at the nodes represent statistical supports for BAY and MP analyses. Bootstrap supports below 50 and Bayesian posterior probabilities below 0.75 are not shown



similarly becoming popular, especially due to its high protein content and low fat and cholesterol content (Tůmová et al. 2013).

T. myocastoris is a common parasite of wild and domestic nutria (Zajíček 1955; Hohner 1961; Babero and Lee 1961; Pastuszko 1991; Rossin et al. 2009; Martino et al. 2012). This parasite was identified by Enigk (1933) and Petrov (1941) and redescribed in detail by Baruš et al. (1975). The fine structures of the cuticle and oesophagus of this species were described by Wright (1968a, b, 1972).

In our study, 60 individuals (young as well-breeding animals) from three localities were described and measured. Morphometrical data of males and females correspond to the description of *Trichuris* nematodes reported by Baruš (1975) in Azerbaijan. The main diagnostic characteristics in *Trichuris* spp. are body length and spicule length and width (Špakulová 1994). With respect to our specimens, the spicule length ranged from 2.55 to 4.9 mm. The other authors reported spicule lengths from 2.7 mm (Enigk 1933) to 3.9 mm (Petrov 1941). The *Trichuris* individuals in this study were slightly longer than those in previous studies. This can be due to the

fact that the number of nutria that was dissected in this study was large individuals culled from breeding programs.

Trichuris spp. are common parasites of rodents. *Trichuris muris* and *Trichuris arvicolae* are the most commonly described species in rodents. The species designation *T. muris* (Schrank, 1788) is commonly used to refer to trichurids, which are found in Murinae; *T. arvicolae* is used to refer to the subfamily Arvicolinae (Feliu et al. 2000).

New *Trichuris* species (*Trichuris pardinasi* n. sp., *Trichuris baina* n. sp., *Trichuris thrichomysi* n. sp.) were recently described in rodents in South America (Robles et al. 2006; 2014; Torres et al. 2011), and *T. muris* and *T. arvicolae* were redescribed in Europe (Feliu et al. 2000). The genus is also commonly found in African rodents, with at least 12 species described to date (Ribas et al. 2013).

In order to describe a new species of the *Trichuris* genus nowadays, it is necessary to utilize morphological and molecular methods. This is necessary because many species, such as *T. arvicolae* and *T. muris* and females of the species *T. ovis* and *Trichuris discolor*, are morphologically indistinguishable (Feliu et al. 2000; Salaba et al. 2013). Conversely, Cutillas

et al. (2004) and Oliveros et al. (2000) could find no differences between sequences of *T. ovis* rDNA and *Trichuris globulosa* rDNA (internal transcribed spacers ITS1-5.8S-ITS2). Similarly, *Trichuris capreoli* is considered a synonym of *T. globulosa* by some authors (Cutillas et al. 2004; Oliveros et al. 2000; Gagarin 1972).

Currently, many *Trichuris* species are only morphologically defined. *T. myocastoris* was morphologically determined by Enigk 1933 and in detail by Baruš et al. (1975). This paper presents the first molecular description of *T. myocastoris*.

The BLASTn program within the GenBank database indicated that *Trichuris* individuals found in *M. coypus* did not match any of the genetically defined *Trichuris* species. Sequences are unique within the phylogenetic tree (Fig. 2), and they are clearly separated from other clusters. In particular, genetic differences between *T. myocastoris* and two trichurids native to the Czech Republic (*T. muris* and *T. arvicolae*) disprove the possibility that nutrias could be infected by local species of rodent trichurids after their translocation to the Czech Republic. Molecular analysis has proved that this is a unique genetic dataset, and it has confirmed that *T. myocastoris* is a separate species within the genus *Trichuris*. *Trichuris* nematodes were isolated from domestic *M. coypus*. The nematodes were identified using morphological and biometrical methods. Subsequently, genomic DNA was isolated and the ITS1-5.8S-ITS2 segment from ribosomal DNA (RNA) was amplified and sequenced using PCR techniques. Molecular analysis has proved the following: (1) this is a unique genetic dataset, which has confirmed that *T. myocastoris* is a separate species within the genus *Trichuris*, and (2) *T. myocastoris* was found to be significantly more closely related to *Trichuris* from rodents than to those from ruminants.

Acknowledgments This study has been funded by the Ministry of Agriculture of the Czech Republic (Nutria Genetic Resources Programme) and the Grant of National Agency for Agricultural Research of the Czech Republic, v.v.i. CIGA No. 20152021. We would also like to thank Mr. Brian Kavalir for his proofreading services.

References

- Babero BB, Lee JW (1961) Studies on the helminths of nutria, *Myocastor coypus* (Molina), in Louisiana with check list of other worm parasites from this host. *J Parasitol* 47:378–390
- Baruš V, Majumdar G, Mikailov TK (1975) Morphology and taxonomy of *Trichocephalus myocastoris* (Enigk, 1933). *Folia Parasitologica Praga* 22:207–213
- Callejón R, de Rojas M, Ariza C, Ubeda JM, Guevara DC, Cutillas C (2008) Cytochrome oxidase subunit 1 and mitochondrial 16S rDNA sequences of *Trichuris skrjabini* (Trichocephalida: Trichuridae). *Parasitol Res* 104:715–716
- Cutillas C, Oliveros R, de Rojas M, Guevara DC (2004) Determination of *Trichuris skrjabini* by sequencing of the ITS1-5.8S-ITS2 segment of the ribosomal DNA: comparative molecular study of different species of *Trichurids*. *J Parasitol* 90(3):648–652
- Dobrovol'skii AV (1952) Parazitarnye zabolevaniya u ondatry (*Ondatra zibethica* L.). *Zoologicheskii Zhurnal* 31(4):640–642
- Enigk K (1933) Einige Nematoden aus der Nutria. *Parasitol Res* 6(3): 326–331
- Feliu C, Spakulova M, Casanova JC, Renaud F, Morand S, Hugot JP, Santalla F, Durand P (2000) Genetic and morphological heterogeneity in small rodent whipworms in southwestern Europe: characterization of *Trichuris muris* and description of *Trichuris arvicolae* n. sp (Nematoda: Trichuridae). *J Parasitol* 86(3):442–449
- Gagarin VG (1972) Analiz vidogo sostava trihicefalov zvačnyh, zaregistrovannyh v Sovetskom Spuze. *Trudy Vsesojuznogo Instituta Gelmintologii K I Skrjabina* 19:39–75
- Gayo V, Cuervo P, Rosadilla D, Birriel S, Dell'Oca L, Trelles A, Cuire U, Sierra RMY (2011) Natural Fasciola hepatica infection in nutria (*Myocastor coypus*) in Uruguay. *J Zoo Wildl Med* 42(2):354–356
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* 41:95–98
- Hohner L (1961) Die Endoparasiten des Sumpfbfibers unter besonderer Berücksichtigung der Kokzidien. *Zoologischen Institut und Veterinär-Parasitologischen Institut der Karl-Marx Universität Leipzig*. pp. 98–151
- Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755
- Martino PE, Radman N, Parrado E, Bautista E, Cisterna C, Silvestrini MP, Corba S (2012) Note on the occurrence of parasites of the wild nutria (*Myocastor coypus*, Molina, 1782). *Helminthologia* 49(3): 164–168
- Oliveros R, Cutillas C, de Rojas M, Arias P (2000) Characterization of four species of *Trichuris* (Nematoda: Enoplida) by their second internal transcribed spacer ribosomal DNA sequence. *Parasitol Res* 86:1008–1013
- Pastuszko J (1991) The examination of intestinal parasitofauna in coypu (*Myocastor coypus*, Molina 1782) from closed husbandries with particular regard to coccidia. *Akademii Rolniczej we Wrocławiu*. p. 41
- Pellérdy LP (1963) Catalogue of Eimeriidea (Protozoa, Sporozoa). *Akademiai Kiadó Budapest*. p. 122
- Pellérdy LP (1974) Coccidia and coccidiosis. *Verlag Paul Parey, Berlin*, p 959
- Petrov AM (1941) Glistnye bolezni pushnykh zverey. *Mosqow*. pp. 1–227
- Ribas A, Lopez S, Makundi RH, Leirs H, de Bellocq JG (2013) *Trichuris* spp. (Nematoda: Trichuridae) from two rodents, *Mastomys natalensis* and *Gerbilliscus vicinus* in Tanzania. *J Parasitol* 99(5): 868–875
- Robles MD, Navone GT, Notarnicola J (2006) A new species of *Trichuris* (Nematoda: Trichuridae) from Phyllotini rodents in Argentina. *J Parasitol* 92(1):100–104
- Robles MD, Cutillas C, Panei CJ, Callejón R (2014) Morphological and molecular characterization of a new *Trichuris* species (Nematoda-Trichuridae), and phylogenetic relationships of *Trichuris* species of cricetid rodents from Argentina. *PLoS One* 11(9):1–11
- Rossin MA, Varela G, Timi JT (2009) *Strongyloides myopotami* in ctenomyid rodents: transition from semi-aquatic to subterranean life cycle. *Acta Parasitologica* 54(3):257–262
- Salaba O, Rylková K, Vadlejch J, Petrtýl M, Scháňková Š, Brožová A, Jankovská I, Jebavý L, Langrová I (2013) The first determination of *Trichuris* sp. from roe deer by amplification and sequencing of the ITS1-5.8S-ITS2 segment of ribosomal DNA. *Parasitol Res* 112(3): 955–960
- Torres EJJ, Nascimento APF, Menezes AO, Garcia J, dos Santos MAJ, Maldonado A, Miranda K, Lanfredi RM, de Souza W (2011) A new species of *Trichuris* from *Thrichomys apereoides* (Rodentia):

- Echimyidae) in Brazil: morphological and histological studies. *Vet Parasitol* 176(2-3):226–235
- Tůmová E, Chodová D, Hrstka Z (2013) Hodnocení masné užitkovosti nutrií. Certifikovaná metodika ČZU v Praze, p. 27
- Woods CA, Contreras L, Willner-Chapman G, Whidden HP (1992) *Myocastor coypus*. *Mamm Species* 398:1–8
- Zajíček D (1955) Parazitární invaze u mladých nutrií. Sborník Vysoké školy zemědělské a lesnické v Brně, řada B, Spisy fakulty veterinární 3–4(224):95–104
- Zhu X, Gasser RB, Jacobs DE, Hung G-C, Chilton NB (2000) Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. *Parasitol Res* 86:738–744