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A frequent roundworm *Baylisascaris transfuga* in overpopulated brown bears *(Ursus arctos)* in Slovakia: a problem worthy of attention

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Abstract

The genus *Baylisascaris* (order Ascaridida) includes numerous relatively host-specific nematodes, which are common in intestines of wild mammals. Some of them may have impact on veterinary and public health, as their larvae have the potential to cause visceral, ocular, and/or neural larva migrans in a wide range of mammals, birds, and humans. *Baylisascaris transfuga* is a parasite occurring in a range of bear species throughout the world. We present the current data on *B. transfuga* occurrence in brown bears from a relatively restricted territory of the Poľana Protected Landscape Area in Central Slovakia, obtained by traditional methods (faecal examination, morphology). Species affiliation was confirmed by employing molecular markers generating nuclear 28S and mitochondrial *cox1* sequences in adult worms. Based on 17 examined samples (15 excrements and two intestines of young bear females), the occurrence of *B. transfuga* in the surveyed area was assessed as 52.9%. Both bear females were infected with adult and juvenile worms. Due to the high density of bears in the locality, the high infection rate with ascarids, and the huge number of eggs produced by the parasites, it is apparent that the respective environment, including the inhabited areas, might be markedly contaminated by *Baylisascaris* eggs. The ability of *B. transfuga* to serve as a zoonotic agent has not been unambiguously proved; however, this attribute should be considered and subjected to further research.

Keywords

European brown bear, *Baylisascaris*, intestinal nematode, morphology, molecular analysis

Introduction

The genus *Baylisascaris* Sprent, 1968 includes ten nematode species with relatively high host-specificity and a facultative heteroxenous life cycle (Sapp *et al.* 2017). Five of them have been reported in Europe so far: *Baylisascaris devosi* (Sprent, 1952) in mustelids (*Martes* spp.), *B. melis* (Gedoelst, 1920) in badger (*Meles meles*), *B. procyonis* (Stefanski and Zarnowski 1951) in raccoon (*Procyon lotor*), *B. transfuga* (Rudolphi, 1819) in bears (*Ursus* spp.), and *B. columnaris* (Leidy, 1856) in skunks (*Mephitis* spp.) (Franssen *et al.* 2013; Bauer 2013). All *Baylisascaris* species are considered to have the potential to cause visceral, ocular, and/or neural larva migrans in a range of mammals, birds and humans, in which the neural larva migrans may manifest as meningoencephalitis (e.g., Huff *et al.* 1984 Kazacos 2016). Although *B. procyonis* and *B. columnaris* are most commonly implicated as zoonotic agents, other *Baylisascaris* species including *B. transfuga* are also considered as potential etiological agents of larva migrans (Schaul 2006).

B. transfuga parasitizes various species of Ursidae on different continents (Papini *et al.* 1996; Foster *et al.* 2004; Guerrero and Castellanos 2016; Sapp *et al.* 2017). Apart from *B. transfuga*, three distinct *Baylisascaris* species are described among the Ursidae representatives: *B. schroederi* (McIntosh, 1939) in giant panda (*Ailuropoda melanoleuca*), *B. ailuri* (Wu, He and Hu, 1987) in red panda (*Ailurus fulgens*), and *B. venezuelensis* (Perez, Garcia and Gauta, 2016) in the South American spectacled bear (*Tremarctos ornatus*) (Bauer *et al.* 2013). In free-ranging brown bear (*Ursus arctos*) in Europe, *B. transfuga* was previously observed in Croatia, with a prevalence of 13.5% detected by the flotation of faecal samples (De Ambrogi *et al.* 2011). In addition, Szczepaniak *et al.* (2012) reported a heavily infected brown bear from the Polish

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part of the High Tatra Mountains, showing granulomatous peritonitis, with 58 *B. transfuga* in intestine. Recently, this nematode was found also in 19% (47/248) of scat samples and samples from organ tissues in brown bears migrating in Eastern Transylvania, Romania (Borka-Vitalis *et al.* 2017). The infection with *B. transfuga* is being often detected in captive bears. For instance, 300 roundworm individuals detected in two captive polar bears (*Ursus maritimus*) living in the Zoo Park of Pistola (Tuscany, Italy) were morphologically and molecularly characterized as *B. transfuga* (Testini *et al.* 2011). Further, in one of eight Dutch Zoo parks, *B. transfuga* eggs were molecularly determined in faeces of one brown bear and one coati (*Nasua nasua*) (Visser *et al.* 2015). In Slovakia, the parasite was relatively often detected in bear scats collected in different areas of the Western Carpathian Mountains of north-eastern and central Slovakia (Goldová *et al.* 2003; Finnegan 2009; Major *et al.* 2009).

During the thirties of the 19th century, the population of brown bears declined in many areas of Europe as a result of human encroachment and habitat destruction and these mammals were close to the extinction for instance in Scandinavia, Latvia, and elsewhere (Servheen 1990; Waits *et al.* 2000). In 1932, the all-year-round protection of bears was officially enacted in Slovakia under both national and international conventions and hunting is currently subjected to the permission by the Ministry of the Environment of the Slovak Republic. Since 1932, the number of bears has been steadily rising and bear populations are now even becoming problematic in several mountainous areas of Slovakia (Krištofík and Danko 2012). At present, 1.256 ± 233 bear individuals were detected in the whole territory of Slovakia using the non-invasive method of DNA determination from bear excrements (Paule *et al.* 2016 a, b). Brown bear is the largest animal in Slovakia and nowadays its frequency in the mountains of the central-northern part the Poľana Protected Landscape Area (PLA), the Veľká Fatra and Malá Fatra National Parks, the Low Tatras, and the High Tatras is very high. A less numerous, spatially separated population of brown bears occurs also in the north-eastern Slovakia in the Poloniny Mountains (Paule *et al.* 2016 a).

The present study was designed to survey the occurrence of *B. transfuga* in brown bears in the territory of the Poľana PLA (Slovakia) by employing the coprology of bear excrements and the dissection of two bear females.

Materials and Methods

Sample collection

During all seasons of the years 2015–2016 (spring – winter), we collected 15 brown bear excrements (scats) in the territory of the Poľana PLA in Central Slovakia. This biosphere reserve was established in 1981 for the purpose of protecting a complex of plant and animal communities and non-living nature. Naturally, the Poľana PLA represents a centre of a wider forest region with settlements in the valleys. It spreads over 20,360 hectares (48°37 N, 19°28 E).

Bear scat samples were collected from different sites, situated also beyond the protected part of Poľana, in order to increase the chance to obtain samples from different bear individuals. We intended to minimize the risk of sample duplicity by collecting scats in distinct locations. Gathered faecal samples were stored at 4°C until microscopic examination.

Further, we dissected gastrointestinal tracts of the two 2-year-old bear females, hunted down in the same area within the permitted regulation of brown bears in the hunting grounds of the Poľana, in collaboration with the state woodland enterprise Forests of the Slovak Republic. Ascaroid nematodes obtained by complete helminthological examination were thoroughly washed in Ringer´s solution, measured, and stored in 70% ethanol. Adult worms were used for the verification of the species affiliation using the worm morphology and molecular analysis.

Faecal examination

Microscopic examination of the faeces was performed using the conventional flotation technique with using Breza and Faust solutions (Manual of Veterinary Laboratory Methods, 1989) and the light microscopy.

Morphological characterization

After the autopsy of small bowel, twelve adult nematodes (2 males and 10 females) were washed in the physiological solution and subjected to detailed morphological analysis and measurements were taken under the Nikon SMZ 1500 stereomicroscope equipped with the Canon EOS 110 D camera. Morphological diagnosis was based on the size, buccal cavity, lips, cephalic alae, number and distribution of precloacal papillae, and spicules (Soulsby 1986).

Molecular analysis

Two nematodes (a male and a female) subjected to genetic analyses were collected from different brown bear females. Adult worms were mechanically disrupted by using sterile pestle. Total genomic DNA was extracted from parasites using a DNeasy tissue kit (Qiagen, Hilden, Germany), following the animal tissue isolation protocol. Fragments of the two genes were targeted for PCR amplifications, namely cytochrome *c* oxidase 1 of mitochondrial DNA (*cox1*, 408 bp) and 28S of nuclear ribosomal DNA (676 bp). For *cox1*, DNA was amplified using primers proposed by Bowles *et al.* (1992):5′-TTTTTTGGGCATCCTGAGGTTTAT-3′/5′-TAACGACATA ACATAATGAAAATG-3′. Primer pairs targeted 28S gene regions were designed by Franssen *et al.* (2013) and contained these nucleotides: 5′-CGAGGATTCCCTTAGTAACT-3′/5′-TCGGATAGGTGGTCAACG-3′. PCR reactions were performed using the following cycling conditions: initial

denaturation step 94°C for 5 min, followed by 35 cycles, 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Amplified products were purified using the Nucleospin Extract II kit (Macherey Nagel, Düren, Germany). Amplicons were directly sequenced using a dye terminator cycle sequencing kit (DYEnamic ET terminator; Amersham Biosciences, UK) and analyzed with the ABI PRISM 377 automated sequencer (Applied Biosystems, USA). Nucleotide sequences were aligned using

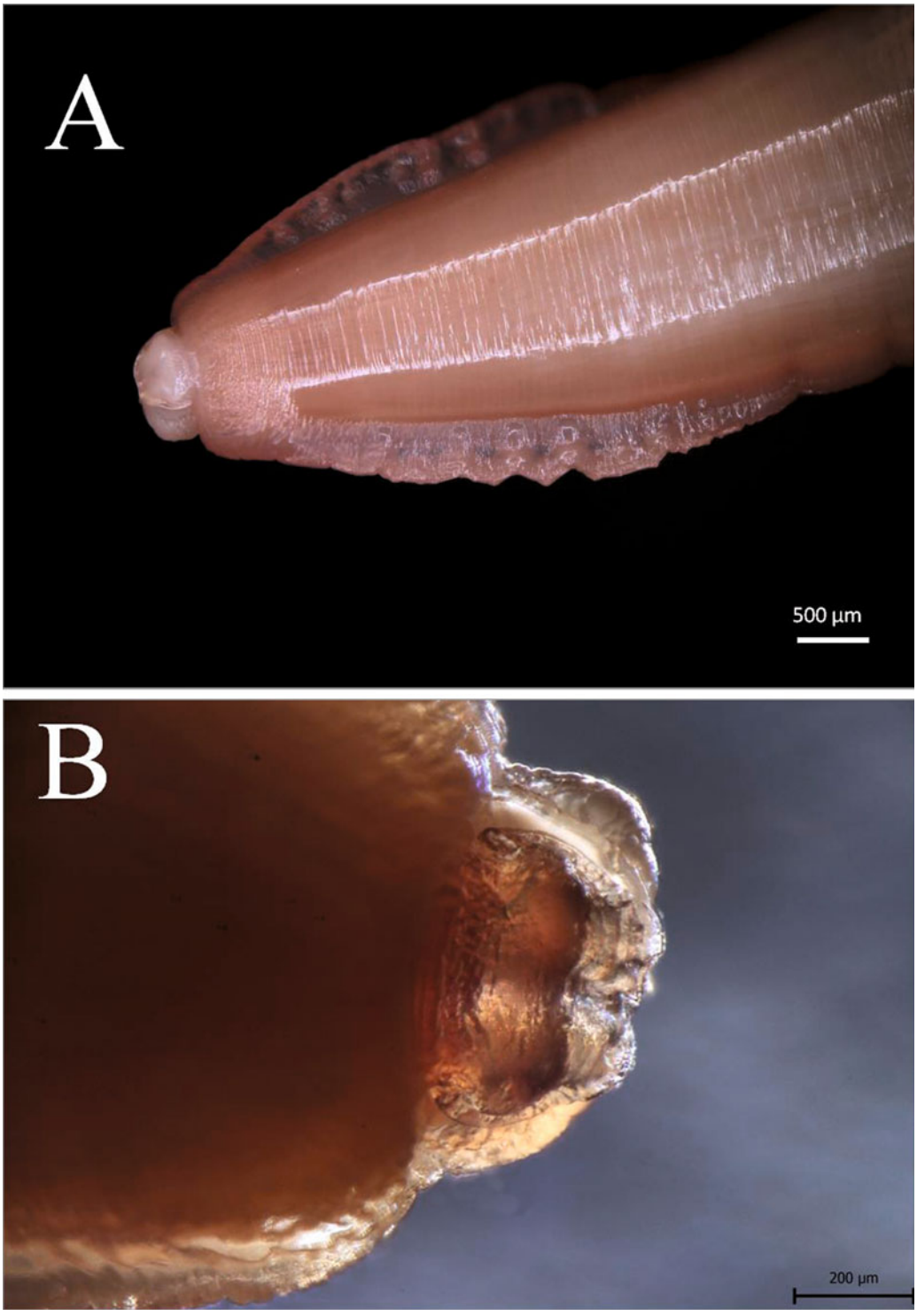

Fig. 1 *Baylisascaris transfuga* female. Anterior end with lips and alae **(A)**, scale bar = 500 µm; dorsal lip with two papillae **(B)**, scale bar = 200 µm

ClustalX2 (Larkin *et al.* 2007) and compared to those stored in the GenBank using the BLAST software. To distinguish synonymous and non-synonymous mutations, nucleotide sequences were translated into a corresponding protein sequence while using the EMBOSS transeq software. The nucleotide sequences for the two genes were deposited in GenBank under accession numbers MF419818 and MF419819.

Results

Parasite occurrence

The flotation procedure of 15 samples of bear scats revealed the occurrence of *B. transfuga* eggs in seven samples (46.7%). In addition, autopsy of gastrointestinal tracts of the two young (2 years old) bear females showed 17 *B. transfuga* individuals (6 females and 1 male and 9 females and 1 male, respectively); 14 ascarids were adult and three were juvenile. Taking these two outcomes together, *B. transfuga* occurred in 52.9% of studied bear samples from the area of Poľana.

Morphological characterization

For morphology, two adult males and ten adult females were used. Males were on average 97.5 mm long, the mean length of females was 228.4 mm. Morphological features of the anterior end include mouthparts with three lips (one dorsal and two subventral) surrounding the mouth opening (Fig. 1 A, B). No buccal capsule was seen; typical filariform oesophagus was 6.9 mm long, cervical alae were in the lateral position (Fig. 2). The female had a blunt tail; vulva opening is located at the ventral side about 74.8 mm from the anterior end (Fig. 3 A, B). Caudal end of the male was slightly rolled with the presence of two spicules 0.9 mm long and 61 precloacal genital papillae (Fig. 4). Detailed data on *B. transfuga* females are presented in Table I.

Table I. Morphometric measurements of *Baylisascaris transfuga* females (n = 10, data are in millimetres)

	Mean ± SD (range)
Body length	228.4 ± 37.3 (179–283)
Oesophagus length	6.9 ± 0.5 (6.2–7.3)
Alae length	7.8 ± 0.4 (7.0–8.4)
Tail length	1.1 ± 0.1 (0.7–1.2)
Distance of anterior end to vulva	74.8 ± 12.4 (56–92)

Molecular analysis

Nuclear 28S rDNA amplicons (676 bp) of the two examined isolates revealed 100% homology with the GenBank sequences previously derived for *B. transfuga* from a brown bear in the Netherlands (GenBank reference KC543471), a Tibetan blue bear in China (JN257009), and a polar bear in China (JN257008). Four nucleotide substitutions were recorded in relation to the most closely related species *B. schroederi* (JN257013) and five substitutions relative to *B. ailuri* (JN257012) in this gene segment.

Alignment of the 408 bp *cox1* sequences showed the nucleotide polymorphism at three sites. Our isolates differed in one base (the C/T synonymous substitution at position 216) from the most common sequence pattern of *B. transfuga*, available for isolates from polar bears in China (EU628683) and Italy (HM594948), and from a sloth bear in the Netherlands (KC543477), thus manifesting 99.7% genetic similarity to the target regions (384 bp, 369 bp, and 394 bp of sequence assemblies matched in the respective fragments). A compari-

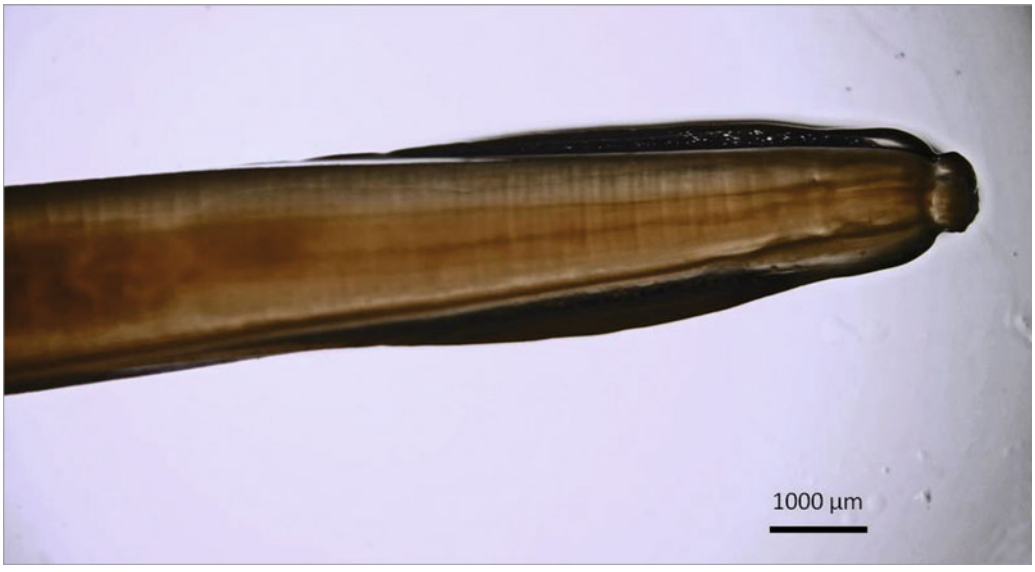

Fig. 2 *Baylisascaris transfuga* female. Filariform esophagus, cervical alae, scale bar = 1,000 µm

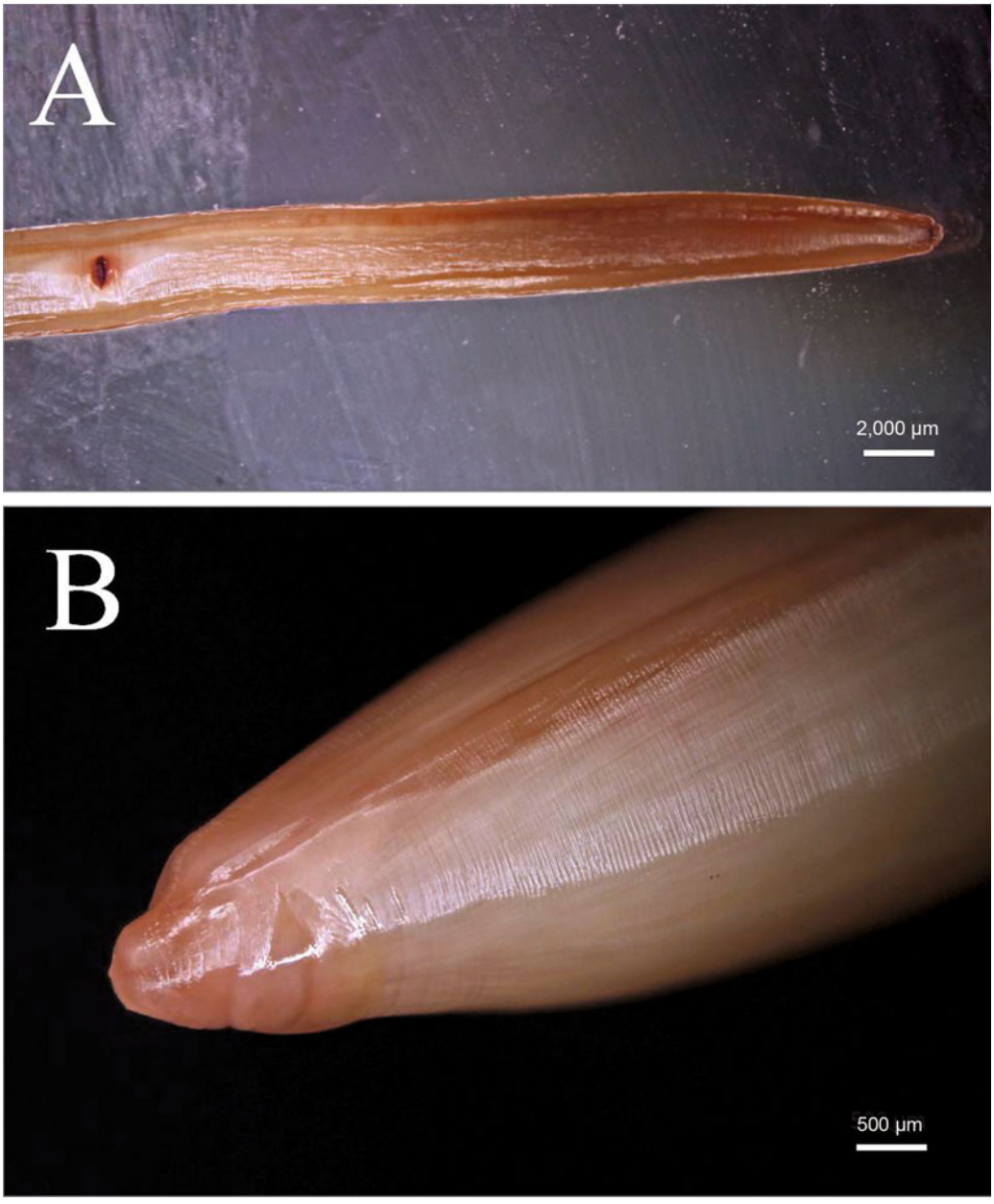

Fig. 3 *Baylisascaris transfuga* female. Genital girdle, vulva opening **(A)**, scale bar = 2,000 µm; blunt tail **(B)**, scale bar = 500 µm

Table II. Nucleotide substitutions detected in *Baylisascaris transfuga* isolates in *cox1* gene

Isolate	Position of substitution			Reference
	9	18	216	
Slovak isolates	G	G	T	current study
KC543477 (Netherlands)	n.a.	n.a.	C	Franssen *et al.* (2013)
EU628683 (China)	n.a.	n.a.	C	–
HM594948 (Italy)	n.a.	n.a.	C	Testini *et al.* (2011)
HQ671079 (China)	T	A	C	Xie *et al.* (2011)

Note: n.a., not analysed in the respective part of the gene portion

Figure 4 *Baylisascaris transfuga* male. Posterior end – spicules, precloacal papillae. Scale bar = 200 µm

son with the entirely overlapping gene portion (408 bp), available for further Chinese isolate from a polar bear (HQ671079), showed two additional synonymous T/G and A/G nucleotide changes at positions 9 and 18, respectively (Table 2). Twelve nucleotide substitutions were recorded in relation to the adjacent *B. ailuri* (HQ671080), accounting for 2.9% interspecific differences, and twenty substitutions (4.9% differences) compared to *B. schroederi* (KJ587842) were detected in the *cox1* gene.

Discussion

In the present study, eggs and/or adult roundworms of *B. transfuga* were found to occur in 52.9% of 17 scat and intestine samples collected from brown bear in the Poľana PLA and the surrounding areas in Central Slovakia. A relatively lower number of investigated samples were due to the complicated collection of field scat samples dispersed in the area; nevertheless, even the used sample size indicates a substantial infection of bears with these ascarids in the locality concerned.

In the central part of the Poľana PLA, 85 brown bears have been recently registered and up to 130–140 bears are estimated to reside in the wider surrounding area (Prof. L. Paule, personal information). Bears commonly migrate to various distances between different mountain ranges of Slovakia and they increasingly enter human settlements and pastures (Lenko *et al.* 2014). Previous data on *B. transfuga* occurrence in Slovakia have been related to the two main areas of the brown bear dispersal, i.e. the central and the north-eastern parts of the Western Carpathian Mountains. The first reports on the bear parasites, including *B. transfuga*, were published in 1970s (for review see Krištofík and Danko 2012). More detailed data were obtained by the examination of 91 bear faecal samples from Western, Low and Belianske Tatra Mountains, which showed *B. transfuga* prevalence of 14.3% (Goldová *et al.* 2003). Further analyses of 188 bear scats collected in another sub-province of the Western Carpathians (The Fatra-Tatra Area) revealed the parasite prevalence of 41.8%, and the survey confirmed seasonal fluctuations, with the lowest values (9.5%) being observed in spring, soon after the winter torpor, and the highest values (84.6%) in autumn (Finnegan 2009). The situation in a separated bear population in the north-eastern Slovakia (Poloniny National Park) was monitored by Major *et al.* (2009) who estimated the prevalence of 63.8% of *B. transfuga* based on examination of 47 faecal samples. The present data from the Poľana Mountains thus fit well with the previous knowledge and it is obvious that the infection rate is steadily considerable throughout the whole area of the brown bear occurrence in Slovakia.

All the above referenced data were obtained exclusively by the faecal flotation; however, the *Baylisascaris* spp. eggs are considered indistinguishable (Sapp *et al.* 2017). To unequivocally categorize species transmitted in bears of the Poľana region, we employed molecular approach that has been previously successfully applied to species determination in both faecal and biopsy *Baylisascaris* samples (e.g., Testini *et al.*

2011; Moudgil *et al.* 2014). In the present study, species identity of the two morphologically characterized specimens, a male and a female of *B. transfuga*, was corroborated by the evidence from nuclear and mitochondrial sequences. Whereas the nucleotide composition of the ribosomal 28S gene was completely identical to the available sequences for *B. transfuga* from the Netherlands and China (comparative data provided by Franssen *et al.* 2013; Li *et al.* 2012), at least one fixed difference was observed in the mitochondrial *cox1* gene for Slovak samples in comparison with isolates from the Netherlands, Italy, and China (Testini *et al.* 2011; Xie *et al.* 2011; Franssen *et al.* 2013). Given that the two examined isolates were collected from brown bears in different sites of the Poľana PLA, the sequence pattern with 216C/T nucleotide substitution seems to be characteristic for the transmitted Slovak *Baylisascaris* population. Nevertheless, this silent mutation does not change the amino acid composition and thus presumably does not provide any selective advantage or disadvantage. According to Blouin *et al.* (1998), the mitochondrial sequences of closely related nematode species typically differ by 10–20%. A level of merely 0.3% of differences in *cox1* sequences herein determined for *B. transfuga* from different continents is far from this presumptive threshold, and is suggestive of striking genetic homogeneity within the species. The present study also corroborated the close relationship between *Baylisascaris* within *Ursidae*, in revealing the highest degree of genetic similarity of the analyzed *B. transfuga* with *B. schroederi* from giant pandas and *B. ailuri* from red pandas.

Baylisascaris roundworms are known to produce a huge number of eggs that are released into the environment with excrements of the host. In fact, the life cycle of *B. transfuga* is not completely recognized; nevertheless, information that captive bears shed as many as 10–20 thousand eggs per gram of bear faeces is available (Papini and Casarosa, 1994). The endogenous development of *B. transfuga* in definitive hosts remains to be more thoroughly elucidated (Bauer, 2013). In contrast to the aggressive *B. procyonis* raccoon roundworm, the bear-affiliated *B. transfuga* has a lower potential to imply health disorders in aberrant, untypical hosts including humans. Its larvae are able to migrate in such host organism, but they are of smaller size, grow slower, and their penetration ability seems to be reduced in comparison with some more thoroughly studied congeners (Schaul 2006). However, some laboratory animals (small mammals and birds), experimentally infected with *B. transfuga*, were found to suffer from visceral, ocular, and even neural larva migrans, but the clinical manifestations were mild, or not apparent (e.g., Matoff and Komandarev 1965; Papini and Casarosa 1994; Sato *et al.* 2004). Nevertheless, a single report is available on a lethal *Baylisascaris* infection of aberrant hosts, Japanese macaques kept together with the American black bears infected with *B. transfuga*, in a Japanese Zoo (Sato *et al.* 2005). The species identity of the larvae from the macaques was not definitively confirmed; however, *B. transfuga* transmission from bears was highly probable. Although the parasite does not cause serious clinical problems to bears in the nature, it might have indirect effects on the condition and nutritional status of the host. The clinical signs associated with larva migrans may be significant in aberrant hosts. Regarding humans, no confirmed reports of larva migrans caused by *B. transfuga* are available; however, this infection is considered to be possible if the intensity of infection is sufficiently strong (Gavin *et al.*, 2005; Sapp *et al.* 2017).

Given that highly infected bears are becoming more common in Slovak settlements, the probability of infecting aberrant hosts (domestic animals, pets, humans) is concomitantly increasing. Indeed, people, including physicians, veterinarians, hunters and wildlife biologists, are not sufficiently aware of the possibility of infection with the bear roundworm and its probable consequences. Therefore, fresh bear excrements found close to human dwellings should be thoroughly disposed and decontaminated, as recommended by Kazacos (2016). In any case, a potential ability of *B. transfuga* to serve as a zoonotic agent should be considered and subjected to further research.

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References

Bauer C. 2013. Baylisascariosis–infections of animals and humans with 'unusual' roundworms. *Veterinary Parasitology*, 193, 404–412. DOI: 10.1016/j.vetpar.2012.12.036

Blouin M.S., Yowell C.A., Courtney C.H., Dame J.B. 1998. Substitution bias, rapid saturation, and the use of mtDNA for nematode systematics. *Molecular Biology and Evolution*, 15, 1719–1727

Borka-Vitalis L., Domokos C., Foldvari G., Majoros G. 2017. Endoparasites of brown bears in Eastern Transylvania, Romania. *Ursus*, 28, 20–30. DOI: 10.2192/URSUS-D-16-00015.1

De Ambrogi M., Aghazadeh M., Hermosilla C., Huber D., Majnaric D., Reljic S. *et al.* 2011. Occurrence of *Baylisascaris transfuga* in wild populations of European brown bears (*Ursus arctos*) as identified by a new PCR method. *Veterinary Parasitology*, 179, 272–276. DOI: 10.1016/j.vetpar.2011.02.025

Finnegan S. 2009. Seasonal dynamics in the prevalence of *Baylisascaris transfuga* ova in the faeces of the brown bear (*Ursus arctos*) in Slovakia. Diploma Thesis, University of Veterinary Medicine and Pharmacy in Košice, Slovakia

Foster G.W., Cunningham M.W., Kinsella J.M., Forrester D.J. 2004. Parasitic helminths of black bear cubs (*Ursus americanus*) from Florida. *Journal of Parasitology*, 90, 173–175. DOI: 10.1645/GE-127R

Franssen F., Xie K., Sprong H., Van der Giessen J. 2013. Molecular analysis of *Baylisascaris columnaris* revealed mitochondrial and nuclear polymorphisms. *Parasite & Vectors*, 6, 124. DOI: 10.1186/1756-3305-6-124

Gavin P.J., Kazacos K.R., Shulman S. 2005. Baylisascariasis. Clinical Microbiology Reviews, 18. 703–718. DOI: 10.1128/CMR.18.4.703-718.2005

Goldová M., Ciberej J., Rigg R. 2003. Medveď hnedý (*Ursus arctos*) a parazitárne zoonózy. (Brown bear (*Ursus arctos*) and parasitic zoonoses). *Folia Venatoria*, 33, 123–127. (In Slovak)

Guerrero R., Castellanos A. 2016. First record of *Baylisascaris transfuga* (Nematoda: Ascarididae) in an Anden Bear (*Tremarctos ornatus*) in Ecuador. *International Bear News*, 25. IBA website: www.bearbiology.org

Huff D.S., Neafie R.C., Binder M.J., De Leon G.A., Brown L.W., Kazacos K.R. 1984. Case 4. The first fatal *Baylisascaris* infection in humans: an infant with eosinophilic meningoencephalitis. *Pediatric Pathology*, 2, 345–352

Kazacos K.R. 2016. *Baylisascaris* Larva Migrans: U.S. Geological Survey Circular 1412, 122 pp. 3 appendixes, http://www.arlis.org/docs/vol1/M/946142348.pdf

Krištofík J., Danko Š. 2012. Cicavce Slovenska. Rozšírenie, bionómia a ochrana (Mammals of Slovakia. Distribution, bionomy and protection). Veda, Bratislava, 712 pp. (In Slovak)

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H. *et al*. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. DOI: 10.1093/bioinformatics/btm404

Lenko P., Tajboš P., Humený M., Celer S., Zwijacz–Kozica T., Zięba F. *et al*. 2014. Problematika a súčasný stav medveďa hnedého v Tatrách (Problems and the current state of the brown bear in the Tatras). OZ Les, Správa Tatranského národného parku, Tatranská Štrba, 91 pp. (In Slovak with English summary)

Li Y., Niu L., Wang Q., Zhang Z., Chen Z., Gu X. *et al*. 2012. Molecular characterization and phylogenetic analysis of ascarid nematodes from twenty-one species of captive wild mammals based on mitochondrial and nuclear sequences. *Parasitology*, 139, 1329–1938. DOI: 10.1017/S003118201200056X

Major P., Molnár L., Štofík J., Goldová M. 2009. Parasitofauna medveďa hnedého v NP Poloniny (Parasitofauna of the brown bear (*Ursus arctos*) in Poloniny National Park). In: Škola – veda – prax I. September 24, 2009, University of Veterinary Medicine and Pharmacy in Košice, Slovakia, 318–322. (In Slovak)

Matoff K., Komandarev S. 1965. Comparative studies on the migration of the larvae *Toxascaris leonina* and *Toxascaris transfuga*. *Parasitology Research*, 25, 538–555

Manual of Veterinary Laboratory Methods. 1989. Ministry of Agriculture, Fisheries and Food. Reference Book 418. Her Majesty´s Stacionary Office, London, UK

Moudgil A.D., Singla L.D., Singh M.P. 2014. First report on molecular identification and fenbendazole resistance against *Baylisascaris transfuga* infection in *Melursus ursinus* (sloth bear). *Helminthologia*, 51, 262–268. DOI: 10.2478/s11687-014-0239-2

Papini R., Casarosa L. 1994. A report on the pathology of *Baylisascaris transfuga* (Ascarididae: Nematoda) for mice. *Revue de Médecine Vétérinaire* (Toulouse), 145, 949–952

Papini R., Renzoni G., Lo Piccolo S., Casarosa L. 1996. Ocular larva migrans and histopathological lesions in mice experimentally infected with *Baylisascaris transfuga* embryonated eggs. *Veterinary Parasitology*, 61, 315–320

Paule L., Krajmerová D., Bakan J. 2016a. Kolik medvědů žije na Slovensku? (How many bears live in Slovakia?) *Svět myslivosti* 3, 17, 14–17. (In Czech)

Paule L., Krajmerová D., Bakan J., Slivková V. 2016b. Odhad veľkosti populácie medveďa hnedého na Slovensku na základe genetických analýz (Estimation of brown bear population size in Slovakia based on genetic analyses). Chapter in the book: Conservation and management of large carnivores in Slovakia, Štátna ochrana prírody SR, Banská Bystrica, Editors: Lešová A, Antal V, pp.73–84. (In Slovak)

Sapp S.G.H., Gupta P., Martin M.K., Murray M.H., Niedringhaus K.D., Pfaff M.A. *et al*.2017. Beyond the raccoon roundworm: The natural history of non-raccoon *Baylisascaris* species in the New World. *International Journal for Parasitology: Parasites and Wildlife*, 6, 85–99. DOI: 10.1016/j.ijppaw.2017.04.003

Sato H., Matsuo K., Osanai A., Kamiya H., Akao N., Owaki S. *et al*. 2004. Larva migrans by *Baylisascaris transfuga*: Fatal neurological diseases in Mongolian jirds, but not in mice. *Journal of Parasitology*, 90, 774–781. DOI: 10.1645/GE-3330

Sato H., Une Y., Kawakami S., Saito E., Kamiya H., Akao N. *et al*. 2005. Fatal *Baylisascaris* larva migrans in a colony of Japanese macaques kept by a safari-style zoo in Japan. *Journal of Parasitology* 91, 716–719. DOI: 10.1645/GE-3374RN

Schaul J.C. 2006. *Baylisascaris transfuga* in captive and free-ranging populations of bears (Family: Ursidae). PhD Thesis, Ohio State University, USA.https://etd.ohiolink.edu/rws_etd/document/get/osu1163172292/inline

Servheen C. 1990. The status and conservation of the bears of the world. *International Conference on Bear Research and Management*, Monograph Series No. 2, 1–32

Soulsby J.F.A. 1986. Helminths, Arthropods and Protozoa of Domesticated Animals. Bailiére-Tindall, London, UK, 809 pp.

Szczepaniak K., Listos P., Lopuszynski W., Skrzypek T., Kazimierczak W. 2012. Granulomatous peritonitis in a European brown bear caused by *Baylisascaris transfuga*. *Journal of Wildlife Diseases* 48, 517–519. DOI: 10.7589/0090-3558-48.2.517

Testini G., Papini R., Lia R.P., Parisi A., Dantas-Torres F., Traversa D. *et al*. 2011. New insights into the morphology, molecular characterization and identification of *Baylisascaris transfuga* (Ascaridida, Ascarididae). *Veterinary Parasitology* 175, 97–102. DOI: 10.1016/j.vetpar.2010.09.017

VisserM.H., van der Giessen J.W.B., Franssen F., van Knapen F., Overgaauw P.A.M. 2015. Survey of *Baylisascaris* sp. in zooanimals in the Netherlands. Master Thesis, Faculty of Veterinary Medicine Theses, Utrecht University, Netherlands

Waits L., Taberlet P., Swenson J.E., Sandegren F., Franzén R. 2000. Nuclear DNA microsatellite analysis of genetic diversity and gene flow in the Scandinavian brown bear (*Ursus arctos*). *Molecular Ecology*, 9, 421–431. DOI: 10.1046/j.1365-294x.2000.00892.x

Xie Y., Zhang Z., Wang C., Lan J., Li Y., Chen Z. *et al.* 2011. Complete mitochondrial genomes of *Baylisascaris schroederi*, *Baylisascaris ailuri* and *Baylisascaris transfuga* from giant panda, red panda and polar bear. *Gene*, 482, 59–67. DOI: 10.1016/j.gene.2011.05.004

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