IDENTIFICATION OF *SARCOCYSTIS COLUMBAE* IN WOOD PIGEONS (*COLUMBA PALUMBUS*) IN LITHUANIA

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Summary. Cysts of *Sarcocystis* were found in two out of 18 wood pigeons (*Columba palumbus*) hunted in Lithuania in 2008 and 2009. Morphologically investigated *Sarcocystis* sp. had type-1 tissue cyst wall and was not distinguishable from *S. calchasi*, *S. columbae* and *S. wobeseri*, parasitizing in birds. According to the DNA analysis, *Sarcocystis* sp. from the wood pigeon was identified as *S. columbae*. On the basis of 18S rRNA and 28S rRNA gene sequences *S. columbae* is phylogenetically most closely related to *Sarcocystis* spp. from birds. According to the phylogenetic and ecologic data, predatory birds are expected to be definitive hosts of *S. columbae*. This is the first report of *Sarcocystis* in birds of the Columbidae family in Lithuania.
Keywords: *Sarcocystis*, identification, phylogeny, wood pigeons, Lithuania.

LIETUVOS KERŠULIŲ (*COLUMBA PALUMBUS*) *SARCOCYSTIS COLUMBAE* IDENTIFIKACIJA

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Santrauka. Iš 2008 ir 2009 metais Lietuvoje sumedžiotų 18 keršulių (*Columba palumbus*) dviejuose aptiktos *Sarcocystis* cistos. *Sarcocystis* sp. turėjo pirmą cistų sienelės tipą ir morfologiškai nesiskyrė nuo *S. calchasi*, *S. columbae* ir *S. wobeseri* rūšių, parazituojančių paukščiuose. Taikant DNR analizę, *Sarcocystis* sp. iš keršulio identifikuota kaip *S. columbae*. Pagal 18S rRNR ir 28S rRNR genų sekas *S. columbae* filogenetiškai giminingiausia *Sarcocystis* rūšims, kurių tarpiniai šeimininkai – paukščiai. Filogenetiniai ir ekologiniai duomenys rodo, jog plėšrieji paukščiai yra labiausiai tikėtini galutiniai *S. columbae* šeimininkai. Šiame straipsnyje pirmą kartą aprašomi *Sarcocystis* genties parazitai Lietuvos karvelinių šeimos paukščiuose.
Raktažodžiai: *Sarcocystis*, identifikacija, filogenija, keršuliai, Lietuva.

Introduction. The genus *Sarcocystis* are apicomplexan parasites of mammals, birds and reptiles. In this genus Odening (1998) counted 189 species and at present their number is estimated to be over 200 species. *Sarcocystis* are characterized by an obligatory prey-predator two-host life cycle with mostly herbivores and omnivores being their intermediate hosts and carnivores as definitive hosts. The intermediate host becomes infected through the ingestion of sporocyts/oocysts found in the faeces of the definitive host and after merogony sarcocysts in muscle tissues finally are formed. Sexual multiplication occurs in the small intestine of the definitive host (Dubey *et al*., 1989; Mehlhorn and Heydorn, 1978). Some *Sarcocystis* species are pathogenic organisms dangerous to humans and domestic animals (Fayer, 2004).

Though approximately 20 named *Sarcocystis* species whose intermediate hosts are birds are known, the life cycle of only some of them has been fully ascertained. *S. falcatula* has a broad range of intermediate hosts that include the Passeriformes, Psittaciformes and Columbiformes orders of birds and the opossum *(Didelphis virginiana)* is a definitive host (Box *et al*., 1984). Intermediate hosts of *S. rileyi* (Stiles, 1893; reviewed by Dubey *et al*., 2003) are the shoveller (*Anas clypeata*) and the mallard duck (*Anas platyrhynchos*), whereas the skunk (*Mephitis mephitis*) serves as its definitive host. The arctic fox (*Alopex lagopus*) is one of the definitive hosts of *Sarcocystis* sp. (cysts type III) from the white-fronted geese (*Anser albifrons*) (Kutkienė *et al*., 2006). *S. calchasi* from the domestic pigeon (*Columba livia* f. *domestica*) is transmitted by the goshawk (*Accipiter gentilis*) (Olias *el al*., 2010b). Likewise birds are definitive hosts of numerous *Sarcocystis* species worldwide (Černá, 1984; Gjerde and Dahlgren, 2010; Yabsley *et al*., 2009).

Unnamed *Sarcocystis* species were reported in different bird species of the family Columbidae almost worldwide (Barrows and Hayes, 1977; Conti and Forrester, 1981; Dylko, 1962; Ecco *et al*., 2008; Kaiser and Markus, 1983a). Experimentally infected domestic pigeons harbored *S. falcatula*, which is a clinical disease agent (Box *et al*., 1984; Smith *et al*., 1990). In the USA *S. falcatula*-like infection caused death in three free-roaming Victoria crowned pigeons (*Goura victoria*) (Suedmeyer *et al*., 2001). Recently a new central nervous system disease in homing pigeons induced by *S. calchasi* has been reported. Clinical signs of this severe infection were depression, polyuria, torticollis, opisthotonus, paralysis, trembling and death (Olias *et al*., 2009, 2010a, b). Subsequently newly characterized non-pathogenic species *S. columbae* were found in wood pigeons (*Columba*

palumbus) in Germany (Olias *et al*., 2010c).

Up till now the structure of the sarcocyst wall was the main taxonomic criteria for *Sarcocystis* species (Dubey *et al*., 1989). Currently the identification and description of new *Sarcocystis* species are based on morphological and DNA investigations (Dahlgren and Gjerde, 2009; Kutkienė *et al*., 2009). The 18S and 28S rRNA genes are generally used to characterize *Sarcocystis* species genetically (Dahlgren and Gjerde, 2009; Mugridge *et al*., 2000). The first internally transcribed spacer (ITS–1) is useful to differentiate closely related species within the genus *Sarcocystis* (Marsh *et al*., 1999; Olias *et al*., 2010c).

It has been demonstrated that more than one *Sarcocystis* species could parasitize in one bird species (Drouin and Mahrt, 1980; Kutkienė and Sruoga, 2004). Furthermore, most of *Sarcocystis* species are strictly specific to the intermediate host; however some *Sarcocystis* species could form cysts in more than one species of the intermediate host (Box *et al*., 1984; Dahlgren and Gjerde, 2010; Kutkienė *et al*., 2010). The possibility that pathogenic species *S. falcatula* and *S. calchasi* parasitize in wood pigeons could not be rejected. So, an investigation of sarcocysts in this bird species is of great interest.

The aim of this study was identification of *Sarcocystis* species detected among wood pigeons hunted in Lithuania using morphological and DNA analysis.

Material and methods. In 2008 and 2009, a total of 18 wood pigeons hunted in three districts (Utena, Trakai and Vilnius) of Lithuania were investigated for *Sarcocystis* cysts.

Light microscopy. Samples of leg muscles of each individual were examined for sarcocysts. For this purpose, 28 oath-size pieces of muscles were cut off, stained with water (1:500) methylene blue solution, lightened with 1.5% water acetic acid solution, pressed into glass compressor and examined by light microscope Nicon ECLIPSE 80i. *Sarcocystis* cysts morphologically were characterized according to the size and shape of sarcocysts, the structure of the cyst wall and morphometric parameters of cystozoites. The morphometric investigations of sarcocysts and cystozoites were carried out using the flexible microscopy imaging tool INFINITY3 in fresh preparations after the cysts had been isolated from the muscle fibres by two preparation needles.

Transmission electron microscopy (TEM). Single mature sarcocyst isolated from leg muscles of one wood pigeon was fixed in Karnovsky‘s fixative, postfixed in 1% osmium tetroxide and dehydrated and embedded in Epon. Ultrathin sections were stained with 2% uranyl acetate, lead citrate and examined by JEOL JEM-100B TEM. Another three sarcocysts isolated from muscle fibres of the same wood pigeon were placed in 1.5 Eppendorf tubes containing 75% ethanol and were prepared for further DNA manipulations.

DNA analysis. Genomic DNA was extracted from sarcocysts using the Qiagen DNeasy tissue kit. ITS–1 region, 18S rRNR gene and 28S rRNR gene fragment were amplified using seven primer pairs P-ITSF\P-ITSR, SarAF\SarAR, SarBF\SarBR, SarCF\SarCR, SarDF\SarDR, KL-P1F\KL-P1R, KL-P2F\KL-P2R (Kutkienė *et al*., 2010). Polymerase chain reactions (PCRs) were performed in the final 25-µl volume consisting of 5 µl 10× PCR buffer, 2.5 µl dNTP (2 mM), 0.2 µM each primer, 1 µl Taq polymerase, 2.5µl $MgCl_2$ and 0.2 µg template DNA. PCRs were carried out with initial denaturing at 95°C for 5 min, 5 cycles at 94°C for 45 s, at 64°C for 60 s, at 72°C for 70 s, followed by 30 cycles at 94°C for 45 s, at 58°C for 60 s, at 72°C for 70 s and ended in with the final extension at 72°C for 10 min. PCR products were visualized using 1.7% agarose gel electrophoresis and purified with the help of exonucleases ExoI and FastAP. PCR products were sequenced directly with an ABI Prism 377 automatic DNA sequencer using the same primers as for the PCR reactions. The identified sequences were compared with the sequences listed in the GenBank database searching for the most similar ones using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences identity values were determined on the European Molecular Biology Open Software Suite (http://www.ebi.ac.uk/emboss/align/) using the default options. Sequences were aligned using ClustalW algorithm implemented in MEGA program version 4.0.1 (Tamura *et al*., 2007). The beginning and the end of some sequences were truncated to have all the sequences beginning with and ending in the same nucleotide positions. The phylogenetic tree of the family Sarcocystidae was constructed from pooled 18S rRNA gene and 28S rRNA gene sequences using the Bayesian method and MrBayes program, version 3. 1. 2 (Ronquist and Huelsenbeck, 2003). *Eimeria tenella* from Eimeridae family was set as an outgroup. The phylogenetic relationships were assessed with the most complex available model, the GTR + I + G evolutionary model, which allows all six possible substitutions to vary with the proportion of invariable sites and a gamma shaped distribution of rates across the sites. Phylogenetic tree was drawn by TreeView version 1.6.6 (Page, 1996).

Results. Cysts of *Sarcocystis* were found in two individuals out of 18 analyzed. The prevalence of infection accounted for 11.1%. Infection intensity in 28 oath-size pieces of leg muscles was 10 and 4 cysts, respectively.

Sarcocystis sp. cysts from the wood pigeons were ribbon-shaped, very long (the largest fragment found reached up to 7mm) and thick (up to 150 µm). They were divided into large compartments by septa. Cystozoites were lancet- or banana- shaped 6.3-7.3 µm in length (n=11) (Fig. 1B). By light microscope the cyst wall seemed smooth or slightly wavy and amounted up to 1.0 µm (Fig. 1A). In both wood pigeons the same type of the cyst wall was found using the flexible microscopy imaging tool. Ultrastructurally, the cyst wall, consisting of primary cyst wall (primary cyst wall is seen as light thin layer) and ground substance reached up to 2.0 µm and was slightly or clearly wavy, without visible protrusions (Fig. 1C-D). The parasitophorous vacuolar membrane had minute undulations in the electron dense layer under it

(Fig. 1E). The ground substance layer extended into the interior of the cyst as septa. These sarcocysts had type-1 tissue cyst wall (Dubey *et al*., 1989).

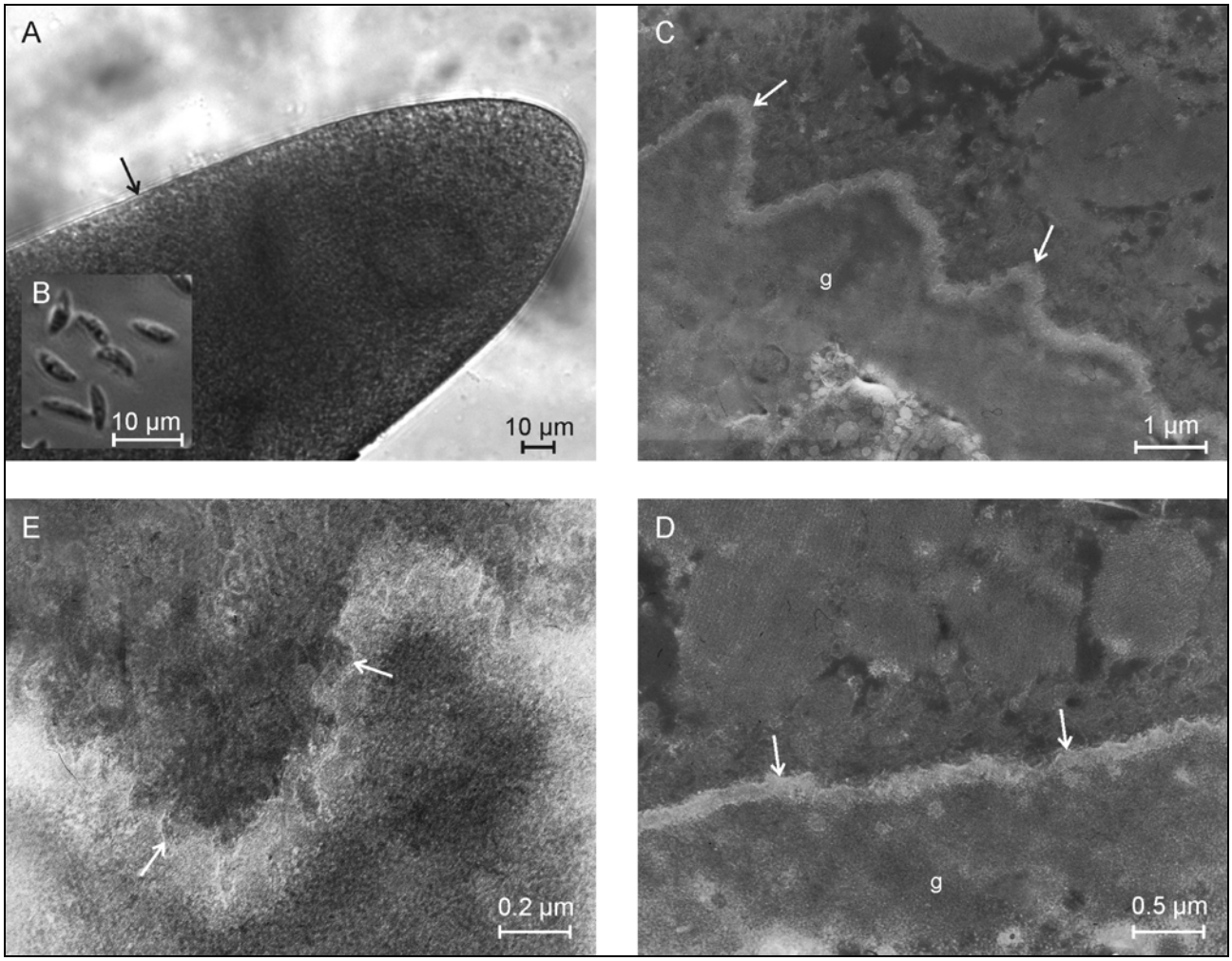

Fig. 1. A-E: **Morphological structure of *Sarcocystis* sp. from the leg muscle of the wood pigeon (*Columba palumbus*).** A, B: Light micrographs (computerized image analysis system). A: Fragment of the cyst; note smooth or slightly wavy cyst wall (arrow). Methylene blue stained preparation. B: Cystozoites. Native preparations. C-E: Electron micrographs of the cyst wall. C: Fragment of the cyst wall with high waves (arrows); g – ground substance. D: Fragment of the almost smooth cyst wall (arrows); g – ground substance. E: High magnification of the cyst wall; note minute invaginations of the parasitophorous vacuolar membrane (arrows).

ITS–1 region (832-bp long), 28S rRNA gene (1,490-bp long) and 18S rRNA gene (1,765-bp long) sequences of *Sarcocystis* sp. from the wood pigeon were deposited in GenBank with accession numbers HM125052–HM125054, respectively. Sequences of *Sarcocystis* sp. from the wood pigeon were the most identical to the sequences obtained from *Sarcocystis* species parasitizing in birds. *Sarcocystis* sp. from wood pigeon was genetically identical to *S. columbae* comparing partial sequences of 18S rRNA and 28S rRNA genes. Identical fragment of these two isolates were 3,082-bp long. Sequences identity of a highly variable region, ITS–1 of *S. columbae* and *Sarcocystis* sp. from the wood pigeon accounted for 99.9%. The comparison of the determined 18S rDNA, 28S rDNA and ITS–1 sequences also showed a very distinct identity of *Sarcocystis* sp. from the wood pigeon to *S. calchasi* and *S. wobeseri* (Table 1). However, sequences identity values within ITS–1 region between *Sarcocystis* sp. from the wood pigeon and *S. calchasi* or *S. wobeseri* were less than 83%. Moreover, *Sarcocystis* sp. from the wood pigeon differed greatly from *S. falcatula* within ITS–1 and differences in sequences were more than 50%. So, the DNA analysis proves that *Sarcocystis* sp. from the wood pigeon hunted in Lithuania belongs to the *S. columbae* species.

ITS–1 region is characterized as having a great variability within the Sarcocystidae family and therefore this molecular marker is inappropriate in drawing the phylogenetic trees of Sarcocystidae. The phylogenetic tree was formed from 3204 aligned nucleotide positions with gaps using a pooled alignment of 18S rRNA and 28S rRNA gene partial sequences. In the phylogenetic tree two subfamilies Sarcocystinae (*Sarcocystis* and *Frenkelia*) and Toxoplasmatinae (*Besnoitia*,

Cystoisospora, *Hammondia*, *Neospora* and *Toxoplasma*) are clearly separated (Fig. 2). *Sarcocystis* species, whose intermediate hosts are birds i.e. *S. calchasi*, *S. columbae*, *S. cornixi*, *S. rileyi*, *S. wobeseri*, *Sarcocystis* sp. ex *Anas platyrhynchos*, *Sarcocystis* sp. ex *Anser albifrons* are united in one well-supported phylogenetic group. *S. columbae* form a sister branch to *S. calchasi* and *S. wobeseri* group in phylogram.

Table 1. **The most genetically closely *Sarcocystis* species to *Sarcocystis* sp. from wood pigeon according to 18S rRNA, 28S rRNA genes and ITS–1 region sequences**

18S rRNR gene	28S rRNR gene	ITS–1 region
S. columbae 100%	*S. columbae* 100%	*S. columbae* 99.9%
S. wobeseri 100%	*S. wobeseri* 99.3 %	*S. calchasi* 82.8%
S. calchasi 99.9%	*S. calchasi* 98.7%	*S. wobeseri* 81.9 %
S. sp. ex *Accipiter nisus* 99.9%	*S.* sp. ex *Accipiter nisus* 98.7%	*S. cornixi* 75.5%
S. cornixi 99.6%	*S. cornixi* 98.3%	*S.* sp. ex *Accipiter nisus* 72.2%

Figures shows percentage values of sequences identity to *Sarcocystis* sp. from wood pigeon.

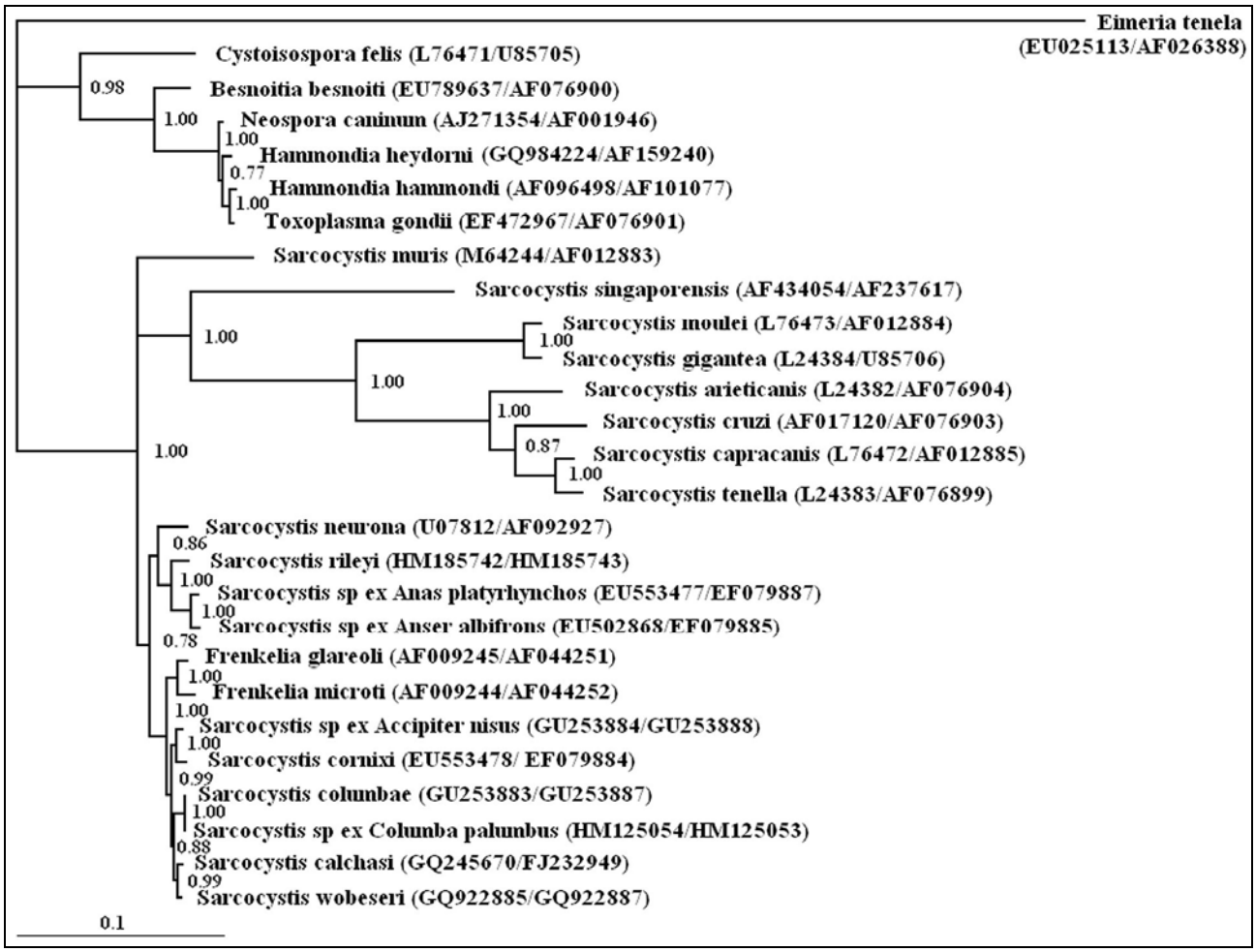

Fig. 2. **Phylogenetic tree of Sarcocystidae family based on fused 18S rRNA and 28S rRNA gene sequences.** Tree was rooted on *Eimeria tenella* and scaled according to the branch length. The numbers in the figure show posterior probability support values. GenBank accession numbers of 18S rRNA and 28S rRNA gene sequences are in brackets respectively.

Discussion. Thus far only several cases of *Sarcocystis* infection in birds of the Columbidae family have been reported. Firstly in Belarus sarcocysts were found in 2 out of 4 pigeons whose species status was not presented (Dylko, 1962). In the southeast of the USA *Sarcocystis* sp. were determined in the striated or cardiac muscles of 32 mourning doves (*Zenaida macroura*) from 255 (12.5%) individuals analyzed (Barrows and Hayes, 1977). Conti and Forrester (1981) detected *Sarcocystis* sp. in whitewinged doves (*Z. asiatica*) and in mourning doves in

Florida in the USA; the prevalence of infection was 7.9% (7 infected birds out of 89 investigated) and 10.4% (7 infected birds out of 67 investigated), respectively. In South Africa sarcocysts were found in 3 out of 70 (4.3%) laughing doves (*Streptopelia senegalensis*) (Kaiser and Markus, 1983a). Little is known about natural *S. falcatula* infection prevalence in doves or pigeons. *S. calchasi* was identified in 47 out of 244 (19.3%) examined homing pigeons in Germany (Olias *et al*., 2009). Interestingly, all five wood pigeons hunted in Germany harbored sarcocysts of *S. columbae* (Olias *et al*., 2010c). It could have been caused by very high breeding densities of wood pigeons breeding in city parks and in other urban and sub-urban habitats of Germany and Poland, with up to 220 pairs/10 ha recorded in the city parks of Poland (Tomialojc, 1976).

Only few cases of morphological examination of *Sarcocystis* spp. in pigeons have been presented thus far. In laughing doves *Sarcocystis* sp. cyst wall had small protrusions with microtubes (Kaiser and Markus, 1983b). Ultrastructurally, *S. calchasi* from the domestic pigeon and *S. columbae* from the wood pigeon had the same sarcocysts wall type-1, which is the most primitive cyst wall type (Dubey and Odening, 2001). *S. calchasi* and *S. columbae* are not distinguishable without a molecular investigation (Olias *et al*. 2010c). Cyst wall type-1 is the mostly distributed cysts wall type among *Sarcocystis* species having taxonomically distant intermediate hosts (Dubey *et al*., 1989). Furthermore, *S. wobeseri* from the birds of the order Anseriformes also demonstrate the same cyst wall type and additionally this species is not rigidly intermediate host specific (Kutkienė *et al*., 2010). By TEM we identified cyst wall type-1 of *Sarcocystis* sp. isolated from the wood pigeon, which was hunted in Lithuania. Hence, morphologically sarcocysts from the wood pigeon could not be separated from *S. calchasi*, *S. columbae* and *S. wobeseri* parasitizing in birds.

The phylogenetic tree of pooled 18S rRNA gene and 28S rRNA gene sequences showed that *Sarcocystis* sp. from the wood pigeon is *S. columbae*. However, branch lengths between *Sarcocystis* species from birds were considerably shorter as compared with those between other *Sarcocystis* species, which use mammals or reptiles as intermediate hosts. Moreover, an intra-specific variability of some *Sarcocystis* species from mammals or reptiles is larger than inter-specific variability of the *Sarcocystis* species from birds (Dahlgren and Gjerde, 2010; Šlapeta et al., 2002). Therefore 18S rRNA and 28S rRNA genes analysis is insufficient to distinguish closely related *Sarcocystis* species whose intermediate hosts are birds. Hence, species status of *S. columbae* under investigation has been established mainly by comparing highly evolving ITS–1 region sequences of *Sarcocystis* species. In conclusion, ITS–1 region is a key taxonomical marker identifying the *Sarcocystis* species parasitizing in birds.

Sarcocystis species, which use birds as intermediate hosts could be subdivided into two phylogenetic groups in phylogram, i.e. the first group unites *S. neurona*, *S. rileyi*, *Sarcocystis* sp. ex *Anas platyrhynchos* and *Sarcocystis* sp. ex *Anser albifrons*, the second one includes *S. calchasi*, *S. columbae*, *S. cornixi*, *S. wobeseri*, *Sarcocystis* sp. ex *Accipiter nisus* and two *Frenkelia* species. If definitive hosts are known, it is a mammal predator in the first group and a bird of prey in the second one. Some researchers hypothesized that phylogenetic relationship between the *Sarcocystis* species depended on the definitive host (Doležel *et al*., 1999; Elsheikha *et al*., 2005). Hence, according to the phylogenetic results, birds of prey are most expected to be a definitive host for *S. columbae*. All wood pigeons analysed in this study were hunted in their breeding sites (in the coniferous forest dominated by the spruce (*Picea abies*)). A high breeding density of wood pigeons (up to 30 pairs/100 ha) was recorded in this area in 2007 – 2008 (Švažas unpubl.). Several breeding pairs of goshawks were also registered in this territory in 2008. Goshawks are main predators of adult wood pigeons in the eastern Baltic region, while hooded crows (*Corvus corone corone*), ravens (*Corvus corax*), magpies (*Pica pica*) and other *Corvidae* species are main predators of the wood pigeon eggs and juveniles (Švažas, 2001). Mammal predators like martens (*Martes martes*) and stoats (*Mustela erminea*) were identified as predators of adult wood pigeons (of incubating females) only in very rare cases (Gorski *et al*., 1998). Therefore the goshawk is likely to be the definitive host of *S. columbae* in Lithuania, as it was found in Germany for the *S. calchasi* forming sarcocysts in the domestic pigeon (Olias *et al*., 2010b). It is also possible that infection with *Sarcocystis* species can be related to wintering grounds of wood pigeons, as huge concentrations of these birds (more than several million individuals) annually concentrate in few wintering sites located in Spain and Portugal (Švažas, 2001).

S. columbae infection in wood pigeons in Lithuania has been reported for the first time in this paper. However it is still not clear whether pathogenic *Sarcocystis* species *S. calchasi* or *S. falcatula* found in pigeons could be presented in Lithuania, therefore we plan to examine other bird species of the family Columbidae when searching for *Sarcocystis*.

Conclusions. Morphologically *Sarcocystis* sp. ex *Columba palumbus* was not distinguishable from *S. calchasi*, *S. columbae* and *S. wobeseri*. According to DNA analysis *Sarcocystis* sp. ex *Columba palumbus* was identified as *S. columbae*.

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References

1. Barrows P. L., Hayes F. A. Studies on endoparasites of the mourning dove (*Zenaida macroura*) in the Southeast United States. J. Wildl. Dis. 1977. Vol. 13(1). P. 24–28.

2. Box E. D., Meier J. L., Smith J. H. Description of

Sarcocystis falcatula Stiles, 1893, a parasite of birds and opossums. J. Protozool. 1984. Vol. 31(4). P. 521–524.

3. Conti J. A., Forrester D. J. Interrelationships of parasites of white-winged doves and mourning doves in Florida. J. Wildl. Dis. 1981. Vol. 17(4). P. 529–536.

4. Černá Ž. The role of birds as definitive hosts and intermediate hosts of heteroxenous coccidians. J. Protozool. 1984. Vol. 31(4). P. 579–581.

5. Dahlgren S. S., Gjerde B. *Sarcocystis* in Norwegian roe deer (*Capreolus capreolus*) molecular and morphological identification of *Sarcocystis oviformis* n. sp. and *Sarcocystis gracilis* and their phylogenetic relationships with other *Sarcocystis* species. Parasitol. Res. 2009. Vol. 104(5). P. 993–1003.

6. Dahlgren S. S., Gjerde B. Molecular characterization of five *Sarcocystis* species in red deer (*Cervus elaphus*), including *Sarcocystis hjorti* n. sp., reveals that these species are not intermediate host specific. Parasitology. 2010. Vol. 137(5). P. 815–840.

7. Doležel D., Koudela B., Jirků M., Hypša V., Oborník M., Votýpka J., Modrý D., Šlapeta J. R., Lukeš J. Phylogenetic analysis of *Sarcocystis* spp. of mammals and reptiles supports the coevolution of *Sarcocystis* spp. with their final hosts. Int. J. Parasitol. 1999. Vol. 29(5). P. 795–798.

8. Drouin T. E., Mahrt J. L. The morphology of cysts of *Sarcocystis* infecting birds in western Canada. Can. J. Zool. 1980. Vol. 58(8) P. 1477–1482.

9. Dubey J. P., Speer C. A., Fayer R. Sarcocystosis of animals and man. CRC Press, Boca Raton, 1989. 215 p.

10. Dubey J.P., Odening K. Toxoplasmosis and related infections. In: Samuel W. M., Pybus M. J., Kocan A. A. (eds) Parasitic diseases of wild mammals. Iowa State University Press, Ames, 2001. P. 478–519.

11. Dubey J. P., Cawthorn R. J., Speer C. A., Wobeser G. A. Redescription of the sarcocysts of *Sarcocystis rileyi* (Apicomplexa: Sarcocystidae). J. Eukaryot. Microbiol. 2003. Vol. 50(6). P. 476–482.

12. Dylko N. I. The occurrence of *Sarcocystis* spp. in Belarus. Doklady Akad Nauk BSSR. 1962. Vol. 6. P. 399–400.

13. Ecco R., Luppi M. M., Malta M. C., Araujo M. R., Guedes R. M., Shivaprasad H. L. An outbreak of sarcocystosis in psittacines and a pigeon in a zoological collection in Brazil. Avian. Dis. 2008. Vol. 52(4). P. 706–710.

14. Elsheikha H. M., Lacher D. W., Mansfield L. S. Phylogenetic relationships of *Sarcocystis neurona* of horses and opossums to other cyst-forming coccidia deduced from SSU rRNA gene sequences. Parasitol. Res. 2005. Vol. 97(5). P. 345–357.

15. Fayer R. *Sarcocystis* spp. in human infections. Clin. Microbiol. Rev. 2004. Vol. 17(4). P. 894–902.

16. Gjerde B., Dahlgren S. S. Corvid birds (Corvidae) act as definitive hosts for *Sarcocystis ovalis* in moose (*Alces alces*). Parasitol. Res. 2010. Vol. 107(6). P. 1445–53.

17. Gorski W., Antczak J., Hetmanski T. The breeding ecology of wood pigeon in urban areas of NW Poland. Acta Zoologica Lituanica 1998. Vol. 8. P. 137–144.

18. Kaiser I. A., Markus M. B. *Sarcocystis* infection in wild Southern African birds. South African J. Sci. 1983a. Vol. 79. P. 470–471

19. Kaiser I. A., Markus M. B. Species of *Sarcocystis* in wild South African birds. Proc. Electron Microscopy Soc. South Africa. 1983b. Vol. 13. P. 103–104.

20. Kutkienė L., Sruoga A. *Sarcocystis* spp. in birds of the order Anseriformes. Parasitol. Res. 2004. Vol. 92(2). P. 171–172.

21. Kutkienė L., Sruoga A., Butkauskas D. *Sarcocystis* sp. from white-fronted goose (*Anser albiforns*): cyst morphology and life cycle studies. Parasitol. Res. 2006. Vol. 99(5) P. 562–565.

22. Kutkienė L., Prakas P., Sruoga A., Butkauskas D. *Sarcocystis* in the birds family Corvidae with description of *Sarcocystis cornixi* sp. nov. from the hooded crow (*Corvus cornix*). Parasitol. Res. 2009. Vol. 104(2). P. 329–336.

23. Kutkienė L., Prakas P., Sruoga A., Butkauskas D. The mallard duck (*Anas platyrhynchos*) as intermediate host for *Sarcocystis wobeseri* sp. nov. from the barnacle goose (*Branta leucopsis*). Parasitol. Res. 2010. Vol. 107(4). P. 879–888.

24. Marsh A. E., Barr B. C., Tell L., Bowmann D. D., Conrad P. A., Ketcherside C., Green T. Comparison of the internal transcribed spacer, ITS-1, from *Sarcocystis falcatula* isolates and *Sarcocystis neurona*. J. Parasitol. 1999. Vol. 85(4). P. 750–757.

25. Mehlhorn H., Heydorn A. O. The Sarcosporidia (Protozoa, Sporozoa): life cycle and fine structure. Adv. Parasitol. 1978. Vol. 16. P. 43–91.

26. Mugridge N. B., Morrison D. A., Jäkel T., Heckeroth A. R., Tenter A. M., Johnson A. M. Effects of sequence alignment and structural domains of ribosomal DNA on phylogeny reconstruction for the protozoan family Sarcocystidae. Mol. Biol. Evol. 2000. Vol. 17(12). P. 1842–1853.

27. Odening K. The present state of species-systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidia). Syst. Parasitol. 1998. Vol. 41. P. 209–233.

28. Olias P., Gruber A. D., Heydorn A. O., Kohls A., Mehlhorn H., Hafez H. M., Lierz M. A novel *Sarcocystis*-associated encephalitis and myositis in

racing pigeons. Avian. Pathol. 2009. Vol. 38(2). P. 121–128.

29. Olias P., Gruber A. D., Kohls A., Hafez H. M., Heydorn A. O., Mehlhorn H., Lierz M. *Sarcocystis* species lethal for domestic pigeons. Emerg. Infect. Dis. 2010a. Vol. 16(3). P. 497–499.

30. Olias P., Gruber A. D., Hafez H. M., Heydorn A. O., Mehlhorn H., Lierz M. *Sarcocystis calchasi* sp. nov. of the domestic pigeon (*Columba livia* f. domestica) and the Northern goshawk (*Accipiter gentilis*): light and electron microscopical characteristics. Parasitol. Res. 2010b. Vol. 106(3). P. 577–585.

31. Olias P., Olias L., Lierz M., Mehlhorn H., Gruber A. D. *Sarcocystis calchasi* is distinct to *Sarcocystis columbae* sp. nov. from the wood pigeon (*Columba palumbus*) and *Sarcocystis* sp. from the sparrowhawk (*Accipiter nisus*). Vet. Parasitol. 2010c. Vol. 171(1-2). P. 7–14.

32. Page R. D. Treeview: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 1996. Vol. 12(4). P. 357–358.

33. Ronquist F, Huelsenbeck J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003. Vol. 19(12). P. 1572–1574.

34. Smith J. H., Neill P. J., Dillard III E. A., Box E. D. Pathology of experimental *Sarcocystis falcatula* infections of canaries (*Serinus canaries*) and pigeons (*Columba livia*). J. Parasitol. 1990. Vol. 76(1). P. 59–68.

35. Suedmeyer W. K., Bermudez A. J., Barr B. C., Marsh A. E. Acute pulmonary *Sarcocystis-falcatula*-like infection in three Victoria crowned pigeons (*Goura victoria*) housed indoors. J. Zoo Wildl. Med. 2001. Vol. 32(2). 252–256.

36. Šlapeta J. R., Kyselová I., Richardson A. O., Modrý D., Lukeš J. Phylogeny and sequence variability of the *Sarcocystis singaporensis* Zaman and Colley, (1975) 1976 ssrDNA. Parasitol. Res. 2002. Vol. 88(9). P. 810–815.

37. Švažas S. Population status of pigeons and doves in the eastern Baltic region. 2001. Naturzale. Vol. 16. P. 71–81.

38. Tamura K., Dudley J., Nei M., Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 2007. Vol. 24(8). P.1596–1599.

39. Tomialojc L. The urban population of wood pigeon in Europe: its origin, increase and distribution. Acta Zoologica Cracoviensis. 1976. Vol. 21. P. 585–631.

40. Yabsley M. J., Ellis A. E., Stallknecht D. E., Howerth E. W. Characterization of *Sarcocystis* from four species of hawks from Georgia, USA. J. Parasitol. 2009. Vol. 95(1). P. 256–259.

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