

The golden jackal *Canis aureus* – a new species in the Baltic countries

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In the Baltic countries, the golden jackal (*Canis aureus*) was first recorded in Estonia on 28 February 2013 and three specimens of golden jackal were hunted in Latvia in 2014. The first golden jackal in Lithuania was hunted on 7 February 2015. The species of the golden jackal was identified using morphological and mitochondrial DNA control region (CR1) analysis. In Lithuania, hunting of these animals is permitted throughout the year. Few studies in the past revealed the potential role of the golden jackal as a carrier of intestinal helminths, parasites, and zoonotic diseases. In this study, the presence of tick-borne pathogens and other parasites in golden jackal specimen were investigated. No pathogens (*Anaplasma phagocytophilum*, *Babesia* sp., *Bartonella* sp.) were found in the spleen of the golden jackal. However, the flukes *Apophallus donicus*, nematodes *Uncinaria stenocephala*, and unidentified individuals of class *Cestoda* were detected. Helminths *A. donicus* and *U. stenocephala* are not new species for Lithuania and neighbouring territories.

Keywords: *Canis aureus*, mtDNA, helminths, *Uncinaria stenocephala*, *Apophallus donicus*, *Cestoda*

INTRODUCTION

The golden jackal (*Canis aureus*) is a native Palearctic species with a historic range extending from North Africa and South-eastern and Central Europe through to Central and East Asia (Jhala and Moehlman, 2008). The population and distribution of the golden jackal within Europe has seen considerable changes over recent decades. Since the mid-twentieth century, the distributional range of golden jackals has expanded significantly across Central and Eastern Europe with occasional animals being documented to the north and the west, far from the established populations

in the areas and countries they had not been recorded before. Golden jackals have been recently sighted as far west as Switzerland and as far north as Estonia (Rutkowski et al., 2015; Trouwborst et al., 2015).

In Estonia and Latvia, golden jackals of unknown origin have been recorded since 2011 (Banea, 2013; Toom, 2014; Trouwborst et al., 2015). The first confirmed case of the golden jackal in the Baltic countries was reported in Estonia in 2013. In February 2013, a golden jackal was killed by hunters in Matsalu National Park, West Estonia (Männil et al., 2014). In 2014, golden jackals were recorded in several other widely dispersed localities across Estonia, including the north, north-east and south of the country. Until May 2015,

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a total of nine individuals were killed either by hunters or in road accidents across Estonia (Stratford, 2015).

First recorded cases of the golden jackal in Latvia were three individuals killed by a hunter in July and December 2013 (Männil et al., 2014). Until 2015, a total of ten golden jackals were shot or found dead in Latvia (Stratford, 2015).

In Lithuania, the first golden jackal was shot in February 2015. The species of the hunted individual was identified through a morphological examination. Since that time, other unconfirmed reports of the golden jackal followed from the districts of Kaunas, Vilnius, Zarasai, Varėna, and Biržai.

In the present study, we used mitochondrial DNA control region data for the confirmation of species identification, and as few studies in the past revealed the potential role of the jackals as carriers of zoonotic diseases, we investigated the presence of tick-borne pathogens and other parasites in the golden jackal specimen from Lithuania.

MATERIALS AND METHODS

Genomic DNA from the specimen spleen was extracted using the Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to manufacturer's instructions. DNA was stored at -20°C until subsequent handlings. A 460 bp of hypervariable left domain of the mitochondrial DNA control region was amplified using PCR and sequencing primers WDLOOP1 5'-TCC-CTGACACCCCTACATTC-3', H519 5'-CGTT-GCGGTCATAGGTGAG-3' (designed on wolf mtDNA CR (Caniglia et al., 2013)) as described in Fabbri et al., 2014. The products were separated on 1.5% agarose gel and visualized by ethidium bromide. PCR amplification product of mtDNA control region was purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Lithuania) and sequenced. The resulting 460-bp-long sequence of the CR mtDNA was revised manually and aligned with golden jackal sequences from GenBank in MEGA 6. The sequence of CR mtDNA was submitted to GenBank and assigned the following accession number: KT123040.

Conventional PCR and nested PCR methods were used for the detection of *Anaplasma phagocytophilum*, *Babesia* spp., *Bartonella* spp. (Masung et al., 1998; Rar et al., 2005; Norman et al., 1995).

Prior to helminthological investigation the material was frozen at -80°C for 10 days for safety reasons (i.e., to avoid possible infection with *Echinococcus* spp.) (WHO/OIE Manual, 2001). The golden jackal was thawed at room temperature and separate organs (entire gastrointestinal tract, lungs, heart, liver, and kidney) were studied helminthologically according to the methodology of Ivashkin et al. (1971). Examination of the content of the intestines and of the stomach was based on the method of consistent flushing. Helminths were picked out and fixed in 70% ethanol for later examination. For the microscopic study, trematodes and nematodes were mounted in glycerin and examined under a Motic BA400 Tension microscope. Identification was based on the key of Kozlov (1977). The diet of the golden jackal was studied using analysis of the stomach content. Each portion of the stomach content was mixed with water and individual components separated into groups.

RESULTS AND DISCUSSION

Comparison of the mtDNA sequence identified in this study with those deposited in GenBank confirmed that the animal hunted in Lithuania in February 2015 was a golden jackal (accession number KT123040). The golden jackal haplotype identified in the present study corresponds with the haplotype previously identified in Italy, Croatia, Bulgaria, Serbia, and the Caucasus (Fig. 1). The golden jackal is a new species in the Baltic region. The confirmed cases of the golden jackal in Latvia and Estonia were recorded in 2013 (Männil et al., 2014; Stratford, 2015), two years earlier than in Lithuania.

The Eurasian golden jackal has been reported as a host of pathogens of zoonotic and veterinary importance, including a range of vector-borne pathogens such as *Ehrlichia canis*, *Anaplasma phagocytophilum*, and others (Waner et al., 1999), and only one *Babesia* species (*B. canis*) was

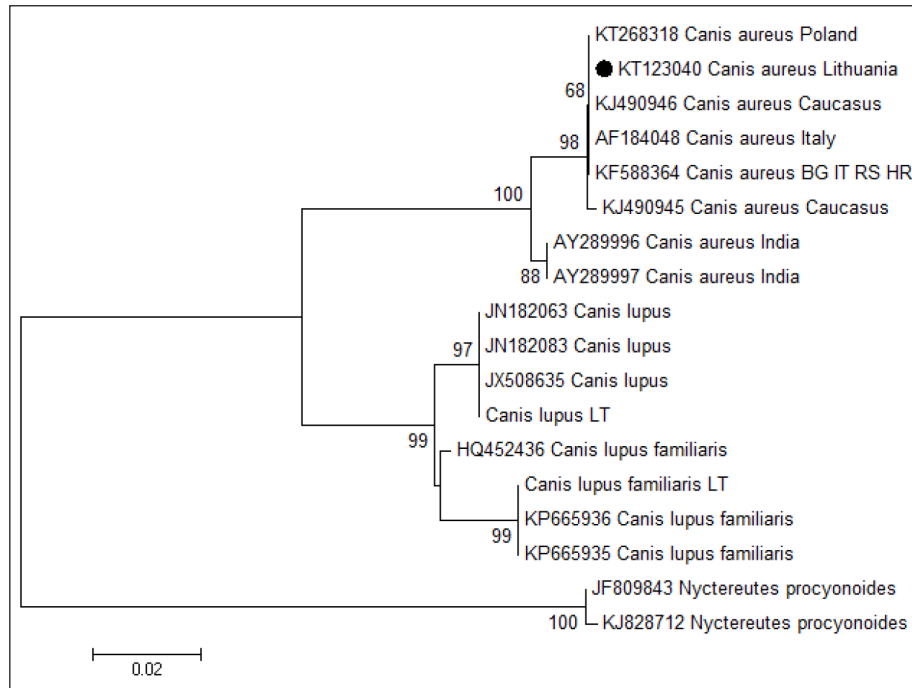


Fig. 1. Phylogenetic tree of mtDNA sequences created using the Neighbor-Joining method and a bootstrap analysis of 1000 replicates. The sample from the present study is marked ●. *Nyctereutes procyonoides* are used as an out-group. Abbreviations: BG – Bulgaria, HR – Croatia, IT – Italy, RS – Serbia

confirmed by molecular methods in golden jackals in Europe, in Romania (Mitkova et al., 2017). However, reports of tick-borne pathogens in *C. aureus* are scarce. In this study, different PCR methods were used for the detection of tick-borne pathogens: *Anaplasma phagocytophilum*, *Babesia* spp., *Bartonella* spp. The results of PCR analysis were negative for all tested pathogens.

Several hookworm (Ancylostomatidae) species have been reported in golden jackals, with

Ancylostoma caninum and *Uncinaria stenocephala* commonly reported across the entire geographical range of these hosts (Gherman, Mihalca, 2017). In the small intestine of the Lithuanian golden jackal, 49 flukes of species *Apophallus donicus*, 11 nematodes of species *Uncinaria stenocephala* (Fig. 2), and 29 unidentified individuals of *Cestoda* were found.

The diet of golden jackal was studied through the stomach content. It was found to consist of

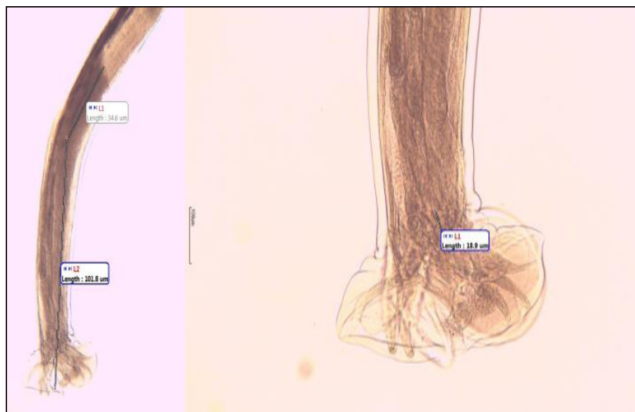


Fig. 2. *Uncinaria stenocephala*, microscopic examination

plants, insects (larvae), birds (feathers), rodents (bones, limbs), and ungulates (hair). In Croatia, both animal and plant components were found in the scat of the golden jackal: the major component was mammals (50.3%), followed by fruit seeds and vegetables (34.1%), insects (29.5%), birds (including eggs; 24.8%), artificial materials (24%), and branches, leaves, and grass (24%) (Radovic, Kovačić, 2010).

CONCLUSIONS

Result of analysis of a sample of unknown animal from Lithuania revealed that it was a golden jackal. Similar genetic patterns based on mtDNA control region sequences were identified in animals from Bulgaria, Croatia, Italy, Serbia, and the Caucasus. No tick-borne pathogens were found in the golden jackal, however, the flukes *Apophallus donicus*, nematodes *Uncinaria stenocephala*, and unidentified individuals of class *Cestoda* were detected.

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PAPRASTASIS ŠAKALAS *CANIS AUREUS* – NAUJA RŪŠIS BALTIJOS ŠALYSE

Santrauka

Pirmą kartą paprastas šakalas (*Canis aureus*) Estijoje buvo pastebėtas 2013 m. vasario 28 d., Latvijoje pirmieji trys paprastieji šakalai buvo sumedžioti 2014 metais. Lietuvoje pirmasis šakalas sumedžiotas 2015 m. vasario 7 d., šio gyvūno rūšies identifikacija atlikta remiantis morfologine ir molekuline (mitochondrinės DNR) analize. Ankstesniais tyrimais nustatyta, kad paprastieji šakalai gali būti žarnyno helmintų ir zoonotinės kilmės ligų pernešėjai. Dabar buvo atlikti erkių pernešamų patogenų ir žarnyno parazitų tyrimai. Erkių pernešamų patogenų (*Anaplasma phagocytophilum*, *Babesia* sp., *Bartonella* sp.) paprastojo šakalo blužnies pavyzdyje nerasta, žarnyne buvo aptiktos siurbikės (*Apophallus donicus*), apvaliosios kirmėlės (*Uncinaria stenocephala*) ir neidentifikuotos rūšies kaspinočių (*Cestoda*) klasės individai.

Raktažodžiai: *Canis aureus*, mitochondrinė DNR, helmintai, *Uncinaria stenocephala*, *Apophallus donicus*, kaspinočiai