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First record of metacestodes of *Mesocestoides* sp. in the common starling (*Sturnus vulgaris*) in Europe, with an 18S rDNA characterisation of the isolate

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Abstract. Metacestodes of *Mesocestoides* sp. were recorded from *Sturnus vulgaris* (Passeriformes: Sturnidae) in the Czech Republic in April 2002. They were found in a cutaneous cyst and in the thoracic region of the body cavity of the bird. This is the first record of metacestodes of *Mesocestoides* sp. in this host species in Europe as well as the first finding of the formation of a cutaneous cyst provoked by this parasite. Additional specimens from *Apodemus agrarius* (Mammalia: Rodentia) from Bulgaria and *Lacerta agilis* (Reptilia: Squamata) from the Czech Republic were compared with that from *S. vulgaris*. Sequence data from the V4 variable region (18S rDNA) were used to compare genetic variability among these and previously characterized isolates of *Mesocestoides* spp. A number of distinct clades were recognized, with metacestodes from *L. agilis* showing the highest degree of relative divergence.

Adult cestodes of the genus *Mesocestoides* Vaillant, 1863 (Cyclophyllidea: Mesocestoididae) are intestinal parasites in carnivorous mammals and birds of prey. Their metacestodes, known as tetrathyridia and recently suggested to be termed as merocercoids (Chervy 2002), were reported from a wide range of mammals, reptiles and amphibians; there are also several records from birds (Chertkova and Kosupko 1978, Prudhoe and Bray 1982, Rausch 1994). Chertkova and Kosupko (1978) listed birds of seven orders, mostly Galliformes and Passeriformes, as intermediate hosts of *Mesocestoides* spp. Among passeriform birds, members of the families Corvidae, Laniidae and Ploceidae were reported as hosts of metacestodes of this genus in Europe.

The present article describes the first record of metacestodes of *Mesocestoides* sp. from the common starling (*Sturnus vulgaris* Linnaeus, 1758) in Europe, provides morphometric data and molecular confirmation of the identity of these larval cestodes.

MATERIALS AND METHODS

In 2002, wild birds were repeatedly trapped with mist nets in the municipal garden in Brno (49°13'N, 16°36'E), Czech Republic. Birds were ringed and released in the same place. On 6 April 2002, an adult common starling with an unusual cutaneous cyst on the abdomen was trapped. The top of the cyst was cut with small scissors and five parasitic 'pouches', approximately 7 mm in length, were easily pushed out and conserved in 70% ethanol. The starling was not released but kept in captivity in the laboratory until 5 May 2002 when it suddenly died. During the month in captivity, the starling showed no sign of disease. The starling was dissected and the dissection revealed 33 specimens of the same parasites as in the cutaneous cyst situated in the thoracic region of the body cavity near the lungs. They were isolated and fixed in 4% formalin.

The parasites were stained by Semichon carmine, Semichon carmine combined with Astra blue or Gomori trichrome or iron acetocarmine and mounted in Canada balsam. Morphometric data are presented below as the range followed by the mean ± standard deviation and the number of measurements taken (n) in parentheses.

As comparative material, metacestodes of *Mesocestoides* spp. from the body cavity of *Lacerta agilis* (Linnaeus, 1758) (sand lizard) collected in Stříbrné Hory, Czech Republic, at 17 July 1997 by D. Modrý, and from the body cavity of *Apodemus agrarius* (Pallas, 1771) (black-striped field mouse) collected near Nova Cherna, Bulgaria, at 20 August 2002 by B.B.G. and P.N. Nikolov, were used for both morphological and molecular studies.

Ethanol-preserved, individual metacestodes from starling, as well as those from *L. agilis* and *A. agrarius*, were used for molecular studies. Ethanol was replaced from the tissues by soaking overnight in 1M Tris-EDTA (pH 8) buffer and the total genomic DNA extracted using a Qiagen® DNeasy™ tissue kit following manufacturer-recommended protocols, with the exceptions that the incubation period with proteinase-K

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was extended to overnight in a rotating incubator and the final elution volume was 200 µl. The V4 region of the 18S ribosomal DNA (rDNA) gene was targeted for sequence analysis due to its high variability and to facilitate comparison with the largest number of publicly available sequences of *Mesocestoides* spp. (from the work of Crosbie et al. 2000). Twenty-five µl PCR amplifications were performed using Ready-To-Go™ (Amersham Pharmacia Biotech) PCR beads, 1 µl of genomic extract and 10 mM of each PCR primer. Poor fixation of the samples resulted in low-yield initial PCR amplifications, and thus the products of the initial PCR were subjected to a second round of amplification using a nested primer. Primary amplifications used primers WormA and 1270R and the following thermocycling conditions: 94°C/5 min denature hold; 40 cycles of 94°C/1 min, 52°C/1 min, 72°C/2 min; 72°C/7 min extension hold. Secondary PCR used nested primer Meso300F (5' GGTGACTCTGGATAATTGTTACAG 3') together with 1270R, and the thermocycling conditions: 94°C/5 min denature hold; 25 cycles of 94°C/30 sec, 55°C/30 sec, 72°C/1 min; 72°C/7 min extension hold. See Littlewood and Olson (2001) for a complete listing of 18S rDNA primers not defined herein.

The secondary PCR products were purified directly using Qiagen Qiaquick™ columns, cycle-sequenced from both strands using ABI BigDye™ chemistry, alcohol-precipitated, and run on an ABI Prism 377™ automated sequencer. Sequencing primers used were Meso300F, 18S-8, 930F, 600R, 1270R and MesoV4R (5' GTGCCATCCGCCCACAGAC ACC 3'). Contiguous sequences (~800 bps) were assembled and edited using Sequencher™ (GeneCodes Corp., ver. 4) and submitted to GenBank under accession numbers AY426256-8. The three sequences were screened using BLAST (Altshul et al. 1997) and aligned manually using MacClade (Maddison and Maddison 2000) together with all available 18S rDNA sequences of *Mesocestoides* spp. (see Fig. 3 for GenBank accession numbers). A total of 779 bps were common among all available *Mesocestoides* 18S sequences in the alignment and were thus used for phylogenetic analysis. Parsimony and bootstrap analyses were performed as described by Olson et al. (2003), with the exception that gaps were treated as a '5th' base.

RESULTS

The cutaneous cyst was located near the abdominal medial line (Fig. 1). The cyst was approximately 8 mm in diameter with the skin hemispherically vaulted. Few parasitic organisms were visible through the tight wall of the cyst. The liquid content of the cyst was slightly fluctuating on palpation.

Parasites from the cutaneous cyst and from the thoracal cavity were determined as metacestodes of *Mesocestoides* sp. and confirmed by molecular analysis. In total, 38 specimens were isolated from the infected starling.

The metacestodes were of variable shape (Fig. 2), mostly longitudinally elongate, 2.30–8.53 mm (5.29 ± 1.62 mm, n = 22) long and 1.31–2.84 mm (1.88 ± 0.38 mm, n = 22) wide. Their scoleces exhibited various degree of invagination. Four suckers with a diameter of

Fig. 1. Cutaneous cyst on the abdomen of *Sturnus vulgaris*. Actual size.

118–206 µm (153 ± 15 µm, n = 42) were distinct on each scolex. The pore of the osmoregulatory system was frequently seen at the posterior end of the body.

For each of the three metacestode sequences characterized herein, the highest BLAST similarity scores were compared to previously accessioned *Mesocestoides* spp. 18S sequences, corroborating the morphological diagnosis of the larval cestodes. It was not necessary to introduce gaps into the alignment with the sole exception of the sequence corresponding to the metacestodes from *L. agilis* which was unique in being 18 bps shorter than all other sequences compared. These missing bases formed a single gap corresponding to a central portion of the V4 variable region, close to stem 21 (see Appendix A in Olson and Caira 1999 for reference). With the exception of this *Mesocestoides* isolate, the sequences among all 18 isolates were highly similar, with only 15 of the 779 bps being parsimony-informative (i.e. varying in more than one sequence). Parsimony analysis resulted in two equally parsimonious trees, the unrooted, strict-consensus of which showed a fair degree of variability among isolates and left the relative positions of the isolates from *S. vulgaris* and *A. agrarius* unresolved (Fig. 3). The isolate from *L. agilis* showed the greatest degree of divergence in part because gaps were treated as unique, rather than missing, data.

DISCUSSION

The general morphology of the metacestodes found corresponds well to that of the comparative specimens and published descriptions (Joyeux and Baer 1936, Neveu-Lemaire 1936, Gvozdev 1958, Chertkova and Petrov 1959, Oshmarin 1963, Chertkova and Kosupko 1978). The present study confirms the great variability of the body shape of the metacestodes of *Mesocestoides* spp.; earlier authors used the binomen "*Tetrathyridium variabile* (Diesing, 1850)" for metacestodes of this genus from avian hosts (see Joyeux and Baer 1936, Neveu-Lemaire 1936, Gvozdev 1958, Chertkova and Petrov 1959).

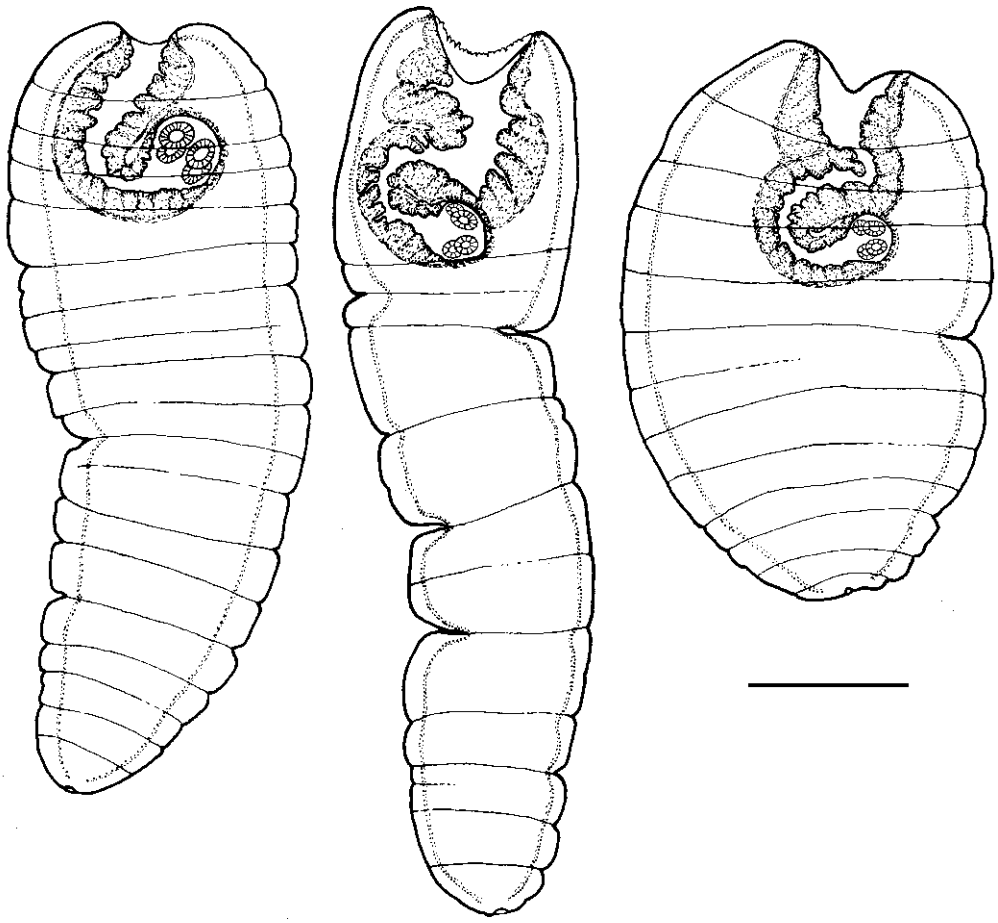

Fig. 2. Metacestodes of *Mesocestoides* sp. from *Sturnus vulgaris*. Scale bar = 1 mm.

There are several reports containing morphometric data on the body size of larval *Mesocestoides* spp. from avian hosts: av. 5 mm long (Joyeux and Baer 1936), 2–4 × 1.5–2 mm (Neveu-Lemaire 1936), 1.4–3.0 × 1.4–2.0 mm (Gvozdev 1958), 2.60–3.63 × 1.07–1.32 mm (Chertkova and Petrov 1959), 1.80–1.95 × 1.35–1.52 mm (Oshmarin 1963), 2–4 × 1.5–2 mm (Movsesyan 1987) or "up to 1 mm in diameter" (Millán et al. 2003). Our specimens, exceeding a length of 8.5 mm and a width of 2.8 mm, exhibit larger measurements than previously reported from avian hosts.

Various sites of infection in birds have been reported previously: peritoneum (Chertkova and Petrov 1959), lungs (Chertkova and Petrov 1959), liver (Chertkova and Petrov 1959, Borgarenko 1981), body cavity (Joyeux and Baer 1936, Chertkova and Petrov 1959, Oshmarin 1963, Borgarenko 1981, Millán et al. 2003), air sacs (Joyeux and Baer 1936) and subcutaneous connective tissue (Borgarenko 1981). According to Chertkova and Kosupko (1978), the most frequent site in birds is in the lung tissue and intercostal musculature. We have not found a publication reporting on the formation of a cutaneous cyst as revealed in the present case. The site within the thoracic cavity, however, is typical of previous reports (Joyeux and Baer 1936, Chertkova and Petrov 1959, Oshmarin 1963, Borgarenko 1981). The only record of metacestodes of *Mesocestoides* sp. from the common starling is from *Sturnus vulgaris porphyronotus* (see Chertkova and Kosupko 1978). This subspecies occurs in Asia only, from Turkmenistan to Nepal and North India (Howard and Moore 1980). Therefore, the present observations show that the common starling (*Sturnus vulgaris vulgaris*) in Europe may also have a role as intermediate or paratenic host of cestodes of the genus *Mesocestoides*.

Crosbie et al. (1998, 2000) examined genetic variability in isolates of *Mesocestoides* spp. primarily from domestic dogs in western USA. Incorporating their data (Crosbie et al. 2000), we recover similar clades of isolates and considerably expand the relative genetic variation by the inclusion of European isolates. The unique 18 bp indel (insertion/deletion) in the isolate from *L. agilis* was particularly interesting in that it may

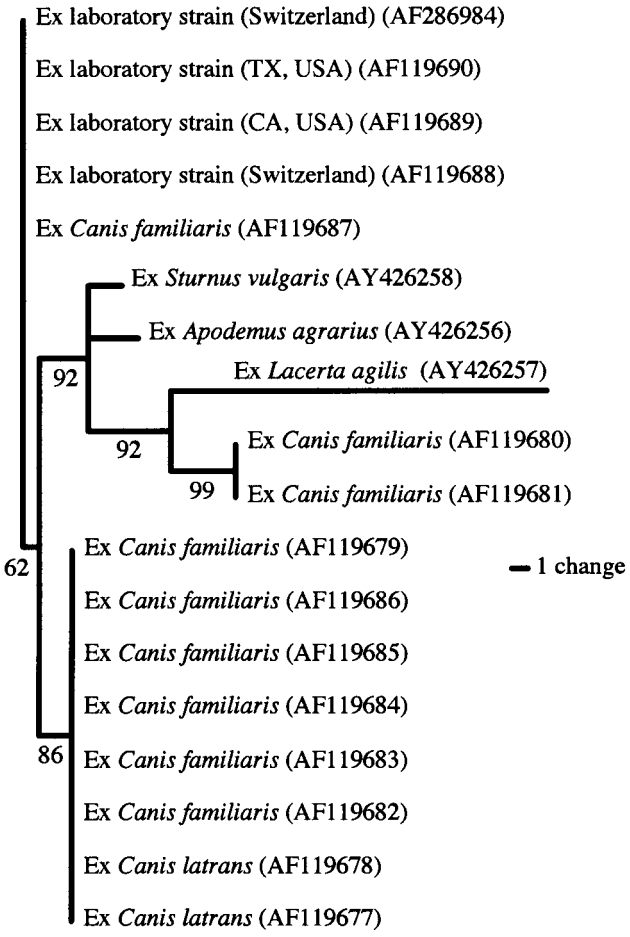

Fig. 3. Unrooted phylogram showing the affinities of the *Mesocestoides* spp. isolates. Nodal support based on bootstrap analysis (1000 replicates). The relative positions of the isolates from *Sturnus vulgaris* and *Apodemus agrarius* were not resolved.

be an appropriate molecular marker applicable for species identification. Due to the morphological uniformity of the metacestodes of *Mesocestoides* spp., their identification to the species level has been difficult. Until now, the only reliable method was the experimental infection of carnivores. Thus, by feeding experiments using cats and captive foxes as final hosts, Sharpilo (1976) revealed that metacestodes from *Vipera ammodytes* (Linnaeus, 1758) belonged to *Mesocestoides lineatus* (Goeze, 1782) and Biserkov (1987) showed that *Podarcis muralis* (Laurenti, 1768) participated in the life cycle of *M. lineatus* while *Lacerta viridis* (Laurenti, 1768) was a host of both *M. lineatus* and *M. litteratus* (Batsch, 1786), all from Bulgaria. Seven species of this genus were recorded in Europe (Chertkova and Kosupko 1978, Yanchev 1986), often sympatrically. Almost nothing is known about the ranges of their intermediate and paratenic hosts in natural conditions. The established genetic variability is promising in view of species identification of metacestodes using molecular sequences and the elucidation of transmission routes of *Mesocestoides* spp. in natural conditions. However, considerable expansion of the molecular data from both adult and larval cestodes from various hosts and geographical areas is necessary to better understand the specific host associations and species circumscriptions.

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REFERENCES

ALTSHUL S.F., MADEN T.L., SCHAFFER A.A., ZHANG Z., MILLER W., LIPMAN D.J. 1997: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. 25: 402.

BISERKOV V.Y. 1987: [Role of reptiles as intermediate and paratenic hosts in the transmission of helminth parasites in Bulgaria.] In: B. Botev (Ed.), Savremenni Postizheniya na Balgarskata Zoologiya. Izdatelstvo na Balgarskata Akademiya na Naukite, Sofia, pp. 81–84. (In Bulgarian.)

BORGARENKO L.F. 1981: Helminth parasites of birds in Tadzhikistan. Book 1. Cestodes. Donish, Dushanbe, 327 pp. (In Russian.)

CHERTKOVA A.N., KOSUPKO G.A. 1978: [Suborder Mesocestoidata Skrjabin, 1940.] In: K.M. Ryzhikov (Ed.), [Principles of Cestodology, Vol. 9.] Nauka, Moscow, pp. 118–229. (In Russian.)

CHERTKOVA A.N., PETROV A.M. 1959: [Helminth parasites of domesticated galliform birds and the diseases caused by them. Volume 1.] All-Union Institute of Helminthology, Moscow, 364 pp. (In Russian.)

CHERVY L. 2002: The terminology of larval cestodes or metacestodes. Syst. Parasitol. 52: 1–33.

CROSBIE P.R., BOYCE W.M., PLATZER E.G., NADLER S.A., KERNER C. 1998: Diagnostic procedures and treatment of eleven dogs with peritoneal infections caused by *Mesocestoides* spp. J. Am. Vet. Med. Assoc. 213: 1578–1583.

CROSBIE P.R., NADLER S.A., PLATZER E.G., KERNER C., MARIAUX J., BOYCE W.M. 2000: Molecular systematics of *Mesocestoides* spp. (Cestoda: Mesocestoididae) from domestic dogs (*Canis familiaris*) and coyotes (*Canis latrans*). J. Parasitol. 86: 350–357.

GVOZDEV E.V. 1958: [Parasitic worms of galliform birds of Kazakhstan.] Izdatel'stvo Akademii Nauk Kazakhskoi SSR, Alma-Ata, 266 pp. (In Russian.)

HOWARD R., MOORE A. 1980: A Complete Checklist of the Birds in the World. Oxford University Press, Oxford, 701 pp.

JOYEUX C., BAER J.G. 1936: Faune de France. 30. Cestodes. P. Lechevalier, Paris, 613 pp.

LITTLEWOOD D.T.J., OLSON P.D. 2001: Small subunit rDNA and the Platyhelminthes: signal, noise, conflict and compromise. In: D.T.J. Littlewood and R.A. Bray (Eds.), Interrelationships of the Platyhelminthes. Taylor & Francis, London, pp. 262–278.

MADDISON D.R., MADDISON W.P. 2000: MacClade 4: analysis of phylogeny and character evolution, ver. 4. Sinauer Associates, Sunderland, Massachusetts.

MILLÁN J., GORTAZAR G., CASANOVA J.C. 2003: First occurrence of *Mesocestoides* sp. in a bird, the red-legged partridge, *Alectoris rufa*, in Spain. Parasitol. Res. 90: 80–81.

MOVSESYAN S.O. (Ed.) 1987: [Cestodes of birds in Transcaucasia.] Izdatel'stvo Akademii Nauk Armyanskoi SSR, Erevan, 547 pp. (In Russian.)

NEVEU-LEMAIRE M. 1936: Traité d'Helminthologie Médicale et Vétérinaire. Vigot Frères, Paris, 421 pp.

OLSON P.D., CAIRA J.N. 1999: Evolution of the major lineages of tapeworms (Platyhelminthes: Cestoidea) inferred from 18S ribosomal DNA and *elongation factor-1α*. J. Parasitol. 85: 1134–1159.

OLSON P.D., CRIBB T.H., TKACH V.V., BRAY R.A., LITTLEWOOD D.T.J. 2003: Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int. J. Parasitol. 33: 733–755.

OSHMARIN G.P. 1963: [Parasitic worms of mammals and birds in Primor'e Region.] Izdatel'stvo Akademii Nauk SSSR, Moscow, 324 pp. (In Russian.)

PRUDHOE S., BRAY R.A. 1982: Platyhelminth Parasites of the Amphibia. British Museum (Natural History) & Oxford University Press, London & Oxford, 217 pp.

RAUSCH R.L. 1994: Family Mesocestoididae Fuhrmann, 1907. In: L.F. Khalil, A. Jones and R.A. Bray (Eds.), Keys to the Cestode Parasites of Vertebrates. CAB International, Wallingford, pp. 309–314.

SHARPILO V.P. 1976: [Parasitic worms of reptiles from the fauna of USSR.] Naukova Dumka, Kiev, 288 pp. (In Russian.)

YANCHEV Y. 1986: On the morphology, taxonomy and distribution of the species of the genus *Mesocestoides* Vailland, 1863 in Bulgaria. Khelmintologiya 21: 45–65. (In Bulgarian.)

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