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Mammomonogamus nematodes in felid carnivores: a minireview and the first molecular characterization

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Abstract

Five of the 13 known species of *Mammomonogamus* have been described in members of the family Felidae, including domestic cats, making felids the most frequent hosts of *Mammomonogamus*. The occurrence of *Mammomonogamus* in felids is geographically scattered and information on the life cycle and other aspects of infections is lacking. The paucity of data opens the questions on possible conspecificity of some of the described species of *Mammomonogamus* and on the existence of possible reservoirs for infections in domestic cats in geographically isolated endemic foci of infection. To test such hypotheses, we compared sequences of mitochondrial and nuclear markers obtained from *Mammomonogamus* adults or eggs collected from domestic cats in three geographically distant localities. Based on morphology, geographic origin and site of infection, the worms examined can be referred to as *Mammomonogamus ierei* and *Mammomonogamus auris*. Phylogenetic analyses of both mitochondrial and ribosomal DNA markers showed monophyly of the genus *Mammomonogamus* and suggested the existence of at least two species in cats. Review of the literature, the existence of several species and the discontinuous geographic distribution of *Mammomonogamus* infections in domestic cats suggest an historical spillover of infection from wild reservoirs, presumably wild felids.

Introduction

Nematodes of the genus *Mammomonogamus* (Ryzhikov, 1948), known also under the common name of gapeworms, are strongylid parasites of the respiratory system of ungulates, elephants, carnivores, non-human primates and humans mainly in tropical areas of the world (Railliet, 1899; von Linstow, 1899; Gedoelst, 1924; Mönnig, 1932; Vuylsteke, 1935; van den Berghe, 1937; Nosanchuk *et al.*, 1995; Červená *et al.*, 2017). Five of the 13 known species of *Mammomonogamus* were described from the members of the family Felidae, including domestic cats, making the felids the most frequent hosts of *Mammomonogamus* (Tables 1–2; Figs 1–2).

In fact, *Mammomonogamus dispar* (Diesing, 1857), found in a puma *Puma concolor* (Linnaeus, 1751), in Brazil was the very first syngamid observed in any mammalian host. Unfortunately, the description of the parasite is deficient, providing only the body length and body width of the nematode, along with simple, little detailed drawings (Diesing, 1857; Tables 1–2). Similarly to most of the *Mammomonogamus* species, *M. dispar* has never been reported again. The second species known from wild felids, *Mammomonogamus felis* (Cameron, 1931), was reported only twice. Interestingly, both animals were captive and imported from Thailand (Tables 1–2; Figs 1–2).

The remaining three *Mammomonogamus* species are known only from domestic cats. *Mammomonogamus ierei* (Buckley, 1934) is quite a common parasite of the nares and nasopharynx of cats on the Caribbean islands (Table 1, Fig. 2) and *Mammomonogamus auris* (Faust and Tang, 1934) parasitizes the feline middle ear, which is a quite exceptional localization for a helminth. The latter species was first described in China (Faust and Tang, 1934) and has been reported on several Asian islands over time (Table 1; Fig. 2). Finally, Seneviratne (1954) described *Mammomonogamus mcgaughei* (Seneviratne, 1954) in the pharynx and

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Table 1. Overview of *Mammomonogamus* spp. described from felid carnivores

Species	Host	Locality	Site of infection	Reference
Mammomonogamus dispar	*Puma concolor*	Brazil	Trachea	Diesing (1857)
Mammomonogamus felis	*Panthera tigris jacksoni*	Edinburgh/Thailand*	Bronchi	Cameron (1931)
	Panthera pardus	Japan/Thailand*	Bronchi	Sakamoto *et al.* (1971)
Mammomonogamus ierei	Domestic cat	Trinidad	Nares	Buckley (1934)
	Domestic cat	Jamaica	Nares	Guilbride (1953)
	Domestic cat	Puerto Rico, San Juan	Nasopharynx	Cuadrado *et al.* (1980)
	Domestic cat	St. Kitts	Eggs in feces	Gattenuo *et al.* (2014)
Mammomonogamus auris	Domestic cat	China, Foochow	Middle ear	Faust and Tang (1934)
	Domestic cat	Sri Lanka, Kandy	Middle ear	Seneviratne (1954)
	Domestic cat	Japan, Saitama	Middle ear	Sugiyama *et al.* (1982)
	Domestic cat	Japan, Okinawa	Middle ear	Asato *et al.* (1986)
	Domestic cat	Saipan	Middle ear	Tudor *et al.* (2008)
Mammomonogamus mcgaughei	Domestic cat	Sri Lanka, Kandy	Frontal sinus	Seneviratne (1954)
Mammomonogamus sp.	Domestic cat	USA, Kansas/Nigeria, Zaria*	–	Lindquist and Austin (1981)
	Prionailurus bengalensis iriomotensis	Japan, Iriomote Island	Middle ear	Hasegawa (1992)
	P. tigris	Thailand, Huai Kha	Eggs in feces	Patton and Rabinowitz (1994)
	P. pardus	Thailand	Eggs in feces	Patton and Rabinowitz (1994)
	Prionailurus bengalensis	Thailand	Eggs in feces	Patton and Rabinowitz, (1994)
	Leopardus pardalis	Not specified	Not specified	Murray and Gardner (1997)
	L. pardalis	Panama	Eggs in feces	Magnaval and Magdeleine, (2004)
	Domestic cat	St. Kitts, Basseterre	Eggs in feces and nasal flushings	Krecek *et al.* (2010)

*The locality on the left is the place, where the parasite was diagnosed and on the right, there is the country of origin.

frontal and nasal sinuses of domestic cats in Sri Lanka. In addition, the same author reported finding *M. auris* in the middle ear of four other cats, distinguishing the two species by the vulva position and size of the spicules. However, *M. mcgaughei* has not been reported ever since and its possible conspecificity with other species remains to be investigated.

Eggs of *Mammomonogamus* were detected in feces or sputum of both domestic cats and free-ranging felids in Asia, Africa, Central and South America (Table 1; Fig. 2). Unfortunately, the exact determination of *Mammomonogamus* species based solely on the egg morphology is not possible, leaving the true identity of the parasite unrevealed. Even though the species of *Mammomonogamus* from the Iriomote cats, *Prionailurus bengalensis iriomotensis* (Imaizumi, 1967), from Japan (Hasegawa, 1992) was not determined, based on the locality and site of infection one can assume that it was probably *M. auris*. Lindquist and Austin (1981) reported a domestic cat originating from Nigeria sneezing out a pair of *Mammomonogamus* nematodes after episodes of sneezing and coughing spasms; unfortunately, identification was not made to the species level. Interestingly, it is the only case of *Mammomonogamus* infection in a felid host known from the African continent.

To date, only a little is known about the transmission of *Mammomonogamus* spp. in felines. The eggs are shed in feces or in nasal discharge and sputum; larvae develop within 10 days to the third stage and emerge from the eggs. The free larvae are not capable of skin penetration; however, they seem more active when the environment temperature is raised (Buckley, 1934; Cuadrado *et al.*, 1980). Buckley (1934) attempted to induce infection in adult cats and kittens using hatched larvae of *M. ierei*, unfortunately without any success. For this reason, he suggested involvement of an intermediate or paratenic host in the life cycle. A similar strategy is known from other strongylids infecting the respiratory system of felids, such as *Aelurostrongylus abstrusus* (Railliet, 1898) or *Troglostrongylus* spp., which use molluscs as intermediate and vertebrates as paratenic hosts (Gerichter, 1949; Anderson, 2000; Bowman *et al.*, 2002).

The diagnosis of *Mammomonogamus* is usually based on the clinical signs such as nasal discharge, coughing and sneezing, loss of weight (*M. ierei*) or head shaking (*M. auris*) and subsequent observation of adults, e.g. through the tympanic membrane during otoscopic examination in case of *M. auris*, or occasionally, the adult worms are expelled by the host (Guilbride, 1953; Cuadrado *et al.*, 1980; Lindquist and Austin, 1981; Tudor *et al.*, 2008). The eggs can be detected in feces or sputum (Cuadrado *et al.*, 1980; Krecek *et al.*, 2010; Gattenuo *et al.*, 2014). Even though the genus *Mammomonogamus* is traditionally assigned to the family Syngamidae, the eggs do not have polar plugs as found in *Syngamus* spp. and their outer shell surface is finely striated. Although the eggs of *Mammomonogamus* superficially resemble those of hookworms, they can be easily distinguished from the latter based on their larger size and the thicker striated

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Table 2. Morphology of *Mammomonogamus* spp. found in felids (based on the original descriptions)

Species	*Mammomonogamus dispar*	*Mammomonogamus felis*	*Mammomonogamus ierei*	*Mammomonogamus auris*	*Mammomonogamus mcgaughei*
Length[a]					
Male	2.5–3	5.5–7	5–6.9	3.3–8.1	3–5.5
Female	10–12	17.5–26.5	20.3–23.8	14–30	9–22
Width					
Male	0.25	–	–	0.39	–
Female	0.5	–	–	0.8–1.4	–
Vulva[b]	–	6	–	–	2.1–5
Vulva ratio[c]	–	–	1/2.9–1/3.6	43–46%	23–28%
Buccal capsule	–				
Length					
Male	–		0.28	0.2	0.31
Female	–	1.1	0.25–0.4	0.362	0.32
Width					
Male	–	1	0.3	0.27	0.27
Female	–	1.3	0.4–0.55	0.580	0.40
Teeth	–	8–9	8	8	6–8
Ribs[d]	–	0 (M); 6 (F)	0	8[e]	Some
Oesophagus	–				
length					
Male	–	1.2	–	–	0.54–0.93
Female	–	1.78	1.1–1.4	–	0.76–1.76
Spicules (μM)	–	25; 30	36–47; 44–58	52	23.5; 27.3
Eggs					
Length (μM)	–	–	84–100	88	86–94
Width (μM)	–	–	48–52	48	46.8–54.6

[a]The measurements are given in millimetres if not stated differently.
[b]Distance of vulva from cranial end.
[c]Ratio of the distance of vulva from cranial end and the length of the body.
[d]See Fig. 1. M, male; F, female.
[e]Ribs were not mentioned in the original description; however, the drawing of buccal capsule shows some structures resembling the ribs (Fig. 1) and their presence was later confirmed by Sugiyama *et al.* (1982).

shell (Cuadrado *et al.*, 1980; Sugiyama *et al.*, 1982; Patton and Rabinowitz, 1994). The egg morphology within the species of *Mammomonogamus* found in felids is quite uniform, differing only slightly in size (Table 2). Also eggs of *Mammomonogamus* found in herbivores have very similar morphology differing only in the pattern of shell striations (Graber *et al.*, 1971; Červená *et al.*, 2017). Treatment of ear infections usually consists of removing the adults after tympanotomy or topical application of selamectin or a combination of thiabendazole, dexamethasone and neomycin (Tudor *et al.*, 2008). Fenbendazole was successfully used to treat *M. ierei* infection in a cat (Gattenuo *et al.*, 2014).

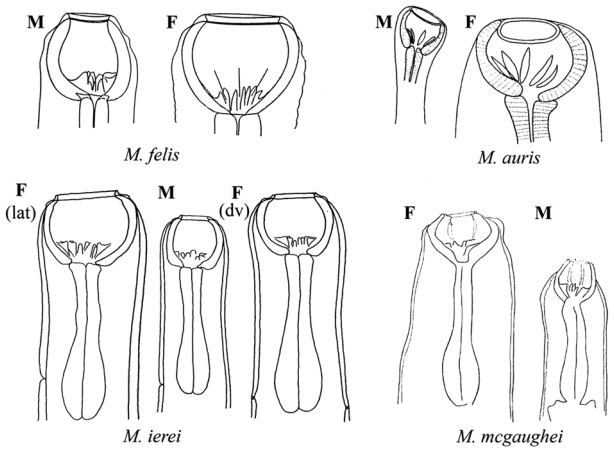

Fig. 1. Morphology of cranial extremities of four *Mammomonogamus* species known from Malay tiger (*M. felis*) and domestic cats (*M. auris*, *M. ierei*, *M. mcgaughei*). The pictures are adjusted from the original descriptions (Cameron, 1931; Buckley, 1934; Faust and Tang, 1934; Seneviratne, 1954). Note the presence/absence of the longitudinal ribs in the buccal capsule. F, female; M, male; lat, lateral view; dv, dorso-ventral view.

In domestic cats, two of the known species, namely *M. ierei* and *M. auris*, are detected on a regular basis. The differences in morphology of both species are rather minor (Table 2). Tudor *et al.* (2008) suggested the capability of *Mammomonogamus* to move between the nasal sinuses, larynx, trachea and middle ear *via* the Eustachian tube, nasopharynx and laryngopharynx, opening a question of the conspecificity of *M. ierei* and *M. auris*. To test such hypothesis, we compared the sequences of mitochondrial and nuclear markers obtained from *Mammomonogamus* adults or eggs collected from domestic cats in three geographically distant areas covering area of occurrence of both species. The resulting phylogenetic analyses showed monophyletic status of the genus *Mammomonogamus*, confirmed the validity of *M. ierei* and *M. auris* and suggested the existence of one more species infecting domestic cats.

Material and methods

Material from cats

Adults of *M. ierei* were collected from carcasses of domestic cats submitted for necropsy to Ross University School of Veterinary Medicine, Saint Kitts (West Indies). The helminths were found in the nasal cavity firmly attached to the mucosa, as is typical for the species (Fig. 3) and immediately preserved in 96% ethanol and 10% formalin. The adults of *M. auris* were collected from the

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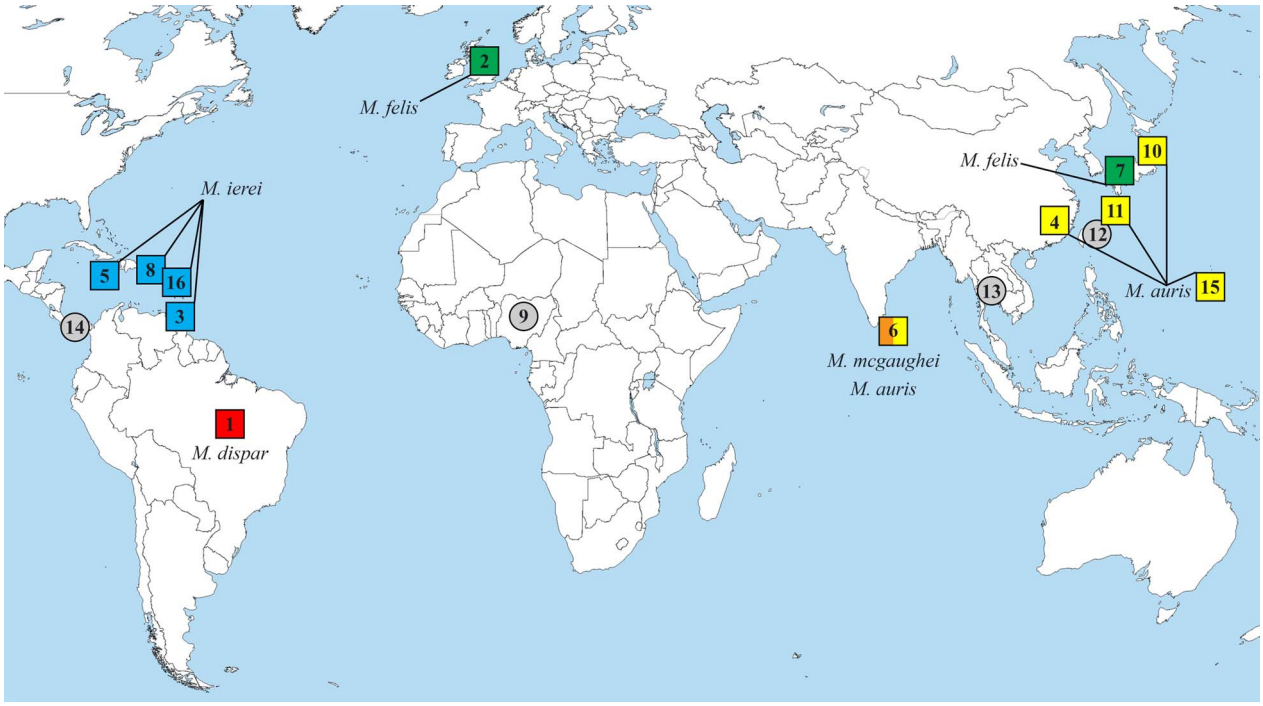

Fig. 2. Map of distribution of *Mammomonogamus* spp. in wild and domestic felids. Square is for determined helminths, the species is written in the map. Grey circles are for undetermined *Mammomonogamus*. 1 – Diesing (1857), 2 – Cameron (1931), 3 – Buckley (1934), 4 – Faust and Tang (1934), 5 – Guilbride (1953), 6 – Seneviratne (1954), 7 – Sakamoto *et al.* (1971), 8 – Cuadrado *et al.* (1980), 9 – Lindquist and Austin (1981), 10 – Sugiyama *et al.* (1982), 11 – Asato *et al.* (1986), 12 – Hasegawa (1992), 13 – Patton and Rabinowitz (1994), 14 – Magnaval and Magdeleine (2004), 15 – Tudor *et al.* (2008), 16 – Krecek *et al.* (2010); Gattenuo *et al.* (2014).

middle ear of a domestic cat referred to Paradise Island Animal Hospital, Saipan (Northern Mariana Islands). As 96% ethanol was not available in Saipan, the helminths were placed in Bacardi 151 (Bacardi Limited, Hamilton, Bermuda; 75.5% of alcohol) and moved to 96% ethanol after arrival at the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic (ca 2 weeks after their collection). Two couples of *M. ierei* and two couples of *M. auris* were examined using an Olympus AX70 microscope equipped with Nomarski interference contrast and a DP 70 digital camera. Basic morphological features were observed and measurements taken. Subsequently, the helminths were separated, washed with physiological saline and the DNA from adults and eggs was isolated using the Genomic DNA Mini Kit GT300 (Tissue) (Geneaid, New Taipei City, Taiwan). Paragenophores of *M. ierei* and cranial and caudal extremities of *M. auris* specimens were deposited in the Helminthological collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic under the numbers IPCAS N-1158 and IPCAS N-1159.

Feces of two domestic cats were collected during a sterilization campaign in Fatu Hiva (French Polynesia) and preserved in 96% ethanol. The cat feces were examined by modified Sheather's flotation and simple sedimentation. The detected eggs of *Mammomonogamus* were collected and mechanically disrupted before the DNA was isolated as described in Červená *et al.* (2017). All procedures leading to the collection of material were performed under approved Institutional Animal Care and Use Committee (IACUC) protocols.

Comparative material

To allow comparison of genetic variability and to extend the phylogenetic analyses, we used the following comparative material: (i) DNA sequences of *Mammomonogamus*, most probably *M. loxodontis* and *M. nasicola* obtained from typical eggs isolated from fecal samples of western lowland gorillas *Gorilla gorilla gorilla* (Savage and Wyman, 1847) and African forest elephants *Loxodonta cyclotis* (Matschie, 1900) and from African forest buffaloes *Syncerus caffer nanus* (Boddaert, 1785), respectively (Červená *et al.*, 2017, 2018), originating from several African localities; (ii) DNA sequences obtained from adults of *Mammomonogamus laryngeus* (Railliet, 1899) collected from a water buffalo *Bubalus bubalis* (Linnaeus, 1758) at a slaughterhouse in the municipality of Soure (Pará State, Brazil); and finally (iii) DNA sequences from two individuals of *Syngamus* spp. nematodes collected from *Turdus merula* (Linnaeus, 1758) and *Corvus corone* (Linnaeus, 1758) in the Czech Republic; and (iv) one *Cyathostoma* cf. *bronchialis* (Mühling, 1844) from a domestic goose from Slovakia. More details about this comparative material can be found in Červená *et al.* (2018) and the GenBank accession numbers are listed in the Supplementary Table S1.

DNA amplification

Primers presented in Table 3 were used to amplify partial sequences of 18S rDNA, internal transcribed spacer 1 (ITS1), 28S rDNA and gene for cytochrome c oxidase subunit I (*cox1*). Fragments of 18S rDNA, ITS, 28S rDNA and *cox1* originating from adult helminths were amplified in 25 μL PCR reactions containing 12.5 μL of PPP Master Mix (Top-Bio, Prague, Czech Republic), 0.8 μM of each primer, 7.5 μL PCR H_2O (Top-Bio, Prague, Czech Republic) and 1 μL of template DNA. PCR conditions were identical for all markers: initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C denaturation for 40 s, 50 °C annealing for 40 s, 65 °C elongation for 40 s, followed by a 5 min post-amplification extension at 72 °C. To amplify 18S rDNA and *cox1* from eggs, multiplex PCR was used. In the first round, 25 μL reaction mixtures contained 12.5 μL of Phusion® High-Fidelity PCR Master Mix HF Buffer (New England Biolabs, Ipswich, UK), 0.5 μM of each of primers NC18SF1,

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Fig. 3. Eggs and adults of examined *Mammomonogamus* spp. in domestic cats. (A) Egg of *Mammomonogamus* sp. from feces of a cat, Fatu Hiva, French Polynesia. (B) Same egg, focus on the surface, showing fine striation of the outer wall. (C) Egg of *Mammomonogamus ierei* from feces of a cat, Saint Kitts. (D) A cross-section through skull of necropsied cat from Saint Kitts with clearly visible adult of *M. ierei* emerging from the nasal cavity (encircled). (E) Two pairs of *Mammomonogamus auris* from middle ear of a cat from Saipan. (F) Two pairs of *M. ierei* from a cat from Saint Kitts. A–C in same scale, scale bar = 50 um; E–F in same scale, scale bar = 10 mm.

NC5BR, HCO2198, coxmamF (Table 3) and 3 μL of template DNA. PCR conditions were as follows: initial denaturation at 98 °C for 30 s followed by 35 cycles of 98 °C denaturation for 10 s, 55 °C annealing for 30 s and 72 °C elongation for 1 min with final post-amplification elongation for 7 min at 72 °C. One microlitre of the PCR product was then used as DNA template for the reamplification PCR round using the primers NC18SF1 and NC5BR for 18S rDNA and HCO2198, coxmamF for *cox1* separately with the same polymerase and conditions as in the first round. Annealing temperature was 54 and 58 °C for 18S

Table 3. Primers used for the amplification of nuclear and mitochondrial DNA of *Mammomonogamus* spp.

Gene	Name		Sequence 5′→3′	Product size	Reference
18S rDNA	NC18SF1	F	AAAGATTAAGCCATGCA	~1800 bp	Chilton *et al.* (2006)
	NC5BR	R	GCAGGTTCACCTACAGAT		
ITS1	NC16	F	AGTTCAATCGCAATGGCTT	~1100 bp	Chilton *et al.* (2003)
	NC2	R	TTAGTTTCTTTTCCTCCGCT		
28S rDNA	NC2R	F	AGCGGAGGAAAAGAAACTAA	~800 bp	Chilton *et al.* (2003)
	NC28R	R	GCATAGTTCACCATCTTTCGGGT		
cox1	coxmamF	F	ATTCTATTATTACAGCTCATG	~500 bp	Červená *et al.* (2017)
	HCO2198	R	TAAACTTCAGGGTGACCAAAAAATCA		Folmer *et al.* (1994)

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rDNA and *cox1*, respectively. The ITS1 and 28S rDNA were amplified in multiplex PCR as well with the NC2R, NC28R, NC16 and NC2 primers using the same protocol described above, only the annealing temperature was 60 °C in both rounds of PCR. PCR products were separated in 1.2–2% agarose, visualized using GoodView™ Nucleic Acid Stain (Ecoli s.r.o., Bratislava, Slovakia) and detected using an UV illuminator. DNA was purified directly from the PCR product or from the bands cut from the gel using Gel/PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan). Products were commercially sequenced in both directions by Macrogen (Amsterdam, The Netherlands). As the ITS1 sequences were mostly of low quality, PCR products were cloned using p-GEM®-T Easy Vector System (Promega, Madison, Wisconsin, USA), and single clones were sequenced separately. Sequences obtained in this study were submitted to GenBank under the accession numbers MF668043–MF668083 (Supplementary Table S2).

Phylogenetic analyses

DNA sequences of 18S rDNA, ITS1, 28S rDNA and *cox1* were trimmed, assembled and manually edited in BioEdit 7.2.3 (Hall, 1999). Alignment of nuclear DNA sequences was done in ClustalW implemented in BioEdit (Larkin *et al.*, 2007); the nucleotide sequences of *cox1* were aligned guided by CLUSTALW amino acid alignment implemented in Geneious (http://geneious.com; Kearse *et al.*, 2012). Pairwise sequence distances for both nucleotides and amino acids (*cox1*) were calculated using Geneious. Concatenated phylogenetic analysis of 18S and 28S sequences was carried out using Bayesian inference (BI) in MrBayes 3.2.2 (Hastings, 1970; Ronquist and Huelsenbeck, 2003). GenBank sequences of selected strongylids (Chilton *et al.*, 2006; Supplementary Table S1) were added into the alignment of 18S rDNA and 28S rDNA for comparison. BI was done in two simultaneous runs of four Metropolis-couple Monte Carlo Markov chains of one million generations sampled each 100 generations and 25% generations discarded as burn-in. The GTR + I + Γ model of sequence evolution used in BI for both nuclear and mitochondrial DNA was chosen with Modeltest 3.7 (Posada and Crandall, 1998).

Results

Morphology

The morphometry of the adult helminths is summarized in Table 4. Based on the morphology, geographical locality and site of infection, the helminths from Saint Kitts were determined as *M. ierei* and specimens from Saipan as *M. auris*. This determination corresponds well with their within-host localization. The eggs of *Mammomonogamus* were detected in one of two fecal samples originating from Fatu Hiva. Their size varied from 95–115 × 50–60 μM and their thick shell was covered in fine parallel wavy line striations (Fig. 3). There was no evident difference when compared with the eggs of *M. ierei* originating from Saint Kitts (Fig. 3).

Nuclear markers: 18S and 28S rDNA, ITS1

Partial sequence of 18S rDNA (~1217 bp) was identical in all eight adults of *M. ierei* and *M. auris*. The sequence obtained from one *Mammomonogamus* egg from Fatu Hiva differed from the others only in a single nucleotide. A fragment of ~748 bp of 28S rDNA was identical in all four individuals of *M. ierei*, while the two couples of *M. auris* differed in one nucleotide. The pairwise sequence distances among the *Mammomonogamus* spp. from cats are summarized in Table 5. The sequences originating from cats differed from the sequences from African herbivores from 6.78 to 8.51% and from *M. laryngeus* from 6.66 to 7.32%.

The ~599 bp alignment of ITS1 sequences originating from four adults of *M. auris* and four adults of *M. ierei* showed two obviously different patterns (Supplementary Fig. S3). Two areas of short tandem repeats resulting in length variability were found in both species. Firstly, the trinucleotide repeat CGT was present in 8–30 repetitions in *M. ierei* and in 10–21 repetitions in *M. auris* (Supplementary Fig. S4). Secondly, CA dinucleotide was present in 9–16 repetitions in *M. ierei*, while in *M. auris* it was only 8–9 repetitions. *Mammomonogamus auris* had one more short area of 5–6 CA repeats, which was not present in *M. ierei*.

The tree resulting from BI of concatenated alignment of 2035 bp of 18S rDNA and 28SrDNA showed a monophyletic clade comprising all *Mammomonogamus* sequences. The sequences of *Mammomonogamus* spp. formed three distinct subclades in cats based on the geographic origin and host species (Fig. 4). The remaining syngamids, represented by *Syngamus* and *Cyathostoma* sequences, cluster in another, well-supported clade rather distant from that of *Mammomonogamus* spp.

Mitochondrial marker: cox1

The ~420 bp fragment of *cox1* was obtained from all eight adults of *M. ierei* and *M. auris* and from one egg of *Mammomonogamus* sp. from Fatu Hiva. The pairwise nucleotide sequence distances are summarized in Table 5B. While all individuals of *M. auris* had an identical *cox1* sequence, the two couples of *M. ierei* differed in six nucleotides. The distance between the feline *Mammomonogamus* spp. and *Mammomonogamus* from African herbivores varied from 15.71 to 22.43%, and when compared with the sequence of *M. laryngeus*, the distance ranged from 24.52 to 24.76%. The pairwise amino acid sequence distance within the *Mammomonogamus* spp. from domestic cats is summarized in Table 5C.

The tree resulting from BI of 420 bp of *cox1* of *Mammomonogamus* and *Necator americanus*, used as an outgroup, showed three distinct branches within the *Mammomonogamus* spp. originating from cats based on the geographic localities (Fig. 5).

Discussion

Since the middle of the 19th century, five species of *Mammomonogamus*-infecting felid carnivores have been described. The two of these nominal taxa, namely *M. dispar* and *M. felis*, were recorded from free-ranging felids and have not been recorded since (Diesing, 1857; Cameron, 1931). The three remaining species, *M. mcgaughei*, *M. ierei* and *M. auris*, are known as parasites of domestic cats; the latter two species are locally detected on a regular basis (Buckley, 1934; Faust and Tang, 1934; Seneviratne, 1954; Cuadrado *et al.*, 1980; Sugiyama *et al.*, 1982; Tudor *et al.*, 2008; Gattenuo *et al.*, 2014). Reports of unidentified *Mammomonogamus* sp. from wild felids in the areas of occurrence of known *Mammomonogamus* species, especially *M. ierei* and *M. auris*, raise questions about their possible conspecificity and the role of wild felids as natural reservoirs of *Mammomonogamus* (Hasegawa, 1992; Patton and Rabinowitz, 1994; Magnaval and Magdeleine, 2004).

While the reports of *Mammomonogamus* in free-ranging felids are restricted to the mainland of South America and Asia, including one Japanese island (Diesing, 1857; Hasegawa, 1992; Patton and Rabinowitz, 1994; Magnaval and Magdeleine, 2004), domestic cats are known to host *Mammomonogamus* spp. on numerous

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Table 4. Morphometry of adults *Mammomonogamus ierei* and *Mammomonogamus auris* available for our study

Species	*M. ierei*		*M. auris*	
Locality	Saint Kitts		Saipan	
Host	Domestic cat		Domestic cat	
Site of infection	Nasal cavity		Middle ear	
	Couple 1	Couple 2	Couple 1	Couple 2
Body length[a]				
Male	3.7	3.0	4.9	5.3
Female	10.8	13.8	25.1	22.0
Body width				
Male	0.42	0.42	0.44	0.49
Female	0.62	0.58	1.2	1.0
Vulva[b]	2.5	4.7	7.7	8.3
Vulva ratio[c]	0.23	0.34	0.31	0.38
Buccal capsule				
Length				
Male	0.20	0.18	0.21	0.18
Female	0.25	0.23	0.35	–
Width				
Male	0.22	0.21	0.23	0.29
Female	0.40	0.40	0.49	–
Oesophagus length				
Male	0.59	0.60	0.79	0.84
Female	0.61	0.83	–	–
Spicules (μm)[d]	–	–	35; 40	30; 37.5

[a]The measurements are given in millimetres if not stated differently.
[b]Distance of vulva from cranial end.
[c]Ratio of the distance of the vulva from cranial end and the body length.
[d]The spicules were not observed in *M. ierei*.

islands in the Caribbean, Southeast Asia and Pacific Ocean islands, with a single case known from continental China (Faust and Tang, 1934; Guilbride, 1953; Seneviratne, 1954; Cuadrado *et al.*, 1980; Sugiyama *et al.*, 1982; Asato *et al.*, 1986; Tudor *et al.*, 2008; Krecek *et al.*, 2010; Gattenuo *et al.*, 2014). It appears that *M. auris* is restricted to the Far East, while *M. ierei* occurs only in the Caribbean region, suggesting the speciation of *M. ierei* after the migration of felids to the Americas (O'Brian and Johnson, 2007). We examined the material collected from domestic cats in two localities, where *Mammomonogamus* typically occurs and, in addition, we found a single cat infected with *Mammomonogamus* in a totally new geographic area. In fact, the finding of *Mammomonogamus* in Fatu Hiva, Marquesas Islands in the French Polynesia, represents the first record of *Mammomonogamus* in domestic cat from the Southern Hemisphere.

Table 5. Pairwise sequence distances of 28S rDNA (A), nucleotide (B) and amino acid sequence (C) of *cox1* for *Mammomonogamus ierei* (Saint Kitts), *Mammomonogamus auris* (Saipan) and *Mammomonogamus* sp. (Fatu Hiva) found in cats

A. 28S rDNA	*M. ierei*	*M. auris*
M. ierei	0	
M. auris	3.07%	0.13%
Mammomonogamus sp.	1.2%	2.67%
B. *cox1* – nucleotide	*M. ierei*	*M. auris*
M. ierei	1.43%	
M. auris	11.90%	0
Mammomonogamus sp.	12.62%	12.14%
C. *cox1* – amino acid	*M. ierei*	*M. auris*
M. ierei	0	
M. auris	5%	0
Mammomonogamus sp.	4.29%	5%

Until now, the taxonomy of the genus *Mammomonogamus* at the specific and higher level has been assessed based only on the morphology. Traditionally, this genus is referred to as a member of the family Syngamidae, due to similar, rather basic, morphological features, site of infection and the state of permanent copulation (Ryzhikov, 1949; Baruš and Tenora, 1972). That was also the reason why we included comparative sequences from *Cyathostoma* and *Syngamus* spp. in our dataset. Our study is the first analysing the DNA sequences of *Mammomonogamus* spp. infecting domestic cats and shows clear monophyly of the genus, involving also the sequences from African herbivores and *M. laryngeus* from water buffalo. Surprisingly, the syngamids infecting birds formed another clade rather distant from *Mammomonogamus*. The tree topology together with differences in egg morphology suggest that the biological and morphological traits used for the definition of Syngamidae are not true synapomorphies and the current grouping of syngamids seems to be rather artificial. Under such a scenario, permanent copulation and affinity to the respiratory tract, including the auditory system, could have evolved independently among strongylids parasitizing avian and mammalian hosts. Poly- or paraphyletic characters are common also in other families within the order Strongylida, as presented by Chilton *et al.* (2006). This issue and its consequences to the taxonomy of the group should be addressed in the future.

Topology of the phylogenetic trees resulting from the analyses of nuclear and mitochondrial DNA confirm *M. ierei* and *M. auris* as two distinct species (Figs 4–5). The identity of

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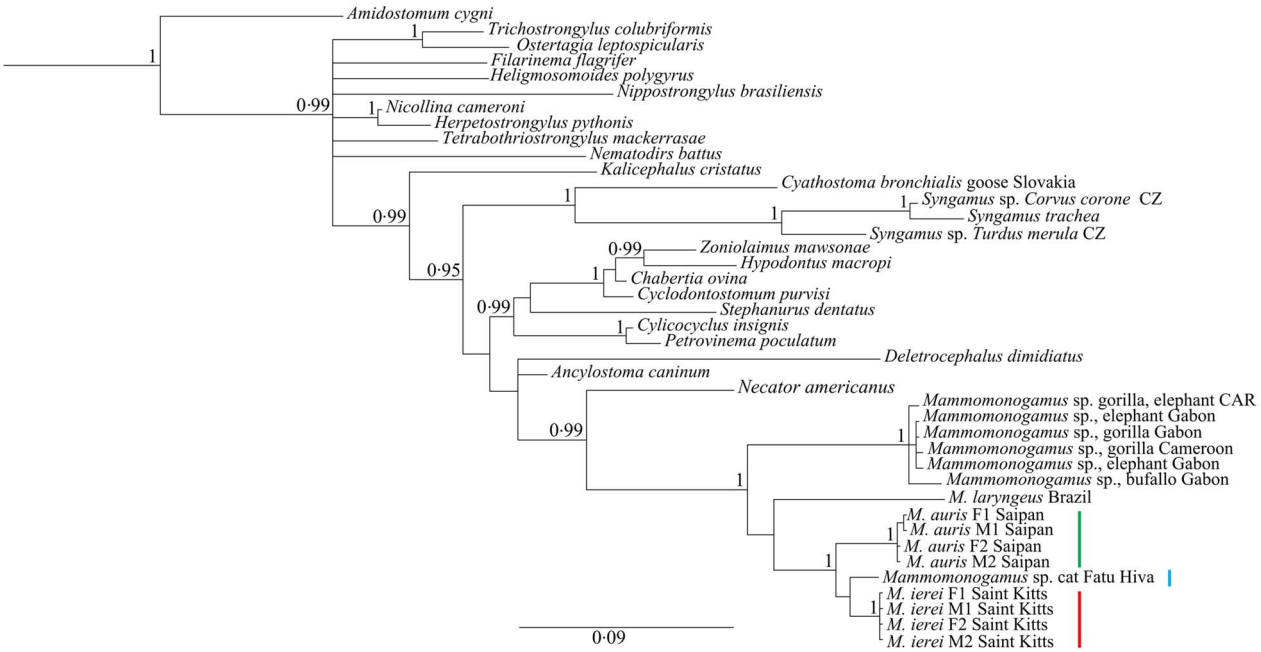

Fig. 4. Phylogenetic tree based on Bayesian inference of concatenated 18S rDNA and 28S rDNA sequences of *Mammomonogamus* spp. originating from domestic cats (*M. ierei*, *M. auris*, *Mammomonogamus* sp. from Fatu Hiva), water buffalo (*M. laryngeus*) and African herbivores, syngamids from birds and other strongylids. Sequences of *Metastrongylus elongatus*, *Protostrogylus rufescens* and *Aelurostrongylus abstrusus* used as an outgroup are not displayed. Node-supporting values refer to BI posterior probability (values above 0.9 displayed only). Red line marks the samples from Saint Kitts, blue line the samples from Fatu Hiva and green line is for the samples from Saipan. CZ, Czech Republic; CAR, Central African Republic; F, female; M, male.

the *Mammomonogamus* isolate from Fatu Hiva, French Polynesia remains questionable. While the *cox1* sequence does cluster with *M. ierei*, the nuclear DNA sequence clusters with *M. auris* but the node is not strongly supported in either case. The pairwise sequence distances comparison of the Fatu Hiva haplotype to the other two feline *Mammomonogamus* species suggests its distinctiveness at species level, as the values are similar to those for interspecific comparison between *M. ierei* and *M. auris* (Table 5) and between African *Mammomonogamus* spp. (Červená *et al.*, 2018).

The origin of *Mammomonogamus* infections in domestic cats represents intriguing question. The areas of its typical occurrence in the Far East and Caribbean region are far away from the domestication centres of the domestic cat in the Near East (Hu *et al.*, 2014; Vigne *et al.*, 2016) and these parasites can hardly be classified as heirloom parasites of the *Felis silvestris* lineage leading to the modern domestic cat. Therefore, the spillover from a wildlife reservoir, presumably felid carnivores, represents a plausible explanation. The existence of two geographically well

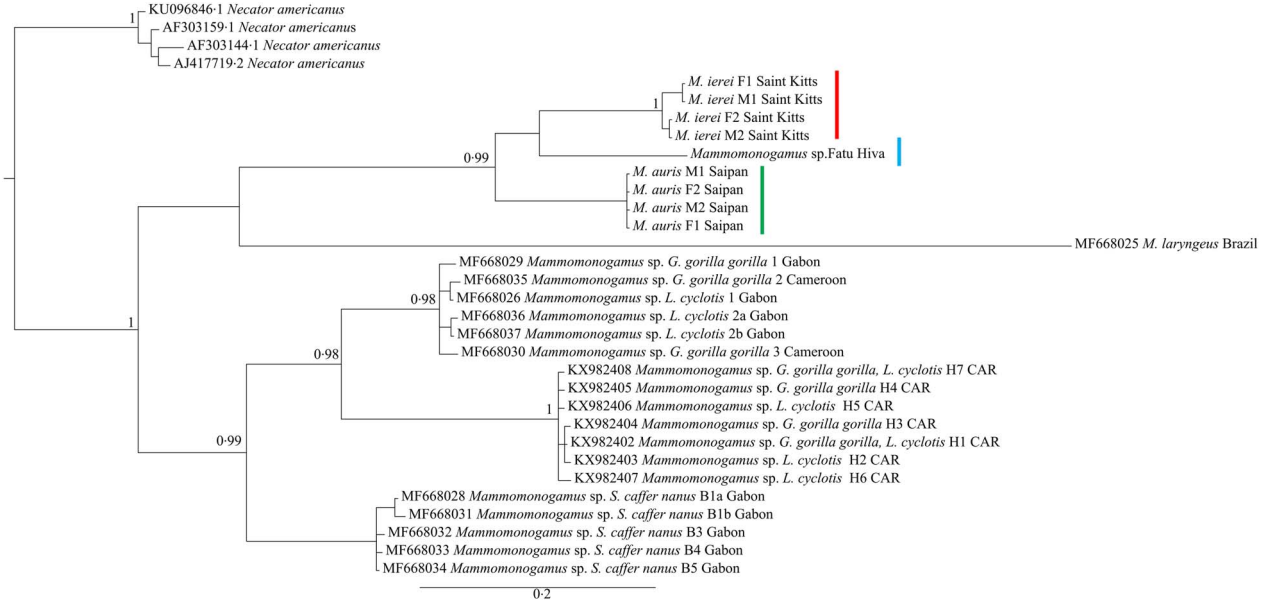

Fig. 5. Phylogenetic tree resulting from Bayesian inference of 420 bp of *cox1* gene sequences of *Mammomonogamus* spp. originating from domestic cats (*M. ierei*, *M. auris*, *Mammomonogamus* sp. from Fatu Hiva), water buffalo (*M. laryngeus*) and African herbivores. Based on the topology of the tree resulting from the analysis of nuclear DNA, *Necator americanus* was used as an outgroup. Node-supporting values refer to BI posterior probability (values above 0.9 displayed only). Red line marks the samples from Saint Kitts, blue line the samples from Fatu Hiva and green line is for the samples from Saipan. F, female; M, male; CAR, Central African Republic.

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separated *Mammomonogamus* species in domestic cats strongly suggests two centres of 'domestication' of *Mammomonogamus* nematodes in cats. Importantly, in both geographic areas, wild felid species are known to host *Mammomonogamus* nematodes, thus representing potential candidates for reservoir hosts. *Mammomonogamus ierei* was first described in a domestic cat from the island of Trinidad, where the ocelots *Leopardus pardalis* (Linnaeus, 1758), known hosts of *Mammomonogamus*, are present (Murray and Gardner, 1997; Magnaval and Magdeleine, 2004). Similarly, *Mammomonogamus* infection is reported from leopard cats *P. bengalensis* in the Far East (Hasegawa, 1992; Patton and Rabinowitz, 1994). Interestingly enough, both these wild cat species were historically commonly kept as pets in the areas of their occurrence, which facilitates contact with domestic cats. On the contrary, the *Mammomonogamus* infections nowadays typically occur in the populations of domestic cats living in island environments where the wild felid species are absent. Spread of the infection over the islands can be explained by the movement of domestic cats in recent times. Such a scenario suggests either a direct life cycle or the presence of rather unspecific intermediate and/or paratenic hosts. The saurian reptiles (geckoes, skinks and anoles) are among the most abundant terrestrial vertebrates in the areas of occurrence of *Mammomonogamus* in cats and are invariably hunted by cats during outdoor activity. The involvement of these hosts in the life cycle deserves more attention, especially because the mode of transmission is not known yet.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182018000768

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Ethical standards. All procedures leading to the collection of material were performed under approved Institutional Animal Care and Use Committee (IACUC) protocols.

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