

## Invited article

## Molecular identification of *Taenia hydatigena* and *Mesocestoides* species based on copro-DNA analysis of wild carnivores in Mongolia

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## ARTICLE INFO

## Keywords:

*Taenia hydatigena*  
*Mesocestoides* sp.  
*cox1* gene  
12S rRNA gene  
Wild carnivores  
Mongolia

## ABSTRACT

Cyclophyllidean tapeworms obligatorily parasitize numerous mammalian species, including herbivores, domestic animals and humans, of which, the genera *Taenia* and *Mesocestoides* are well characterized. However, little is known about these parasitic infections in wild animals. This study aims to investigate the prevalence and distribution of *Taenia* sp. and *Mesocestoides* sp. in wild carnivores in Mongolia by identifying tapeworm species based on mtDNA analysis. The field survey was carried out in 2012–2013 in 19 provinces located in different ecological regions. A total of 405 fecal samples from wild carnivores were collected. Specific DNA markers in fecal samples was detected via copro-DNA analysis and tapeworm species were identified by DNA sequencing. From 27.7% (112/405) of samples, *cox1* and 12S rRNA genes of tapeworms were amplified. Further, *Taenia hydatigena* (50.0%, 56/112) and two *Mesocestoides* species, including *Mesocestoides* sp.-1 (36.6%, 41/112) and *Mesocestoides* sp.-2 (13.4%, 15/112) were identified by DNA sequencing. The prevalence of *T. hydatigena* was 19.9% (27/136), 13.8% (23/167), 4.8% (3/62), and 7.5% (3/40) in wolves, red foxes, corsac foxes, and snow leopards, respectively. The prevalence of *Mesocestoides* sp.-1 was 14.7% (20/136), 9% (15/167), 9.7% (6/62) in wolves, red foxes, and corsac foxes, while the prevalence of *Mesocestoides* sp.-2 was 4.4% (6/136), 1.8% (3/167), 3.2% (2/62), and 10.0% (4/40) in wolves, red foxes, corsac foxes, and snow leopards, respectively. *T. hydatigena* was found throughout all ecological regions, while *Mesocestoides* sp.-1 was in the mountain taiga, forest-steppe, steppe, desert-steppe, and desert, and *Mesocestoides* sp.-2 in the alpine, forest-steppe, steppe, and desert-steppe ecoregions. This study revealed the prevalence and distribution of cyclophyllidean tapeworms in wild carnivores in Mongolia; while also confirming that wolves, red foxes, corsac foxes, and snow leopards serve as definitive hosts for unidentified *Mesocestoides* species.

## 1. Introduction

Mongolia is a land locked country located in Central and East Asia bordering with the Russian Federation in the north and the People's Republic of China in the west, south, and east, and consists of 21 provinces. Great diversities characterize the geography of the country. Currently, from north to south, it can be divided into four regions (Western, Khangai, Central, and Eastern), and six ecological regions: alpine, mountain taiga, forest-steppe, steppe, desert-steppe, and desert

(Table 2 and 3, Fig. 1). The total population of Mongolia is 3.238.479 (NSO, 2019); of which nearly 40% of the rural population is nomadic or semi-nomadic herdsmen (Myadagsuren et al., 2007).

Tapeworms of the genus *Taenia* include over 100 species (Gonzalez et al, 2018) that affect dogs, cats, goats, sheep, cattle, pigs, and other livestock, domestic and wild animals as well as humans. The lifecycle of the *Taenia* species relies on a vertebrate intermediate host in which the infective larvae develop, as well as on a definitive host that ingests the uncooked flesh of the intermediate host (Gonzalez et al, 2018). *Taenia*

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**Table 1**  
Number of PCR amplified in 405 fecal samples and prevalence of *T. hydatigena* and *Mesocestoides* sp. infections by wild carnivores in Mongolia.

PCR amplified/Infecting parasite	Number and prevalence (%)			
	Wild carnivore			
	Wolf (n = 136)	Red fox (n = 167)	Corsac fox (n = 62)	Snow leopard (n = 40)
PCR amplified	53/112 (47.3)	41/112 (36.6)	11/112 (9.8)	7/112 (6.3)
Infecting parasite				
<i>T. hydatigena</i>	27/136 (19.9)	23/167 (13.8)	3/62 (4.8)	3/40 (7.5)
<i>Mesocestoides</i> sp.-1	20/136 (14.7)	15/167 (9.0)	6/62 (9.7)	0/40 (0.0)
<i>Mesocestoides</i> sp.-2	6/136 (4.4)	3/167 (1.8)	2/62 (3.2)	4/40 (10.0)

**Table 2**  
Prevalence of *T. hydatigena* and *Mesocestoides* sp. by region and province in Mongolia.

Region	Province	Number and prevalence (%)		
		<i>T. hydatigena</i>	<i>Mesocestoides</i> sp.-1	<i>Mesocestoides</i> sp.-2
Western	Bayan-Ulgii	2/16 (12.5)	0/16 (0.0)	2/16 (12.5)
	Govi-Altai	0/16 (0.0)	1/16 (6.3)	2/16 (12.5)
	Zavkhan	3/19 (15.8)	6/19 (31.6)	3/19 (15.8)
	Uvs	4/21 (19.0)	2/21 (9.5)	1/21 (4.8)
	Khovd	2/16 (12.5)	0/16 (0.0)	2/16 (12.5)
Khangai	Arkhangai	3/26 (11.5)	2/26 (7.7)	0/26 (0.0)
	Bayankhongor	2/21 (9.5)	2/21 (9.5)	1/21 (4.8)
	Bulgan	5/23 (21.7)	5/23 (21.7)	0/23 (0.0)
	Uvurkhangai	3/14 (21.4)	2/14 (14.3)	1/14 (7.1)
	Khuvsgul	0/8 (0.0)	1/8 (12.5)	0/8 (0.0)
	Orkhon-Uul	5/28 (17.9)	1/28 (3.6)	0/28 (0.0)
Central	Gobi-Sumber			
	Dornogobi	2/49 (4.1)	2/49 (4.1)	1/49 (2.0)
	Dundgobi	5/28 (17.9)	3/28 (10.7)	0/28 (0.0)
	Umnugobi	2/25 (8.0)	2/25 (8.0)	2/25 (8.0)
	Selenge	2/23 (8.7)	4/23 (17.4)	0/23 (0.0)
	Tuv	7/25 (28.0)	1/25 (4.0)	0/25 (0.0)
	Darkhan-Uul	3/12 (25.0)	2/12 (16.7)	0/12 (0.0)
Eastern	Dornod			
	Sukhbaatar	4/22 (18.2)	4/22 (18.2)	0/22 (0.0)
	Khentii	2/13 (15.4)	1/13 (7.7)	0/13 (0.0)

sp. is distributed worldwide, whereby abundance and incidence in different regions depend on each particular species (Rostami et al., 2015; Poglayen et al., 2017; Kubečka et al., 2018).

*T. hydatigena* is an omnipresent tapeworm found in domestic animals worldwide (Nguyen et al., 2016; Miran, 2017), exerting a significant constraint on the development of the livestock industry in developing countries. Furthermore, in Mongolia it has been reported that carnivores such as gray wolves, red fox, corsac fox, and dogs are the definitive hosts for *T. hydatigena* (Dubinin and Dubinina, 1951; Danzan, 1978; Tinnin et al., 2002); whereas, the metacestodes found in sheep, goat, cattle, argali, ibex deer, and roe deer, serve as intermediate hosts (Danzan, 1978; Sharkhuu, 2001; Sharkhuu and Sharkhuu, 2004). These intermediate hosts may become infected by environments that are contaminated by infected wild carnivores or dogs, primarily wolves, which become infected following consumption of infected wild and domestic livestock. In fact, within dogs in Mongolia, the prevalence of *T. hydatigena* was reported as 61.3% (Danzan, 1978). Intermediate hosts containing *T. hydatigena* cysticerci in the migratory phase, present with hemorrhaging in the liver parenchyma and beneath the liver surface (Blazek et al., 1985). Moreover, cysticerci are permitted to develop in hosts when immature immune systems are incapable of defeating the parasite. Mass infection of *T. hydatigena* cysticerci may cause the death of infected animals during the migration of cysticerci (Scala et al., 2016; Sgroi et al., 2019).

*T. hydatigena* accounts for one of the most prevalent tapeworm infections in intermediate hosts, causing significant negative impacts to the health of infected animals (Getaw et al., 2010; Dumitri et al., 2011; Oryan et al., 2012; Debas and Ibrahim, 2013), and significantly impacting the livestock industry in developing countries such as Mongolia (Jenkins et al., 2014; Nguyen et al., 2016; Miran, 2017). Specifically, *T. hydatigena* infected livestock can result in meat condemnation (Oryan et al., 2012; Debas and Ibrahim, 2013; Rashid et al., 2019). Since animal husbandry accounts for approximately 20–30% of Mongolia's GDP (MoFALI, 2018), tapeworm infections represent a significant economic burden on herders who practice a nomadic lifestyle. However, *T. hydatigena* is not only detrimental to developing countries, but has also been reported to cause significant economic losses, reaching 330,000 euros, in developed nations (Scala et al., 2015).

*Mesocestoides* sp. exhibit unique characteristics compared to other groups of cyclophyllideans (Cho et al., 2013). For instance, the life cycle of *Mesocestoides* is complex, requiring two or three intermediate hosts to complete its development (Zalesny and Hildebrand, 2012; McAllister et al., 2013; Poglayen et al., 2017). The oncosphere *Mesocestoides* develops into a second-stage larva in the first intermediate host (arthropod); while in the second intermediate host, such as small mammals (Loos-Frank, 1991) and reptiles, the larva develops into third-stage (tetrathyridium). Among the *Mesocestoides* isolates, *M. litteratus* and *M. lineatus* were commonly isolated from red foxes, dogs, coyotes

**Table 3**  
Prevalence of *T. hydatigena* and *Mesocestoides* sp. infections in wild carnivores by ecoregions in Mongolia.

Ecoregion/ Wild carnivore	No. of fecal sample	Number and prevalence (%)		
		<i>T. hydatigena</i>	<i>Mesocestoides</i> sp.-1	<i>Mesocestoides</i> sp.-2
<b>Alpine (n = 38)</b>				
Wolf	10	1/10 (10.0)	0/10 (0.0)	0/10 (0.0)
Snow leopard	28	3/28 (10.7)	0/28 (0.0)	4/28 (14.3)
<b>Mountain taiga (n = 26)</b>				
Wolf	14	2/14 (14.3)	3/14 (21.4)	0/14 (0.0)
Snow leopard	12	0/12 (0.0)	0/12 (0.0)	0/12 (0.0)
<b>Forest-steppe (n = 113)</b>				
Wolf	41	10/41 (24.4)	7/41 (17.1)	0/41 (0.0)
Red fox	49	7/49 (14.3)	3/49 (6.1)	1/49 (2.0)
Corsac fox	23	0/23 (0.0)	2/23 (8.7)	0/23 (0.0)
<b>Steppe (n = 89)</b>				
Wolf	40	8/40 (20.0)	2/40 (5.0)	6/40 (15.0)
Red fox	36	5/36 (13.9)	5/36 (13.9)	2/36 (5.6)
Corsac fox	13	0/13 (0.0)	4/13 (30.8)	0/13 (0.0)
<b>Desert-steppe (n = 101)</b>				
Wolf	23	5/23 (21.7)	6/23 (26.1)	0/23 (0.0)
Red fox	59	11/59 (18.6)	0/59 (0.0)	0/59 (0.0)
Corsac fox	19	1/19 (5.3)	0/19 (0.0)	2/19 (10.5)
<b>Desert (n = 38)</b>				
Wolf	8	1/8 (12.5)	2/8 (25.0)	0/8 (0.0)
Red fox	23	0/23 (0.0)	7/23 (30.4)	0/23 (0.0)
Corsac fox	7	2/7 (28.6)	0/7 (0.0)	0/7 (0.0)

and wolves, which served as definitive hosts (Hrčková et al., 2011; Zalesny and Hildebrand, 2012). Alternatively, in Mongolia domestic or wild animals such as sheep, goats, cattle, argali, ibex deer, and roe deer serve as the second intermediate hosts (Danžan, 1978; Sharhuu and

Sharhuu, 2004). Although there are few studies regarding the identity of definitive hosts in Mongolia, it has been reported that definitive host becomes infected after eating meat contaminated with tetrathyridia (CDC, 2017). Once infected, the intermediate host may experience hemorrhagia within the liver (Blazek et al., 1985), while the definitive hosts have their small intestines colonized by *Mesocestoides* sp., which can prove to be very dangerous (Jabbar et al., 2012) for rare species such as the snow leopard.

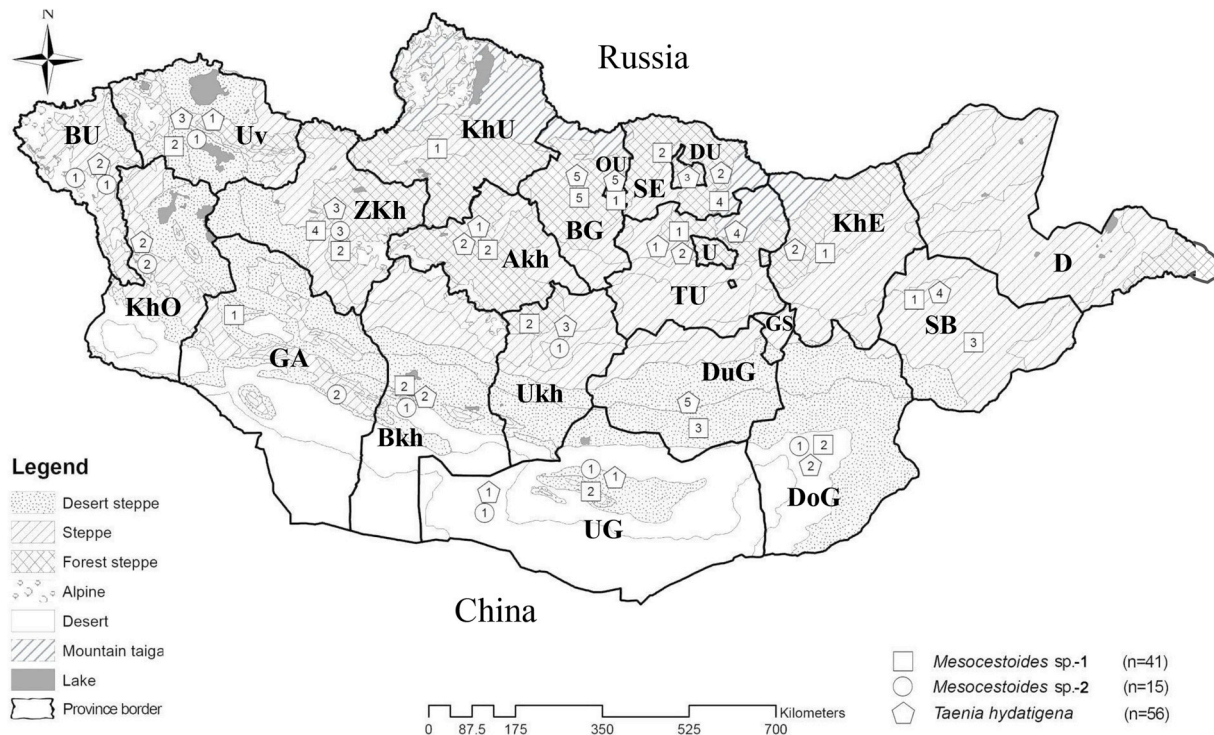
Cestodes represent a significant risk to the health of both humans and their livestock. However, little is known about these tapeworm infections in wild animals. Within Mongolia, soil-transmitted cestode infections are increasing in prevalence, making the need for accurate molecular characterization, and mapping of the geographic distribution of the parasites, of utmost importance if efficient prevention and control options are to be designed (Ebright et al., 2003; McFadden et al., 2016).

This study aims to investigate the prevalence and distribution of *Taenia* sp. and *Mesocestoides* sp. in wild carnivores in Mongolia by identifying tapeworm species based on copro-DNA analysis.

## 2. Materials and methods

### 2.1. Collection of canid and felid fecal samples

A total of 405 fecal samples were randomly collected from 167, 136, 62, and 40 wolves, red foxes, corsac foxes, and snow leopards, respectively, from 19 of the 21 Mongolian provinces, save for Dornod and Gobi-Sumber, representing all ecological regions of Mongolia (Fig. 1, Table 1). Each fecal sample weighed 18 g. The feces were collected using standard techniques (Lawrence and Brown, 1967; Strachan et al., 1996). The species for each feces sample was visually identified based on color, shape, location, pugmarks, scrapes, and the nearby remains of



**Fig. 1.** Map of Mongolia showing the distribution of *Taenia hydatigena* (pentangle), *Mesocestoides* sp.-1 (square), and *Mesocestoides* sp.-2 (circle) by province detected by molecular identification of fecal samples from wild carnivores. Mongolia consists of 21 provinces: Arkhangai (Akh), Bayankhongor (Bkh), Bayan-Ulgii (BU), Bulgan (BG), Darkhan-Uul (DU), Dornogobi (DoG), Dundgobi (DuG), Govi-Altai (GA), Khentii (KhE), Khovd (KhO), Khuvsgul (KhU), Orkhon-Uul (OU), Selenge (SE), Sukhbaatar (SB), Tuv (TU), Umnu-Gobi (UG), Uvs (Uv), Uvurkhangai (Ukh), Zavkhangai (ZKh), Dornod (D), and Gobi-Sumber (GS). The field survey was conducted in all provinces, unless Dornod (D), and GS (Gobi-Sumber). Ulaanbaatar (U) is the capital city of Mongolia located in Tuv Province. The field survey was conducted in all provinces, unless Dornod (D), and GS (Gobi-Sumber).

**Table 4**  
Pairwise genetic distance in *cox1* gene among *Mesocostoides* isolates from Mongolia and related taxa.

Mesocostoides isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1 <i>Mesocostoides</i> sp.-1 AB792715 (red fox) Mongolia	0.005																							
2 <i>Mesocostoides</i> sp.-1 AB792713 (wolf) Mongolia	0.003	0.003																						
3 <i>Mesocostoides</i> sp.-1 AB792716 (corsac fox) Mongolia	0.160	0.163	0.160																					
4 <i>Mesocostoides</i> sp.-2 AB792718 (red fox) Mongolia	0.160	0.164	0.160	0.013																				
5 <i>Mesocostoides</i> sp.-2 AB792720 (wolf) Mongolia	0.192	0.196	0.192	0.174	0.165																			
6 <i>Mesocostoides</i> sp.-2 AB792719 (snow leopard) Mongolia	0.125	0.133	0.129	0.166	0.170	0.171																		
7 <i>Mesocostoides</i> sp. KP941432 (dog) Germany	0.121	0.129	0.125	0.162	0.166	0.167	0.003																	
8 <i>Mesocostoides</i> sp. KY434329 (wolf) Germany	0.137	0.145	0.141	0.180	0.174	0.181	0.037	0.034																
9 <i>Mesocostoides</i> sp. JQ740884 (cat) Italy	0.125	0.132	0.129	0.203	0.189	0.229	0.155	0.151	0.170															
10 <i>M. lineatus</i> JF268501 (red fox) Slovakia	0.131	0.139	0.135	0.211	0.196	0.242	0.162	0.158	0.177	0.008														
11 <i>M. lineatus</i> JF268500 (red fox) Slovakia	0.164	0.172	0.168	0.156	0.164	0.157	0.154	0.153	0.153	0.166	0.178													
12 <i>M. lineatus</i> KX962362 (wolf) Germany	0.164	0.172	0.168	0.156	0.156	0.164	0.157	0.154	0.153	0.166	0.178	0.000												
13 <i>M. lineatus</i> KX962361 (wolf) Germany	0.164	0.172	0.168	0.156	0.156	0.164	0.157	0.154	0.153	0.166	0.178	0.000	0.000											
14 <i>M. lineatus</i> KX962367 (wolf) Germany	0.173	0.181	0.176	0.161	0.161	0.162	0.157	0.154	0.153	0.175	0.187	0.022	0.022	0.022										
15 <i>M. lineatus</i> JF268514 (red fox) Slovakia	0.172	0.180	0.176	0.160	0.160	0.161	0.154	0.150	0.149	0.175	0.187	0.019	0.019	0.019	0.003									
16 <i>M. lineatus</i> JF268513 (red fox) Slovakia	0.049	0.056	0.053	0.172	0.172	0.172	0.183	0.114	0.110	0.125	0.131	0.142	0.155	0.155	0.155	0.167	0.163							
17 <i>M. canislagopodis</i> KT232149 (gyrfalcon) Iceland	0.049	0.056	0.053	0.172	0.172	0.172	0.183	0.114	0.110	0.125	0.131	0.142	0.155	0.155	0.155	0.167	0.163	0.005						
18 <i>M. canislagopodis</i> KT232147 (gyrfalcon) Iceland	0.049	0.056	0.053	0.172	0.172	0.172	0.183	0.114	0.110	0.125	0.131	0.142	0.155	0.155	0.155	0.167	0.163	0.005	0.000					
19 <i>M. canislagopodis</i> KT232145 (rock ptarmigan) Iceland	0.049	0.056	0.053	0.172	0.172	0.172	0.183	0.114	0.110	0.125	0.131	0.142	0.155	0.155	0.155	0.167	0.163	0.005	0.005	0.005				
20 <i>M. canislagopodis</i> KT232144 (arctic fox) Iceland	0.156	0.164	0.161	0.195	0.185	0.188	0.126	0.123	0.133	0.168	0.175	0.150	0.150	0.150	0.149	0.145	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162
21 <i>M. corit</i> AP017667	0.157	0.165	0.161	0.194	0.194	0.197	0.125	0.122	0.142	0.169	0.176	0.158	0.158	0.158	0.158	0.154	0.170	0.170	0.170	0.170	0.170	0.170	0.170	0.170
22 <i>M. corit</i> AB033413	0.156	0.164	0.161	0.195	0.185	0.188	0.126	0.123	0.133	0.168	0.175	0.150	0.150	0.150	0.149	0.145	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162
23 <i>M. corit</i> LMS33943	0.156	0.164	0.161	0.195	0.185	0.188	0.126	0.123	0.133	0.168	0.175	0.150	0.150	0.150	0.149	0.145	0.162	0.162	0.162	0.162	0.162	0.162	0.162	0.162

prey species, by biologists, ecologists and local hunters (Oli et al., 1993; Bachi and Mishra, 2006). Each sample was stored in individually labeled zip-lock plastic bags to prevent contamination, and were stored at -80 °C until processing.

2.2. DNA analysis

The copro-DNA samples were extracted from frozen feces using the QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The cytochrome c oxidase subunit 1 (*cox1*) gene and 12S rRNA was amplified by polymerase chain reaction (PCR) using previously published primers (Bowles and McManus, 1993; von Nickisch-Roseneck et al., 1999). Amplicons were sequenced in both directions on a 3100-Advant Genetic Analyzer (ABI PRISM, Applied Biosystems, Hitachi, Japan).

2.3. Phylogenetic analysis

Sequence data were assembled and edited using BioEdit (version 7.2.5) and were compared to the reference sequences in GenBank® by using BLAST. Phylogenetic trees were constructed based on the partial nucleotide sequences of *cox1* and 12S rRNA, together with reference sequences available in GenBank and using the MEGA software, version 7.0 (<http://www.megasoftware.net/mega7>). The evolutionary distances were computed using the maximum likelihood estimation method (HKY + G + I substitution model) and neighbor joining (TN93 + G substitution model) was presented as the number of base substitutions per site (Kumar et al., 2016).

2.4. Data analysis

Infection prevalence was determined for the study years. A comparison of infection prevalence was conducted using the Pearson's chi-squared test. All analyses were performed using SPSS v.22 (IBM). Arlequin 3.1 (Excoffier et al., 2005) was used to calculate population diversity indices (haplotype (h) and nucleotide (π) diversities), neutrality indices, Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997). A p-value < 0.05 was considered statistically significant.

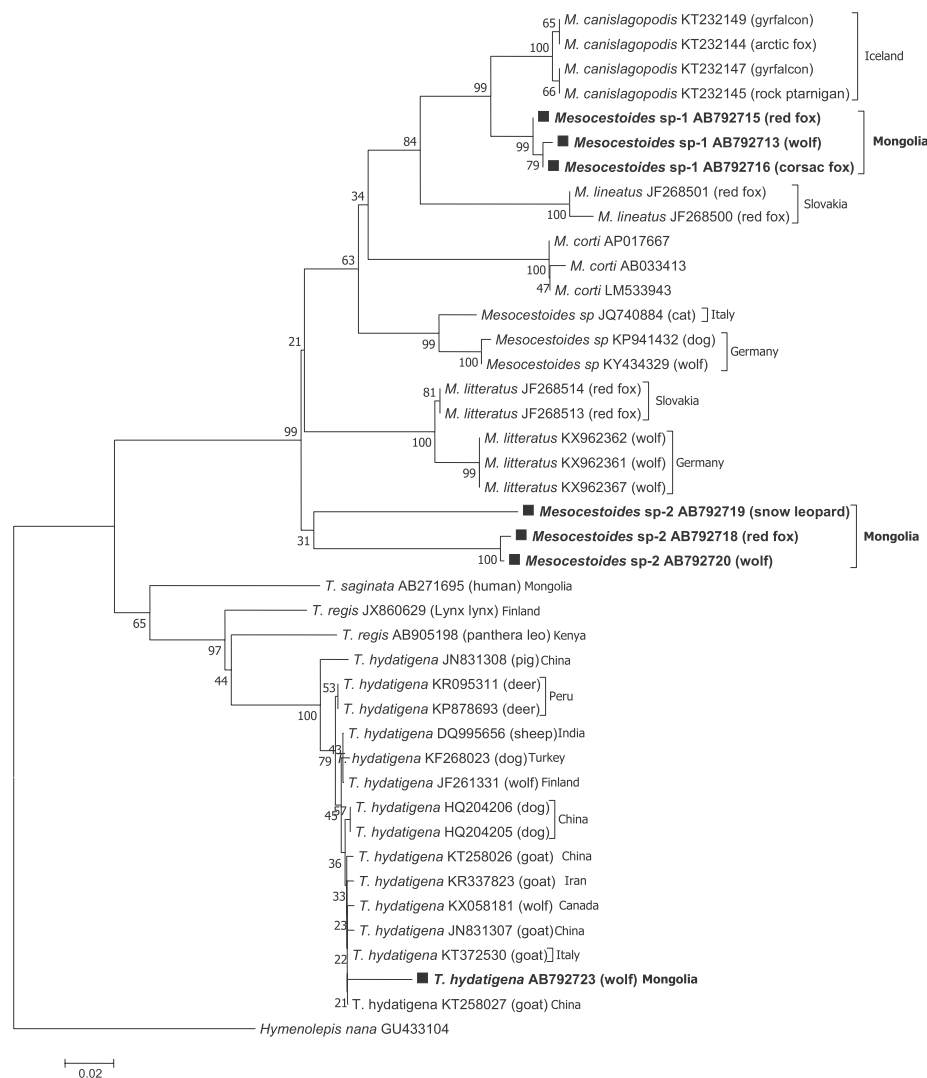
This study was approved by the Research Ethics Committee of the Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia (Number 2012/3–4, dated November, 24).

3. Results

Table 1 shows results from PCR amplification of 405 wild carnivores fecal samples, which indicate that 27.7% (112/405) of samples were amplified with molecular sizes of 400 bp and 314 bp for *cox1* and 12S rRNA, respectively. Tapeworm DNA was detected in wolves (47.3%, 53/112), red foxes (36.6%, 41/112), corsac foxes (9.8%, 11/112), and snow leopards (6.3%, 7/112). GenBank® accession numbers for cyclophyllidean tapeworms identified in Mongolia are presented in Table 6, along with a breakdown by DNA marker, parasite species/isolate code, wild carnivore, and coordinate. Of the 112 amplified samples, 54 and 56 different sequences were detected in the *cox1* and 12S rRNA genes, respectively, and were classified as *T. hydatigena* (50.0%, 56/112), *Mesocostoides* sp.-1 (36.6%, 41/112), and *Mesocostoides* sp.-2 (13.4%, 15/112) via the sequence homology search. Phylogenetic tree analysis revealed that *T. hydatigena* from Mongolia was included in the clade composed of *T. hydatigena* from other countries (Table 4, Fig. 2a, 2b, 3a, and 3b).

*T. hydatigena* infection in wild carnivores was detected throughout all regions, 19.6% (11/56), 32.1% (18/56), 37.5% (21/56), and 10.7% (6/56) in Western, Khangai, Central, and Eastern, respectively (Table 2, Fig. 1). *T. hydatigena* infection in wild carnivores was detected in 17 provinces. The highest prevalence was observed in Tuv (28.0%, 7/25), while the lowest was in Dornogobi (4.1%, 2/49). No *T. hydatigena* was





**Fig. 2.** Phylogenetic tree based on a partial sequence of tapeworms obtained by the neighbor joining method was conducted using the TN93 + I nucleotide substitution model. Numbers above branches are percent bootstrap values based on 1,000 replicates. Bootstrap value > 70% are shown.

(a) Phylogenetic tree based on the *cox1* sequences of *T. hydatigena* and *Mesocoestoides* sp. isolates available in the GenBank® database were included. *Hymenolepis nana* served as an out-group.

(b) Phylogenetic analysis of the 12S rRNA partial sequence of *T. hydatigena*, *Mesocoestoides* sp., and *M. lineatus* inferred using the sequence distance method and maximum likelihood. *Hymenolepis nana* was used as an out-group.

isolated in samples from Govi-Altai and Khuvsgul (Table 2). Furthermore, the prevalence in wolf samples from Tuv was significantly higher than in other provinces (12.9%, 49/380) ( $p = 0.034$ ).

The prevalence of *T. hydatigena* infection in wild carnivores was 19.9% (27/136), 13.8% (23/167), 4.8 (3/62), and 7.5% (3/40) in wolves, red foxes, corsac foxes, and snow leopards, respectively (Table 1). Prevalence in wolves was significantly higher compared to other wild carnivores (10.7%, 29/269) ( $p = 0.012$ ).

The prevalence of *T. hydatigena* infection in wolves was 10.0% (1/10), 8.3% (2/24), 24.4% (10/41), 20.0% (8/40), 21.7% (5/23), and 12.5% (1/8) in alpine, mountain taiga, forest-steppe, steppe, desert-steppe, and desert, respectively. The prevalence in red foxes was 14.3% (7/49), 13.9% (5/36), and 18.6% (11/59) in forest-steppe, steppe, and desert-steppe, and the prevalence in corsac foxes was 5.3% (1/19) and 28.6% (2/7) in desert-steppe and desert, respectively. The prevalence of *T. hydatigena* infection in the snow leopards was 10.7% (3/28) in alpine region (Table 3).

Using the *cox1* and 12S rRNA sequence data we observed that *Mesocoestoides* species from Mongolia formed two genetically distant

clades designated *Mesocoestoides* sp.-1 and *Mesocoestoides* sp.-2, which are not similar to the reference sequences present in GenBank (Fig. 2a, 2b, 3a, and 3b). Moreover, the genetic distances (d value) of the *cox1* gene between *Mesocoestoides* sp.-1 and *M. lineatus* from Slovakia were as large as 0.107–0.120 (Table 4). These results were also supported by the 12S rRNA sequence analysis and genetic distance data (Table 5). Haplotype diversity high compared to the nucleotide diversity was observed for *cox1* and 12S rRNA gene. Due to Tajima's D was > 0 for the *cox1* and 12S rRNA sequences, rare alleles scarce in the population. The statistically significant Fu and Li's D and F values observed for the *cox1* sequences showed  $P < 0.02$ , Fu and Li's D value observed for the 12S rRNA sequence showed  $P < 0.02$  and Fu and Li's F value showed  $P < 0.05$  (Table 7).

*Mesocoestoides* sp.-2 isolated from snow leopards differed slightly from those isolated from red foxes and wolves. However, it did not form any clades with known *Mesocoestoides* species in *cox1* genes (Fig. 2a, 2b, 3a, and 3b), and the d values between *Mesocoestoides* sp.-2 and European isolates were as large as 0.149–0.164 (Table 4). Similarly, *Mesocoestoides* sp.-2 did not form any clades with known *Mesocoestoides* species, and the

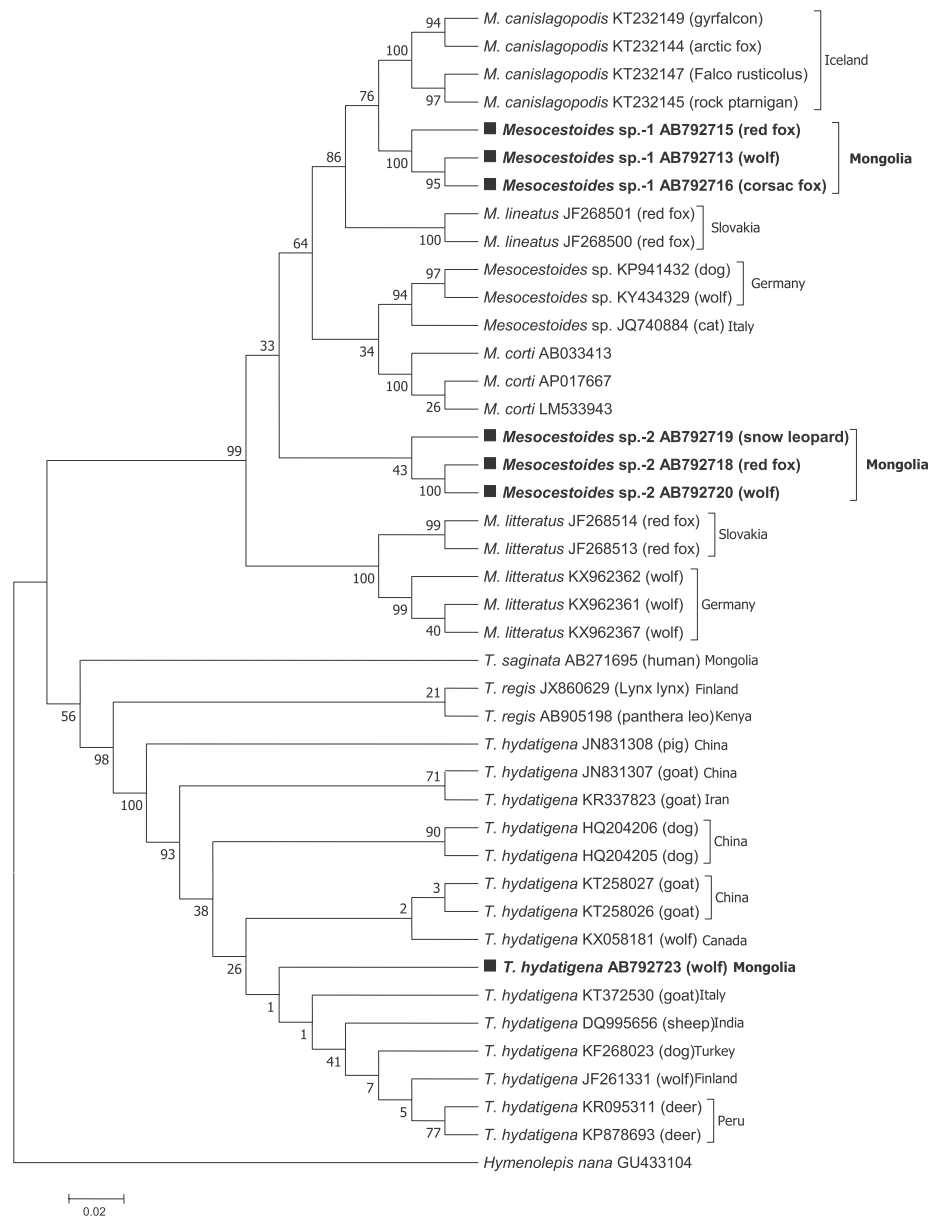


Fig. 2. (continued)

d values were also large (Table 5).

*Mesocystoides* sp.-1 infection in wild carnivores was detected in all regions, 22.0% (9/41), 31.7% (13/41), 34.1% (14/41), and 12.2% (5/41) in Western, Khangai, Central, and Eastern, respectively (Table 2, Fig. 1). The prevalence of *Mesocystoides* sp.-1 infection in wild carnivores was 14.7% (20/136), 9.0% (15/167), and 9.7% (6/62) in wolves, red foxes, and corsac foxes, respectively. However, no *Mesocystoides* sp.-1 infection was observed in the snow leopard (Table 3). Additionally, *Mesocystoides* sp.-1 infection was detected in wild carnivores from 17 of the 21 provinces (Table 2). The highest prevalence was observed in Zavkhan (31.6%, 6/19), while the lowest was in Dornogobi (4.1%, 2/49). No *Mesocystoides* sp.-1 infection was identified in Bayan-Ulgii or Khovd (Table 2).

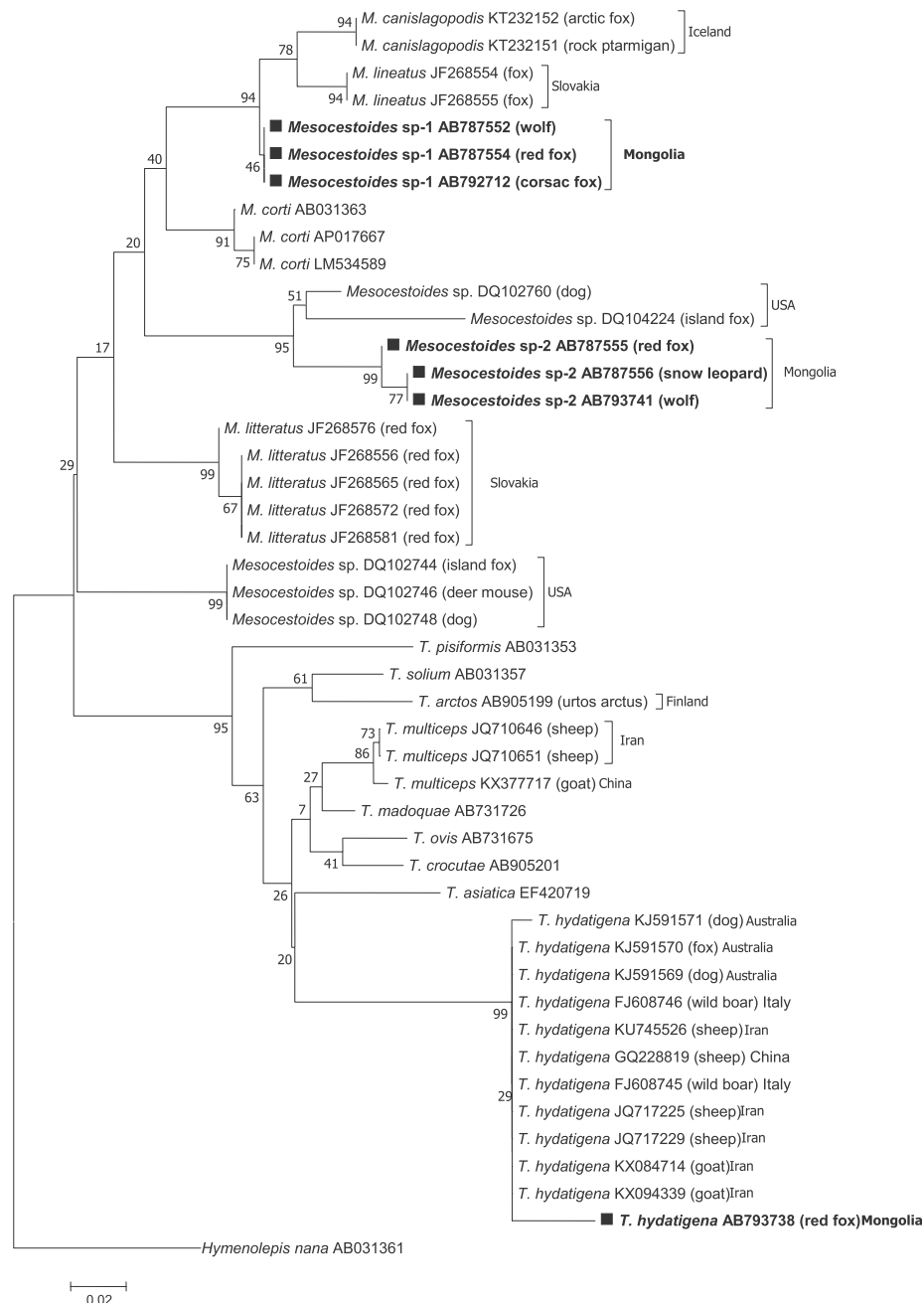
The prevalence of *Mesocystoides* sp.-1 infection in wolves was 21.4% (3/14), 17.0% (7/41), 5.0% (2/40), 26.1% (6/23), and 25.0% (2/8) in mountain taiga, forest-steppe, steppe, desert-steppe, and desert (Table 3). The prevalence in red foxes was 6.1% (3/49), 13.9% (5/36), and 30.4% (7/23) in forest-steppe, steppe, and desert, and the prevalence in corsac foxes was 8.7% (2/23) and 30.8% (4/13) in forest-

steppe and steppe, respectively. There was no *Mesocystoides* sp.-1 infection observed in snow leopards (Table 3).

*Mesocystoides* sp.-2 infection in wild carnivores was detected in three regions, 66.7% (10/15), 13.3% (2/15), and 20.0% (3/15) in Western, Khangai, and Central, respectively (Table 2, Fig. 1). The prevalence of *Mesocystoides* sp.-2 infection in wild carnivores was 15.0% (6/40), 3.5% (3/85), 10.5% (2/19), and 14.3% (4/28) in wolves, red foxes, corsac foxes and snow leopards, respectively (Table 3).

The prevalence of *Mesocystoides* sp.-2 infection was 10.5% (4/38), 0.9% (1/113), 9.0% (8/89), and 2.0% (2/101) in alpine, forest-steppe, steppe, and desert-steppe. The prevalence in wolves was 15.0% (6/40) in steppe. The prevalence in red foxes was 2.0% (1/49) and 5.6% (2/36) in forest-steppe and steppe. The prevalence in corsac foxes was 10.5% (2/19) in desert-steppe, and the prevalence in snow leopards was 14.3% (4/28) in alpine (Table 3).

*Mesocystoides* sp.-2 infection in wild carnivores was detected in nine provinces. The prevalence of which is shown in Table 2. The highest was in Zavkhan (15.8%, 3/19), and the lowest in Dornogobi (2.0%, 1/49) (Table 2).



**Fig. 3.** Phylogenetic tree based on a partial sequence of tapeworms obtained by the maximum likelihood method was conducted using the HKY + G + I nucleotide substitution model. Numbers above branches are percent bootstrap values based on 1,000 replicates. Bootstrap value > 70% are shown.

(a) Phylogenetic tree based on the *cox1* sequences of *T. hydatigena* and *Mesocoestoides* sp. isolates available in the GenBank® database were included. *Hymenolepis nana* served as an out-group.

(b) Phylogenetic analysis of the 12S $r$ RNA partial sequence of *T. hydatigena*, *Mesocoestoides* sp., and *M. lineatus* inferred using the sequence distance method and maximum likelihood. *Hymenolepis nana* was used as an out-group.

#### 4. Discussion

Ecological regions, the existence of wild carnivores and the behavior of local herders all serve to create favorable conditions for parasites to complete their life cycle in Mongolia. The steppe region represents a typical broad grassland that supports stable herds of larger vertebrates. Currently, more than 180,000 herders (34%) in Mongolia live a nomadic or semi-nomadic live style (NSO, 2019).

Animal husbandry is essential for Mongolia's economy. The number of livestock has reached 66.5 million consisting primarily of goats and sheep, followed by horses, cattle, and camels. (MoFALI, 2018). Most

herders in rural areas of Mongolia own livestock, which are kept in common open pastures (Myadagsuren et al., 2007). The majority of the 32 million head of livestock are located within the grassland in the steppe ecoregion, which accounts for about 70% of the country's territory. However, herein, we did not investigate *T. hydatigena* infection in livestock, which serve as the intermediate host for these tapeworm species, but rather this study is the first to investigate the prevalence and distribution of *Taenia* sp. and *Mesocoestoides* sp. using specific molecular tools in wild carnivores in 19 provinces of Mongolia, covering all ecological regions. The tapeworm eggs in the fecal samples were not examined microscopically due to the difficulty of morphologically

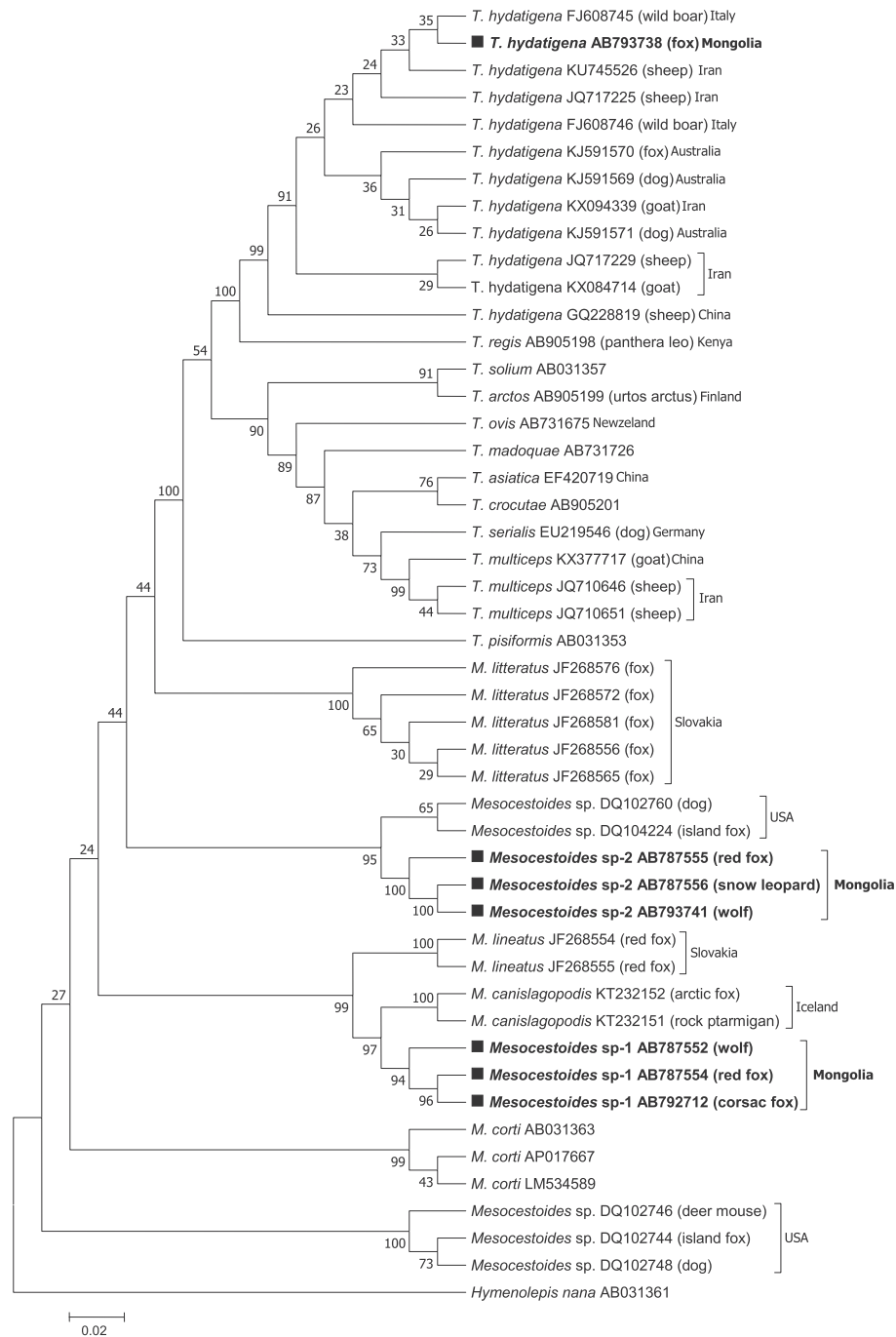


Fig. 3. (continued)

identification.

This study demonstrated that parasitic infections were identified in wild carnivores from all ecosystems in Mongolia. Geographically these animals were distributed from western to eastern regions in all ecoregions and provinces, save for Dornod and Gobi-Sumber, from which there was no available. The infections were primarily detected in wolves (47.3%) followed by red foxes (36.6%), corsac foxes (9.8%), and snow leopards (6.3%) (Table 2).

Phylogenetic tree analysis revealed that *T. hydatigena* from Mongolia was included in the clade composed of *T. hydatigena* from other countries (Table 4, Figs. 2 and 3). Moreover, wild carnivores infected with this parasite were detected in 17 provinces, with the highest prevalence observed in Tuv province ( $p < 0.05$ ), where the capital city of Mongolia, Ulaanbaatar, is located. The population of Tuv province is

approximately 1.2 million (37.5%) (NSO, 2019), and this province provides suitable conditions that support nomadic pastoralism. Additionally, we show that the prevalence of *T. hydatigena* infection is most common in wolves ( $p < 0.05$ ) (Table 1). Similar results were reported in Iran (Nabavi et al., 2014), Italy and Serbia (Ćirović et al., 2015), where wild and domestic hoofed animals were reported to serve as intermediate hosts. Alternatively, no *T. hydatigena* infection was observed in the Govi-Altai and Khuvsgul provinces of Mongolia (Table 2).

In contrast, *Mesocostoides* sp.-1, and *Mesocostoides* sp.-2 isolated from wild carnivores in Mongolia were not found to be similar to reference sequences in GenBank, nor with known *Mesocostoides* species (Figs. 2 and 3), suggesting that *Mesocostoides* sp.-1 is a unique species from *M. lineatus*. These results were also supported by 12S rRNA



**Table 5**  
Pairwise genetic distance in 12S rRNA gene among *Mesocostoides* isolates from Mongolia and related taxa.

Mesocostoides isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
1 <i>Mesocostoides</i> sp.-1 AB792712 (corsac fox) Mongolia																								
2 <i>Mesocostoides</i> sp.-1 AB787554 (red fox) Mongolia	0.004																							
3 <i>Mesocostoides</i> sp.-1 AB787552 (wolf) Mongolia	0.008	0.004																						
4 <i>Mesocostoides</i> sp.-2 AB787555 (red fox) Mongolia	0.174	0.168	0.163																					
5 <i>Mesocostoides</i> sp.-2 AB787556 (snow leopard) Mongolia	0.192	0.186	0.180	0.012																				
6 <i>Mesocostoides</i> sp.-2 AB793741 (wolf) Mongolia	0.198	0.192	0.186	0.004	0.004																			
7 <i>Mesocostoides</i> sp. DQ102748 (dog) USA	0.161	0.156	0.151	0.154	0.172	0.178																		
8 <i>Mesocostoides</i> sp. DQ102760 (dog) USA	0.190	0.184	0.178	0.067	0.081	0.086	0.173																	
9 <i>Mesocostoides</i> sp. DQ102746 (deer mouse) USA	0.155	0.150	0.145	0.148	0.166	0.171	0.004	0.166																
10 <i>Mesocostoides</i> sp. DQ104224 (island fox) USA	0.212	0.207	0.201	0.104	0.120	0.125	0.224	0.072	0.232															
11 <i>Mesocostoides</i> sp. DQ102744 (island fox) USA	0.155	0.150	0.145	0.161	0.179	0.185	0.004	0.173	0.008	0.224														
12 <i>M. lineatus</i> JF268554 (red fox) Slovakia	0.052	0.047	0.043	0.169	0.187	0.193	0.150	0.183	0.144	0.213	0.144													
13 <i>M. lineatus</i> JF268555 (red fox) Slovakia	0.037	0.033	0.029	0.169	0.187	0.193	0.150	0.183	0.144	0.213	0.144	0.000												
14 <i>M. canislagopodis</i> KT232152 (arctic fox) Iceland	0.037	0.033	0.029	0.169	0.187	0.193	0.150	0.183	0.144	0.213	0.144	0.000	0.056											
15 <i>M. canislagopodis</i> KT232151 (rock ptarmigan) Iceland	0.159	0.154	0.148	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174										
16 <i>M. litteratus</i> JF268556 (red fox) Slovakia	0.159	0.154	0.148	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174	0.000									
17 <i>M. litteratus</i> JF268565 (red fox) Slovakia	0.159	0.154	0.148	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174	0.000	0.000								
18 <i>M. litteratus</i> JF268572 (red fox) Slovakia	0.159	0.154	0.148	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174	0.000	0.000	0.000							
19 <i>M. litteratus</i> JF268576 (red fox) Slovakia	0.154	0.148	0.143	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174	0.000	0.004	0.004	0.004						
20 <i>M. litteratus</i> JF268581 (red fox) Slovakia	0.159	0.154	0.148	0.131	0.148	0.153	0.155	0.137	0.155	0.188	0.155	0.174	0.174	0.174	0.000	0.000	0.000	0.000	0.004					
21 <i>M. corri</i> AP017667	0.112	0.107	0.102	0.116	0.132	0.138	0.077	0.131	0.082	0.156	0.077	0.124	0.124	0.118	0.118	0.116	0.116	0.116	0.121	0.116				
22 <i>M. corri</i> LM534589	0.112	0.107	0.102	0.116	0.132	0.138	0.077	0.131	0.082	0.156	0.077	0.124	0.124	0.118	0.118	0.116	0.116	0.116	0.121	0.116	0.000			
23 <i>M. corri</i> AB031363	0.106	0.101	0.096	0.121	0.137	0.143	0.082	0.137	0.087	0.162	0.082	0.130	0.124	0.124	0.124	0.110	0.110	0.110	0.116	0.110	0.004	0.004		

**Table 6**  
GenBank® accession numbers for cyclophyllidean tapeworms identified in Mongolia.

DNA marker	Parasite species/isolate code	Wild carnivore	Accession number <sup>a</sup>
<i>cox1</i>	<i>Mesocostoides</i> sp.-1		
	Isolate MI01	Wolf	AB792713
	Isolate MI03	Red fox	AB792715
	Isolate MI04	Corsac fox	AB792716
	<i>Mesocostoides</i> sp.-2		
	Isolate Msp04	Wolf	AB792720
	Isolate Msp02	Red fox	AB792718
	Isolate Msp03	Snow leopard	AB792719
	<i>Taenia hydatigena</i>		
	Isolate Th03a	Wolf	AB792723
12S rRNA	<i>Mesocostoides</i> sp.-1		
	Isolate MI01	Wolf	AB787552
	Isolate MI03	Red fox	AB787554
	Isolate Msp04	Corsac fox	AB792712
	<i>Mesocostoides</i> sp.-2		
	Isolate Msp03	Wolf	AB793741
	Isolate Msp01	Red fox	AB787555
	Isolate Msp02	Snow leopard	AB787556
	<i>Taenia hydatigena</i>		
	Isolate Th03b	Red fox	AB793738

<sup>a</sup> Accession numbers reported in this study.

sequence analysis and genetic distance data (Table 5). Phylogenetic analysis of *T. hydatigena*, *Mesocostoides* based on *cox1* and 12S rRNA was performed with a 410 bp long alignment comprising 48 sequences. Phylogenetic trees obtained by the use two tree-bulding methods have the same topology (Fig. 2a, 2b, 3a, and 3b). Further, *Mesocostoides* sp.-2 isolated from snow leopards differed slightly from red fox and wolf isolates, and did not form clades with known *Mesocostoides* species in *cox1* genes (Fig. 2a, 3a). In fact, the d values between *Mesocostoides* sp.-2 and European isolates were as large as 0.149–0.164 (Table 4). Similarly, *Mesocostoides* sp.-2 did not form any clades with known *Mesocostoides* species, and the d values were also quite high (Table 5), indicating that these two unidentified *Mesocostoides* species from Mongolia likely represent new species or species unregistered in GenBank. Using two mitochondrial genes, the *cox1* and the 12S rRNA we molecularly confirmed the presence of *Mesocostoides* in canids from Mongolia. The overall high haplotype, low nucleotide diversities (Table 7) and the mainly significant negative neutrality indices detected for both *cox1* and 12S rRNA sequences for Mongolian *Mesocostoides* sp.-1 and *Mesocostoides* sp.-2 from wolf, red fox, corsac fox, snow leopard from different ecological regions. This is similar to results reported for *Mesocostoides* from many regions worldwide (Foronda et al., 2007; Hrkčková et al., 2011; Zalešný and Hildebrand, 2012; Skirnisson et al., 2016; Varcasia et al., 2018; Montalbano et al., 2018).

Based on the study results, *Mesocostoides* sp.-1 infection in wild carnivores was detected primarily (34.1%) in the Central region, with the highest prevalence observed in Zavkhan (31.6%, 6/19), and the lowest in Dornogobi (4.1%, 2/49) (Table 2). Moreover, the highest prevalence was in corsac foxes (30.8%), followed by red foxes (30.4%), and wolves (26.1%) in steppe, desert, and desert-steppe, respectively. There was no *Mesocostoides* sp.-1 infection seen in the snow leopard (Table 3).

*Mesocostoides* sp.-2 infection in wild carnivores was detected primarily in the Western (66.7%) region where the highest prevalence was again in Zavkhan (15.8%) and the lowest in Dornogobi (2.0%) (Table 2). *Mesocostoides* sp.-2 infection was found to be more prevalent in wolves (15.0%) and snow leopards (14.3%) in steppe and alpine ecoregions, respectively (Table 3).

The distribution of *Mesocostoides* sp.-1 and *Mesocostoides* sp.-2 infections in wild carnivores may be related to the natural habitat of these

**Table 7**  
Diversity and neutrality analysis for *Mesocestoides* isolates from Mongolia.

Genes	n	Hn	Hd	VarHd	nd	SD of Hd	TajimaD	SigD	FuLiD*	SigD	FuLiF*	SigF
12S rRNA <sup>a</sup>	56	6	0.775	0.00098	0.23468	0.031	0.3935	Not	2.28560	**	1.85576	*
cox1 <sup>b</sup>	56	6	0.775	0.00098	0.23975	0.031	1.6513	Not	2.28452	**	2.44219	**

12S rRNA<sup>a</sup> sequences of *Mesocestoides* from canids in Mongolia.

cox1<sup>b</sup> sequences of *Mesocestoides* from canids in Mongolia.

Abbreviation: n-number of isolate; H-haplotype; Hd-haplotype diversity; nd-nucleotide diversity; SD – standard deviation; SigD-statistical significance D; FuLiD- Fu and Li's D test statistic; FuLiF- Fu and Li's F test statistic; SigF- statistical significance F12S rRNA gene for Tajima's D test statistic: Not significant, P > 0.10; Fu and Li's D\* test statistic: Significance \*\*, P < 0.02; Fu and Li's F\* test statistic: Significance: \*, P < 0.05.

cox1 gene for Tajima's D test statistic: Not significant, P > 0.10; Fu and Li's D and F\* test statistic: Significance: \*\*, P < 0.02.

wild carnivores in Mongolia. The prey of these wild animals primarily comprise large herbivorous hoofstock including blue sheep, wild sheep, mountain goat, deer, Siberian ibex, and domestic animals such as goats, sheep, cattle, and horses (McCarthy, 2000; McCarthy et al., 2005; Shehzad et al., 2012). However, they also hunt birds, marmots, rodents, and other small mammals (Shehzad et al., 2012). We, therefore, assumed that the relationship between wolves, corsac foxes, and snow leopards and livestock has a central role in the lifecycle of *Mesocestoides* sp.-1, *Mesocestoides* sp.-2 in Mongolia. Hence, although felids and canids often act as definitive hosts for tapeworm species, they may also serve as secondary intermediate hosts (Venco et al., 2005; Elini et al., 2007; Jabbar et al., 2012).

The increase in the prevalence of parasitic diseases in wildlife poses a significant challenge to endangered species conservation (Aguirre, 2009; Pedersen and Greives, 2008; Smith et al., 2009). Therefore, it is imperative that appropriate measures be taken to prevent the spread of potentially fatal organisms, such as *T. hydatigena* and *Mesocestoides* sp., to the endangered snow leopard. The snow leopard is included on the International Union for Conservation of Nature's (IUCN) red list and the Mongolian Red List of Mammals (Clark et al., 2006) due to its endangered status. Approximately 20% (over 1000 animals) of the world's population of snow leopards are found in Mongolia (Fox ., 1994; McCarthy et al., 2010).

Moreover, as pasture lands are likely extensively contaminated with the feces of wild animals infected with *T. hydatigena*, *Mesocestoides* sp.-1 and *Mesocestoides* sp.-2, feral herbivores, livestock, herders, and the local people are at risk of acquiring these tapeworm infections. Thus, preventive measures that limit the spread of these parasites are necessary. The results of this study serve as a baseline with the potential inform the development of improved practices to better control the spread of parasitic infections in domestic and wild animals, particularly those considered to be endangered.

The limitation of this study, PCR-positive products were detected in only 112 samples (27.7%). This low isolation rate may have been caused by improper preservation conditions of feces (old and dry) or the presence of inhibitors. In addition, DNA extraction of mtDNA from fecal samples serves to preserve fragments less than 200 bp and are, therefore, more reliably amplified compared to fragments longer than 300 bp (Frantzen et al., 1998). Moreover, these DNA products employ short mtDNA markers that ensure effective and accurate genotyping.

This study reports the prevalence of *T. hydatigena* and *Mesocestoides* species in wild carnivores based on copro-DNA analysis. The most salient finding is that *Mesocestoides* sp.-1, and *Mesocestoides* sp.-2 from Mongolia likely account for new species, or species that are currently unregistered on GenBank. Furthermore, *T. hydatigena* was detected throughout all ecoregions was *Mesocestoides* sp.-1, save for in the alpine; while *Mesocestoides* sp.-2 was isolated from samples in the alpine, forest-steppe, steppe, and desert-steppe regions. Finally, wolf, red fox, corsac fox, and snow leopard were confirmed as new definitive hosts for *Mesocestoides* sp.-1 and *Mesocestoides* sp.-2.

## Funding

This study was partially supported by the Fellowship Awards 2012 from the Japanese Association of University Women to MN.

## Declaration of competing interest

The authors have no conflict of interest regarding the contents of this manuscript.

## Acknowledgments

We are grateful for the support of the Mongolian National University of Medical Sciences and staff of the Laboratory of Helminthology, Department of Parasitology, National Institute of Infectious Diseases for their kind cooperation.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2019.12.004>.

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