Morphological and molecular analyses of *Taenia* and *Mesocestoides* species from red foxes (*Vulpes vulpes*) in northwestern China

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**ABSTRACT**

In this study, 263 tapeworms were collected from eight road-killed red foxes in Xinjiang Uygur Autonomous Region (XUAR, northwestern China). The tapeworms were analyzed based on morphological characters and mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene sequences. Eighty-three *Taenia* and 180 *Mesocestoides* tapeworms were identified according to the presence or absence of rostellar, and the number, the length and the shape of the large rostellar hooks. The morphological and molecular analyses revealed that i) 180 *Mesocestoides* tapeworms, here named as *Mesocestoides* sp. (*Vulpes vulpes*), showed 99.21% (378/381 bp) identity to *Mesocestoides* sp. reported from red fox in Mongolia; and ii) 83 *Taenia* tapeworms belonged to three species. The first *Taenia* species (*n* = 16, named as *Taenia* sp. (*Vulpes vulpes*)), based on the length of large rostellar hooks (337–342 µm) and its *cox1* sequence, was identified as a potentially novel species, which is phylogenetically close to *Taenia laticollis*. The second species (*n* = 54, named as *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*)), was morphologically similar to *Taenia endo thoracicus* according to the number (*n* = 52), the length (319–332 µm) and the shape of the large rostellar hooks. This species, infecting three red foxes, shared 100% *cox1* sequence identity with *Taenia* sp. (*Rhombomys opimus*) genotype C found previously in great gerbils (*Rhombomys opimus*) in the same region. The third species (*n* = 13, named as *Taenia polyacantha-like*), had shorter large rostellar hooks (178–180 µm) and showed 96.27% (361/375 bp) sequence identity to *Taenia polyacantha* reported from red fox in Italy. The “great gerbil-red fox” life cycle of *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*), belonging to the mitochondrial lineage of T. *endo thoracicus*, is confirmed. The *T. polyacantha-like* species was firstly found in red fox in China. *Taenia* sp. (*Vulpes vulpes*) is a potentially novel species, which is close to *T. laticollis* based on its phylogenetic properties.

1. Introduction

The red fox (*Vulpes vulpes* Linnaeus, 1758), that is the largest species of true foxes (Carnivora: Canidae: *Vulpes*), is widespread in North-America, Asia, Europe and North Africa (Ablimiti, 2013). Its habitats overlap with activity areas of humans and domestic animals, leading to risks of infectious diseases (e.g. rabies and echinococcosis) relevant to public and veterinary health (CDC, Zheng et al., 2008).

Tapeworms (cestodes) have a domestic or sylvatic life cycle between definitive hosts and intermediate hosts (Thompson, 2013). Previously, the red fox was reported to harbor many species of cyclophyllidean cestodes, such as *Taenia taeniformis*, *Taenia pisiformis*, *Taenia polyacantha*, *Taenia hydatigena*, *Taenia multiceps*, *Taenia serialis*, *Taenia crassiceps*, *Taenia ovis*, *Echinococcus multilocularis*, *Echinococcus

**ARTICLE INFO**

**Keywords:**
- Cestoda
- Tapeworms
- Cyclophyllidea
- *Taeniidae*
- *cox1*

**ABBREVIATIONS:**
- *cox1*, cytochrome c oxidase subunit 1; XUAR, Xinjiang Uygur Autonomous Region.
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**https://doi.org/10.1016/j.ijppaw.2021.11.003**

Received 14 October 2021; Received in revised form 16 November 2021; Accepted 17 November 2021

Available online 19 November 2021

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2. Material and methods

2.1. Sample collection

In this study, eight red foxes were examined. These animals were road-killed in the Gurbantunggut Desert, the northern region of XUAR in North-western China (Ji et al., 2021; Liu et al., 2021). The aim of the present study was to identify tapeworms from road-killed red foxes in XUAR.

2.2. Morphological identification

The staining procedure was performed as previously reported (Li et al., 2009). In brief, the scolex of representative tapeworms were stained with acetate carmine and observed with an Olympus DP70 digital camera (Olympus, Tokyo, Japan).

2.3. DNA extraction and molecular analysis

After detailed morphological examination, 40 tapeworm specimens were selected for molecular analysis (as shown in Table 1). DNA was extracted from a small part of the immature strobila using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). A conventional PCR was performed to amplify an approximately 450-bp-long part of the mitochondrial cytochrome c oxidase subunit I (cyt b) gene, BLASTN analysis indicated that i) sixteen Mesocestoides spp. were most closely related to Mesocestoides sp. (accession number: AB792718) from red fox in Mongolia, sharing 99.21% (378/381 bp) sequence identity to it. Provisionally, this species was named as Mesocestoides sp. (Vulpes vulpes) Cox1 (MZ575884); ii) the first Taenia sp. was most closely related to Taenia sp. (Rambomys opimus) (accession numbers: MW321849 and MW321850) detected in great gerbils in our previous work (Ji et al., 2021), but sharing only 88.03% (309/351 bp) sequence identity with it. Provisionally, this species was named as Taenia sp. (Vulpes vulpes) (MZ529998), and it belonged to Clade B of the Cox1 phylogenetic tree (Fig. 1); iii) the second Taenia sp. was 100% identical in its Cox1 sequence to Taenia sp. (Rambomys opimus) genotype C (accession numbers: MW321849 and MW321850) detected in great gerbils, originating from the same region as the relevant three red-killled red foxes (#5, #7 and #8) in this study. Provisionally, this species was named as Taenia sp. (Vulpes vulpes & Rambomys opimus) (MZ529999), and belonged to Clade A of the cox1 phylogenetic tree (Fig. 1); and iv) the third Taenia species was most closely related to T. polyacantha (accession number: MT806362) from red fox in Italy, sharing 96.27% (361/375 bp) cox1 sequence identity. Provisionally, this species was named as “T. polyacantha-like” (MZ513000), and it belonged to Clade C of the cox1 phylogenetic tree (Fig. 1).

Analysis of COX1 amino acid sequences showed that i) Mesocestoides sp. (Vulpes vulpes) has 100% (127/127 aa) identity to Mesocestoides sp. (accession number: AB792718) from red fox in Mongolia; ii) Taenia sp. (Vulpes vulpes) shares 93.16% (109/117 aa) identity with Taenia sp. (Rambomys sp.) (MW321849) (Fig. 2A); and iii) “T. polyacantha-like” (from Clade C in Fig. 1) has 100.00% (125/125 aa) identity to T. polyacantha (MT806362) (Fig. 2B).

4. Discussion

The previously known geographical range of T. endothoracicus, with

| Table 1 |
|---|
| Sampling data of eight red foxes (Vulpes vulpes) in XUAR, North-western China. |
| Serial number | Red fox |
|---|---|
| Age | adult |
| Habitat type | desert |
| Genus Taenia | T. sp. (Vulpes vulpes) |
| Genus Mesocestoides | T. sp. (Vulpes vulpes & Rambomys opimus) |
| | T. polyacantha-like |
| Mesocestoides sp. (Vulpes vulpes) | 80 (8)* |
| (a)*: Number of molecular analysis. |

Six foxes (#1, #3, #5, #6, #7 and #8) were found infected with 263 tapeworms. According to the presence or absence of rostellum, 83 were identified as Taenia spp., while 180 belonged to the genus Mesocestoides spp. According to the number, the length and measurement data (total length, total width, posterior length, anterior length and guard length) of large rostellar hooks, 83 Taenia tapeworms were assigned to three species. The first species (n = 16, removed from red fox #1) and the second species (n = 54, from red foxes #3, #5, #7 and #8) were similar to Taenia endothroacicus, Taenia krepkogorski and Taenia macrocephalus in the number and length of large rostellar hooks (Tables S1 and S2). The third species (n = 13, from red foxes #5, #6 and #8) belonged to Taenia polyacantha according to the number and length of large rostellar hooks.
red fox as its definitive host, included Iran and North Africa. Polycephalic larvae occur in the thoracic cavity of rodents from the genera *Meriones*, *Gerbillus* and *Rhombomys*. In our previous work, *Taenia* sp. (*Rhombomys opimus*) genotypes A, B and C were demonstrated from the thoracic cavity of great gerbils in Gurbantungut Desert with 5.3% (10/188) prevalence (Ji et al., 2021). A lot of protoscoleces were observed in large white and web-shaped cysts, which caused shrinking and stiffness in the lung parenchyma (Ji et al., 2021). Interestingly, here adults of *Taenia* sp. (*Vulpes vulpes* & *Rhombomys opimus*) were found in four red foxes, and the corresponding *cox1* sequence had 100% identity to that of *Taenia* sp. (*Rhombomys opimus*) genotype C in great gerbils. Further-more, the sampling sites of the three red foxes (#5, #7 and #8) and of the two great gerbils (#27 and #31) infected with *Taenia* sp. (*Rhombomys opimus*) genotype C are situated in the same area (44°67′-44°70′ N, 86°34′-86°39′ E) (Ji et al., 2021). Previously, Lin et al. (2010) reported that great gerbils are important in the diet composition of red foxes in Gurbantungut Desert, and accounted for 16.48% of prey items based on 273 fecal samples of red foxes. These findings suggest that the life cycle of *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*) involves the great gerbil and the red fox (Fig. S3). In addition, the shape of large rostellar hooks was compared among *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*), *T. endothoracicus*, *T. krepkogorski* and *T. macrocystis* (as shown in S pptx). The results indicated that the shape of large rostellar hooks is similar between *T. endothoracicus* and *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*). Interestingly, the shape of cysts showed significant difference between *T. endothoracicus* reported from Iran and *Taenia* sp. (*Rhombomys opimus*) in previously in North-western China (Fig. S4) (Ji et al., 2021; Mowlavi et al., 2004; Dautel et al., 2013), suggesting that *Taenia* sp. (*Vulpes vulpes & Rhombomys opimus*) belongs to a genetic lineage separate from *T. endothoracicus*. However, unfortunately, the *cox1* sequence of the latter species is not available in GenBank to confirm this.

According to the length of the large rostellar hooks and definitive host (*Vulpes*, *Taenia* sp. (*Vulpes vulpes*) is similar to *T. endothoracicus*, *T. krepkogorski* and *T. macrocystis*. However, when the shape of the large rostellar hooks was compared among them, *Taenia* sp. (*Vulpes vulpes*) showed obvious differences from these three tapeworm species (as shown in S pptx). Due to the lack of *cox1* data of *T. endothoracicus*, *T.
Vulpes vulpes, and a potentially novel species, is phylogenetically closely related to T. laticeps based on the cox1 gene.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.11.003.

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