

Prevalences and characteristics of *Trichuris* spp. infection in sheep in pastoral areas of the Tianshan, Xinjiang, China

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Abstract

Introduction: Nematodes of the *Trichuris* genus are commonly reported parasites that can cause trichuriasis in many animals, which leads to inflammation, intestinal bleeding and reductions of productivity in livestock. Knowledge of the prevalence of *Trichuris* infestation in the Tianshan ovine population and of the nematode species parasitising the population is not exhaustive, and this study aimed to expand the knowledge. **Material and Methods:** A total of 1,216 sheep slaughtered in five pasture areas in the Tianshan Mountains of Xinjiang were investigated and a phylogenetic analysis based on the mitochondrial *cox1* gene was performed to clarify the genetic relationships of the various *Trichuris* species. **Results:** Sheep totalling 1,047 were infected with *Trichuris* spp. establishing the rate at 86.1%. Using a morphological protocol, six documented and one undefined species were identified, namely *T. gazellae*, *T. lani*, *T. ovina*, *T. longispiculus*, *T. concolor*, *T. discolor* and *Trichuris* sp. Among them, *T. gazellae* and *T. lani* were the dominant species, accounting for 34.5% and 31.0% of *Trichuris* spp., respectively. Phylogenetic analysis divided the detected species of *Trichuris* spp. into two genetic clades (clade I and clade II). The six documented species that can infect sheep and the undefined species were clustered into clade I, with inter- and intra-species genetic diversity apparent. **Conclusion:** This survey described in detail the morphological characteristics of six known and one undefined species of *Trichuris*, which not only enriched the taxonomic information on record regarding *Trichuris* spp., but also provided valuable epidemiological data for the prevention and control of trichuriasis in sheep.

Keywords: sheep, *Trichuris* spp., prevalence and characteristics, phylogenetic analysis.

Introduction

Nematodes of the *Trichuris* genus can parasitise the cecum and colon of humans, cattle, sheep, pigs and other animals, causing trichuriasis (also known as whipworm disease), which is harmful to human and livestock health and livestock production (2, 15, 23, 28). In ruminants, *Trichuris* spp. infection can cause chronic cicatricial inflammation of the cecum and colon, resulting in wasting, anaemia and even death (7, 24). Currently, the disease is widespread and globally distributed, seriously threatening the sustainability and expansion potential of the farming industry (20).

The *Trichuris* genus includes several species such as *T. trichiura*, *T. suis* and *T. vulpis*, which can infect

humans and various animals and are rightfully accorded medical and veterinary importance (9, 11, 14, 18). Since Roederer first named the genus in 1761, so far at least 24 *Trichuris* species have been documented in ruminants (26, 27). In 2012, 415 domesticated adult ruminants were investigated for evidence of gastrointestinal protozoan and helminthic infections in India and a 9.15% infection rate of *T. trichiura* was noted (3).

Xinjiang is one of the most important sheep farming regions in China, with a current stock of 36 million sheep. Digestive tract parasitic infections are very common in sheep and result in serious economic losses to farmers who graze their flocks in the pasture areas of Xinjiang. However, the infection status of the region's sheep and the prevalent species of *Trichuris* in them are

still unknown. Therefore, the main objective of this study was to investigate the prevalences of *Trichuris* spp. in sheep in the Tianshan pasture areas of Xinjiang and characterise *Trichuris* spp. using morphological and molecular biological protocols. The investigation's findings are hoped to provide valuable epidemiological data for the prevention and control of trichuriasis.

Material and Methods

Overview of the surveyed areas. The five pastoral areas in the Xinjiang Tianshan Mountains investigated in this survey are Urumqi, Yili, Bole, Tacheng and Altay (Supplementary Fig. S1). The five pastoral areas are located between 44° and 48°N and 83° and 88°E. The region has a temperate continental climate with an annual precipitation of 500 to 600 mm and an average annual temperature of -4 to 9°C. The average temperatures in summer range from 20 to 25°C and in winter range from -15 to -25°C.

Animals surveyed. During 2019–2021, 1,216 sheep were investigated, of which approximately 70% were under one year old and which had all been slaughtered in five pastoral slaughterhouses. All of the sheep had been reared by grazing. All slaughtered sheep were sorted, registered and numbered. After slaughter and evisceration, the large intestine was collected and transported at low temperature to the Xinjiang Key Laboratory of Animal Disease Prevention and Control.

Methods of investigation. Briefly, the collected large intestine was placed in a basin pre-filled with saline, the intestine was dissected and the contents washed out into the liquid, and then the mucosa was examined carefully in a sequential manner. Meanwhile, the washed-out contents were repeatedly sedimented with saline until the liquid ran clear. The sediment was then taken and the worms carefully examined. Adult *Trichuris* nematodes were collected and placed in 75% alcohol supplemented with 5% glycerol (18, 29).

Morphological identification of *Trichuris* spp. The morphological identification of *Trichuris* spp. was based on the size of the adult male worm, the ratio of whip to body, the length of the copulatory spine, and the shape and size of the spiny sheath (6, 11, 20, 22, 26, 27). All individuals of *Trichuris* spp. were subjected to morphometrical identification using an Olympus BX53 microscope (Olympus, Tokyo, Japan).

Molecular and phylogenetic analysis of *Trichuris* spp. Primers were designed based on the conserved sequence of a mitochondrial marker, the cytochrome c oxidase I (*cox1*) gene. Their sequences were FP: 5'-ACYACATAGTAGGTRTCATG-3' and RP: 5'-TGATTTTTTGGTCACCCTGAAGTTTA-3'. Total genomic DNA was isolated from an individual male specimen of different *Trichuris* species with a DNA extraction kit (TaKaRa, Shiga, Japan) according to the instructions. Then, a PCR was performed to amplify the *cox1* gene. The PCR reaction system

consisted of 21 µL of water, 1 µL of each FP1 and RP1 primer (0.2 µmol/L), 25 µL of 2× Premix Ex Taq and 2 µL of DNA template. The reaction was carried out for 35 cycles of 94°C for 40 s, 56°C for 40 s and 72°C for 35 s followed by extension at 72°C for 10 min. The PCR products were detected using 1.6 % agarose gel electrophoresis, purified using a PCR purification kit (TaKaRa), and sequenced at the Huada Biological Company (Shenzhen, China). Each product was sequenced three times. Sequences that were identical were used for comparisons. Subsequently, a phylogenetic tree based on the *cox1* gene was constructed using the neighbour-joining method using MEGA software version 7.0 (12).

Statistical analysis of data. All data were statistically analysed using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). Continuous and categorical variables were analysed using one-way analysis of variance and chi-squared tests, respectively. Differences were considered significant when $P < 0.05$ and highly significant when $P < 0.01$.

Results

Of the 1,216 sheep examined, 1,047 were infected by *Trichuris* spp., which equated to an infection rate of 86.1%. Of the five pasture areas, Altai had the sheep with the highest infection rate (91.4%), while Urumqi had the animals with the lowest (80.7%) (Table 1). The infection rates were as high as 99.8% for lambs less than one year old and were accompanied in these youngest animals by an average infection intensity of 17.8 *Trichuris* spp. (All feces in the large intestine). However, the infection in sheep aged one to four years was less prevalent and weaker, with an 80.7% infection rate and an average intensity of infection of 9.7. Sheep over four years old had a prevalence of 38.7% and an average intensity of 6.7 *Trichuris* spp. The survey showed that there were no significant differences between infection rates in different grazing areas ($P > 0.05$), while both infection rates and intensity varied significantly ($P < 0.05$) between lambs less than one year old and adult sheep over four years old (Table 2).

A total of 16,849 *Trichuris* spp. (7,548 males and 9,301 females) from sheep were collected in this survey. Six documented species and one undefined species of *Trichuris*, namely *T. gazellae*, *T. lani*, *T. ovina*, *T. longispiculus*, *T. concolor*, *T. discolor* and *Trichuris* sp. (Fig. 1), were morphologically identified based on key characteristics such as the ratio of whip to body, the length of the copulatory spine and the shape and size of male spiny sheaths (Table 3). It was indicated that the undefined species of *Trichuris* obtained from sheep in the pastoral areas of the Tianshan in Xinjiang was different in its morphological characteristics from previously reported *Trichuris* species. The spicule sheath of *Trichuris* sp. was covered with small spines and shaped such that the beginning of the sheath flared

and then gradually became thinner, with no spines at the end. *Trichuris gazellae* and *T. lani* were the dominant species, accounting for 34.5% and 31.0% of all *Trichuris*, respectively. The other species of *Trichuris*, which were *T. ovina*, *T. longispiculus*, *T. concolor*, *T. discolor* and *Trichuris* sp., accounted for 15.8%, 6.9%, 5.2%, 3.9% and 2.7%, respectively.

After amplification (Fig. 2) and sequencing of the *cox1* gene of the six known species, it was found to share 99.1–99.8% identities with other *Trichuris* spp. After the same steps, the undefined species' *cox1* gene transpired to share only 93.21% identity with other *Trichuris* spp. The *cox1* genes of the investigated species of *Trichuris* were submitted to GenBank and given these accession numbers: *T. gazellae*, ON190112, ON190105 and ON190107; *T. lani*, ON190106 and ON213215; *T. ovina*, ON190109;

T. longispiculus, ON190110; *T. concolor*, ON190108; *T. discolor*, ON184014; and *Trichuris* sp., ON189049, ON189053 and ON189054. Phylogenetic analysis revealed that *Trichuris* spp. divided into two genetic clades, namely clade I and clade II. The six known species and the undefined species investigated in this study were all classified into clade I. However, these species shared a higher genetic relatedness with *T. muris* and *T. pampeana*, with the exception of *T. ovina*, which displayed a closer genetic relationship with *T. navonae*.

Moreover, the phylogenetic tree based on *cox1* gene also indicated that those *Trichuris* spp. derived from sheep had closer genetic relationships with *T. trichiura*, while they were genetically far from *T. suis* and *T. vulpis*, which supported the morphological classification in terms of molecular taxonomy.

Table 1. Survey on the prevalences of *Trichuris* spp. infection in sheep from the Tianshan Mountains pastoral areas in China

Pastoral area	Year						Total	
	2019		2020		2021		Number of sheep surveyed	Number of infected sheep (%)
Urumqi	86	69 (80.2)	84	66 (78.6)	79	66 (83.5)	249	201 (80.7)
Yili	78	70 (89.7)	85	73 (85.9)	82	70 (85.4)	245	213 (86.9)
Bole	85	74 (87.0)	88	74 (84.1)	75	65 (86.7)	248	213 (85.9)
Tacheng	75	65 (86.7)	78	68 (87.2)	88	74 (84.1)	241	207 (85.9)
Altai	72	66 (91.7)	77	71 (92.2)	84	76 (90.5)	233	213 (91.4)
Total	396	344 (86.9)	412	352 (85.4)	408	351 (86.0)	1,216	1,047 (86.1)

Table 2. Infection of *Trichuris* spp. in sheep at different ages

Sheep age (years)	<1	1–4	≥4
Number of sheep surveyed	862	119	235
Number of infected sheep (%)	860 (99.8) ^a	96 (80.7) ^a	91 (38.7) ^b

Different superscript letters (^{a, b}) in the same row indicate significant difference ($P < 0.05$)

Table 3. Key characteristics used in the identification of *Trichuris* spp. in sheep from the Tianshan Mountains pastoral areas in China

Species of <i>Trichuris</i> of which a male was examined	Length of body (mm)	Whip length/body length	Length of spicule (mm)	Length of the spicule sheath (mm)	Morphology of the spicule sheath
<i>T. lani</i>	46.5 ± 10.3	1.8/1	3.2–4.5	0.18–0.2	Tubular; the spicule sheath surface densely covered with small spines, the outer shape resembling a baseball bat
<i>T. gazellae</i>	52.1 ± 6.6	2.2/1	1.5–2.2	0.2–1.2	Proximal 3/4 of the sheath densely covered with small spines; spiny part forming an expanded part as it approaches the unarmed part; distal 1/4 thinner, unarmed
<i>T. ovina</i>	47.4 ± 8.7	2.5/1	0.8–1.2	0.18–0.32	Proximal 1/2 of sheath densely covered with small spines; distal 1/2 unarmed
<i>T. discolor</i>	45.2 ± 1.8	1.9/1	1.2–1.5	0.18–0.20	Surface densely covered with small spines, sheath end dilated and spherical
<i>T. longispiculus</i>	45.6 ± 10.4	2.0/1	1.3–2.5	0.21–0.66	Surface densely covered with small spines, sheath ends flared and enlarged
<i>T. concolor</i>	36.8 ± 7.4	2.2/1	1.1–1.5	0.25–0.3	Rod-shaped, densely covered with small spines
<i>Trichuris</i> sp.	40.5 ± 5.6	2.0/1	1.1–1.5	0.25–0.3	Surface covered with small spines; the beginning of the sheath flares and then gradually becomes thinner, with no spines at the end

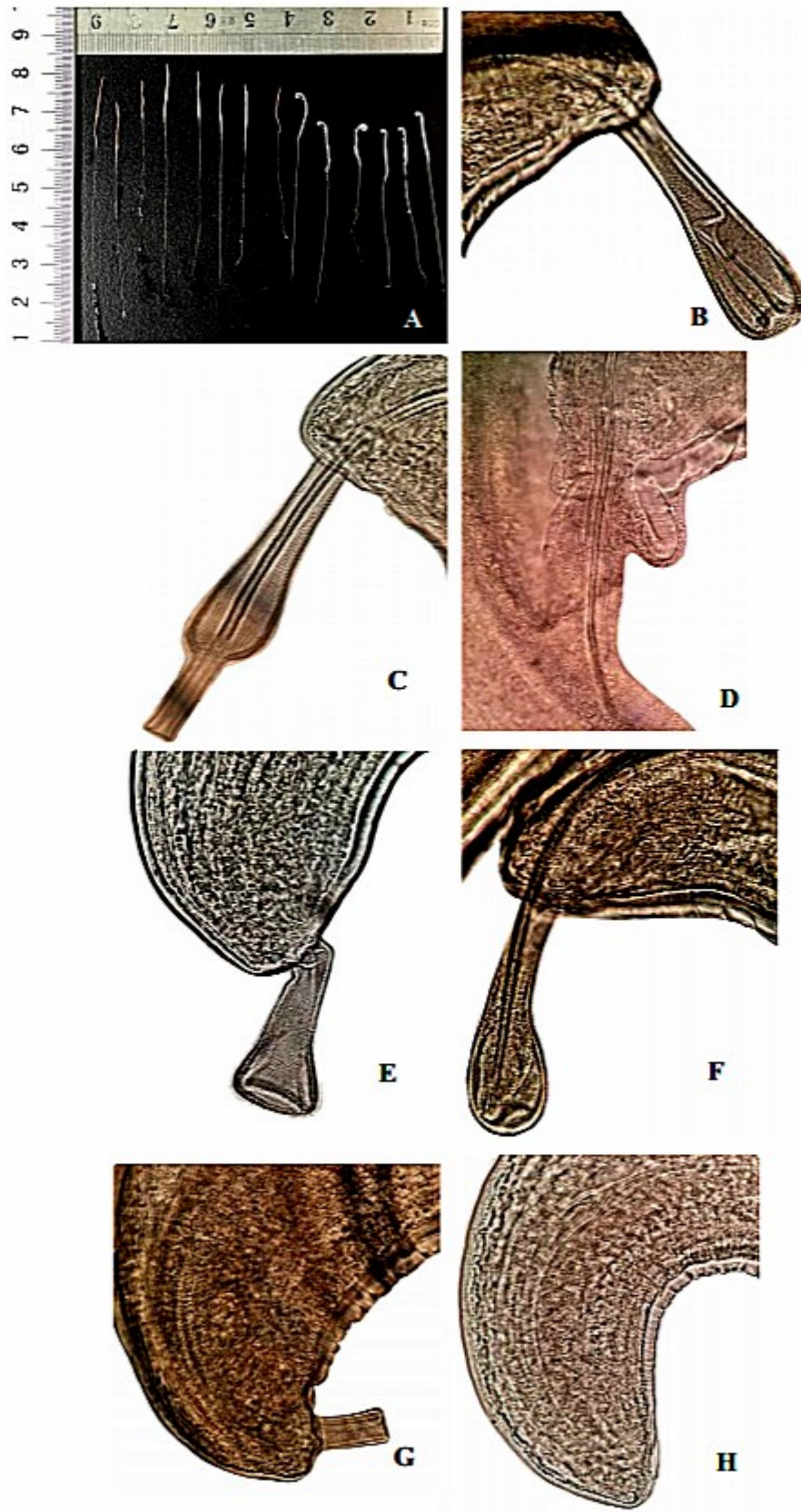


Fig.1. Morphological characteristics of the spine and spicule sheath of male specimens of *Trichuris* spp. from sheep from the Tianshan Mountains pastoral areas in China. A – gross morphology of *Trichuris* spp.; B – *T. lani*; C – *T. gazellae*; D – *T. ovina*; E – *T. discolor*; F – *T. longispiculus*; G – *T. concolor*; H – *Trichuris* sp.

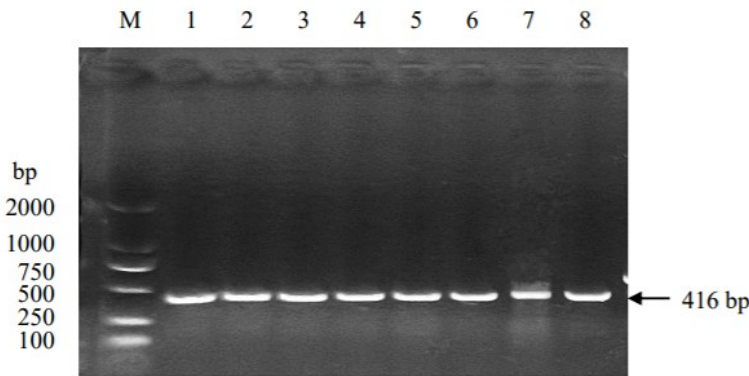


Fig. 2. Amplification of the mitochondrial *cox1* gene of *Trichuris* spp. M – standard DNA marker DL-2000 (2,000, 1,000, 750, 500, 250 and 100 bp); 1–7 – positive samples detected by RT-PCR; 8 – positive control

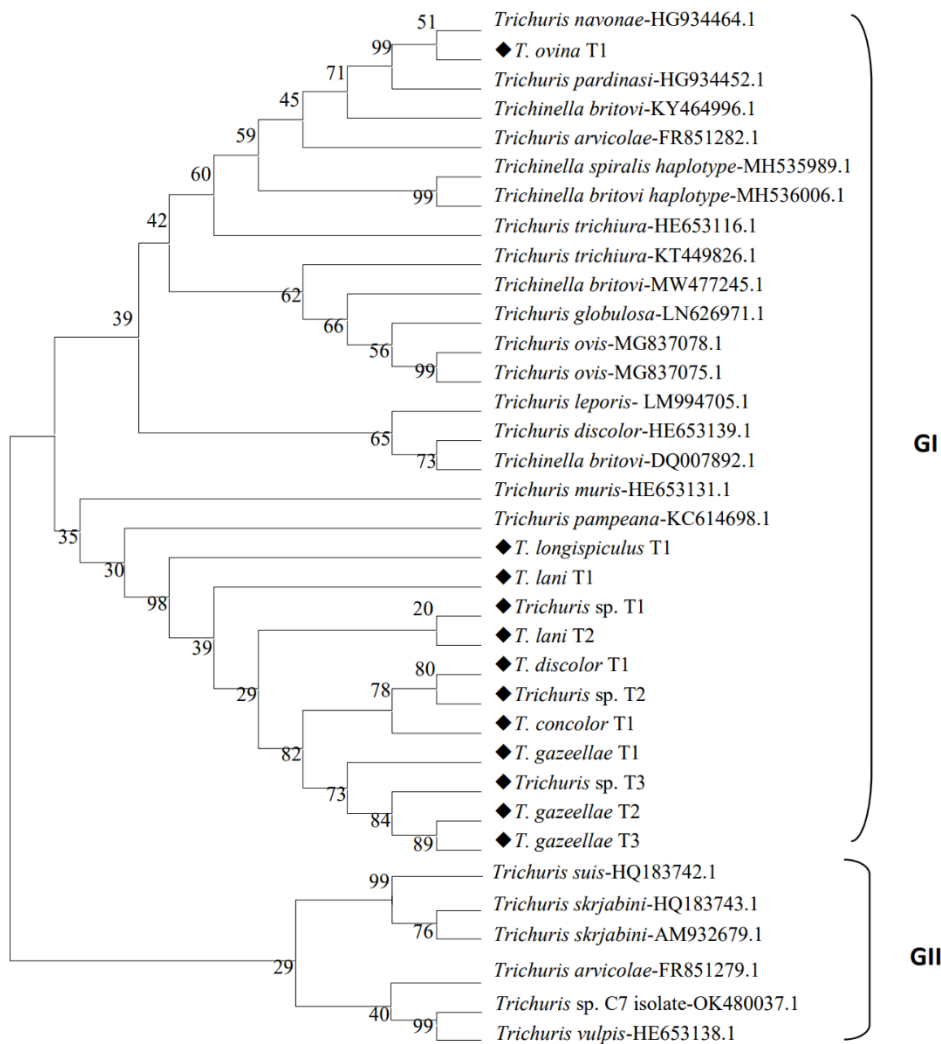


Fig. 3. Phylogenetic analysis of different species of the *Trichuris* genus based on the *cox1* gene using MEGA 7.0 software (neighbour-joining method, bootstrap values 1,000)

Discussion

Trichuris spp. are soil-transmitted helminths, which can naturally infect a wide range of hosts including rodents, cats, dogs, pigs, ruminants, humans, and non-

human primates and cause trichinosis (5, 25, 28). These helminths have strong resistance to degrading factors in the external environment because of the thick shells of their eggs, thus they can spread widely in domestic animals grazing on pasture (5, 7). Since most sheep in

Xinjiang are mainly grazed, the sheep are very heavily infected with digestive parasites. However, the infection status of sheep and prevalences of *Trichuris* spp. in them are still unknown.

The prevalences of *Trichuris* species and their infection intensities in sheep were investigated in the northern Xinjiang region. Existing studies have confirmed that there are approximately 24 species of *Trichuris* which parasitise ruminants (11, 26, 27). In this epidemiological survey, 6 documented species and 1 undefined species of *Trichuris* were found in sheep in Xinjiang and characterised. It is assumed that this breadth of species variety may be related to the frequency of trade in sheep and the geography. Among the nematodes detected, *T. gazellae* and *T. lani* were the dominant species, which accounted for 34.5% and 31.0% of the total number of parasites detected, respectively. The overall infection rate in sheep in the five grazing areas was 86.1% and the highest infection rate of 91.4% was in Altai. The prevalences and infection intensity of *Trichuris* spp. were significantly higher in lambs than those in adult sheep, which may be related to the incomplete development of the immune system in lambs or the immunity to the parasites of adult individuals.

Accurate identification of *Trichuris* spp. is essential for epidemiological investigations (15, 21). Generally speaking, the features of *Trichuris* spp. in females are indistinguishable for various species (17). In the present study, distinguishing *Trichuris* females corresponding to each species of male was difficult because of co-infection with several species. Therefore, females were not identified in this study, and the identification of *Trichuris* species was based on the key characteristics of the reproductive organs of adult males as is common practice (6, 11, 15, 20). Studies have revealed that the features such as the body length, the whip length to body length proportion, the length of the spicule and its sheath, and the morphometric characteristics of the spines of the spicule sheath are of high discriminatory value in differentiating *Trichuris* species (6, 11). The morphological characteristics of males of six known species and one undefined species of *Trichuris* were detailed through this survey, which enriched the body of taxonomic information of different species of *Trichuris* in ruminants. However, key characteristics are not present in some *Trichuris* species in the typical form, so it is necessary to combine morphological and molecular protocols for definitive and comprehensive identification (1, 8, 10, 12, 19, 21).

Phylogenetic analysis based on the *cox1* gene revealed that *Trichuris* species can be divided into two genetic clades. Clade I was where the six known species and the single undefined species investigated in this study were classified. However, obvious inter- and intra-species genetic diversity was displayed by *Trichuris*. Considering that the single *cox1* gene provides very limited genetic information, a new molecular typing method based on multiple genes, such as MLST, should

be developed for the identification of species and elucidation of the genetic diversity of *Trichuris* spp. in the future (14, 29).

In summary, this study revealed for the first time that six documented species and one undefined species of *Trichuris* were prevalent in sheep in the Tianshan pasture areas of Xinjiang, China. These observations provided valuable morphological and molecular data for the control of trichuriasis in sheep.

* These authors contributed equally to this study and should be considered co-first authors.

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