Parasitology

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Phylogenetic evidence of a possible *Trichuris globulosa* species complex in Arabian camels from Kuwait

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Research Article

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Abstract

During a 1-year study, *Trichuris* adults were obtained after necropsy of Arabian camels (*Camelus dromedarius*) from a slaughterhouse in Kuwait. Morphological and molecular identification was performed to confirm the identity of the *Trichuris* specimens obtained from *C. dromedarius*. Fifteen male *Trichuris* specimens were selected, and molecular identification was performed using mitochondrial cytochrome *c* oxidase subunit I, 12S ribosomal RNA, 16S ribosomal RNA genes and the nuclear internal transcribed spacer 2 (ITS2) region. Through phylogenetic analysis, 2 distinct groups were obtained using the mitochondrial genes, where group 1 showed a close relationship to *Trichuris globulosa* while group 2 showed a close relationship to *Trichuris ovis*, providing molecular evidence of a possible *T. globulosa* species complex. Additionally, the nuclear ITS2 region did not provide enough resolution to distinguish between the 2 groups of *Trichuris* specimens. Observation of morphological characters revealed variations in the shape of the male spicule sheath, where specimens present either a globular posteriorly truncated swelling or the absence of posteriorly truncated swelling. Moreover, the variations in male spicule sheath does not corroborate with the results of molecular data, suggesting the limited use of this character for identification of *T. globulosa*. In conclusion, molecular analysis suggests a possible species complex in *T. globulosa*, with the mitochondrial genetic markers successfully differentiating between the 2 groups. The limited use of the male spicule sheath as a diagnostic character for identification of *T. globulosa* is suggested.

Introduction

The genus *Trichuris* Roederer, 1761 includes numerous species that parasitize humans and animals, and have a cosmopolitan distribution (Doležalová *et al*., 2015). Species identification has generally been based on morphological characters and morphometrical measurements, including host species as a guide (García-Sánchez *et al*., 2019). Molecular phylogenetics are also providing evidence of species complexes within *Trichuris*, where it is highly possible that *Trichuris* can harbour cryptic species due to their wide geographic distribution and capability to parasitize variety of host species (Callejón *et al*., 2012; Ravasi *et al*., 2012; Robles *et al*., 2014; Rivero *et al*., 2021). In ruminants, more than 23 species of *Trichuris* have been described, including *Trichuris globulosa* (Linstow, 1901), *Trichuris ovis* (Abildgaard, 1795), *Trichuris skrjabini* Baskakov, 1924 and *Trichuris discolor* (Linstow, 1906) (Knight, 1974; Cutillas *et al*., 1995). In camels specifically, 9 species of *Trichuris* have been found. They are *Trichuris barbetonensis* Ortlepp, 1937, *T. globulosa*, *Trichuris infundibulus* (Linstow, 1906), *Trichuris lani* (Artjuch, 1948), *T. skrjabini*, *Trichuris tenuis* Chandler, 1930, *Trichuris vulpis* (Froelich, 1798), *Trichuris raoi* Alwar and Achutan, 1960 and *Trichuris cameli* (Chandler, 1930; Knight, 1971; Sazmand and Joachim, 2017). Aside from parasitizing camels, some species have also been found in other hosts, for example, *T. globulosa* have also been found in sheep and goats, while *T. skrjabini* have been reported in other large and small ruminants (sheep, deer, goats, elk) (Knight, 1971). Similarly, *T. ovis* and *T. discolor*, which are parasites of sheep and cattle respectively, were also found in many hosts globally (Chandler, 1930; Knight, 1971; Cutillas *et al*., 1995; Oliveros *et al*., 2000; Wang *et al*., 2012).

Based on male morphology, the spicule sheath, spicule lengths and spines on the spicule sheath are used as the main criteria for differentiating *T. globulosa* and *T. ovis*. *Trichuris globulosa* was described as having the spicule sheath with a globular posteriorly truncated swelling, with spines longer on the swelling than the rest of the spicule sheath, while in *T. ovis*, 2 morphological types of spicule sheath were observed: one was having a spherical bulge in the distal part of the spicule sheath (Yevstafieva *et al*., 2018) and the other with a globular posteriorly truncate sheath (Sarwar, 1945; Cutillas *et al*., 1995). Although spicule lengths have been cited as a useful criterion by Chandler (1930), overlapping lengths were previously reported

from specimens isolated from various hosts, limiting their discriminatory power (Chandler, 1930; Callejón *et al.*, 2015*a*). In *Trichuris* females, the structure of the vulva is mainly used for species differentiation. Baylis (1932) found consistent differences between the vulva structure of *T. globulosa* and *T. ovis* (Baylis, 1932; Yevstafieva *et al.*, 2018). However, other studies further revealed that the vulva structure cannot be used as a distinguishing morphological character for species differentiation of *T. globulosa* from *T. ovis* (Callejón *et al.*, 2015*a*). Thus, the vagueness of morphological characters and overlap of morphometrical measurements render species identification challenging. Additionally, the existence of *T. globulosa* and *T. ovis* in similar hosts can further complicate morphological identification due to host-induced variation and phenotypic plasticity.

With the challenges in morphological identification, molecular genetic markers have aided in *Trichuris* species identification and have been used in the investigation of genetic variability among different hosts. These include the mitochondrial cytochrome *c* oxidase subunit I (*COI*), cytochrome b (*cytB*), 16S ribosomal RNA (rRNA), complete mitochondrial genomes and nuclear internal transcribed spacer 1 and 2 regions (ITS1 and ITS2) (Callejón *et al.*, 2012, 2015*b*; Hawash *et al.*, 2015; Rivero *et al.*, 2020; Rivero *et al.*, 2022). Among *T. globulosa* and *T. ovis* isolated from sheep and goats, Oliveros *et al.* (2000) revealed that these 2 species are synonymous using the ITS2 region. However, through a morpho-biometrical and molecular study using mitochondrial *COI* and *cytB* markers, it was concluded that *T. globulosa* constitute a different genetic lineage to *T. ovis* (Callejón *et al.*, 2015*b*). Additionally, Salaba *et al.* (2013) revealed that specimens initially morphologically identified as *T. globulosa* were later molecularly identified as *T. discolor* using the nuclear ITS regions (Salaba *et al.*, 2013).

Here, to increase the clarity of the species status of *T. globulosa*, 3 mitochondrial genetic markers (*COI*, 12S and 16S rRNA) and the nuclear ITS2 region were used for the molecular identification of *Trichuris* species from *Camelus dromedarius* (Arabian camel) in Kuwait. Through molecular analyses, we provide phylogenetic evidence to suggest a probable *Trichuris* species complex with the specimens obtained from *C. dromedarius* and suggest that the morphology of the male spicule sheath is not a reliable character for species discrimination.

Materials and methods

Morphological measurements and identification

Fifteen *Trichuris* males obtained after necropsy of 3 *C. dromedarius* hosts from a slaughterhouse in Kuwait were used as representative specimens for morphological and molecular identification. *Trichuris* males were selected as representatives for this study as the morphological characters are useful for species identification. In the Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, individual specimens were washed thoroughly in sterile distilled water, and morphological measurements of the body length and body width were obtained using a stereomicroscope (Olympus SZ51). The posterior and anterior portions of each specimen were separated, and the posterior portion was subsequently mounted on a microscope slide with lactophenol. Under an inverted compound microscope (ZEISS Primovert), morphological measurements and characters of the posterior portion (spicule length and width, spicule sheath shape, spicule sheath length and width) were performed following Callejón *et al.* (2015*a*). The middle portion of the specimen that was not mounted on a microscope slide with lactophenol was maintained in 70% ethanol and at −20°C for preservation prior to molecular identification.

Molecular identification and phylogenetic analyses

Genomic DNA extraction and polymerase chain reaction (PCR) amplification

Each specimen was individually washed in sterile water and transferred into a 1.7 mL microcentrifuge tube. Total genomic DNA (gDNA) was extracted using the DNeasy® Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations.

PCR amplification was performed for 4 genetic markers – the mitochondrial *COI*, 12S rRNA, 16S rRNA genes and the nuclear ITS2 region. A T100™ thermocycler (Bio-Rad, California, USA) was used for amplification with a final PCR volume of 30 µL. Each reaction contained 15 µL of 2× i-Taq™ mastermix (iNtRON Biotechnology, Gyeonggi, South Korea), 5–10 µm of each primer and 2 µL of template DNA. The thermocycling profiles and primers used follow the protocol by Callejón *et al.* (2015*a*) for the *COI* gene and ITS2 region, while the protocols for the mitochondrial 12S and 16S rRNA genes follow Chan *et al.* (2020). Amplicons were visualized on 1% agarose gel stained with SYBR™ Safe (Life Technologies, California, USA). Successful amplicons were purified with the Geneaid PCR Purification kit (Geneaid Biotech Ltd, Taipei, Taiwan) using the manufacturer's recommendations. The purified DNA samples were sent for Sanger sequencing by Macrogen (Seoul, South Korea) with the same primers used for PCR amplification.

Phylogenetic analyses

Electropherograms obtained after Sanger sequencing were checked using Bioedit 7.0, and the sequences were aligned using ClustalX 2.1 with reference *Trichuris* and *Trichinella* (used as outgroup) sequences obtained from the NCBI database (Hall, 1999; Thompson *et al.*, 2002). The reference sequences used are presented in Supplementary Table 1. The aligned sequences were checked using Bioedit 7.0 and used to construct neighbour-joining (NJ) and maximum-likelihood (ML) phylogenetic trees in MEGA X (Kumar *et al.*, 2018). NJ phylogenetic tree construction used the pairwise nucleotide distance (*p*-distance) model while the ML phylogenetic tree used a best-fit nucleotide substitution model with 1000 bootstrap iterations for tree support. FigTree 1.3.1 was used for phylogenetic tree aesthetics (Rambuat, 2009). The sequences obtained in this study with their NCBI accession numbers, and their corresponding specimen and host identity numbers are listed in Table 1.

Genetic distance calculation was performed in MEGA X, where the *p*-distance values for each genetic marker were calculated using the aligned sequences (Kumar *et al.*, 2018). The values were converted into percentage distance based on sequence dissimilarity.

Statistical analysis of morphological measurements

Statistical analysis of the morphological measurements was performed to determine the suitability of the morphological characters for identification between the 2 molecular groups (based on the results after molecular identification, 2 molecular groups were identified). The 7 morphological characters used were – posterior body length, posterior body width, spicule length, spicule width, spicule width at the proximal, spicule sheath length and the maximum spicule sheath width. Comparisons between groups for each morphological character were visualized with a boxplot. The suitability of the characters was then examined by the statistical significance in the means between the 2 groups using either the Mann–Whitney or the independent *t* test (depending on whether the data were normally distributed). A P value <0.05 indicates that the means were significantly different. All statistical analysis and boxplot visualization were performed in R Studio version 1.2.5033 (RStudio Team, 2021).

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Table 1. *Trichuris* specimens from *Camelus dromedarius* used for analysis

		NCBI accession number			
Host ID	Specimen ID	*COI*	12S	16S	ITS2
T3	T3-7M	OQ535010	OQ550226	OQ534994	OQ550211
	T3-8M	OQ535011	OQ550227	OQ534995	OQ550212
	T3-9M	OQ535012	OQ550228	OQ534996	OQ550213
T4	T4-7M	OQ535013	OQ550229	OQ534997	OQ550214
	T4-8M	OQ535014	OQ550230	OQ534998	OQ550215
	T4-9M	OQ535015	OQ550231	OQ534999	OQ550216
T5	T5-8M	OQ535016	OQ550232	OQ535000	OQ550217
	T5-9M	OQ535017	OQ550233	OQ535001	OQ550218
	T5-10M	OQ535018	OQ550234	OQ535002	OQ550219
	T5-11M	OQ535019	OQ550235	OQ535003	OQ550220
	T5-12M	OQ535020	OQ550236	OQ535004	OQ550221
	T5-13M	OQ535021	OQ550237	OQ535005	OQ550222
	T5-14M	OQ535022	OQ550238	OQ535006	OQ550223
	T5-15M	OQ535023	OQ550239	OQ535007	OQ550224
	T5-16M	OQ535024	OQ550240	OQ535008	OQ550225

Results

Morphology of Trichuris *male spicule sheath*

The specimens obtained from Arabian camels were morphologically identified as *Trichuris*, with morphological variations observed in the male spicule sheath. The 2 different types of spicule sheaths are – the first type presents the globular posteriorly truncated swelling (Fig. 1a and b), while the second type does not present a globular posteriorly truncated swelling (Fig. 1c and d). Specimens with both types of spicule sheath were present in host T5, whereas hosts T3 and T4 had only specimens with the spicule that did not present a globular posteriorly truncated swelling.

Molecular identification of Trichuris *species from camels*

Based on the molecular phylogenies of the *Trichuris* specimens obtained from camels, the specimens were grouped into 2 distinct clusters using the mitochondrial *COI*, 12S rRNA and 16S rRNA genes. The phylogenies across the 3 mitochondrial genetic

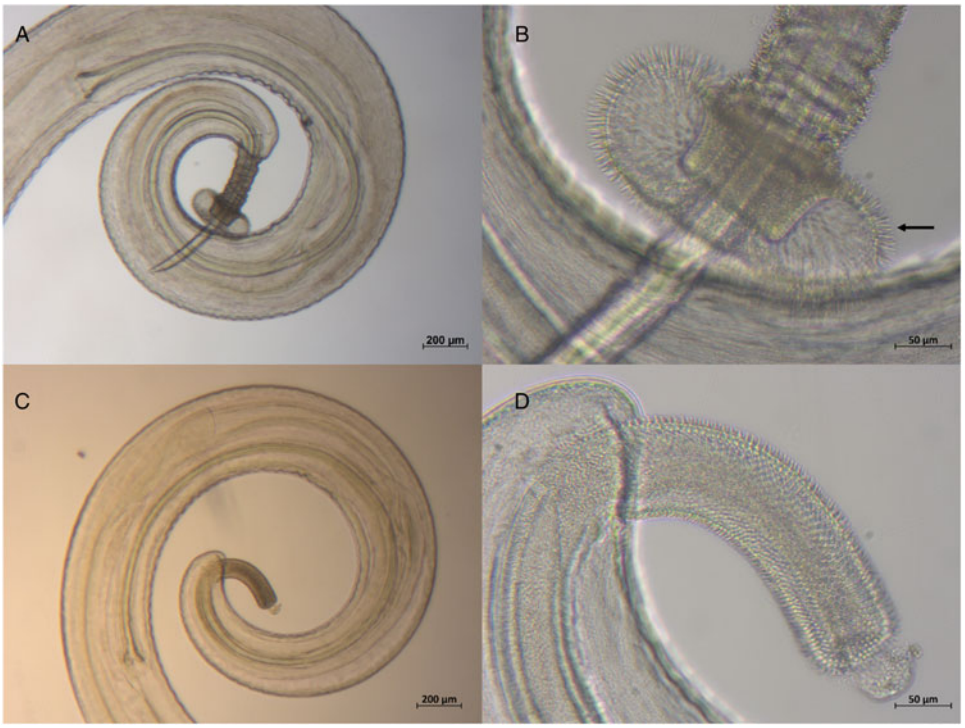

Figure 1. Male spicule sheath morphology of *Trichuris* species from camels. In (a and b), a globular posteriorly truncated swelling with spines on the swelling (indicated by the black arrow) longer than the rest of the spicule sheath is shown. In (c and d), the spicule sheath does not have a globular posteriorly truncated swelling.

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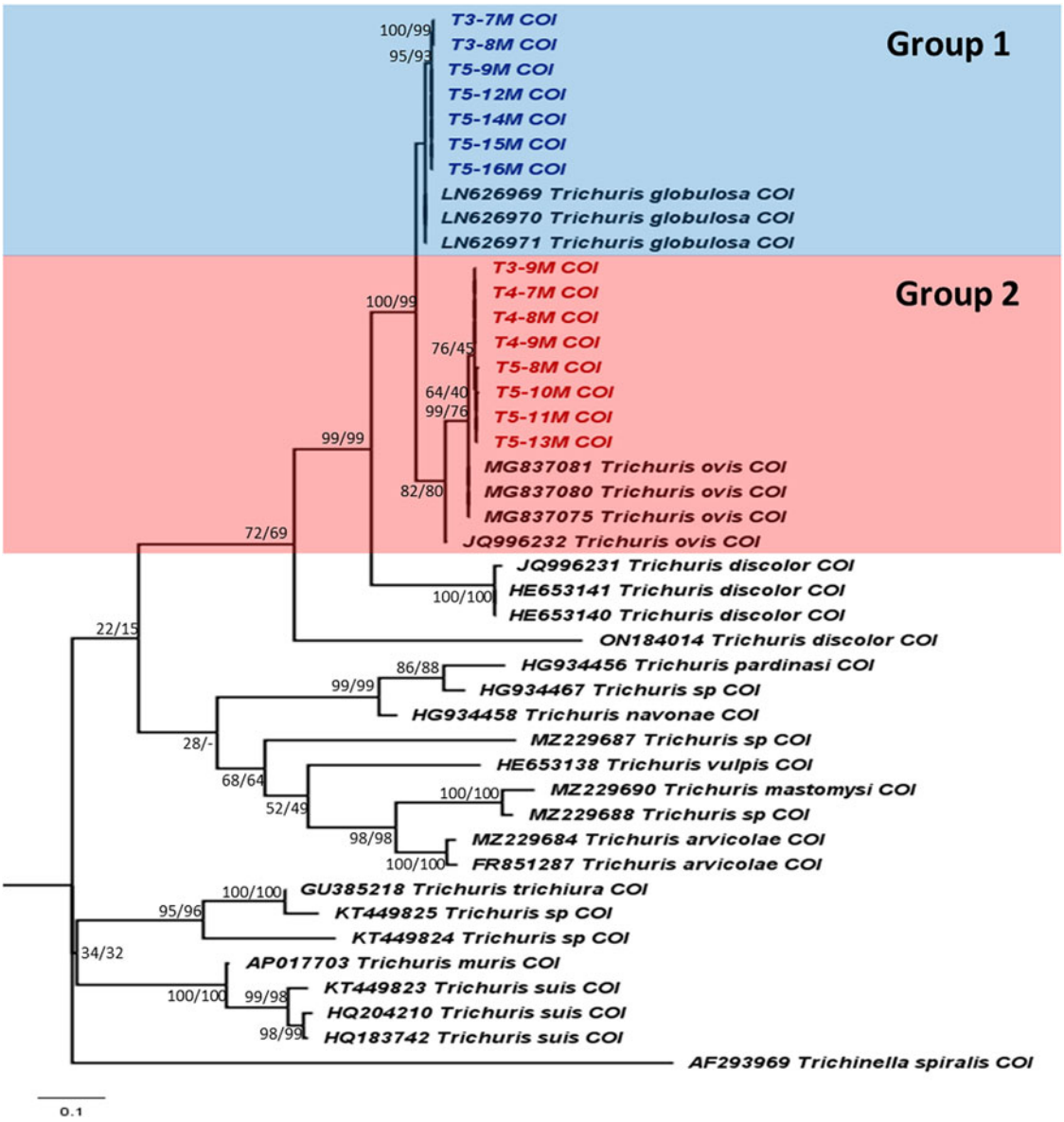

Figure 2. Phylogeny of *Trichuris* spp. based on the mitochondrial *COI* gene. The phylogenetic tree was inferred using the ML and NJ algorithms in MEGA X. The numbers at the nodes indicate bootstrap support obtained through 1000 replications (ML/NJ). *Trichuris* specimens in this study are indicated in blue (group 1) and red (group 2) colours. The final alignment used for phylogenetic tree construction was 390 bp in length.

markers were congruent, revealing 2 distinct genotypes with strong bootstrap support. Figs 2–4 present the phylogenies inferred from the mitochondrial genetic markers. In the first cluster (group 1), the specimens were phylogenetically placed together with *T. globulosa*, while in the second cluster (group 2), the specimens were closely placed with *T. ovis*. Additionally, 3 *Trichuris* specimens isolated from the same host (T5) were found in groups 1 and 2. Of the 9 specimens isolated from T5, 5 were phylogenetically placed in group 1 while 4 were placed in group 2. Conversely, no distinct groups were obtained based on the nuclear ITS2 phylogeny (Fig. 5). All 15 specimens were clustered together with the reference *T. globulosa* sequences, with *T. ovis* in a separate clade.

Compared among the other *Trichuris* species, the nuclear and mitochondrial phylogenies obtained showed that *T. discolor* was a sister group to *T. globulosa* and *T. ovis*, supporting a clade of *Trichuris* species belonging to ruminants.

Genetic variability among T. globulosa

As shown in Table 2, the genetic distance obtained from the ITS2 region ranged from 0 to 0.49% within our *Trichuris* specimens and the reference *T. globulosa* sequences, while the genetic distance between *T. globulosa* and *T. ovis* ranged from 3.47 to 3.96%. For the mitochondrial genetic markers, genetic distances between groups 1 and 2 ranged from 6.32 to 7.42% with the *COI* gene. The genetic distances for the 12S and 16S rRNA genes were smaller, ranging from 3.03 to 3.86% and 1.35 to 2.70%, respectively. Within group genetic distances were also smaller than between group genetic distances for each of the mitochondrial genetic markers.

Comparison of morphological characters between groups 1 and 2

Based on the molecular results from the *Trichuris* specimens obtained in this study, 2 molecular groups were present. Table 3 presents the morphological characteristics and measurements of the *Trichuris* specimens used in this study, allocated based on their molecular groupings. Of the 7 morphological characters that had continuous data, statistical analysis revealed that there was significant difference ($P = 0.038$) between the means of the spicule sheath length, with group 1 averaging a length of 0.656

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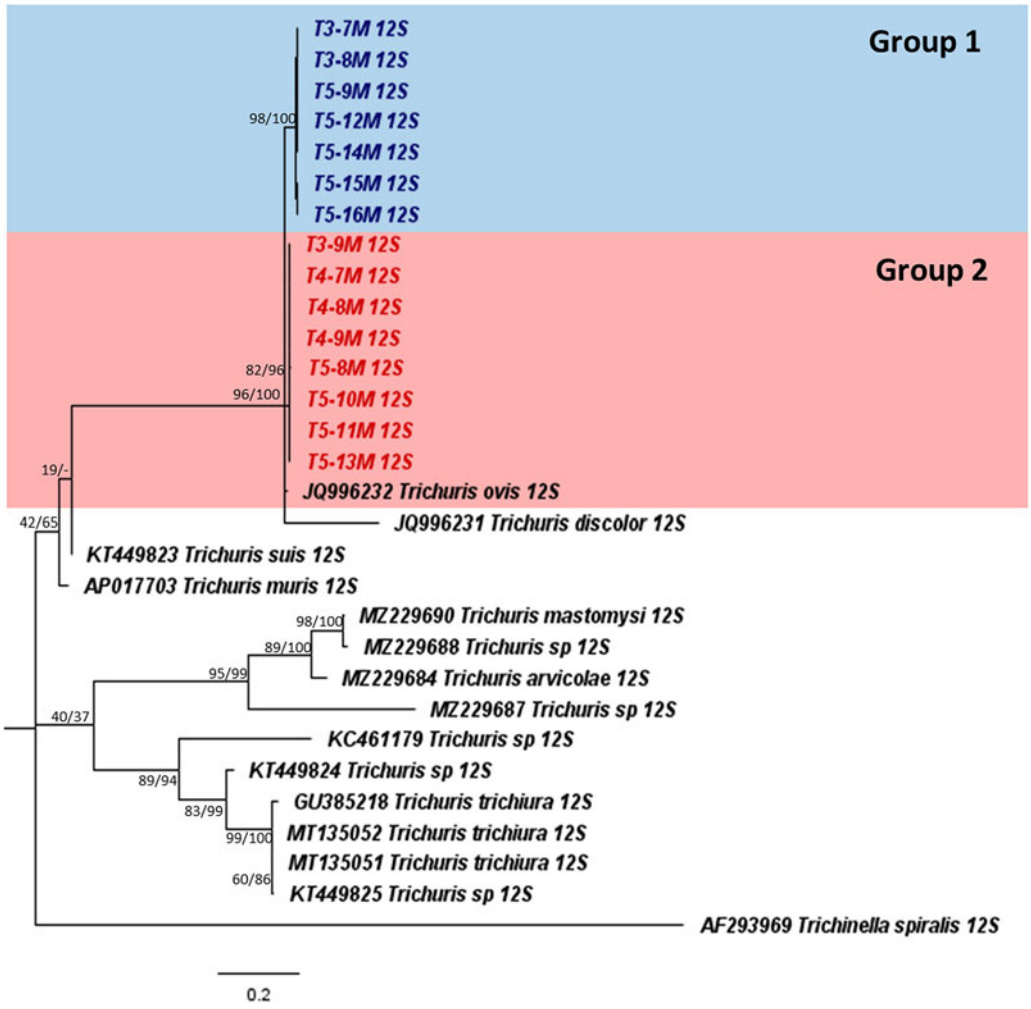

Figure 3. Phylogeny of *Trichuris* spp. based on the mitochondrial 12S rRNA gene. The phylogenetic tree was inferred using the ML and NJ algorithms in MEGA X. The numbers at the nodes indicate bootstrap support obtained through 1000 replications (ML/NJ). *Trichuris* specimens in this study are indicated in blue (group 1) and red (group 2) colours. The final alignment used for phylogenetic tree construction was 420 bp in length.

mm and group 2 with 0.364 mm (Fig. 6). No statistical significance was observed with the other 6 morphological characters.

Additionally, both types of male spicule sheath were found in each molecular group. The morphological variations of the male spicule sheath did not corroborate with the molecular phylogenies, thus limiting the use of the male spicule sheath as a diagnostic character for species identification of *T. globulosa*.

Discussion

Our findings demonstrated the use of the mitochondrial *COI*, 12S rRNA, 16S rRNA genes and the nuclear ITS2 region for molecular identification of *Trichuris* from *C. dromedarius*. Two distinct clusters of *Trichuris* were obtained from the mitochondrial phylogenies (group 1 closer to *T. globulosa* while group 2 closer to *T. ovis*), providing molecular evidence of a possible *T. globulosa* species complex. Also, 2 forms of male spicule sheath that did not corroborate with molecular data were observed, suggesting the limited use of the male spicule sheath as a morphological character for species identification.

Molecular evidence of possible T. globulosa *species complex*

With the *T. globulosa* specimens obtained in this study, molecular phylogenies suggest the possibility of a *T. globulosa* species complex. Firstly, the phylogenies obtained from the mitochondrial genes revealed 2 distinct clusters within *T. globulosa*. Secondly, no distinction between groups 1 and 2 was observed in the nuclear ITS2 phylogeny.

The species status of *T. globulosa* has been questioned by some authors, with mitochondrial genetic markers revealing that *T. globulosa* and *T. ovis* are different species, while morphology, isoenzymatic and the nuclear ITS2 region support the synonymy of both species (Cutillas *et al.*, 1995; Oliveros *et al.*, 2000). Based on the nuclear ITS2 region, Oliveros *et al.* (2000) demonstrated that no sequence variation was present between *T. ovis* and *T. globulosa* isolated from sheep and goats (Oliveros *et al.*, 2000). Contrarily, using the mitochondrial *cytB* and *COI* genes, Callejón *et al.* (2015*a*) showed that *T. globulosa* isolated from *C. dromedarius* in Iran and *T. ovis* isolated from *Ovis aries* in South Africa were 2 distinct species (Callejón *et al.*, 2015*a*). Moreover, the overlap in morphological characters such as spicule lengths and size between the 2 species render accurate species identification challenging (Knight, 1974; Callejón *et al.*, 2015*a*).

Here, with the 2 distinct groups present based on the mitochondrial genetic markers, the *T. globulosa* specimens obtained from this study could present a possible species complex. Specimens obtained from the same host were also found in groups 1 and 2. Moreover, specimens from each group could not be differentiated based on their morphological characters. Species complexes are no stranger among helminths, and molecular evidence has also facilitated the discovery of closely related species that are morphologically similar but genetically different (Callejón *et al.*, 2013; Xie *et al.*, 2018). Among *Trichuris*, Rivero *et al.* (2021)

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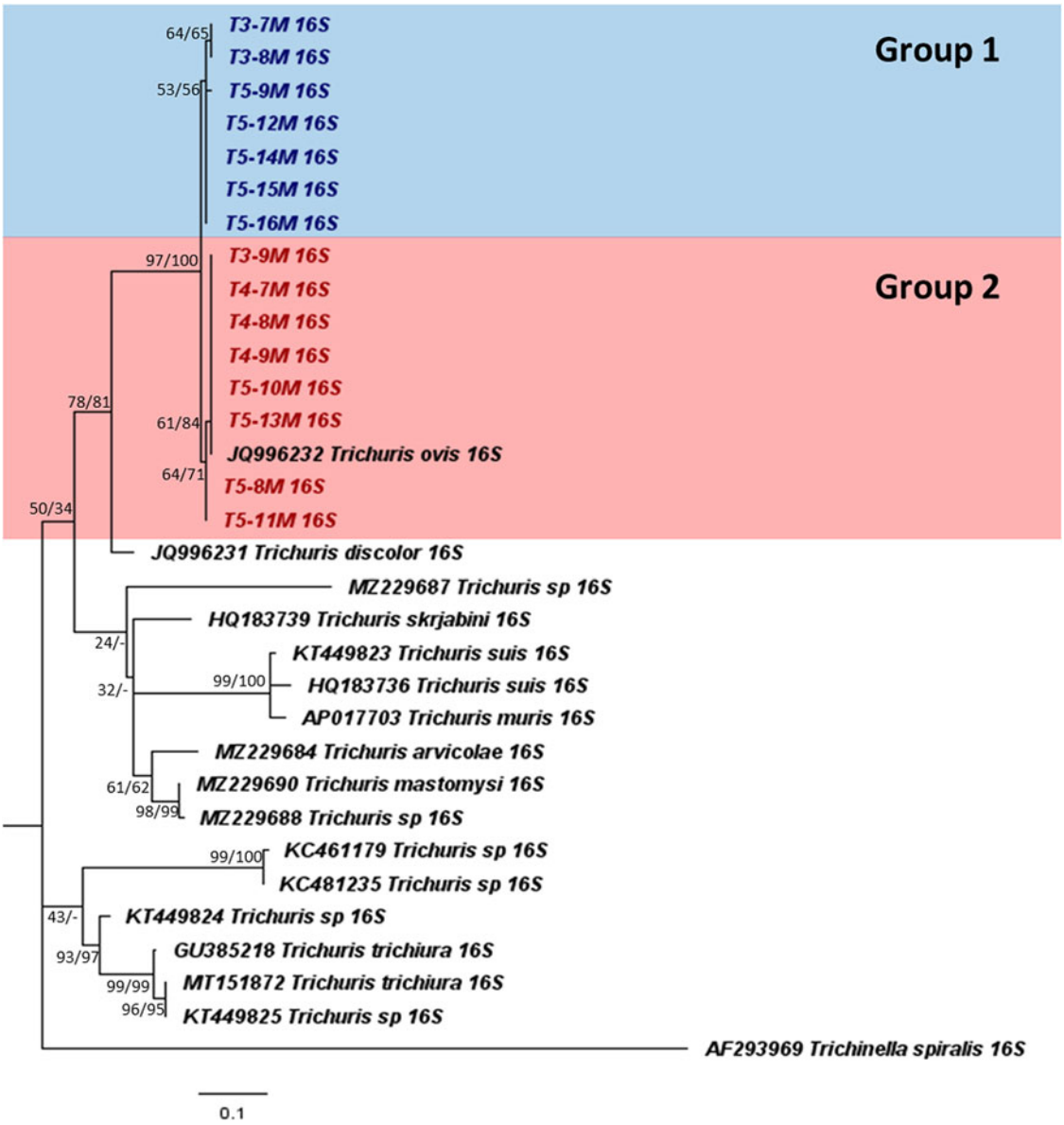

Figure 4. Phylogeny of *Trichuris* spp. based on the mitochondrial 16S rRNA gene. The phylogenetic tree was inferred using the ML and NJ algorithms in MEGA X. The numbers at the nodes indicate bootstrap support obtained through 1000 replications (ML/NJ). *Trichuris* specimens in this study are indicated in blue (group 1) and red (group 2) colours. The final alignment used for phylogenetic tree construction was 190 bp in length.

revealed the presence of 2 different genotypes corresponding to different lineages within *Trichuris trichiura* obtained from humans and non-human primates using the mitochondrial *COI* and *cytB* genetic markers, suggesting the existence of a *T. trichiura* species complex (Rivero *et al.*, 2021). The mitochondrial genetic markers are known to contain high sequence variation for cryptic species delimitation, with the ability to differentiate closely related members within species complexes (Thaenkham *et al.*, 2022). Moreover, they have proven to be useful for *Trichuris* species differentiation in instances where specimens were unable to be morphologically identified to the species level (Callejón *et al.*, 2016; Di Filippo *et al.*, 2020). Contrarily, although the nuclear ITS2 region can be used for species differentiation, the genetic marker is relatively conserved among members of species complex and closely related species partly due to concerted evolution in play (Thaenkham *et al.*, 2022). Likewise, low levels of sequence variation were observed for our specimens using the ITS2 region, with no distinction between groups 1 and 2, supporting the possibility that the 2 groups are genetically closely related. Also, genetic differences obtained using the full-length ITS2 region between *T. globulosa* and *T. ovis* ranged from 3.47 to 3.96%, disagreeing with previous studies suggesting that *T. globulosa* and *T. ovis* are synonymous.

Through congruence of the mitochondrial phylogenies supporting distinction between the 2 groups of *T. globulosa*, insufficient ITS2 sequence variation to differentiate between groups, along with the complicated species status between *T. globulosa* and *T. ovis* based on previous studies, a species complex is thus plausible.

Limited use of morphological characters for T. globulosa *species identification*

From the *T. globulosa* specimens used in this study, 2 morphological variations of the male spicule sheath were observed, and they did not correspond to the groups based on the mitochondrial phylogenies (groups 1 and 2). The shape and spines of the male spicule sheath has been used as a criterion distinguishing *T. globulosa* from other *Trichuris*, particularly with the closely related *T. ovis* (Sarwar, 1945; Cutillas *et al.*, 1995). Previous studies

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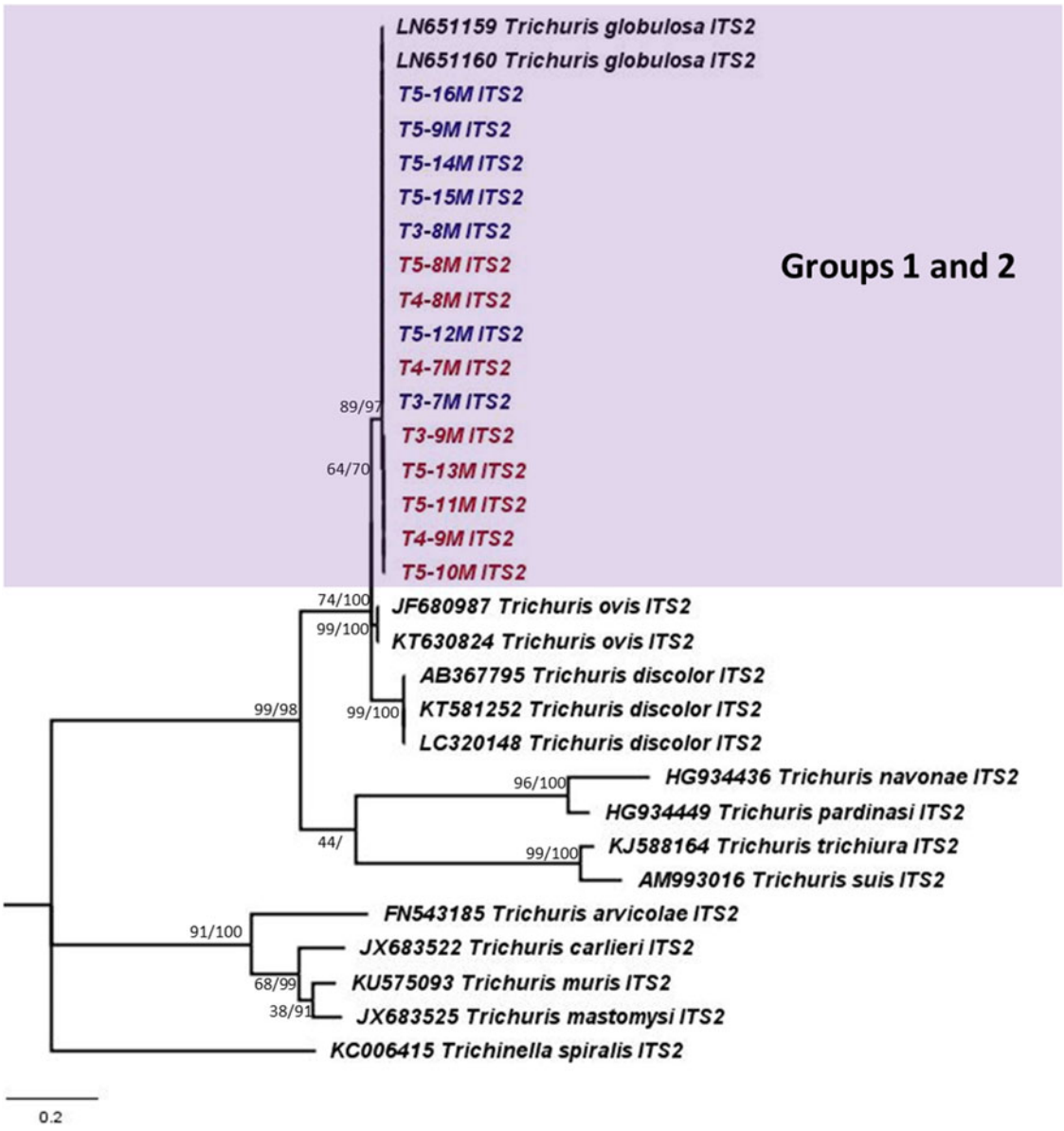

Figure 5. Phylogeny of *Trichuris* spp. based on the nuclear ITS2 region. The phylogenetic tree was inferred using the ML and NJ algorithms in MEGA X. The numbers at the nodes indicate bootstrap support obtained through 1000 replications (ML/NJ). *Trichuris* specimens in this study are indicated in blue (group 1) and red (group 2) colours. The final alignment used for phylogenetic tree construction was 320 bp in length.

Table 2. Genetic distance (% difference) comparison between genetic markers for *Trichuris globulosa* and *Trichuris ovis*

Genetic marker/group	*COI*	12S	16S	ITS2
Group 1	0–1.10 (0.48)	0–0.55 (0)	0–1.35 (1.00)	NA
Group 2	0–4.396 (1.18)	0–1.93 (1.00)	0–0.68 (0)	NA
Group 1 vs group 2	6.32–7.42 (6.82)	3.03–3.86 (3.40)	1.35–2.70 (2.17)	NA
Groups 1 and 2 vs *T. globulosa*	NA	NA	NA	0–0.49 (0)
Groups 1 and 2 vs *T. ovis*	NA	NA	NA	3.47–3.96 (3.61)

The average genetic distances are indicated in parentheses, and NA indicates not applicable.

have reported that *T. globulosa* isolated from camels, sheep and goats present a male spicule sheath with a globular posteriorly truncated swelling with spines on the swelling longer than the rest of the spicule sheath (Sarwar, 1945; Cutillas *et al.*, 1995; Callejón *et al.*, 2015*a*; Yevstafieva *et al.*, 2018). Cutillas *et al.* (1995) also added that while *T. ovis* does not have a posterior swelling, some may also present a globular posteriorly swelling. However, the spines on the globular posterior swelling are shorter than or equal to the rest of the spicule sheath, rendering *T. ovis* being morphologically different from *T. globulosa*.

Species identification of *Trichuris* using morphological characters is often challenging for taxonomists due to similar and overlapping characters for both males and females. Distinct *Trichuris* genotypes were found from morphologically similar *Trichuris* species isolated from rodents (Robles *et al.*, 2014). For *T. globulosa*, aside from spicule sheath, spicule lengths have also been used as another criterion. However, overlapping lengths have been observed between *T. globulosa* and *T. ovis* (Callejón *et al.*,

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Table 3. Morphological measurements of *Trichuris* from each group used in this study

Morphological characters	Group 1	Group 2	*P* value
Posterior body length	14.241 (11.684–17.926)	13.070 (9.771–17.461)	0.397
Posterior body width	0.477 (0.336–0.598)	0.424 (0.334–0.563)	0.536
Spicule length	4.003 (3.437–4.575)	3.811 (3.199–5.007)	0.472
Spicule width	0.038 (0.030–0.048)	0.040 (0.030–0.056)	0.679
Spicule width at proximal	0.107 (0.083–0.134)	0.088 (0.066–0.119)	0.088
Spicule sheath length	0.656 (0.447–1.027)	0.364 (0.256–0.508)	0.038*
Spicule sheath width	0.114 (0.058–0.218)	0.103 (0.075–0.242)	0.222
Spicule sheath with no globular posteriorly truncated swelling	T3-7M, T3-8M, T5-9M, T5-12M, T5-15M, T5-16M	T3-9M, T4-7M, T4-8M, T4-9M, T5-8M, T5-10M, T5-13M	NA
Spicule sheath with globular posteriorly truncated swelling	T5-14M	T5-11M	NA

Measurements are given in mm. The mean values are shown, while the minimum and maximum values are in parentheses.
An asterisk (*) indicate statistical significance ($P < 0.05$) between the means of groups 1 and 2.

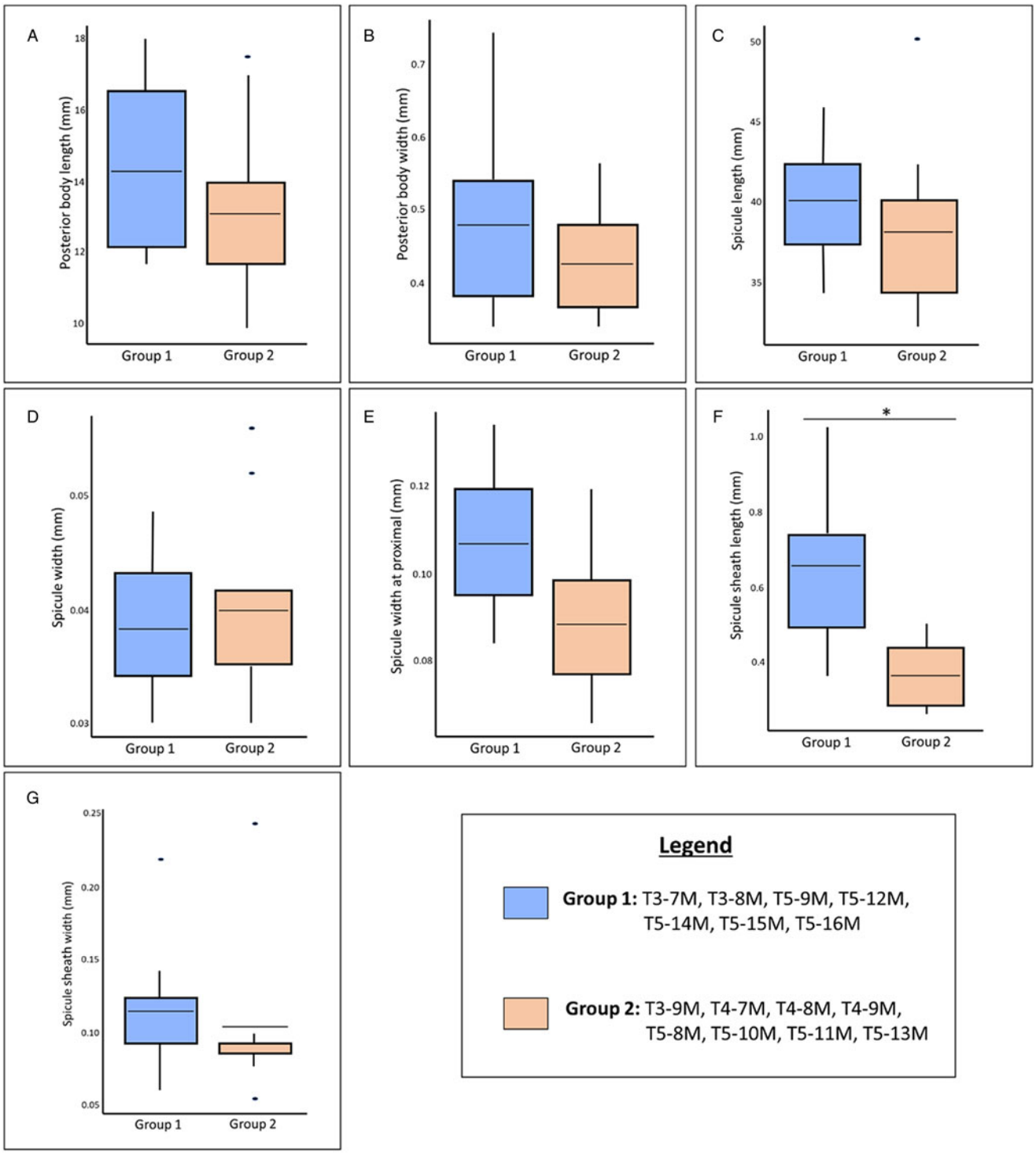

Figure 6. Boxplot of the 7 morphological characters of the *Trichuris* specimens. The morphological characters are (a) posterior body length, (b) posterior body width, (c) spicule length, (d) spicule width, (e) spicule width at proximal, (f) spicule sheath length and (g) spicule sheath width. An asterisk (*) indicates statistical significance ($P < 0.05$) between the means of groups 1 and 2. The black solid dots indicate outliers. The solid line of the boxplot indicates the mean value obtained from the morphological measurements.

2015*a*). Additionally, assumptions of species identity are often based on the types of hosts that they are found in, but evidence has revealed their capacity to infect wide varieties of hosts for some species. As both *T. globulosa* and *T. ovis* have been found in similar hosts such as camels, sheep and goats, host-induced phenotypic plasticity can be present, thus limiting the use of morphological characters. Here, with the morphological variations of the male spicule sheath observed among *T. globulosa*, along with their capability to infect various hosts, identification of *T. globulosa* requires reliance on molecular genetic markers for accurate species identification.

Limitations

Firstly, only male *Trichuris* specimens were selected for morphological and molecular analysis in this study. The inclusion of *Trichuris* females in the analysis could provide more information regarding the genetic variation among the *Trichuris* specimens

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obtained. Secondly, similar to any molecular study, the accuracy of species identification is subjected to the accuracy of reference sequences in the database. The sequences included for comparison were thus limited due to the small number of sequences and molecular studies that have been performed on *T. globulosa*. Thirdly, as compared to the phylogeny obtained using the ITS region by Betson *et al.* (2015) and Cavallero *et al.* (2015), *Trichuris suis* and *T. trichiura*, although being morphologically similar and closely related, they each formed their own subclade within the monophyletic clade containing both species (Betson *et al.*, 2015; Cavallero *et al.*, 2015). Contrarily in our ITS2 phylogeny, no genetic difference was observed between the *T. globulosa* groups 1 and 2 specimens. Thus, a nuclear genetic marker from another loci or the whole genome can be utilized to further investigate the phylogenetic relationships among *T. globulosa*. Lastly, as the *Trichuris* specimens were obtained from Arabian camels from the slaughterhouse, sequence variation of *Trichuris* between localities could not be compared.

Conclusion

Mitochondrial phylogenies revealed 2 groups of *T. globulosa*, while the nuclear ITS2 region did not have sufficient sequence variation to discriminate between the 2 *T. globulosa* groups. Molecular evidence thus suggests the possibility of a *T. globulosa* species complex, with potential implications on the administration of the appropriate anthelmintic treatment for ruminants. Additionally, 2 morphological variations of the male spicule sheath were revealed, suggesting the limited use of the male spicule sheath as a diagnostic character for species identification of *T. globulosa*.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182024000374.

Data availability statement. The data that support the findings of this study are available from the first and corresponding authors upon reasonable request. Nucleotide sequences of the 12S rRNA (OQ550226 to OQ550240), 16S rRNA (OQ534994 to OQ535008), COI (OQ535010 to OQ535024) and ITS2 (OQ550211 to OQ550225) genetic markers for the specimens used in this study have been deposited in GenBank.

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Author's contribution. U. T. and A. A. A. conceived, supervised and designed the study. A. H. and A. H. E. C. investigated and performed formal analysis. All authors reviewed and edited the article.

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