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Original article

Spiculopteragia mathevossiani Ruchliadev, 1948 is the minor morph of *Spiculopteragia spiculoptera* (Gushanskaya, 1931): molecular evidence

Emmanuel LIÉNARD[a,b]*, Jérôme DEPAQUIT[a], Hubert FERTÉ[a]

[a] EA 3800, UFR de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims, France
[b] 2C2A-CERFE, 08240 Boult-aux-Bois, France

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Abstract – Although *Spiculopteragia spiculoptera* is primarily a parasite of cervids, it can also but less often contaminate domestic livestock. Little is known about its epidemiology and its pathogenicity in domestic ruminants and other unusual cervid species. Its taxonomic status remains unclear since the hypothesis of morphological polymorphism among males has been proposed. However, accurate taxonomy is fundamental in the identification and survey of potentially pathogenic species of parasites. The second internal transcribed spacer of rDNA (ITS-2) and the mitochondrial (mt) DNA-derived ND4 gene were used to study the polymorphism hypothesis for *S. spiculoptera*. ND4 evolves more quickly than ITS-2 and is considered to be more discriminant in the characterization of closely related species. DNA sequences of ITS-2 and ND4 were studied in 18 individual males of morphological type *spiculoptera* and in 3 of morphological type of *mathevossiani* from Red deer (*Cervus elaphus*), Roe deer (*Capreolus capreolus*) and Chamois (*Rupicapra rupicapra*). Intraindividual ITS-2 variations were detected within and between each morphotype of *Spiculopteragia* but these differences did not separate the two morphs *mathevossiani* and *spiculoptera*. Similarly, although ND4 showed a high level of nucleotide substitution, the morphotypes *S. mathevossiani* and *S. spiculoptera* were clustered together. Our genetic data support the dimorphic male hypothesis for the species *S. spiculoptera*.

mitochondrial DNA / second internal transcribed spacer (ITS-2) / *Spiculopteragia spiculoptera* / *Spiculopteragia mathevossiani* / polymorphism

1. INTRODUCTION

The composition of the helminthofauna of Roe deer (*Capreolus capreolus*) and Red deer (*Cervus elaphus*) shows a similar community of the abomasal nematode parasites. Accurate identification of trichostrongyle nematodes in wild ungulates is important for studying their susceptibility to contamination from or towards domestic ruminants. Moreover, the status of some taxa remains controversial since the description was made of morphologically distinct but genetically identical adult male Ostertagiinae [13, 24, 26]. Morphological polymorphism was only detected in males and affects the morphology and length of spicules and the genital cone [13]. Several approaches have supported the hypothesis of male polymorphism in different species of Ostertagiinae as seen with cross-breeding experiments [27, 38],

* Corresponding author:
emmanuel.lienard@cerfe.com

morphological observations [13, 28, 30], and allozyme studies [17]. Comparisons of genetic sequences from both Internal Transcribed Spacers (ITS-1 and ITS-2) which are known as specific level markers are now used to detect polymorphism [18, 23, 42]. Yet, no ITS sequence difference has been displayed within species morphs [9, 32, 36]. Mitochondrial DNA has also been investigated to resolve the polymorphic issue [42] or to assess cryptic species [7].

Among the Ostertagiinae with type 2-2-1 copulatory bursa [14], the polymorphism hypothesis has been previously genetically studied for *Spiculopteragia asymmetrica* [33], *Teladorsagia circumcincta* [37] using ITS and *Teladorsagia boreoarcticus* [22] using mitochondrial DNA. No substantial difference in genetic sequences was found to discriminate between minor and major morphs to justify minor morphotype species status.

Primarily regarded as a pathogen of wild ruminants, *Spiculopteragia spiculoptera* is also reported, but less often, as a parasite of sheep, goats and cattle [2, 10, 32, 34]. The hypothesis of male polymorphism, however, renders its taxonomy and systematics confusing, especially for the potential survey of its distribution pattern among domestic livestock and reservoir hosts. According to the synonyms reviewed by Durette-Desset [15], *Spiculopteragia mathevossiani* Ruchliadev, 1948 (= *Rinadia schulzi* (Grigoryan, 1951) = *S. caucasica* (Assadov, 1955) = *S. pavloskyi* (Kadenatsii and Andreeva, 1957) = *S. quadrifurcata* (Andrews, 1964)) has been suggested as a minor morph of *Spiculopteragia spiculoptera* (Gushanskaya, 1931) (= *S. boehmi* (Gebauer, 1932) = *S. kutkascheni* (Assadov, 1952) = *S. pigulevski* (Ruchliadev, 1961)) [13, 24, 27]. Consequently, the objective of this study was to test whether individuals representing each of these morphological forms could be consistently differentiated by their sequences of either ND4 or ITS-2.

2. MATERIALS AND METHODS

Worms were collected in different hosts during the hunting season or from dead animals. The origins of our 26 specimens are given in Table I. All samples (*Spiculopteragia spiculoptera* morph *spiculoptera* n = 18, *Spiculopteragia spiculoptera* morph *mathevossiani* n = 3, *Ostertagia leptospicularis* morph *leptospicularis* n = 2 and *Ostertagia leptospicularis* morph *kolchida* n = 2) have been collected from different areas in France, except *Teladorsagia circumcincta* morph *circumcincta* which has been collected in Cyprus. The abomasa were removed and stored frozen at –20 °C before analysis. Adult worms were collected by washing the defrosted abomasum with water. Only adult male nematodes were used. The anterior part of each worm was cut off and placed individually into a sterile vial and stored in 95% ethanol before DNA extraction. The posterior part of the worm was cleared and preserved in Amman lactophenol on a glass side. They were identified as *S. spiculoptera* morph *spiculoptera* or morph *mathevossiani*, and *O. leptospicularis* morph *leptospicularis* or morph *kolchida* by using morphological features according to Skrjabin et al. [35] and Dróżdż [10, 13]. These specimens are available upon request to the authors.

DNA extraction was performed by means of a Qiamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Before the tissue lysis (first step), bodies were individually crushed using a piston pellet (Treff, Degersheim, Switzerland), and the DNA was eluted in 400 μL of the elution buffer provided by the manufacturer. Polymerase Chain Reactions (PCR) were performed in a 50 μL volume using 5 μL

Table I. Sample origins. All samples were collected from different areas in France except the sheep ones.

Host	Department	Locality	Location	Identification[a]	Reference
Roe deer	Deux-Sèvres	Chizé	46°07'34" N 0°25'28" W	*Ostertagia leptospicularis (leptospicularis)*	DS
				Spiculopteragia spiculoptera (spiculoptera)	DS1 DS2
				S. s. (mathevossiani)	DS3 m
	Marne	Trois-Fontaines	48°41'35''N 4°55'37"E	*O. l (leptospicularis)*	MA
	Marne	Humbécourt	48°35'05''N 4°57'25"E	*O. l. (kolchida)*	MA k
				S. s. (spiculoptera)	MA1 MA2
	Marne	Courtagnon	49°08'23''N 3°58'49"E	*S. s. (spiculoptera)*	MA3 MA4
	Marne	Saint-Imoges	49°05'48''N 3°58'32"E	*S. s. (mathevossiani)*	MA5 m
	Ardennes	Signy l'Abbaye	49°40'32''N 4°25'21"E	*S. s. (spiculoptera)*	AR3 AR4
	Dordogne	Fraisse	44°53'21''N 0°20'04"E	*S. s. (spiculoptera)*	DO1 DO2
				O. l. (kolchida)	DO k
	Yonne	Val de Mercy	47°40'50''N 3°35'42"E	*S. s. (spiculoptera)*	YO1 YO2
Red deer	Ardennes	Belval-Bois-des-Dames	49°29'32''N 5°02'26"E	*S. s. (spiculoptera)*	AR1 AR2
	Haut-Rhin	Saint-Amarin	47°53'25'N 7°01'10"E	*S. s. (mathevossiani)*	HR1 m
				S. s. (spiculoptera)	HR2 HR3
Chamois	Haut-Rhin	Oderen	47°55'11''N 6°59'11"E	*S. s. (spiculoptera)*	HR4 HR5
Sheep	Cyprus			*Teladorsagia circumcincta (circumcincta)*	CYP

[a] Morphs are in parentheses.

of extracted DNA solution and 50 pmol of each of the primers. The PCR mixture contained (final concentrations) 10 mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$, KCl 50 mM, Triton X 100 0.01%, 200 μM dNTP each, and 0.25 μL (1.25 units) of *Taq* polymerase (Qiagen). Two different sequences were amplified by PCR: the ITS-2 sequences of ribosomal DNA, and the ND4 gene of the mitochondrial DNA. ITS-2 fragment was amplified with NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') [19] and C1 (5'-ATGCTTAAGTTCAGCGG GT-3') and the ND4 fragment with LNC (5'-CGACAAACCACCTTGAT-3') and ND4Nem2 (5'-CAAAGTGATTCCAAGT CATTGGC-3') [4]. For the ITS2 fragment, the thermocycling conditions used were the following: initial denaturation at 94 °C for 4 min 30 s, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 45 s and extension at 72 °C for 1 min with a final elongation time of 10 min at 72 °C. For the ND4 fragment, the PCR conditions were the following: initial denaturation at 94 °C for 4 min 30 s, followed by 40 cycles of denaturation at 94 °C for 45 s, annealing at 45 °C for 1 min 30 s and extension at 72 °C for 2 min with a final elongation time of 10 min at 72 °C. Amplicons were analysed by electrophoresis in 1.5% agarose gel containing ethidium bromide. Negative and positive controls were provided in each set of reactions. Direct sequencing in both directions was performed by Qiagen using the primers used for DNA amplification.

Sequence alignments were performed by ClustalW included in the BioEdit version 7.0.1 software package [21]. Corrections were performed manually. Average ND4 nucleotide diversity with the Tajima-Nei model for each *S. spiculoptera* morphotype was computed according to the method of Lynch and Crease [31]. Computation of ND4 sequence mean distances and standard errors within and between species and morphs of *S. spiculoptera* and *O. leptospicularis* was performed according to the Tajima-Nei model. Standard errors were computed from 1 000 bootstrap replicates. All substitutions were included and rates were assumed to be uniform among sites. ND4 data were analyzed without modification. Phylogeny reconstruction using Neighbor-Joining (NJ) were performed with the Tajima-Nei model and using uniform rates among sites. MEGA version 3.1 software was used to compute average ND4 nucleotide diversity, ND4 sequence mean distances and to build a Neighbor-Joining tree [25]. Bootstrap analyses were performed using PAUP* version 4.0 [40]. No correction was performed for multiple mutations and 1 000 bootstrap replicates were made. ITS-2 and ND4 sequence alignments and trees were undertaken, both with and without inclusion of outgroup taxa. Outgroups were taken among the Haemonchinae: *Haemonchus contortus* and *Haemonchus placei*. The nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the following accession numbers: DQ354309 to DQ354360.

3. RESULTS

3.1. Analysis of the rDNA ITS-2 dataset of *S. spiculoptera*

Alignment of ITS-2 sequences was performed including the ITS-2 sequences of the species of the *Spiculopteragia* genus: *S. spiculoptera* (= *S. boehmi*) already available in GenBank™ under the accession number AJ577460, *S. asymmetrica* (Ware, 1925) morph *asymmetrica* (AF480617) and *S. asymmetrica* morph *quadrispiculata* (AF480618). ITS-2 sequences of *Spiculopteragia* were either 241 (morph *spiculoptera*/*mathevossiani*) or 243 (morph *asymmetrica*/*quadrispiculata*) nucleotides

in length. Their GC content was 34%. Five nucleotides were different between the two species *S. spiculoptera* and *S. asymmetrica*. Out of the 21 specimens of *S spiculoptera*, we observed a mixed base in both morphs (one transition C/T), two transitions (C/T, A/G) for *spiculoptera* and one transition (A/G) from *mathevossiani* (Fig. 1). In addition, these sites of intra-individual variations in all samples were not correlated with their geographical origin as well as with the host species.

Moreover, *Ostertagia leptospicularis* morph *leptospicularis* and morph *kolchida* shared identical 238 nucleotides in length ITS-2 sequences. Three polymorphic sites were found (two transversions and one transition) which were independent of the morphotype (data not shown).

The topology of the tree obtained by neighbor joining is given in Figure 2. *Haemonchus contortus* (X78803) and *Haemonchus placei* (X78812) were defined as the outgroup. Despite alignment gaps, no modification occurred in the topology of the tree without outgroup taxa. *S. spiculoptera* morph *mathevossiani* and *S. spiculoptera* morph *spiculoptera* belonged to the same cluster while morphs of *S. asymmetrica* (AF480617, AJ251125 and AF480618) were found in another cluster. Morphs of *O. leptospicularis* were also in only one cluster. They were separated from *T. circumcincta* while the members of the *Spiculopteragia* genus belonged to the same block.

		2 2	5 7	5 9	6 9	1 1	1 2	1 6	2 0	2 1	2 3
AJ251125		T	T	T	A	G	T	C	C	G	A
AF480617		.	.	Y	.	.	.	.	.	.	.
AF480618		.	.	Y	.	.	.	.	.	.	.
AJ577460		C	.	.	.	-	-	.	T	.	T
DO1		C	.	.	.	-	-	.	T	.	T
DO2		C	.	.	.	-	-	.	T	.	T
DS3	m	C	.	.	.	-	-	.	T	.	T
DS1		C	.	.	.	-	-	.	T	.	T
DS2		C	.	.	G	-	-	.	T	.	T
YO1		C	.	.	.	-	-	.	T	.	T
YO2		C	.	.	.	-	-	T	T	.	T
MA1		C	.	.	.	-	-	T	T	.	T
MA2		C	C	.	.	-	-	.	T	.	T
MA3		C	.	.	.	-	-	T	T	.	T
MA4		C	.	.	.	-	-	T	T	.	T
HR2		C	.	.	.	-	-	Y	T	.	T
HR1	m	C	.	.	.	-	-	T	T	A	T
HR3		C	.	.	G	-	-	.	T	.	T
HR4		C	.	.	.	-	-	.	T	.	T
HR5		C	.	.	.	-	-	T	T	.	T
MA5	m	C	.	.	.	-	-	.	T	.	T
AR1		C	.	.	.	-	-	.	T	.	T
AR2		C	.	.	.	-	-	T	T	.	T
AR3		C	.	.	.	-	-	Y	T	.	T
AR4		C	.	.	.	-	-	T	T	.	T

Figure 1. Sequence variations observed in the second ribosomal internal transcribed spacer (ITS-2) of *Spiculopteragia asymmetrica* (*asymmetrica*) (AJ251125 and AF480617), *Spiculopteragia asymmetrica* (*quadrispiculata*) (AF480618), *Spiculopteragia boehmi* (AJ577460), and our 21 *Spiculopteragia spiculoptera* (minor and major morphs). Geographical abbreviations are as follows: AR: Ardennes, DO: Dordogne, DS: Deux-Sèvres, HR: Haut-Rhin, MA: Marne, YO: Yonne, followed by worm numbers; m = *mathevossiani*. Identical bases are represented by a dot (.) and gap by a minus sign (–). Nucleotide nomenclature: Y = C or T. Only variable sites, with sequence positions given above, are shown.

3.2. Analysis of the mtND4 dataset of *S. spiculoptera*

The alignment was performed according to published partial ND4 sequences of *H. placei* (AF070825), *H. contortus* (AF070736). The mitochondrial sequences were aligned unambiguously without gap.

Within *S. spiculoptera* morph *spiculoptera* and morph *mathevossiani*, all 21 partial ND4 sequences were 396 bp in length. Their GC content was 21% for the two morphs of *S. spiculoptera*. The two morphotypes shared 326 (84.5%) conserved sites over the 396 alignment

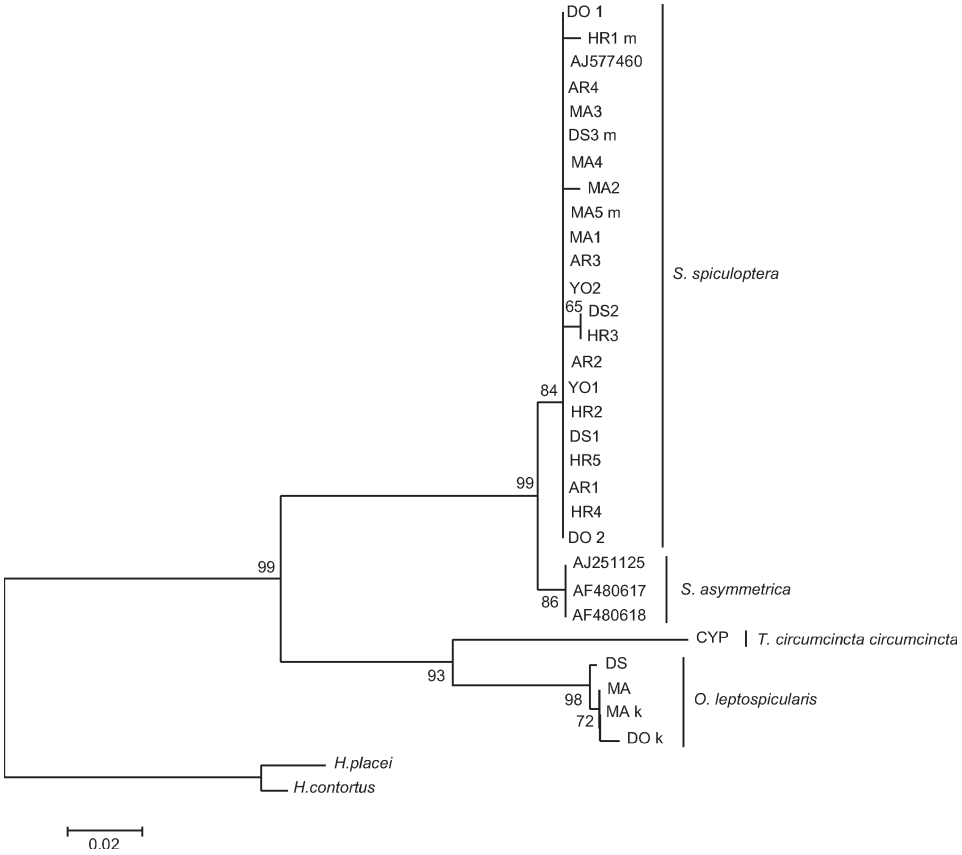

Figure 2. Neighbor-joining tree (Tajima-Nei model), based on ITS-2 sequence, showing the position of *Spiculopteragia spiculoptera* (minor and major morphs), with *Spiculopteragia boehmi* (AJ577460), *Spiculopteragia asymmetrica* (minor and major morphs), *Ostertagia leptospicularis* (minor and major morphs) and *Teladorsagia circumcincta* (*circumcincta*). Geographical abbreviations are as follows: AR: Ardennes, DO: Dordogne, DS: Deux-Sèvres, HR: Haut-Rhin, MA: Marne, YO: Yonne, followed by worm numbers; m = *mathevossiani*, k = *kolchida*. *S. spiculoptera* (*mathevossiani*) were clustered together with *S. spiculoptera* (*spiculoptera*) whereas *S. asymmetrica* (*asymmetrica*) (AF480617 and AJ251125) and *S. asymmetrica* (*quadrispiculata*) (AF480618) were found in another cluster. There was virtually no nucleotide difference between *S. spiculoptera* (= *boehmi*) [15] and *S. spiculoptera* (*mathevossiani*). *Ostertagia leptospicularis* (*leptospicularis*) and *O. leptospicularis* (*kolchida*) were clustered together in a different clade from the *Spiculopteragia* genus. *T. circumcincta* (*circumcincta*) was closer to *Ostertagia leptospicularis* than *S. spiculoptera. Haemonchus contortus* (X78803) and *Haemonchus placei* (X78812) were used as outgroup. The scale shows the number of nucleotide substitutions per site between DNA sequences. The numerals in the tree indicate the bootstrap values of the nodes.

Table II. ND4 sequences mean distances and standard errors within and between species and morphs of two Ostertagiinae. Values in bold along the diagonal are intra-morph mean distances; values below the diagonal are mean distances between each morph. All values were computed according to the Tajima-Nei model and values between parentheses are standard errors computed by 1000 bootstrap replicates.

	S. spiculoptera	(lato sensu)	*O. leptospicularis*	(lato sensu)
Species[a]	(1)	(2)	(3)	(4)
(1) *S. s.* (*spiculoptera*)	**0.043**			
	(0.006)			
(2) *S. s.* (*mathevossiani*)	0.031	**0.017**		
	(0.004)	**(0.006)**		
(3) *O. l.* (*kolchida*)	0.285	0.286	**0.029**	
	(0.032)	(0.033)	**(0.009)**	
(4) *O. l.* (*leptospicularis*)	0.286	0.289	0.053	**0.087**
	(0.031)	(0.032)	(0.008)	**(0.016)**
Mean distance within species	0.040 (0.005)		0.055 (0.009)	
Mean distance between species	0.286 (0.030)			

[a] Abbreviations for the species (morphs are in parentheses): *S. s.* (*spiculoptera*) = *Spiculoptera spiculopteragia* (*spiculoptera*), *S. s.* (*mathevossiani*) = *Spiculoptera spiculoptera* (*mathevossiani*); *O. l.* (*kolchida*) = *Ostertagia leptospicularis* (*kolchida*); *O. l.* (*leptospicularis*) = *Ostertagia leptospicularis* (*leptospicularis*).

positions. As expected, the partial ND4 sequence exhibited a high degree of polymorphism. The fact that one haplotype was shared by two worms of each morph was particularly striking. Table II (based on ND4 sequence mean distances) shows that *S. spiculoptera* morph *mathevossiani* individuals vary less from one another than do *S. spiculoptera* (lato sensu) overall. Moreover, the mean distance between the two morphotypes of *S. spiculoptera* did not exceed the variation among individuals of the *spiculoptera* morphotype. Similar results were obtained between *O. leptospicularis* morphotypes.

The neighbor joining tree was rooted on sequences of *H. placei* and *H. contortus* (Fig. 3). Similar topology of the tree occurred without outgroup taxa. The tree showed that mtDNA haplotypes of morphs *spiculoptera* and *mathevossiani* were not separated into two distinct clusters but were coupled in a single cluster different from that of *O. leptospicularis* and *T. circumcincta* (Fig. 3). As noted with *S. spiculoptera*, ND4 sequences of *O. leptospicularis* morph *kolchida* and morph *leptospicularis* were 396 nucleotides in length. The minor morph *kolchida* belonged to the same cluster of the *O. leptospicularis* major morph. This cluster was near that of *T. circumcincta* (Fig. 3).

4. DISCUSSION

The two neighbor joining trees show some similarity with regards to the relative position of *O. leptospicularis*, *S. spiculoptera* and *T. circumcincta*. This result was consistent with the phylogeny of the Trichostrongylina [8, 16, 20].

Among the *Spiculopteragia* genus, only one genetic study based on the comparison of ribosomal DNA ITS-1 and

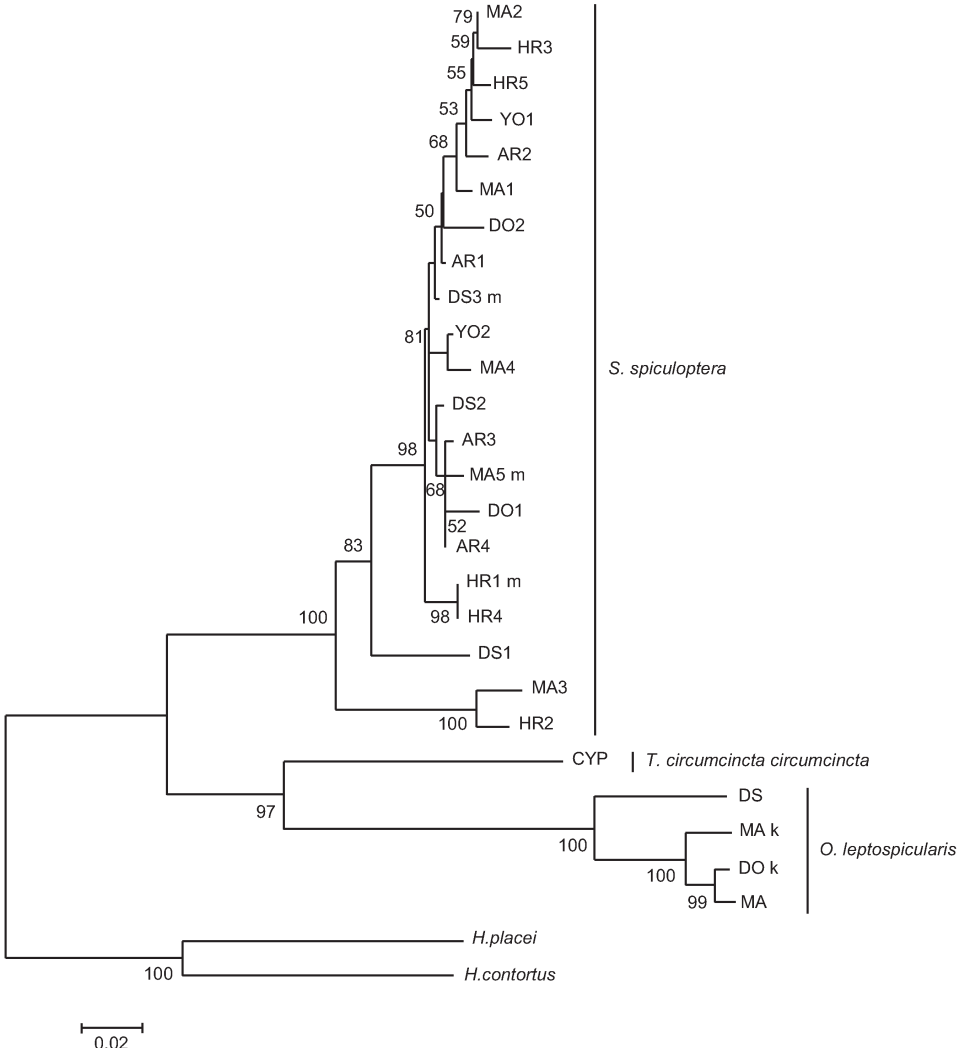

Figure 3. Neighbor-joining tree (Tajima-Nei model), based on ND4 partial sequence, rooted on *Haemonchus contortus* (AF070736) and *Haemonchus placei* (AF070825), showing the position of *Spiculopteragia spiculoptera* (minor and major morphs), *Ostertagia leptospicularis* (minor and major morphs) and *Teladorsagia circumcincta* (*circumcincta*). Geographical abbreviations are as follows: AR: Ardennes, DO: Dordogne, DS: Deux-Sèvres, HR: Haut-Rhin, MA: Marne, YO: Yonne, followed by worm numbers; m = *mathevossiani*, k = *kolchida*. *S. spiculoptera* (*mathevossiani*) were clustered together with *S. spiculoptera* (*spiculoptera*) whereas *O. leptospicularis* (*leptospicularis*) and *O. leptospicularis* (*kolchida*) were found in another cluster. *T. circumcincta* (*circumcincta*) from Cyprus was closer to *Ostertagia leptospicularis* than *S. spiculoptera*. The scale shows the number of nucleotide substitutions per site between DNA sequences. The numerals in the tree indicate the bootstrap values of the nodes.

ITS-2 has shown identical sequences between *S. asymmetrica* morph *asymmetrica* and *S. asymmetrica* morph *quadrispiculata* [33]. The alignments performed with our results confirmed the specificity of the ITS-2 domain: five repeated different nucleotides for ITS-2 between *S. spiculoptera* and *S. asymmetrica* and only three between *H. contortus* and *H. placei* [36].

According to previous studies on trichostrongylid nematodes, especially for *Teladorsagia circumcincta* [37], *Spiculopteragia spiculoptera* (lato sensu) ITS-2 exhibits intraspecific variation. This intraspecific diversity was thus not surprising in our study and did not affect the identifiability of Ostertagiinae species by the ITS-2 domain. Intraindividual variations were also detected. However, these two levels (intraindividual and intraspecific) of ITS-2 nucleotide diversity were not correlated with morphological polymorphism. Based on this genetic marker, the two morphs cannot be separated. Our results were in agreement with those seen in other polymorphic Ostertagiinae species [9, 33, 42].

Mitochondrial DNA are independent of nuclear DNA and are known to evolve faster than ITS [7]. Among mitochondrial genes, Cytochrome Oxydase I (COX-1) assessed the polymorphism within *Teladorsagia* spp. [41]. We used mtND4 which exhibits less conservation sites than COX-1 [7] and is often suggested for prospecting closely related species [5, 29]. The average nucleotide diversity for *S. spiculoptera* morph *spiculoptera* and *S. spiculoptera* morph *mathevossiani* together is consistent with values computed for various Haemonchidae species such as *Ostertagia ostertagi*, *Haemonchus contortus*, *Haemonchus placei* and *Teladorsagia circumcincta* from domestic hosts and *Mazamastrongylus odocoilei* from white-tailed deer (*Odocoileus virginianus*) [3, 6]. We suggest that the average nucleotide diversity difference between the two morphs may be due to the low sampling of *mathevossiani* in light of its low occurrence [11]. The diversity between the two morphs does not influence the ND4 sequence mean distances: each distance between minor and major morphs is substantially lower than the distances between species.

Despite the high ND4 nucleotide diversity, the hypothesis of *S. spiculoptera* being a dimorphic species is strongly supported since we noted one haplotype shared by two worms of each morphotype. The identity of ND4 sequences between minor and major morphs has already been reported for *Teladorsagia boreoarcticus* [22].

Red deer and Roe deer are primarily reservoir hosts of *S. spiculoptera* [10] but domestic livestock and other wild ungulates can be infected [2, 32, 34]. Recent contamination of other deer species such as white-tailed deer (*Odocoileus virgianus*) has also been reported [32]. Cross contaminations from reservoirs to unusual ungulate species hosts can occur by grazing in common pastures. Our genetic data support this assumption because of the common haplotype found within a red deer and a chamois from the same location. Classified as a specialist parasite, *S. spiculoptera* is suggested as a marker of interaction between hosts with little medical importance [41]. Its infectivity and its pathogenicity have never been investigated in non-reservoir ruminants. However, with regards to *O. leptospicularis*, mixed infections of *O. leptospicularis* and *O. ostertagi* lead to more severe clinical signs than single infections in sheep and cattle [1]. It is unlikely that infection persists durably because of breeding management and anthelmintic treatments. In addition, the percentage usually found in reservoir hosts of the minor morph could change in domestic livestock and newly infected deer species, being related to variation of host susceptibility, longevity of infective larvae on pasture and season [12, 39]. *S. spiculoptera*

could then provide a biological model to study the significance of male polymorphism as an adaptation strategy in host-parasite interactions. Further investigations are needed.

Based on ITS-2 and ND4 sequences, our molecular data give a new support to the results of Suarez and Cabaret [38] who have shown, by crossbreeding, that *O. leptospicularis* and *O. kolchida* constitute a single species. Similarly, our genetic results suggest that male polymorphism occurs in *S. spiculoptera*.

In conclusion, we recommend using the nomenclature *S. spiculoptera* morph *mathevossiani* rather than *Rinadia mathevossiani* or *S. mathevossiani*. Studies on the population genetic structure of *S. spiculoptera* lato sensu should be critical to determine the rates of gene flow and thus the risk of anthelmintic resistance spread among wild ruminants.

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