

# Is ITS-2 rDNA suitable marker for genetic characterization of *Sarcoptes* mites from different wild animals in different geographic areas?

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## A B S T R A C T

The present study examined the relationship among individual *Sarcoptes scabiei* mites from 13 wild mammalian populations belonging to nine species in four European countries using the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) as genetic marker. The ITS-2 plus primer flanking 5.8S and 28S rDNA (ITS-2+) was amplified from individual mites by polymerase chain reaction (PCR) and the amplicons were sequenced directly. A total of 148 ITS-2+ sequences of 404 bp in length were obtained and 67 variable sites were identified (16.59%). UPGMA analyses did not show any geographical or host-specific clustering, and a similar outcome was obtained using population pairwise *F*<sub>st</sub> statistics. These results demonstrated that ITS-2 rDNA does not appear to be suitable for examining genetic diversity among mite populations.

### Keywords:

Europe  
Genetic characterization  
Genotypes  
Ribosomal DNA (rDNA)  
Second internal transcribed spacer (ITS-2)  
*Sarcoptes scabiei*  
Wildlife

## 1. Introduction

*Sarcoptes scabiei* was probably originated as a human parasite and man spread the infection to domestic animals (Fain, 1978). Various wildlife species in turn were infected, often from contact with their domestic counterparts (Pence and Ueckermann, 2002). *Sarcoptes* mite infections are endemic in many European wild mammal populations, causing devastating mortality (up to >90%), reported especially from Alpine and Pyrenean chamois, Iberian ibex, aoudad and red fox, whereas only few cases have been reported in the stone marten, badger and roe deer

(Ryser-Degiorgis et al., 2002; González-Candela et al., 2004; Rossi et al., 2007; Oleaga et al., 2008). Apparently, no epidemiological relationship exists, in Europe, between mange foci affecting wild ruminants, wild boars and carnivores. A well-known example is the Alpine chamois in the Western Alps, whose populations have never been affected by this severe condition in spite of long lasting sympatry with affected foxes and wild boars (Berrilli et al., 2002).

The question as to whether *Sarcoptes* mites infecting different hosts belong to different species or whether they are, in fact, monospecific, has been the subject of an ongoing debate for many years (Zahler et al., 1999; Bornstein et al., 2001; Berrilli et al., 2002; Gu and Yang, 2008). Using microsatellite markers, Walton et al. (1999) substantiated previous data that gene flow between sympatric scabies mite populations on humans and dogs is extremely rare in northern Australia. Likewise, genetic

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differences were detected by microsatellites between geographically distinct populations (Walton et al., 2004; Soglia et al., 2007). Also, microsatellite markers were used by Alasaad et al. (2008a) to describe a new phenomenon of genetic structuring among *S. scabiei* at individual host skin-scale level. On the other hand, Zahler et al. (1999) analysed 23 pooled samples of mite isolates (not single mites) from nine host species in four continents, using the second internal transcribed spacer (ITS-2) of ribosomal DNA (rDNA) as genetic marker, and concluded that *Sarcoptes* consists of a single, heterogeneous species.

Conversely, using ribosomal and mitochondrial DNA sequences of 28 single *Sarcoptes* mites from three wild species in Spain and Italy, Berrilli et al. (2002) detected genetic differences between mites derived from red foxes in North Italy and Northwest Spain. The contradiction between the above-mentioned two studies may be due to the fact that Zahler et al. (1999) used pooled samples, so genetic variation among individuals may have been underestimated.

The objective of the present study was to examine the genetic characteristics of *S. scabiei* individuals collected from 13 wild mammalian populations representing nine wild animal species in four European countries using ITS-2 as a genetic marker, and to test the effectiveness of ITS-2 rDNA for studying genetic diversity of *Sarcoptes* mites using relatively a large number of *Sarcoptes* specimens.

## 2. Materials and methods

### 2.1. Collection of *S. scabiei*

Using Postponed Isolation (Post-frozen Isolation) and Direct Isolation (Live Isolation) methods, as described by Alasaad et al. (2008c), 148 *Sarcoptes* mites were collected from the skin of 75 wild animals belonging to nine European wild mammalian species from Switzerland, Italy, France, and Spain (Table 1). *Rupicapra rupicapra*, *Cervus elaphus*, *Sus scrofa*, *Ovis aries musimon*, *Capra ibex*, and *Vulpes vulpes* were sympatric in Northeast Italian Alps. *V. vulpes* and *Martes foina* were sympatric in Northwest Italian Alps. All mites were identified as *S. scabiei* based on known morphological criteria (Fain, 1968).

Table 1

Countries, geographical locations and host species used in this study, together with the number of host animals and *Sarcoptes* mite samples, followed by GenBank™ accession numbers for ITS-2+ sequences.

Codes	Countries and codes	Geographical locations and codes	Host species and codes	No. of animals	No. of mites	GenBank™ accession number		
SwVv	Switzerland	Sw	Different locations	<i>Vulpes vulpes</i>	Vv	13	15	AM980676–AM980690
ItNERr	Italy	It	Northeast	<i>Rupicapra rupicapra</i>	Rr	11	33	AM980691–AM980723
ItNECe	Italy	It	Northeast	<i>Cervus elaphus</i>	Ce	1	2	AM980724–AM980725
ItNESs	Italy	It	Northeast	<i>Sus scrofa</i>	Ss	2	6	AM980726–AM980731
ItNEOam	Italy	It	Northeast	<i>Ovis aries musimon</i>	Oam	2	6	AM980732–AM980737
ItNECi	Italy	It	Northeast	<i>Capra ibex</i>	Ci	2	5	AM980738–AM980742
ItNEVv	Italy	It	Northeast	<i>Vulpes vulpes</i>	Vv	5	14	AM980743–AM980756
ItNWVv	Italy	It	Northwest	<i>Vulpes vulpes</i>	Vv	10	26	AM980757–AM980782
ItNWMf	Italy	It	Northwest	<i>Martes foina</i>	Mf	1	3	AM980783–AM980785
FrNESs	France	Fr	Northeast	<i>Sus scrofa</i>	Ss	3	4	AM980786–AM980789
SpNEVv	Spain	Sp	Northeast	<i>Vulpes vulpes</i>	Vv	1	4	AM980790–AM980793
SpNWRp	Spain	Sp	Northwest	<i>Rupicapra pyrenaica</i>	Rp	3	9	AM980794–AM980802
SpSCp	Spain	Sp	South	<i>Capra pyrenaica</i>	Cp	21	21	AM980803–AM980823

### 2.2. Preparation of *Sarcoptes* gDNA

HotSHOT Plus ThermalSHOCK technique (Alasaad et al., 2008b) was applied to prepare genomic DNA for all single *Sarcoptes* mites from Iberian ibex (Sierra Nevada-Spain), while the DNA of single *Sarcoptes* mite samples from the other hosts were extracted with the NucleoSpin Tissue kit procedure (Macherey-Nagel, Düren, Germany).

### 2.3. Amplification and sequencing of the ITS-2+ rDNA

The ITS-2 rDNA plus primer flanking 5.8S and 28S sequences (ITS-2+) was amplified by PCR using primers RIB-18 and RIB-3 as reported previously (Zahler et al., 1999) in a 2720 thermal cycler (Applied Biosystems, Foster City, California). The amplicons were examined on 1.5% agarose gel, stained with ethidium bromide for DNA visualization under UV light. The purified PCR products were directly cycle-sequenced from both directions, on ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, California) using the BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Foster City, California).

Individual mite consensus sequences were manually trimmed of primer sequences, aligned, compared and edited using BioEdit v7.0.9.0 (Hall, 1999). Polymorphic sites were designated with IUPAC codes.

### 2.4. Molecular analysis

Genetic relationships among individual mites was examined using the unweighted pair group method based on arithmetic means (UPGMA; Sneath and Sokal, 1973) in MEGA v. 4 (Tamura et al., 2007), starting from a distance matrix based on the Kimura 2-parameter index (Kimura, 1980), measured by bootstrapping over 1000 replicates, using *Notoedres cati* (GenBank™ accession number AF251801) as the out-group, because it is the most closely related taxon to *S. scabiei*. The pairwise comparisons were made of the level of sequence differences (Chilton et al., 1995). Arlequin v.3.1 (Excoffier et al., 2005) was used to calculate the population pairwise  $F_{st}$  (Distance method),  $F_{st}$  P values, and the matrix of significant  $F_{st}$  P values

(significance level = 0.05) (Weir, 1996; Excoffier et al., 1992). The dendrogram was visualized in the TreeIllustrator v. 0.52 Beta software format (Trooskens et al., 2005). Likewise, MEGA v.4 was used to establish the UPGMA tree, showing the genetic relationships among the 13 *Sarcoptes* populations. The number and frequency of variable sites in different numbers of samples in each population were calculated with F-stat (Goudet, 2001).

### 3. Results and discussion

Individual ITS-2+ sequences were obtained from 148 *S. scabiei* samples, and deposited in the GenBank™ (accession numbers AM980676–AM980823). The mean G+C content of the sequences was 38.4%. All of the sequences were 404 bp in length, with the exception of two sequences from Italian foxes: ItNWVv1b had two deletions at the bases number 97 and 98, and ItNEVv2b had one deletion at the base number 321. Sixty-seven variable sites

were identified in the sequences obtained (16.59%, 67/404), representing 101 different ITS-2+ sequences (sequence data available from the authors upon request). The number of the variable sites was significantly more than that obtained in other studies where a small number of mites were analyzed (Berrilli et al., 2002; Zahler et al., 1999). It appears that the number of variable sites is related to the magnitude of sample diversity.

The genetic relationships among the individual mites, constructed by UPGMA analysis, showed no specific clustering comprising mites from single host species, nor geographical relation among the 21 resulting clusters (Fig. 1 and Table 2). Mites from different host species and different geographical locations clustered together. As a consequence, mites from the same host species or geographical locations were scattered in different clusters. Mites from the same individual host were even found in different clusters. Likewise, using the population pairwise Fst and the UPGMA tree among populations, no significant

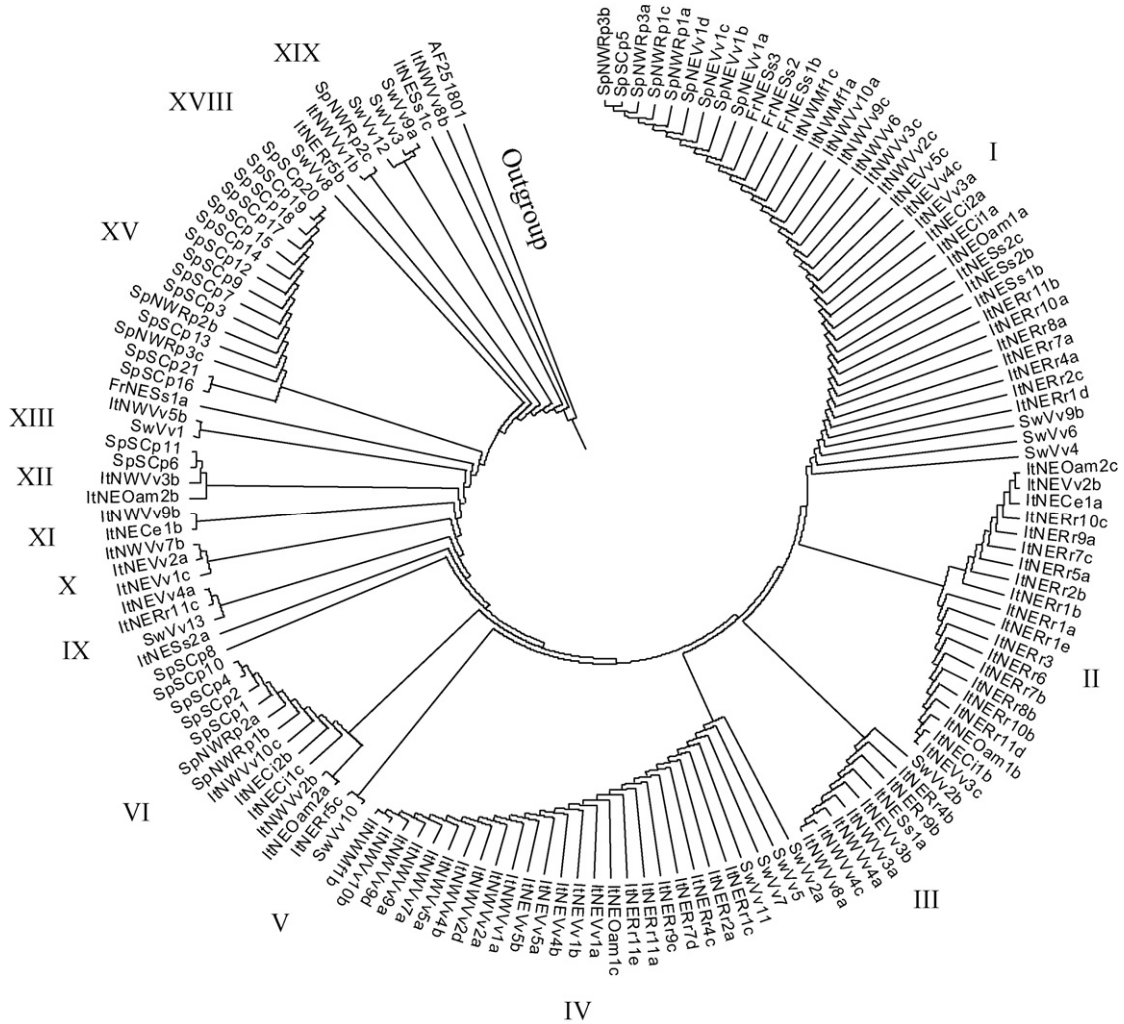


Fig. 1. UPGMA tree showing clustering of the 148 *Sarcoptes* mites from 13 wild animal populations belonging to nine species in four European countries, based on ITS-2 ribosomal DNA sequences, using *Notoedres cati* (GenBank™ accession number AF251801) as the out-group. Clusters are in clockwise order (only clusters with more than one mite are marked). *Sarcoptes* mites belonging to each cluster are detailed in Table 2. Codes in this figure represent the sample codes in Table 1.

Table 2

Twenty-one clusters of the UPGMA tree representing 148 Sarcoptes mites, from 13 wild animal populations representing nine species in four European countries, based on ITS-2 ribosomal DNA sequences, using *Notoedres cati* (GenBank™ accession number AF251801) as the out-group. Codes in this table represent the sample codes in Table 1.

Clusters	Sarcoptes mites
I	SpNWRp3b, SpSCp5, SpNWRp3a, SpNWRp1c, SpNWRp1a, SpNEVv1d, SpNEVv1c, SpNEVv1b, SpNEVv1a, FrNESs3, FrNESs2, FrNESs1b, ItNWMf1c, ItNWMf1a, ItNWVv10a, ItNWVv9c, ItNWVv6, ItNWVv3c, ItNWVv2c, ItNEVv5c, ItNEVv4c, ItNEVv3a, ItNECi2a, ItNECi1a, ItNEOam1a, ItNESs2c, ItNESs2b, ItNESs1b, ItNERr11b, ItNERr10a, ItNERr8a, ItNERr7a, ItNERr4a, ItNERr2c, ItNERr1d, SwVv9b, SwVv6, SwVv4
II	ItNEOam2c, ItNEVv2b, ItNECe1a, ItNERr10c, ItNERr9a, ItNERr7c, ItNERr5a, ItNERr2b, ItNERr1b, ItNERr1a, ItNERr1e, ItNERr3, ItNERr6, ItNERr7b, ItNERr8b, ItNERr10b, ItNERr11d, ItNEOam1b, ItNECi1b, ItNEVv3c
III	SwVv2b, ItNERr4b, ItNERr9b, ItNESs1a, ItNEVv3b, ItNWVv3a, ItNWVv4a, ItNWVv4c, ItNWVv8a
IV	SwVv2a, SwVv5, SwVv7, SwVv11, ItNERr1c, ItNERr2a, ItNERr4c, ItNERr7d, ItNERr9c, ItNERr11a, ItNERr11e, ItNEOam1c, ItNEVv1a, ItNEVv1b, ItNEVv4b, ItNEVv5a, ItNEVv5b, ItNWVv1a, ItNWVv2a, ItNWVv2d, ItNWVv4b, ItNWVv5a, ItNWVv7a, ItNWVv9a, ItNWVv9d, ItNWVv10b, ItNWMf1b
V	ItNERr5c, SwVv10
VI	SpSCp10, SpSCp4, SpSCp2, SpSCp1, SpNWRp2a, SpNWRp1b, ItNWVv10c, ItNECi2b, ItNECi1c, ItNWVv2b, ItNEOam2a
VII	SpSCp8
VIII	ItNESs2a
IX	ItNEVv4a, ItNERr11c, SwVv13
X	ItNWVv7b, ItNEVv2a, ItNEVv1c
XI	ItNWVv9b, ItNECe1b
XII	SpSCp11, SpSCp6, ItNWVv3b, ItNEOam2b
XIII	ItNWVv5b, SwVv1
XIV	FrNESs1a
XV	SpSCp20, SpSCp19, SpSCp18, SpSCp17, SpSCp15, SpSCp14, SpSCp12, SpSCp9, SpSCp7, SpSCp3, SpNWRp2b, SpSCp13, SpNWRp3c, SpSCp21, SpSCp16
XVI	SwVv8
XVII	ItNERr5b
XVIII	SpNWRp2c, ItNWVv1b,
XIX	SwVv9a, SwVv3, SwVv12
XX	ItNESs1c
XXI	ItNWVv8b
Outgroup	AF251801

distance among populations was detected, in agreement with findings of Zahler et al. (1999) and, up to a point, with Berrilli et al. (2002), who reported that only Pyrenean chamois-derived mites formed a separated group. However, in the present study, Pyrenean chamois-derived mites were randomly distributed in different clusters. The discrepancy is likely due to differences in sample sizes among studies.

Forty-six out of the 67 variable sites in the ITS-2+ sequences were observed only in single populations (unique variable sites). The number of variable sites was very different among populations: the population ItNWVv was the most variable (30 mutations), whereas the ItNWMf (4 mutations) and SpNEVv (5 mutations) were the most uniformed mite populations. With the exception of wild boar-derived mites (FrNESs and ItNESs), all populations had two variable sites, namely a R transition in position 68 with an average frequency of 0.21 (0.00–0.5) and a W

transversion in position 272 with an average frequency of 0.34 (0.00–0.82). In addition, in the French and Italian wild boar-derived mite populations there were some unique variable sites, a transition R (0.25) and a transversion W (0.125) for the FrNESs, while three R transitions (0.17 each), a Y transition (0.17), a K transversion (0.08), and a S transversion (0.17) for ItNESs.

Both populations of Spanish wild ruminant mites (SpNWRp and SpSCp) were characterized by two R transitions and three different transversions MWK, all showing intermediate frequencies, being higher in SpSCp. Italian and Switzerland fox derived mite populations were very variable and shared three transitions (R, Y, Y) and three transversions (K, M, S) with frequencies clearly higher in SwVv (average 0.16; 0.03–0.26), all absent in Spanish fox derived mites.

ITS-2 rDNA appears to be suitable genetic marker for distinguishing closely related species and examining phylogenetic relationships within genera but not suitable for genetic characterization within a species for mites (Navajas et al., 1994, 1998, 2000; Navajas and Fenton, 2000; Zahler et al., 1998, 1999; Essig et al., 1999; Berrilli et al., 2002). For example, Navajas et al. (1998, 2000) found very low variation in ITS-2 sequences within *Tetranychus* urticae. Similar patterns of variation were found by Navajas et al. (1994) for *Mononychellus progresivus* and, as mentioned above, by Zahler et al. (1999) and Berrilli et al. (2002) for *Sarcoptes*. In addition, Zahler et al. (1998) and Essig et al. (1999) were not able to find clear differences within the worldwide distributed genera *Psoroptes* and *Chorioptes*, respectively. However, Navajas et al. (1998) showed extensive polymorphism in sequences of mitochondrial cytochrome oxidase I in *T. urticae*. Some sorts of geographical or host specificity in *Sarcoptes* populations have been shown in studies in which microsatellites were used as markers (Walton et al., 2004; Soglia et al., 2007).

In conclusion, ITS-2 rDNA does not appear to be suitable marker for examining genetic diversity among *Sarcoptes* mite populations. For characterization of sub-populations or strain typing within *S. scabiei*, genetic markers other than ITS-2 rDNA, such as microsatellite DNA and/or mitochondrial DNA should be used.

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