Phylogeography of *Trichuris* populations isolated from different Cricetidae rodents

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SUMMARY

The phylogeography of *Trichuris* populations (Nematoda) collected from Cricetidae rodents (Muroidea) from different geographical regions was studied. Ribosomal DNA (Internal Transcribed Spacers 1 and 2, and mitochondrial DNA (*cytochrome c- oxidase* subunit 1 partial gene) have been used as molecular markers. The nuclear internal transcribed spacers (ITSs) 1 and 2 showed 2 clear-cut geographical and genetic lineages: one of the Nearctic region (Oregon), although the second was widespread throughout the Palaearctic region and appeared as a star-like structure in the minimum spanning network. The mitochondrial results revealed that *T. arvicolae* populations from the Palaearctic region were separated into 3 clear-cut geographical and genetic lineages: populations from Northern Europe, populations from Southern (Spain) and Eastern Europe (Croatia, Belarus, Kazahstan), and populations from Italy and France (Eastern Pyrénean Mountains). Phylogenetic analysis obtained on the basis of ITS1-5·8S-ITS2 rDNA sequences did not show a differential geographical structure; however, these markers suggest a new *Trichuris* species parasitizing *Chionomys roberti* and *Cricetulus barabensis*. The mitochondrial results revealed that *Trichuris* populations from arvicolinae rodents show signals of a post-glacial northward population expansion starting from the Pyrenees and Italy. Apparently, the Pyrenees and the Alps were not barriers to the dispersal of *Trichuris* populations.

Key words: phylogeography, *Trichuris arvicolae*, Nematoda, ribosomal DNA, mitochondrial DNA, Cricetidae, rodents.

INTRODUCTION

Arvicoline rodents (voles and lemmings) are numerically and functionally the dominant mammalian herbivores in the Northern parts of the Holarctic regions (Western Nearctic and the Western half Palaearctic regions). The Arvicolinae subfamily (Cricetidae) consists of 26 genera and 140 species, the most diverse genus being *Microtus* with 60 recognized species.

Previous studies (Tenora, 1967; Merkusheva and Bobkova, 1981) reported that *Trichuris muris* is a nematode parasite found mainly in Murinae and Arvicolinae rodents. Nevertheless, based on isoenzymatic techniques, Feliú *et al*. (2000) suggested that trichurids parasitizing hosts of the family Arvicolidae (presently regarded as a subfamily in Cricetidae, Wilson and Reeder, 2005) constitute a separate species of *Trichuris* and they described a new species, *T. arvicolae*, as a parasite of the Arvicolidae rodent family. Cutillas *et al*. (2002) amplified and sequenced the ITS1-5·8S-ITS2 region of the ribosomal DNA (rDNA) of *T. muris* and *T. arvicolae* using conserved primers; they reported that PCR molecular techniques differentiated *T. muris* and *T. arvicolae* as two well-defined species.

Comparative analysis of coding and noncoding regions of ribosomal DNA has become a useful tool for the construction of phylogenetic trees of many organisms including nematodes (Subbotin *et al*. 2001). The internal transcribed spacers 1 and 2 (ITS1 and ITS2) located in the ribosomal DNA are considered appropriate molecular markers to resolve relationships at the *Trichuris* species (Cutillas *et al*. 2009, 2007, 2004, 2002).

Mitochondrial DNA (mtDNA) has proven useful in molecular phylogenetics due to its presumed maternal inheritance, rapid rate of divergence and lack of recombination (Arrivillaga *et al*. 2002). The first subunit of the mtDNA *cytochrome c-oxidase* (*cox*1) gene has been used to study evolutionary relationships among recently diverged rapidly evolving taxa and also to resolve deep branch phylogenies in which multiple substitutions are a critical problem (Bowles and McManus, 1993;

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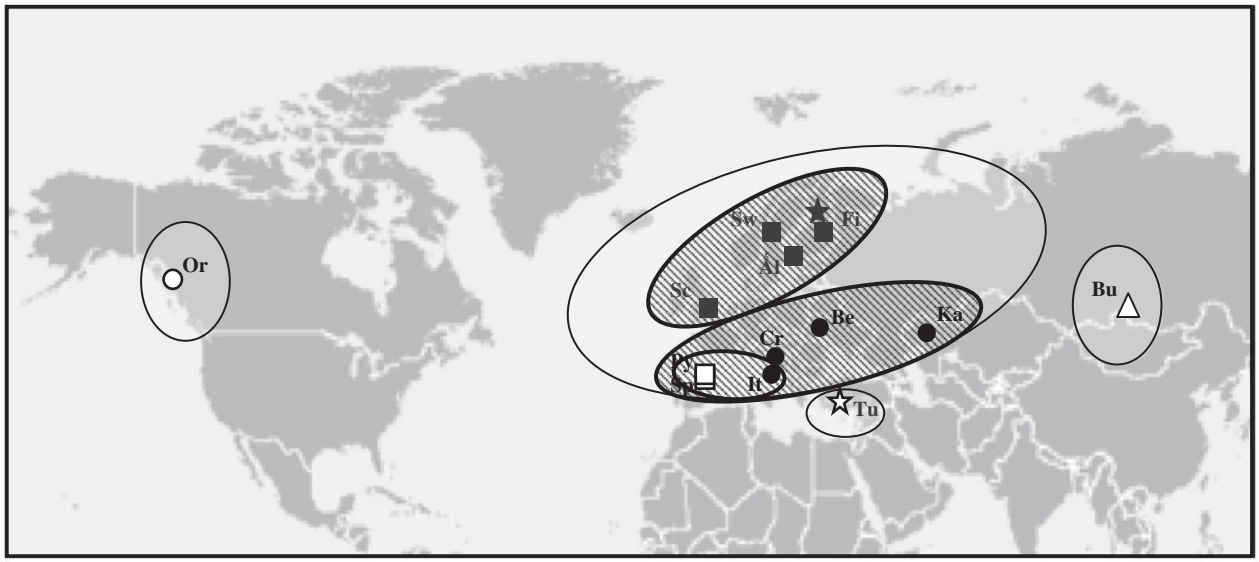

Fig. 1. Geographical distribution of *Trichuris* populations and the extension of their genetic clades. Host species: black square: *Microtus agrestis*; black star: *Microtus levis*; black circle: *Microtus arvalis*; white square: *Myodes glareolus*; white circle: *Microtus townsendii*; white star: *Chionomys roberti*; white triangle: *Cricetulus barabensis*. Localities: *Or:* Oregon, *Fi:* Finland (South), *Ål:* Finland (Åland island), *Sc:* Scotland, *Sw:* Sweden, *Py:* France (Eastern Pyrenean Mountains), *Sp:* Spain (Montseny, Barcelona), *It:* Italy, *Cr:* Croatia, *Ka*: Kazakhstan, *Be:* Belarus, *Tu:* Turkey, *Bu:* Buryatia. ○Indicate the subdivision of populations in geographical clades based on ribosomal DNA marker. ▨Indicate the subdivision of populations in geographical clades based on mitochondrial DNA marker.

Kumazawa and Nishida, 1993; Sukhdeo *et al*. 1997; Morgan and Blair, 1998).

The number of phylogeographical studies on animals and hominids has increased greatly during recent years, particularly in Europe (Taberlet *et al*. 1998; Hewitt, 1999; Avise, 2000; Jaarola and Searle, 2004; Folinsbee and Brooks, 2007), but are mainly concerned with vertebrate taxa (fish, amphibians, birds and mammals) while invertebrate taxa, particularly parasite species, have been hardly studied (Burban *et al*. 1999; Attwood, 2001; Wikstrom *et al*. 2003; Haukisalmi and Henttonen, 2001; Haukisalmi *et al*. 2001, 2004, 2006, 2007, 2008, 2009, 2010*a*, *b*). Conversely, the phylogeography of different species of nematodes has been studied avidly recently by several authors (Nieberding *et al*. 2005; Miranda *et al*. 2008; Traversa *et al*. 2008 and Zhou *et al*. 2011).

The present work was an attempt to study the phylogeography of *T. arvicolae* isolated from different rodent hosts from different geographical regions testing whether host specificity or geography play a role in structuring the parasite phylogeography. To discriminate between the alternative hypotheses of co-speciation (host-parasite) versus geographical differentiation, we carried out a molecular study based on the amplification and sequencing of the ITS1-5·8S-ITS2 fragment of the ribosomal DNA and the first subunit of the *cytochrome c oxidase* (*cox*1) partial gene mitochondrial DNA, looked on species of *Trichuris* isolated from *Microtus agrestis*, *Microtus arvalis*, *Microtus levis*, *Microtus townsendii*, *Myodes glareolus*, *Cricetulus barabensis* and *Chionomys roberti* sampled from different geographical areas (North America, Europe and Asia).

MATERIALS AND METHODS

Collection of samples

Although Feliú *et al*. (2000) suggested that trichurids parasitizing hosts of the Arvicolidae family (presently subfamily Arvicolinae) are *T. arvicolae*, we considered different populations of *Trichuris* isolated from 7 species of rodent hosts (*Microtus agrestis*, *Microtus arvalis*, *Microtus levis*, *Microtus townsendii*, *Myodes glareolus*, *Chionomys roberti* and *Cricetulus barabensis*) from different geographical regions as Operational Taxonomic Unit (OTUs) (Chilton *et al*. 1995). A total of 38 adult *Trichuris* sp. were collected from 11 *Microtus agrestis* (Cricetidae: Arvicolinae), 4 *Microtus arvalis* (Cricetidae: Arvicolinae), 1 *Microtus levis* (Cricetidae: Arvicolinae), 1 *Microtus townsendii* (Cricetidae: Arvicolinae), 6 *Myodes glareolus* (Cricetidae: Arvicolinae) and 2 *Chionomys roberti* (Cricetidae: Arvicolinae) from different localities from Europe: Turkey, Spain (Montseny, Barcelona), Eastern Pyrenean Mountains (France), Finland (South), Finland (Åland Island), Sweden, Scotland, Italy, Belarus and Croatia; from Asia: Kazakhstan; and from America: Oregon (Fig. 1, Table 1). Furthermore, 1 *Trichuris* sp. from *Cricetulus barabensis* (Cricetidae: Cricetinae) from Buryatia, Siberia, was analysed. Worms were washed extensively in 0·9% saline solution and stored in 70%

alcohol until required for PCR and sequencing. The identification of species of *Trichuris* found in the caecum of these rodent hosts was made according to Feliú *et al*. (2000).

Sequence data

Genomic DNA from individual worms was extracted using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol. Genomic DNA was detected using 0·8% agarose gel electrophoresis and ethidium bromide.

The ribosomal DNA (rDNA) region ITS1-5·8S-ITS2 was amplified by PCR using a Perkin Elmer thermocycler and the following PCR mix: 10 μl 10 × PCR buffer, 2 μl 10 mM dNTP mixture (0·2 mM each), 3 μl 50 mM $MgCl_2$, 5 μl primer mix (0·5 mM each), 5 μl template DNA, 0·5 μl *Taq* DNA polymerase (2·5 units) and autoclaved distilled water to 100 μl. The following conditions were applied: 94 °C for 3 min (denaturing), 35 cycles at 94 °C for 1 min (denaturing), 55 °C for 1 min (annealing), 72 °C for 1 min (primer extension), followed by 10 min at 72 °C. DNA sequences of the forward primer NC5 (5′-GTAGGTGAACCT-GCGGAAGGATCATT-3′) and reverse primer NC2 (5′-TTAGTTTCTTTTCCTCCGCT-3′) corresponded to the conserved 3′–5′ ends of the ITS1-5·8S-ITS2 flanking the 18S and 28S gene regions (Gasser *et al*. 1996). For each set of PCR reactions and extraction of the DNA, samples without DNA (negative) and a known (positive) control DNA samples were also included.

The mitochondrial DNA (mtDNA) *cytochrome c-oxidase* subunit 1 gene (*cox*1) was amplified by PCR using a Perkin Elmer thermocycler. PCR conditions and oligonucleotide primers were those designed for amplification of *cox*1 from *Trichinella* isolates (Nagano *et al*. 1999); it was anticipated that the molecular approach employed for Trichinellidae nematodes could also be applied to the Trichuridae group.

Thus, DNA sequences of the forward primer FORCOXI: 5′-TTTGGGCATCCTGAGGTT-TA-3′; (L6625 modified from Nagano *et al*. 1999) and reverse primer H7005: 5′-ACTACGTAG-TAGGTATCATG-3′ (Nagano *et al*. 1999) corresponded to the conserved regions of the *cytochrome c-oxidase* subunit 1 gene. For each set of PCR reactions and extraction of the DNA, samples without DNA (negative) and a known (positive) control DNA sample were also included.

The PCR products were checked on ethidium bromide-stained 2% Tris-Borate-EDTA (TBE) agarose gels. Bands were eluted from the agarose by using the QIAEX II Gel Extration Kit (Qiagen). The isolated DNA was cloned into *Escherichia coli* DH5α using pGEM-T Easy vector system (Promega). Transformed cells were selected by overnight incubation at 37 °C on LBB/Amp/X-gal/IPTG plates. In order to check for successful cloning and to study the intra-individual variation, at least 10 single recombinants (clones) were screened for the DNA insert and sequenced. The 10 clones containing the correct insert were used to inoculate 5 ml of LBB/Amp broth and incubated, shaken at 37 °C for 12 h. Plasmid was purified using a Wizard Plus SV (Promega) and sequenced by MWG-Biotech (Germany) with a universal primer (M13).

The intra-individual variation was determined by sequencing between 3 and 5 clones of 1 individual per population of *Trichuris*. The inter-individual variation was determined by sequencing at least 3 individuals of each locality and host.

Phylogenetic analysis

All analyses were performed on the mtDNA and rDNA datasets, *cox*1 partial gene and ITS1-5·8S-ITS2 sequences were aligned using the Clustal X program version 2.0 (Larkin *et al*. 2007).

The ribosomal phylogenetic analysis was carried out using sequences of *Trichuris muris* isolated from European murine rodents (Callejón *et al*. 2010) (Table 2) as an outgroup, while the mitochondrial phylogenetic analysis was carried out using the *cytochrome c oxidase* 1 partial sequence of *Trichuris muris* (GenBank, Accession number: CB013185.1, Blaxter *et al*. 2000, *unpublished*) as an outgroup.

Phylogenetic relationships were analysed by maximum parsimony (MP) methods using the MEGA 5 program (Tamura *et al*. 2011), maximum likelihood (ML) using the PHYML package (Guindon and Gascuel, 2003) and Bayesian-based inference as implemented in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). MrModel Test 2.3 (Nylander, 2008) was used to choose a best-fit model of sequence evolution (Posada, 2008). Models of evolution were chosen for subsequent analyses according to the Akaike Information Criterion (Huelsenbek and Rannala, 1997; Posada and Buckley, 2004). A general time-reversible (GTR + I) model with a proportion of invariable sites was chosen as the optimal model of evolution for *cox*1 partial gene and a Hasegawa-Kishino-Yano (HKY85) model with gamma-distributed rate variation for ITS1-5.8S-ITS2 fragment. Three independent runs of 4 Markov chains for 10 million generations, were run, sampling every 500 generations. Adequacy of sampling and run convergence were assessed using the effective sample size diagnostic in TRACER 1.5 (Rambaut and Drummond, 2007). Trees from the first million generations were discarded based on an assessment of convergence.

Furthermore, NETWORK (version 4.5.1.0) was used to create intraspecific median-joining networks

Table 1. Distribution of 39 individuals of *Trichuris* isolated from 12 populations of Arvicolinae and Cricetinae (Cricetidae, Muroidea) rodent hosts collected from different localities and their haplotypes (ITS)

(*Trichuris muris* haplotypes (Callejón *et al*. 2010) have been used as outgroups in the phylogenetic studies. Intra. V. = Intraindividual variation; Inter. V. = Interindividual variation. Symbols: Fi: Finland; Al: Finland (Åland Island); Sc: Scotland; Sw: Sweden; Cr: Croatia; It: Italy; Ka: Kazakhstan; Be: Belarus; Py: France (Eastern Pyrénean Mountains); Sp: Spain; Or: Oregon; Tu: Turkey; Bu: Buryatia; Ro: Romania; Go: Spain (Gomera, Canary Island); Pa: Spain (La Palma, Canary Island); Hi: Spain (Hierro, Canary Island); Te: Spain (Tenerife, Canary Island). Host: M.ag: *Microtus agrestis*; M.ar: *Microtus arvalis*; M.ro: *Microtus levis*; M.gl: *Myodes glareolus*; M.to: *Microtus townsendii*; C.ro: *Chionomys roberti*; C.ba; *Cricetulus barabensis*; A.sy: *Apodemus sylvaticus*; A.fl: *Apodemus flavicollis*; M.do: *Mus domesticus*; R.ra: *Rattus rattus*.)

Host	Number of individuals Host/ Parasite size	Locality	ITS2		ITS1		Haplotypes (Number of sequences)	Sample symbol	Accession numbers
			Intra. V.%	**Inter. V.%**	**Intra. V.%**	**Inter. V.%**	**44 (86)**		
Cricetidae									
Microtus agrestis	**4/6**	**South Finland**	**0·9**	**0·4**	**0**	**0·7**	**H1 (1)**	**Fi,M.ag**	**FR849652**
							H2 (1)		**FR849653**
							H3 (1)		**FR849654**
							H4 (1)		**FR849655**
							H5 (1)		**FR849656**
							H6 (1)		**FR849657**
							H7 (1)		**FR849658**
							H8 (1)		**FR849659**
	3/4	Åland Island (South Finland)	0·4	0·4	0·2	0·4	H9 (3)	Al,M.ag	FR849660
							H13 (1)		FR849664
							H14 (1)		FR849665
							H15 (1)		FR849666
							H16 (1)		FR849667
							H17 (1)		FR849668
							H18 (1)		FR849669
	3/5	Scotland	0·2	0	0·4	0·2	H9 (9)	Sc,M.ag	FR849660
							H10 (1)		FR849661
							H11 (1)		FR849662
							H12 (1)		FR849663
	1/1	Sweden	0·6	–	0	–	H8 (3)	Sw,M.ag	FR849659
Microtus arvalis	1/3	Croatia	0·2	0	0	0·2	H9 (4)	Cr,M.ar	FR849660
							H19 (1)		FR849670
	1/3	Italy	0·4	0·4	0	0	H9 (2)	It,M.ar	FR849660
							H20 (1)		FR849671
							H21 (1)		FR849672
							H22 (1)		FR849673
	1/2	Kazakhstan	0·4	0	0·2	0	H9 (3)	Ka,M.ar	FR849660
							H23 (1)		FR849674

							H24 (2)		FR849675
	1/1	Belarus	–	–	–	–	H9 (1)	Be,M.ar	FR849660
Microtus levis	1/3	South Finland	0·2	0	0	0	H8 (7)	Fi,M.ro	FR849659
							H28 (1)		FR849679
							H29 (1)		FR849680
							H30 (1)		FR849681
Microtus townsendii	1/1	Oregón	0·4	–	0·4	–	H25 (2)	Or,M.to	FR849676
							H26 (1)		FR849677
							H27 (1)		FR849678
Myodes glareolus	3/3	Spain (Montseny)	0	0·2	0·2	0·2	H9 (5)	Sp,M.gl	FR849660
							H35 (1)		FR849686
							H36 (1)		FR849687
	3/3	France (Eastern Pyrénean Mountains)	0·2	0·2	0·2	0·2	H31 (3)	Py,M.gl	FR849682
							H32 (1)		FR849683
							H33 (1)		FR849684
							H34 (1)		FR849685
Chionomys roberti	2/3	Turkey	1·1	0·2	1·1	0·7	H37 (1)	Tu,C.ro	FR849688
							H38 (2)		FR849689
							H39 (1)		FR849690
							H40 (2)		FR849691
Cricetulus barabensis	1/1	Buryatia	0	0	0·4	–	H41 (1)	Bu,C.ba	FR849692
							H42 (1)		FR849693
							H43 (1)		FR849694
							H44 (1)		FR849695
Muridae								11 (14)	
Apodemus sylvaticus		Turkey					H 28	Tu,A.sy	FN543152 (Callejón 2010)
		Spain (Montseny)					H 32	Sp,A.sy	FN543156
Apodemus flavicollis		Turkey					H 24	Tu,A.fl	FN543148
		Croatia					H 45	Cr,A.fl	FN543169
		Romania					H 48	Ro,A.fl	FN543172
Mus domesticus		Denmark					H 7	De,A.fl	FN543131
		Spain (Calafell)					H 51	Sp,M.do	FN543175
		Spain (La Riera)					H 58	Sp,M.do	FN543182
		Spain (Canary Islands)					H 6	Go,Pa,Hi, Te,M.do	FN543130
Rattus rattus		France (Eastern Pyrénean Mountains)					H 54	Py,R.ra	FN543178
		Spain (Tenerife)					H 24	Sp,R.ra	FN543148

Table 2. Percentages of similarity observed in the ITS1 and ITS2 sequences of *Trichuris* populations isolated from different hosts

(*M. agrestis: Microtus agrestis; M. levis: Microtus levis; M. arvalis: Microtus arvalis; M. glareolus: Myodes glareolus; M. townsendii: Microtus townsendii; C. roberti: Chionomys roberti; C. barabensis: Cricetulus barabensis*).

ITS1 (% Similarity)								
				Clade 1		Clade 2	Clade 3	Clade 4
	Host	*M. agrestis*	*M. levis*	*M. arvalis*	*M. glareolus*	*M. townsendii*	*C. roberti*	*C. barabensis*
Clade 1: Palaearctic region	*M. levis*	99·9						
	M. arvalis	99·9	99·9					
	M. glareolus	99·7	99·8	99·7				
Clade 2: Nearctic region	*M. townsendii*	91·6	91·7	91·6	91·5			
Clade 3: Palaearctic region	*C. roberti*	93	93·1	93	93·1	93·8		
Clade 4: Palaearctic region	*C. barabensis*	92·2	92·2	92·2	92	93·1	94·7	
Outgroup	*A. sylvaticus*			84·3		85·5	86·6	86·5

ITS2 (% Similarity)								
				Clade 1		Clade 2	Clade 3	Clade 4
	Host	*M. agrestis*	*M. levis*	*M. arvalis*	*M. glareolus*	*M. townsendii*	*C. roberti*	*C. barabensis*
Clade 1	*M. levis*	98·8						
	M. arvalis	99·6	98·7					
	M. glareolus	99·6	98·7	99·7				
Clade 2	*M. townsendii*	93·8	93·2	93·8	93·2			
Clade 3	*C. roberti*	93·7	93·8	93·8	93·8	93·3		
Clade 4	*C. barabensis*	91·3	91·4	91·4	90·6	90·6	91·6	
Outgroup	*A. sylvaticus*			88·2		88·7	87·9	90·8

(Bandelt *et al*. 1999; available at www.fluxus-engineering.com), to visualize evolutionary relationships between haplotypes. This approach has been shown to yield the best resolved genealogies relative to other rooting and network procedures (Cassens *et al*. 2003).

Phylogeographical analysis

The phylogeographical analysis was performed on the mtDNA datasets. Nucleotide diversity (*pi*) and haplotype (*h*) diversities were estimated using the DnaSP version 5.0 (Rozas and Rozas, 1997). Nucleotide diversity (*pi*) and haplotype (*h*) diversities were calculated at level of clade defined by the phylogenetic and networks analyses. The estimations of nucleotide (*pi*) and haplotypes (*h*) diversities were calculated between different clades and genetic groups.

To discriminate between the alternative hypotheses of co-speciation (host-parasite) versus geographical differentiation, we performed an analysis of molecular variance (Arlequin ver. 3.5; Excoffier and Lischer, 2010). This method estimates the proportion of genetic variation assignable to differences between pre-defined hierarchical groups, among populations within these groups, and among populations throughout the entire study area (Turner *et al*. 2000). These AMOVA analyses were performed at different hierarchical levels using information from the geographical distribution (among the 3 major geographical groups of populations) and host species.

RESULTS

Ribosomal DNA: ITS1-5.8S-ITS2

A single PCR product (about 1100 base pairs) was amplified from the genomic DNA of *Trichuris* sp. isolated from different localities and hosts. The sequences of different populations of *Trichuris* from different *Microtus* species and *Myodes glareolus* were of 1035–1093 base pairs (bp), corresponding with 433-448 bp of the ITS1; 173 bp of the 5·8S; 423–473 bp of the ITS2, while the sequences of *Trichuris* sp. isolated from *Chionomys roberti* and *Cricetulus barabensis* were of 1093–1094 and 1086–1096 base pairs, respectively, corresponding with 442 and 443–574 bp of the ITS1; 173 and 168 bp of the 5·8S; 478–479 and 474–475 bp of the ITS2, respectively. In total, 40 haplotypes were observed for the 82 (ITS1-5·8S-ITS2) sequences obtained from *Trichuris* populations from Arvicolinae hosts (GenBank Accession numbers FR849652 to FR849691) (Table 1), while 4 haplotypes were observed for the ITS1-5·8S-ITS2 sequences of *Trichuris* sp. isolated from *Cricetulus barabensis* (Cricetinae) (GenBank Accession numbers FR849692 to FR849695) (Table 1).

Intra-individual and intra-specific variations were observed in the ITS1 and ITS2 sequences of different individuals isolated from different hosts and regions (Table 1).

Different repetitive nucleotide sequences called microsatellites were found in the ITS1 and ITS2 sequences of *Trichuris* sp. isolated from different localities and hosts. Thus, in the ITS1 sequences of all species of *Trichuris* analysed, Poly (GC) and Poly (CTG) were observed at positions 26 and 83 respectively, while Poly (TA) was only observed at position 419 in the ITS1 sequences of *Trichuris* from *Microtus agrestis*, *M. arvalis*, *M. levis* and *Myodes glareolus*. Furthermore, in the ITS2 sequences, Poly (AGT), Poly (GCT) and Poly (CG) were observed at positions 36, 223 and 407, respectively.

The percentage of similarities observed by the comparative study of the ITS1 and ITS2 sequences of *Trichuris* populations isolated from different rodent hosts collected from different geographical regions are shown in Table 2.

Mitochondrial DNA: cytochrome c-oxidase *subunit 1 partial gen*

A single PCR product was amplified from each of the genomic DNA of *Trichuris* species isolated from different localities and hosts. C*ytochrome c-oxidase* subunit 1 (*cox*1) partial gene sequences of *Trichuris* sp. were of 409–410 base pairs (bp) and the AT% content ranging from 63·1 to 64·7% (Table 3).

A total of 16 haplotypes (Table 3) were observed for the 38 *cox*1 partial gene sequences obtained from *Trichuris* populations from Arvicolinae hosts (GenBank Accession numbers, Table 4). Intraspecific variations were observed in the *cytochrome c-oxidase* subunit 1 gene sequences of different individuals isolated from different hosts and regions (Tables 3 and 4). It is noteworthy that the highest variability was observed among the individuals from *Myodes* isolated from Eastern Pyrenees (1·2%, see Table 4). Different repetitive nucleotide sequences called microsatellites were found in the *cox*1 partial gene sequences of *Trichuris* populations isolated from different localities and hosts. Thus, Poly (TA) and Poly (TTA) were observed at positions 162 and 384.

When the *cox*1 partial gene sequences of *Trichuris* species from *Microtus agrestis*, *Microtus arvalis*, *Microtus levis* and *Myodes glareolus* isolated from different Palaearctic regions (Fig. 1) were compared, the percentages of similarities ranged from 96·8% to 100%, while a 85·1% to 87·1% (not shown) of homology was observed when these *cox*1 gene sequences were compared with *Trichuris* sp. of *Chionomys roberti*. Furthermore, when *cox*1 partial gene sequences of *Trichuris* populations from voles were compared with those of *Trichuris muris*, the percentages of similarity were about 82·1% to 82·7%.

Table 3. Interhaplotype differences found in the mtDNA *cox1* partial gene sequences of *Trichuris* populations

(Bp = Basis pair.)

	Cox 1																								
	Bp length	% AT	Nucleotide position																						
			27	31	33	42	52	57	64	68	93	114	150	153	159	170	186	210	234	255	265	276	327	342	345	354
T. arvicolae haplotypes (n = 14)																									
H1	410	63·4	C	T	G	C	T	G	T	T	T	C	G	A	T	C	T	A	G	G	A	T	G	A	G	T
H2	410	63·4	C	T	G	T	A	G	T	T	T	C	G	A	T	C	T	A	G	G	A	T	G	A	G	T
H3	410	63·1	C	T	A	C	T	G	T	T	T	C	G	G	C	G	A	A	G	G	A	T	G	A	G	T
H4	409	63·3	C	T	A	C	T	G	T	T	T	C	G	G	T	C	A	A	G	G	A	T	G	A	G	T
H5	409	63·1	C	T	A	C	T	G	T	T	T	C	G	G	T	C	A	A	G	G	G	T	G	A	G	T
H6	410	63·6	C	T	G	C	T	G	T	T	T	C	G	A	T	C	T	A	G	A	A	T	G	A	G	T
H7	410	64·7	T	T	A	C	T	A	T	T	T	T	A	A	T	C	A	G	G	G	A	T	G	A	A	T
H8	410	64·4	T	T	A	C	T	A	T	T	C	T	G	A	T	C	A	A	A	G	A	T	A	A	G	C
H9	410	64·1	T	C	A	C	T	A	T	T	C	T	G	A	T	C	A	A	A	G	A	T	A	A	G	C
H10	410	64·2	T	T	A	C	T	A	T	T	C	T	G	A	T	C	A	A	G	G	A	T	A	A	G	C
H11	410	63·2	C	T	G	C	T	G	T	T	T	C	G	G	T	C	A	A	G	G	A	T	G	A	G	T
H12	410	64·4	T	T	A	C	T	A	A	A	T	T	A	A	T	C	A	G	G	G	A	C	G	A	A	T
H13	410	64·7	T	T	A	C	T	A	T	T	T	T	A	A	T	C	A	A	G	G	A	T	G	A	G	T
H14	410	63·9	T	T	A	C	T	A	T	T	T	T	G	A	T	C	A	A	G	G	A	T	A	G	G	C
	Cox 1																								
	Bp length	% AT	Nucleotide position																						
			280																						
Chionomys roberti haplotypes(n = 2)																									
H15	410	63·4	A																						
H16	410	63·2	G																						

Table 4. Distribution of *Trichuris* populations isolated from different localities and hosts (Cricetidae, Arvicolinae)

(Different haplotypes (*cox*1) are shown. IInter. V. = Interindividual variation. Symbols: Fi: Finland; Al: Finland (Åland Islands); Sc: Scotland; Sw: Sweden; Cr: Croatia; It: Italy; Ka: KKazakhstan; Be: Belarus; Py: France (Eastern Pyrenean Mountains); Sp: Spain; Tu: Turkey; Host: M.ag: *Microtus agrestis*; M.ar: *Microtus arvalis*; M.ro: *Microtus levis*; M.gl: *Myodes glareolus*; C.ro: *Chionomys roberti*.)

Host	Host/ Parasite size	Locality	*Cox*1	Haplotype/ (Number of sequences)	Sample symbol	Accession numbers
			Inter. V.%	16/ 38		
Microtus agrestis	4/9	South Finland	0·5	H1 (8)	Fi, M.ag	FR851275
				H2 (1)		FR851276
	2/3	Åland Island (South Finland)	0·7	H3 (1)	Al, M.ag	FR851277
				H4(1)		FR851278
				H5 (1)		FR851279
	2/3	Scotland	0	H4 (3)	Sc, M.ag	FR851278
	2/2	Sweden	0·2	H1 (1)	Sw, M.ag	FR851275
				H6 (1)		FR851280
Microtus arvalis	1/3	Croatia	0·2	H8 (2)	Cr, M.ar	FR851282
				H9 (1)		FR851283
	1/3	Italy	0	H7 (3)	It, M.ar	FR851281
	1/2	Kazakhstan	0	H10 (2)	Ka, M.ar	FR851284
	1/1	Belarus	–	H8 (1)	Be, M.ar	FR851282
Microtus levis	1/3	South Finland	0·5	H1 (2)	Fi, M.ro	FR851275
				H11 (1)		FR851285
Myodes glareolus	3/3	Spain (Montseny)	0·7	H10 (2)	Sp, M.gl	FR851284
				H14 (1)		FR851288
	3/3	Py	1·2	H12 (1)	Py, M.gl	FR851286
				H13 (2)		FR851287
Chionomys roberti	1/3	Turkey	0·2	H15 (1)	Tu, C.ro	FR851289
				H16 (2)		FR851290

Unfortunately, we could not obtain any sequence of the *cox*1 partial gene of *Trichuris* populations from *Microtus townsendi* from Oregon (USA) and *Trichuris* from *Cricetulus barabensis* from Buryatia. Thus, a comparative study could not be carried out with these two species.

Phylogenetic reconstruction of Trichuris *populations isolated from Arvicolinae hosts*

Phylogenetic and network relationships of ITS1-5.8S-ITS2 fragment sequences. The *Trichuris* species data matrix was composed of 86 rDNA sequences (44 haplotypes) (Table 1) and 38 mtDNA sequences (16 haplotypes) (Table 4). The ML (−ln = 3389·1), MP (Length = 320 steps; Consistency Index (CI) = 0·934169; Retention Index (RI) = 0·986850; Rescaled Consistency Index (RCI) = 0·921885) and Bayesian reconstruction analyses (The potential scale reduction factor (PSRF) were all close to 1·0 for all parameters) were performed on the sequences obtained from *Trichuris* species collected from 7 species of rodent hosts (Table 1) isolated from different geographical regions. *Trichuris muris* sequences from murid rodents (*Apodemus sylvaticus*, *A. flavicollis*, *Mus domesticus* and *Rattus rattus*) were used as outgroups (Callejón *et al*. 2010, Table 1).

The topology was congruent across the 3 methods assayed. Four well-supported genetic groups (Fig. 2) appeared: clade 1 (Bootstrap values (BP) of 100%, 99% and 100% in ML, MP and Bayesian analyses, respectively) was a large, widely distributed clade corresponding with *Trichuris* populations from *Microtus* species and *Myodes glareolus* from the Palaearctic zone; clade 2: *Trichuris* populations from *Microtus townsendii* (BP of 100%, 100% and 100%, respectively) corresponding with the Nearctic zone; clade 3: *Trichuris* populations from *Chionomys roberti* (BP of 100%, 100% and 100%, respectively) and clade 4: *Trichuris* populations from *Cricetulus barabensis* (BP of 100%, 100% and 100%, respectively).

Networks of the 44 haplotypes of *Trichuris* sp. from voles showed a general congruence with the phylogenetic reconstruction (Fig. 3). The minimum spanning network showed the 4 main groups defined above: clades 2, 3 and 4 appeared well separated with 40, 28 and 36 mutational steps, respectively. Clade 1 clustered all the haplotypes of *Trichuris* populations from the Western and Eastern European regions. H9 haplotype was the most frequent haplotype observed

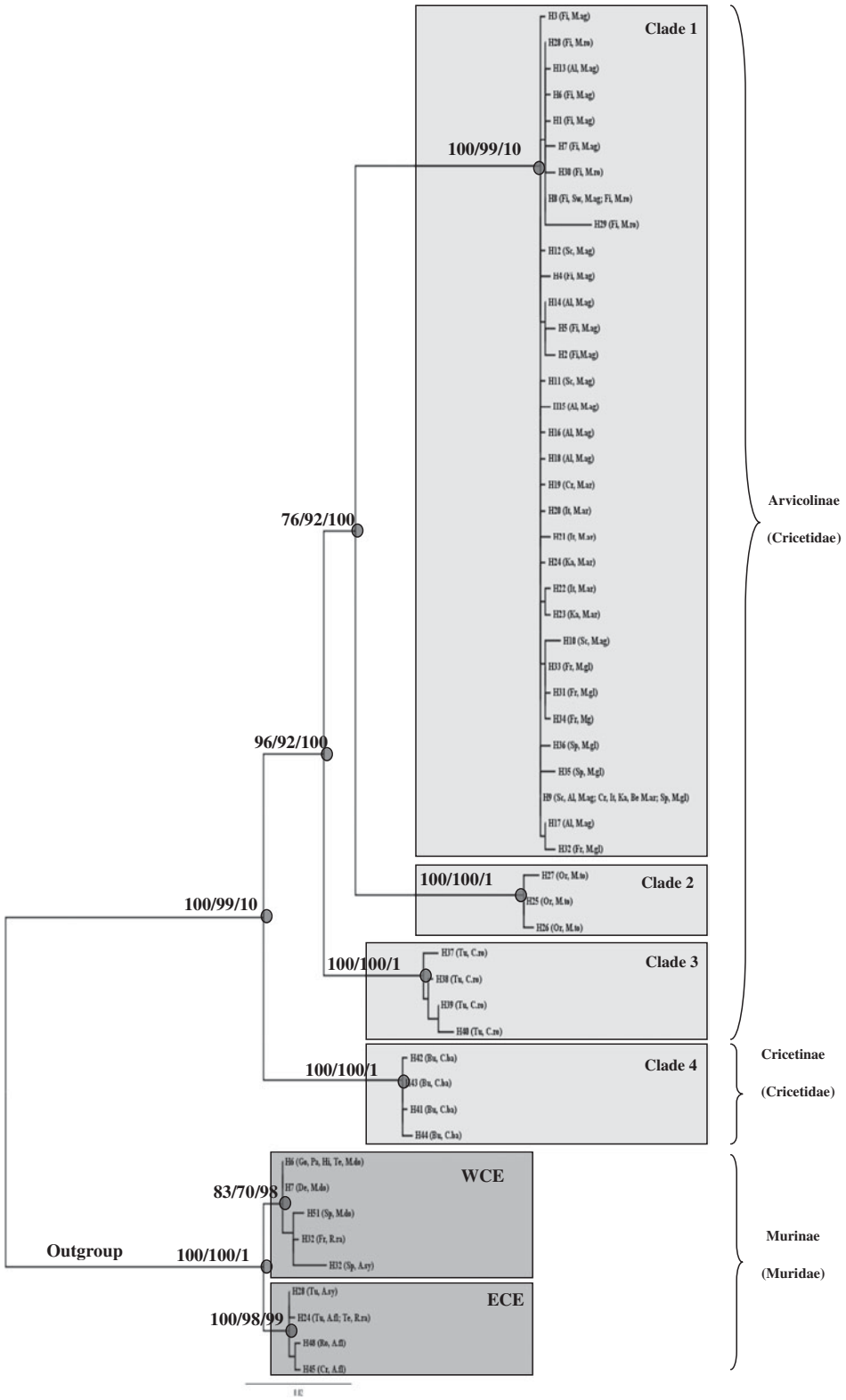

Fig. 2. Most likely tree of the PHYML for the 44 haplotypes observed for the ITS1-5.8S-ITS2 sequences of *Trichuris* sp. isolated from different voles (Cricetidae: Arvicolinae and Cricetinae). Geographical origins and hosts (see Tables 1 and 2 and Fig. 1) are shown in parentheses. Numbers on branches indicate, from left to right (a) bootstrap support obtained in the PHYML analysis (HKY85); (b) bootstrap support obtained in one tree of 307 trees of the MP reconstruction; (c) bootstrap support obtained in the Bayesian analysis. Note that Bootstrap values under 70% were not considered.

in Palaearctic populations (showed by 27 taxa) distributed throughout a wide extension of regions (Åland Island, Scotland, Croatia, Italy, Kazakhstan, Belarus and Spain) (Fig. 3). The haplotypes network of clade 1 revealed star-like patterns around haplotype 9. Nevertheless, phylogenetic analysis obtained

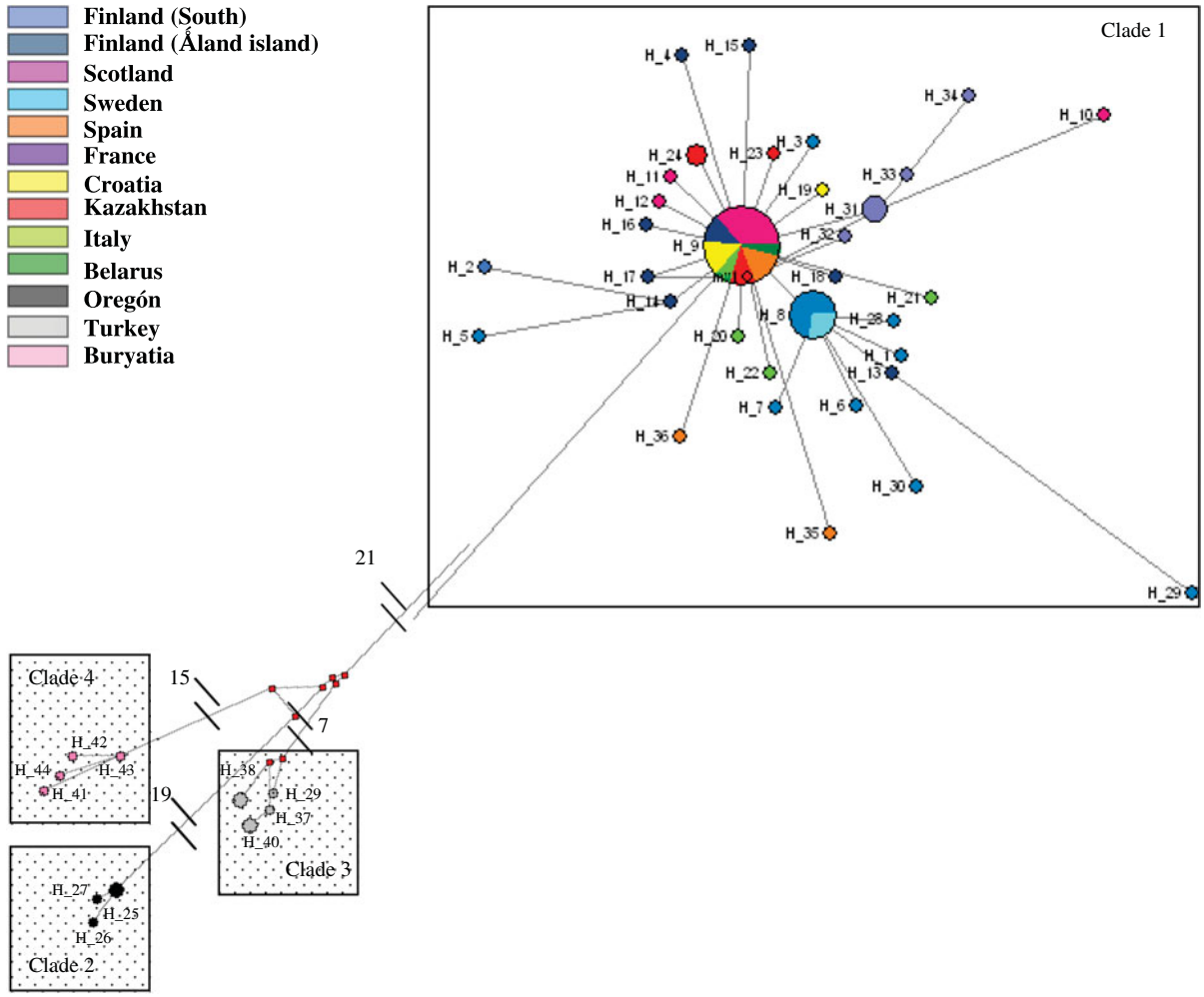

Fig. 3. A minimum spannig network constructed using the 44 haplotypes of ribosomal ITS1-5·8S-ITS2 fragment sequences. The geographical origin for each haplotype is shown in Table 1. The size of the circle is proportional to the numbers of haplotypes represented. The numbers correspond to the mutational steps observed between clades and groups.

on the basis of ITS1-5.8S-ITS2 rDNA sequences did not show a differential geographical structure because all of the populations from the Palaearctic region were clustered in clade 1.

Phylogenetic relationships of cytochrome c-oxidase *subunit 1 partial gene sequences*. The ML (−ln = 1121·79), MP (Length = 126 steps; CI = 0·920635; RI = 0·921875; RCI = 0·848710) and Bayesian reconstruction analyses (the potential scale reduction factor (PSRF) were all close to 1·0 for all parameters) were performed using *Trichuris muris* sequences as outgroups (Table 4). The phylogenetic tree (Fig. 4) of *Trichuris* populations from *Microtus* sp., *Myodes glareolus* and *Chionomys roberti* from Western or Eastern Europe showed four well-supported genetic groups: (1) clade 1: *Trichuris* populations isolated from *Microtus agrestis* and *Microtus levis* from Northern Europe (South Finland, Åland Islands (SW Finland), Scotland and Sweden) (Bootstrap values BP of 95%, 94% and 97% ML, MP and Bayesian analyses respectively); (2) clade 2: *Trichuris* populations isolated from *Microtus arvalis* and *Myodes glareolus* from the southwestern, southeastern and eastern Europe (Spain, Croatia, Belarus) and Central Asia (Kazakhstan) (BP of 91%, 86% and 86% respectively); (3) clade 3: *Trichuris* populations isolated from *Microtus arvalis* and *Myodes glareolus* from France (Pyrenees) and Italy (BP of – %, 87% and 68% respectively); (4) clade 4: *Trichuris* populations from *Chionomys roberti* from Turkey (BP of 87%, 100% and 100% respectively). *Trichuris* populations clustered in clade 1, clade 2 and clade 3 showed high BP values (96%, 100% and 100% respectively), separated from *Trichuris* populations from *Chionomys roberti*. Furthermore, in the phylogenetic tree populations of *Trichuris* isolated from Northern Europe (clade 1) they appeared clustered in 2 main groups including the South of Finland and Sweden populations (subclade 1a) supported by high bootstrap values (91%, 77% and 100% respectively) another group clustering populations of *Trichuris* from South Finland, Åland Islands SW Finland and Scotland (Fig. 4).

Table 5. Percentages of similarity observed in *cox*1 partial gene sequences between different clades of *Trichuris* populations isolated from different hosts (Arvicolinae, Cricetidae)

*Cox*1 sequences: Geographical origin	Clade 1	Clade 2	Clade 3	Clade 4
Clade 1: Northern Europe	98·3–100			
Clade 2: Southern and Eastern Europe	97·3–98·1	99·3–100		
Clade 3: Italy and France	96·8–98·3	97·3–99	98·8–100	
Clade 4: Turkey	85·1–86·1	85·9–86·6	86·6–87·1	99·8–100

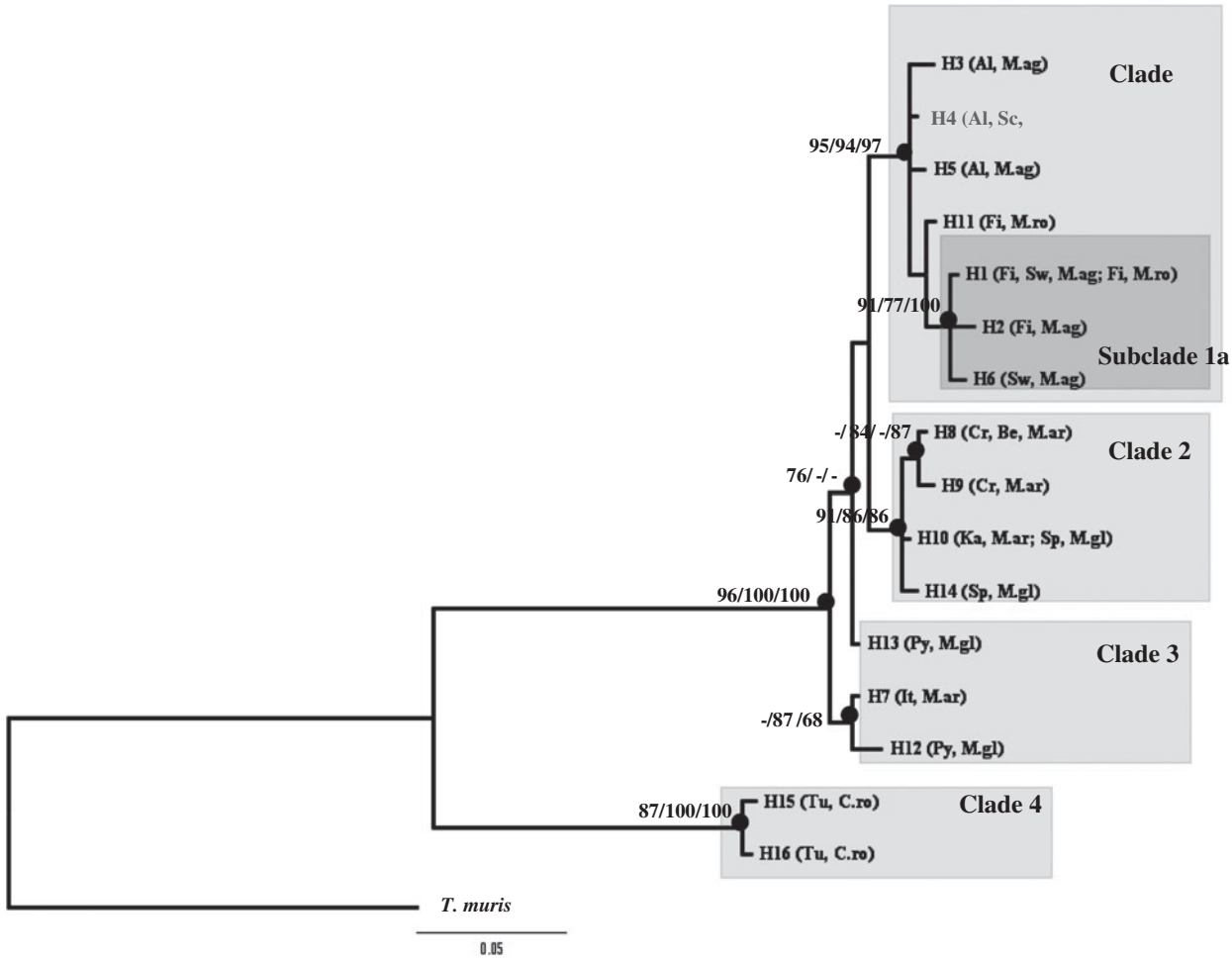

Fig. 4. Mayority-rule consensus tree for the 16 haplotypes observed for the *cox*1 partial sequences of *Trichuris* sp. isolated from different voles (Cricetidae: Arvicolinae) derived from Bayesian inference. Geographical origins and host (see Table 3 and Fig. 1) are shown in parentheses. Numbers on branches indicate, from left to right (a) bootstrap support obtained in the PHYML analysis (GTR + G + I); (b) bootstrap support obtained in one tree of 51 trees of the MP rescontruction; (C) bootstrap support obtained in the Bayesian analysis. Note that Bootstrap values under 65% were not considered. Symbols: Fi: Finland; Al: Finland (Åland Island); Sc: Scotland; Sw: Sweden; Cr: Croatia; It: Italy; Ka: Kazakhstan; Be: Belarus; Py: France (Eastern Pyrénean Mountains); Sp: Spain; Tu: Turkey. Host: M.ag: *Microtus agrestis*; M.ar: *Microtus arvalis*; M.ro: *Microtus levis*; M.gl: *Myodes glareolus*; C.ro: *Chionomys roberti*.

The percentages of similarity intra- and inter-clade are shown in Table 5. The network of the 14 haplotypes of *Trichuris* populations showed a general congruence with the phylogenetic reconstruction. The minimum spanning network showed the 3 main groups defined above and separated from each other by a genetic distance of 4–7 mutational steps (Fig. 5). Clade 1 clustered 1 distinct group (subclade 1a), linked by 2 mutational steps. A typical haplotype (H1) observed in the clade 1 was the most frequent haplotype (showed by 11 taxa). On the other hand, a typical haplotype (H10) observed in the clade 2 was the most frequent (showed by 4 taxa) in *Trichuris* populations from the South and East of Europe. Furthermore, clade 4 clustered *cox*1 sequences of *Trichuris* isolated from *Chyonomis roberti* from

Table 6. Intra-clade and inter-clade genetic variability based on *cox*1 partial gene sequences among *Trichuris* populations

	Sample size	Number of haplotypes	Nucleotide diversity (*pi*) ± S.D.	Haplotype diversity (*h*) ± S.D.
Inter-clades (Palaeartic zone)	34	14	0·013 ± 0·0136	0·872 ± 0·044
Intra-clades				
Clade 1 (North Europe)	20	7	0·00490 ± 0·0009	0·679 ± 0·102
Subclade 1a (Sweden and Finland)	11	3	0·00130 ± 0·00075	0·345 ± 0·172
Clade 2 (Southern and Eastern Europe)	9	4	0·00244 ± 0·00059	0·750 ± 0·112
Clade 3 (Italy and France)	6	3	0·00504 ± 0·00163	0·733 ± 0·155

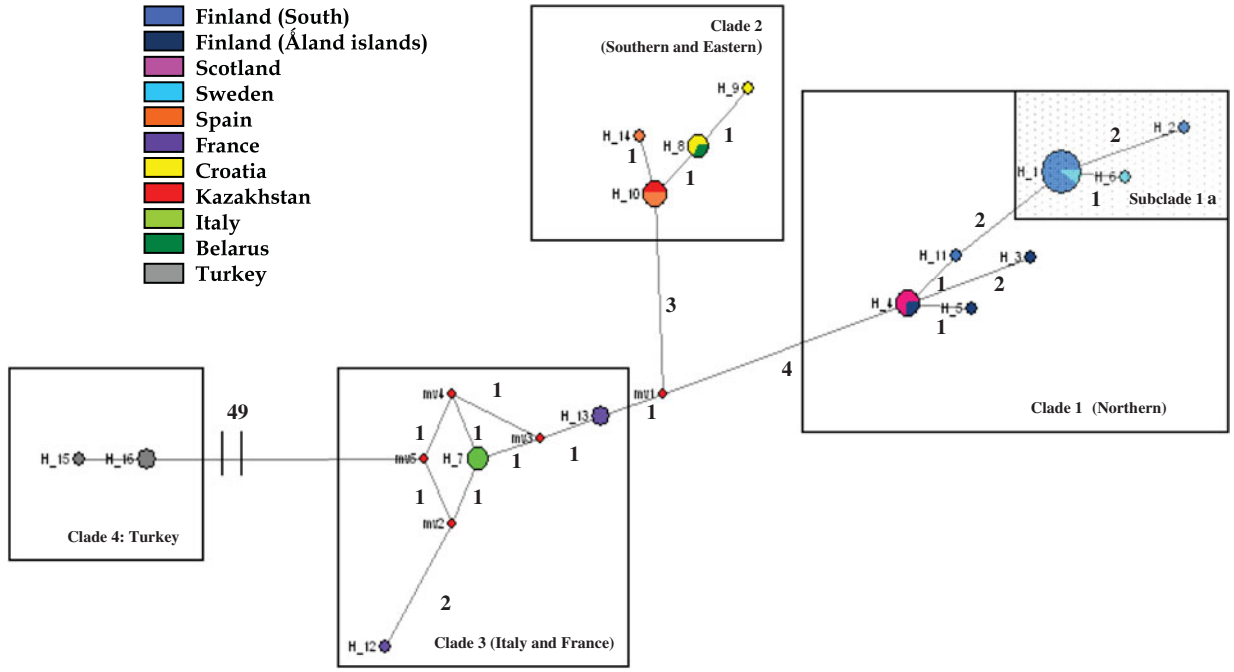

Fig. 5. A minimum spanning network constructed using 16 haplotypes of mitochondrial *cox*1 partial gene sequences of *Trichuris arvicolae* and *Trichuris* sp. The geographical origin for each haplotype is shown in Table 3. The size of the circle is proportional to the numbers of haplotypes represented and the numbers correspond to the mutational steps observed between clades and/or groups.

Turkey and this was separated from the other 3 clades by 49 mutational steps.

The phylogenetic analyses of the mtDNA *cox*1 partial gene sequences (Figs 4 and 5) revealed patterns of genetic differentiation within populations of *Trichuris* parasitizing rodent hosts (Arvicolinae) from the Palaearctic region.

Phylogeographical analysis of cytochrome c-oxidase *subunit 1 partial gene sequences (mtDNA).* The estimation of nucleotide (*pi*) and haplotypes (*h*) diversities was performed on populations of *Trichuris* isolated from Arvicolinae hosts from the Palaearctic region (Table 6). Despite the lowest sample size, clade 3 (Italy and France) had a nucleotide diversity (*pi*) higher than the other 2 clades. On the other hand, clade 2 (Southwestern and Eastern Europe), with a similar sample size to clade 3, presented the lowest *pi* value, while, surprisingly, clade 1 (Northern Europe) showed a higher value than clade 2 (Table 6). Within this clade 1, nucleotide diversity was, contrary to expectations, higher in the Åland Islands than in the rest of the Northern continental region.

AMOVA analysis (Table 7) showed the influence of geographical factors versus co-speciation (host-parasite) in the biogeography of *Trichuris* species. Thus, attending to the co-speciation hypothesis, the molecular variance was about 37·3% whereas the geographical differentiation showed 69·2%. Therefore and according to these results the geographical model would explain the 3 genetic lineages (Italy and France, Northern Europe and Southern and Eastern Europe) by analysis of the molecular variance (Table 7). Furthermore, within none of these groups was a low percentage variation (19·9%) observed among populations.

Table 7. Analysis of molecular variance of *cox*1 partial gene of *Trichuris* populations

(D.F.: degrees of freedom; Fsc: measures of differentiation among populations within group; Fct: measures differentiation among individuals within populations; Fst: measures of the genetic variation between populations; P: P-value.)

Barrier	Source of variation	D.F.	Sum of squares	Percentage of variation
Geographical differentiation	Among groups	2	71·9	69·2
	Among populations within groups	7	24·3	19·9
	Within populations	29	13·5	11
	Total	38	109·7	
: $P<0·01$; ***: $P<0·001$. D.F. = degrees of freedom. Fixation indices: Fsc = 0·6; Fst = 0·9***; Fct = 0·7***				
Co-speciation (host-parasite)	Among groups	2	24·7	37·3
	Among populations within groups	4	23·1	45·4
	Within populations	19	10·6	17·3
	Total	25	58·4	
: $P<0·01$; ***: $P<0·001$. D.F. = degrees of freedom. Fixation indices: Fsc = 0·7; Fst = 0·8***; Fct = 0·4***				

DISCUSSION

The ITS1 and ITS2 sequences observed for *Trichuris* populations isolated from *Microtus agrestis*, *M. arvalis*, *M. levis* and *Myodes glareolus* were identical to those obtained by Cutillas *et al.* (2002) for *T. arvicolae* isolated from *Myodes glareolus*. Nevertheless, when these sequences were compared with those of *Trichuris* populations from *Microtus townsendii* from Oregon (USA, Nearctic region), *Chionomys roberti* (Arvicolinae) and *Cricetulus barabensis* (Cricetinae) the percentages of similarity showed less than 94%. This observation indicates that there may be a second species of *Trichuris* in arvicoline rodents.

There are no models which define the level of nucleotide differences required to distinguish between closely related parasite species (Stevenson *et al.* 1995), nevertheless, the range of percentages of variation observed between different *Trichuris* populations was higher than those observed intraindividually.

Although mitochondrial DNA marker (*cox*1 sequences) corroborated ribosomal markers results, the percentages of similarity observed between different populations of *Trichuris* by ribosomal markers were higher than those observed by the sequencing of *cox*1 partial gene. This observation has been explained by several other authors. Thus, Blouin (2002) and Hu *et al.* (2003) cited that the within-nematode species variation in protein-genes of mtDNA are greater than in ribosomal spacers probably due to the absence of cyto-nuclear disequilibrium or to epistatic effects or drift across genomes (Asmussen *et al.* 1987). Intraspecific divergence in *cox*1 gene is usually less than 5% (Blouin, 2002; Hu *et al.* 2002; Otranto *et al.* 2005), whilst closely related congeneric species display a range of variation of 10–20% (Blouin, 2002). Thus, if 2 individuals differ by 10% or more, one might question whether they really are conspecific (Blouin *et al.* 1998; Blouin, 2002). Thus, Blouin *et al.* (1998) found a mitochondrial DNA sequence variation among individuals of the same species (intra-specific variation) of nematode averaging a fraction of a percent up to 1·2% and the maximum difference ever observed between a pair of individuals that were clearly members of the same interbreeding population of *Ostertagia ostertagi* was 6%. According to these authors, the percentages of similarity observed in the *cox*1 partial gene and ITS1-5.8S-ITS2 sequences of *Trichuris* populations could suggest other species of *Trichuris* than *Trichuris arvicolae* parasitizing *Microtus townsendi* and *Chionomys roberti*. Further morphological and molecular studies could test this hypothesis. It is well known for cryptic/sibling species to be described initially by molecular, karyotypic, ecological or behavioral characters and for minor morphological features to be detected subsequently (Jaarola and Searle, 2004; Haukisalmi *et al.* 2008). Nevertheless, we must be careful, since only 1 individual could be collected from the Nearctic zone.

The phylogenetic analysis carried out on the basis of ribosomal DNA molecular markers suggested the existence of two genetic lineages (Nearctic and Palaearctic lineages) of *Trichuris* populations and the minimum spanning network showed all the haplotypes from European regions (clade 1) clustered together and with star-like pattern around haplotype 9. Based on coalescent theory (Slatkin and Hudson, 1991) this star topology showed that *Trichuris* populations had experienced a significant population

expansion. At the centre of the network is haplotype 9, which is distributed widely and takes over the highest proportion in the population. This suggests that the haplotype 9 should be the ancestral haplotype. This same topology was found by Zhou *et al.* (2011) in the *cox*1 gene haplotypes of *Ascaris* populations from humans and pigs from China.

These results are not in agreement with previous studies in other species of *Trichuris* (Callejón *et al.* 2010). Thus, a phylogeographical study carried out on *Trichuris muris*, nematode parasitizing Murinae rodents from the Muridae family, isolated from 4 different hosts and from different geographical regions of Europe by amplification and sequencing of the ITS1-5.8S-ITS2 fragment of the ribosomal DNA, revealed 2 clear-cut geographical and genetic lineages: one of them was widespread from Northern Spain (Catalonia) to Denmark (Western European region), while the second was widespread in the Eastern and Southeastern European region (Croatia, Romania, and Turkey).

Mitochondrial results based on *cox*1 partial gene sequences revealed that *T. arvicolae* populations from the Palaearctic region are separated into 3 clear-cut geographical and genetic lineages corresponding to the Northern Europe (Finland, Scotland and Sweden), Southwestern and Southeastern Europe, and Central Asia (Spain, Croatia and Kazakhstan) and Italian and French populations. Thus, we can conclude that mitochondrial genome sequences clearly present data for analysing a phylogeographical pattern of *Trichuris* populations whereas the ribosomal genome sequences are not informative enough for this analysis. Nevertheless, previous results (Callejón *et al.* 2010) established the phylogeographical pattern of *Trichuris muris* based on ribosomal DNA.

Wu *et al.* (2009) cited that the mitochondrial marker showed stronger genetic structure than the ribosomal marker because mtDNA is haploid, so that the effective population size is only one-quarter that of nuclear DNA (Page and Holmes, 1998; Ballard and Whitlock, 2004). In addition, they suggest that *cox*1 is substantially more differentiated than ITS1 rDNA in the studied populations, and that nematode mtDNA evolves more quickly than the mtDNA of other taxa (Blouin *et al.* 1995; Anderson *et al.* 1998). Mitochondrial DNA has been widely used in studies of population genetics, phylogeography and phylogeny because it provides easy access to an orthologous gene set with rapid evolution and with little or no recombination (Mas-Coma and Bargues, 2009). Derycke *et al.* (2007) concluded that both *cox*1 and ITS data revealed high levels of molecular diversity, yet, the ITS data revealed the same 5 lineages, but divergence values between the populations were lower than in the mitochondrial *cox*1 gene.

Haplotype diversity (*h*) and nucleotide diversity (*pi*) are important indices to evaluate genetic diversity and differentiation, and a high value of the indices usually indicates a wealth of genetic diversity in the studied population (Huang *et al.* 2007; Liu *et al.* 2006). The estimation of nucleotide (*pi*) and haplotype (*h*) diversities performed on populations of *Trichuris* isolated from Arvicolinae hosts revealed higher nucleotide diversity than expected in *Trichuris* populations from the Åland Islands. According to Fernández-Palacios (2010), one of the most important island features that make them a biologically interesting study system is the lower biological complexity of island communities when compared to equivalent mainland ones. Furthermore, Delicado *et al.* (2010) cited that insular species were less variable genetically than continental species suggesting a more recent divergence of the former. Nevertheless, islands in the Baltic Sea are unique because inter-island distances are generally small, salinity is low, and seasonality is pronounced (Järvingen and Ranta, 1987). Furthermore, there is a long history of research on many of these islands rendering them suitable for studies in population and community ecology and conservation (Niemelä *et al.* 1985; Järvinen and Ranta, 1987; Ås *et al.* 1997; Nieminen and Hanski, 1998).

Nieberding *et al.* (2005) carried out the phylogeography of *Heligmosomoides polygirus* in the Western half of the Palaearctic region. The analysis of nucleotide diversity (*pi*) showed values above 0·012 obtaining maximum values of 0·026 corresponding to high genetic diversity. In our case, the low genetic variability observed in our material could be explained by the appearance of genetic bottlenecks during one of the last Ice Ages (Michaux *et al.* 2003; Nieberding *et al.* 2004). This hypothesis is corroborated by 2 results: the very short branch length between haplotypes within this group in the distance analysis and the star-like topology in the minimum spanning network suggesting a rapid expansion from a small number of founder animals (Michaux *et al.* 2003).

From a biogeographical point of view, Europe has some distinctive features. It is a large peninsula connected to Asia, with an east-west orientation. The Mediterranean Sea in the south constitutes a strong barrier, and has limited the possibility of southern displacement of biota during cold periods (Taberlet *et al.* 1998). Furthermore, the east-west orientation of the main mountain ranges of the Alps and the Pyrenees acted as a barrier to northward expansion of species during warm periods. The effects of the ice ages on European species has been examined in detail by Hewitt (1999): during the Quaternary, each species went through many contractions/expansions of range, characterized by extinctions of northern populations when the temperature decreased, and a northward expansion from refuges (e.g. in Carpathians) involving spreading from the leading edge. Such a colonization process implies successive

bottlenecks that may lead to a loss of genetic diversity in the northern populations, with the exception of cold-tolerant taxa.

Furthermore, studies on the comparative phylogeny of taxa strongly linked by an ecological factor such as parasitism have shown that the degree of phylogenetic congruence increases with the obligate character of the host-parasite relationships (Nieberding *et al*. 2004). At an intra-specific level, it can be assumed that the phylogeographical patterns observed between species linked by a parasitic relationship are likely to be congruent in time as well as space, providing the parasite is specific and obligate (Price, 1980). Thus, the degree of genetic differentiation among parasite populations depends on gene flow, which is generally determined by host mobility, effective (i.e. breeding) population sizes, which is determining the rate of genetic drift and therefore the rate of independent differentiation of populations, and reproductive mode (Blouin *et al*. 1995; Nadler *et al*. 1995). Huyse *et al*. (2005) concluded that parasite population ecology and population genetics are closely linked. More specifically, they argue that the structure of parasite populations correlates with (i) host mobility, (ii) mode of reproduction of the parasite, (iii) complexity of the parasite life cycle, (iv) parasite infrapopulation size and (v) host specificity. The importance of these factors varies from one parasite species to the next. Therefore, a comparative approach with a phylogenetic perspective is crucial to disentangle the various processes that drive parasite diversification.

Trichuris arvicolae is a parasite of the caecum of specific hosts (Arvicolinae) and has a direct life cycle; therefore, the biogeography of this parasite is closely linked to the biogeography of its host. Jaarola and Searle (2002) studied the phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences and found 3 phylogenetic lineages corresponding with 3 phylogeographical groups with strict geographical distributions labelled as 'southern', 'eastern' and 'western'. Furthermore, they cited that the western and eastern lineages entered Fennoscandia from the south and northeast, respectively.

These results were similar to those observed by Nieberding *et al*. (2005) of *Heligmosoides polygyrus* from western Palaearctic region, and Wu *et al*. (2009) in *Camallanus cotti* from China.

The mtDNA analysis of *Trichuris* populations from voles shows signs of a post-glacial northward population expansion starting from the Pyrenees and Italy. Apparently, the Pyrenees and the Alps were not barriers to the dispersal of *Trichuris arvicolae* populations. Similar results were obtained by Seddon *et al*. (2001) for *Erinaceus europaeus*. Thus, mtDNA data showed signals of a post-glacial northward population expansion starting from 3 refugia: Iberia, Italy and the Balkans.

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