Host taxon-derived Sarcoptes mite in European wild animals revealed by microsatellite markers

Host taxon-derived Sarcoptes mite in European wild animals

R. Rasero[*], L. Rossi[*], D. Soglia[*], S. Maione[*], P. Sacchi[*], L. Rambozzi[*], S. Sartore[*], V. Spalenza[*] and S. Alasaad[*†]

[*]Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia, Università degli Studi di Torino, Via Leonardo da Vinci 44, I-10095, Grugliasco, Italy

[†]Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas (CSIC), Avda. Americo Vespucio s/n 41092 Sevilla, Spain

Correspondence (1): Samer Alasaad, fax: +34953211873;

E-mail: samer@ebd.csic.es

Correspondence (2): Luca Rossi, fax: +39116709000;

E-mail: luca.rossi@unito.it

Abstract Microsatellite genotyping was applied on individual Sarcoptes mites from 15 wild mammalian populations belonging to 10 host species in three European countries, using 10 Sarcoptes mite specific markers. The results showed that the geographical separations had real biological significance for the definition of mite sub-populations, and that the degree of genetic exchange occurring between mites from different localities was related to the geographical distance between locations. Wild host-derived mite populations were clustered into three main groups: herbivore-, carnivore- and omnivore-derived Sarcoptes mite populations. Omnivore-derived was halfway between herbivore- and carnivore-derived Sarcoptes mite populations. The separation between the three mite groups (herbivore-, carnivore- and omnivore-derived mite) was more

supported than that by the geographical separations; nevertheless a kind of sub-clustering was detected within each group (carnivore-, omnivore- and herbivore-), scattering mite populations up to their geographical localities (countries). The lack of gene flow between Sarcoptes populations may have improved parasitic adaptations and led to, what we called host taxon-derived (carnivore host-, herbivore host- and omnivore host-derived) Sarcoptes mite populations in European wild animals. Our results demonstrated that Sarcoptes is not a single panmictic population even within locations, which will have important ramifications on the study of population genetic structure, life cycle, diagnosis and monitoring protocols, and could contribute to the better understanding of the epidemiology of the ubiquitous Sarcoptes mite.

Keywords: Sarcoptes scabiei; Genetic epidemiology; Genetic structure; Microsatellite markers; Omnivore-derived; Carnivore-derived; Herbivore-derived; Host taxon-derived.

Introduction

Predicting the spread of a disease to wildlife is critical in order to identify populations at risk, target surveillance, and design proactive management programmes (Blanchong et al. 2008). Recently, there has been an increased interest in diseases of free-living animals and the ecological role of diseases in populations, particularly their ability to regulate animal abundance (Scott 1988; Lyles & Dobson 1993; Robinson 1996; Daszak et al. 2000). There is also an awareness that free-living species may act as reservoirs of diseases of man and domestic animals (Robinson 1996; Daszak et al. 2000).

Although neglected as a pathogen, the ectoparasite Sarcoptes scabiei continues to affect humans and a wide range of mammalian hosts on a worldwide scale (Bornstein et al. 2001; Pence & Ueckermann 2002; Walton et al. 2004a). The introduction of infected domestic animals and the succeeding adaptation of Sarcoptes mite to a new highly susceptible and receptive wild host have been proposed to give rise to sarcoptic mange epizootics in previously mange-free wildlife populations (Arlian 1989).

In several European wild mammal populations, Sarcoptes mite infections are endemic and cause devastating mortality as reported especially for Alpine and Pyrenean chamois, Iberian ibex, aoudad, and red fox (Fandos 1991; Mörner 1992; Pérez et al. 1997; León-Vizcaino et al. 1999; González-Candela et al. 2004; Rossi et al. 2007). Notwithstanding, only few cases have been reported in other sympatric host species, like stone marten, badger, lynx, and roe deer (Ryser-Degiorgis et al. 2002; Oleaga et al. 2008).

Morphological studies have failed to identify any significant differences among mite populations (Fain 1978), and experimental cross contamination of Sarcoptes mite between hosts of different species is commonly unsuccessful (Arlian et al. 1984; Arlian

1989). Apparently no epidemiological relationship exists, in Europe, between mange foci affecting wild ruminants, wild boars, and carnivores (Berrilli et al. 2002).

The question as to whether Sarcoptes mites might be divided into different species or whether they are, in fact, monospecific has been the subject of an ongoing debate (Zahler et al. 1999; Burgess 1999; Berrilli et al. 2002; Gu & Yang 2008; Alasaad et al. 2009c). Zahler et al. (1999) and Berrilli et al. (2002), using the ITS-2 sequences as genetic markers, did not detect clear-cut evidence of genetic separation related to host species or geographic location. As well as, in our previous study, we have shown that ITS-2 rDNA does not appear to be suitable marker for examining genetic diversity among Sarcoptes mite populations from different wild host species and/or geographical localities (Alasaad et al. 2009c). In phylogenetic analyses bootstrapping support for the closest relationships may be relatively poor due to reduced time to accumulate informative changes in the sequences examined. Further resolution is therefore provided in faster evolving hypervariable sequences such as nuclear polymorphic microsatellite loci (Walton et al. 2004b).

Walton et al. (1999; 2004b), using multi-locus genotyping applied to microsatellite markers, substantiated previous data that gene flow between scabies mite populations on human and dog hosts is extremely rare in northern Australia. As well as, genetic differences were detected between geographically distinct populations, even between householders. Microsatellite markers were used by Alasaad et al. (2008b) to describe a new phenomenon of genetic structuring among S. scabiei at individual host skin-scale.

Taking into account all the above-mentioned information, the aim of the present study was to test the extent of genetic relationship between sympatric wild host-derived Sarcoptes mite populations, and to study the influence of the geographical isolation on

89 the genetic structuring of Sarcoptes. This is pivotal for wildlife health management in
90 order to understand the geographic variation among bordered mite populations, and to
91 measure the patterns of host specific differences, especially in sympatric hosts.
92
93 **Materials and methods**
94
95 Collection of S. scabiei
96
97 Using Postponed Isolation (Post-frozen Isolation) and Direct Isolation (Live Isolation)
98 methods, as described by Alasaad et al. (2009b), 251 Sarcoptes mites were collected
99 from the crusted skin of 100 animals belonged to 15 populations of 10 European wild
100 mammalian species, as listed in Table 1, which were sampled in Italy, France and Spain
101 (Fig. 1). Rupicapra rupicapra rupicapra, Cervus elaphus, Martes martes, Ovis
102 musimon, Capra ibex, and Vulpes vulpes were sympatric in Northeast Italian Alps. V.
103 vulpes, Martes foina and Sus scrofa were sympatric in Northwest Italian Alps. Taking
104 into account the topography of Sierra Nevada Mountain and that the first case of
105 Sarcoptes mite infection was reported in Dílar Valle (East Sierra Nevada) (Pérez et al.
106 1997), mites from Sierra Nevada were divided into two different groups, East and West
107 populations. All mites were identified as S. scabiei based on known morphological
108 criteria (Fain 1968).
109 In Table 1, term 'Code' refers to all mites belonging to the same geographical
110 and/or host species-derived population, from now onwards called 'component
111 population' or, simply, population (Bush et al. 1997).
112
113 Preparation of Sarcoptes gDNA

The DNA of individual Sarcoptes mites was extracted with the NucleoSpin Tissue kit procedure (Macherey-Nagel, Düren, Germany) with some modifications proposed by Soglia et al. (2009), and recently with HotSHOT Plus ThermalSHOCK technique (Alasaad et al. 2008a).

Fluorescent-based polymerase chain reaction analysis of microsatellite DNA

From the panel described by Walton et al. (1997), ten microsatellites (Sarms 33-38, 40, 41, 44, and 45) were selected and analysed with one 10× multiplex PCR. Each 15 µl PCR reaction mixture consisted of 3 µl of the single mite DNA, together with the PCR mixture containing all primer pairs (ranged from 0.04 to 0.1 µM per primer), 200 µM of each dATP, dCTP, dGTP, and dTTP, 1.5 µl of 10× PCR buffer (200 mM KCl and 100 mM Tris-HCl, pH 8.0), 1.5 mM MgCl2, and 0.15 µl (0.5 U/reaction) HotStartar Taq (QIAGEN, Milano, Italy). Samples were subjected to the following thermal profile for amplification in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA): 15 min at 95°C (initial denaturing), followed by 37 cycles of three steps of 30 s at 94°C (denaturation), 45 s at 55°C (annealing) and 1.5 min at 72°C (extension), and a final elongation of 7 min at 72°C.

Microsatellite analysis

Using 96-well plates, aliquots of 12 µL of formamide with Size Standard 500 Liz (Applied Biosystems, Foster City, CA, USA) and 2 µl PCR product were prepared. Then, the plates were heated for 2 min at 95°C and chilled to 4°C. Fluorescent PCR

amplification products were analyzed by ABI PRISM 310 Genetic Analyzer with pop4. Allele calling was performed using the GeneMapper v. 4.0 software (Applied Biosystems, Foster City, CA, USA). To track and minimize the amount of error associated with genotyping, the genetic data were collected twice by SA and DS.

Descriptive statistics and cluster analysis

CONVERT 1.31 software (Glaubitz 2004) was used to reformat files for the statistical softwares. Descriptive statistics and diversity analyses were carried out with GenAlEx v. 6.2 (Peakall & Smouse 2006), Genepop v. 4.0 (Raymond & Rousset 1995), Fstat v. 2.9.3 (Goudet 1995), and Arlequin v. 3.1 (Excoffier et al. 2005) softwares, i.e. allelic richness (R), number of private alleles, allele frequencies, unbiased expected (He) and observed (Ho) heterozygosity, test for Hardy-Weinberg equilibrium (HWE), test for linkage equilibrium (LE), and F statistics. All pairs of the component populations were compared for homogeneity of genetic variation using the Wilcoxon's matched-pairs signed-rank test (GraphPad InStat software).

Analysis of structure and relationships among host-specific mite populations were studied using two different approaches:

(i) Multilocus proportion of shared alleles (Dps) was computed between all possible pairs of individual mites using the Microsat software (Minch 1997) ignoring any preliminary information on origins of parasites. One thousand datasets were generated by resampling the input data (bootstrapping), the Neighbor-Joining algorithm was used as implemented by the Phylip v. 3.6 package (Felsenstein 1989), and a consensus dendrogram was obtained. The dendrogram was visualized using the Dendroscope v. 2.2.2 software (Huson et al. 2007).

(ii) The analysis of relationships among mites was then improved by a Bayesian assignment test using the method implemented by the Structure v. 2.2 software (Pritchard et al. 2000). We performed 50000 MCMC (Markov chain Monte Carlo) replicates following a burn-in period of 10000 steps. This parameter set was run 20 times for each of different numbers, K, of genetic clusters of multilocus genotypes; all values of K from 1 to 20 were tested. The probability of the multilocus genotype of any individual mite to occur in each of the K clusters was computed. We used the admixture model (each mite drew some fraction of its multilocus genotype from each of the K clusters) allowing the allele frequencies to be correlated among clusters. This configuration has been considered the best in the case of subtle population structure (Falush et al. 2003). We used the height of the modal value of the distribution of ΔK in order to estimate the uppermost number of clusters capturing the overall mite sample structure, as suggested by Evanno et al. (2005). We then associated any individual mite with the cluster that corresponded to its greatest membership, q, that is fraction of its multilocus genotype; a threshold value $q \geq 0.9$ was used. Finally, each of the inferred clusters was associated with the component populations of its mites. If a cluster was labelled with multiple mite populations, an additional substructure analysis for K values from 1 to 5 was performed testing only the mites assigned to that cluster.

Results

Descriptive statistics

Ten marker loci were analysed on 251 mites belonging to 15 populations of 10 European wild mammalian species from Italy, France and Spain; 101 alleles were detected. Allele count for each of the 10 loci ranged from six (Sarms 37) to 15 (Sarms

34). Proportion of missing genotypes was as low as 0.04 and it did not affect single locus or population. Forty two private alleles were detected in 11 wild host-derived mite populations, ranging from 1 (ItNWMf, SpNEVv and SpNWRp) to 10 (FrNESs). Whereas in ItNECe, SpEMf, ItNEOam, and ItNEMm no private alleles were identified (Table 2). The highest within-population genetic variability was observed for the two *S. scrofa* mite populations, in spite of their small size, whereas little variation was found in ItNWVv and SpNWRp (Table 3).

Allelic richness (R) and heterozygosity (He) were used as most informative parameters of diversity. In particular, allelic richness provided a measure of the number of alleles independent of sample size, hence allowing comparison among different populations. Level of genetic diversity varied both across loci and among populations. Wilcoxon's test stated ($P<0.001$) that *C. ibex* mites showed more variability ($R = 2.6$, $He = 0.339$) than mites from the other ruminants whereas *S. scrofa* mites were the most variable at all ($R = 3.4$, $He = 0.667$).

LE test (Lewontin 1964; Slatkin 1994; Slatkin & Excoffier 1996) was performed for all loci and significant linkage disequilibrium ($P<0.05$) was observed for 18 pairs when all the mite populations were pooled. In no cases disequilibrium was observed at the same loci in more than two populations individually analysed. HWE estimates were assessed of 70 locus-by-population comparisons, 40 (57%) showed significant heterozygosity deficiencies. Deviations from HWE did not point at any locus in particular. All populations deviated from HWE across loci after sequential Bonferroni correction ($P<0.001$).

Population differentiation based on allele frequencies for all 15 populations gave an overall $Fst = 0.721$. Each locus significantly ($P<0.001$) contributed to distribution of variability among populations with per-locus values ranging from 0.290 to 0.821. This

very high estimate means that most of the global Sarcoptes genetic variability resided among rather than within component populations different in geographical and host-derived distribution of mites.

Structure and relationships among mite populations

(i) Multilocus proportion of shared alleles (Dps) as a measure of genetic similarity between all pairs of mites.

Genetic variability among populations of Sarcoptes mites collected from the same host species from different localities.

The proportion of shared alleles between pairs of individual mites from the two C. pyrenaica mite populations in Spain (SpSWCp and SpSECp) were scattered randomly with no evidence of distribution based on geographical location of hosts. Individual Sarcoptes mites belonging to the three V. vulpes mite populations from the Northeast and Northwest Italian Alps as well as from Northeast Spain showed clear clustering up to their original populations. V. vulpes mite population from Spain was the most different one, supported by 980/1000 bootstraps. The V. vulpes mites from Northeast and Northwest Italian Alps were relatively more similar between each other, their distribution across two distinct clusters being very poorly supported (169/1000 bootstraps) (Fig. 2). Regarding mites from S. scrofa populations from Northwest Italian Alps and from Northeast France, strong separation was detected between them (1000/1000 bootstraps, data not shown).

Genetic variability among populations of Sarcoptes mites collected from sympatric host

239 species.

240 The dendrogram of individual mites from six sympatric host-derived populations from
241 Northeast Italian Alps, and three sympatric host-derived populations from Northwest
242 Italian Alps allowed the clustering of the mites into three groups (Fig. 3). The first
243 group was formed by all carnivore-derived mites from East and West Italian Alps,
244 mainly mites from V. vulpes and, in additon, M. foina (Figure 3, a) and M. martes
245 (Figure 3, b), nevertheless it included also a mite from C. ibex (Figure 3, c). The second
246 group included the herbivore-derived mite populations from Northeast Italian Alps,
247 mainly mites from C. ibex and R. Rupicapra, all scattered across the cluster. In addition,
248 the cluster included O. aries musimon (Figure 3, d) and C. elaphus (Figure 3, e) mites.
249 The separation of herbivore- and carnivore-derived mites was then quite clear-cut
250 (470/1000 bootstraps). The S. scrofa mites from Northwest Italian Alps were near the
251 carnivore-derived mites but distinct from them (572/1000 bootstraps).

252

253 Genetic variability among Sarcoptes populations distributed according to both host
254 species and geographical localities.

255 Five clusters resulted from the analysis of the overall 15 wild host-derived mite
256 populations (Fig. 4). Cluster I included almost all the herbivore-derived mites from
257 Spain (SpNWRp, SpSWCp and SpSECp) and the C. ibex mite which clustered with
258 carnivore-derived parasites in Figure 3 (c). Although some mites scattered around,
259 evidence of separation between SpNWRp and SpSWCp-SpSECp was observed.
260 Clusters IIa and IIb contained all the omnivore-derived mites, ItNWSs and FrNESs
261 respectively, and one ItNECi (included in cluster IIb, Figure 4, arrow). Cluster III
262 included all the carnivore-derived mites, i.e. ItNEMm, ItNWMf, ItNWVv, ItNEVv as
263 well as SpNEVv (Figure 4, a) and SpEMf (Figure 4, b). Cluster IV contained almost all

264 the herbivore-derived mites from Italy (ItNERr, ItNECi, ItNECe, and ItNEOam,) and
265 one SpNWRp.
266
267 (ii) Analysis of mite population structure by the Bayesian method.
268
269 The modal value of the statistic ΔK for the overall dataset (251 mites) stated that the
270 uppermost cluster value was K = 4 (Evanno et al. 2005). Each of the four inferred
271 clusters was then associated with the information of its mites. For each cluster the
272 average membership and number of mites assigned with the greatest membership were
273 computed (Fig. 1). Cluster I shared all mites with the cluster I in Fig. 4, i.e. nearly all
274 mites of Spanish ruminants (SpNWRp, SpSECp and SpSWCp) and one ItNECi. High
275 proportions of membership were always obtained ($q > 0.97$). Cluster II grouped all the
276 S. scrofa mites (ItNWSs and FrNESs) (see Fig. 4, clusters IIa and IIb) and their
277 membership fraction was $q > 0.98$. Two ItNECi (for one of them see Fig. 4, cluster IIb)
278 and three SpNEVv mites were also added but they lacked to show high membership in
279 this cluster ($q < 0.70$). Cluster III shared most mites with the cluster III in Fig. 4
280 grouping all mites of Italian V. vulpes (ItNEVv and ItNWVv), M. martes (ItNEMm), and
281 M. foina (ItNWMf and SpEMf) with very robust membership ($q > 0.95$ for all mites but
282 SpEMf parasite with $q = 0.86$). One SpNEVv mite showed some similarity to this cluster
283 although with poor membership ($q = 0.56$). Cluster IV grouped the majority of the mites
284 of Italian ruminants (ItNERr, ItNECi, ItNECe, and ItNEOam) and one SpNWRp with
285 very high membership ($q > 0.97$). Its members corresponded to those of the cluster IV
286 in Fig. 4.
287 The computation of the statistic ΔK was repeated separately for four subsets of
288 samples made up of the main geographical and host-specific mite groups, i.e. mites

belonging to S. scrofa (ItNWSs and FrNESs), Italian ruminants (ItNERr, ItNECi, ItNECe, and ItNEOam), V. vulpes (ItNEVv, ItNWVv, and SpNEVv), and Spanish ruminants (SpNWRp, SpNECp and SpNWCp). No evidence of substructure was detected in any case.

Spanish V. vulpes mites received ambiguous assignment as in case of mixed ancestry. However, they were collected from a single host animal and lacked a substantial component population as a reference. Two ItNECi were misplaced in S. scrofa mite cluster with low membership. One of them was also assigned to the sympatric V. vulpes mite cluster (Fig. 3, c) or to the allopatric Spanish ruminant mite cluster (Fig. 4, Cluster I) depending on the populations used for the comparison. Such individual parasites seemed to be randomly assigned since they carried multilocus genotypes infrequent in their population, so the algorithm could not recognize their ancestry.

In synthesis, the proportion of shared alleles as a similarity measure among mites and the assessment of structure using the Bayesian method provided patterns in agreement with each other. When the full data set was used, four distinct genetic clusters of mites were inferred, i.e. omnivore-, Italian herbivore-, carnivore-, and Spain herbivore-derived parasites.

Discussion

Differentiation of host-specific mites using morphological traits, apart from being very difficult and time consuming, proved to be impossible to implement when mites of the same host-specific variant, but belonging to different geographical component populations, have to be compared (Arlian et al. 1984; Arlian 1989). Short fragments of

314 mitochondrial or ribosomal DNA spacer regions have been shown not to be suitable
315 markers for examining genetic diversity among Sarcoptes mite populations (e.g.
316 Alasaad et al. 2009c). Further resolution is therefore provided in faster evolving
317 hypervariable sequences such as nuclear polymorphic microsatellite loci. Microsatellites
318 have previously demonstrated to provide strong support for geographically discrete
319 populations, they showed congruence with evolutionary patterns at the population level,
320 and reported genetic differentiation at the skin-scale of individual mangy hosts
321 (Bowcock et al. 1994; Walton et al. 2004b; Alasaad et al. 2008b).

322 The number of the mites utilized in this study (251 samples) has to be
323 considered high as compared with previous studies in this field (see Alasaad et al.
324 2009a for review).

325 All the component populations showed a strong deficiency of heterozygosity
326 over all loci and mites belonging to the same component population showed to be
327 scattered through the same cluster more than subdivided across individual host animals.

328 Sarcoptes mites lack free-living stages. Individual hosts, depending on their
329 susceptibility and behaviour, are more or less ephemeral habitats and may provide
330 patchy environments which hamper random mating (Price 1980; Criscione et al. 2005).
331 All mites on an individual host could form an 'infrapopulation' (Bush et al. 1997) with
332 some recurrent generations on that host. Number of generations is affected by short
333 generation interval of the parasite, about three weeks, as well as by life expectancy of
334 the infested host, depending on its susceptibility.

335 In our data set, the reduced gene pools made mites alike each other and hid
336 possible equilibrium between dispersive process and gene flow among infrapopulations.
337 This may be due to rapid diffusion of few genotypes as for an epidemic population
338 structure (Oura et al. 2005).

339 Another evident feature of our results is the lack of homogeneity of genetic
340 diversity across populations. S. scrofa mites were the most variable at all. Wild boar
341 populations are widespread and growing, and generally show higher resistance to
342 parasites than other mammalian species (Rodrigues & Hiraoka 1996; Nejsum et al.
343 2009). Consequently, a single host can be affected by repeated infestation events
344 through mites from other infrapopulations or, even, from other component populations.
345 The other major determinants of gene flow among mites are the degree of host
346 specificity and geographical structure of host populations. Previous investigations
347 showed that approaches to individual clustering provide appropriate characterisation of
348 population structure at high Fst values (Rosenberg et al. 2001; Manel et al. 2002; Latch
349 et al. 2006). In the presence of very diverging taxa, few loci are needed to achieve high
350 performance, regardless of the sample sizes (Manel et al. 2002; Tadano et al. 2008). In
351 fact, the ideal marker locus for our purposes should be monomorphic within any taxon
352 and polymorphic across taxa (Reed 1973).
353 In our data set, the unusually high value of Fst and the high number of private
354 alleles, in most populations, indicated that the mite component populations were very
355 unlike each other. All the 10 loci provided a significant component of among-
356 population diversity. As a consequence, our marker panel provided good accuracy for
357 analysis of the genetic characteristics of Sarcoptes populations.
358 Sarcoptes scabiei (1) from different host species belonging to different
359 geographical localities, (2) from the same host species belonging to different
360 geographical localities, and (3) from closely related host species belonging to different
361 geographical localities clustered up to their original populations. Clear genetic diversity
362 among mite populations from different geographical localities exists. The differences
363 show to be as stronger as the geographical separation between host populations is

364 larger. In the case of short geographical separations (East and West Sierra Nevada)
365 mites from C. pyrenaica scattered randomly in the dendrogram and no clear separation
366 was detected. The differentiation between V. vulpes mites from Spain and Italy was
367 highly supported whereas the genetic separation between V. vulpes mites from East and
368 West Italian Alps was poorly supported. This finding suggests that gene flow occurring
369 among mites from different localities is related to the geographical distances.

370 The individual mites belonged to the six sympatric host-derived mite
371 populations from East Italian Alps, and the three sympatric host-derived mite
372 populations from West Italian Alps clustered into three main groups (Fig. 1, 3, and 4):
373 herbivore-derived mites (ItNECi, ItNERr, ItNEOm and ItNECe), carnivore-derived
374 mites (ItNEVv, ItNEMm, ItNWMf and ItNWVv), and omnivore-derived mites (ItNWSs).
375 In particular, mites from S. scrofa were distinct from both herbivore- and carnivore-
376 derived mites and they did not cluster with the sympatric Northwest Italian populations.
377 In other words, the host-specific separation among the three clusters was stronger than
378 that by the geographical separation between East and West Italian Alps.

379 Similar results were obtained when the overall mite samples of our investigation
380 were analysed (Fig. 1 and 4). For example, Cluster III contained all the carnivore-
381 derived mites regardless of their geographical origins from different European countries
382 under study.

383 Our results from the sympatric wild animals in Italy and from the general
384 analysis of all mite populations show unambiguously lack of gene flow or recent
385 admixture among Sarcoptes populations carnivore-, herbivore-, and omnivore-derived.
386 Mite transmission may occur within each mite cluster herbivore-, carnivore-, and
387 omnivore-derived but it seems to be extremely rare or absent among them. This might

388 improve parasite adaptations and led to, what we called, host taxon-derived (carnivore,
389 herbivore, and omnivore host-derived) Sarcoptes mite populations.
390 Population structure of Sarcoptes would be that of a species subdivided into
391 genetically small populations with restricted gene flow among local demes (Price 1980;
392 Martínez et al. 1999; Nadler & Hafner 1990). Strong specialisation could be the result
393 of a host taxon-derived shift and, even if two host taxon-derived species are sympatric
394 as for their host species, they should be considered as allopatric if the parasites have no
395 possibility of host choice. In other words, the host sympatry is not the same as the
396 parasite sympatry.
397 The probability of disease transfer between sympatric host taxon-derived
398 species could be reduced by evolved intrinsic mechanisms, this means that the
399 behaviour has been selected to impede crosses between individuals from two different
400 host taxon-derived, and this could represent the first step of sympatric speciation
401 (McCoy 2003). Host taxon-derived effect is stronger than the geographical separation in
402 the definition of speciation events.
403 The existence of host taxon-derived Sarcoptes mites could be the explanation of
404 the mange-free wildlife populations in sympatry with other mangy wild animals, like
405 the mange-free C. ibex and R. rupicapra of West Italian Alps which are in sympatry
406 with the endemically mangy population of V. vulpes. This effect could be the reason
407 behind the successful of the cross-transmission/infection in some Sarcoptes varieties
408 e.g. S. scabiei var vulpes/canis readily infect dogs and other canids as well as felids
409 including domestic European cats, as all of them belong to the same host taxon-derived
410 (Bornstein 1995).
411 We have no clear explanation of this taxonomic affiliation. Further studies on
412 dispersal capability of host animals and their disposition to interact with each other, host

behaviour and parasite adaptation are needed to explain the host taxon-derived *Sarcoptes*. Characterization of host genetic structure in addition to mite population genetic structure would contribute additional valuable information.

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Table 1 Countries, geographical locations and host species used in this study, together with the number of host animals and Sarcoptes mite samples.

Code	Countries	Geographical	Host taxon	Host species	Nº. of animals	Nº. of mites
ItNERr	Italy	Northeast	Herbivore	Rupicapra rupicapra	20	63
ItNECi	Italy	Northeast	Herbivore	Capra ibex	10	25
ItNECe	Italy	Northeast	Herbivore	Cervus elaphus	1	1
ItNEOam	Italy	Northeast	Herbivore	Ovis aries musimon	1	2
ItNEVv	Italy	Northeast	Carnivore	Vulpes vulpes	7	23
ItNEMm	Italy	Northeast	Carnivore	Martes martes	1	3
ItNWVv	Italy	Northwest	Carnivore	Vulpes vulpes	11	30
ItNWMf	Italy	Northwest	Carnivore	Martes foina	1	2
ItNWSs	Italy	Northwest	Omnivore	Sus scrofa	1	3
FrNESs	France	Northeast	Omnivore	Sus scrofa	4	5
SpNEVv	Spain	Northeast	Carnivore	Vulpes vulpes	1	4
SpNWRp	Spain	Northwest	Herbivore	Rupicapra pyrenaica	9	26
SpSECp	Spain	Southeast	Herbivore	Capra pyrenaica	21	33
SpSWCp	Spain	Southwest	Herbivore	Capra pyrenaica	11	30
SpEMf	Spain	West	Carnivore	Martes foina	1	1

Table 2 Private alleles detected at the 10 microsatellite loci for the host-associated mite populations, together with their frequencies.

Pop (N°mites)	Locus	Allele	Freq	Pop (N° mites)	Locus	Allele	Freq
ItNERr (63)	ms33	224	0,008	ItNEVv (23)	ms35	150	0,065
	ms33	244	0,025		ms41	232	0,022
	ms34	170	0,016	ItNWVv (30)	ms35	146	0,200
	ms34	192	0,190		ms41	264	0,033
	ms41	214	0,008	SpNEVv(23)	ms38	205	1,000
	ms41	250	0,083	FrNESs(5)	ms33	266	0,800
	ms38	290	0,008		ms33	268	0,100
ItNECi (25)	ms34	188	0,104		ms33	270	0,100
	ms34	208	0,042		ms34	182	0,200
	ms35	138	0,022		ms35	126	0,200
	ms37	176	0,045		ms35	128	0,300
	ms41	244	0,026		ms36	287	0,400
	ms38	223	0,043		ms37	178	0,900
SpSWCp(30)	ms35	160	0,333		ms41	228	0,600
	ms36	263	0,017		ms44	274	0,700
	ms36	273	0,017	ItNWSs(3)	ms33	274	1,000
	ms40	217	0,100		ms34	200	0,250
	ms40	225	0,067		ms40	235	1,000
SpSECp(33)	ms35	158	0,015		ms45	176	0,500
	ms36	277	0,015	ItNWMf(2)	ms38	219	0,250
	ms45	164	0,030	SpNWRp (26)	ms45	198	0,679
ItNECe(1); SpEMf(1); ItNEOam(2) and ItNEMm(3): no private allele detected							

Table 3 Descriptive statistics for the main mite populations (R=allelic richness; He=expected heterozygosity; Ho=observed heterozygosity)

	ItNERr	ItNECi	ItNEVv	ITNWVv	SpNWRp	SpSWCp	SpSECp	FrNESs-ItNWSs
R	1.9	2.6	2.0	1.5	1.6	1.8	1.8	3.4
He	0.215	0.339	0.232	0.119	0.119	0.217	0.216	0.667
Ho	0.048	0.097	0.126	0.020	0.051	0.077	0.103	0.340

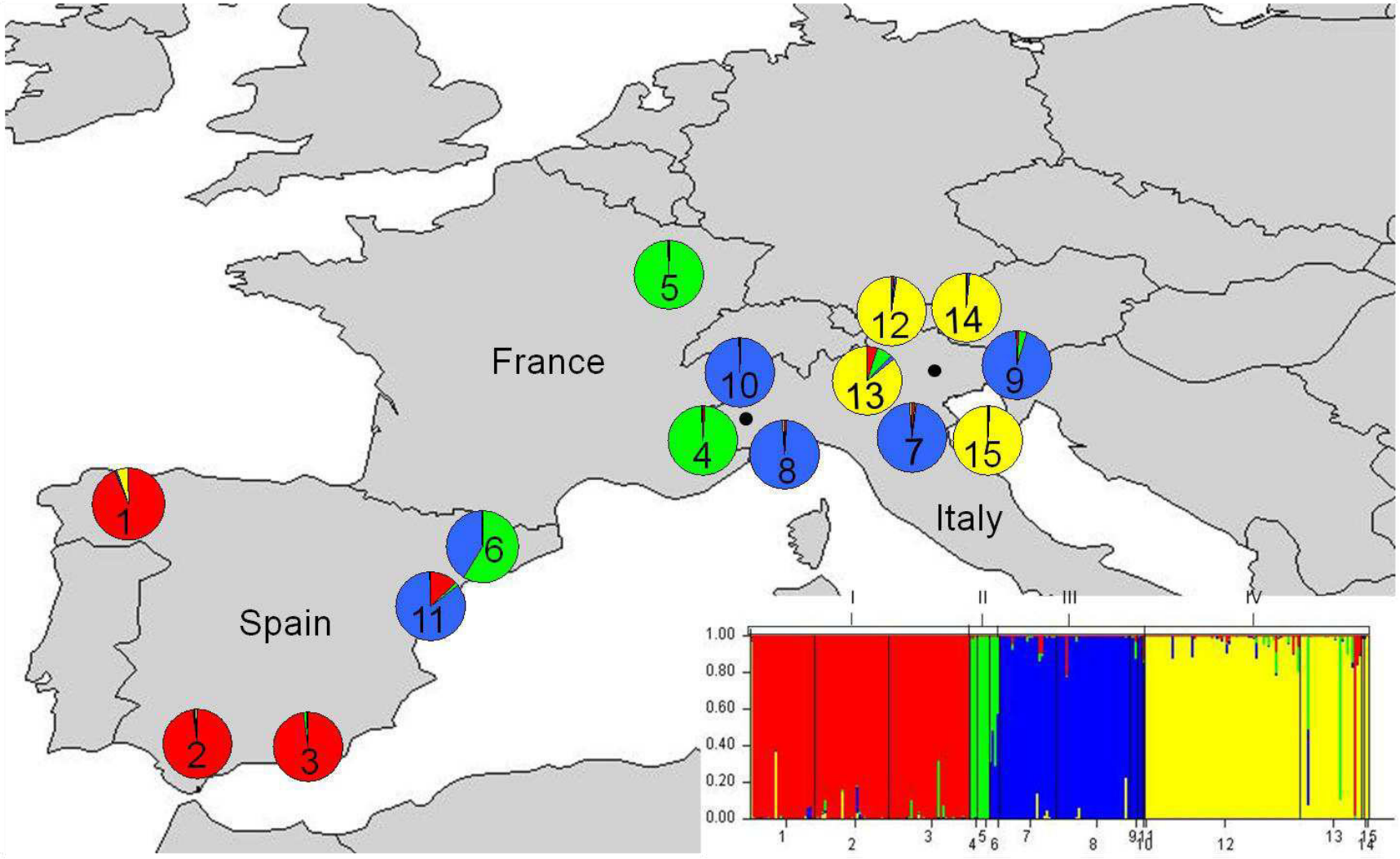
Fig. 1 Europe map showing approximate sites for sample collection, together with structure clusters. The colours within bars show the proportion of membership of each individual to the genetic clusters for each Sarcoptes population separately. The pie charts give the genetic membership per Sarcoptes population. 1=SpNWRp, 2=SpSWCp, 3=SpSECp, 4=ItNWSs, 5=FrNESs, 6=SpNEVv, 7=ItNEVv, 8=ItNWVv, 9=ItNEMm, 10= ItNWMf, 11=SpEMf, 12=ItNERr, 13=ItNECi, 14=ItNECe, and 15=ItNEOam. For site abbreviations see Table 1.

Fig. 2 Unrooted Dps consensus dendrogram for individual Sarcoptes mites from three V. vulpes-derived mite populations from Northwest and Northeast Italian Alps, and from Northeast Spain. Numbers at the nodes are percentage values of 1000 bootstraps supporting the same branching structure. Codes in this figure (bold for Northwest Italian Alps, italic for Northeast Italian Alps, grey for Northeast Spain) represent the sample codes in Table 1 and Fig.1.

Fig. 3 Unrooted Dps consensus dendrogram for individual Sarcoptes mites from six sympatric host-derived mite populations in Northeast Italian Alps, and three sympatric host-derived mite populations in Northwest Italian Alps (Table 1). Numbers at the nodes are percentage values of 1000 bootstraps supporting the same branching structure. Thick branches for all carnivore-derived mites, thin branches for all herbivore-derived mites, grey branches for S. scrofa-derived mites. Carnivore-derived mites: V. vulpes and Herbivore-derived mite: C. ibex and R. Rupicapra. a: M. Foina, b: M. martes, c: C. ibex, d: O. aries musimon and e: C. elaphus.

Fig. 4 Unrooted Dps consensus dendrogram for individual Sarcoptes mites from the 15 wild host derived populations (Table 1) using a similarity matrix based on the proportion of shared alleles. Thick branches for all carnivore-derived mites, thin branches for all herbivore-derived mites, grey branches for S. scrofa-derived mites. Cluster I (SpNWRp, SpSWCp and SpSECp), Clusters IIa and IIb (ItNWSs and FrNESs), Cluster III (ItNEMm, ItNWMf, ItNWVv and ItNEVv) and Cluster IV (ItNERr, ItNECi, ItNECe, and ItNEOam). a: SpNEVv, b: SpEMf and arrow: ItNECi.

Fig. 1


France
Spain
Italy
1
2
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6
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10
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12
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14
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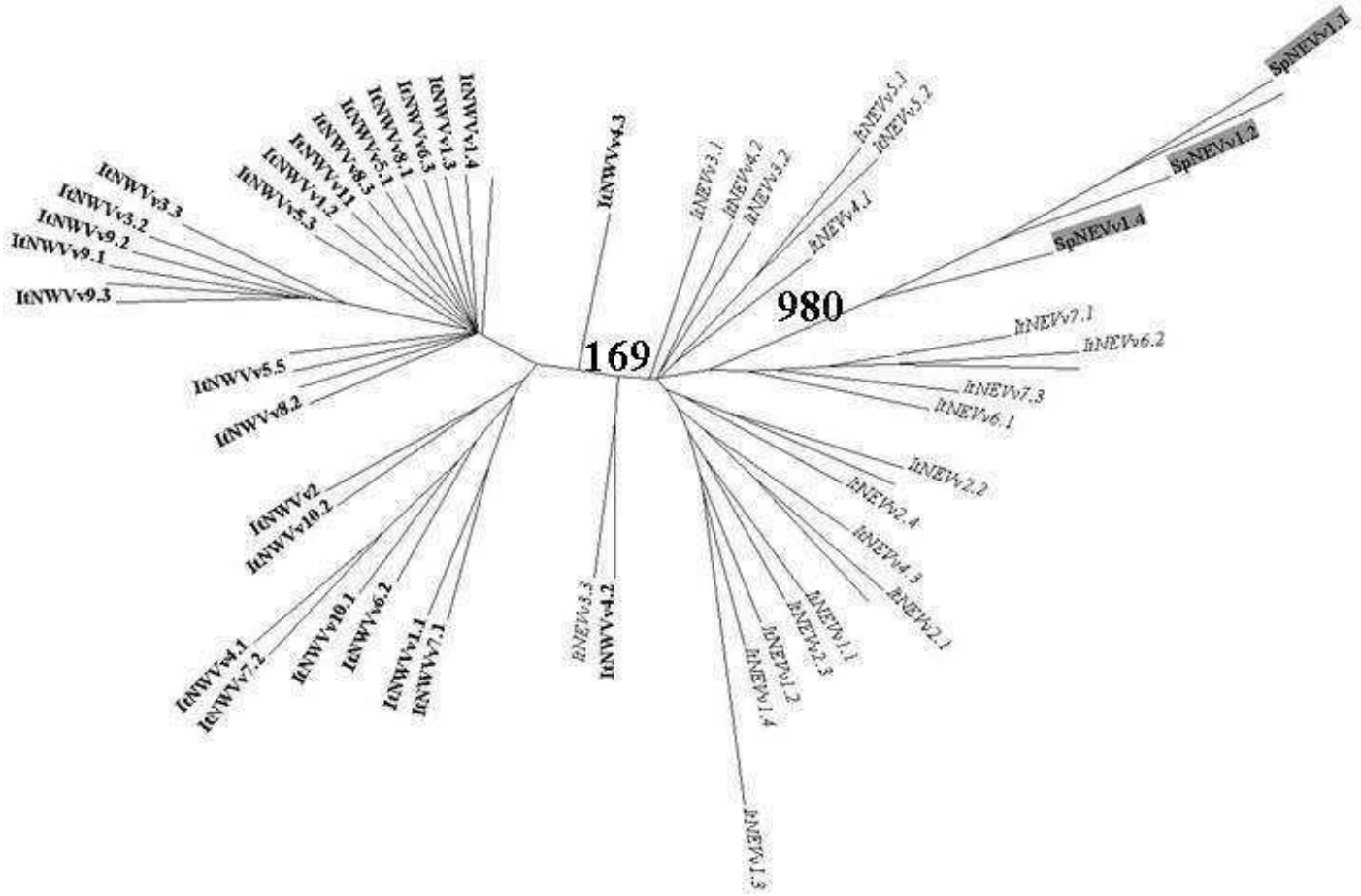
I
II
III
IV
1.00
0.80
0.60
0.40
0.20
0.00

Fig. 2


ItNWVv3.3
ItNWVv3.2
ItNWVv9.2
ItNWVv9.1
ItNWVv9.3
ItNWVv5.5
ItNWVv8.2
ItNWVv2
ItNWVv10.2
ItNWVv4.1
ItNWVv7.2
ItNWVv10.1
ItNWVv6.2
ItNWVv1.1
ItNWVv7.1
ItNWVv5.3
ItNWVv1.2
ItNWVv8.3
ItNWVv5.1
ItNWVv8.1
ItNWVv6.3
ItNWVv1.3
ItNWVv1.4
ItNWVv4.3
169
ItNWVv4.2
ItNEVv3.5
ItNEVv3.1
ItNEVv4.2
ItNEVv3.2
ItNEVv4.1
ItNEVv5.1
ItNEVv5.2
980
SpNEVv1.1
SpNEVv1.2
SpNEVv1.4
ItNEVv7.1
ItNEVv6.2
ItNEVv7.3
ItNEVv6.1
ItNEVv2.2
ItNEVv2.4
ItNEVv4.3
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ItNEVv2.3
ItNEVv1.2
ItNEVv1.4
ItNEVv1.3

Fig. 3

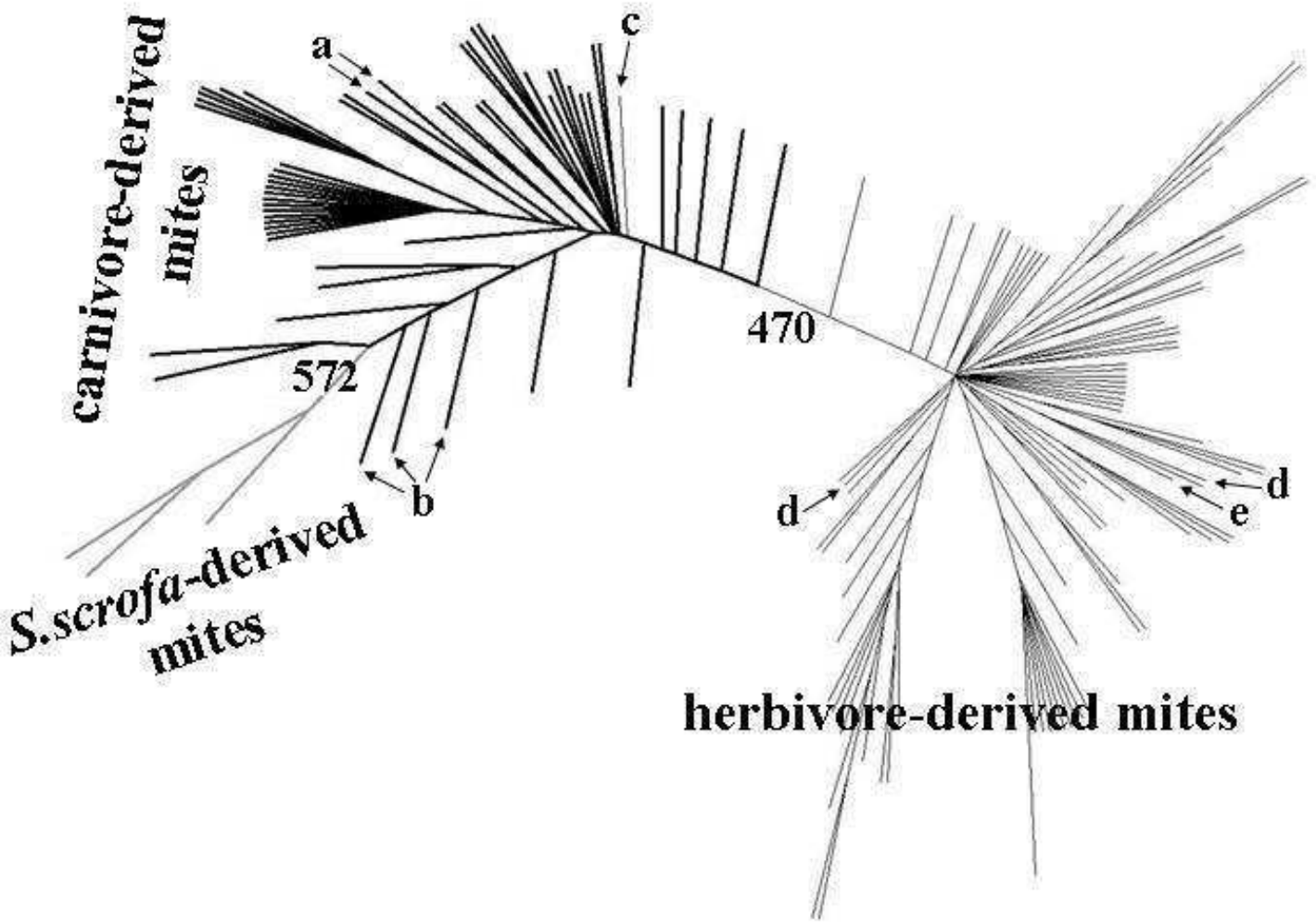
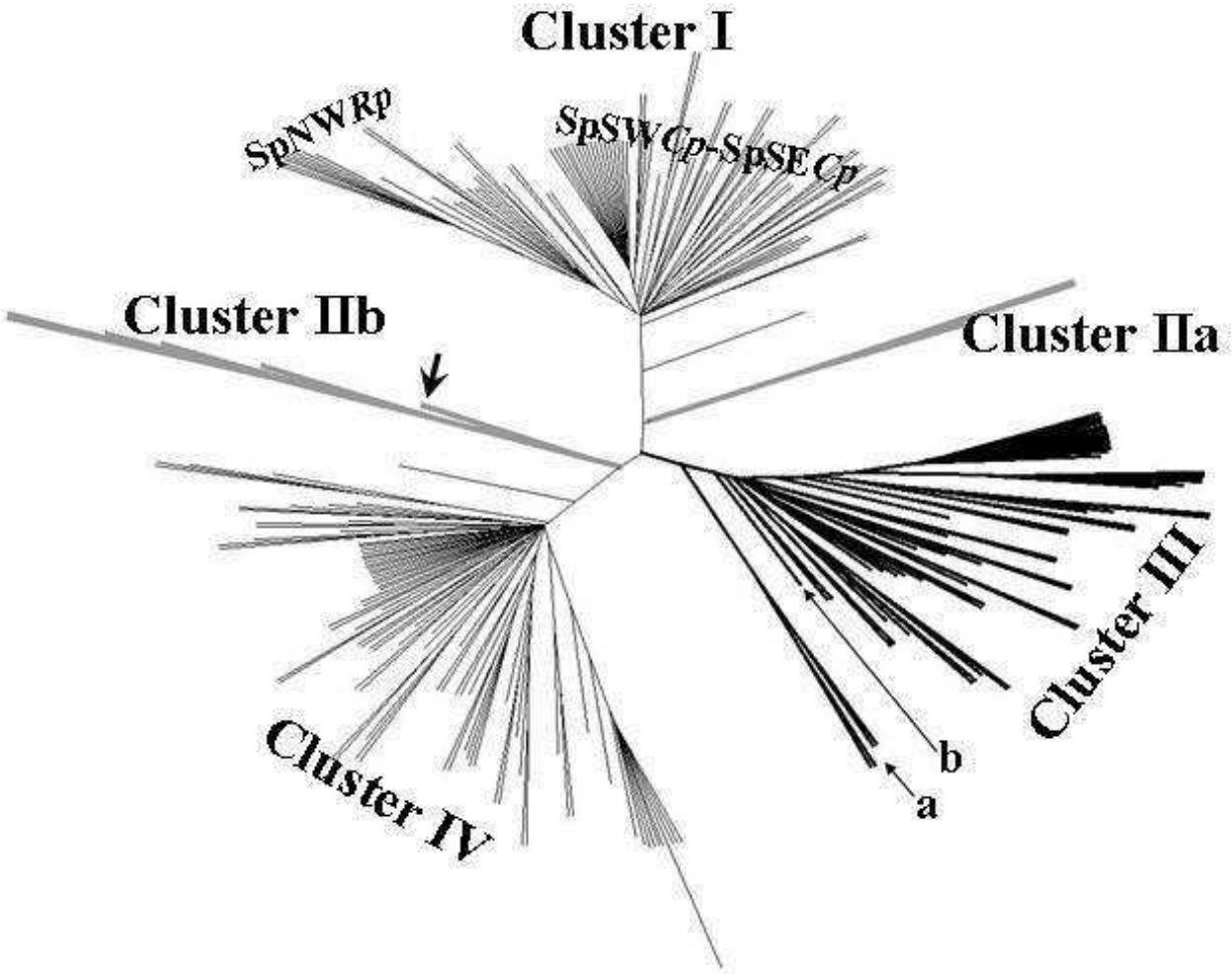

carnivore-derived mites
a
c
572
470
b
d
d
e
S.scrofa-derived mites
herbivore-derived mites

Fig. 4


Cluster I
SpNWRp
SpSWCp-SpSECp
Cluster IIb
Cluster IIa
Cluster III
Cluster IV
b
a