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Original article

Genetic structure and phylogeography of pine shoot beetle populations (*Tomicus destruens* and *T. piniperda*, Coleoptera Scolytidae) in Italy

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Abstract – *Tomicus* are among the most dangerous pine pests. In this paper we assess the genetic structure of some Italian *Tomicus* populations, and the possible sympatry of *T. destruens* and *T. piniperda*. A fragment 358 bp long of the mitochondrial DNA relative to the COI was investigated in eight populations by SSCP analyses and sequencing. In the sampled populations *T. destruens* and *T. piniperda* were not found to be sympatric. *T. destruens* populations of southern and central Italy strongly differ from a population of northern Italy. The phylogeographic analysis of *T. destruens* populations in Europe is geographically structured, probably due to the fragmentation of the host pine ranges. The populations of *T. piniperda* are polymorphic, with haplotypes occurring also in Europe and Asia. *T. piniperda* populations seem to be genetically unstructured because of both the continuous distribution area of its main host (*Pinus sylvestris*) and the international trade of pine timber.

mtDNA / SSCP / Scolytidae / pine / phylogeography

Résumé – Structure génétique et phylogéographie des populations de *Tomicus destruens* et *T. piniperda* (Coleoptera Scolytidae) en Italie. Le genre *Tomicus* figure parmi les espèces les plus dangereuses pour les forêts de pins. La structure génétique de huit populations italiennes de *T. destruens* et *T. piniperda* a été étudiée, ainsi que la sympatrie possible des deux espèces. Un fragment de 358 pb de l'ADN mitochondrial relatif au COI a été amplifié, séquencé et soumis à une analyse par SSCP. Aucun cas de sympatrie n'a été observé entre *T. destruens* et *T. piniperda*. Les populations de *T. destruens* d'Italie méridionale et centrale sont très différentes d'une population d'Italie du Nord. *T. destruens* montre une structuration géographique probablement liée à la distribution fragmentée des pins hôtes. Par contre, les populations de *T. piniperda* sont très polymorphes, sans doute à cause de la distribution continue de son principal hôte, le pin sylvestre, ainsi qu'au commerce international de bois.

1. INTRODUCTION

The pine shoot beetles belonging to the genus *Tomicus* Latreille (Coleoptera Scolytidae) are among the most dangerous insects living in Eurasian pine forests [23]. These bark beetles have a major role in the decline of many pine forests growing in both Europe and Mediterranean countries, including Northern Africa. Among the six species belonging to the genus *Tomicus*, only *T. piniperda* (Linnaeus, 1758), *T. destruens* (Wollaston, 1865) and *T. minor* (Hartig, 1834) occur in Europe [21]. *Tomicus piniperda* is widespread in Eurasia and it has been recently introduced in North America [13]. *Tomicus destruens* occurs in all circum-Mediterranean regions and Madeira Islands, whereas *Tomicus minor* occurs in Europe and Asia.

For a long-time *T. piniperda* and *T. destruens* were considered as synonyms [27], even if attempts to separate the two species were done [18, 21]. However, recent papers reported both morphological and genetic characters useful for the separation of the two siblings [11, 15, 16]. Nevertheless, species determination by morphological analysis is still extremely difficult, and the genetic support is needed. In addition, the sympatry of the two *Tomicus* in some Mediterranean countries [11, 15] makes the identification even more difficult.

The species confusion has made it possible that the largest part of data published in Mediterranean region, where *T. destruens* is more common, reports *T. piniperda* as the investigated species [3–5, 12, 19, 25, 26, 38]. From this point of view, data concerning many populations of *T. destruens* are confused and would need reconsideration. Finally, the lack of specificity of

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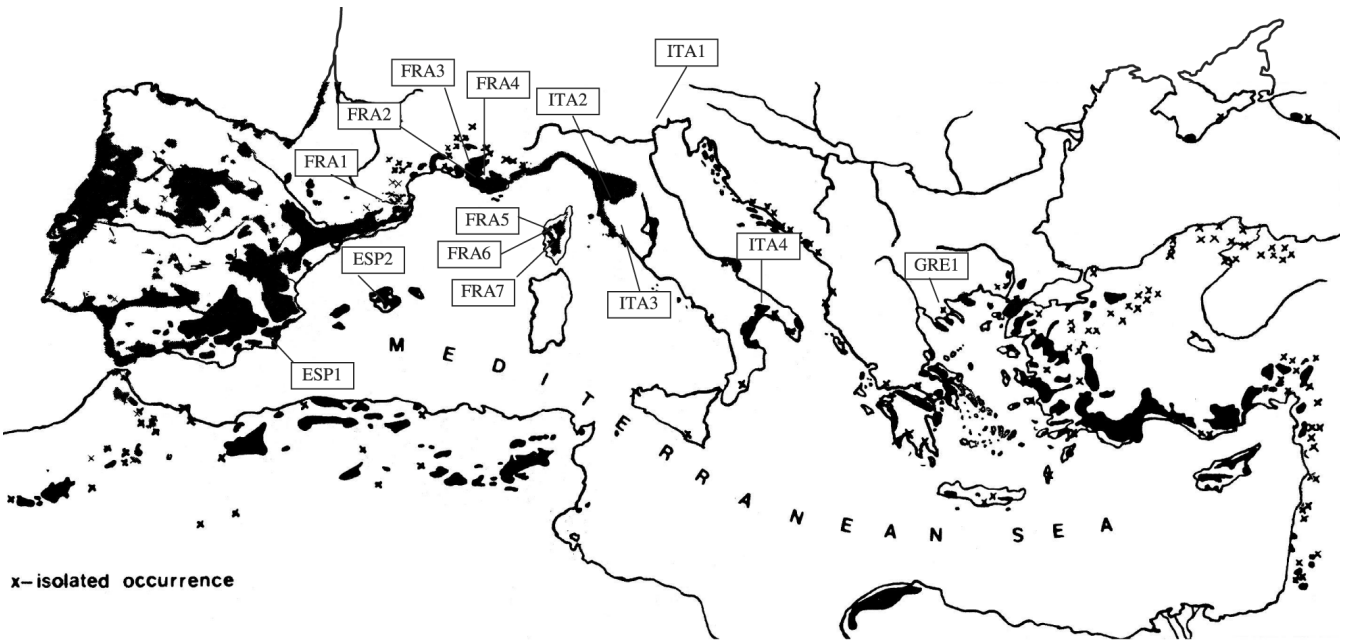

Figure 1. Sampling sites of Italian populations of *T. destruens* (ITA1-4) and locations of the populations used for the phylogeographic analysis. The natural range of the potential host pine species *P. pinea*, *P. pinaster*, *P. brutia* and *P. halepensis* (modified from Critchfield and Little [7]) is given in the background. The actual range is wider for the artificial spread of pine in plantations, as it is evident for the population ITA1. A three letters abbreviation corresponding to the country and a locality identification number indicates the sites where the populations were sampled.

Tomicus species for a host plant does not allow to use host species as a tool for insect identification, although *T. destruens* was found only on Mediterranean pines so far, whereas *T. piniperda* was collected from both continental (*Pinus sylvestris* and *P. nigra*) and Mediterranean pines (*P. pinaster*) [11, 15].

Recently, Ritzerow et al. [24] have used a phylogeographic approach to the analysis of mtDNA genetic data from several Eurasian populations of *T. piniperda*. The study suggests that only very long distances or important geographic barriers, like the Pyrenees, are relevant to the separation of the populations and the consequent differentiation of haplotypes. In this respect, comparative phylogeography permits the investigation of biogeographic questions on spatial and temporal scales that are smaller than those typically addressed with other approaches [2]. Moreover, the phylogeography is useful in elucidating contemporary patterns of evolutionary subdivision within species and species complexes, providing novel insights into the understanding of biotic diversification [1].

In this paper, we want to use this approach to study evolutionary history and the origin of Italian populations of *T. destruens* and *T. piniperda*, by genetic markers of mtDNA. The central geographic position of Italy in the Mediterranean could be important for understanding the genetic structure of *Tomicus* populations occurring in southern Europe. For example, during the last glaciation Italian pine forests could have been a refuge area for *Tomicus* populations, as suggested for other bark beetle species [31]. In addition, we want to test the possible sympatry of *T. destruens* and *T. piniperda* in Italian transition areas between continental and Mediterranean pine forests.

2. MATERIALS AND METHODS

2.1. Sample collection and DNA isolation

Adults of *Tomicus* were collected from eight different populations living in pine forests growing in Italy (Fig. 1 and Tab. I). The insects were collected from recently infested pine logs, and firstly identified by morphological characters [21]. The genomic DNA was extracted following a salting out protocol [20].

2.2. Mitochondrial DNA analysis

A fragment of about 490 bp of the Cytochrome Oxidase I (COI) was amplified using the lepidopteran mitochondrial primers C1J2441 [29] for both the *Tomicus* species, and C1N2934, and C1N2937 for *T. destruens* and *T. piniperda*, respectively [16]. Once sequenced, the COI fragments were used to design a *Tomicus* specific primer TMC2 (5'-ATTGATGAAATAATATTTCATATAAAATATGC-3').

The SSCP analysis was performed on a COI fragment 358 bp long, which was amplified using the primers C1J2441 and TMC2. Template DNA (4 µL) were used in 12.5 µL of PCR reaction mix, containing 0.5 U of Taq DNA polymerase (Promega®), 1× reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP's and 0.5 µM of each primer. The target region was amplified by 37 cycles of PCR on a Perkin Elmer DNA Thermal Cycler 9600®. Following a pre-denaturation step of 3 min at 94 °C, the thermal profile included denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C, and extension for 1 min at 72 °C. A final elongation step (5 min at 72 °C) was also performed. Then, 3 µL of the PCR reaction were heat denatured and electrophoresed through a 11% polyacrylamide gel (29:1 acrylamide:bisacrylamide). The runs were performed at 10 °C for 10 000 V/h in a 23 cm long vertical apparatus. The samples were classified into distinct mobility classes

Table I. Characteristics of the *Tomicus* populations sampled in Italy. *N*: number of insects collected in each population; Host pine: P.P.: *Pinus pinaster*, P.D.: *Pinus pinea*, P.H.: *Pinus halepensis*, P.N.: *Pinus nigra*, P.S.: *Pinus sylvestris*.

Species	Code	*N*	Locality	Lat. N	Long. E	Altitude m a.s.l.	Host pine	Date
T. destruens	ITA1	19	Valle Vecchia (VE)	45° 54'	12° 36'	3	P.P.	03/2001
	ITA2	17	Poggio Valicaia (FI)	43° 34'	11° 13'	370	P.P.	04/2002
	ITA3	18	Alberese (GR)	42° 40'	11° 06'	42	P.D.	03/2002
	ITA4	16	Ginosa (TA)	40° 34'	16° 45'	240	P.H.	03/2002
T. piniperda	ITA5	20	Villasantina (UD)	46° 25'	12° 55'	363	P.S.	04/2001
	ITA6	16	Passo del Bocco (GE)	44° 20'	9° 23'	956	P.N.	04/2002
	ITA7	12	Rocciamelone (TO)	45° 10'	7° 08'	1600	P.S.	05/2002
	ITA8	20	Sonico (BS)	46° 11'	10° 23'	1010	P.S.	11/2002

(haplotypes) according to their SSCP pattern. In addition, as two mobility classes were difficult to distinguish, the attribution of individuals to each class was confirmed by digesting the amplified DNA with the restriction enzyme *Bst*1107I, which cuts at a diagnostic site, followed by an agarose gel electrophoresis.

All the rare haplotypes and a random sample of the more frequent mobility classes, for a total of 40 individuals (16 of *T. destruens*, 24 of *T. piniperda*), were sequenced. The PCR products were then purified using a pre-sequencing kit (Amersham-Pharmacia Biotech®). Finally, purified DNA was sequenced using a BygDye Terminators 3.0 Cycle Sequencing kit (Applied Biosystems®) and it was run in an ABIPRISM 3700 DNA Analyser (Applied Biosystem®).

2.3. Analysis of the genetic data

The genetic sequences of each specimen were aligned using the ClustalX® program [37] and then analysed by MEGA 2.1® [17]. We also investigated genetic variation within single populations of the two species. The degree of polymorphism of each population was determined using the program Arlequin 2.0® [28], and expressed as haplotype (h) and nucleotide diversity (π). The sequences were aligned with those of *T. destruens* and *T. piniperda* available in GenBank.

In order to evaluate the neutrality and mutation/drift equilibrium of the investigated sequences, i.e. the degree of homogeneity of the population, we also tested the distance from the neutrality expectations using both Tajima (*D* value) [32] and Ewens-Watterson tests (*F* value) [10, 39], as implemented in Arlequin 2.0®. For mt DNA, high values of *F* and negative values of *D* indicate the occurrence of few common haplotypes [6]; low *F* values and positive *D* values may be expected in the presence of subdivided populations or migration [30]. The haplotype networks for the COI data was constructed using the software TCS 1.18. The TCS program creates a haplotype network using Statistical Parsimony [34], which outputs the 95% plausible set of most parsimonious linkages among haplotypes. Ambiguous linkages are depicted by "loops" in the haplotype network.

To have a comparison in a wider phylogeographic context, the *T. destruens* sequences were compared with those deposited in GenBank. Because the COI region sequenced in our analysis was not the same of those deposited in GenBank, the alignment was reduced to 185 bp. Differently, the whole Italian sequences of 358 bp of *T. piniperda* were compared with the corresponding part of those reported by Ritzerow et al. [24].

The haplotype network was then nested into a series of clades following Templeton et al. [33] and Templeton and Sing [35] and used for nested clade analysis using the GeoDis 2.0 program [22]. Nested clade analysis provides an objective statistical framework for discriminating among historical (e.g., range expansion and fragmentation) and recurrent (e.g., gene flow and drift) processes that may explain the observed distribution of genetic variation. The geographical data were quantified as *Dc* (geographical spread of a particular clade) and *Dn* (distribution of a given clade relative to the sister clades). This analysis tested the association of clades with geographical locations, the significance of the distances *Dc* and *Dn*, as well as the contrasts between interior-tip subgroups. The statistical significance of these measures was determined using random permutation tests, which simulate the null hypothesis of a random geographical distribution for all clades within a nesting category, given the marginal clade frequencies and sample sizes per locality. The interpretation of the observed distances was carried out using the revised inference key by Templeton [36] (accessible at: http://InBio.byu.edu/Faculty/kac/crandall_lab/geodis.htm).

3. RESULTS

The morphological and genetic analysis of the eight Italian populations allowed to attribute all the individuals within a population to one of the two species, excluding the sympatric occurrence of the two *Tomicus* in the study sites (Tab. I). The investigated populations showed a polymorphism associated to several classes of mobility, i.e. haplotypes. The SSCP analyses carried out on 159 specimens clearly distinguished seven haplotypes for *T. destruens* and eleven for *T. piniperda* (Tab. II). The sequencing of all the variants confirmed the presence of at least one nucleotide substitution from one to another. Moreover, the sequence analysis of five (*T. destruens*) and eight (*T. piniperda*) individuals sharing the same haplotype in different populations confirmed the accuracy of the SSCP method. The sequence of each different haplotype has been deposited in GenBank under accession numbers AY796318 - AY796332. The analysed fragment of COI (358 bp) revealed seven (7 transitions and 1 transversion) and thirteen variable sites (13 transitions and no transversion) for *T. destruens* and *T. piniperda*, respectively (Tab. III). For both species all the mutations were synonymous.

The number of private haplotypes was 3 in *T. destruens* (A, C, G) and 6 in *T. piniperda* (4, 5, 6, 8, 10, 11) (Tab. II).

Haplotype diversity (*h*) and nucleotide diversity (π) for each population are showed in Table IV.

Table II. Mitochondrial haplotypes found in *Tomicus destruens* and *Tomicus piniperda* collected from different pine stands. *N*: total specimens per population.

Species	Population	*N*	Haplotypes										
			A		B	C		D	E	F			G
			AY796318		AY796319	AY796320		AY796321	AY796322	AY796323			AY796324
T. destruens	ITA1	19	12		6	–		–	–	1			–
	ITA2	17	–		13	1		1	1	1			–
	ITA3	18	–		14	–		2	1	1			–
	ITA4	16	–		10	–		–	–	4			2
			1	2	3	4	5	6	7	8	9	10	11
					AY796325	AY796326	AY796327	AY796328	AY796329	AY796330		AY796331	AY796332
T. piniperda	ITA5	20	18	1	1	–	–	–	–	–	–	–	–
	ITA6	16	9	6	–	–	–	–	1	–	–	–	–
	ITA7	12	6	–	1	–	1	–	–	–	1	2	1
	ITA8	20	10	–	–	3	–	4	1	1	1	–	–

Table III. Variable nucleotide sites in the 7 haplotypes of *T. destruens* and 11 haplotypes of *T. piniperda* defined on the basis of 358 bp of the mitochondrial DNA Cytochrome Oxidase I. The numbers indicate variable positions corresponding to positions of haplotype B for *T. destruens* and haplotype 1 for *T. piniperda.*

	T. destruens								*T. piniperda*														
	Position								Position														
Haplotype	85	175	217	250	328	340	349	**Haplotype**	28	46	111	150	175	181	223	229	250	320	331	351	354
B	C	A	G	C	C	A	C	**1**	T	T	G	C	A	T	C	A	C	T	G	T	C
G	.	.	.	T	.	.	.	**9**	.	.	.	.	.	.	.	.	T	.	.	.	.
D	.	.	A	.	.	.	.	**2**	.	.	.	.	.	C	.	.	.	.	.	.	.
E	.	.	T	.	.	.	.	**3**	.	.	.	.	.	.	.	.	.	C	.	.	.
F	.	.	.	.	T	.	.	**5**	C	.	.	.	G	.	.	.	.	C	.	.	.
C	.	G	.	.	.	.	T	**7**	C	.	A	T	G	.	.	.	.	C	.	.	.
A	T	.	.	.	T	G	T	**11**	.	.	.	.	G	.	.	.	.	C	.	.	.
								8	.	.	.	.	G	.	T	G	.	C	.	.	.
								6	.	C	.	.	G	.	.	.	.	C	A	C	.
								4	.	.	.	.	.	.	.	.	.	C	A	.	.
								10	.	.	.	.	.	.	.	.	.	C	A	.	T

Table IV. Haplotype and nucleotide diversity with result of the Tajima and Ewens-Watterson neutrality tests on the analysed COI segment in *Tomicus* Italian population. $F_{obs} - F_{exp}$ is the difference between the observed and expected homozygosity (* $P < 0.05$, ** $P < 0.01$).

Populations		Haplotype diversity (*h*)	Nucleotide diversity (π)	Tajima's *D* statistic	$F_{obs} - F_{exp}$
T. destruens	ITA1	0.5263 (±0.0887)	0.005449 (±0.003609)	2.02065*	–0.05421
	ITA2	0.4265 (±0.1468)	0.001630 (±0.001542)	–1.55781*	0.25791**
	ITA3	0.3987 (±0.1379)	0.001172 (±0.001250)	–0.68482	0.1927*
	ITA4	0.5667 (±0.1090)	0.001775 (±0.001636)	0.12996	–0.07042
T. piniperda	ITA5	0.1947 (±0.1145)	0.000560 (±0.000809)	–1.51284*	0.25559**
	ITA6	0.5750 (±0.0799)	0.003175 (±0.002433)	–1.2933	–0.01183
	ITA7	0.7576 (±0.1221)	0.004990 (±0.003487)	–0.41098	0.06363
	ITA8	0.7158 (±0.0875)	0.007681 (±0.004743)	–0.44013	0.02585

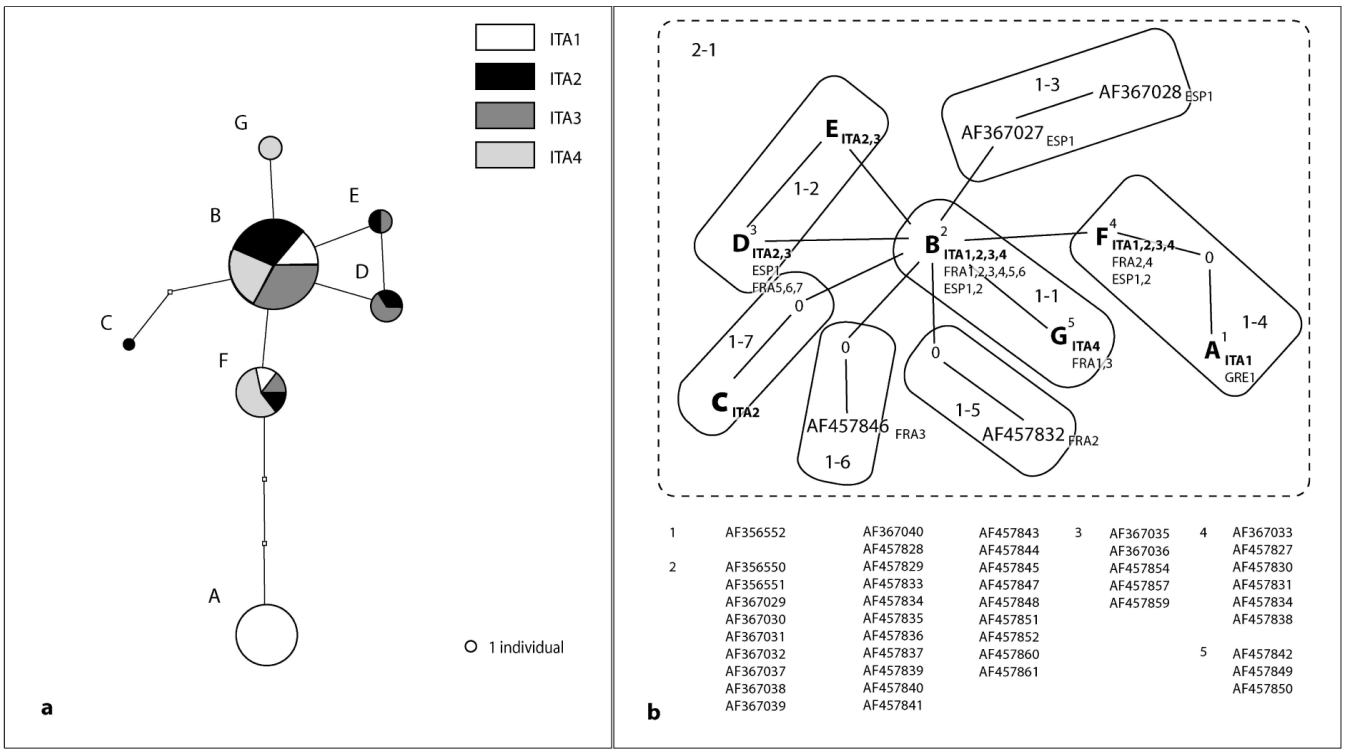

Figure 2. (a) Italian haplotype network of *T. destruens* based on 358bp. Each line represents a mutational step; small squares refer to missing or theoretical haplotypes. Circles represent haplotypes with size proportional to relative frequencies; sectors of different colours refer to absolute number of haplotype counts for each population. (b) Haplotype network based on mutational differences in the COI mtDNA sequences of *T. destruens* populations. Italian haplotypes are reported as bold letters, whereas haplotypes from GenBank are indicated by their accession numbers. The list below the figure reports the haplotypes of *T. destruens* deposited in GenBank and matching the Italian ones for the 185bp allowed by the overlapping. The sites in which haplotypes were found are indicated with the country abbreviation and the locality identification number (see Fig. 1). In addition, the nested clade analysis is reported: the clades are identified using a two numbers system with the first number referring to the nesting hierarchy and the second is the clade identification number. "0" means a missing or theoretical haplotype.

For *T. destruens* the Tajima's *D* value was lower than expected in two populations (ITA2 and ITA3), but statistical significance was reached only in ITA2 (Tab. IV). However, integrating the Tajima's test with the Ewens-Watterson test (*F* value), also the ITA3 population showed a homozygosity degree significantly higher than in expected neutrality conditions (Tab. IV). *D* value was significantly higher than expected only for the ITA1 population (Tab. IV). Both tests indicated that ITA4 was in equilibrium (*F* and *D* value close to zero).

Concerning *T. piniperda* all populations show Tajima's *D* values lower than expected, but only the ITA5 population shows statistical significance (Tab. IV). In addition the population ITA6 seems to be in equilibrium having both *F* and *D* value close to zero.

The haplotypes B and F of *T. destruens* were the most common. The haplotype A was the most differentiated from the haplotypes B and F (three and four mutational steps respectively) (Fig. 2a). The haplotype G was occurring only in the southern population of *T. destruens* living on *Pinus halepensis*. Haplotypes C, D and E were found only in central Italy. The analysis of European sequences showed that the haplotypes B and F were the most common found in populations of *T. destruens* sampled in western Mediterranean basin (Italy, France and Spain) (Fig. 2b). The haplotype A was similar to a haplotype found in Greece (accession number AF356552) (Fig. 2b), suggesting its probable eastern origin. The comparison with sequences deposited in GenBank showed that the haplotype G was already found in France on the same host tree [15]. Finally, the haplotypes C and E were found only in Italy. Other four private haplotypes occurred in continental France and Spain (Fig. 2b).

The nested clade design included 11 haplotypes across two nesting levels (Fig. 2b). At the total cladogram level the analysis showed that there was a restricted gene flow excepted for the clade 1-4, which includes the haplotype A occurring only in North Italy and Greece, showing a long-distance dispersal (Fig. 2b).

In *T. piniperda,* the haplotype 1 was the most common, whereas the other haplotypes occurred in very few individuals (Fig. 3 and Tab. IIb). The populations sampled at the southern edge of host's range (ITA5 and ITA6) were much less heterogeneous than populations collected within the range (ITA7 and ITA8), which shared most of the rare haplotypes. On a wider scale, Italian haplotypes 1, 2 and 9 occurred also in Europe, in particular as fragments of haplotypes I, II, VII for 1, IV for 2, and VIII, IX for 9 [24]. The other eight haplotypes were found

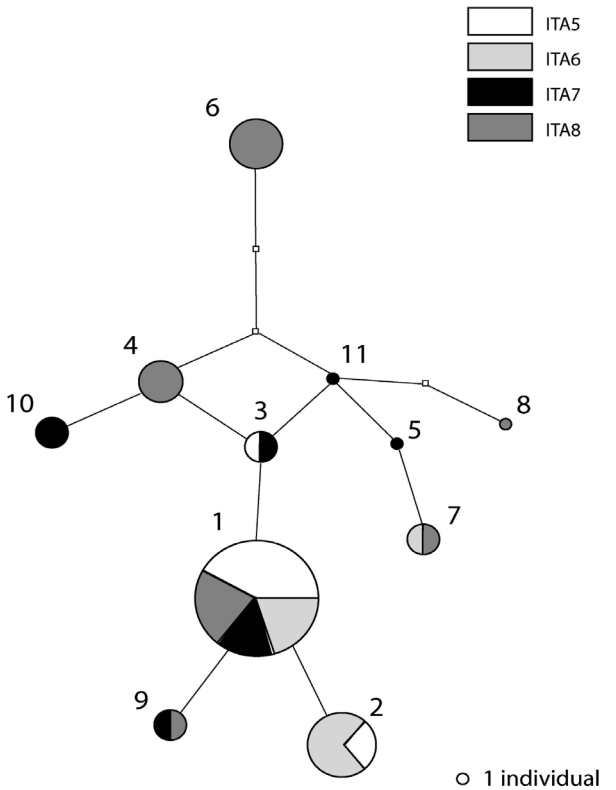

Figure 3. Italian haplotype network of *T. piniperda* based on 358 bp (for figure explanation see Fig. 2).

only in Italy. The nested clade analysis gave as result an inconclusive outcome, showing a low phylogeographic structure for *T. piniperda.*

4. DISCUSSION

In this paper we show that the two sibling species of pine shoot beetle, *Tomicus destruens* and *Tomicus piniperda*, differ strongly in relation to the genetic structure of their populations in Italy as well as in Europe. *T. destruens* is characterized by a strong phylogeographic structure, whereas this was not observed for *T. piniperda*. As *T. destruens* has been shown to be associated exclusively with Mediterranean pine species [21], the high fragmentation of the hosts range seems to be the most likely factor explaining the separation of the populations. Conversely, the continuous range of the main host of *T. piniperda* (*P. sylvestris*), and the possibility for this species to colonize other hosts and even Mediterranean pines [11, 15], appear to be the main reason for the lack of genetic structure [24], in spite of the very numerous private haplotypes found in Italy as well as in other regions.

In this study *Tomicus destruens* and *T. piniperda* were not found to be sympatric, not even in the transition areas from coastal to alpine stands, such as in ITA1 and ITA5 populations. *T. destruens* was found only on Mediterranean pine species (*Pinus pinea*, *P. pinaster* and *P. halepensis*), whereas *Tomicus piniperda* was collected only from continental pine species such as *P. nigra* and *P. sylvestris*. The phylogeographic analysis of *T. destruens* shows that the populations of France, Italy and Spain are well structured, having their own haplotypes. However, it was possible to verify that some Italian haplotypes were already found in Europe. In particular, the haplotypes B and F were the most common haplotypes found in all the investigated Italian populations, as well as in many French and Spanish populations [11, 15, 16], suggesting their older origin. Besides, the haplotype G found in insects sampled from *Pinus halepensis* (ITA4), was found also in France from the same host tree [15], indicating a possible differentiation in relation to this host or its habitat, which is typical of warm and dry Mediterranean areas.

The genetic structure of the northern population (ITA1) is quite different from those of the other investigated populations (Fig. 2b). The haplotype A was the only found in four specimens of a Greek population [16], suggesting a Balkan origin of the ITA1 population. In fact, this population originates from a coastal plantation of Mediterranean pines about 50 years old, belonging to a system of plantations created to protect the Adriatic coasts since the Roman time, about 2000 years ago [8]. It appears that part of *Tomicus* population probably came from the eastern coast of Adriatic sea (haplotype A), whereas a part came from the south-western Adriatic coast, through the coastal system of pine plantations (haplotypes B and F). In this respect, the ITA1 population seems to occur in a contact area between western and eastern parts of Mediterranean basin. This hypothesis is confirmed by the high *D* value (Tab. IV), which is usually expected either from populations affected by strong immigration or divided in sub-populations having a high number of haplotypes and a heterogeneous genetic pool [30].

Finally, the haplotypes C and E (from ITA2 and ITA3) seem to be characteristic of central Italy. In this respect, the results obtained with Tajima's test and the Ewens-Watterson test for the ITA2 and ITA3 populations (Tab. IV) can be expected in populations having had either a bottleneck [32] or a rapid growth from a fewer number of individuals [30], as already suggested by Kerdelhué et al. [15]. However, populations from central and southern Italy share most haplotypes with French populations [15], to which they are connected through the continuous distribution of pine stands along the coast.

The analysis of new populations from the eastern Mediterranean and northern Africa could shed more light on the genetic structure of *T. destruens*, as well as the analysis of populations established in artificial plantations of pines along the Mediterranean coasts could provide useful information on the insect dispersal and associated gene flow.

Concerning *T. piniperda*, the high number of haplotypes (11) found in the Italian populations can be explained by the fact that many individuals were analysed for each population, increasing the probability to find new haplotypes. Another possible explanation of the high number of isolated haplotypes (8 out of 11) can be found in the role played by the Alps during the last glaciation, as they were a refuge area for many European insect populations forced to move southward, looking for more suitable climatic conditions [14]. This migration had therefore increased the genetic pool of *T. piniperda* in Italy, as it

has been observed also for the spruce bark beetle *Ips typographus*, which also has a large Palaearctic diffusion [31]. Similar considerations were reported by Ritzerow et al. [24], who suggest the high polymorphism of *T. piniperda* as due to the existence of several distinct refugial areas during the last ice age. A fragmentation and prolonged genetic isolation of European populations during the last glacial period could have led to the origin of new haplotypes. The same authors identify the area of Southern France, the Iberian Peninsula and the area south of S. Petersburg as refugial areas. Based on our results, it seems that Italy could have been a refuge area as well. The available data did not allow to delineate a clear geographic characterisation of *T. piniperda* populations, as shown by Ritzerow et al. [24]. This can be explained by both the lower number of bp used in our analysis and by the addition of the new Italian haplotypes. The only consideration deals with the haplotypes 1 and 2, which are the most common in Europe and probably the most ancient haplotypes of the taxon.

Little information exists about the migration routes of most *T. piniperda* haplotypes. It has been suggested that a parallel evolution occurred between *T. piniperda* and its main host, *P. sylvestris*, which has a very large and continuous distribution covering all Europe and Asia [9, 24]. That gives to the insect the possibility to move along both east-west and north-south directions. In addition, we suggest that the increase of the international trade of pine wood is responsible of insect movement among countries and even continents. In this concern, Ritzerow et al. [24] reported that the populations of *T. piniperda* found in North America were introduced from Europe, based on haplotype identity. Following the previous considerations, the low genetic structure of *T. piniperda* seems to be due to both the large and continuous range of its main host and to the intensive trade of timber among different countries, which led to a general mixing of haplotypes coming from different populations.

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