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Phylogeography of the ladybird *Iberorhyzobius rondensis*, a potential biological control agent of the invasive alien pine bast scale *Matsucoccus feytaudi*

Catarina Tavares · Alain Roques · Béatrice Courtial · Manuela Branco · Hervé Jactel · Carlos Lopez-Vaamonde

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Abstract Understanding the genetic structure of natural enemies is an important step to develop efficient biocontrol programs. The ladybird *Iberorhyzobius rondensis* Eizaguirre (Coleoptera: Coccinellidae) from the Iberian Peninsula, is a specialized predator and potential biological control of *Matsucoccus feytaudi* Ducasse (Hemiptera: Matsucoccidae), an invasive alien species in Southeastern France and Italy. *M. feytaudi* is specialized on *Pinus pinaster* Aiton. The beetle is also restricted to this habitat. Genetic structure of *I. rondensis* populations was analysed with the barcode region (COI). It revealed two main refugia areas for the beetle in Iberian Peninsula. Comparative phylogeography of the three trophic levels, plant-herbivore-predator, is discussed. Similar population structure was found for the prey and to some extent for the pine. Indications are given on where to collect the beetles for their use in biocontrol of *M. feytaudi,* in order to obtain the highest genetic diversity and match with the origin of the invasive prey.

Keywords Invasive alien species · mtDNA · *Pinus pinaster* · Matsucoccidae · Coccinellidae

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C. Tavares (✉) · M. Branco
Centro de Estudos Florestais (CEF), Instituto Superior de Agronomia, University of Lisbon (ISA-UL), 1349-017 Lisbon, Portugal
e-mail: catarina.a.tavares@gmail.com

A. Roques · B. Courtial · C. Lopez-Vaamonde
UR 633 Zoologie Forestière, INRA, 2163 Avenue de la Pomme de Pin, CS 40001 Ardon, 45075 Orléans, France

H. Jactel
UMR 1202 Biodiversité Gènes et Ecosystèmes, INRA, 69 Route d'Arcachon, 33612 Cestas Cedex, France

Introduction

Invasive alien species presently constitute a major worldwide threat to biodiversity on the planet (Shea and Chesson 2002) and can generate severe ecological and economic impacts (Kenis et al. 2009; Perrings et al. 2002). The lack of natural enemies of exotic species in the invaded region is considered one of the main causes of their establishment, spread and impact (Sax et al. 2007). By introducing natural enemies, such as predators and parasitoids, from the native region of the pest in its invaded area, classical biological control programmes try to achieve a self-regulatory control of the pest (Hoelmer and Kirk 2005).

Molecular markers are increasingly being used in biological control studies to identify the target pest species and its natural enemies (Gariepy et al. 2007; Hoelmer and Kirk 2005). In addition, molecular data is also being used to determine the areas of origin of invasive alien species (Lees et al. 2011; Valade et al. 2009) and to search for natural enemies in the native region (Gebiola et al. 2013; Hernandez-Lopez et al.

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2011). It is expected that natural enemies, which came from the areas of origin of the pest, are more specific and efficient, as a result of long periods of co-evolution (Hufbauer and Roderick 2005; Roderick and Navajas 2003).

The maritime pine bast scale, *Matsucoccus feytaudi* Ducasse (Homoptera: Matsucoccidae) is a highly specialized sap-sucker insect, feeding exclusively on the tree trunk of maritime pine, *Pinus pinaster* Aiton (Pinales: Pinaceae) (Foldi 2004). *P. pinaster* is native from the Mediterranean Basin, including South western France, Corsica and North Italy (Burban and Petit 2003; Carrión et al. 2000; Bucci et al. 2007). The bast scale is a recently invasive alien species in the South eastern part of France, northern Italy (Covassi et al. 1991) and Corsica (Jactel et al. 1996, Rigot et al. 2014). In these invaded areas outbreaks of *M. feytaudi* cause serious damage on the maritime pine trees. Sap sucking results in great decline of tree vigor and ultimately mortality due to attacks of secondary forest pests such as bark beetles (Foldi, 2004).

The bast scale is endemic to the Iberian Peninsula and North Africa, and mitochondrial data has shown three distinct lineages occurring in different geographical areas: Western and Central Iberian Peninsula; Andalusia region and the Atlas Mountains (Burban et al. 1999). A recent study (Kerdelhué et al. 2014) using microsatellite markers and an approximate bayesian computation approach corroborate this pattern: the native range is geographically structured. During the 1950s the pine bast scale expanded its range to Southeastern Europe. The maritime pine trees from this region have not coevolved with the pine bast scale, and therefore are more susceptible to its effects (Burban and Petit 2003). The invasive process of the bast scale took place in three steps: *M. feytaudi* first arrived to the pine planted region of Landes in South-Eastern France, after which it reached the forests of Liguria and Tuscany in Italy. Later, the island of Corsica was colonized with individuals from the forests of South-Eastern France and Liguria (Kerdelhué et al. 2014). In its native range *M. feytaudi* has several natural predators, some specialized on this prey, including *Elatophilus crassicornis* Reuter (Heteroptera: Anthocoridae), *Hemerobius stigma* Stephens (Neuroptera: Hemerobiidae), *Aplocnemus brevis* (Coleoptera: Dasytidae) and *Iberorhyzobius rondensis* Eizaguirre (Coleoptera: Coccinellidae) (Branco et al. 2006a; Mendel et al. 2004). In the invaded range, other natural enemies are present, e.g. *Elatophilus nigricornis* Zetterstedt, predator of *Matsucoccus pini* Green, and the generalist beetle *Rhyzobius chrysomeloides* Herbst (Covassi et al. 1991; Fabre et al. 2000), but none of them have proved effective enough to slow the spread of the invasive scale insect. The release of native natural enemies of *M. feytaudi* in its invaded range has been therefore envisaged as a possible solution for biocontrol (Jactel and Menassieu 2005).

Iberorhyzobius rondensis is a recently discovered species (Raimundo et al. 2006), from the Iberian Peninsula. This ladybird is highly specialized on the pine bast scale. It occurs only in pine woodlands of *P. pinaster,* the habitat of its prey (Tavares et al. 2014) and cannot complete its life cycle without egg-masses of *M. feytaudi* as feeding resources. Both the adults and the larvae are attracted to the sex pheromone of the prey *M. feytaudi*, especially the larval instars (Branco et al. 2006b). Therefore *I. rondensis* is regarded as a potential biocontrol agent against *M. feytaudi*.

According to Smith et al. (2011) the extent to which co-distributed species share a common biogeographic history depends on the strength and specificity of their ecological relationships. Thus, it is expected that specialized natural enemies display similar phylogeographic patterns to that of their host or prey (Barbosa et al. 2012). Most of the studies on the genetic structure of the third trophic level are focused on parasitoids (e.g. Gebiola et al. 2013; Hernandez-Lopez et al. 2011; Lozier et al. 2008; Nadel et al. 2012) but only few studies have addressed predators (e.g. Coll et al. 1994; Omondi et al. 2011). Given the high degree of prey and habitat specialization of the ladybird beetle *I. rondensis* (Tavares et al. 2014), and the strict species-specific association between *M. feytaudi* and *P. pinaster* (Burban and Petit 2003), the phylogeographic pattern of the predator in the Iberian Peninsula is expected to match that of its prey and prey's host plant.

Mitochondrial DNA has been widely used in phylogeographic studies due to extensive intraspecific polymorphism, rapid mutation rate, maternal inheritance and absence of recombination (Avise 2000). Mitochondrial genes have recently been used in several genetic studies of ladybird beetles (e.g. Brown et al. 2008; Greenstone et al. 2011; Gregory et al. 2003; Kim et al. 2012). In this study we used DNA sequence data to reconstruct the phylogeography of *I. rondensis* in the Iberian Peninsula and to compare it

with that of its prey, *M. feytaudi*, and host plant, *P. pinaster*. Our main objective was to provide information to allow decision making for the biocontrol of the bast scale, and especially for the selection of areas from which the predators should be collected for rearing and release (Lozier et al. 2008; Roderick and Navajas 2003). Our results show that *I. rondensis* has a similar phylogeographic pattern to that of its prey. In addition, we identify areas as sources for ladybird specimens to be potentially used in biocontrol programs.

Material and methods

Insect sampling

Twelve locations were sampled from six different regions of the Iberian Peninsula (Table 1). In each location at least three different stands of *P. pinaster*, and 30–50 trees per stand were surveyed. Beetles were attracted with a rubber dispenser impregnated with 200 μg of the female sex pheromone of *M. feytaudi* (UPMC, INRA Versailles, France). The lure was pinned to the trunk 1 m above the ground level where the bark surface had been smoothed to produce an 'arena' which allowed for the detection of *I. rondensis* larvae. The arena was monitored for 1 h since the larvae respond quickly to the kairomone (Branco et al. 2006a, b). Only one beetle per tree was collected in order to prevent the collection of siblings, and immediately killed and stored in 98 % alcohol. Sampling was carried out in 2010–2012, in the months of March, April and May, which corresponds to the peak period of *I. rondensis* larval activity (Tavares et al. 2014).

A total of 104 ladybird beetles were collected (all larvae except for three adults). Due to its geographical proximity and low number of individuals, Sierra d'Espada and Cova de l'Aigua were grouped together. Similarly, the three locations in Algarve were grouped in a single population (Table 1). Usually individuals from the same populations were no more than 130 km apart, except in Sintra and Setúbal (two localities separated by a large estuary).

Molecular procedures

Genetic variation of *I. rondensis* was examined by amplifying the barcode fragment (658 bp) of the mitochondrial gene, cytochrome oxidase c subunit 1 (COI) (Hebert and Gregory 2005). The whole genomic DNA was extracted from the abdomen of individual specimens preserved in alcohol, using a Nucleospin®-Tissue XS Kit (Macherey–Nagel, Düren, Germany). DNA was amplified with the COI primers LCO1490 and HCO2198 (Folmer et al. 1994). Reactions were performed in 25 μl volumes containing 2 μl of DNA template (concentration around 30 ng μl^{-1}), 1 × PCR Buffer without $MgCl_2$ (Sigma, Saint Louis, MS, USA), 2.5 mM $MgCl_2$, 400 μM of each dNTP, 1 U REDTaq Genomic DNA polymerase (Sigma, Saint Louis, MS, USA), and 1 μM each of the forward and reverse primers. PCR was carried out using a 2,720 Thermal Cycler (Applied Biosystems, Foster City, California, USA) with the following settings: 5 min at 94 °C; followed by five cycles of 30 s at 94 °C, 40 s at 47 °C, and 1 min at 72 °C; followed by a further 40 cycles of 30 s at 94 °C, 40 s at 52 °C, and 1 min at 72 °C; and a final extension of 5 min at 72 °C. Successful amplification was confirmed by agarose gel electrophoresis and PCR products were subsequently cleaned by using a Nucleospin® Gel and PCR Clean-up Kit (Macherey–Nagel, Düren, Germany). PCR fragments were then sequenced in both directions using the ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). Sequencing reactions were purified by ethanol precipitation, loaded on a 3,500 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and analyzed with Sequencing Analysis v5.4 software. Sequences were aligned by ClustalW multiple alignment in BioEdit 7.0.9.0 (Hall 1999) and primers sequences were removed from the analysis. Ambiguities were edited by eye. COI sequences were translated by using the EMBOSS-Transeq website (http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) to confirm the absence of nuclear pseudogenes (Song et al. 2008). All sequences have been deposited in GenBank (KJ637343–KJ637446). DNA barcodes have been deposited in the published projects section of the Barcode of Life Data systems (BOLD) project code: IBERH (http://www.barcodinglife.org). Information on specimen vouchers (field data and GPS coordinates) and sequences (nucleotide composition, trace files) can be found in this project. The *I. rondensis* dataset is registered under DOI number http://dx.doi.org/10.5883/DS-RONDEN.

Table 1 Sampled regions and locations, with the different populations and corresponding geographic coordinates

Regions	Locations	Populations	Geographic coordinates (Lat, Long)	Samples	Haplotypes	Haplotype richness	(Hd ± SD)	(π ± SD)	Haplotype distribution
North of Portugal	Lousã	1	40.07, −8.22	16	4	2.0 ± 0.60	0.62 ± 0.096	0.07 ± 0.0419	H1 (9), H3 (5), H4 (1), H5 (1)
	Vila Real	1	41.28, −7.86	16	4	1.6 ± 0.62	0.35 ± 0.148	0.016 ± 0.0133	H3 (1), H11 (1), H26 (13), H27 (1)
Center of Portugal	Setúbal	1	38.57, −9.14	12	2	1.8 ± 0.40	0.53 ± 0.08	0.04 ± 0.0269	H2 (7), H3 (5)
	Sintra	1	38.80, −9.45	11	5	2.4 ± 0.60	0.76 ± 0.107	0.065 ± 0.0402	H1 (1), H2 (1), H10 (3), H19 (5), H20 (1)
South Portugal (Algarve)	Vila Real St. Ant.		37.18, −7.41	5		1.7 ± 0.46	0.47 ± 0.132	0.062 ± 0.0391	H1 (1), H2 (4)
	Vila Bispo	1	37.08, −8.92	4	2				H1 (1), H2 (3)
	Odeceixe		37.38, −8.78	1					H1 (1)
Central System	Sierra de Gredos	1	40.38, −4.58	17	13	2.9 ± 0.32	0.96 ± 0.033	0.039 ± 0.0256	H6 (1), H7 (1), H8 (2), H9 (1), H10 (2), H11 (3), H12 (1), H13 (1), H14 (1), H15 (1), H16 (1), H17 (1), H18 (1)
Iberian System	Sierra d'Espadá	1	40.01, −0.38	2	4	3.0 ± 0.00	1.00 ± 0.177	0.132 ± 0.0939	H11 (1), H21 (1)
	Cova de l'Aigua	1	38.86, −0.11	2					H22 (1), H23 (1)
Betic System	Ronda	1	36.58, −5.19	3	2	2.0 ± 0.00	0.67 ± 0.314	0.126 ± 0.1018	H24 (1), H25 (1)
	Sierra Nevada	1	37.08, −3.52	15	6	2.5 ± 0.56	0.83 ± 0.064	0.07 ± 0.0422	H28 (4), H29 (5), H30 (2), H31 (2), H32 (1), H33 (1)

Haplotype richness was calculated with the rarefaction method

π nucleotide, *Hd* haplotype diversity, *SD* standard deviation, for each population, sampled for COI

Data analyses

Because the number of beetles studied differed between the different populations, a rarefaction method was used to compare the haplotype diversity across populations using the Rarefaction Calculator (http://www.biology.ualberta.ca/jbrzusto/rarefact.php) (Kalinowski 2004). A correlation between number of samples and haplotypes was calculated. Haplotype (Hd) and nucleotide diversity (π) were estimated for each population (Table 1) with Arlequin 3.5 (Excoffier and Lischer 2010). A parsimony haplotype network with 95 % support (Templeton et al. 1992) was constructed using TCS version 1.21 (Clement et al. 2000). Loops, representing less probable mutational steps, were kept (Crandall and Templeton 1993).The genetic diversity of the different populations was visualized by plotting genetic data as pie charts on a map with *P. pinaster* and *M. feytaudi* distributions using ArcGIS 2012 (ESRI 2011). Genetic structure was studied with a spatial analysis of molecular variance, which is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance due to differences between groups of populations (SAMOVA 1.0; Dupanloup et al. 2002). The program was run for 10,000 iterations for $k = 2$–7 (groups of populations) from each of 100 random initial conditions. Analyses were repeated three times for consistency. The genetic differentiation among groups was computed as genetic molecular distance between DNA sequences using pairwise differences. A Mantel test with 1,000 permutations was performed to test if the genetic structure of the population follows isolation by distance model (Slatkin 1993) using pairwise *Fst* values and straight-line geographical distances in km estimated by using Google Maps Distance Calculator. Neutrality tests, Tajima's D test (Tajima 1989) and Fu's Fs (Fu 1997) test, were used to test for selective neutrality and population equilibrium, with 1,000 simulations. Since some populations had a very low number of sampled individuals, they were enclosed by geographic proximity and the neutrality tests were applied to these groups: Portugal (Vila Real, Lousã, Sintra, Setúbal, Algarve); Central System (Sierra de Gredos) and Betic System + Iberian System (Sierra Nevada, Sierra Ronda, Sierra d'Espadá and Cova de l'Aigua).

Results

A total of 33 different haplotypes were obtained, with 20 unique haplotypes (each one found in a single individual) (Table 1, Fig. 1). The highest haplotype diversity was observed in Sierra de Gredos, Sierra Nevada and the Iberian System, although in this last region only four individuals were sampled (Table 1). These results were confirmed after the rarefaction analysis. Haplotype diversity was not correlated with sampling effort ($r = 0.526$, n = 9, $P = 0.156$). The highest nucleotide diversity was found in Sierra Ronda and the Iberian System (Table 1).

In the spatial analysis of molecular variance (SAMOVA) differentiation was maximized between four groups of populations and minimized between populations within these four groups ($\varphi_{CT} = 0.466$, $P = 0.013$) (Fig. 2). Parsimony network analysis separated populations in four distinct groups: I) Western and Central Iberia (Portugal and Central System), and then Eastern Iberia separated in II) Sierra Nevada + Betic System, III) Sierra Nevada and IV) Sierra Ronda. Sierra Nevada region consists of two very distant groups of haplotypes, one of them shared with the Betic System (Fig. 1b). The mutual distance between groups II, III and IV has a bigger number of mutation steps than from the cluster I. This division in four groups was supported by the SAMOVA.

The ancestral haplotype, defined by the parsimony network analysis as H8, originates from Sierra de Gredos populations, with two individuals represented. The haplotype H11 (n = 5), may also be considered an ancestral haplotype and it is shared between Sierra de Gredos (Central System), Sierra d'Espadá (Iberian System) and North Portugal. Only one other haplotype is shared between Sierra de Gredos and Portugal (center): H10 (n = 2). The populations from Central System, Iberian System and Betic System are well separated. However haplotypes from Sierra de Ronda are split between Western and Central Iberia, and with Eastern Iberia, respectively, H24 (n = 2) and H25 (n = 1) (Fig. 1). The most shared haplotypes are found in Portugal: H1 (n = 13), H2 (n = 15), H3 (n = 11) and H26 (n = 13).

The Mantel test revealed a significant correlation between geographic distance and pairwise *Fst*

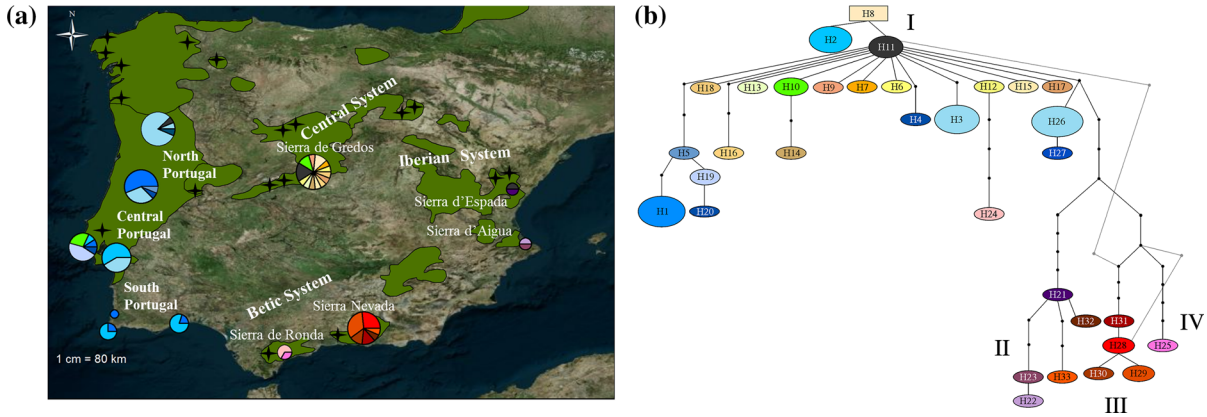

Fig. 1 Geographical distribution of mtDNA haplotypes of 104 *Iberorhyzobius rondensis* specimens. Tones of red and rose correspond to the Betic System, tones of violet to the Iberian System, tones of yellow to the Central System and tones of blue to Portugal. Group I, Western and Central Iberian comprising Atlantic Coast (Portugal), Sierra de Gredos and partially Sierra Ronda; Group II, Sierra Nevada and the Betic System, Group III, Sierra Nevada and Group IV, Sierra Ronda. These three last groups correspond to Eastern Iberia. **a** Haplotype distribution of the 33 haplotypes found among the six regions sampled in the Iberian Peninsula. Size of pies is correlated with number of sampled individuals. Green layer corresponds to the *Pinus pinaster* distribution on Iberian Peninsula, black stars present the known distribution of *Matsucoccus feytaudi* populations (Burban et al. 1999); **b** COI statistical parsimony network (95 %) of *Iberorhyzobius rondensis*. Haplotypes frequencies are represented by the size of the circles. Each line corresponds to a mutational step. Black dots represent possible haplotypes. Less probable mutational steps are drawn in grey. (Color figure online)

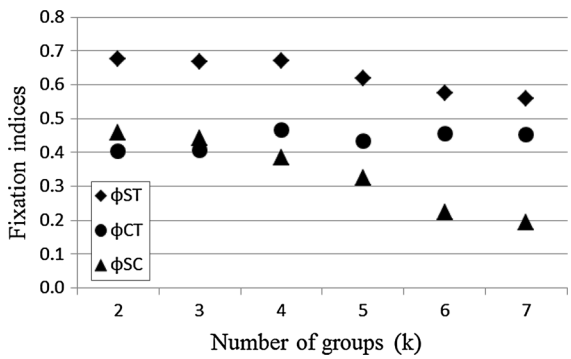

Fig. 2 Population structure results for COI. Values of fixation indices (φ_{CT} = among groups differentiation; φ_{SC} = among populations within groups differentiation; φ_{ST} = total differentiation among populations) obtained by SAMOVA from a predefined number of groups (K) ranging from two to seven

($r = 0.512$, $P = 0.011$), which is indicative of isolation by distance. Values of Tajima's D test and Fu's Fs were negative for the Betic System and Sierra de Gredos ($D = -0.68$, $Fs = -1.013$ and $D = -2.06$, $Fs = -11.13$) but were only significant ($P < 0.05$) for Sierra de Gredos. Positive and non significant values of Tajima's and Fu's F test were found for Portugal populations ($D = 0.6075$, $Fs = 1.11$).

Discussion

Phylogeography of *Iberorhyzobius rondensis*

The mitochondrial DNA revealed geographically structured populations for *I. rondensis*. Also, a relatively high number of haplotypes (33) was found compared with other studies of ladybird species using COI (Greenstone et al. 2011; Kobayashi et al. 2011; Zakharov and Shaikevich 2013). COI haplotype diversity varies greatly among coccinellid species: e.g. 15 haplotypes were found in 127 individuals of *Adalia bipunctata* (L.), an eurytopic predator, whereas 44 haplotypes were found in 70 individuals of *Henosepilachna pustulosa* (Kôno), a stenotopic and phytophagous species (Kobayashi et al. 2011; Zakharov and Shaikevich 2013).

The highest genetic diversity was found in Sierra de Gredos and Sierra Nevada, where most of the unique haplotypes occur. A high genetic diversity was also found for the Iberian System (Table 1). Yet, this is possibly due to an effect of pooling the two populations (Sierra d'Espadá and Cova de l'Aigua) together, since they have a high genetic divergence, which then inflates the level of nucleotide diversity.

Independent refugia may explain the division of the populations in the four different groups. For instance, Sierra de Ronda and Sierra Nevada are part of the Betic System, which is considered an important glacial refugium for several species. In fact they display the highest plant biodiversity and level of endemism in continental Europe (Gómez and Lunt 2007; González-Martínez et al. 2007). Sierra Ronda and Sierra Nevada are different mountain ranges, and thus they may have served as different refugia. Different clusters of pines are also found in these mountains (González-Martínez et al. 2007). Further, Sierra Nevada is an area of high biogeographical diversity (Feliner 2011). The existence of microrefugia (Dobrowski et al. 2010) may explain the presence of those distant haplotypes within near distance in Sierra Nevada. However, since a low number of individuals were collected from Ronda and the Iberian System, interpretation on its phylogeography must be addressed with caution. Another important refugium is Central System, to which belongs Sierra de Gredos (Gómez and Lunt 2007). This refugium is supported by the high polymorphism and genetic diversity of *I. rondensis* populations found on those areas.

Haplotypes from Portugal present a low genetic diversity and are closely related with the ones from Sierra de Gredos. Usually populations in the more recently colonized areas exhibit lower genetic diversity following rapid expansion of refugial populations leading to series of bottlenecks (Hewitt 1996). Thus, we hypothesize that Sierra de Gredos populations expanded their range to the Atlantic coast (Portugal), which is also supported by the negative values of Tajima's D and Fu's F tests (Fu 1997; Tajima 1989). Another hypothesis is that the restricted group of haplotypes in the Atlantic area may be evidence that this region acted as a refugium. This has been suggested for other Mediterranean species, such as *Tomicus destruens* (Vasconcelos et al. 2006), which also feeds on *P. pinaster* trees.

The isolation by distance may be explained by the fragmentation of *P. pinaster* forests that could have prevented the spread of the beetles. Indeed, until recent historical times, *P. pinaster* remained restricted to specific areas in the Iberian Peninsula, which would probably reflect the post-glacial refugia areas (Bucci et al. 2007). During the twentieth century large areas were planted with this pine species especially in the Northwest and the Center of Iberia accounting for its present distribution (Figueiral 1995). Thus, the phylogeographic structure of *I. rondensis* populations could reflect the isolation of ancient *P. pinaster* natural forests, despite the recent afforestation programs. A low capacity of dispersal of the beetle could also explain the isolation by distance pattern. Studies on the flight performances of this ladybird beetle would help to test this hypothesis.

Comparative phylogeography of the tritrophic interaction

Matsucoccus feytaudi phylogeography has been studied using RFLP fragments of COI + COII and cytB + 12S rDNA, and 22 mitotypes were found (Burban et al. 1999). Although the genetic markers used were partly different, the predator *I. rondensis* shows a remarkably similar phylogeographical pattern with that of its prey *M. feytaudi* (Burban et al. 1999) and that of the prey's host, *P. pinaster* (Bucci et al. 2007; Burban and Petit 2003; Carrión et al. 2000; González-Martínez et al. 2007; Salvador et al. 2000) (Fig. 3). A comparison across the phylogeography of the three trophic levels highlights some common patterns:

i) Western and Central Iberian Peninsula group: It includes the populations of *I. rondensis* from Sierra de Gredos and Portugal (Fig. 3c), which match the group of *M. feytaudi* found in Portugal and the Central System (Burban et al. 1999) (Fig. 3b). This lineage presents high polymorphism and is considered a center of origin for the bast scale. The *M. feytaudi* group present in Portugal was considered less polymorphic than the one of Central System which suggested an expansion to Portugal (Burban et al. 1999). However for *P. pinaster*, the Central System is considered a zone of admixture of divergent lineages from different refugia (Bucci et al. 2007; Carrión et al. 2000; Salvador et al. 2000) (Fig. 2a). Bucci et al. (2007) suggested that Portugal is an Atlantic refugium for *P. pinaster.*

ii) Eastern Iberian Peninsula group. The *M. feytaudi* group in this area is constituted by populations from Málaga and Granada (Fig. 3b), which correspond to the Sierra de Ronda and Sierra Nevada populations of *I.*

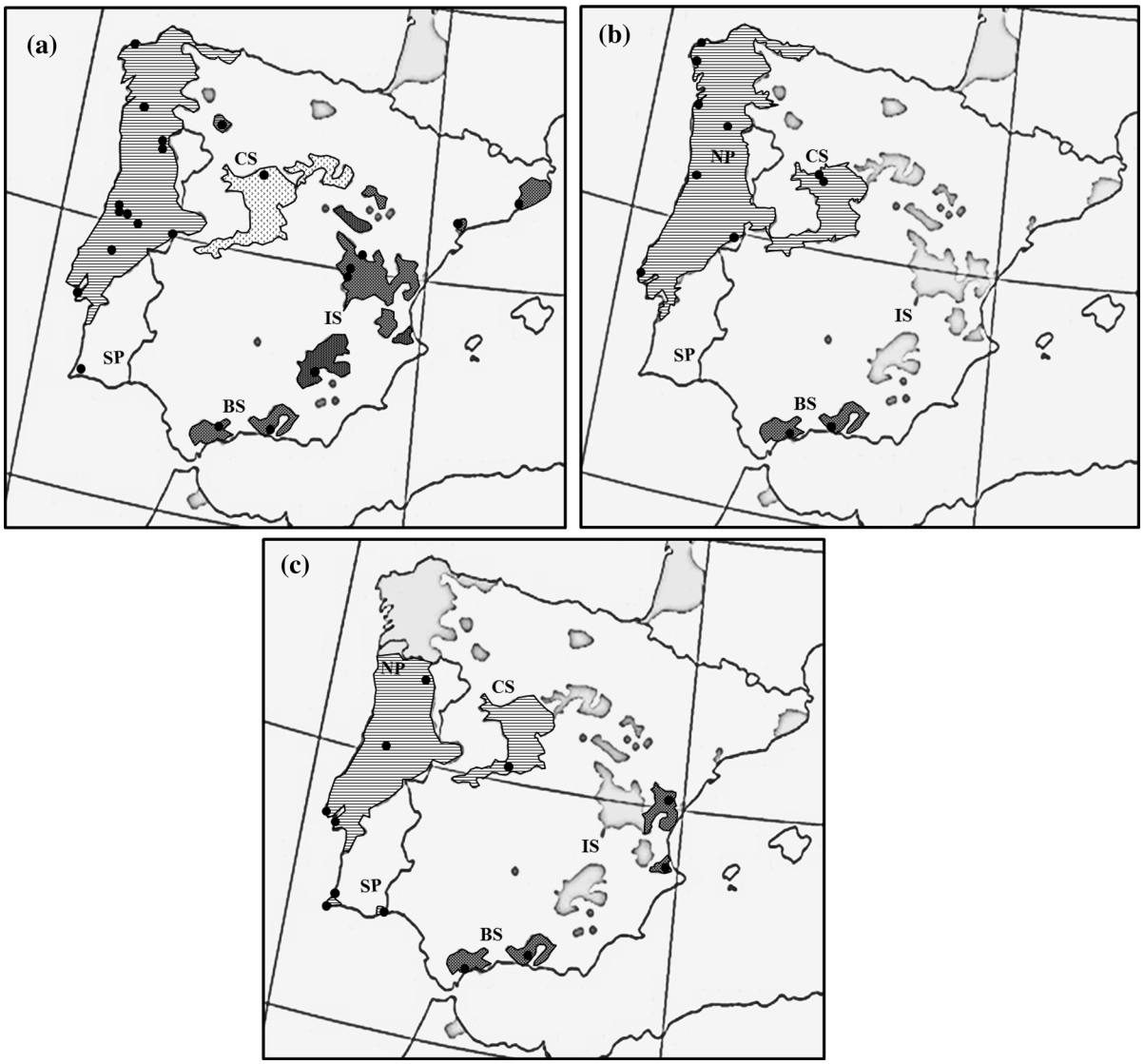

Fig. 3 Distribution maps of maritime pine (*Pinus pinaster*)—EUFORGEN 2009, http://www.euforgen.org—with the main genetic groups of the three throphic system: *Pinus pinaster*–*Matsucoccus feytaudi*– *Iberorhyzobius rondensis*. **a** *Pinus pinaster* (Bucci et al. 2007); two main genetic groups with admixture in Central Spain based on 16 spatially interpolated haplotype frequencies from five chloroplast microsatellites, Western (horizontal lines) and Central Iberian (light pattern) and Eastern (dark pattern). **b** Distribution of *Matsucoccus feytaudi* mitotypes with RFLP fragments of COI + COII and cytB + 12S rDNA (Burban et al. 1999), focusing on two phylogeographic lineages, Western and Central Iberian (horizontal lines) and Southern Iberian (dark pattern). **c** *Iberorhyzobius rondensis* distribution with the two major genetic lineages based on mtDNA: Western and Central Iberian (horizontal lines) and Eastern (dark pattern). The greyish area corresponds to the natural distribution of maritime pine in Iberian Peninsula. Black dots correspond to sampled areas. Regions: *NP* north of Portugal; *SP* south of Portugal; *CS* central System; *BS* Betic system; *IS* Iberian system. (Color figure online)

rondensis (Fig. 3c). No genetic information is available for populations of *M. feytaudi* from the Iberian System. The Betic System is also considered a refugium for *P. pinaster* (Fig. 3a), supported by several authors (Bucci et al. 2007; Burban and Petit 2003; González-Martínez et al. 2007; Salvador et al. 2000). Carrión et al. 2000 suggested that Sierra de Ronda and Sierra Nevada were different refugia areas of *P. pinaster,* which may also explain the presence

of three different lineages of *I. rondensis* in the Betic area. The Iberian System is considered another refugium of *P. pinaster* (Carrión et al. 2000; Salvador et al. 2000), from where populations migrated to the center and south of Iberia (Fig. 2a). It is possible that the Iberian System, may have been also a refugial area for *I. rondensis*, given the polymorphism and distribution of the haplotypes found (Fig. 1).

In conclusion, the three trophic-linked species, *P. pinaster*, *M. feytaudi* and *I. rondensis*, share, to some extent, the same phylogeographic pattern in the Iberian Peninsula. In particular, the phylogeography of the predator is well explained by the one of the prey, which agrees with our hypothesis of prey-predator phylogeographic match. There is a clear cleavage between south-eastern Spain and the rest of Iberian Peninsula. The Betic system was a shared refugium for the three species.

Since we only used a fragment of the mtDNA, this pattern should be confirmed with nuclear markers. Further, it would be quite interesting to see whether the ladybird is present in the Atlas region (Morocco), where a different lineage of *M. feytaudi* and *P. pinaster* occurs (Burban et al. 1999; Burban and Petit 2003).

Implications for biological control

Matsucoccus feytaudi has expanded to France, Italy and Corsica, and these populations present only a few haplotypes, which originated from the Western and Central part of Iberia (Burban et al. 1999). Apparently the predator beetle has not yet followed its prey's expansion. Hence, when considering the use of *I. rondensis* for biocontrol of *M. feytaudi* in its new range, two important points should be taken into consideration: (i) laboratory rearing colonies to be used in biocontrol should have a high genetic diversity (Hufbauer and Roderick 2005; Roderick and Navajas 2003), and (ii) the ladybirds to be used in biocontrol in the invaded areas should be collected in the same native areas as their invasive prey (Hoelmer and Kirk 2005). Because most haplotypes of *M. feytaudi* in the invaded regions are coming from the Central System and western of Iberian Peninsula (Burban et al. 1999), it would be advisable to use *I. rondensis* beetles collected from those areas. This would allow the capture of a high genetic diversity, and match *I. rondensis* haplotypes with the ones of *M. feytaudi* from the same region. Further, it would be interesting to test whether different lineages of *I. rondesis* also differ in their prey specialization to different lineages of *M. feytaudi* and in their predatory effectiveness.

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References

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge, USA

Barbosa AM, Thode G, Real R, Feliu C, Vargas JM (2012) Phylogeographic triangulation: using predator-prey-parasite interactions to infer population history from partial genetic information. PloS ONE 7:e50877

Branco M, Franco JC, Dunkelblum E, Assael F, Protasov A, Ofer D, Mendel Z (2006a) A common mode of attraction of larvae and adults of insect predators to the sex pheromone of their prey (Hemiptera: Matsucoccidae). Bull Entomol Res 96:179–185

Branco M, Lettere M, Franco JC, Binazzi A, Jactel H (2006b) Kairomonal response of predators to three pine bast scale sex pheromones. J Chem Ecol 32:1577–1586

Brown P, Adriaens T, Bathon H, Cuppen J, Goldarazena A, Hägg T, Kenis M, Klausnitzer B, Kovář I, Loomans A (2008) *Harmonia axyridis* in Europe: spread and distribution of a non-native coccinellid. BioControl 53:5–21

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía R, Vendramin GG (2007) Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. Mol Ecol 16:2137–2153

Burban C, Petit RJ (2003) Phylogeography of maritime pine inferred with organelle markers having contrasted inheritance. Mol Ecol 12:1487–1495

Burban C, Petit RJ, Carcreff E, Jactel H (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. Mol Ecol 8:1593–1602

Carrión JS, Navarro C, Navarro J, Munuera M (2000) The distribution of cluster pine (*Pinus pinaster*) in Spain as derived from palaeoecological data: relationships with phytosociological classification. The Holocene 10:243–252

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659

Coll M, de Mendoza G, Roderick GK (1994) Population structure of a predatory beetle: the importance of gene flow for intertrophic level interactions. Heredity 72:228–236

Covassi M, Binazzi A, Toccafondi P (1991) Studies on the entomophagous predators of a scale of the genus *Matsucoccus* Cock. in Italy. I. Faunistical-ecological notes on species observed in pine forests in Liguria and Tuscany. Redia 74:575–597

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. Genetics 134:959–969

Dobrowski SZ (2010) A climatic basis for microrefugia: the influence of terrain on climate. Glob Change Biol 17:1022–1035

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. Mol Ecol 11:2571–2581

ESRI (2011) ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, USA

EUFORGEN (2009) Distribution map of maritime pine (*Pinus pinaster*). In: European Forest Genetic Resources Programme. Available online at http://www.euforgen.org. Cited 7 Apr 2014

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Fabre J-P, Menassieu P, Foing J-J, Chalon A (2000) Biology and ecology of *Elatophilus nigricornis* Zetterstedt (Hemiptera Anthocoridae) predator of *Matsucoccus feytaudi* Ducasse (Homoptera Matsucoccidae) in the South-East of France. Ann For Sci 57:777–792

Feliner GN (2011) Southern European glacial refugia: a tale of tales. Taxon 60:365–372

Figueiral I (1995) Charcoal analysis and the history of *Pinus pinaster* (cluster pine) in Portugal. Rev Palaeobot Palynol 89:441–454

Foldi I (2004) The Matsucoccidae in the Mediterranean basin with a world list of species (Hemiptera: Sternorrhyncha: Coccoidea). Ann Soc Entomol Fr 40:145–168

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Fu Y-X (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Gariepy TD, Kuhlmann U, Gillott C, Erlandson M (2007) Parasitoids, predators and PCR: the use of diagnostic molecular markers in biological control of Arthropods. J Appl Entomol 131:225–240

Gebiola M, Lopez-Vaamonde C, Nappo A, Bernardo U (2013) Did the parasitoid *Pnigalio mediterraneus* (Hymenoptera: Eulophidae) track the invasion of the horse chestnut leafminer? Biol Invasions 16:843–857

Gómez A, Lunt D (2007) Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In: Weiss S, Ferrand N (eds) Phylogeography of southern European refugia. Springer, Dordrecht, The Netherlands, pp 155–188

González-Martínez S, Gómez A, Carrión J, Agúndez D, Alía R, Gil L (2007) Spatial genetic structure of an explicit glacial refugium of maritime pine (*Pinus pinaster* Aiton) in southeastern Spain. In: Weiss S, Ferrand N (eds) Phylogeography of southern European refugia. Springer, Dordrecht, The Netherlands, pp 257–269

Greenstone MH, Vandenberg NJ, Hu JH (2011) Barcode haplotype variation in north American agroecosystem lady beetles (Coleoptera: Coccinellidae). Mol Ecol Resour 11:629–637

Gregory TR, Nedvěd O, Adamowicz SJ (2003) C-value estimates for 31 species of ladybird beetles (Coleoptera: Coccinellidae). Hereditas 139:121–127

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hebert PDN, Gregory TR (2005) The promise of DNA barcoding for taxonomy. Syst Biol 54:852–859

Hernandez-Lopez A, Rougerie R, Augustin S, Lees D, Tomov R, Kenis M, Cota E, Kullaj E, Hansson C, Grabenweger G, Roques A, Lopez-Vaamonde C (2011) Host tracking or cryptic adaptation? Phylogeography of *Pediobius saulius* (Hymenoptera, Eulophidae), a parasitoid of the highly invasive horse chestnut leafminer. Evol Appl 5:256–269

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. Biol Linn Soc 58:247–276

Hoelmer KA, Kirk AA (2005) Selecting arthropod biological control agents against arthropod pests: can the science be improved to decrease the risk of releasing ineffective agents? Biol Control 34:255–264

Hufbauer RA, Roderick GK (2005) Microevolution in biological control: mechanisms, patterns, and processes. Biol Control 35:227–239

Jactel H, Menassieu P (2005) La cochenille du pin maritime *Matsuccocus feytaudi*: nouvelles données épidémiologiques et perspectives de lutte. For Méditerr 26:37–46

Jactel H, Ménassieu P, Burban C (1996) Découverte en Corse de *Matsucoccus feytaudi* Duc (Homoptera: Margarodidae), cochenille du pin maritime. Ann For Sci 53:145–152

Kalinowski S (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conserv Genet 5:539–543

Kenis M, Auger-Rozenberg M-A, Roques A, Timms L, Péré C, Cock MW, Settele J, Augustin S, Lopez-Vaamonde C (2009) Ecological effects of invasive alien insects. Biol Invasions 1:21–45

Kerdelhué C, Boivin T, Burban C (2014). Contrasted invasion processes imprint the genetic structure of an invasive scale insect across southern Europe. Heredity 1–11

Kim MJ, Wan X, Kim I (2012) Complete mitochondrial genome of the seven-spotted lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae). Mitochondrial DNA 23:179–181

Kobayashi N, Kumagai M, Minegishi D, Tamura K, Aotsuka T, Katakura H (2011) Molecular population genetics of a host-associated sibling species complex of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachninae). J Zoo Syst Evol Res 49:16–24

Lees DC, Lack HW, Rougerie R, Hernandez-Lopez A, Raus T, Avtzis ND, Augustin S, Lopez-Vaamonde C (2011) Tracking origins of invasive herbivores through herbaria

and archival DNA: the case of the horse-chestnut leaf miner. Front Ecol Environ 9:322–328

Lozier JD, Roderick GK, Mills NJ (2008) Evolutionarily significant units in natural enemies: identifying regional populations of *Aphidius transcaspicus* (Hymenoptera: Braconidae) for use in biological control of mealy plum aphid. Biol Control 46:532–541

Mendel Z, Assael F, Dunkelblum E (2004) Kairomonal attraction of predatory bugs (Heteroptera: Anthocoridae) and brown lacewings (Neuroptera: Hemerobiidae) to sex pheromones of *Matsucoccus* species (Hemiptera: Matsucoccidae). Biol Control 30:134–140

Nadel RL, Wingfield MJ, Scholes M, Lawson S, Noack A, Neser S, Slippers B (2012) Mitochondrial DNA diversity of *Cleruchoides noackae* (Hymenoptera: Mymaridae): a potential biological control agent for *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae). BioControl 57:397–404

Omondi B, van den Berg J, Masiga D, Schulthess F (2011) Phylogeographic structure of *Teretrius nigrescens* (Coleoptera: Histeridae) predator of the invasive post harvest pest *Prostephanus truncatus* (Coleoptera: Bostrichidae). Bull Entomol Res 101:521–532

Perrings C, Williamson M, Barbier EB, Delfino D, Dalmazzone S, Shogren J, Simmons P, Watkinson A (2002) Biological invasion risks and the public good: an economic perspective. Conserv Ecol 6:1

Raimundo A, Canepari C, Mendel Z, Branco M, Franco C (2006) *Iberorhyzobius* Raimundo & Canepari gen. nov., for *Coccidula rondensis* Eizaguirre (Coleoptera: Coccinellidae). Zootaxa 1312:49–58

Rigot T, van Halder I, Jactel H (2014) Landscape diversity slows the spread of an invasive forest pest species. Ecography 37:648–658

Roderick GK, Navajas M (2003) Genes in new environments: genetics and evolution in biological control. Nat Rev Genet 4:889–899

Salvador L, Alía R, Agúndez D, Gil L (2000) Genetic variation and migration pathways of maritime pine (*Pinus pinaster* Ait) in the Iberian Peninsula. Theor Appl Genet 100:89–95

Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings A, Holt RD, Mayfield MM, O'Connor MI, Rice WR (2007) Ecological and evolutionary insights from species invasions. Trends Ecol Evol 22:465–471

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends Ecol Evol 17:170–176

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279

Smith CI, Tank S, Godsoe W, Levenick J, Strand E, Esque T, Pellmyr O (2011) Comparative phylogeography of a coevolved community: concerted population expansions in Joshua trees and four yucca moths. PLoS ONE 6:e25628

Song H, Buhay JE, Whiting MF, Crandall KA (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proc Natl Acad Sci USA 105:13486–13491

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Tavares C, Jactel H, van Halder I, Mendel Z, Branco M (2014) A case of ecological specialization in ladybirds: *Iberorhyzobius rondensis* (Coleoptera: Coccinellidae), potential biocontrol of *Matsucoccus feytaudi* (Hemiptera: Matsucoccidae). Bull Entomol Res 104:367–375

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633

Valade R, Kenis M, Hernandez-Lopez A, Augustin S, Mari Mena N, Magnoux E, Rougerie R, Lakatos F, Roques A, Lopez-Vaamonde C (2009) Mitochondrial and microsatellite DNA markers reveal a Balkan origin for the highly invasive horse-chestnut leaf miner *Cameraria ohridella* (Lepidoptera, Gracillariidae). Mol Ecol 18:3458–3470

Vasconcelos T, Horn A, Lieutier F, Branco M, Kerdelhué C (2006) Distribution and population genetic structure of the Mediterranean pine shoot beetle *Tomicus destruens* in the Iberian Peninsula and Southern France. Agric For Entomol 8:103–111

Zakharov I, Shaikevich E (2013) Comparative study of mtDNA in species of the genus *Adalia* (Coleoptera: Cocinellidae) and origin of ancient mitochondrial haplotypes in the gene pool of *Adalia bipunctata*. Eur J Entomol 110:427–433