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Long-term survival and diversification of an endemic *Melitaea* species in mountains of Iran and adjacent areas

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Abstract

Disjunct distribution patterns regularly resulted in the separation of different genetic lineages in glacial refugia. Recent patterns of survival and expansion have been often revealed by climatic niche modelling. We used the combination of genetic markers, geometric morphometry and climatic niche modelling to clear up the taxonomy and reconstruct the potential range of an endemic Iranian, taxonomically disputed *Melitaea* species in climatically different epochs. Our results show that this species (*Melitaea abbas* Gross & Ebert, 1975, *comb. n.* = *M. zagrosi* Tóth & Varga syn. nova) is clearly separated from all taxa of the *Melitaea phoebe* species group and only occurs in Iran and Azerbaijan but was also predicted for some adjacent regions. Molecular markers and distribution modelling show consistently that this species should have had a long-term survival in this area and its range could have been slightly larger during the LGM than currently. Based on the studied molecular markers three main groups in *M. abbas* can be recognised: those of steppic area of Azerbaijan, Western Iran and North-Eastern Iran. Each group is characterised by own mitochondrial haplotypes, but also a high level of genetic diversity appears in the central part of the distribution area (Zagros Mts.).

Key words: MaxEnt, LGM, Mid-Holocene, geometric morphometry, Bayesian phylogeny

Introduction

Earth's climate is characterised by series of glacial and interglacial periods. These well-pronounced climatic change events left clear effects on the distribution of animal and plant

species. According to the most generally accepted contraction-expansion model, temperate species experienced contractions of ranges in glacial periods and expanded their distribution during inter- and postglacial periods (de Lattin 1967; Hewitt 1996). Thus, the majority of these species survived the glacial periods in southern Mediterranean regions such as the Iberian, the Apennine or the Balkan Peninsula. Their disjunct glacial distribution patterns regularly resulted in the separation of different genetic lineages in the three major Mediterranean peninsulas combined with different basic patterns of postglacial expansion referred to as 'paradigms' (Comes and Kadereit 1998; Habel et al. 2005; Hewitt 2000; Hewitt 1999; Taberlet et al. 1998). Although repetitive patterns have been recognised, recent investigations support that the responses of a species might be species-specific (Stewart et al. 2010) and may also depend on life history traits and habitat preference (Bhagwat and Willis 2008).

Additionally, the methodological improvements of the past few decades contribute to the reconsideration of the paradigms. Phylogeography has become an important integrative field of evolutionary biology due to the recent development of molecular analytical tools (Hickerson et al. 2010). Nowadays, the results of molecular analyses such as haplotype networks or phylogenetic inference analysis are often combined with predictions of Species Distribution Models (SDM) to reveal biogeographic dynamics and identify refugial areas (Habel et al. 2011a; Habel et al. 2011b; Schorr et al. 2012; Wielstra et al. 2013). The application of these new techniques reshaped our knowledge on biogeography. For example, recent studies have located numerous extra-Mediterranean refugia, strongly modifying the biogeographical view of Europe. These favourable but geographically limited extra-Mediterranean areas could have served as refugia during the Last Glacial Maximum even for some Mediterranean species (Varga and Schmitt 2012).

The majority of phylogeographical studies focus on North American and European species. On the contrary, the Middle East is less studied from biogeographical points of view. Few studies have focused on this field, despite that complex landscapes including a number of mountain chains located at the contact zone of the European, Asian and African continents has led to complex phylogenetic relationships between taxa. Moreover, several areas in the Middle East are considered as refugia for several temperate species during climatic oscillations (Gündüz et al. 2007; Gvozdik et al. 2010; Veith et al. 2008; Wielstra et al. 2013).

Besides, the application of molecular methods (frequently complemented with modern geometric morphometrics) have considerable effects on taxonomical research, since it often leads to the recognition of cryptic species within putatively well-known taxa such as

Zerynthia polyxena (Dapporto 2010), *Polyommatus icarus* (Dinca et al. 2011a) or *Leptidea sinapis* (Dinca et al. 2011b). Furthermore, it became clear that the traditional view on a given species is based on the misinterpretation of morphological variants as it has been shown in *Maculinea rebeli* (Bereczki et al. 2005; Fric et al. 2007) some *Maniola* species (Kreuzinger et al. 2015) or in the case of *Melitaea scotosia* (Leneveu et al. 2009; Tóth and Varga 2011). The *Melitaea phoebe* species-group is also a typical example of how improvements of scientific methods modified our view on species boundaries. Using morphological and molecular data, several subspecies of *Melitaea phoebe* proved to be distinct species such as *Melitaea punica* (Leneveu et al. 2009; Tóth et al. 2014; Tóth and Varga 2011), *Melitaea ornata* (Russell et al. 2007; Tóth et al. 2014; Tóth and Varga 2011; Varga et al. 2005) and *Melitaea zagrosi* (Tóth et al. 2014; Tóth and Varga 2011). Recently, it has also been indicated that *M. telona* sensu stricto from Israel and *M. ornata* are different taxa (Tóth et al. 2014). Previous morphometrical studies have already revealed small differences in the genital structures of the males ((Tóth et al. 2013; Tóth and Varga 2011) but the authors interpreted the difference as a well-pronounced intra-specific difference. In contrast, molecular data clearly showed that the two taxa are genetically distinct from each other. Based on the results of the analysis of seven genes, Tóth et al. (2014) concluded that *M. telona* is not a subspecies of *M. ornata* but a species in its own right. *Melitaea zagrosi* Tóth & Varga, 2011 was described from Iran based on the significant difference in male and female genitalia and the distinct wing pattern. Later, the analysis of DNA reinforced the species level of this taxon. These findings did not allow the synonimisation of *M. ornata* and *M. zagrosi* with *M. telona* or each other as suggested by von Oorschot (2014).

The general confusion between these morphologically similar species has prevented the accumulation of biological information. This is especially true in the case of *M. zagrosi*.

In this study, we provide a wide range of information on *Melitaea zagrosi*, including of its taxonomy, distribution and phylogeography.

Material and Methods

Sample material

The samples were obtained from the Hungarian Natural History Museum, the Zoological State Collection of Munich, Staatliches Museum für Naturkunde, Karlsruhe, the collection of Jagellonian University of Krakow and the Lepidoptera collection of University of Debrecen.

DNA

DNA was extracted from the head or the proximal end of the abdomen following the protocol in Bereczki et al. (2014) from 30 specimens (**Table 1.**). The cytochrome c oxidase subunit I gene (*COI*), which is commonly used in barcoding animal life (Hebert et al., 2003;Wiemers & Fiedler, 2007), offers an adequate tool to obtain insight into the phylogeny of taxa at species level. We therefore sequenced this section of the mitochondrial genome together with the nuclear elongation factor 1 (*EF-1*), malate dehydrogenase (*MDH*), ribosomal protein S5 (*RpS5*) and wingless (*wg*). These genes were amplified by specific primers modified at their 5′-end to include the universal sequencing primer T7 promoter (Wahlberg & Wheat, 2008). Amplification from 1 µl of DNA extracts was carried out in 25 µl final reaction volumes containing 5× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.02 units/µl of Taq DNA polymerase (Phusion Hot Start II High-Fidelity, Thermo Scientific) and 0.3 µM of each primer. Amplification was carried out in an ABI Veriti thermal cycler programmed for: initial denaturation for 3min at 94°C; 35 cycles of 30 s at 94 C, 30 s at the locus specific annealing temperature, 1 min at 72 C; final elongation of 10min at 72 C. The success of PCR amplification was checked by running 2 µL of product on 1% agarose gels stained with GelRed Nucleic Acid Stain (Biotium Inc.). PCR-products were sequenced by commercial service provider Macrogen Inc. (Seoul, South Korea).

Although COI proved to be highly informative in phylogenetic and phylogeographic studies and most of the cases it is suitable for species identification, recently it became clear that it could be manipulated by the intracellular bacteria genus *Wolbachia*. This microorganism could induce 'two barcodes – one species' (Kodandaramaiah et al. 2013) or its opposite, 'one barcode – two species' phenomenon (Jiggins 2003). In these cases, the results of mitochondrial DNA based analyses could be misleading. Thus, a phylogeographic or a phylogenetic study is inconceivable without the screening of *Wolbachia* presence in the sample material. The presence of *Wolbachia* was checked by polymerase chain reaction (PCR) by the amplification of the highly conservative 16S ribosomal RNA gene with *Wolbachia* specific primers W-Spec of Werren and Windsor (2000), following the protocols described by these authors. The success of PCR amplification was checked by running 2 µl of product on 1% agarose gels.

DNA sequences were edited and revised manually by Chromas Lite v. 2.01, then aligned using MEGA v. 6 (Tamura et al., 2011). For statistical analysis of the aligned datasets Bayesian analyses was conducted using MrBayes 3.2.5 (Ronquist et al., 2012) on single-gene, nuclear genes only and all-gene datasets. The multiple genes datasets were partitioned by

136 genes. The different models of molecular evolution were sampled for each gene (both single
137 and combined data) and the model-jumping feature was used through the command 'lset
138 applyto=(all) nucmodel=4by4 nst=mixed rates=gamma covarion=no;'
139 Two independent MCMC runs each with four simultaneous chains (one cold and three heated)
140 for each analysis were run for 10 million generations and the sampling of trees and parameters
141 was set to every 1000 generations. Convergence of the two runs was determined by the
142 stationary distribution plot of the log-likelihood values against number of generations and
143 confirmed by the average standard deviation of split frequencies which were lower than 0.05
144 in all cases. We discarded the first 2 500 000 generations as burn-in and trees were
145 summarized under the 50% majority rule method. The summarised tree with posterior
146 probabilities were plotted using FigTree (http://tree.bio.ed.ac.uk/software/*figtree*/)
147 Additionally, a haplotype network was constructed using the R computing environment (R-
148 CoreTeam, 2014) with pegas package v. 0.81 (Paradis, 2010). For haplotype analysis we used
149 COI sequences without ambiguities. Haplotypes distribution in geographical space was
150 visualised using QGIS (QGIS Development Team, 2015).
151
152 *Morphometry*
153 Geometric morphometry was used to determine morphological relationships among *Melitaea*
154 *phoebe*, *M. ornata*, *M. zagrosi* and some paratypes of *M. phoebe abbas* Gross & Ebert, 1975
155 (described from South-Iran, Fars province, 50 km NW Ardekan Tange Surkh, 2250m). In
156 total, 107 specimens have been measured. The analysed material is partly identical with the
157 specimens used in Tóth & Varga (2011) but it has been completed with a significant amount
158 of new material (**Appendix Table 1**).
159 We analysed the shape of the processus posterior (male genitalia) which has already proved to
160 be useful in the *Melitaea phoebe* species group. We followed a standard genital preparation
161 method. The abdomens were removed and heated in 15% KOH solution in 80 C° for 30
162 minutes. Next, genitalia were cleaned and dehydrated in ethanol and mounted in euparal.
163 Genitalia slides were digitalized using a combination of a stereo microscope and a digital
164 camera.
165 TpsDig2 was used to record 9 landmarks at the tips and origin of the main processi (**Fig. 1**)
166 similarly to Tóth & Varga (2011). The raw coordinates were transformed using Procrustes
167 Generalised Least Squares. Based on the transformed coordinates we used Canonical
168 Variance Analysis (CVA) to determine the morphological relationships between the studied
169 taxa. We visually inspected the shape changes along the axes using landmark warp function.

Jack-knife grouping was also used to quantify the classification success. In Jack-knife, specimens are alternately excluded for each run and assigned using the CVA axes. The significance of the visible pattern was analysed by pair-wise MANOVA using Bonferroni corrected significance levels. All the morphometrical analyses were carried out by PAST 2.17 (Hammer et al. 2001).

Although DNA analysis indicated that none of our Iranian specimens belongs to *Melitaea ornata,* all of them turned out to represent *Melitaea zagrosi* including the North Eastern Iranian samples from the Eastern Elburs and the Kopeth-Dagh. At the same time, a previous morphometrical study (Tóth &Varga 2011) showed that *M. ornata* also occurs in the Kopeth-Dagh Mountain range. , Most of these specimens are unfortunately worn, thus we were not able to detect the identification traits described in Tóth & Varga 2011. Therefore, the specimens from North-Eastern Iran were classified as ungrouped cases even if additional information was available based on DNA (ZAIR16, ZAIR18) or based on the well-preserved wing colouration pattern (P111, P103).

SDM

The MaxEnt modelling method was used to predict the potential distribution of *Melitaea zagrosi* using BIOCLIM variables (Busby 1991). Among a broad variety of distribution modelling tools, MaxEnt is one of the most commonly used method for predicting species distributions and environmental tolerances using presence-only data (Warren and Seifert 2010). MaxEnt's predictive performance is consistently competitive with the highest performing methods (Elith et al. 2011), moreover MaxEnt shows little sensitivity to small sample sizes (Sinclair et al. 2010). Based on the examined specimens in this article we could use 17 non-overlapping presence points for modelling (**Fig. 4**). The climate variables were downloaded from WoldClim database (www.worldclim.com).

Although MaxEnt is more robust in controlling for correlations between variables than stepwise regression (Elith et al. 2011), strongly correlated variables ($r>0.75$) recommended to excluded from the analysis (see: Elith et al. 2010, Stohlgren et al. 2010). ENMtools 1.3 was used to calculate the level of correlations (Warren & Seifert 2010).To assess which predictors provide the most useful information by itself we applied jackknife test using MaxEnt.

Since the biology of *M. zagrosi* is basically unknown, the results of jackknife and the correlation tests were considered during variable selection. Finally, four variables were selected: bio11 (mean temperature of coldest quarter), bio18 (precipitation of warmest quarter), bio4 (temperature seasonality) and bio15 (precipitation seasonality).

The discrimination ability of the model was evaluated by Area Under the Curve (AUC) metric. The value of AUC ranges between 0.0 and 1.0 where 1.0 is considered perfect prediction and for values <=0.5 prediction is considered not significantly deviating from random distribution (Fielding & Bell 1997, Franklin 2009).

The results were visualised on a logarithmic scale, where MaxEnt provides an estimate ranging between 0 and 1 as a metric of climatic suitability for the species.

The distribution model was also projected to the Mid-Holocene climate optimum (MH), i.e. ~6000 years before present (yBP) and the Last Glacial Maximum (LGM), i.e. ~21000 years before present (yBP). For the projections we used the predictions of two different global circulation models (MIROC and CCSM).

Results

DNA

In total, 30 specimens were sequenced for five genes (a mitochondrial and four nuclear genes) (**Table 1**). The final concatenated sequences involved 3818 base pairs (bp), of which 1414 bp were from COI and 2404 bp from the nuclear regions. None of the specimens were infected with *Wolbachia*. The consensus phylogeny from the Bayesian inference analysis clearly separated the species. The sequenced specimens from Iran clustered with *Melitaea zagrosi* except for the single *M. phoebe* specimen (PHIR) which was well-separated (**Fig. 2**). Based on the combined gene phylogram, three main groups of *M. zagrosi* were identified: those belonging to steppic area of Azerbaijan, Western Iran and North-Eastern Iran.

In total, 9 unique haplotypes were identified based on 1414 bp COI sequences which contained 17 parsimony informative sites. The COI-based unique haplotypes were plotted as a network and additionally as geo-referenced pie charts. The distribution of the haplotypes shows a strong geographic pattern. Similarly to the combined gene analysis, the distribution of the haplotypes indicates the separation of the three geographical regions: the steppic area of Azerbaijan, Western Iran and the North-Eastern Iran which all exhibited unique haplotypes (see in **Electronic Appendix Fig. 1**).

Morphometry

The shape of the processus posterior of the studied *Melitaea* species was significantly different with high discriminatory power (Wilks $\lambda=0.05$, $p<0.001$). The first axis explained 79% while the second 17% of variance between groups. On the CVA plot *M. ornata* and *M.*

phoebe are slightly overlapping while the *M. zagrosi* is well-separated from the other two species (**Fig. 3**). The paratypes of *M. phoebe abbas* that were set as one of our apriori groups were mixed together with *M. zagrosi* specimens. The pairwise MANOVA with Bonferroni corrected significance levels showed the same pattern. All the apriori groups were significantly different ($p<0.001$) with the exception of '*abbas'* and '*zagrosi'* (**Table 2**).

The jackknifed classification assigned 77.5% of the individuals correctly. Most of the misclassifications occurred between *abbas* and *zagrosi.* Besides, few misclassifications could be found between *ornata* and *phoebe* (**Table 3**).

The ungrouped specimens from North-Eastern Iran were classified into the *zagrosi* and *ornata* groups. It is remarkable that the two specimens which were also sequenced (ZAIR16, ZAIR18) were classified into *zagrosi*. Interestingly, the two specimens from the same locality (Transkaspian region, Arwas) were classified into different groups and one of the specimens was positioned between *ornata* and *zagrosi* (B66) which is proved to be *zagrosi* based on mtDNA.

Distribution modelling

Despite the low number of presence data, the MaxEnt's prediction for current climatic conditions seems to be realistic. It is remarkable that the model predicted a very small suitable area in Azerbaijan located only in the southern mountainous region and not in steppic areas, although specimens were available from that region. In addition to Iran, relatively large suitable areas were predicted within Turkey, Turkmenistan and Afghanistan (**Fig. 4**).

Model projections fitted to the Last Glacial Maximum suggest relatively small area fluctuations. The two circulation models predicted very similar environments for this period. The predictions of both models (CCSM, MIROC) showed larger potential distribution of the species in the steppic area of Azerbaijan. The prediction for the Mid-Holocene showed a slightly smaller potential area.

The predictions of MaxEnt for different time scales show that the species was able to survive the recent climate fluctuations in the mountainous regions of Iran.

Discussion

In this study we provide a wide range of information on the recently described taxa *M. zagrosi* based on the analysis of five genes, geometric morphometrics and distribution modelling. The shape of the processus posterior proved to be a useful character to separate *M. zagrosi* from *M. phoebe* or *M. ornata*. The results of genitalia morphometry are generally in agreement

with the five-gene-based phylogeny reconstruction. Concerning morphometry, we obtained the same results as provided by Tóth & Varga (2011). It also became clear that genitalia morphology is a more reliable character than wing colouration in those cases when the specimen is worn or lost its typical colouration due to long-time storage. The jackknife classification results show that *Melitaea zagrosi* can be identifiable in nearly 100% based on the male genitalia.

Our findings based on four nuclear (EF-1a, MDH, RpS5 and wg) and a single mitochondrial (COI) genes support that differences in the shape of the processus posteriores qualify for species-level differences. The strong correlations between genitalia morphometry and the results of DNA analyses allow us to draw conclusions based on morphometry. This is crucial for this study since we failed to amplify genes from the type material due to the age of the specimens.

We examined the male genitalia of the type material of *M. phoebe abbas*. The CVA was not able to separate the paratypes of *M. phoebe abbas* from *M. zagrosi* but it clearly separated the taxa from *M. phoebe* and *M. ornata*. Therefore, the taxa *Melitaea phoebe abbas* Gross & Ebert, 1975 is neither a subspecies of *Melitaea phoebe* nor of *Melitaea ornata*, but conspecific with the recently described *Melitaea zagrosi* Tóth & Varga, 2011. Thus, in concordance with the rule of the priority *Melitaea zagrosi* Tóth & Varga, 2011 is a junior subjective synonym of *Melitaea abbas* Gross & Ebert, 1975, *comb. n*. Henceforth, we use the valid name of the species.

It is remarkable that all the sequenced specimens from Iran were identified as *M. abbas* or *M. phoebe* and none of them belongs to *M. ornata*. It seems that the distribution of *Melitaea ornata* is very limited in Iran. Probably it occurs only in Northern Iran: in certain parts of the Kopeth-Dagh Mountains and the adjacent areas in Turkmenistan. However, further studies are necessary to clarify this question. The results of MaxEnt modelling suggest that *M. abbas* has a relatively narrow ecological tolerance. The predicted potential distribution is restricted to a relatively small geographical area. The known present distribution is located in Iran and adjacent areas. All the Iranian populations were typically found in the forest-steppic biotopes of Iran. Generally, the occurrences of *M. abbas* show an interesting co-incidence with two different semi-open vegetation formations: *Pistacia-Amygdalus* forest-steppe and *Quercus brantii* forest-steppe in Western Iran (mostly in Zagros Mts.) and *Juniperus* forest-steppe in the Northern and North-Eastern regions of the country (mainly in Elburs and Kopeth-Dagh Mts.) (Djamali 2008). Therefore, *M. abbas* is increasing the number of narrowly distributed

endemic *Melitaea* species in the Middle East (e.g. *M. turkmanica*, *M. collina*, *M. sarvistana*, *M. tangighaurensis, M. interrupta, M. persea*).

Interestingly, most of the species belonging to the *phoebe*-group are adapted to dry and warm climatic conditions. These species occupy clearly separated areas. *M. punica* is only found in North-Western Africa. The situation is very similar in the case of *M. telona* which is distributed exclusively in the Levant region. *M. abbas* is only distributed in Iran and adjacent areas. Molecular results imply that *M ornata*, which can be characterised by a relatively large range, does not occur or only rarely in these areas. In contrast, *M. abbas* is unknown from Turkey where *M. ornata* is widely distributed (Hesselbarth et al. 1995; Tóth and Varga 2011). All three species, *M. ornata, M. telona* and *M. abbas* are strictly monovoltine with larval aestivation in the hottest and driest periods of the summer. Thus, they are predicted to have similar adaptation mechanisms to prevent the damages due to extreme temperatures and aridity. Correspondingly, we found that caterpillars' nests of *M. ornata* in forest-steppic habitats in Hungary are located in semi-shadowed spots of the habitat. Thus, the survey for ecological constraints in the life-cycle of *M. abbas* would be a fascinating task for the future.

Paleoclimatic predictions showed that suitable areas for the species continuously existed during the Last Glacial Maximum and Mid-Holocene. Based on recent results of Species Distribution Modelling and also molecular results, several mountain ranges (e.g. Elburz, Zagros, Kopeth-Dagh) in Iran have already been considered as refugia during climatic oscillations (Ahmadzadeh et al. 2013; Gvozdik et al. 2010; Rajaei et al. 2013). These results suggest that *M. abbas* was not able to significantly expand its distribution. This phenomenon could be explained by various hypotheses. It is possible that the species is strongly limited by certain environmental conditions as well as strict food plant specialisation. These factors could strongly limit the distribution of a species (Hanspach et al. 2014; Wisz et al. 2013).

The molecular analysis found three well separated groups in *M. abbas* which are localised in three different regions (Azerbaijan, Western Iran including Zagros Mts. and North-Eastern Iran, mostly Khorasan region). All of these areas were characterised by unique haplotypes. According to the consensus phylogeny based on five genes, clear diversification was shown between the specimens from the steppic areas of Azerbaijan and the rest of the sequenced *M. abbas* material. Unfortunately, we only had few specimens from this region thus detailed morphometrical analysis was not possible but this could be an interesting aim for the future research.

The result of distribution modelling indirectly indicated that the populations in Azerbaijan occur in a different climatic regime than in Iran, since MaxEnt did not predict suitable climate

for the steppic areas of Azerbaijan. Although this pattern is obviously the result of the fact that we had only one presence point from this area, it clearly indicates climatic isolation. The adaptation to steppic conditions could lead to pronounced genetic differentiation of these populations.

The other two groups showed more close genetic relation to each other based on the combined gene analysis. The specimens from Western Iran (Zagros Mts., Talysh Mts. and Western part of Elburs Mts.) could be considered as another unit which shows close relationships with the North-Eastern-Iranian group (Kopeth-Dagh, Eastern Elburs). Besides, the COI-based haplotype network separates the previously described three groups although it shows different relatedness. The genetic diversity of the Zagros Mountains is remarkable which could be the outcome of the long-time survival of the species in this region. It would not be right to make conclusions on the other two groups' genetic diversity since only few specimens were analysed in comparison to the Western-Iranian group.

In summary, our results confirmed the previous studies demonstrating the taxonomic distinctness of *M. abbas* from the other *Melitaea* species. Moreover, three well-separated lineages were identified applying molecular markers which are also supported by the results of species distribution modelling methods. This study also highlights the biogeographical importance of the Middle East since this region provided an opportunity of long-term survival for different organisms such as *M. abbas* which demonstrates essentially different area dynamics as compared to most European species.

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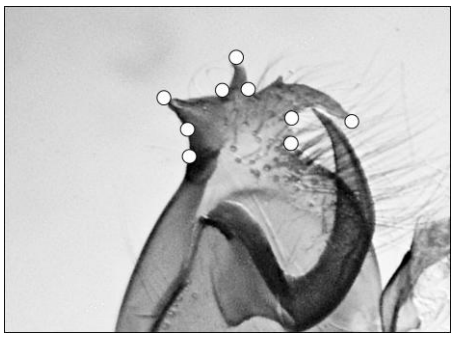
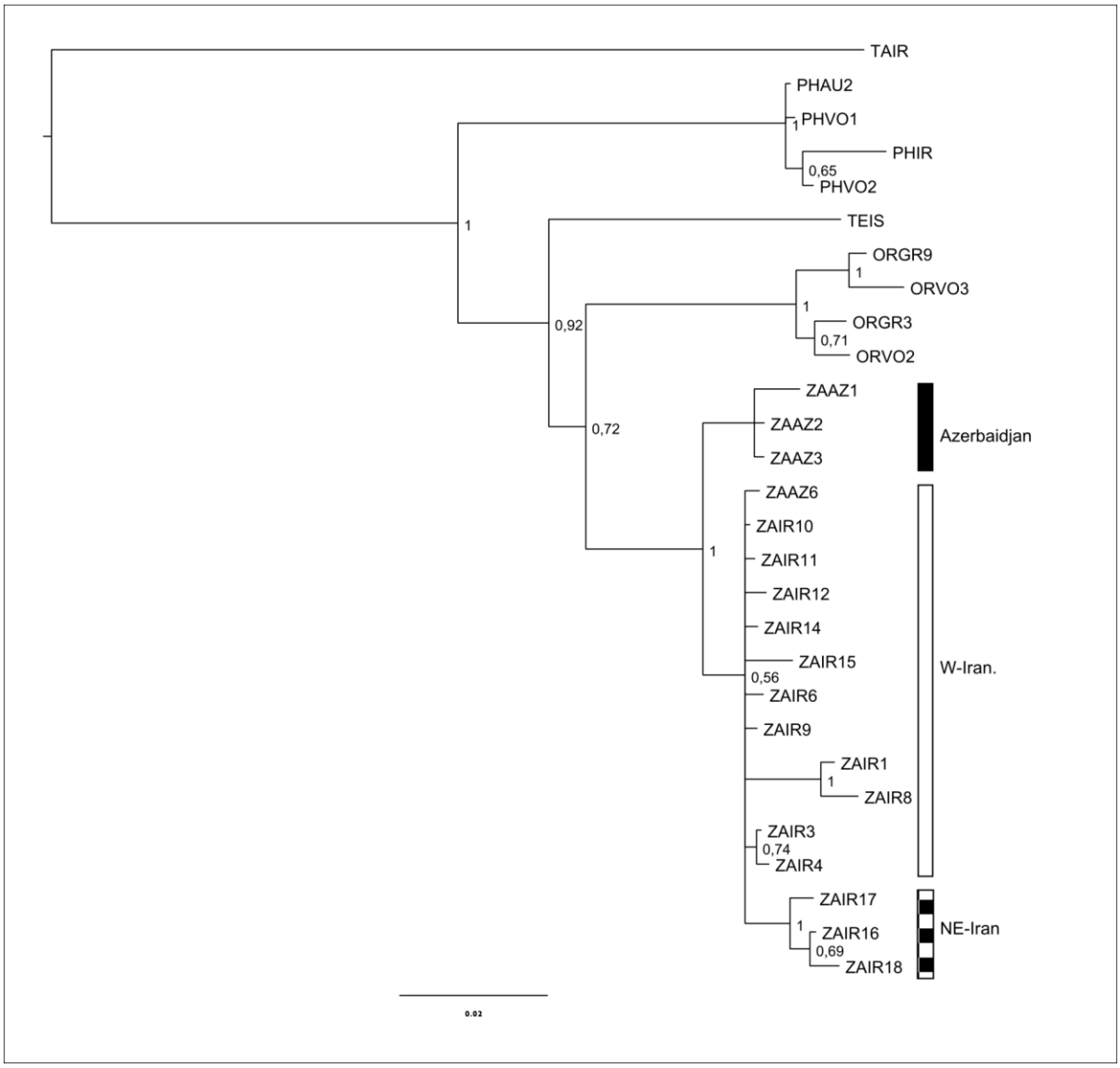
528
529
530 **Figures**
531

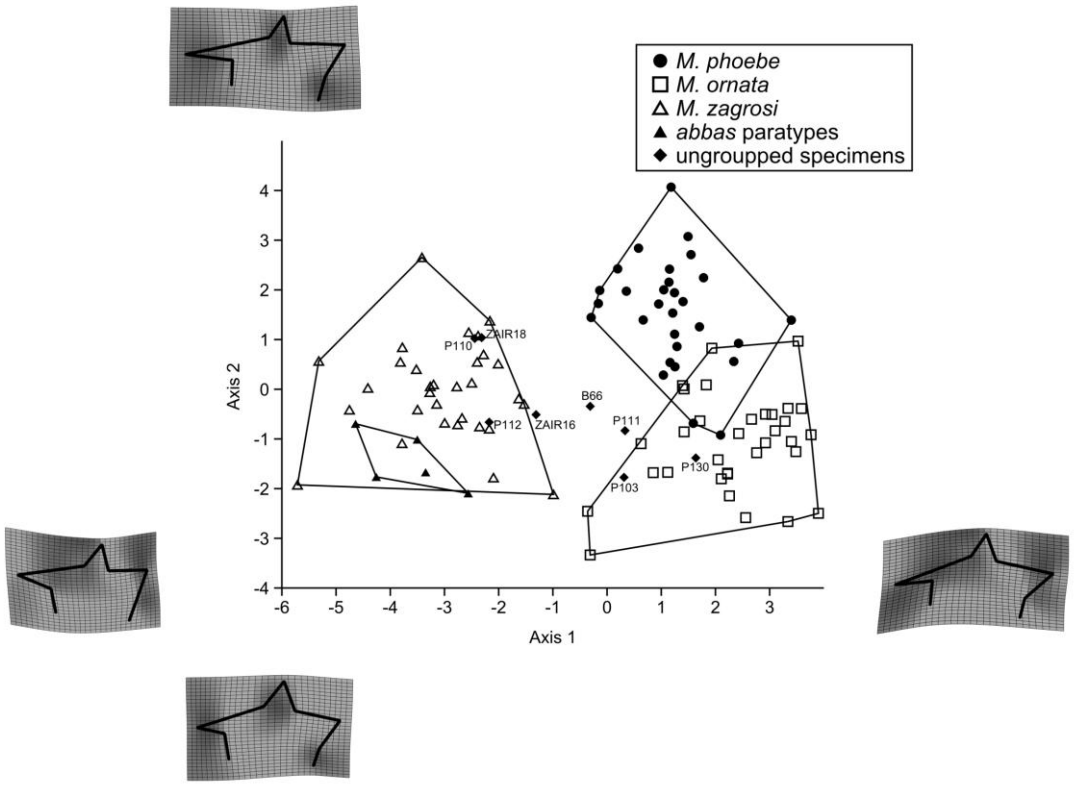


532
533 **Fig. 1**. Landmarks on the processus posterior.



534

535 **Fig 2.** Consensus phylogeny from the Bayesian inference analysis based on five genes (*COI,*
536 *EF-1 , MDH, RpS5, wg).*



537

538 **Fig. 3**. CVA scatterplot for the studied taxa. Shape deformations along discriminant axes
539 shown on thin-plate splin.

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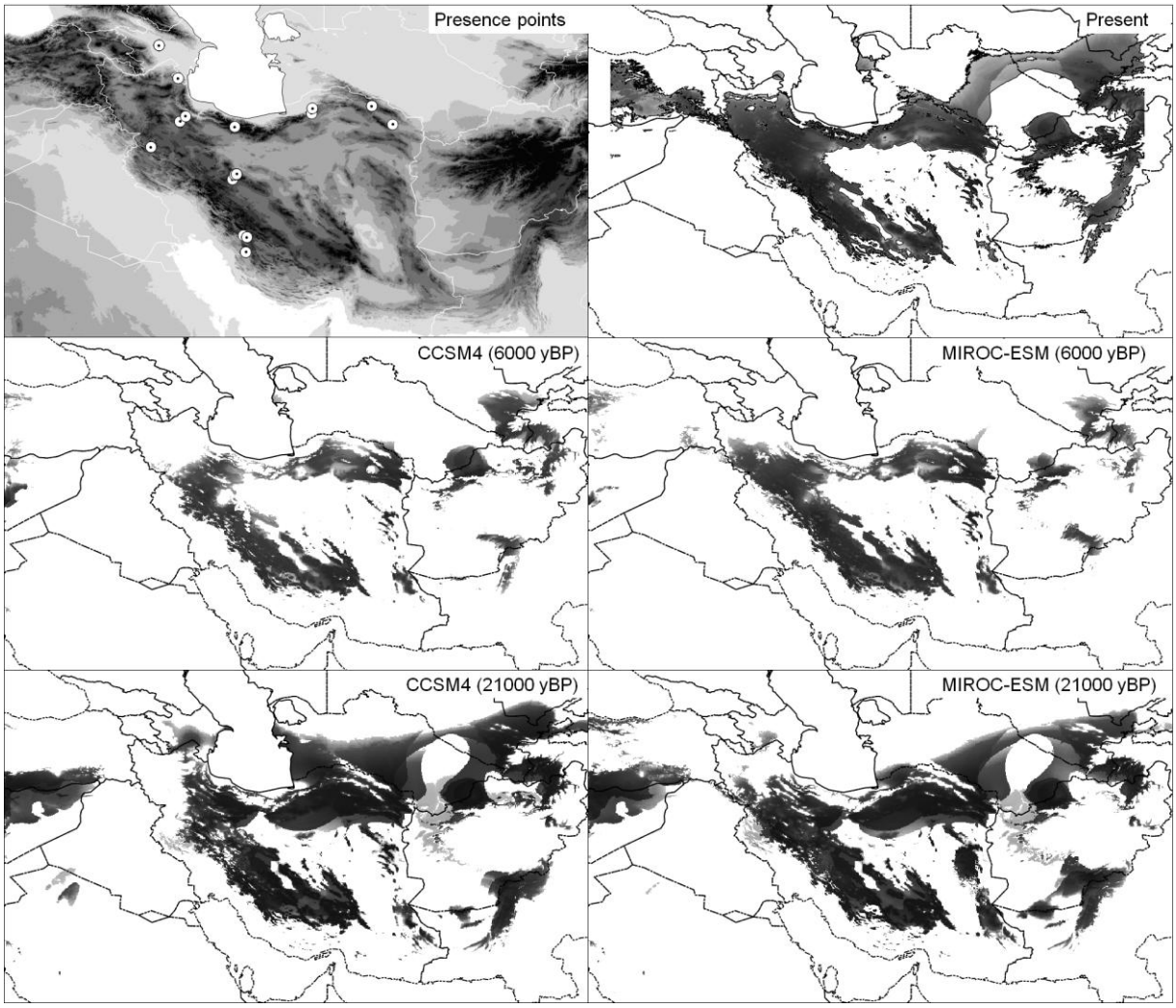
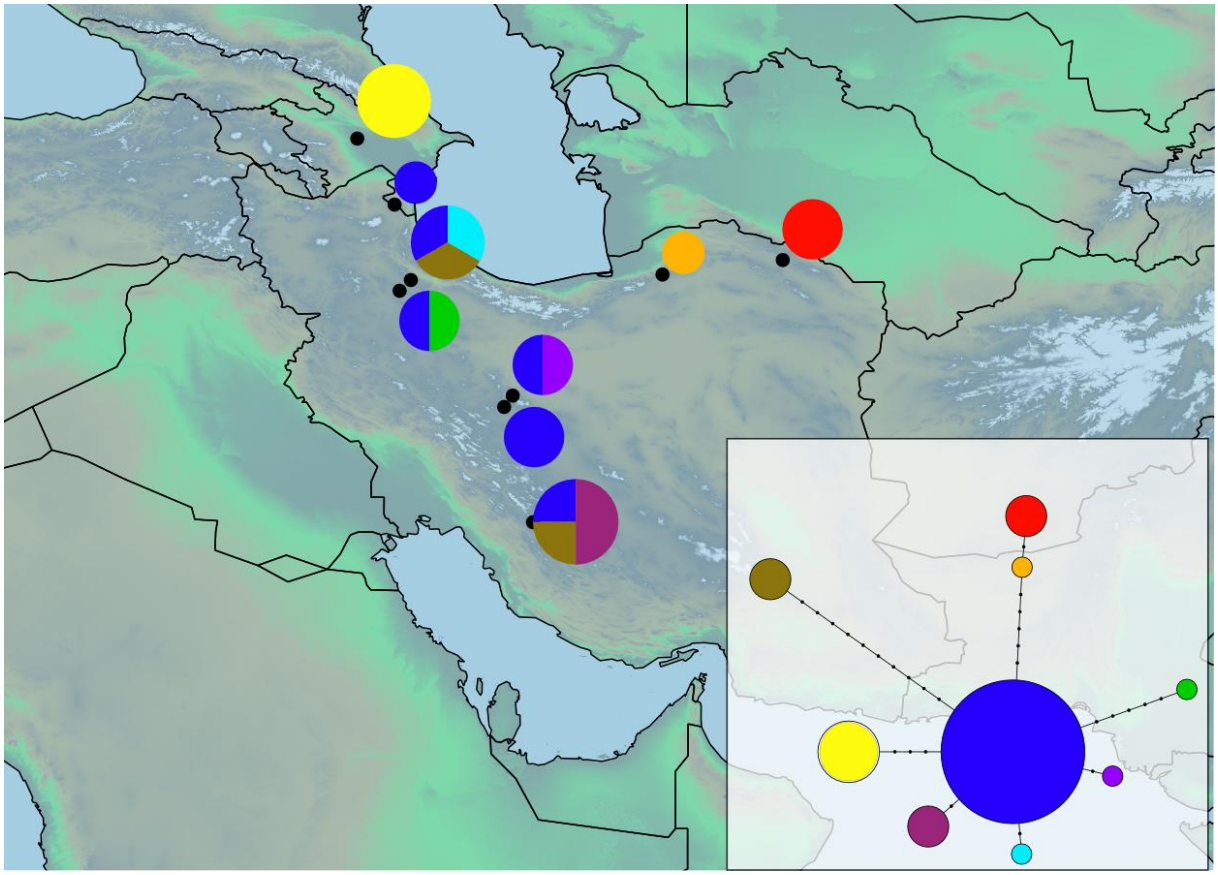


Fig. 4. Distribution of *Melitaea zagrosi* in different time scale. 16 non replicated present points were used to predict the distribution of the species for present climate conditions. Presence points plotted on terrain map. The model was also projected to the Mid-Holocene (6000 yBP) and to the Last Glacial Maximum (21000 yBP) using two global circulation models (CCSM, MIROC). Darker colours show higher climatic suitability for species.

548 **Supplementary**



549

550 **Figure S1**. Geographical distribution of mitochondrial haplotypes (pie charts) of Melitaea
551 abbas Gross & Ebert, 1975, comb. n. (= M. zagrosi Tóth & Varga syn. nova) and haplotype
552 network.
553

554 **Appendix**

555

556 *Melitaea* specimens used in morphometrical analysis. Abbreviations as follows:
557 HNHM=Hungarian Natural History Museum, Budapest, ZSM=Zoologische Staatssammlung,
558 München, SMNK= Staatliches Museum für Naturkunde, Karlsruhe, ZMJU= Zoological
559 Museum of the Jagellonian University, Krakow.

ID	taxa	Country	Location	Museum
B072	ornata	Kazakhstan	Sopka Sauskan-Hg.	HNHM
B073	ornata	Turkey	Angora (Ankara)	HNHM
B076	ornata	Turkey	Angora (Ankara)	HNHM
B078	ornata	Turkey	Ak-Chehir (Aksahir)	HNHM
B080	ornata	Turkey	Angora (Ankara)	HNHM
B082	ornata	Turkey	Angora (Ankara)	HNHM
B086	ornata	Turkey	Amaysia	HNHM
B087	ornata	Turkey	Marasch(Kahranmanmaras), Taurus-Mts.	HNHM
B088	ornata	Turkey	Marasch(Kahranmanmaras), Taurus-Mts.	HNHM
B089	ornata	Turkey	Marasch(Kahranmanmaras), Taurus-Mts.	HNHM
B090	ornata	Turkey	Marasch(Kahranmanmaras), Taurus-Mts.	HNHM
N01	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
N02	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
N18	ornata	Turkey	Egerdir,	ZSM
P100	ornata	Turkey	Egerdir,	ZSM
P103	ornata	Iran	Shahküh Mt., Elburz Mts.	ZSM
P111	ornata	Iran	Mashhad, Kuh-i-Mirabi	ZSM
P113	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P114	ornata	Turkey	Marash (Kharamanmaras), Taurus Mt.	ZSM
P115	ornata	Turkey	Bossanti, Taurus Mt.	ZSM
P130	ornata	Turkmenistan	Transcaspian Region,	ZSM
P132	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
P173	ornata	Turkey	Maras, Achyr Dagi, Taurus	ZSM

P184	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P23	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P32	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
P33	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
P78	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P79	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P80	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P81	ornata	Kazakhstan	Djarkent, Ili (region)	ZSM
P82	ornata	Russia	Kisilskaja, Ural Mts.	ZSM
P85	ornata	Turkey	Marash (Kahramanmaras), Ahir Dagi, Taurus Mts.	ZSM
P86	ornata	Turkey	Marash (Kahramanmaras), Ahir Dagi, Taurus Mts.	ZSM
P87	ornata	Turkey	Marash (Kahramanmaras), Taurus Mts.	ZSM
X34	ornata	Russia	Ural Mts.	HNHM
B100	phoebe	Russia	Sarepta	HNHM
B405	phoebe	Iran	Askaran, Kordestan province	HNHM
B406	phoebe	Iran	Askaran, Kordestan province	HNHM
B407	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B408	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B409	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B410	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B411	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B412	phoebe	Iran	35Km N from Anarak, Esfahan province, Dasht-e Zir Dom	HNHM
B52	phoebe	Russia	Sarepta,	HNHM
B64	phoebe	Russia	Koksu, Altai Mts.	HNHM
B68	phoebe	Russia	Kaukazus,	HNHM
B69	phoebe	Russia	Kaukazus,	HNHM
B70	phoebe	Russia	Kaukazus,	HNHM
B71	phoebe	Russia	Kaukazus,	HNHM
P136	phoebe	Russia	Kislovodsk, Kaukasus	ZSM
P137	phoebe	Russia	Kaukasus	ZSM

P148	phoebe	Russia	Kisilskaja, Ural Mts.	ZSM
P15	phoebe	Russia	Itkol-Hg., Elbrus Mt.	ZSM
P150	phoebe	Russia	Kisilskaja, Ural Mts.	ZSM
P152	phoebe	Russia	Itkol-Hg., Elbrus Mt.	ZSM
P17	phoebe	Russia	Sarepta,	ZSM
P19	phoebe	Russia	Ural Mts.	ZSM
P58	phoebe	Russia	Itkol-Hg., Elbrus Mt.	ZSM
P60	phoebe	Russia	Teberda, Kaukasus	ZSM
P62	phoebe	Russia	Itkol-Hg., Elbrus Mt.	ZSM
P64	phoebe	Russia	Teberda, Kaukasus	ZSM
P75	phoebe	Russia	Sayan Mts., Altai province	ZSM
P76	phoebe	Russia	Anos	ZSM
abbas1	phoebe abbas	Iran	Fars province, 50Km NW Ardekan Tange Surkh	SMNK
abbas2	phoebe abbas	Iran	Fars province, 50Km NW Ardekan Tange Surkh	SMNK
abbas3	phoebe abbas	Iran	Fars province, 50Km NW Ardekan Tange Surkh	SMNK
abbas4	phoebe abbas	Iran	Fars province, 50Km NW Ardekan Tange Surkh	SMNK
abbas5	phoebe abbas	Iran	Fars province, 50Km NW Ardekan Tange Surkh	SMNK
B386	zagrosi	Iran	Esfahan province Kuhha-ye-Qohrud	HNHM
B388	zagrosi	Iran	Askaran, Kordestan province	HNHM
B390	zagrosi	Iran	Askaran, Kordestan province	HNHM
B392	zagrosi	Iran	Askaran, Kordestan province	HNHM
B394	zagrosi	Iran	Zanjan, Zanjan province	HNHM
B396	zagrosi	Iran	Askaran, Kordestan province	HNHM
B398	zagrosi	Iran	Askaran, Kordestan province	HNHM
B399	zagrosi	Iran	Sepidan, Fars province	HNHM
B400	zagrosi	Iran	Sepidan, Fars province	HNHM
B401	zagrosi	Iran	Sepidan, Fars province	HNHM
B403	zagrosi	Iran	Zanjan, Zanjan province	HNHM
B404	zagrosi	Iran	Askaran, Kordestan province	HNHM
B66	zagrosi	Iran	Bognurd (Bojnūrd), Aladag Mt. (Reshteh-ye Ala dagh Mt.)	HNHM

MZAIR10	zagrosi	Iran	Koshrowabad, Esfahan province	HNHM
MZAIR6	zagrosi	Iran	Koshrowabad, Esfahan province	HNHM
MZAIR7	zagrosi	Iran	Koshrowabad, Esfahan province	HNHM
MZAIR8	zagrosi	Iran	Chatar	HNHM
MZAIR9	zagrosi	Iran	Chatar	HNHM
ZAIR16	zagrosi	Iran	N from Qucan, Kopeth-Dagh 2000m, Khorasan province	ZMJU
ZAIR18	zagrosi	Iran	Khoshyeylaq, Shah kuh 2000m, Golestan province	ZMJU
P106	zagrosi	Iran	Kazeroun - Buschir, Konar Takhteh	ZSM
P108	zagrosi	Iran	Kazeroun - Shiraz, Mian Kotal	ZSM
P109	zagrosi	Iran	Kazeroun - Shiraz, Mian Kotal	ZSM
P110	zagrosi	Iran	Kandovan, Elburz Mts.	ZSM
P112	zagrosi	Turkmenistan	Transcaspian Region,	ZSM
P48	zagrosi	Iran	Kazeroun - Buschir, Konar Takhteh	ZSM
P50	zagrosi	Iran	Kazeroun - Shiraz, Mian Kotal	ZSM
P51	zagrosi	Iran	Kazeroun - Buschir, Konar Takhteh	ZSM
ZAAZ2	zagrosi	Azerbaidjan	Akhdash Turyanchay	UD
ZAAZ3	zagrosi	Azerbaidjan	Akhdash Turyanchay	UD
ZAAZ5	zagrosi	Azerbaidjan	Akhdash Turyanchay	UD
ZAAZ6	zagrosi	Azerbaidjan	Akhdash Turyanchay	UD
ZAIR10	zagrosi	Iran	Alazg, Qohrud Mts., Esfahan province	ZMJU
ZAIR6	zagrosi	Iran	Sepidan, Fars province	ZMJU
ZAIR7	zagrosi	Iran	Sepidan, Fars province	ZMJU
ZAIR8	zagrosi	Iran	Sepidan, Fars province	ZMJU
ZAIR9	zagrosi	Iran	Alazg, Qohrud Mts., Esfahan province	ZMJU