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Taxonomy, distribution and ecology of *Melitaea ornata* (Lepidoptera: Nymphalidae)

A *Melitaea ornata* (Lepidoptera: Nymphalidae) taxonómiája, elterjedése és ökológiája

Egyetemi doktori (PhD) értekezés

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DEBRECENI EGYETEM
Természettudományi Doktori Tanács
Juhász-Nagy Pál Doktori Iskola
Debrecen, 2012

Ezen értekezést a Debreceni Egyetem Természettudományi Doktori Tanács Juhász-Nagy Pál Doktori Iskola *Biodiverzitás programja keretében készítettem a Debreceni Egyetem természettudományi doktori (PhD) fokozatának elnyerése céljából.*

Tóth János Pál

Debrecen, 2012. 07. 04.

Tanúsítom, hogy Tóth János Pál doktorjelölt 2006-2009. között a fent megnevezett Doktori Iskola *Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javasolom.*

Dr. Varga Zoltán

Debrecen, 2012. 07. 04.

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Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében
a biológia tudományágban

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(**Biodiverzitás** programja) keretében

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A doktori szigorlati bizottság:
elnök: ………………………… …………………………
tagok: ………………………… …………………………
………………………… …………………………
A doktori szigorlat időpontja: …………………………
Az értekezés bírálói:
………………………… …………………………
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A bírálóbizottság:
elnök: ………………………… …………………………
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A *Melitaea ornata* (Lepidoptera: Nymphalidae) taxonómiája, elterjedése és ökológiája

1. Bevezetés

Az elmúlt évszázad végére komoly kihívássá vált a biodiverzitás csökkenése. Közösségi irányelvek, mint például az Élőhelyvédelmi Irányelv (92/43/EEC), és nemzetközi egyezmények, mint az EU Fenntartható Fejlődés Stratégia (2001) vagy a Johannesburgi Konvenció a biológiai diverzitásról (2002) foglalkoztak a témával, és célul tűzték ki a folyamat megállítását. Az Európai Környezetvédelmi Ügynökségnek a 10. Biodiverzitási Konvenció Konferencia (2010. október 18-29., Nagoya) számára készített jelentése azonban megerősítette, hogy Európa nem tudta megvalósítani a kitűzött célt, a biológiai sokféleség csökkenésének megállítását.

A rovarok a legdiverzebb élőlénycsoport, amely az ismert növény- és állatfajok együttes fajszámának több mint 60%-át teszik ki. Bár a nappali lepkék alkotják a rovarokon belül az egyik leginkább vizsgált taxont, mégis az elmúlt évtizedben több rejtett fajt fedeztek fel a tudományos módszerek fejlődésének köszönhetően. Ilyen például a *Zerynthia cassandra* Olaszországban (Dapporto 2010), a *Polyommatus celina* Spanyolországban és Észak-Afrikában (Wiemers 2003; Wiemers et al. 2010), vagy a *Leptidea juvernica*, ami meglepő módon egész Európában előfordul, kivéve Spanyolországot, ahol a *L. reali* váltja fel (Dinca et al. 2011).

Bár a *Cinclidia* subgenus (*Melitaea*) első jelentős taxonómiai revízióját Higgins már 1941-ben leközölte, a csoport még mindig az egyik legproblematikusabb taxonnak számít. Nemrégiben újra a figyelem középpontjába került (Wahlberg & Zimmermann 2000; Russell et al. 2005, 2006, 2007; Varga et al. 2005; Varga 2007; Leneveu et al. 2009).

A legjobban ismert faj ebben a csoportban a *Melitaea phoebe* ([Denis & Schiffermüller], 1775) (TL: Bécs, Ausztria), melynek elterjedési területe szinte folyamatosan tart Észak-Afrikától Dél- és Közép-Európán keresztül egészen Észak-kelet Kínáig. Ennek a politipikus fajnak számos alfaját írták le, főként a szárny színének mintázata és színezete alapján. Nemrégiben a leírt alfajok közül néhányat önálló fajként kezdtek el kezelni.

A *Melitaea telona* Fruhstorfer, 1908 (TL: Jeruzsálem, Izrael) faji rangját nemrég ismerték fel (Russell et al. 2005; Russell et al. 2007). A faj ismert áreája diszjunkt: a Levantei régiótól Kis-Ázsián és a Balkán-félszigeten át Olaszországig, a Kárpát-medencéig és a Podóliai Platóig terjed (Russell et al. 2005, 2007; Varga et al. 2005; Varga 2007). A *M. telona* és a *M. phoebe* elkülönítése nagyrészt a lárvák morfológiáján alapul (Russell et al. 2005, 2007). A *Melitaea telona* lárvák feje téglavörös a negyedik lárvastádiumtól kezdve. Mono- vagy oligofágok, regionálisan különböző Asteraceae fajokon táplálkoznak. Ezzel szemben a *M. phoebe* oligofág, amely gyakran különböző tápnövényeket szimultán használ (*Centaurea, Cirsium, Carduus*), és a lárváinak mindig fekete a fejkapszulája.

A *Melitaea punica* Oberthür, 1876 (TL: Lambessa, Algéria) faji rangját már maga Oberthür (1914) is javasolta, de genitália vizsgálatok nélkül. Később a „*punica*” nevet mint *M. phoebe* alfajt használták (Higgins 1941) vagy külön fajként, a ”*telona*”-val kombinálva (Gorbunov and Kosterin 2007), a *M. phoebe telona* szinonimájaként. Russel és mts. (2005) rámutattak arra, hogy ezek a kombinációk helytelenek, és a *M. telona* félrehatározásán alapulnak, mivel a *M. punica* kizárólag É-Afrikában fordul elő. Ezeket a megállapításokat később a molekuláris eredmények is megerősítették: két nukleáris és egy mitokondriális gén vizsgálata különböző *Melitaea*-fajokban (Leneveu et al. 2009), illetve enzim elektroforézis vizsgálat a *M. telona kovacsi* és a *M. phoebe* esetében (Pecsenye et al. 2007).

Sok szerző a *Melitaea scotosia* Butler, 1878-t (TL: Japan) és a *M. sibina*-Alphéraky, 1881-t (TL: Kuldja, China) önálló fajoknak tekinti. Az újabb molekuláris kutatások azonban nem találtak szignifikáns különbséget a *M. phoebe*, a *M. scotosia* és a *M. sibina* között (Wahlberg & Zimmermann 2000). Ebből azt a következtetést vonták le, hogy az említett taxonok ugyanannak a fajnak a különböző populációi vagy a környezet által befolyásolt formái. (Leneveu et al. 2009).

A dolgozat (Tóth & Varga 2011) egyik eredményeként kimutattuk, hogy a *Melitaea telona* Fruhstorfer, 1908 és a *M. ornata* Christoph, 1893 konspecifikus (v. ö. Eredmények és értékelés), így a prioritás elvét követve a következő részekben a *M. ornata* nevet használom.

A tézis három fő témakörben tárgyalja az igen érdekes „*M. phoebe*-csoport" néhány vonatkozását:

(i) Palearktikus szintű genitália morfometriai vizsgálatokat végeztünk a „*Melitaea phoebe*-csoport" taxonómiájának tisztázása érdekében. Ugyanakkor elkülönítő diagnosztikus bélyegeket kerestünk a genitáliákon és a szárnyakon a megbízható határozáshoz.

(ii) Többet szerettünk volna tudni a *Melitaea ornata* elterjedéséről és történetéről. Ennek a kutatásnak a főbb célkitűzései az alábbiak voltak: (1) a potenciális elterjedés prediktálása klímamodellek segítségével, (2) a lehetséges refúgiumok azonosítása az utolsó glaciális maximum idején, (3) a különböző klímamodellek összehasonlítása a 2080-ra prediktált elterjedési területek alapján és a klíma kockázat becslése.

(iii) Mivel a *Melitaea ornata* előfordul Magyarországon, lehetőségünk volt rá, hogy terepi vizsgálatot tervezzünk a faj alapvető populációökológiai vonatkozásainak felderítésére, úgymint a (1) faj diszperziós képessége, (2) a különböző távolságra levő élőhely-foltok közötti kapcsolat erőssége, illetve (3) a lárvális tápnövény és a fő nektárforrás kapcsolata a populációmérettel.

2. Módszerek

2.1 Taxonómia

365 hím és 203 nőstény egyedet vizsgáltunk a Magyar Természettudományi Múzeum, a Müncheni Állami Zoológiai Gyűjtemény és a Debreceni Egyetem (Varga Z.) gyűjteményéből. A példányok a Palearktisz számos pontjáról származnak. A *M. ornata* minták gyakorlatilag a faj teljes ismert elterjedését lefedik, ráadásul néhány új helyről is sikerült egyedeket bevonni az analízisbe. A fajok azonosításához egy egyszerű határozókulcsot készítettünk a hátsó szárny fonákán található jellegek alapján.

A genitália preparátumok készítésénél a standard protokollt követtük. A potrohot eltávolítottuk, majd 15%-os KOH oldatban hevítettük. A genitáliát ezután megtisztítottuk, etanollal dehidratáltuk, Euparal-ba ágyaztuk, majd a tárgylemezen lefedett tartós preparátumot digitalizáltuk.

A hímeknél landmark alapú geometriai és tradicionális morfometriai vizsgálatokat végeztünk, hogy számszerűsíteni tudjuk a genitália alakjában fellépő varianciát. Mivel a nőstény genitáliákon nem találtunk jó landmarkokat, tradicionális morfometriát használtunk.

2.2 Elterjedés

Mivel a *M. ornata*-ról eddig nem készültek megbízható elterjedési térképek, elterjedési modellt használtunk a potenciális area becslésére. Az elemzésekhez 255 duplikáció nélküli jelenlét adatot használtuk fel. A jelenlegi klímára vonatkozó információkat, a paleoklimatikus szimulációkat és a jövőre vonatkozó klímaszcenáriókat a WorldClim adatbázis 1.4-es verziójából kölcsönöztük ((Hijmans et al. 2005); http:// www.worldclim.org). A *Melitaea ornata* elterjedését különböző idősíkokban analizáltuk a MaxEnt programkörnyezet segítségével (http://www.cs.princeton.edu/ _shapire/Maxent). A genitália morfometria eredményeit és a prediktált refúgiumokat felhasználva rekonstruáltuk a lehetséges rekolonizációs utakat, ugyanakkor a potenciális area változását is prediktáltuk 2080-ra.

2.3 Ökológia

2009. május 15. és 25. között 3, ill. 4 alkalommal végeztünk jelölés-visszafogás vizsgálatot tíz 50×40 m-es mintavételi területen. Egy időben 5 területen dolgoztunk egyszerre, 9:00 és 13:00 között 3 órán keresztül, megfelelő időjárási körülmények esetén. Az elfogott lepkéket vízálló XF filctollal jelöltük meg. Ez a jelölés lehetővé tette az egyedek elkülönítését, így visszafogáskor következtetni lehetett a mozgási mintázatukra.

A mintavételi területeket GPS segítségével bemértük, majd Google Earth szatellitképekre illesztettük fedvények formájában. A mintavételi területek közepét egy egyenessel kötöttük össze, ezek adták a mintavételi területek közötti távolságot. A mozgásmintázatokat a terepnapló adatai alapján rajzoltuk meg.

A jelzés-visszafogás vizsgálat után megbecsültük a *Cirsium pannonicum* (lárvális tápnövény) és a *Dianthus pontederae* (fő nektárforrás) denzitását. Öt db (2×2 m) random kvadrátot jelöltünk ki minden mintavételi helyen. Ezekben a kvadrátokban mindkét növényt leszámoltuk, majd a kapott értékekből kiszámítottuk a m^2-re eső tőszámot. A növények és a megfigyelt egyedszámok közötti kapcsolatot Pearson-féle korrelációs teszttel vizsgáltuk.

3. Eredmények és értékelésük

3.1 Taxonómia

A hátulsó szárny fonákán alapuló határozókulcs hasznosnak bizonyult (lásd: II. közlemény). A genitália morfometria megbízható eredményeket mutatott a „*phoebe*-csoport” fajainak elkülönítésében. A hímeknél a processus posterior alakja értékes jellegnek bizonyult a fajok határozásában. A nőstényeknél a posterior lamella bizonyult jó karakternek, bár ez alapján nem lehetett elkülöníteni a *M. punica*-t a *M. phoebe*-től és a *M. ornata*-tól. Összevetve a hím és a nőstény genitálián alapuló eredményeket elmondható, hogy a nőstény genitália kevésbé megbízható.

Egyes szerzők a *M. telona*-t a *M. punica* alfajának tekintették (Hesselbarth et al. 1995; Abadjiev 2001), míg mások a *M. ornata*-t hozták a *M. punica*-val összefüggésbe (Korshunov & Gorbunov 1995). Az eredményeink viszont azt mutatják, hogy a *M. ornata* a *M. punica*-tól eltérő faj. Ezzel szemben, a *Melitaea ornata* (eredetileg *Melitaea phoebe* var. *ornata* Christoph, 1893; Iris VI: 87) konspecifikus a *M. telona* Fruhstorfer, 1908-val, így a prioritás elvét követve az előbbiekben *M. telona*-ként említett faj érvényes neve *Melitaea ornata* Christoph, 1893 (stat. revid.).

A Zagrosz hegységből származó *Melitaea* egyedek karakterisztikus szárnymintázatuk alapján új fajnak bizonyultak a hím és a nőstény genitália alapján egyaránt. *Melitaea zagrosi* sp. n. néven írtuk le (lásd: II. közlemény).

3.2 Elterjedés

A *Melitaea ornata* jelenre prediktált elterjedése jól illeszkedik az eddigi ismereteinkhez, kivéve az Ibériai-félszigetet és Észak-Afrikát, ahol a faj nem fordul elő. A predikció alapján elmondható, hogy a nem-mediterrán közép-európai területek kevéssé alkalmasak a faj számára.

Az Utolsó Glaciális Maximum (LGM) idejére (~21 000 éve) prediktált elterjedési területek nagyrészt egybeesnek a széles körben elfogadott kelet-mediterrán refúgiumokkal (pl. Reinig 1950; de Lattin 1967; Bennett et al. 1991; Hewitt 2000). Európa rekolonizációja valószínűleg két fő területről történt meg: az Appennini-félszigetről és a Balkánról, utóbbi valószínűleg kapcsolatban állt az Anatóliai refúgiumokkal. A levantei régió és az Elburz hegység populációi nem mutatnak jelentős expanziót. További vizsgálatok szükségesek a prediktált közép-ázsiai refúgiumok tisztázására.

A 2080-ra vonatkozó predikciók a faj észak felé terjedését mutatják, és ezzel egy időben extinkciót valószínűsítenek a Mediterránemuban. Dél-Oroszországban, a Kárpát-medencében és esetleg még a Provence régióban, Franciaországban azonosítottunk olyan gócterületeket, amelyek a faj túlélése és terjedése szempontjából fontos szerepet játszhatnak a jövőben. A prediktált északi irányú area-eltolódás azonban csak akkor lehetséges, ha sikerül megőriznünk a potenciális „leading edge” populációkat és élőhelyeiket.

3.3 Ökológia

A *Melitaea ornata* mérsékelten helyhez kötött. A faj képes elérni az egymástól több száz méterre levő élőhely-foltokat. A *M. phoebe* minden mintavételi területen előfordult, sőt néhány mintavételi helyen nagyobb egyedszámban fordult elő, mint a *M. ornata*. Ezek olyan területek voltak, amelyek növényzete a „jó” *ornata*-élőhelyekhez képest degradált volt. A *M. phoebe* képes ugyan kifejlődni a *Cirsium pannonicum*-on is, de figyelemre méltó, hogy azokon a helyeken, ahol az említett növény denzitása magas volt, a *M. phoebe* kis egyedszámban volt jelen.

Az adataink azt mutatják, hogy a lárvális tápnövény fontosabb az élőhely választásban, mint a nektárforrás.

4. Köszönetnyilvánítás

Szeretnék köszönetet mondani mindazoknak, akik segítették ennek a munkának az elkészítését. Elsősorban témavezetőmnek, Varga Zoltánnak a munka koordinálásáért és hasznos tanácsaiért, amivel segítette a dolgozat elkészítését. Köszönöm szerzőtársaim, Bereczki Judit, Nigel Spring, Végvári Zsolt és Varga Katalin együttműködését. Köszönet illeti Bálint Zsoltot (Magyar Természettudományi Múzeum) és Axel Hausmann-t (Zoological State Collection in Munich), amiért lehetővé tették a vizsgálatokhoz szükséges múzeumi példányok kölcsönzését. Nagyon köszönöm Boldogh Sándornak, Huber Attilának és Farkas Rolandnak, az Aggteleki Nemzeti Park munkatársainak a terepi munka szervezésében nyújtott segítségét. Köszönöm Nigel Spring-nek és Kathy Henderson-nak (European Conservation Action Network), hogy támogatták a nemzetközi konferenciákon való részvételemet. Nagyra értékelem Leonardo Dapporto, Thomas Schmitt és az anonim bírálók hasznos javaslatait és javításait az értekezésem alapjául szolgáló kéziratokon. Köszönöm Tanszékünk vezetőjének, Barta Zoltánnak, hogy támogatásával lehetővé tette a dolgozat befejezését. A vizsgálatok anyagi feltételeinek biztosítása a K84071 OTKA-pályázat alapján volt lehetséges.

Taxonomy, distribution and ecology of *Melitaea ornata* (Lepidoptera: Nymphalidae)

1. Introduction

The decline of biodiversity has become a serious challenge for nature conservation at the end of the last century. Community directives as the Habitats' Directive (92/43/EEC) and international agreements such as the EU Sustainable Development Strategy (2001) or the Johannesburg Convention on Biological Diversity (2002) has been addressed to halt this process. However, the report of the European Environmental Agency for the 10th meeting of the Conference of the Parties to the Convention on Biological Diversity (18-29 October 2010, Nagoya, Japan) has confirmed that Europe has not achieved its target of halting biodiversity loss by 2010.

The insects form the most diverse group of animals which contribute to more than 60% of the number of known plant and animal taxa. Despite the fact that butterflies constitute one of the most intensively surveyed groups, several cryptic species has been discovered in Europe in the past decades, owing to the improvement of scientific methods. For example, *Zerynthia cassandra* in Italy (Dapporto 2010), *Polyommatus celina* in Spain and North Africa (Wiemers 2003; Wiemers et al. 2010), *Leptidea juvernica* which occur surprisingly in most part of Europe and is replaced by *L. reali* in Spain (Dinca et al. 2011).

However, since the first significant taxonomic revision of the subgenus *Cinclidia* (*Melitaea*) published by Higgins in 1941, it has remained one of the most problematic groups in terms of taxonomic relatedness. Recently, it has become the focus of interest again (Wahlberg & Zimmermann 2000; Russell et al. 2005, 2006, 2007; Varga et al. 2005; Varga 2007; Leneveu et al. 2009).

The best known species in the group is *Melitaea phoebe* ([Denis & Schiffermüller], 1775) (TL: Wien, Austria) which occupies an almost continuous area from North Africa across southern and Central Europe to

North-Eastern China. This polytypic species shows considerable variation with several described subspecies. Diagnostic characters used for identification are mostly the colouration and pattern of the upper side of wings. Moreover, some of the subspecies have recently been claimed to be distinct species.

The species rank of *Melitaea telona* Fruhstorfer, 1908 (TL: Jerusalem, Israel) has only been recognised recently (Russell et al. 2005; Russell et al. 2007). Its known area is disjunct ranging from the Levant across Asia Minor, the Balkans to southern Italy and Sicily as well as the Carpathian Basin and the Podolian plateau on the north (Russell et al. 2005; Russell et al. 2007; Varga et al. 2005; Varga 2007). The distinction between *M. telona* and *M. phoebe* is predominantly based on larval characters (Russell et al. 2005; Russell et al. 2007). Larvae of all *M. telona* subspecies have brick red head from the 4th larval instar onwards, which feed mono- or oligophagously on regionally different Asteraceae. In contrast, *M. phoebe* is oligophagous often using different food plants simultaneously (*Centaurea*, *Cirsium*, *Carduus*). Its larvae always have black head capsulae. Some external morphological differences also recognised (Varga 1967).

The species rank of *Melitaea punica* Oberthür, 1876 (TL: Lambessa, Algeria) has already been suggested by Oberthür (1914), although without surveying genitalia characteristics. Later the name '*punica'* was either used as a distinct subspecies of *M. phoebe* (Higgins 1941) or in combination with '*telona'* (Gorbunov & Kosterin 2007) as a synonym of *M. phoebe telona.* Russell et al. (2005) have pointed out that this combination was based on the misidentification of *Melitaea telona*. Namely, *M. punica* occurs exclusively in North Africa. These results have also been confirmed by genetic surveys analysing one mitochondrial and two nuclear genes in several *Melitaea* species (Leneveu et al. 2009) as well as by enzyme electrophoresis in *M. telona kovacsi* and *M. phoebe* (Pecsenye et al. 2007).

Many authors consider *Melitaea scotosia* Butler, 1878 (TL: Tokyo, Japan) and *Melitaea sibina* Alphéraky, 1881 (TL: Kuldja, China) to be distinct species. However, recent molecular surveys could not find any significant difference between *M. phoebe*, *M. sibina* and *M. scotosia* (Wahlberg & Zimmermann 2000). Therefore, it has been suggested that they may represent populations or environmental forms of one species rather than separate species (Leneveu et al. 2009).

Recently, we have pointed out that *Melitaea telona* is conspecific with *M. ornata* Christoph, 1893. Therefore, according to the priority rule, we will use the name *M. ornata* for this species in the following parts of this thesis (see: Results and Discussion).

To answer some of the questions concerning this fascinating '*phoebe* group' three main topics are discussed in my thesis:

(i) A Palearctic-level morphological and morphometric survey has been applied to genital structures to clarify the taxonomy of the '*Melitaea phoebe* group'. We were also searching for reliable characters for genitalia and wing pattern showing differential diagnostic traits among species.

(ii) We aim to obtain more information on the distribution of *Melitaea ornata* and on the history of this species. The main objectives of this investigation were to (1) predict the potential distribution using climatic models, (2) identify possible refugia during the Last Glacial Maximum and combine them with the results of morphometrical studies and (3) compare the consistency of models predicting future distribution areas for 2080 and derive conclusions for climatic risk assessment.

(iii) Since *Melitaea ornata* (as *M. ornata kovacsi* Varga, 1967) occurs in Hungary it was possible to plan a field survey to study some basic population ecological concerns: (1) the dispersion ability of the species and (2) the strength of the connection between the habitat patches at different distances apart (3) the relationship between the supply of the larval food plant, the main nectar sources and the population size.

2. Methods

2.1 Taxonomy

365 male and 203 female individuals were analysed from the material of the Hungarian Natural History Museum, the Zoological State Collection, Munich and the collection of the University of Debrecen (Z. Varga). The specimens involved in this study originated from many locations of the Palaearctic region. *M. ornata* samples cover almost the entire known distribution area of the species. In addition, we also have individuals from new localities. To identify the species, a simple key was constructed on the basis of the characters of the underside of wings.

We followed the standard way of genital preparation. The abdomen was removed and boiled in 15% KOH solution. Genitalia were cleaned and dehydrated in ethanol and mounted in Euparal between microscope slides and cover slips. Genital slides were digitalized.

Landmark-based geometric morphometric analysis and traditional morphometrics were used to quantify the variation in the shape of the male genitalia. We could not find good landmarks on female genitalia thus traditional morphometrics was applied in the case of them.

2.2 Distribution

Since there is no reliable distribution map available for *M. ornata* we used the species distribution modelling approach to predict the potential area. To do so, we used 255 non-duplicate observations of *Melitaea ornata*, with maximum one record in each 2.5 arc minutes cell. Information on current climate, paleoclimatic simulation data of the LGM and future climate scenarios were obtained from the WorldClim database, version 1.4 ((Hijmans et al. 2005); http:// www.worldclim.org). The distribution of *M. ornata* was analysed on different time scales by MaxEnt software (http://www.cs.princeton.edu/

_shapire/Maxent). Using the results of genitalia morphometry and the predicted potential refugia during the Last Glacial Maximum (LGM), the probable re-colonisation routes have been reconstructed. We also predicted changes in the potential area for 2080.

2.3 Ecology

Our data were collected through capture-recapture surveys on three or four occasions in 2009 15-25 May from ten sample quadrats (sample sites) with dimensions of 50×40 m. We worked on five sites simultaneously between 9:00 and 13:00 for three hours, only in optimal weather conditions. Captured butterflies were marked with a water resistant XF marker pen. This code enabled us to recognise butterflies during recapture and to derive their movement patterns.

The coordinates of the sample sites were recorded by GPS equipment, which were consequently overlaid on Google Earth satellite image. The centres of the sample quadrats were connected with lines and we measured these. The value was used as the distance between the quadrats. Movement patterns were drawn from the field sheets data.

After the capture-recapture survey, we estimated the density of *Cirsium pannonicum* (the larval food plant) and *Dianthus pontederae* (the most important nectar source). Five quadrates (2×2 m) were marked randomly at each sample site, and the two plant species were counted in these quadrats. We calculated the density of plants per m^2. The relation between the observed numbers of butterflies and the density of the nectar source and the larval food plant were analysed using Pearson’s correlation.

3. Results and Discussion

3.1 Taxonomy

The identification key based on the underside of the hind wing proved to be useful (see: Study II). Genital morphometry yielded reliable results in the separation of the species of the ‘*phoebe* group’. In males, the shape of the processus posterior proved to be a valuable character in species identification. In females, the shape of the posterior lamella was a plausible character although it could not separate *M. punica* from *M. telona* and *M. phoebe*. Comparing the results of males and females, we concluded that female genitalia provided less power to characterise the differentiation among the given taxa.

Some authors suggested that *M. telona* is a subspecies of *M. punica* (Hesselbarth et al. 1995; Abadjiev 2001). Others (Korshunov & Gorbunov, 1995) used *M. ornata* as subspecies of *M. punica*. We demonstrated that *M. ornata* is not conspecific with *M. punica*. On the contrary, *M. ornata* (originally *Melitaea phoebe* var. *ornata* Christoph, 1893; Iris VI:87) proved to be conspecific with *M. telona* Fruhstorfer, 1908, hence *Melitaea ornata* Christoph (stat. revid.) is the valid name according to the priority rule for the species mentioned earlier as *M. telona.* A *Melitaea* sample from the Zagros Mts. (Iran) with characteristic wing pattern elements proved to be a distinct species on the basis of both male and female genitalia. We described it as *Melitaea zagrosi* Tóth & Varga, 2011 (sp. n.).

3.3 Distribution

The predicted present distribution of *Melitaea ornata* fits well the known occurrence data except for the Iberian Peninsula and North-Africa where the species is missing. Based on our predictions, extra-Mediterranean European areas seem to be less suitable for the species.

Predicted distribution during the LGM (~21 000 years ago) mainly fits to widely accepted eastern Mediterranean refugia (e.g. Reinig 1950; de Lattin 1967; Bennett et al. 1991; Hewitt 2000). Europe was probably re-colonised from two main sources, from the Apennine peninsula and from the Balkans which has been probably connected to the Anatolian refugia. Populations of the Levant region and also of the Elburs Mts. do not show any significant expansion. Further studies are necessary in the case of the predicted Central Asian refugia.

Predictions for 2080 show a possible northward shift and some extinction events in the Mediterranean region. Core areas are identified which might have a potential for expansion including southern Russia, Pannonian region and possibly Provence in France. Predicted northward area shifts are only possible if the potential leading edge populations and habitats of the species can be preserved.

3.2 Ecology

M. ornata is moderately localized: the species is able to reach habitat patches in distances of several hundred metres. *M. phoebe* occurred in all sample sites. Moreover, in some quadrats, greater numbers of *M. phoebe* were recorded than in *M. ornata*. These were the sites with degraded vegetation compared with the strongly populated habitats of *M. ornata*.

M. phoebe has the ability to develop on *Cirsium pannonicum* as well, but interestingly *M. phoebe* tends to be found in lower numbers where this plant is abundant. Our data suggest that the density of the larval food plant in the habitat selection of *M. ornata* is more important than the abundance of nectar sources.

4. Acknowledgements

I am extremely grateful to all the people who helped me to finish this work. First of all, I would like to thank my supervisor Zoltán Varga and co-authors Judit Bereczki, Nigel Spring, Zsolt Végvári and Katalin Varga. Many thanks to Zsolt Bálint (Hungarian Natural History Museum) and Axel Hausmann (Zoological State Collection in Munich) for borrowing valuable museum specimens for our studies. Many thanks go to Sándor Boldogh, Attila Huber and Roland Farkas, the members of Aggtelek National Park who helped us to organize the field works in Aggtelek National Park. I am also grateful to Nigel Spring and Kathy Henderson (European Conservation Action Network) who supported the participation at international conferences. Useful suggestions and corrections of Leonardo Dapporto, Thomas Schmitt and anonymous referees on the articles are highly appreciated. Thanks a lot the head of our department, Zoltán Barta who supported the research in the last year. Our surveys were supported by the OTKA (K84071).

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Study I: Morphometric study on the genitalia of sibling species *Melitaea phoebe* ([Denis & Schiffermüller], 1775) and *M. telona*[1] Fruhstorfer, 1908 (Lepidoptera: Nymphalidae)

Tóth JP & Varga Z (2010) Morphometric Study on the Genitalia of Sibling Species *Melitaea phoebe* and *M. telona* (Lepidoptera: Nymphalidae). *Acta Zoologica Academiae Scientiarum Hungaricae* 56 (3): 273-282.

ABSTRACT

Taxonomy and phylogeny of the diverse nymphalid genus *Melitaea* was often considered during the last decade. *Melitaea phoebe* (Knapweed Fritillary) and *M. telona* has been considered as conspecific until the last few years. Morphometric characters of genital structures were analysed by traditional morphometric method. Significant but slightly overlapping differences were found in both sexes. In male genitalia we observed that *M. telona* can be characterised by a more notched saccus, and more symmetric processi posteriore than *M. phoebe.* In females, *Melitaea phoebe* has more circle shaped, while *M. telona* has more elliptic shaped posterior lamella. The furca is usually smaller in *M. phoebe*. *M. phoebe ornata* specimens from the South Ural, Russia, were clustered together with *M. telona* in the analyses.

Keywords: morphometrics, *Melitaea phoebe, Melitaea telona,* taxonomic separation, male and female genitalia

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[1] *Melitaea telona* Fruhstorfer, 1908 is a junior synonym of *M. ornata* Christoph, 1893 (see: Study II).

INTRODUCTION

Molecular taxonomy and phylogeny of the nymphalid genus *Melitaea* was thoroughly studied during the last decade (WAHLBERG & ZIMMERMANN 2000, WAHLBERG *et al.* 2003, WAHLBERG *et al.* 2005, LENEVEU *et al.* 2009). The taxonomy of the "*phoebe* group" (= subg. Cinclidia Hübner, [1819]) was also discussed in several recent papers (RUSSELL *et al.* 2005, VARGA *et al.* 2005, RUSSELL *et al.* 2007, VARGA 2007).

Melitaeae phoebe ([DENIS & SCHIFFERMÜLLER], 1775) is a polytypic species with several described subspecies and infra-subspecific forms (HIGGINS 1941). Its range extends from North Africa over Eurasia to the Far East. *M. phoebe* and its subspecies are generally polyphagous, their host-plants are different species of Asteraceae (*Cirsium*, *Centaurea*, *Serratula, Saussurea, Stemmacantha*) and Plantaginaceae (KORSHUNOV&GORBUNOV 1995, TUZOV *et al.* 2000, RUSSELL *et al.* 2007, VARGA 2007, TOLMAN & LEWINGTON 2008). They are mostly bivoltine (except some high mountain populations), and its L4 larvae have black head capsule. The western Mediterranean populations also share these characters.

	Melitaea phoebe	*Melitaea telona*
Distribution	Euro-Siberian Nearly continuous	Ponto-Mediterranean Disjunct (insular)
Habitats	Euryoecious; Wide range in altitude (lowland to sub-alpine)	Stenoecious; nature-like xeric or mesic grasslands; narrow range of altitude (lowland to montane)
Larval food plants	Polyphagous on Asteraceae (*Cirsium*, *Centaurea*, *Serratula*, *Saussurea*, *Stemmacantha*) and *Plantago*	Oligophagous on local (endemic) Asteraceae
Voltinism	Generally bivoltine, in high altitudes monovoltine	Strictly monovoltine (even in Mediterranean habitats)
Morphology of larvae	Variable but usually with black head capsule of hibernated larvae	Generally black with brick-red head capsule of hibernated larvae

Table 1. Ecological traits of *Melitaea telona* and *phoebe*.

Several taxa from the Eastern Mediterranean region were also described as subspecies of *M. phoebe*: *Melitaea phoebe telona* FRUHSTORFER, 1908 from Jerusalem (Israel), *Melitaea phoebe ogygia* FRUHSTORFER, 1908 from Poros (Greece), *Melitaea phoebe totila* STAUDER, 1914 from southern Italy, *Melitaea phoebe amanica* REBEL, 1917 from Amanus Mt. (Turkey, Asia Minor), *Melitaea phoebe emipunica* VERITY, 1919 from Palermo (Sicily), *Melitaea phoebe nigrogyia* VERITY, 1938 from Opatija (Croatia). All of these taxa have, however, some important common, distinctive characters: they are usually univoltine, the L4 larvae have a red head capsule, and they are feeding on some, regionally different specific, often endemic *Cirsium* or *Centaurea* host-plants (RUSSELL *et al.* 2007). Also the Hungarian subspecies *Melitaea phoebe kovacsi* VARGA, 1967 described from Central Hungary (Budakeszi) shows the same characters. RUSSEL *et al.* (2005, 2007) and VARGA *et al.* (2005) suggested, based on these similarity, the separation of these taxa as *Melitaea telona* FRUHSTORFER, 1908 (syn: *M. ogygia* FRUHSTORFER, 1908). *M. phoebe* *ornata* Christoph, 1893 described from Guberlya, South Ural was mentioned as a subspecies (KORSHUNOV&GORBUNOV 1995) but recently it was used as a synonym of *M. phoebe phoebe* (TUZOV *et al.* 2000). However, this taxon shows the typical wing pattern on the undersides of the wings and the shape of antennae like *M. telona* figured already by Varga (1967).

LENEVEU *et al*. (2009) analysed a mitochondrial and two nuclear genes from many taxa belonging to the *Melitaea* genus. Their results suggest that *Melitaea phoebe*, *M. telona* and *M. punica* are three well-differentiated species. Enzyme electrophoretic study of Hungarian populations has also shown obvious differences between *M. phoebe* and *M. telona* without any mark of hybridisation (PECSENYE *et al.* 2007).

Genitalia are often species-specific, and their forms are often more divergent among closely related species than are the forms of other traits. This relatively rapid divergence of genitalia is extremely widespread taxonomically, and has made them especially useful in distinguishing closely related species (EBERHARD 1985, SHAPIRO & PORTER 1989). A combination of morphometric studies and multivariate analysis has been used in the last few years and is growing in importance as an approach (WAKEHAM-DAWSON *et al.* 2004, SIMONSEN 2005, MUTANEN 2006b, SIMONSEN 2006, SIMONSEN *et al.* 2006, HERNÁNDEZ-ROLDÁN&MUNGUIRA 2008, PRIETO *et al.* 2008).

M. phoebe and *M. telona* have been analysed until yet by molecular analysis only. In this paper we would like to focus to the morphological differences on genitalia using traditional morphometrics.

MATERIAL AND METHODS

In our study we surveyed 115 specimens to identify the potential morphological differences allowing the separation of these two species. The genital characters are much more conservative than external characters, and they are insignificantly influenced by environmental factors (CESARONI *et al.* 1994, DAPPORTO *et al.* 2009).

M. phoebe (23♂, 26♀) and *M. telona* (32♂, 34♀) specimens originated mostly from Hungary (72) but the sample also includes some museum specimens from Greece (5), Macedonia (12), Albania (2), Romania (1), Bulgaria (6), Turkey (3), Serbia (1) and Russia (6). The specimens belong to the Hungarian Natural History Museum, Zoological State Collection in Munich and to the collection of the University of Debrecen.

We made genitalia slides and digitalised those by the combination of an Olympus camera and a Canon stereo microscope. Set of distance measurements from the structures of male and female genitalia were taken using the ImageJ 1.34 Java image processing program.

In permanent genital slides some parts of the genitalia can be deformed in different way, so they are unsuitable for the measures even if they possibly bear important traits. However, the preparation is necessary because the sclerotised parts must be fixed in a standard way in order to measure at least some characters. Mereover, this process can be repeated in any time. For females structures we could find only a few characters what we could measure.

We grouped the specimens according to the characters of the wing pattern and colouration, morphology of antennae, forelegs (VARGA 1967), the occurrence and collection time.

Bilaterally symmetrical features were measured on the left side of the genitalia. Seven characters were measured on males genitalia (Fig. 1a) and three on females (Fig. 1b) Two ratio was calculated from these measurements.

We used ratio G to describe the curvature of the harpe, it was calculated as follows: $G=HI/((HH\times\pi)/2$ and ratio $P=PLH/PLW$ to demonstrate how close to oval the posterior lamella is in females.

Measurement error was calculated by the following formula: $ME=S^2_{within}/(S^2_{within}+S^2_{among})\times100$ (LESSELLS & BOAG 1987) using nested ANOVA (BAILEY & BYRNES 1990, YEZERINAC *et al.* 1992). The repeatability of measurements was then calculated as 100%-ME.

Most of the characters were highly repeatable ME<1% in both sex, but some traits have little bit more measurement error: PW (ME=7.13%), PI (ME=4.02%) and HE (ME=3.25%).

Univariate and multivariate analysis were used in statistics. Statistical analysis was carried out using the statistical software package SPSS 16.0. The measurements were tested independently by ANOVA (Analysis of Variance). Multiple Discriminant Analysis (MDA) was used to unravel the relationship between the previously sorted groups (expected species) and find the most discriminative traits. All variable entered simultaneously. Wilks's lambda (λ) measures the discriminatory power of the model. Its value ranges from 0 (perfect discriminatory power) to 1 (no discriminatory power). Some specimens (3♂, 3♀) were grouped to *M. telona* from a new locality: Magnitogorsk and Guberlya in S Urals Russia. MDA was also used to classify these specimens. These were set as ungrouped cases. PCA (Principal Component Analysis) was also used to demonstrate the relationship between the specimens.

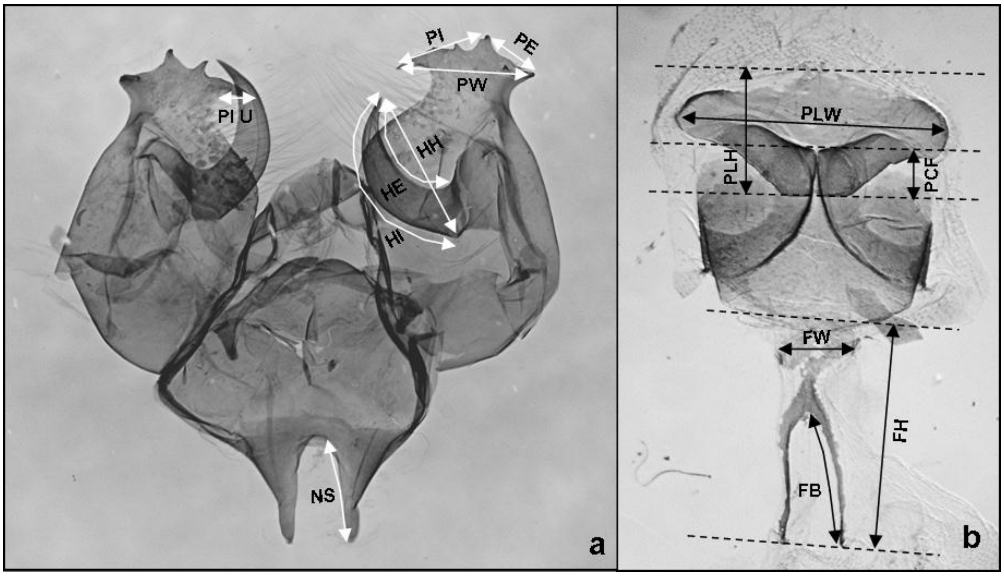

Fig. 1. Male (a) and female (b) measured characters.
Abbreviation:NS= length of the central notch of saccus, PE= external process of the processus posterior PI= inner process of the processus posterior PW= width of the processus posterior, HE= external arc of the harpe, HI= internal arc of the harpe HE= external arc of the harpe, HH= height of the harpe, PIU= underside length of the inner process of the processus posterior PLW=width of the posterior lamella, PLH=height of the posterior lamella, PCF=cover flap of the posterior lamella, FB=left branch of the furca, FH=height of the furca, FW=width of the base of furca.

RESULTS

Shapiro-Wilks test supported that all the measured variables showed normal distributions. MDA demonstrated significant differences between the two species in males ($p<0.001$, Wilks' $\lambda=0.19$) and in females ($p<0.001$, Wilks' $\lambda=0.29$).

Males

The MDA correctly classified 96.2% of male specimens (we got the same results with the cross validation procedure), only two specimens were misclassified. *M. "phoebe" ornata* specimens were set as ungrouped cases and they all were classified as *M. telona*. The NS show the biggest correlation with the discriminative function. The univariate ANOVA tests showed significant differences except some trait: PI, PW and HH. Trait HE shows just marginal significance (Table 2).

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
NS	0.449	61.284	1	50	0.000
PE	0.884	6.550	1	50	0.014
PI	0.984	0.818	1	50	0.370
PW	0.960	2.086	1	50	0.155
PIU	0.816	11.298	1	50	0.001
HI	0.840	9.527	1	50	0.003
HE	0.929	3.836	1	50	0.056
HH	0.992	0.423	1	50	0.518
G	0.662	25.530	1	50	0.000

Table 2. Results of the univariate ANOVA of the male characters.

PCA (Principal Component Analysis) plot revealed an overlap between the two species, but we also the occurrence of some well segregated objects. Two surveyed specimens from Guberlya, Russia felt into the *telona* “cloud” well separated from the *M. phoebe* and one in the intermediate zone (Fig. 2). The first two axes accounted for 58.36 % of the variance. In Axis 1, traits of the harpe (HI, HE, HH) have the largest importance. In Axis 2 what is more important in species separation NS G showed the highest loadings.

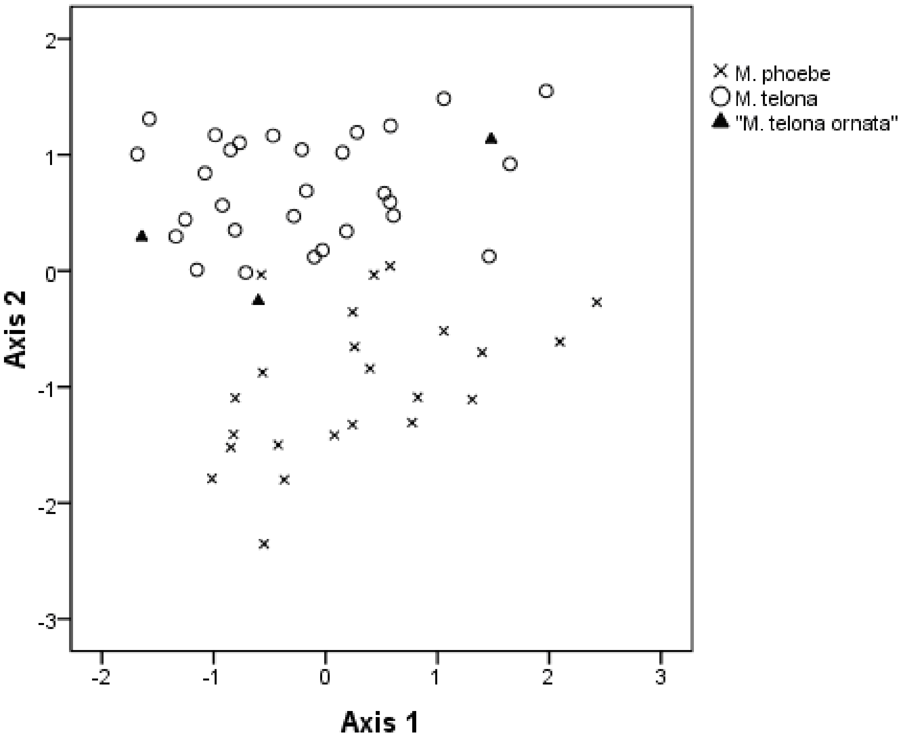

Fig. 2. PCA plot of males. We can see the separation of the two species. The triangles are mark the specimens from Russia (*M. telona ornata*).

Females

In females, we get very similar results. MDA correctly classifies 93.0% (86.2 % with the cross validation procedure) of original grouped cases. All specimens from Southern Urals were classified as *M. telona*. The P, PCL, PLH and FL show the largest correlation with the discriminant function. Except PLW all character showed statistically significant differences ($p<0.05$) between the two species when tested separately (Fig. 4).

Tests of Equality of Group Means

	Wilks' Lambda	F	df1	df2	Sig.
PLW	.980	1.121	1	55	.294
FW	.915	5.092	1	55	.028
FL	.761	17.299	1	55	.000
FBL	.619	33.885	1	55	.000
PCL	.624	33.209	1	55	.000
PLH	.664	27.772	1	55	.000
P	.541	46.684	1	55	.000

Table 3. Results of the univariate ANOVA of the female characters.

Using PCA method, we obtained quite similar results to males (Fig. 5) also revealing an overlapping between the two species. The animals from Magnitogorsk and Guberlya (Russia) are in the *telona* “cloud” (Fig. 5). The first two axis accumulated 73.7% of the variance. In species discrimination axis 1 had the largest importance. Traits P, PCL, PLH, FL, and FBL revealed the largest loadings in this axis.

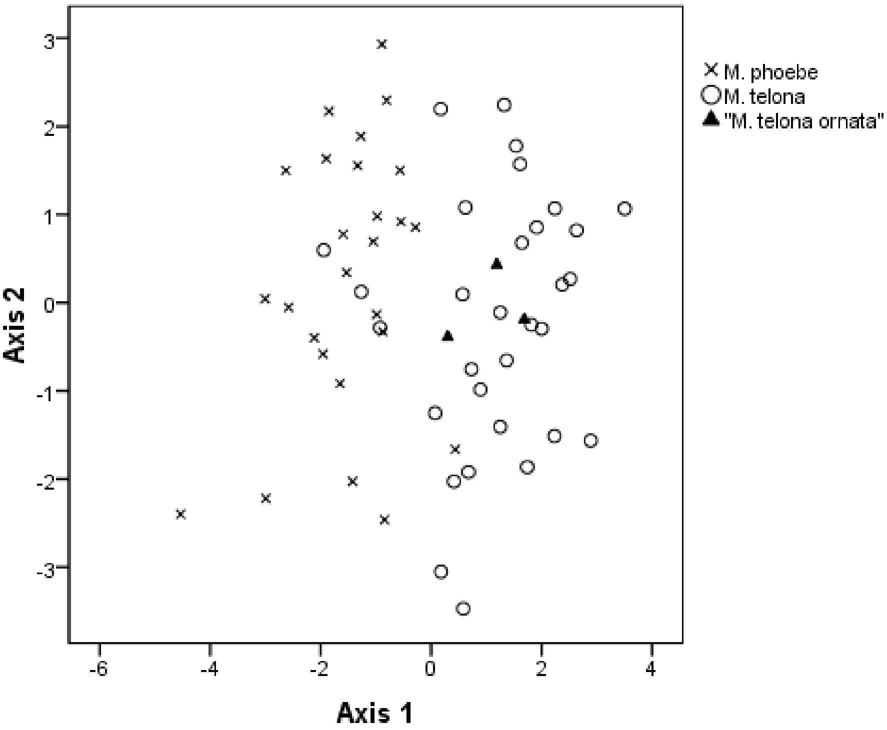

Fig. 3. PCA plot of females. The specimens from Russia (*M. telona ornata*) were marked by triangles.

DISCUSSION

In complete agreement with the distinctive characters between *M. phoebe* and *M. telona* reviewed in the Introduction, we found significant differences in univariate and multivariate level in the genital structures between the two species in both sexes. Using MDA method we could classify correctly most of the specimens and the low Wilks' λ also demonstrate this two species mostly identifiable based on some genitalia traits. The PCA plots are also demonstrate separation in the case of most of the specimens. However, PCA do not always give good enough results when it is applied to when two species show overlap (MUTANEN 2006a).

In males the depth of the central notch of the saccus proved to be the most important difference. Except for two characters all show significant differences, but if we separately consider these differences we found a major overlap. In general we observed that in *M. telona* we can see a more notched saccus, and more symmetric shape of processus posterior (in same side) because the inner process of the processus posterior is shorter than *M. phoebe* (Fig. 6). These two characters are essentially the same as the distinctive characters in the original description of *M. "phoebe" kovacsi* (VARGA 1967).

The case of females we could measure only few characters. Although the width of the posterior lamella did not show a significant difference only in its height, its shape proved to be different significantly. *Melitaea phoebe* has a more rounded shape of lamella, thus its shape is closer to a circle. Oppositely, *M. telona* shows a more extended elliptic shape of the lamella. These are the most important differences between the females. The furca is usually shorter in *M. phoebe* (Fig. 7).

Surprisingly, the specimens from Magnitogorsk and Guberlya, South Ural (Russia) seem to belong to *M. telona* based on external and genital traits too. Possibly they can be classified as an own subspecies (*M. telona ornata* CHRISTOPH, 1893 comb. n.), because the conspicuous external colouration (dichroism) and also due to its widely separated ocurrence from the nominotypical and other eastern Mediterranean forms of *M. telona*. We have to study more material to clarify this enigmatic question, but these new finding draw our attention to the fact that the species is distributed much more to the East.

Despite of our results which demonstrate significant differences between the surveyed species, there is some overlap in both sexes. This picture could be the consequence of a very recent wave of diversification in this species group (LENEVEU *et al.* 2009). We think the slightly different genital structures cannot exclude the mating between these sibling species. Since the allozyme surveys have not shown any mark of hybridisation between *M. phoebe* and *M. telona* *kovacsi,* these results can be interpreted by two different ways (or a combination of both).

Either there are some prae-mating barriers e.g. the different habitat and food plant preferences combined with different behavioural or chemical (pheromone) signals. The different shape of the tip of antennae may be a signal of such differences. The other possibility is the lower fertility or survival chance of hybrids. The solution of this question might be very significant for the Hungarian nature conservation because *M. telona kovacsi* is protected in Hungary.

ACKNOWLEDGEMENTS

We thank to Dr. Axel Hausmann (Zoological State Collection in Munich), Dr. Zsolt Bálint (Hungarian Natural History Museum) for borrowing valuable museum specimens for our studies. Thanks are due to László Peregovits, László Ronkay and Leonardo Dapporto for useful suggestions and corrections on the manuscript.

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Study II: Inter- and intraspecific variation in the genitalia of the ‘*Melitaea phoebe* group’ (Lepidoptera, Nymphalidae)

Tóth JP & Varga Z (2011) Inter- and intraspecific variation in the genitalia of the '*Melitaea phoebe* group' (Lepidoptera, Nymphalidae). *Zoologischer Anzeiger – A Journal of Comparative Zoology* 250 (3): 258-268.

Abstract:

The ecology and phylogeny of the Melitaeini butterflies have received considerable attention in the last few years. Several publications have been based on research using molecular methods. Genital morphometry has already been published on two sibling species but without any accompanying taxonomic revision of related species or their intraspecific taxonomy. In this study the morphology of genitalia was analysed by landmark-based geometric morphometrics in males and by traditional morphometrics in females. Based on these morphological characters, the whole *M. phoebe* group (subg. *Cinclidia*) was revised. According to our results *M. telona* Fruhstorfer, 1908 is conspecific with *M. ‘phoebe’ ornata* Christoph, 1893. Thus, we consider *M. telona* a junior synonym of *M. ornata*, and we propose new combinations: *M. ornata ornata* Christoph, 1893, *M. ornata telona* Fruhstorfer, 1908, *M. ornata amanica* Rebel, 1917, *M. ornata capreola*Varga, 1967, *M. ornata emipunica*Verity, 1919, *M. ornata enoch* Higgins, 1941, *M. ornata kovacsi* Varga, 1967, *M. ornata nigrogygia* Verity, 1938, *M. ornata ogygia* Fruhstorfer, 1907 and *M. ornata totila* Stauder, 1914 based on the rule of priority. The validity of the species status of *M. punica* and *M. scotosia* was confirmed, whereas *M. sibina* and *M. pseudosibina* could not be separated from *M. phoebe* using morphometrics. A new species from Iran (Zagros Mts.) was described as *M. zagrosi* sp. n. In addition, the whole geographical range of *M. ornata* was reconsidered and some biogeographical connections were discussed. In *M. ornata* four morphotypes were found based on genitalia: 1. ‘Western type’:

including the Italian, Hungarian and SW Ukrainian populations; 2. ‘Eastern type’: with a much extended distribution ranging from the Balkan Peninsula through Asia Minor to Kazakhstan; 3. ‘The Levant type’ (=*M. ornata telona* comb. nov.); 4. ‘Northern Iranian type’ (Kopeth Dagh and Elburz Mts.). The distribution of these morphotypes can be attributed to historic separation in distinct glacial refugia.

Keywords: *Melitaea*; Palaearctic; *Melitaea ornata* stat. revid.; Morphometrics; Biogeographic subdivision; Identification key

Introduction

The first significant taxonomic revision of *Melitaea* was published by Higgins in 1941. His taxonomical concept has shaped the knowledge on *Melitaea* for several decades. The '*phoebe* group' (=subg. *Cinclidia* Higgins, 1981) of the Melitaeini tribe proved to be one of the most problematic groups taxonomically. Several papers have been published on this tribe using molecular methods (Wahlberg, 2000; Wahlberg and Zimmermann, 2000; Leneveu et al., 2009). Nevertheless, genital morphometry has only been analysed in two sibling species without revising related species and their intraspecific taxonomy (Tóth and Varga, 2010).

The best known species of the group *Melitaea phoebe* ([Denis & Schiffermüller], 1775) (TL: Wien, Austria) occupies an almost continuous area from North Africa across southern and central Europe to north-eastern China. This polytypic species shows considerable variation, with several described subspecies. The basis of these descriptions is mostly the colouration and pattern of the upper side of wings. Moreover, some of the subspecies have recently been claimed to be distinct species.

Here we would like to highlight some taxa which have been associated with *Melitaea phoebe* but they taxonomical relegation is unsolved or questionable. *Melitaea phoebe enoch*, Higgins, 1941 (TL: Transcaspia (Turkmenistan), Jablonowka (?), Achal Tekke) was characterised by Higgins: '. . .there is a small difference in the male genitalia of two specimens examined, in the heavier and rather differently shaped posterior process of the clasp. It may be that *enoch* should be ranked as a subspecies, but without more extensive material it is not possible to be sure of this' (Higgins, 1941). *Melitaea phoebe pseudosibina* Alberti, 1969 (TL: Itkol Mt., Elbrus, Russia) being externally similar to *M. sibina* Alpheraky, 1881 was described from the Elbrus region of

Great Caucasus above 2200m altitude. Its taxonomical status is controversial (Korshunov and Gorbunov, 1995).

The species rank of *Melitaea telona* Fruhstorfer, 1908 (TL: Jerusalem, Israel) has only been recognised recently (Russell et al., 2005, 2007). Its known area is disjunct ranging from the Levant across Asia Minor, the Balkans to southern Italy and Sicily as well as the Carpathian Basin and the Podolian plateau on the north (Russell et al., 2005, 2007; Varga et al., 2005; Varga, 2007). The distinction between *M. telona* and *M. phoebe* is mostly based on larval characters (Russell et al., 2005, 2007). Larvae of some *M. telona* subspecies have brick red head from the 4th larval instar. They are mono- or oligophagous feeding on different Asteraceae, mainly on regionally different, often endemic *Centaurea* species. In contrast, *M. phoebe* is oligophagous often using different food plants simultaneously (*Centaurea*, *Cirsium*, *Carduus*). Its larvae always have black head capsule.

The species rank of *Melitaea punica* Oberthür, 1876 (TL: Lambessa, Algeria) has already been suggested by Oberthür (1914), though without surveying genital organs. Later the name '*punica'* was either used as a distinct subspecies of *M. phoebe* (Higgins, 1941) or in combination with '*telona'* (Gorbunov and Kosterin, 2007) as a synonym of *M. phoebe telona.* Russell et al. (2005) have pointed out that this combination was based on the misidentification of *Melitaea telona*. Thus, *M. punica* only occurs in North Africa. These results have also been confirmed by genetic surveys analysing one mitochondrial and two nuclear genes in several species of the *M. phoebe* group (Leneveu et al., 2009), by enzyme electrophoresis in *M. telona kovacsi* and *M. phoebe* (Pecsenye et al., 2007) and recently by the survey of male and female genitalia (Tóth and Varga, 2010). In this latter study a few specimens of *M. 'phoebe' ornata* Christoph, 1893 (described from Guberlya, South Ural), were also involved and they were clustered together with *M. telona.*

Many authors consider *Melitaea scotosia* Butler, 1878 (TL: Tokyo, Japan) and *Melitaea sibina* Alphéraky, 1881 (TL: Kuldja, China) to be a distinct species. However, recent molecular surveys could not find any significant difference between *M. phoebe*, *Melitaea sibina* and *M. scotosia* (Wahlberg and Zimmermann, 2000). Therefore, it has been suggested that they may represent populations or environmental forms of one species rather than separate species (Leneveu et al., 2009).

According to earlier studies there are no generally known discriminating characters either on the wings or on the genital organs in the '*Melitaea phoebe* group'. This is especially true in *M. phoebe, M. telona* and *M. punica*. Although genitalia often showspecies-specific characters, and demonstrate more distinct divergence even among closely related species than other traits, e.g. wing patterns and colouration (Shapiro and Porter, 1989), they were hardly used in these butterflies.

Thus, our goal was to perform a survey on male and female genitalia of the above taxa at a Palaearctic scale. We were looking for reliable characters on the genitalia showing clear differences between the species. We also wanted to get an overview on the geographical pattern of *M. phoebe* and *M. telona*. A combination of morphometry and multivariate statistical analysis has been frequently used in the last few years (Wakeham-Dawson et al., 2004; Wakehan- Dawson et al., 2004; Simonsen, 2005; Mutanen et al., 2006; Mutanen and Pretorius, 2006; Simonsen, 2006; Prieto et al., 2008; Dapporto, 2010; Tóth and Varga, 2010) and proved to be almost as successful in species identification as DNA barcoding (Dinca et al., 2011).

2. Materials and methods

2.1. Sample

365 male and 203 female individuals were analysed from the material of the Hungarian Natural History Museum, the Zoological State Collection, Munich and the collection of the University of Debrecen. The detailed list of the individuals is below. The sex of the specimens was marked (MM= males, FF = females). The same symbols were used in the UPGMA trees. From some countries (for example France, Bulgaria) we had sufficient material to form regional subgroups, while from others (for example Spain) the sample was small and was treated as a single group.

1. *M. phoebe*:

Spain: 7MM, 3FF; **France 1** (Alpes Maritimes): 12MM; **France 2** (Burgundy): 14MM; **France 3** (Hautes- Alpes): 4MM; **France 4** (Burgundy + Hautes-Alpes): 15FF; **Italy**: 8MM, 4FF; **Hungary 1** (North-East): 15MM, 7FF; **Hungary 2** (Middle-North): 9MM, 20FF; **Hungary 3** (Mátra Mt.): 7MM; **Hungary 4** (West): 16MM; **Albania**: 8MM, 9FF; **Montenegro**: 13MM, 7FF; **Macedonia**: 4MM, 4FF; **Bulgaria 1** (Pirin Mt.): 11MM; **Bulgaria 2** (Vitosa): 3MM, **Bulgaria 3** (Central Rhodope): 7MM, **Bulgaria 4** (Pirin Mts. +Vitosa): 8FF; **Turkey**: 5MM, 5FF; **Iran**: 8MM; **Ukraine** (Krim): 12MM, 4; **Russia** (Caucasus): 10MM, 6FF; **Mongolia**: 16MM, 8FF; **China**: 3MM.

2. *M. pseudosibina*: Russia Itkol Mt.: 4MM, 3FF.

3. *M. punica*: N-Africa: 6MM, 3FF.

4. *M. scotosia*: China, Korea, Japan: 8MM, 4FF.

5. *M. telona*:

Sicily: 9MM, 7FF; **Italy**: 5MM, 4FF; **Hungary 1** (Aggtelek area): 19MM, 18 FF; **Hungary 2** (Bükk Mt.): 9MM, 6FF; **Hungary 3** (Middle-North): 18MM, 9FF; **Greece**: 4MM, 3FF; **Kazakhstan**: 7MM; **Macedonia**: 18MM, 7FF; **Russia** (South Ural, Guberlya, Uralsk): 7MM, 7FF; **Turkey 1** (Middle): 4MM;

Turkey 2 (East): 8MM; **Turkey:** 10FF; **Ukraine**: 4MM; **Levant**: 9MM, 6FF; **Iran**: 5MM.

6. *M. sibina*: Afghanistan, Uzbekistan, Tajikistan: 9MM, 4FF.

7. *M. zagrosi **sp. n.***: **Iran 1** (Northern Zagros Mts.): 11 MM, 6 FF; **Iran 2** (Southern Zagros Mts.): 9MM, 2FF.

The specimens involved in this study originated from many locations of the Palaearctic. *M. telona* samples cover almost the entire known distribution of the species. In addition, we also included individuals from some new localities. To identify the species, a simple key was constructed on the basis of the characters of the underside of wings, (see also Higgins, 1941; Varga, 1967; Korshunov and Gorbunov, 1995; Varga et al., 2005; Russell et al., 2006; Gorbunov and Kosterin, 2007; Varga, 2007; Tolman and Lewington, 2008) and personal experience (see Supplement).

2.2. Preparation method

We followed the standard way of genital preparation. The abdomen was removed and boiled in 15% KOH solution. The genitalia were cleaned and dehydrated in ethanol and mounted in euparal between microscope slides and cover slips. Genitalia slides were digitalized using a combination of an Olympus C-4000 Zoom camera and a Nikon 102 stereo microscope.

2.3. Landmark based geometric morphometrics

Landmark based geometric morphometric analysis was used to quantify the variation in the shape of processus posterior on the valvae. We recorded 9 landmarks at the tips and origin of the main processi (Fig. 1a). TpsDig 2.1 was used to digitalise landmarks and IMP software family for the analyses (Zelditch et al., 2004). Procrustes generalized least squares (GLS) was used to get the shape coordinates. CVA (canonical variates analysis) was applied to unravel the relationship between the original groups and those obtained in Jack-knife grouping to quantify the validity of the visible pattern. In Jack-knife, one specimen is left out at a time, and assigned using the CVA axes. The analyses were carried out by CVAgen 6.0. The average processus posterior was drawn by PCAgen 6.0 in each group.

The differences in centroid sizes (the square root of the summed squared distances of each landmark from the center of the form) were analysed by univariate ANOVA. Levene test was applied to indicate homogeneity. Since it failed Games–Howell post hoc test was used to compare the differences between the given taxa. SPSS 16.0 was used to construct plots and perform ANOVA.

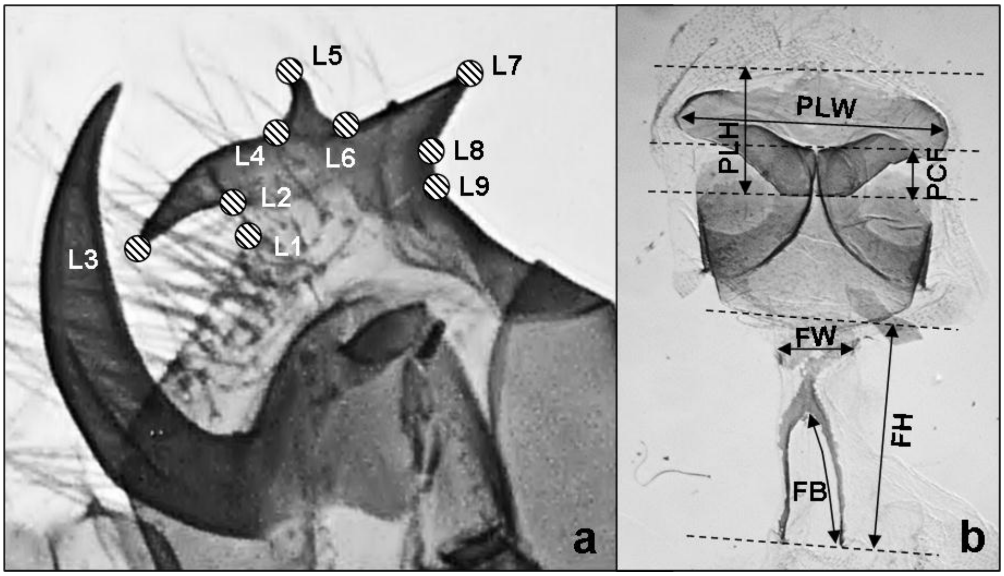

Fig. 1. The landmarks on males (a) and the measured characters on females (b) PLW= width of the posterior lamella, PLH = height of the posterior lamella, PCF = cover flap of the posterior lamella, FB = left branch of the furca, FH = height of the furca, FW= width of the base of furca.

2.4. Traditional morphometrics

We could not find good landmarks on female genitalia thus traditional morphometrics was applied. Six characters were measured on the posterior lamella and furca described by Tóth andVarga (2010). PLW= width of the posterior lamella, PLH = height of the posterior lamella, PCF = cover flap of the posterior lamella, FB = left branch of the furca, FH = height of the furca, FW= width of the base of furca (see in Fig. 1b). A ratio was used: P = PLH/PLWto demonstrate how close to oval the shape of posterior lamella is in females.

Burbanaby approach (Burnaby, 1966) was used, in order to remove the effect of size for further size independent data analyses. PAST (Hammer et al., 2001) programme package was used to perform this analysis. CVA (canonical variance analysis) was used to unravel the relationship between the groups and find the most discriminative traits. All variables were entered simultaneously. Cross-validated classification was used to allocate the specimens. The statistical analyses were carried out with SPSS 16.0.

2.5. Tree construction

In both sexes of *M. phoebe* and *M. telona*UPGMAtreewas constructed on the basis of Mahalanobis distances using the “agnes” function in R programme package (R Development Core Team, 2010). Since interspecific characters could be different from intraspecific ones these analyses were performed separately in *M. phoebe* and *M. telona*.

2.6. Measurement error

Repeatability was calculated by the following formula: ME = $S2$ within/($S2$ within + $S2$ among) × 100 (Lessells and Boag, 1987) where $S2$ within is the within measurement component of variance and $S2$ among is the among-measurement component. ANOVA was used to calculate these values (Bailey and Byrnes, 1990; Yezerinac et al., 1992). In females the measurement error was very low, less than 5% in all cases and less than 1% in the case of PWL, PLH, FL. In males it was less than 5% for most landmarks. It was around 10% in the case of L1, L2 and L8.

3. Results

3.1. Males

The previously calculated shape coordinates were analysed using CVA. The first six discriminant functions were significant ($p < 0.001$) and the first three ones could explain 74.8% of variance. In the scatter plot (Fig. 2) the first three axes were used (3D) since the third function could still explain 14% of variance. The most separated taxa were *M. scotosia* and *M. punica* as well as the *Melitaea* sample from the Zagros Mountains on this plot. *M. phoebe* and *M. telona* centroids formed two separate groups. *Melitaea sibina* and *M. pseudosibina* were involved in the 'phoebe cloud' though the Iranian sample seemed to be little more isolated. The Iranian and the Levantine samples of *M. telona* also seemed to be isolated from the '*telona* cloud'. At the same time '*ornata*' belonged to the '*telona* cloud' on the CVA plot.

We set the previously described groups for the Jack-knife groupings (Table 1). 74.7% of the specimens were correctly classified. In cases of *M. punica*, *M. scotosia* and the Iranian *M. phoebe* we did not observe any misclassification. Based on the results of classification it seemed that the clouds of *M. phoebe* and *M. telona* were overlapping (11.8% and 14.3% of individuals were classified to the other group, respectively). Only one specimen from the Zagros Mts. (Iran (Z)) groupwas misclassified (5%) to the Northern Iranian (Iran (N)) group. It is remarkable that none of the specimens from the other groups were classified to this group.

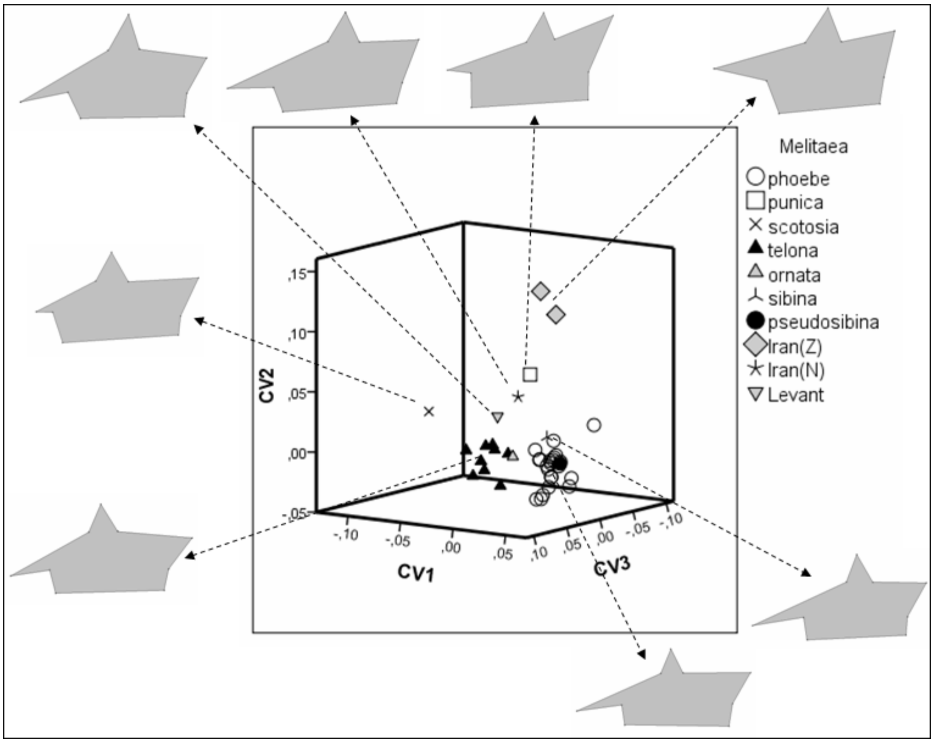

Fig. 2. Differences in the shape of the processus posterior. The grey figures represent the average form of the given taxa. The symbols shows the group centroid.

	phoebe	punica	scotosia	telona	Levant(t)	Zagros	Iran(t)	Iran(ph)
phoebe	71.4%	0	0	11.8%	3.0%	0	7.9%	5.9%
punica	0	100.0%	0	0	0	0		0
scotosia	0	0	100.0%	0	0	0		0
telona	14.3%	0	0	71.4%	4.5%	0	9.8%	0
Levant	0	0	0	11.1%	77.8%	0	11.1%	0
Zagros	0	0	0	0	0	95.0%	5.0%	0
Iran(t)	0	0	0	40.0%	0	0	60.0%	0
Iran(ph)	0	0	0	0	0	0	0	100.0%

Table 1. Result matrix of the Jack-knife assignment grouping, based on 9 landmark point configuration. Original groups along rows, CVA groups along columns. 74.7% of cases correctly assigned.

The univariate analysis of centroid size of processus posterior showed significant differences between some of the groups ($F = 13.4$, $p < 0.05$) (Table

2). *M. scotosia* had the largest processus posterior and itwas separated from all other surveyed taxa significantly. *M. punica, M. sibina* and *M. pseudosibina* had the smallest processi posteriores. No significant difference between *M. phoebe* and *M. telona* was found.

UPGMA trees were constructed on the basis of Mahalanobis distances and they indicated a very similar picture (Fig. 3). In *M. phoebe* the geographical pattern was not clear. *M. sibina* and *M. pseudosibina* were not separated from the *M. phoebe* populations while the Iranian sample of *M. phoebe* was well separated from all the others (Fig. 3b).

	Iran (ph)	**phoebe**	**punica**	**scotosia**	**telona**	**sibina**	**ps.sibina**	**Iran (Z)**	**Iran (t)**	**Levant**
Iran (ph)	-	0.7324	p<0.05	p<0.05	1	p<0.05	0.1743	0.5437	0.9609	1
phoebe	0.7324	-	p<0.05	p<0.05	0.6774	0.0615	0.295	0.9809	1	0.8037
punica	p<0.05	p<0.05	-	p<0.05	p<0.05	0.4241	0.9294	p<0.05	p<0.05	p<0.05
scotosia	p<0.05	p<0.05	p<0.05	-	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05
telona	1	0.6774	p<0.05	p<0.05	-	p<0.05	0.1643	0.4944	0.9449	1
sibina	p<0.05	0.0615	0.4241	p<0.05	p<0.05	-	1	0.5017	0.1612	p<0.05
ps.sibina	0.1743	0.295	0.9294	p<0.05	0.1643	1	-	0.5655	0.3246	0.1435
Iran (Z)	0.5437	0.9809	p<0.05	p<0.05	0.4944	0.5017	0.5655	-	0.9955	0.5623
Iran (t)	0.9609	1	p<0.05	p<0.05	0.9449	0.1612	0.3246	0.9955	-	0.9426
Levant	1	0.8037	p<0.05	p<0.05	1	p<0.05	0.1435	0.5623	0.9426	-

Table 2. Results of the Games-Howell post hoc test. The grey fields mark the significant differences at the 0.05 level.

M. telona samples from the Levantine area and Iran were clearly separated from the others (Fig. 3a). The remaining samples composed two main branches: the “western group” including the Hungarian, Italian and Ukrainian populations and the “eastern group” involving the Greek, Macedonian, Turkish, Russian (marked as ornata in the CVA plot) and Kazakh populations (Fig.3a).

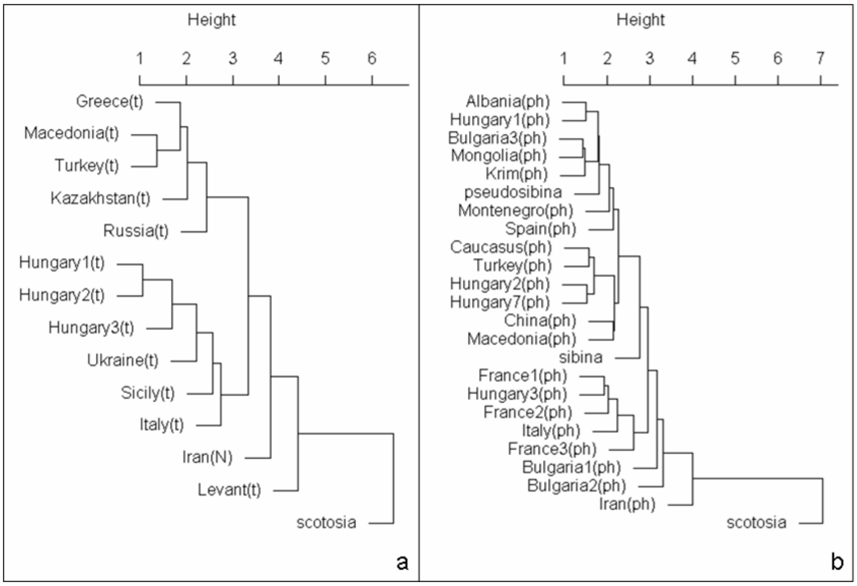

Fig. 3. UPGMA tree of the males, based on Mahalanobis distances. *Melitaea telona* (a) and *Melitaea phoebe* (b). Further explanation of the legends see material and methods.

3.2. Females

The adjusted variables were entered together in the CVA. The first four discriminant functions were significant ($p < 0.001$). The ratio of the posterior lamella (P) had the largest absolute correlation with the first function which explained 51.1% of the variance. The second function accounted for 23.6% of the variance; FBL showed the highest correlation with this function. We used the first two axes to plot the results (Fig. 4). *M. scotosia* and the *Melitaea* sample from the Zagros Mountains were separated from the '*telona-phoebe* cloud'. *M. punica* was located between the *M. telona* and *M. phoebe* clouds. *M. sibina* and *M. pseudosibina* were localised within the '*phoebe* cloud'. As in males, the sample from the south Ural region (*ornata*) appeared in the '*telona* cloud'. Cross validation process was applied to classify the specimens into their original groups (Table 3).

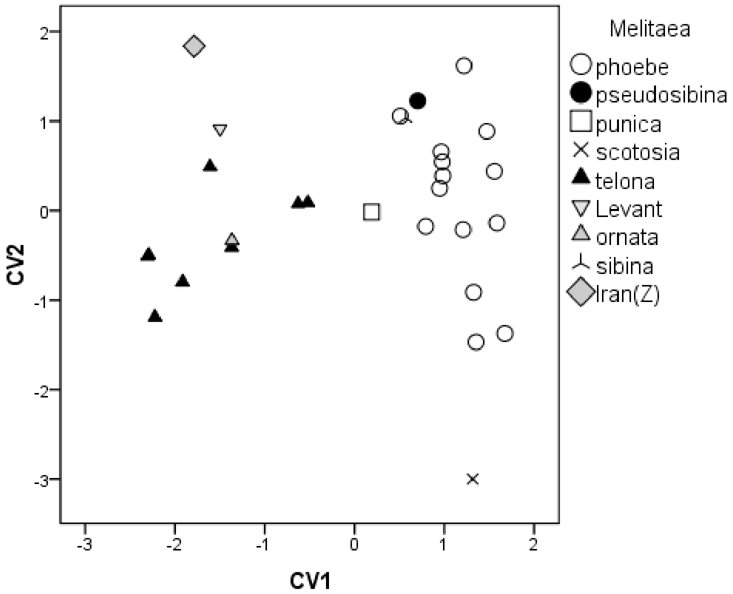

Fig. 4. Scatter plot of the females. The symbols represent the group centroid.

	phoebe	**punica**	**scotosia**	**telona**	**Iran(Z)**
phoebe	63.2%	23.9%	5.1%	5.1%	2.6%
punica	75%	0	0	25%	0
scotosia	0	0	100%	0	0
telona	5.3%	11.8%	0	71.1%	11.8%
Iran(Z)	0	0	0	0	100%

Table 3. Cross-validated classification results of the females based on distance measurements. Original groups along rows,CVAgroups along columns. 67.1% of grouped cases correctly classified.

M. telona a very similar picture was obtained to that of males. The two main groups were also recognisable although the sample from Southern Russia (ornata in the CVA plot) was clustered to the ‘western group’ and the Levant population was not separated from the ‘eastern group’.

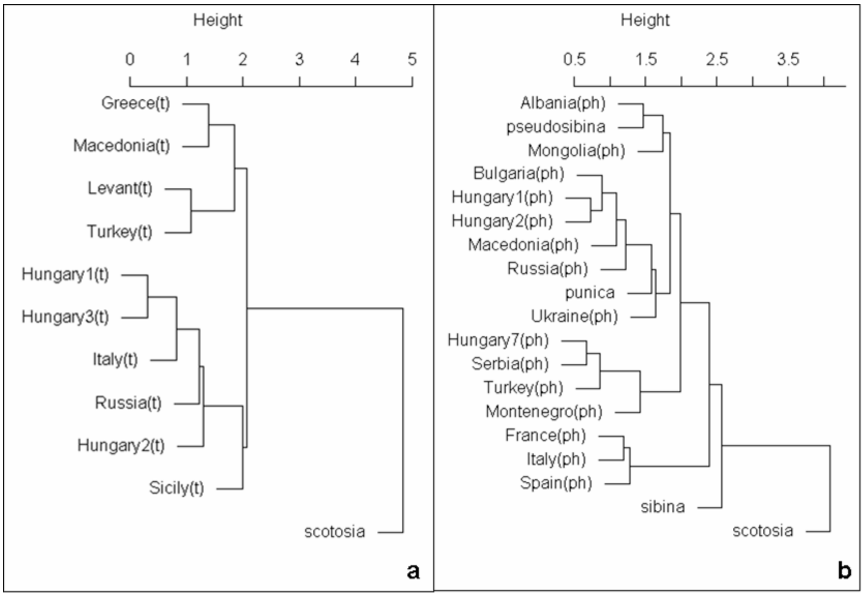

Fig. 5. UPGMA tree of the females based on Mahalanobis distances. *Melitaea telona* (a) and *Melitaea phoebe* (b). Further explanation of the legends see material and methods.

4. Discussion

Genital morphometry yielded reliable results in the separation of the species of the '*phoebe* group'. In males, the shape of the processus posterior proved to be a valuable character in species identification. In females, the shape of the posterior lamella was a good character although it could not separate *M. punica* from *M. telona* and *M. phoebe*. Comparing the results of males and females, we concluded that female genitalia provided less power to characterise the differentiation among the given taxa. Although this view is generally accepted in butterflies it has rarely been tested (Shapiro and Porter, 1989).

4.1. Validation of the species *Melitaea ornata* (comb. n.) Christoph, 1893

Our present results confirm and even extend our previous work where it was shown that *M. ornata* does not belong to *M. phoebe* (Tóth and Varga, 2010). Some authors suggested that *ornata* is a subspecies of *M. punica* (Hesselbarth et al., 1995; Korshunov and Gorbunov, 1995). In this study, it was demonstrated that *ornata* does not belong to *M. punica*. On the contrary, *Melitaea phoebe* var. *ornata* Christoph, 1893 (Iris VI: 87) is the oldest valid name for the species mentioned earlier as *Melitaea 'telona'*. Thus the name '*ornata*' has a clear priority as species name over *M. telona* Fruhstorfer, 1908. Therefore, following the rule of priority, we introduce here two new combinations: *Melitaea ornata ornata* Christoph, 1893 (comb. n., stat. revid.) and *Melitaea ornata telona* Fruhstorfer, 1908 (comb. n.) for these cases. In Table 4 we summarized the new combinations of the surveyed *M. ornata* and we used these new combinations in the following parts.

4.2. Morphometry vs. molecular taxonomy

Our morphometric results are in agreement with those of molecular studies, with the exception of *M. scotosia*.

Morphometric analyses, similarly to DNA studies (Leneveu et al., 2009), suggest that *M. sibina* is just a well separated group (subspecies) within *M. phoebe*. Our results suggest the same solution for *M. pseudosibina*, however, there are no published molecular data for this taxon. These taxa have only been separated from *M. phoebe* on the basis of the pattern and colouration of the upperside of the wings. The present results clearly demonstrate how unreliable this kind of identification in *Melitaea* is because some of the wing traits might be highly influenced by environmental factors (Cesaroni et al., 1994; Dapporto et al., 2009) e.g. temperature and humidity. Unfortunately most descriptions of subspecies and sometimes even species are based on such characters (see in Higgins, 1941).

M. ornata specimens from Northern Iran (Kopeth-Dagh and Elburz) are probably identical to *M. "phoebe" enoch* Higgins, 1941, but this statement will have to be confirmed by surveying the type specimens. In agreement with the molecular results, *Melitaea punica* is a distinct species on the basis of male genitalia but the analysed traits of genital organs of females were not suitable to separate this species from *M. phoebe*.

As opposed to the DNA results (Leneveu et al., 2009), *M. scotosia* can be clearly separated from all the other members of the *phoebe* species group based on the genitalia or wing pattern of both sexes. We note that the specimen depicted as *M. scotosia* in the previously mentioned DNA analysis (http://www.nymphalidae.utu.fi/Vouchers.htm) does not show the typical *M. scotosia* wing pattern. Thus, it may represent a *M. phoebe* specimen from the overlapping area of these two species. In this context, we have to note that a significant overlap exists between these species in south eastern Siberia, China and perhaps also in Mongolia.

New combinations introduced in the present publication

name	type locality	taxonomical notes
M. ornata amanica Rebel, 1917	Ammanus Mt., Turkey	Hesselbarth et al. 1995 synonimised with ogygia
M. ornata capreola Varga, 1967	Kiwerce, Ukraine	
M. ornata emipunica Verity, 1919	Palermo, Sicily	
M. ornata enoch Higgins, 1941	Achal Tekke, Turkmenistan	should be confirmed by checking type specimens
M. ornata kovacsi Varga, 1967	Budakeszi, Hungary	
M. ornata nigrogygia Verity, 1938	Opatija, Macedonia	
M. ornata ogygia Fruhstorfer,1908	Poros, Greece	
M. ornata ornata Christoph, 1893	S-Ural, Russia	
M. ornata telona Fruhstorer, 1908	Jerusalem, Israel	
M. ornata totila Stauder, 1914	Monte Cocuzzo, S-Italy	

Table 4. The surveyed *M. ornata* taxa.

4.3. Identification of the species *Melitaea zagrosi* sp. n

Melitaea sample from the Zagros Mts. (Iran) with characteristic wing pattern elements, proved to be a distinct species on the basis of both male and female genitalia.

Holotype: Male, Iran, between Kazeroun and Buschir (Konar Takhteh), III. 1938, coll. Pfeiffer; in Zoological State Collection, Munich (genitalia ID: P106).

Paratypes: Two males from Iran, between Kazeroun and Shiraz (Mian Kotal), III. 1938, coll.: Pfeiffer; in Zoological State Collection, Munich (genital slides ID: P108, P109). Three males from Iran, (Fars) Sepidan (30.3407◦ North, 51.948733◦ East). 13. VI. 2007, coll.: T. Hácz & J. Babitcs; in Hungarian Natural History Museum (genitalia ID: B399, B400, B401).

Two females from the same collection data as the holotype: coll.: Pfeiffer; in Zoological State Collection, Munich (genitalia ID: P49, P107).

11 males and 6 females were not included in the paratype series due to the large geographical distance from the locality of the holotype.

4.4. Diagnosis and description

Smaller than *M. phoebe*, wingspan 33–40 (males), 39–42 mm (females). Upperside of the wing is yellowish-brownish orange with partially reduced black markings. Most important differential characters are visible on the underside of hind wings: as opposed to other species of the *phoebe* group the inner and outer parts of the discal band dichrous, inner part light ochreous, outer part silvery shiny white. Submarginal band shiny white and the marginal (‘capillar’) stripe light ochreous (in fresh specimens with orange hue). Black marginal lunules disconnected, more or less triangular. These latter three characters never occur simultaneously in any species of the ‘*M. phoebe* group’.

Genital characters allow a clear separation from the related species. Male genitalia: the shape of processus posterior is distinctive (see in Supplement Fig. 9). Inner process is much longer than in *M. punica* and apically less curved downwards than in *M. phoebe* and *M. ornata*. The outer spine of the processus is rather robust and directed more upwards than in the related species. Female genitalia are the most similar to *M. ornata* but the posterior lamella is more sclerotised (Supplement Fig. 9).

4.5. Geographical pattern

We have relatively large samples from *M. phoebe* and *M. ornata*. The within-group variability of these species is remarkable. We did not find a clear geographical pattern in *M. phoebe* although some of the clusters fit well to some geographical trends. A possible explanation of this pattern could be that the emergence of the species is relatively recent (Leneveu et al., 2009). The possibility of character displacement between *M. ornata* and *M. phoebe* can also be suggested as an alternative explanation of the considerable variation among *M. phoebe* populations with little geographical relevance (Tóth et al. unpublished).

In *M. ornata* male genitalia show four morphotypes (Fig. 6). The ‘Western type’ includes the Italian, Hungarian and south-western Ukrainian populations. The ‘Eastern type’ shows a much more extended distribution from the Balkan Peninsula through Asia Minor to the Eastern border of Kazakhstan. Further surveys are required to explain the relatively poor differentiation of the steppic populations of Southern Russia and Kazakhstan from those of the Balkans (Macedonia, Greece) and Asia Minor. The Levant region type and the Northern Iranian (Kopeth Dagh and Elburz Mts.) type seem to be separated from this “Eastern type”. A possible explanation of their separation can be that they were separate refugia isolated by lowland deserts unsuitable for *M. ornata*.

The results suggest that there were at least two main refugia of *M. ornata* in Europe: the Adriatic region including the hilly areas of Italian Peninsula and Sicily and the southern part of the Balkan Peninsula most probably extending into Asia Minor. The ‘eastern’ branch of *M. ornata* (*M. ornata ogygia* comb. nov.) has reached the Struma valley and the Pontic regions in Bulgaria and also

Dobrogea in Romania (see Rákosy and Varga, 2002; Varga et al., 2005). Further refugia can be assumed in Asia Minor as in grasshoppers (Çiplak, 2003) and other butterfly species (Wahlberg and Saccheri, 2007), in the Levant region and also in the Elburz Mts. in Iran. can be assumed in Asia Minor as in grasshoppers (Çiplak, 2003) and other butterfly species (Wahlberg and Saccheri, 2007), in the Levant region and also in the Elburz Mts. in Iran.

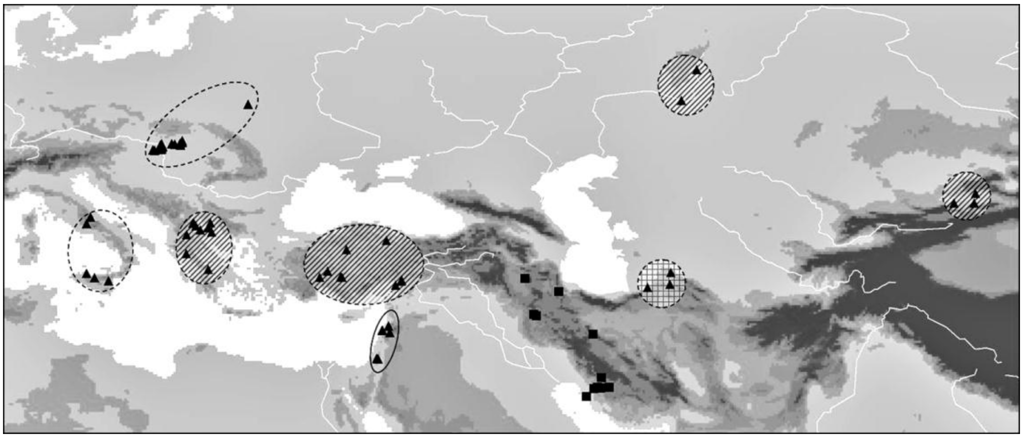

Fig. 6. Sample localities of *M. ornata* (filled triangle) and *M. zagrosi sp. n.* (squares). The ovals show the main clusters based on the male processus posterior. Empty oval with dashed line: 'Western type', empty oval with continuous line: 'Levant type', striped oval: 'Eastern type', squared oval: 'Northern Iranian type'.

4.6. Range and habitats of *M. ornata*

The northernmost populations of *M. ornata,* e.g. *M. ornata kovacsi* (comb. n.) in Central and Northern Hungary, show a patch-like distribution in warm forest-steppic habitats, strictly associated with the mass occurrence of the food-plant *Cirsium pannonicum*. They are surrounded by the more continuously distributed and generalist populations of *M. phoebe* (Tóth et al., 2011). The situation is probably the same in Southern Slovakia according to specimens kept in the Hungarian Natural History Museum.

The coexistence of *M. ornata* and *M. phoebe* should be surveyed in Western Ukraine (Podolia) and in Transylvania (steppic habitats). In the southern Balkans and in Asia Minor *M. ornata ogygia* (comb. n.) seems to be the generally distributed and more frequent taxon (Hesselbarth et al., 1995, revised museum materials) being better adapted to summer aridity by the aestivation of larvae and the monovoltine development. The westernmost border of the range of *M. ornata* is questionable. There is a dubious record about the occurrence of *M. ornata* near Fayance (France), but it was reported only once (Russell et al., 2007).

More surveys are necessary to unravel the whole distribution of *M. ornata* especially in its Asian part. The known easternmost populations are situated near to the eastern border of Kazakhstan but they may also occur in the adjunct areas of Western China.

Obviously, *M. ornata* should be considered as a polytypic species with several core areas of distribution. However, the available information on the genetic structure of the populations is insufficient to unravel the phylogeographic background of this distribution pattern.

In summary, we could outline a much clearer taxonomic and biogeographic description of the '*M. phoebe* group'.We have revised nearly all taxa of this species group, described a new species and introduced several new combinations of names. On the basis of our results, the taxonomical meaning and also the known distribution range of *M. ornata* has been essentially extended. We think that these results serve as a good starting point for further studies and support essentially the reliable identification of the members of '*M. phoebe* group'. Morphometry combined with multivariate statistics proved to be very useful again. It is especially important as there are huge amount of specimens conserved in museums most of which are not suitable for DNA surveys. The 'modern' genitalia morphometry can be an alternative method to get more information about these specimens.

Acknowledgements

We thank to Dr. Axel Hausmann (Zoological State Collection in Munich), Dr. Zsolt Bálint (Hungarian Natural History Museum) for borrowing valuable museum specimens for our studies. Thanks to Katalin Pecsenye, Mihály Földvári and Judit Bereczki for useful suggestions on the manuscript. We thank a lot to Thomas Schmitt and an anonymous referee for the useful critics and suggestions. Last but not least thanks to Nigel Spring for the linguistic corrections on the abstract. The survey was supported by the OTKA (K75696).

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Appendix

Key for identification of the species of the '*Melitaea phoebe* group'

1a. The reddish spots in the postdiscal band of the hind-wing underside are strongly marked, not vague. These reddish spots fill a relatively large part of the "caps" (Fig. 8c). It only rarely has black spots (the so called "cinxoid" forms some time appear in *M. phoebe* and in *M. telona*). In latter case the black spots are situated in the sharply outlined reddish spots. --**2.**

1b. The reddish spots are not visible well, or a black spot situated there (Fig. 8b) or it is very vague, just slightly different from the background of the spots (Fig. 8a). The spots fill a much smaller part of the "caps" than the previous group. The ground colour of the hind wing's underside is pale reddish. This large species (first wing length expanse 23-33 mm) is distributed in the Far East: East China, Korea, East Russia and Japan --*Melitaea scotosia*

2a. On the hind-wing underside the marginal ("capillar") stripe has a more yellowish colour compared to the discal submarginal band (similarly to *Melitaea britomartis* or *M. aurelia*). This colour is paler in females. The ground colour of the outer part of the discal band is shiny white, especially in males (Fig. 8d). This species exclusively occurs in North-Africa. -- *Melitaea punica*

2b. On the hind-wing underside the marginal ("capillar") stripe has darker (ochre) colour. This colour is paler in females. The ground colour of the outer part of the discal band is shiny white especially in males (Fig. 8e, Fig. 10). From the previous species the genitalia characters and the provenance are clearly separate. – *Melitaea zagrosi* ***sp. n.*** (see the description of the new species)

2c. On the hind-wing underside the marginal band has the same colour as the submarginal band. The ground colour is not shiny white --**3**

3a. The lunules in the marginal region of the hind-wing underside are forming a black zigzag line, because they are usually connected with each other. Rarely the lunules are disconnected but in this case they are not triangle or oval like. Sometimes they are seems like triangle but in this case they are always connected. The underside ground colour is usually yellowish white. On the fore-wing underside the lunules are seems like lines especially between nerve m3 and cu1. The club of the antennae is elongated and the flagellum segments continuously forming the club (Fig. 9a). -- *Melitaea phoebe*

3b. The underside of the hind-wing is the same like *M. phoebe*. On the upper side of the wings the colouration is extremely vivid reddish-orange and the black patterns are reduced. Distributed in Ghissar-Darvaz, W Pamirs and Tian-Shan. -- *Melitaea sibina*

3c. The lunules in the marginal region of the hind-wing underside are disconnected. They are triangle or oval like, not connected with each other. The light parts of the underside are usually white, more whitish than in *M. phoebe*. On the fore-wing underside the lunules are seems like triangles especially between veins m3 and cu1 .The club of the antennae is broad elliptic like and the flagellum segments are abruptly forming the club (Fig. 9b). -- *Melitaea ornata* comb. n. (syn.: *M. telona* syn. n.)

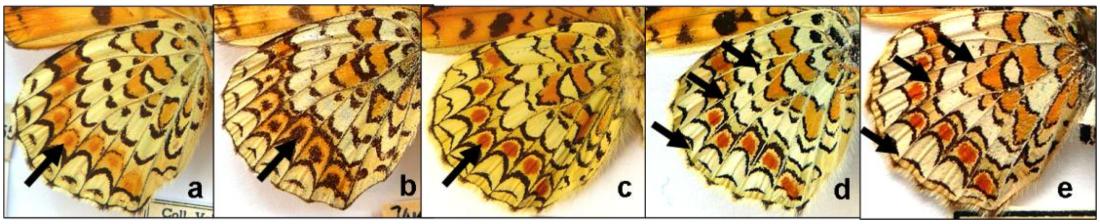

Fig. 8. Hind-wings underside (males). The reddish spots are just slightly visible in *M. scotosia*, or a black spot is situated there (b) or it is blurred and its colour just slightly differs from the background of the spots (a). The reddish spots are different in the other members of the "phoebe group" (c). In *M. punica* the marginal band has a different colour (yellowish) than the submarginal band (d). The arrows show the same coloured regions. *Melitaea zagrosi* ***sp. n.*** from Western Iran shows a similar pattern but the marginal colour is darker (e).

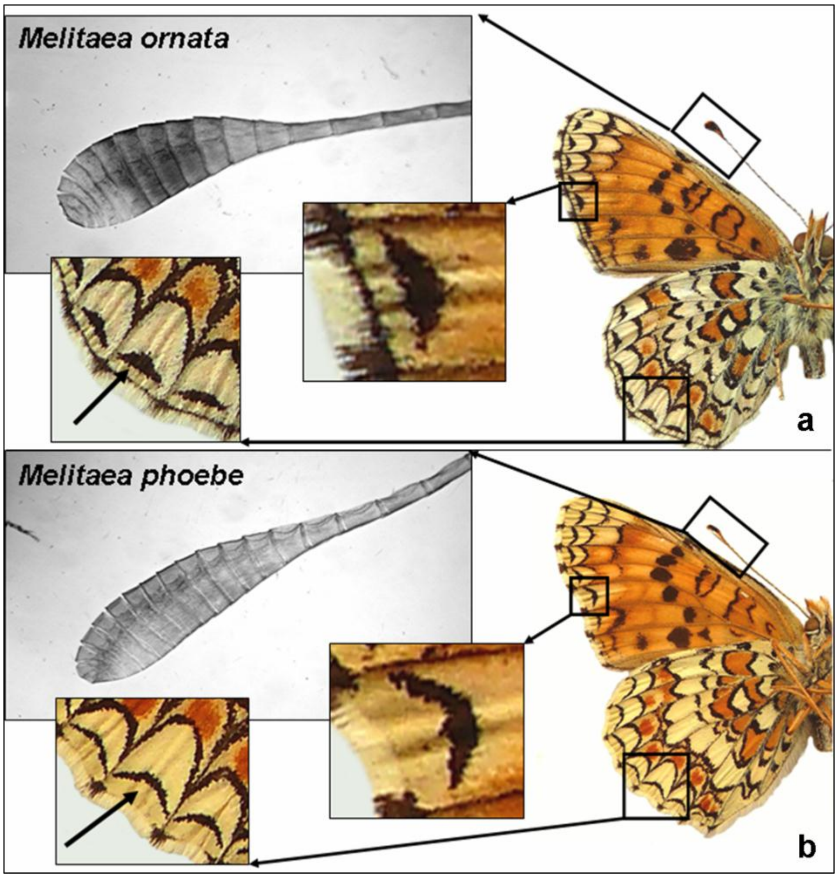

Fig. 9. Discriminative characters for *Melitaea phoebe* and *M. ornata*. In the marginal region the lunules are triangle like (a) in *M. ornata*, and they form a zigzag line (b) in *M. phoebe*.

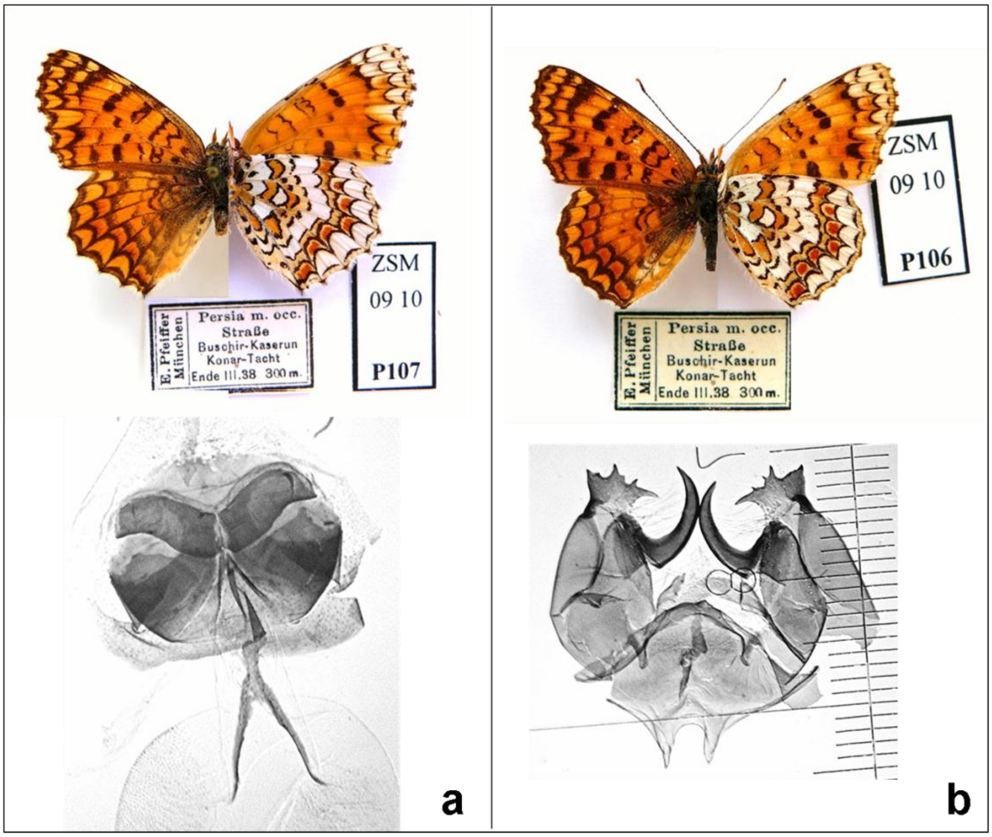

Fig. 10. Male (a) and female (b) specimen of *Melitaea zagrosi* ***sp. n.***

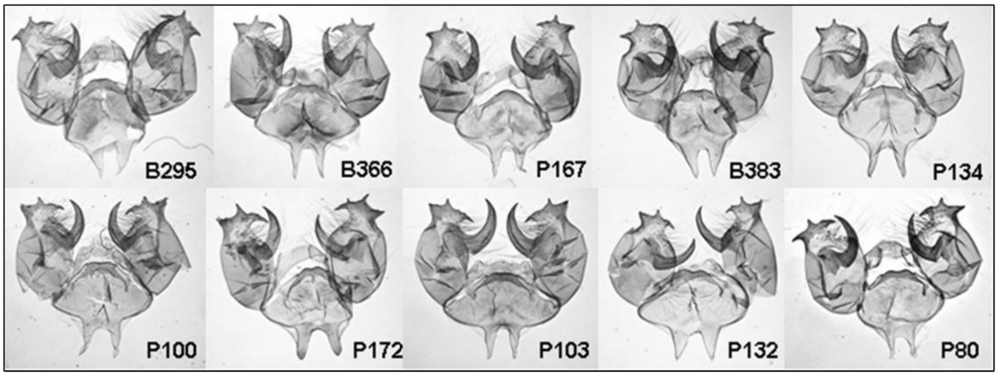

Fig. 11. *Melitaea ornata* male genitalia. **B295**: Kiwerce, Ukraine; **B366**: Bükk Mt., Hungary; **P167**: Pellegrino Mt., Sicily; **B383**: Matka Mt., Macedonia; **P134**: Delphi, Greece; **P100**: Egerdir, Turkey; **P172**: Bscharre, Lebanon; **P103**: Shahküh Mt. (Elburz Mts.), Iran; **P132**: Magnitogorsk, Russia; **P80**: Djarkent, Kazakhstan.

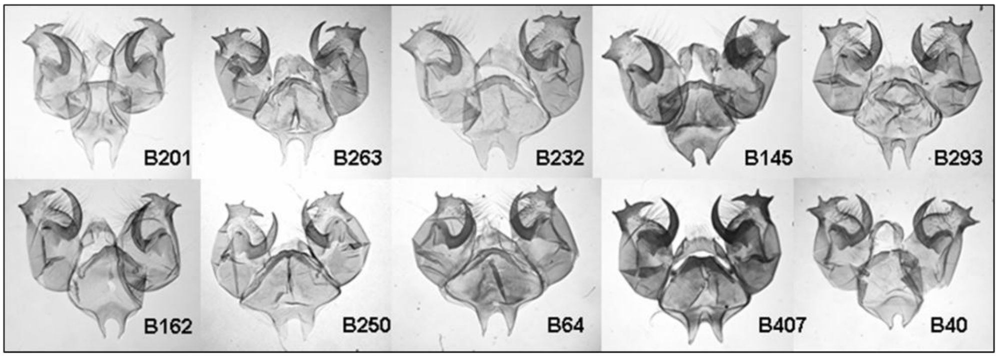

Fig. 12. *Melitaea phoebe* male genitalia. **B201**: Bronchales, Spain; **B263**: Saint-Martin-Vesubie, France; **B232**: Fenestrelle, Italy; **B145**: Sutorine, Montengro; **B293**: Kő-Hill, Hungary; **B162**: Razlog, Bulgaria; **B250**: Simferopol, Ukraine; **B64**: Teberda, Russia; **B407**: Quamu, Iran; B40: Khovd, Mongolia.

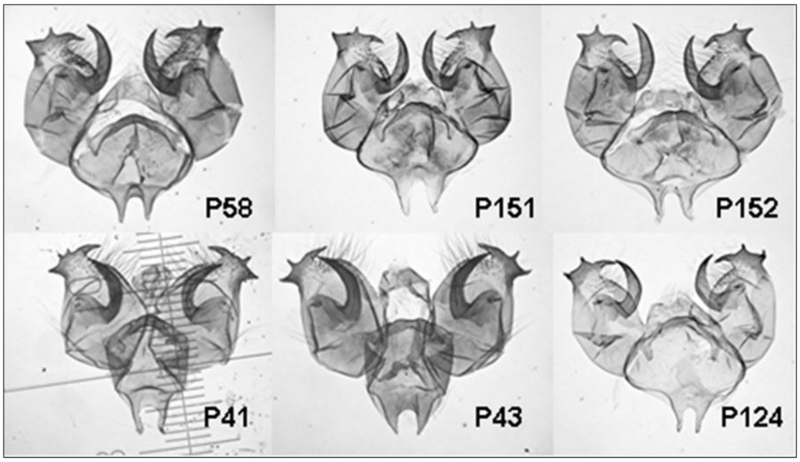

Fig. 13. Male genitalia of *Melitaea pseudosibina*: **P58**, **151**, **152** from Itkol Mt. (Elburz Mts.), Russia; and *M. sibina*: **P41**: Margelan, Uzbekistan; **P43**: Khinich-e-Andarab (Badakhashan), Afghanistan; **P124**: Djarkent, Kazakhstan.

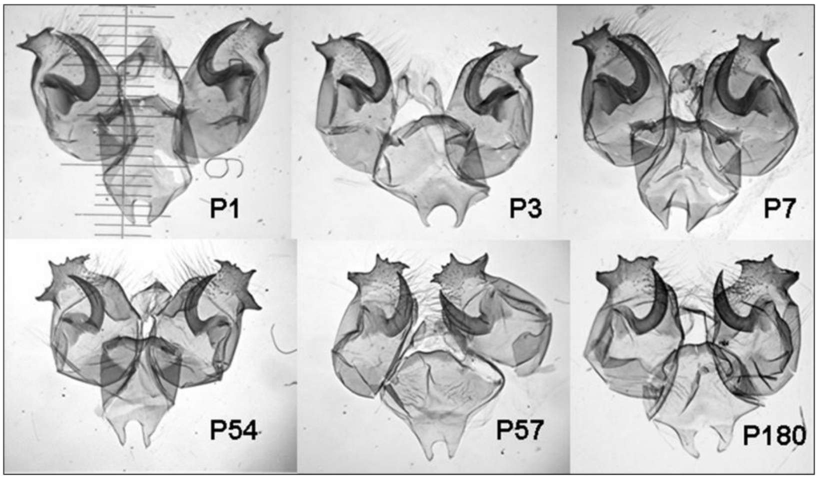

Fig. 14. Male genitalia of *Melitaea punica*: **P1**: ?, Morocco; **P3**: Lambése, Algeria; **P7**: Mischliffen, Algeria; and *Melitaea scotosia*: **P54**: Pyongyang, Korea; **P57**: Azumaya Mt., Japan; **P180**: Pjön Jang, Korea.

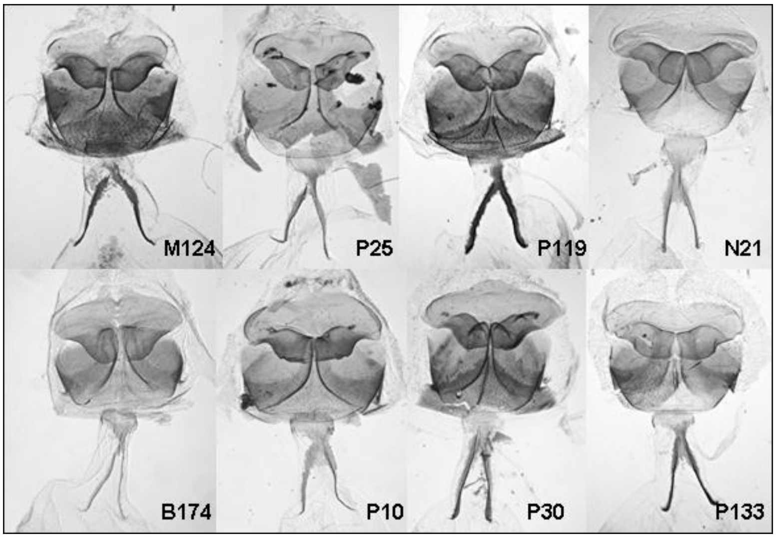

Fig. 15. Some genitalia of *Melitaea ornata* females. **M124**: Aggtelek area, Hungary; **P25**: Madonie Mt. (Sicily), Italy; **P119**: Paradiso Mt., Italy; **N21**: Skopje, Macedonia; **B174**: Ioannina, Greece; **P10**: Beirut, Lebanon; **P30**: Marasch, Turkey; **P133**: Magnitogorsk, Russia.

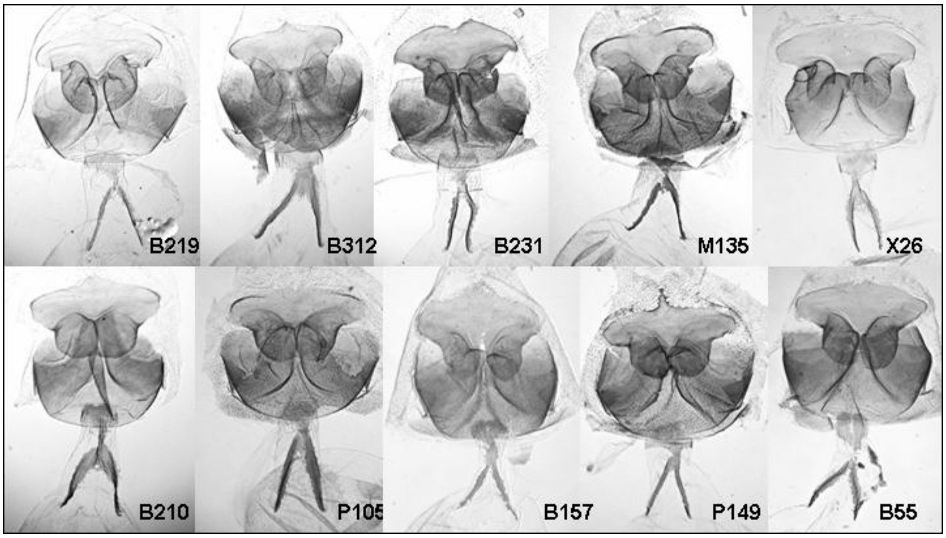

Fig. 16. Some genitalia of the *Melitaea phoebe* females. **B219**: Barcelona, Spain; **B312**: Sassy, France; **B231**: Fenestrelle, Italy; M135: Hetefejércse, Hungary; **X26**: Rogozina, Albania; **B210**: Szandansky-Valley, Bulgaria; **P105**: Brodec, Macedonia; **B157**: Simferopol, Ukraine; **P149**: Magnitogorsk, Russia; **B55**: Sarkha Nur, Mongolia.

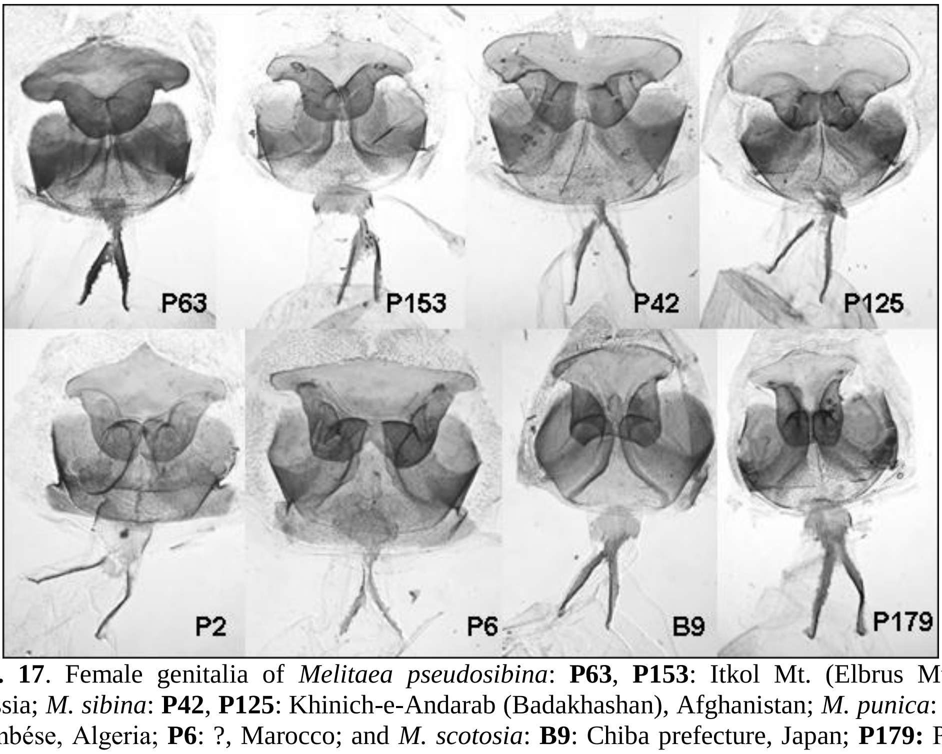

Fig. 17. Female genitalia of *Melitaea pseudosibina*: **P63**, **P153**: Itkol Mt. (Elbrus Mts.), Russia; *M. sibina*: **P42**, **P125**: Khinich-e-Andarab (Badakhashan), Afghanistan; *M. punica*: **P2**: Lambése, Algeria; **P6**: ?, Marocco; and *M. scotosia*: **B9**: Chiba prefecture, Japan; **P179:** Pjön Jang, Korea.

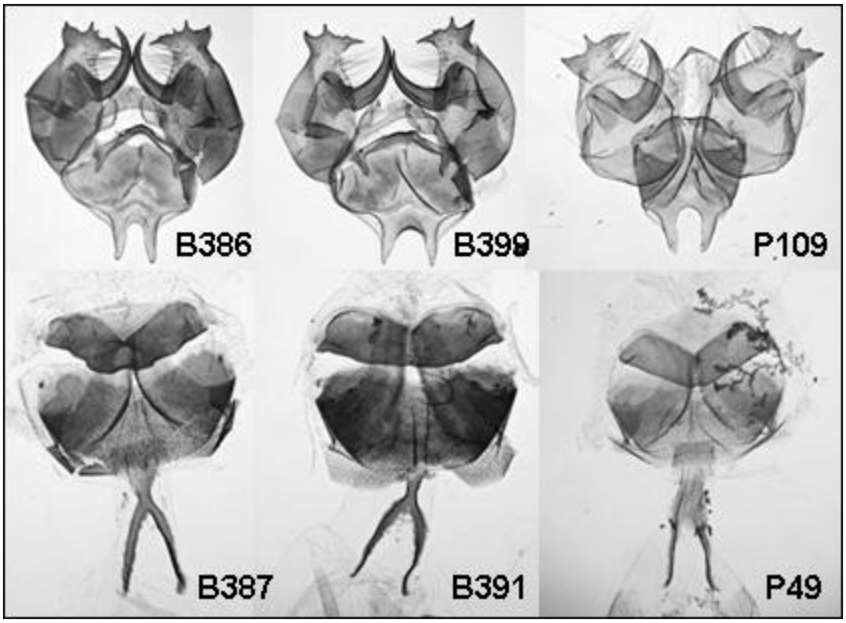

Fig. 18. Genitalia of *Melitaea zagrosi* ***sp.n.*** **B386**: Quamu, Iran; **B399**: Sepidan, Iran; **P109**:between Kazeroun and Shiraz, Iran; **B387**: Quamu, Iran; **B391**: Askaran, Iran; **P49**: between Kazeroun and Buschir, Iran.

Study III: Distribution of the Eastern knapweed fritillary (*Melitaea ornata* Christoph, 1893) (Lepidoptera: Nymphalidae): past, present and future

Tóth JP, Varga K, Végvári Zs & Varga Z (2012) Distribution of the Eastern knapweed fritillary (*Melitaea ornata* Christoph, 1893) (Lepidoptera: Nymphalidae): past, present and future. *Journal of Insect Conservation* (*in press*) DOI: 10.1007/s10841-012-9503-2

Abstract

Climatic change during the Quaternary resulted in periodical range restrictions and expansions in most temperate species. Although some repetitive patterns have been supported, it became obvious that species' responses might be rather specific and may also depend on habitat preferences of the species in question.

Distribution of *Melitaea ornata*, a little known fritillary species is analysed on different time scales using MaxEnt software. Using the results of genitalia morphometry and the predicted potential refugia during the Last Glacial Maximum (LGM), we reconstructed probable re-colonisation routes. We also predicted changes in the potential area for 2080. The present distribution fits well the known occurrence data except for the Iberian Peninsula and North-Africa where the species is missing. Based on our predictions, temperate areas seem to be less suitable for the species. We proposed two hypotheses to explain this pattern: a less probable recent extinction from Iberia and a more supported historical explanation. Predicted distribution during the LGM mainly fits to widely accepted refugia. Europe was probably re-colonised from two main sources, from the Apennine peninsula and from the Balkans which was probably connected to the Anatolian refugia. Populations of the Levant region and in the Elburs Mts. do not show any significant expansion. Further studies are necessary in the case of the predicted Central Asian refugia.

Predictions for 2080 show a northward shift and some extinction events in the Mediterranean region. Core areas are identified which might have a potential for expansion including southern Russia, Hungary and possibly Provence in France. Predicted northward area shifts are only possible if the potential leading edge populations and habitats of the species can be preserved.

Keywords: Palearctic - Last - Glacial - Maximum MaxEnt - Climate change - Species distribution model

Introduction

Climatic changes during the Quaternary have resulted in periodical range restrictions and expansions in most temperate species. During the Last Glacial Maximum (LGM) they have been constrained into southern European refugia, i.e. mostly to climatically sheltered parts of South-European peninsulas (e.g. Reinig 1950; de Lattin 1967; Bennett et al. 1991; Hewitt 2000). Later, during postglacial warming, these taxa were able to advance northwards following general expansion tracks (paradigms) (e.g. Hewitt 1996; Taberlet et al. 1998; Hewitt 1999; Schmitt 2007) with the exception of several sedentary species. Recent surveys propose a number of different possibilities for survival, e.g. in “cryptic” or “extra-Mediterranean” refugia suggesting that the former idea of the LGM landscape in Europe of being largely treeless needs to be revised. (Stewart and Lister 2001; Willis and van Andel 2004; Schmitt 2007; Provan and Bennett 2008; Svenning et al. 2008; Varga 2010). Although some repetitive patterns have been recognised, it became obvious that the responses of a species might be rather specific (Stewart et al. 2010) and may also depend on habitat preference of the species in question (Bhagwat and Willis 2008). It follows that it is necessary to apply different climate modelling approaches to predict likely refugia of temperate plants and animals (e.g. Benito Garzón et al. 2007; Leroy and Arpe 2007; Waltari et al. 2007; Svenning et al. 2008; Fløjgaard et al. 2009) and estimate the degree to which species have been able to track climate during the late glacial and postglacial periods (Normand et al. 2011). These models might provide predictions on possible range changes as consequences of recent and future climate changes.

Predicted effects of current global climate change on various ecosystems and taxa have recently been reported (Walther et al. 2002; Root et al. 2003; Parmesan 2006). Climatic hypotheses consistently forecast extinction and colonization events, poleward shifts of latitudinal ranges and upslope shifts of elevational components of distribution areas in many species with fragmented distribution ranges (Loarie et al. 2009; Jackson and Sax 2010). As significant changes in land use have been documented in the past two decades (de Chazal and Rounsevell 2009), interactions between climatic drivers and range fragmentation might lead to heavy population declines and increase the risk of extinction in threatened animals (Dawson et al. 2011).

Butterflies represent a group of invertebrates with increased levels of climatic responsiveness including a number of taxa vulnerable to the combined effects of climatic processes and human-induced habitat fragmentation (Cormont et al. 2011). Thus, butterfly species are especially advantageous to test the consistency of long-term climatic predictions and to identify the importance of various climatic predictors in driving distributional changes (Wilson et al. 2009).

Recently, climatic scenarios have successfully been used in investigating the impacts of global change on biodiversity (Nakićenović et al. 2000; Heller and Zavaleta 2009). Additionally, this approach yielded consistent patterns in climatic effects across various ectotherms and biogeographical regions (Deutsch et al. 2008). Specifically, climate modelling has been applied for studies on risk assessment and behavioural adaptations of butterflies (Settele et al. 2008; Tobin et al. 2008).

Modelling species distribution is a principal problem in conservation biology (Phillips *et al.*, 2004). In the last two decades, many developments have been observed in the field of species distribution modelling with the availability of multiple methods (Elith et al. 2006). One of the most popular approaches is the MaxEnt software which applies a presence-only niche modelling technique based on the maximum entropy principle (Phillips et al. 2006). MaxEnt’s predictive performance is consistently competitive with the highest performing methods (Elith et al. 2006).

In this study Species Distribution Modelling (SDM) was applied to predict the potential distribution of the little known species *Melitaea ornata* Christoph, 1893 (syn=*Melitaea telona* Fruhstorfer, 1908). The species is not included in the Climatic Risk Atlas of European Butterflies (Settele et al. 2008) missing from the Distribution Atlas of European butterflies (Kudrna et al. 2011) and mentioned as Data Deficient species in the European red list of butterflies (van Swaay et al. 2011).

The main focus of this investigation was to (1) predict the potential distribution of *M. ornata* using climatic models to (2) identify the possible refugia during the Last Glacial Maximum and compare these with the results of morphometrical studies and to (3) compare the consistency of models predicting future distribution areas for 2080 and derive conclusions for climatic risk assessment.

Material and methods

Study species

Knowledge on *Melitaea ornata* Christoph, 1893 (syn. = *Melitaea telona* Fruhstorfer, 1908; (see: Tóth and Varga 2011) has been improved significantly in the past few years. The species rank of *M. ornata* has only been recognised recently (Russell et al. 2005; Russell et al. 2007) supported by DNA (Leneveu et al. 2009), enzyme polymorphism (Pecsenye et al. 2007) and genitalia morphometric investigations (Tóth and Varga 2010; Tóth and Varga 2011). Its separation from other closely related species is possible based on morphological characters of caterpillars (Russell et al. 2007), genitalia, antennae, and wing pattern (Varga 1967; Tóth and Varga 2011).

Up to now, this species has been found in Sicily, southern part of the Apennine Peninsula, Balkan Peninsula, Carpathian Basin, Anatolia, Levant, Kopeth Dagh and Elburz Mountains in Iran, Podolian Plateau in Ukraine, Volgograd region and southern Urals in Russia and in the eastern part of Kazakhstan close to the Chinese border. (Hesselbarth et al. 1995; Korshunov and Gorbunov 1995; Russell et al. 2005; Russell et al. 2007; Kuznetsov 2010; Tóth and Varga 2010; Tóth and Varga 2011; Jakšić 2011; Russell and Pamperis 2011). Although it has been demonstrated that *Melitaea ornata* is rather a Ponto-Mediterranean-Turkestanic species than a ponto-Mediterranean one, there are many unanswered questions about its distribution still remaining.

Melitaea ornata is a mono- or oligophagous species feeding on different Asteraceae, mainly on regionally different, sometime endemic *Centaurea* species (Russell et al. 2007). However, according to recent data, the species feeds exclusively on *Cirsium pannonicum* in the Carpathian Basin (Varga 1967; Varga et al. 2005; Tóth et al. 2011).

Four morphotypes were found in *M. ornata* based on genitalia morphometry: (1) 'Western type': including the Italian, Hungarian and SW Ukrainian populations; (2) 'Eastern type': with a much extended distribution ranging from the Balkan Peninsula through Asia Minor to Kazakhstan; (3) 'The Levant type'; (4) 'Northern Iranian type' (Kopeth Dagh and Elburz Mts.). The distribution of these morphotypes can be attributed to historic separation in distinct glacial refugia (Tóth and Varga 2011).

Presence points

In this study, 255 non-duplicate observations were used, with maximum one record in each 2.5 arc minutes cell (**Fig. 1a**). Most of these data are originated from the Hungarian Natural History Museum, Collection of the University of Debrecen and the Zoological State Collection in Munich. Records from literature were also used if specimen photos or genitalia were available or the head capsule colour of the fourth state or older caterpillars were mentioned.

Additionally, internet records were used if the pictures were suitable for identification and the exact collection site was known (**Table 1**).

Country	**Validation based on**	**citation**
Italy	larva head capsule colour	(Russell *et al.*, 2007)
Serbia	genitalia photo	(Jakšić, 2011)
Greece	larva head capsule colour	(Russell *et al.*, 2007; Russell & Pamperis, 2011)
	picture from specimen	http://www.euroleps.ch/seiten/s_art.php?art=nym_telona
Turkey	photo from specimen	(Hesselbarth *et al.*, 1995)
Syria	larva head capsule colour	(Russell *et al.*, 2007)
Israel	larva head capsule colour	(Russell *et al.*, 2007)
Russia	larva head capsule colour photo from specimen photo from genitalia	(Gorbunov & Kosterin, 2007; Kuznetsov, 2010, 2011)

Table 1. Presence data of *Melitaea ornata* from literature and the internet.

Statistics

Information on current climate was obtained from the WorldClim database, version 1.4, which is based on weather conditions recorded from 1950 to 2000 with a grid cell resolution of 2.5 arc minutes ((Hijmans et al. 2005); http://www.worldclim.org).

Paleoclimatic simulation data of the LGM (Last Glacial Maximum ca. 21000 BP) with a resolution of 2.5 arc minutes were downloaded from WorldClim (http://www.worldclim.org/past) where derived PIMP2 data (Braconnot et al. 2007a; Braconnot et al. 2007b) are available.

For projections under future global warming scenarios, a set of different families of emission predictions were formulated by the IPCC based on the future production of greenhouse gases and aerosol precursor emissions. For projections under future global warming scenarios, a set of different families of emission scenarios was formulated by the IPCC based on the future production of greenhouse gases and aerosol precursor emissions. Future climate scenarios were also obtained from the WorldClim database (www.worldclim.org/futdown.htm). Three climate models were used for the years 2080 developed by (1) the Canadian Centre for Climate Modeling and Analysis (CCCMA), (2) Hadley Centre Coupled Model v3 (HadCM3) and (3) Commonwealth Scientific and Industrial Research Organization (CSIRO). Each of these climate models project two emission scenarios predicting conservative (a2a) and liberal (b2a) estimations. All climate models and the associated emission scenarios were analysed independently.

Based on the biological background information of the species, six BioClim variables (Busby, 1991) were pre-selected and used for model fitting, as follows.

As the studied species is a Mediterranean one for which warm and dry summers could be crucial, mean temperature of late spring and the summer (Bio 10) might be important environmental predictors. Further, winter coldness seems to be less important as a limiting factor in shaping the distribution range (when caterpillars reach a critical weight they start to aestivate independently from the environmental conditions). In this period caterpillars are very resilient and can survive even in -18 C° (unpublished laboratory experiment (Tóth *et al.*)).

Since *M. ornata* prefer semidry and dry habitats due its food-plant preference and its life-cycle adapted to summer aridity we think this species might be sensitive to humidity. Thus, precipitation of the wettest quarter of the year (Bio16) might be a plausible predictor, as it excludes areas with permanently higher humidity levels where the species is not able to survive.

Precipitation of the warmest quarter (Bio18) is used to identify regions with arid summers. Mean temperature of the wettest quarter (Bio8) and precipitation seasonality (Bio15) are added to fine-tune of the effects of Bio18 and Bio16 (for example how balanced is the distribution of the precipitation across the year).

To control for the presence of hilly- and mountainous regions in the distribution area, altitude was also used in model fitting.

Pearson correlations were used to test the relationships between selected variables using ENMtools 1.3 (Warren et al. 2010).

Default settings were used when running MaxEnt 3.3.3k (http://www.cs.princeton.edu/ _shapire/Maxent). MaxEnt solutions are affected by landscape (region) used for the background sample (VanDerWal et al. 2009). The western and central part of the Palearctic region was used as background layers since our interest was limited to this region. The output grid is on logistic scale with each pixel value representing the estimated probability that the species can be present in that pixel (Phillips and Dudík 2008). Although all models were fitted on the complete data set, a 10-fold cross-validation method was also employed to estimate errors around fitted functions. Models were fitted and projected onto past and future climates.

To generate a sampling bias grid, the Hawtools package of ArcGIS and SPSS were used. The weighting surface was based on the species records weighted by a Gaussian kernel with a standard deviation. Methods for obtaining this grid are detailed in Elith et al (2010) which makes the sampling bias counterbalanced.

When projecting, the “fade by clamping” function was used to treat variables outside the training range as if they were at the limit of the training range.

Further, the DIVA-GIS software environment was used to evaluate the impact of climate change on the distribution of the studied species. Applying threshold rule (10 percentile training presence), presence (1) and absence (0) rasters were produced. To provide future predictions, presence values have been changed from 1 to 2 followed by grid overlaying (subtract method) which results in four possible situations for each cell: i. *High impact areas*: areas where a species potentially occurs in the present climate but which will not be suitable any longer in the future. ii. *Areas outside of the realized niche*: areas that are neither suitable under current conditions nor under future conditions (as modelled). iii. *Low impact areas*: areas where the species can potentially occur in both present and future climates. iv. *New suitable areas*: areas where a species could potentially occur in the future, but which are not suitable for natural occurrence under current conditions (Scheldeman and Zonneveld 2010). Based on these results we could calculate the area change as follows: $\mathbf{A_n}\% = \mathbf{A_n} / \mathbf{A_p} \times 100$, $\mathbf{A_d}\% = \mathbf{A_d} / \mathbf{A_p} \times 100$, $\mathbf{A_s}\% = \mathbf{A_s} / \mathbf{A_p} \times 100$ where $\mathbf{A_n}$ is the new suitable areas, $\mathbf{A_p}$ is the suitable areas in the present, $\mathbf{A_d}$ is the area decrease and $\mathbf{A_s}$ is the stable areas.

Geometric morphometric data on the processus posterior of male genitalia from Tóth & Varga (2011) were analysed using geographic information system (GIS) based method. Consequently, discriminate analysis was carried out and values of the first axis were interpolated using inverse distance weighting (IDW) (see: (Dapporto and Bruschini 2012) The interpolation was made by QGIS 1.7.

Results

SDM

Correlation levels between preselected variables were less than the critical level ($r^2 < 0.75$) for all cases. The models received substantial support (mean AUC=0.918, standard deviation= 0.021) values following the definitions of Sweets (1988).

Based on the analysis of variable contributions Bio10 (mean temperature of the warmest quarter), altitude and Bio16 (precipitation of the wettest quarter) were the most important factors defining distribution on Palearctic scale. The jackknife test of variable importance showed that Bio10 appears to have the most useful information by itself while Bio16 appears to have the most information not included in other variables (**Table 2**).

variable	percent contribution	permutation importance	training gain without	training gain with only	min	max	mean	standard deviation
altitude (m)	28.6	15.6	0.787	0.394	18	2880	973.1	602.3
Bio08 (C°)	1.5	2.2	0.85	0.179	-2.8	22.4	8.8	6.5
Bio10 (C°)	30.6	34.1	0.78	0.395	10.9	27	20.2	2.8
Bio15 (mm)	2.1	6.1	0.834	0.144	12.8	116.7	51.1	20.8
Bio16 (mm)	27.7	36	0.738	0.231	74	704	241.6	102.4
Bio18 (mm)	9.4	6.1	0.844	0.252	0	280	85.7	72.7

Table 2. Variables have been used in modelling. Bio 10 (mean temperature of the warmest quarter), altitude and Bio 16 (precipitation of the wettest quarter) were the most important variables. The training gains show the results of jackknife test.

Present distribution

The predicted potential distribution fits well the known occurrence data with new potential localities appearing. The supported model shows that temperate localities inside the distribution area are less suitable for the species (**Fig. 1**).

The model predicts the Iberian Peninsula to be a highly suitable area for the species. Further, our model predicts suitable areas in South-Eastern France including Provence, southern Germany, eastern Austria, southern Slovakia and southern Ukraine with the Krim-peninsula. So far, there are no occurrence data from these localities.

Although, only few occurrence data are known from the eastern border of Kazakhstan but the model predicts large areas which climatically suitable in Altai, Tarbagataj Mts., Dzhungarian-Alatoo near Alma-Ata. The model also predict suitable areas to the lower parts of the Kuh-e-Baba (less than 2600 m) in Afganistan, North-Western Pakistan, Eastern Uzbekistan, Western Tajikistan, Western Kyrgyzstan (Tien-Shan, Alai, Kyrgyz-Mts. and Talass-Alatau).

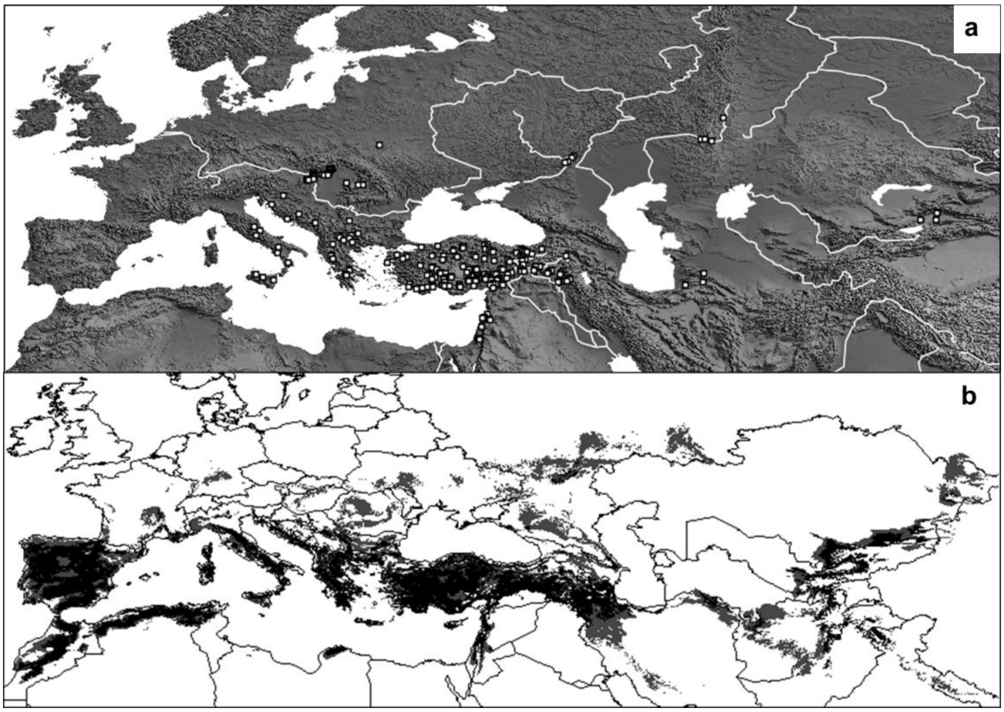

Fig. 1. Occurrence points and the predicted present distribution. (a) 253 presence localities (white squares) from the Palaearctic region used in the modelling process. (b) The potential distribution of the *Melitaea ornata*: lighter colours indicate less suitable areas.

Potential refugia during the LGM

IDW interpolation revealed a pattern very similar to that of UPGMA tree (**Fig 3a**), where four groups the “Western”, “Eastern”, “Iranian” and the “Levant” types are to be recognised (**Fig. 2)**.

Refugia predicted by model results are situated in the southern part of the Apennine Peninsula and Sicily, southern Balkan Peninsula, Asia Minor, the

Levant region, the Elburs Mts. and the Northern Zagros in Iran (**Fig. 3b**), which is also well supported by recent distribution and taxonomical subdivision. Model predictions include less probable refugia in the Iberian Peninsula, North Africa and Central Asia.

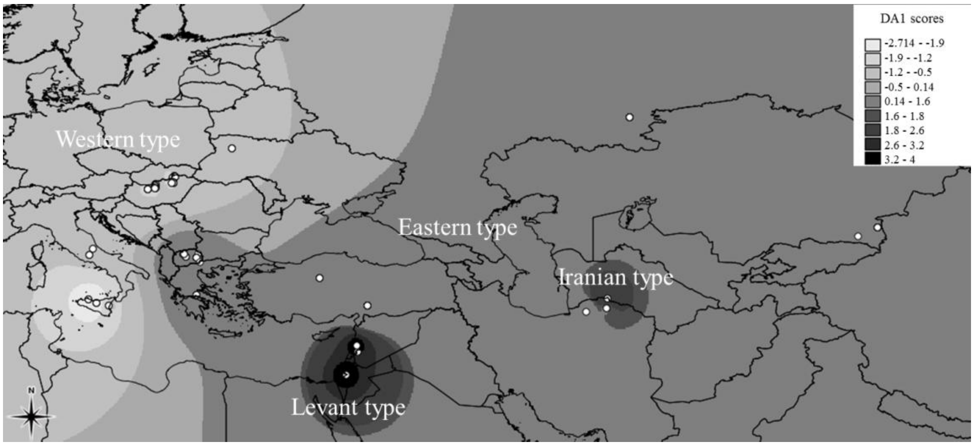

Fig. 2. Inverse distance weighting (IDW) interpolation of first axis of discriminant analysis. The first axis can explain 51.7% of the total variance between groups. Four main group visible on the map: Western, Eastern, Levant and North Iranian group.

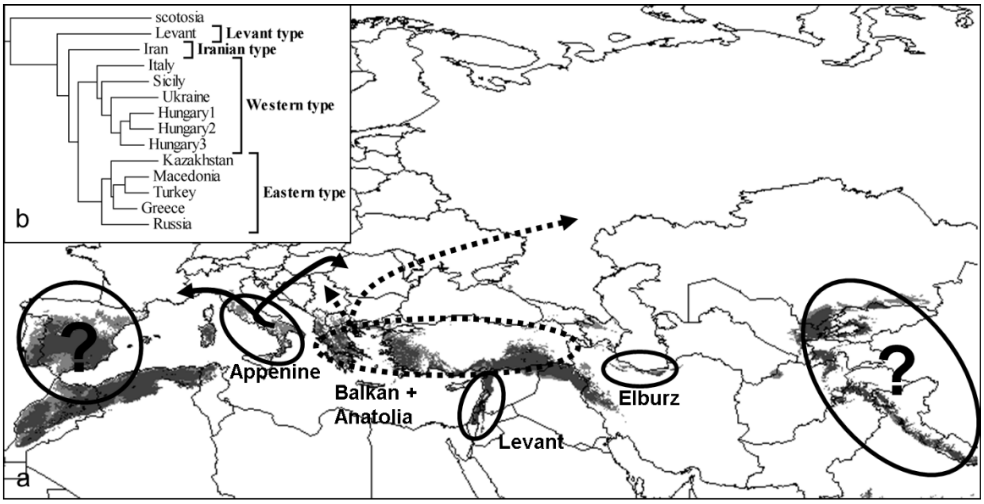

Fig. 3. Potential refugees of the Last Glacial Maximum based on MaxEnt modelling (**a**) and the theoretical ways of the recolonisation based on the present distribution and the results of the genitalia morphometrics. The UPGMA tree (phenogram) (**b**) based on Mahalanobis distance, *Melitaea scotosia* used as out-group (based on Tóth & Varga 2011).

Predictions for 2080

All scenarios support the expansion of the potential area to North (**Table 3**) with a significant loss of the potential area changing from 30% to 63% depending on climatic scenario. As there are no occurrence data from the Iberian Peninsula and North Africa, these records were excluded when calculating the area of decrease/increase to get more realistic results. The most dramatic extinction is predicted in the Ponto-Mediterranean region (**Fig. 3**) and in Central Asia.

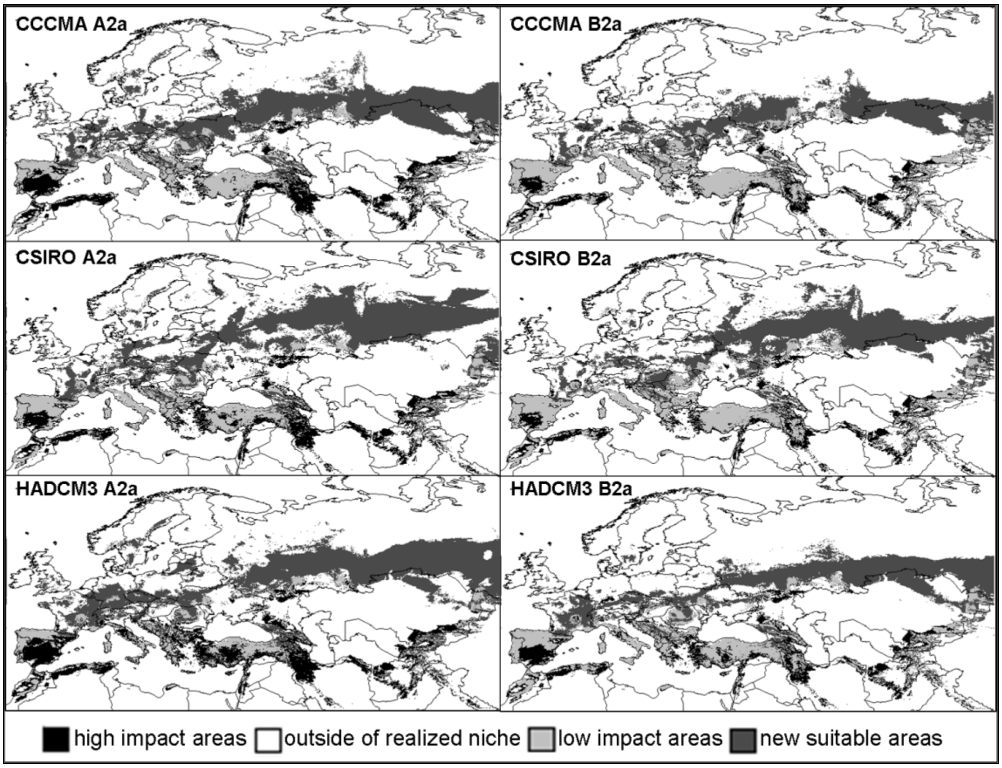

Fig. 4 The impact of climate change on the distribution of *Melitaea ornata* based on the different climate scenarios. **High impact areas**: areas where a species potentially occurs in the present climate but which will not be suitable anymore in the future. **Areas outside of the realized niche**: areas that are neither suitable under current conditions nor under future conditions (as modelled). **Low impact areas**: areas where the species can potentially occur in both present and future climates. **New suitable areas**: areas where a species could potentially occur in the future, but which are not suitable for natural occurrence under current conditions.

Trend of change	**Climate scenario for 2080**											
	CCCMA A2a		**CCCMA B2a**		**CSIRO A2a**		**CSIRO B2a**		**HDCM3 A2a**		**HDCM3 B2a**	
	area (km^2)	%	area (km^2)	%	area (km^2)	%	area (km^2)	%	area (km^2)	%	area (km^2)	%
decrease	1342483	-48	925378	-33	1420014	-50	1003378	-36	1781422	-63	1225010	-43
stable	1474566	52	1894106	67	1397469	50	1816359	64	1036079	37	1593914	57
increase	3994344	42	2987601	6	4194228	49	3868355	137	4198289	49	3301039	17

Table 3. The predicted area (km^2) of suitable habitat for *Melitaea ornata* from three different climate models; CCCMA, HadCM3 and CSIRO. Predicted changes in area for 2080 show the calculated areas of stable, increasing and decreasing habitat compared to current climate model. The Iberian Peninsula and northern Africa were excluded when calculating (see in discussion).

Discussion

In this study we predict potential distribution areas for *Melitaea ornata* on three different time scales. Our model predictions can be discussed in detail for present, past and possible future distribution ranges, as follows.

Present rage

Although the predicted range of *Melitaea ornata* for the present is larger than the known distribution (**Fig. 1**), new occurrence data are expected from southern Russia and Central Asia and also from some insufficiently surveyed parts of Europe, such as parts of the Balkan Peninsula or western Ukraine. Our model also predicts climatically suitable areas in southern France from where a single unreinforced occurrence data (one female) was reported from Provence (Russell et al. 2007) suggesting the need of further surveys in this region.

The lack of this species from the Iberian Peninsula is rather difficult to explain. However, we suggest two approaches: **(1)** a historical explanation considering the inaccessibility of this area for *Melitaea ornata*, and alternatively **(2)** a recent ecological explanation hypothesising the absence of this species due to extinction. Several phylogeographical surveys have shown (Hewitt 1996; Taberlet et al. 1998; Hewitt 1999; Schmitt 2007) that the Pyrenees functioned as an effective barrier both for the expansion of many Iberian taxa and also for blocking the immigration of central and/or eastern European species. The connection between the Iberian Peninsula to north-western Africa via Gibraltar can also be excluded, as the Maghreb region is populated by the closely related *Melitaea punica* Oberthür, 1876, which is also missing from the Iberian Peninsula. In addition, results of an extended survey of European plant species (Normand et al. 2011) demonstrated that for one-sixth of the surveyed species not climate but accessibility was the strongest predictor of the recent range. Accessibility could particularly be important for species with e.g. limited long-distance dispersal ability, southern glacial ranges and for small-range species of southern Europe. *Melitaea ornata* can meet most of these criteria, being an oligophagous or food-plant specialist and as a ponto-Mediterranean-Turkestanic species connected to xerothermic grasslands and forest-steppe habitats. However, the alternative explanation might also not be excluded. In sum, we think that the historical explanation of inaccessibility has more *pro*'s than the more hypothetic extinction driven by climate change.

Glacial refugia and range changes

The next issue is the predicted retreat of *M. ornata* during LGM into isolated refugia and its footprints in recent geographical and taxonomical patterns (Tóth and Varga 2011). The genitalia are considered by Dapporto (2011b) as neutral genetic marker with respect to ecological influence.

The colonisation history of a species could be very complex including repeated retreat, expansion and replacement as it has been shown in *Melanargia galathea* (Habel et al. 2011) or in *Maniola jurtina* (Dapporto and Bruschini 2012; Dapporto et al. 2011a). In these species genetic and/or morphological data supported recent invasion of a linage which modified the original re-colonisation pattern. As in the present study the geographical pattern of genitalia morphometrics do not indicate such events the simple explanation seems to be more probable ('Occam razor'). Although, more data are needed especially from the Eastern part of the distribution to get more detailed information, but we believe the quiet stable and clear patterns allow us to conclude general implications.

We follow here the suggestions of Stewart et al. (2010) stating that Quaternary refugia should be defined as "the geographical region or regions representing the species' maximum contraction in geographical range", in our case during the LGM. This concept applies to our case clearly expressing that species, adapted to different climatic conditions, in general respond to climatic changes independently of each other (Hewitt 1996; Taberlet et al. 1998; Bhagwat and Willis 2008; Stewart et al. 2010). Large parts of the predicted refugia are also populated mostly by strong populations of *M. ornata* (e.g. in the southern part of the Balkan Peninsula, nearly the whole Asia Minor and Levant, etc.). On the other hand, the information is scarcer on a number of distant predicted refugia in Central Asia. The results of genitalia morphometrics support the existence of four different refugia in the Apennine Peninsula, Balkan Peninsula and Anatolia, Levant and the Elburs Mts. in Iran (**Fig 3**). As separate refugia in southern Russia and Central Asia have not been found in morphological patterns of genitalia, more data are needed to clarify this question.

Similarly, larval morphology shows definite geographical trends: while caterpillars from southern Italy and Hungary have the same monomorphic colouration of hibernated larvae and characterised by brick red head capsule, unicolorous black body without ochreous or brownish thorns or stripes, while those of southern Balkans, Levant and southern Russia are more polymorphic (Varga et al. 2005; Varga 2007; Russell et al. 2007; Kuznetsov 2011).

Combining the results of genitalia morphometrics with those of species distribution modelling, four main refugia are supported: the populations of the Levant and the Elburs Mts. were not able to expand significantly, unlike the cases of Apennine Peninsula, Balkan Peninsula and Anatolia.

The postglacial re-colonisation of the Carpathian Basin from south-west is well supported not only by recent distribution data but also by genitalia morphometrics. It is noteworthy that similar conclusions were drawn also for the phylogeography of the Chalk-hill Blue, *Polyommatus coridon* (Schmitt and Seitz 2001; Schmitt and Seitz 2002) and similar patterns can be hypothesised for several xerothermic, south-eastern European butterflies, such as *Spialia* *orbifer*, *Polyommatus thersites, Brenthis hecate, Arethusana arethusa* which are fairly common in the western and central hilly regions of the Carpathian Basin but more scattered in its eastern part (Varga et al. 2005; Varga 1995). Similar patterns have been outlined for some orchid species e.g. *Ophrys bertolonii*, *O. flucifora* and *Hymantoglossum adriaticum*. Obviously, the range of *M. ornata* is much scantier but it is not only climatically limited but also constrained by the patchy distribution of suitable habitats. These constraints might also restrict the survival and expansion chances.

South-East Europe was probably re-colonized mainly from the Balkan Peninsula and Anatolia. These populations seem to be the most expansible since morphometric results indicate that the populations in southern Russia and eastern Kazakhstan possibly originated from this region (**Fig 3**). Similarly, several steppic (including xeromontane) species widely distributed in Asia Minor, Iran and often also in Central Asia (e.g. *Euxoa basigramma, Dichagyris squalorum, D. vallesiaca, Chersotis elegans, Ch. capnistis, Eugnorisma chaldaica, E. ignoratum*) have also cumulated occurrences in Lower Volga and Southern Urals regions (Fibiger 1990, 1997; Varga 2010; Varga 1996).

The future of Melitaea ornata: Implications for conservation

Predictions for 2080 imply important species-specific conservation issues. Although climate change scenarios contain uncertainties (Beaumont et al. 2007) we can use them to formulate hypotheses for future conservation planning, already demonstrated to be effective in several cases (e.g. Davies et al. 2006; Willis et al. 2009). Although climatic scenarios consistently predict northward shifts of the potential areas the distributions of these new suitable patches are not equally distributed in space. To analyse this pattern we have to take the accessibility (Normand et al. 2011) into consideration. Populations in marginal (and sub-optimal) positions are likely to expand northwards in the future, especially in the steppic and forest-steppic areas of Russia and Ukraine. In Central and Western Europe two core areas could be very important: the not confirmed (but highly possible) southern French and the Hungarian populations. Clearly, climate change alone cannot drive area expansions and other factors could be very important (Menéndez et al. 2007). Further, it is evident that suitable habitats are also necessary for the species in accessible distances for spreading. The Hungarian subspecies *Melitaea ornata kovacsi* Varga, 1967, which is a well-studied and protected butterfly, serves as a good example, being food plant specialist and associated to dry and semidry grasslands. Although our predictions clearly forecast increasingly favourable climates in Hungary, *M. ornata kovacsi* populations are declining in most part of the country, (Tóth et al. 2011) probably driven by habitat loss and not by climate change. In summary, however, northward area shifts predicted for the future are not possible without the effective protection of suitable habitats and potential core populations of this species.

Acknowledgements

Thanks for Gennadiy V. Kuznetsov for the useful information and the coordinates form the Volgograd region (Russia). We thank to Dr. Axel Hausmann (Zoological State Collection in Munich), Dr. Zsolt Bálint (Hungarian Natural History Museum) for borrowing valuable museum specimens for our studies. Useful suggestions and corrections of Leonardo Dapporto and an anonymous referee are highly appreciated.

The survey was supported by the OTKA (K75696).

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Study IV: Dispersal ability and habitat selection in *Melitaea telona*[2] kovacsi Varga, 1967 (Lepidoptera: Nymphalidae) in steppe grassland

Tóth JP, Bereczki J, Spring N & Varga Z (2011) Dispersal ability and habitat selection in *Melitaea telona kovacsi* Varga, 1967 and *M. phoebe* (Denis & Schiffermüller, 1775) (Nymphalidae) in steppe grassland. *Nota Lepidopterologica* 33 (2):199-207.

Abstract

Melitaea telona is a protected species in Hungary. The known range has shown significant regression in the last decades. In order to conserve this species it is important to have information on its dispersal ability and movement patterns. Hence, mobility of *M. telona* was studied using capture-recapture methods. 307 *M. telona* and 139 *M. phoebe* individuals were marked and 12% recaptured during May 2009 in three study areas with ten sample sites. We estimated the densities of the larval food plant (*Cirsium pannonicum*) and of the most important nectar source (*Dianthus pontederae*). *M. telona* is moderately localized: the species is able to reach habitat patches several hundred metres distant. It tends to occur in high numbers where the food plant is abundant. In these sites, fewer *M. phoebe* were found, while there were higher numbers in the more degraded patches. Our data suggests that the density of the food plant is more important than the abundance of nectar sources in habitat selection by *M. telona*.

Keywords: capture-recapture, food plant, *Melitaea telona*, *Melitaea phoebe*, mobility, nectar source.

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[2] *Melitaea telona* Fruhstorfer, 1908 is a junior synonym of *M. ornata* Christoph, 1893 (see: study 2).

Introduction

Melitaea telona kovacsi Varga, 1967 is a protected forest-steppe species in Hungary. It lives only in habitats where *Cirsium pannonicum* grows in significant density. Evidence of museum specimens suggests that in the 1960‘s this species was widely distributed in the central and northern hills of Hungary. Nowadays it is known from only a few localities in the Budai Hills, the region of the Aggtelek-Karst and the Borsodi Hills. Maybe the most drastic decline has been observed in the Bükk Mts. There are many museum specimens from this region, but in the last few years we could not confirm the species' presence. The Aggtelek region seems to be the only locality where this species has still strong populations and has any chance of a long term conservation (Varga 2007; Varga et al. 2005). In this region there are many suitable habitats for the species in close proximity to each other. The situation in the Bükk Mts. is totally different where the known suitable habitats are sometimes tens of kilometres apart.

Habitat isolation depends on both the hostility of the environment in between and the organism's gap-crossing ability (Ricketts 2001; Schmitt et al. 2000; Tischendorf and Fahrig 2000; Wratten et al. 2003). Thus to understand isolation, one must consider the distance between all potential source populations and also the landscape between the habitat fragments. Landscape connectivity does not need to imply structural connectivity, but rather functional connectivity. Overall, depending on the species concerned, the landscape context may facilitate or impede movements of insects between habitat patches (Tscharntke and Brandl 2004). The movement ability of the species can vary considerably even within a relatively small group like butterflies (Scott 1975).

We have carried out a capture-recapture survey on *Melitaea telona kovacsi*. Our questions were: how localized are the populations, and how strong is the connection between the habitat patches at different distances apart? In addition we were interested in other basic biological concerns: what plant species are the main nectar sources? What is the relationship between the supply of the larval food plant and the population size?

Material and methods

Target species. *M. telona* Fruhstorfer, 1908 is distributed in the Ponto-Mediterranean belt. Its range extends from the eastern part of the Mediterranean Sea coast across Asia Minor to the north-western part of Balkan Peninsula and southern Italy including Sicily. The subspecies in the Carpathian basin has become isolated from the main distribution area and it has patchy habitats. In Hungary the only known food plant is *Cirsium pannonicum* while in other regions it feeds on different types of Asteraceae, mainly on *Centaurea* species, mostly local endemics (Russell et al. 2007). The species has one brood, flying from mid-May to mid-June. The females lay their eggs near to the ground on the undersides of the food plant leaves. The young caterpillars live in a web, spreading out over the plant as they feed, leaving only the upper epidermis remaining. When the warmer part of the summer arrives, the caterpillars go into a diapause and overwinter in this, the third instar. The next spring they continue feeding, gregariously at first, but later solitarily. When they are fully developed, they start to pupate in the grass litter. The imagos emerge one or two weeks later (Varga et al. 2005).

Melitaea phoebe ([Denis & Schiffermüller], 1775) has the biggest distribution in the tribus Melitaeini, with many described subspecies. Its range is nearly continuous, extending from North Africa over Eurasia to the Far East. It is widely distributed in Hungary, and was found at all of the sample sites. It is known to use several larval food plant species: *Centaurea, Carduus, Cirsium* spp. and is specialised to absorb irido-glycosids from them like all member of the tribus Melitaeini (Wahlberg 2000). It is possible to feed the caterpillars with *Cirsium pannonicum.* Moreover, we collected young caterpillars from this plant and later realised they were *Melitaea phoebe*. Generally it is only possible to separate the caterpillars once they have reached the 4th instar, based on the coloration of the head capsule. *M. phoebe* has a black and *M. telona* has a brick red larval head capsule (Russell et al. 2007). The young caterpillars feed together like *M. telona,* but *M. phoebe* has two broods. The first brood flies nearly at the same time as *M. telona* and the second flies from the end of June/beginning of July to the end of August/beginning of September. To summarise the comparison of these two species, we can say that while *M. phoebe* is a widely distributed euryoecious species, *M. telona* is a narrowly distributed food plant specialist.

Even though these two species are very similar; we are able to separate them with a good degree of accuracy based on the morphology of wings and the shape of the tips of the antennae (Varga 2007; Varga et al. 2005).

Data sampling. Our study areas were in three well-known *M. telona* localities: Szőlőhegy near to Jósvafő, and Zabanyik and Borház-tető between Tornakápolna and Varbóc in the Aggtelek-Karst area. The vegetation of the study areas belongs to the alliance *Polygalo majori-Brachypodietum pinnati.* The choice of these study areas was based on three main considerations: the presence of the focal species, a variety of distances between the sample sites, and a variation in the density of the food plant in the different sites.

The Szőlőhegy study area has been extensively cultivated for a long time, most typically for fruit production, using widely spaced fruit trees with seasonally mown herbaceous vegetation. Thanks to this sensitive management, the vegetation on this hill has become very similar to that of forest steppe. We had five sample sites here.

A: is good condition steppic grassland with *Stipa tirsa*, *S. joannis* and *Iris pumila*. *Cirsium pannonicum* has a high density here. It is rich in dicots (*Jurinea mollis*, *Inula ensifolia*, *Polygala major*, *Scorzonera purpurea*, many tall forb Asteraceae and Apiaceae etc).

B: with degraded vegetation, dominated by grasses. There is little evidence of the food plant here.

C: formerly used as a forestry hayfield. Nowadays, this site is mown irregularly by the staff of the national park. It is characterized by many different grass and herbaceous species, including some tall forbs and polycormon-forming species (*Echium maculatum*, *Peucedanum cervaria*, *Centaurea* spp., and *Inula hirta*, *I. ensifolia*, *Thymus* spp., *Dorycnium germanicum*).

L: is cut every year. Some Fabaceae are abundant here, e.g. *Onobrychis arenaria*, *Hippocrepis comosa* and *Vicia tenuifolia*.

N: is a slightly bushy patch, poor in dicotyledonous plants.

On the Zabanyik hill we can see the remains of fruit trees, indicating its former use as an orchard, similar to the Szőlőhegy. Compared with the Szőlőhegy, this hill is drier and warmer. Probably the species-rich patches are edaphically treeless. However, close to the top we can see some old Italian pubescent oaks (*Quercus virgiliana*) with a species-rich fringe vegetation (*Anemone sylvestris*, *Cytisus procumbens*, *Dracocephalum austriacum*, *Euphorbia polychroma*). We had three sample sites here.

G: This site is very near the oak forest on the south-east slope of the hill. It is a species-rich semi-natural grassland.

H: Facing Szőlősardó on the south-west slope of the hill, this site is drier and a little poorer in species. *Stipa joannis* and *S. tirsa* are characteristic here.

I: is very bushy and poor in species. The south-east and north-east parts border *Pinus nigra* plantations.

The Borház-tető used to be a fruit production area too. Nowadays we can only see a few relict vines which bear witness to this activity. The north-east slope with *Bromus erectus* has been burned every year. We chose two sample sites here on the western slope very close to each other:

J: is a slightly bushy and dry patch with clastic soil. Some parts of this are very rich in *Stipa tirsa* and *S. joannis*.

K: is near to the forest with many bushes; the dominant grass species is *Brachypodium pinnatum*.

Methods. Our data were collected through capture-recapture surveys on three or four occasions (Table 1.) in 2009 May 15–25 from ten sample quadrats (sample sites) with dimensions 50 × 40 m. We worked on five sites simultaneously between 9:00 and 13:00 for 3 hours, only in good weather conditions. Captured butterflies were marked with a water resistant XF marker pen. We wrote a code on the underwing, consisting of a letter and a number. This code enabled butterflies to be recognized on recapture and their movement pattern deduced. The following information was registered on the field sheets: the sex, the species, and the activity before capture (or recapture). This last is important in identifying nectar sources. As we mentioned, *M. phoebe* and *M. telona* are very similar, so we re-identified all specimens on recapture to double-check. Misidentifications were not noticed. From the daily distribution of the marked animals we concluded that the sampling was made at the second part of the flight period (Table 1). It may have been better if we had started the survey before the peak of the flight period. However, it came earlier than we had anticipated because of the hot and dry weather conditions.

The coordinates of the sample sites were measured with GPS, and after it were matched with Google Earth to a satellite image. The middles of the sample quadrats were connected with lines and we measured these. The value was used as the distance between the quadrats. Movement patterns were drawn from the field sheets data.

After the capture-recapture survey, we estimated the density of *Cirsium pannonicum* and *Dianthus pontederae.* Five 2×2 m quadrates were marked randomly at each sample site, and the two plant species were counted in these sample sites. We calculated the density of plants per m^2. The relation between the observed numbers of butterflies and the density of the nectar source and the larval food plant were analysed using Pearson's correlation.

Results

307 *M. telona* and 139 *M. phoebe* were marked and recaptured 12% of telona and 14% of phoebe . The sex ratio was 56% male and 44% female in the case of *M. telona* and 70% male and 30% female in case of *M. phoebe* individuals.

The most *Melitaea* individuals were observed in the sites "A" and "G", and the least in sites "N" and "B" (Table 1 and 2). *M. telona* and *M. phoebe* were observed at every sample site but in very varying proportions (Figure 2). The biggest differences were in the Szőlőhegy. The ratio changed from 92% to 36%. The smallest differences were observed in the Borháztető 46% and 44%, respectively, but in this place the two sample sites were very close to each other. On the Zabanyik Hill this ratio varied between 93% and 77%, respectively.

	A		**L**		**N**		**B**		**C**	
date	t	ph	t	ph	t	ph	t	ph	t	ph
V.16	28	4	-	-	-	-	10	8	22	0
V.20	24	5	16	3	7	9	11	5	8	4
V.22	12	1	1	1	3	5	0	4	6	4
V.25	6	1	7	6	1	4	1	1	4	0
sum	70	11	24	10	11	18	22	18	40	8
	J		K		G		H		I	
	t	ph	t	ph	t	ph	t	ph	t	ph
V.15	14	13	9	1	27	0	-	-	-	-
V.18	11	22	8	11	15	9	8	2	13	2
V.21	3	5	4	0	15	4	6	0	4	3
V.25	1	1	0	1	2	0	0	0	0	0
sum	29	41	21	13	59	13	14	2	17	5

Table 1: Marked individuals of *M. telona* (t) and *M. phoebe* (ph) with date. Some of the sites we could not sample on the first day (-).

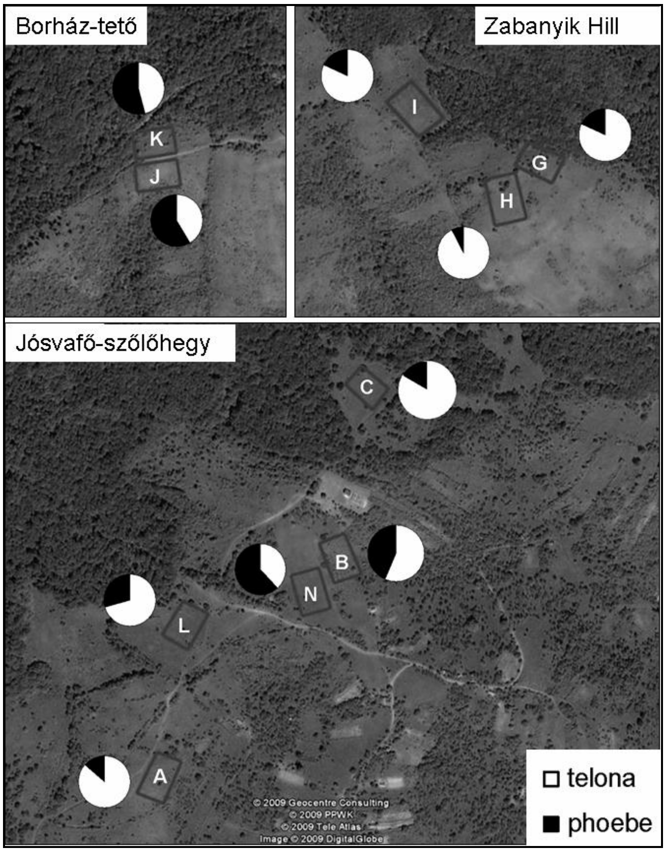

Figure 1: Topography of sample sites, as well as the observed ratios of *M. phoebe* (black) and *M. telona* (white).

Feeding *M. telona* individuals (51% males, 49% females) were observed on 64 occasions during the capture-recapture survey. This sex ratio is not significantly different from the sex ratio of the marked butterflies. Feeding was recorded 62 times on *Dianthus pontederae* (96%), once on *Ajuga reptans* and once on *Polygala major*.

We found a significant ($p<0.05$) but slightly different level of correlation between the density of *Dianthus pontederae* and *Cirsium pannonicum* and the observed number of individuals: $r=0.7$ for the nectar source, and $r=0.9$ for the larval food plant. The connection between the two sources is not statistically significant. If we compare the ratio of *M. telona* and *M. phoebe* with the density of the food plant we can see *M. phoebe* never has a significant ratio where *Cirsium pannonicum* grows densely (Table 2.).

	N ph	N t	C.p./m²	D.p./m²
A	7	42	18.9	6.2
L	10	24	5.4	3.2
N	18	11	2.2	1.75
B	10	12	0	1.05
C	8	18	5.35	1.85
J	28	15	0.15	1.3
K	12	12	4.75	0.05
G	13	32	18.65	4.95
H	2	14	4.95	2.55
I	5	17	0.35	6.75

Table 2: The sum of the observed *M. telona* (N t) and M. phoebe (N ph) individuals on the last three days, as well as the density of *C. pannonicum* (C.p.) and *Dianthus pontederae* (D.p.) in the sample sites.

From the 37 movement events discernible of *M. telona* we conclude that the individuals can fly several hundred metres (Figure 2). The longest registered distance was 420 m. The greater the distance between two sample sites, the less likelihood there is of observing flights between them. Naturally these distances are just approximate values, calculated from a map. However, as butterflies do not fly in straight lines, in reality their routes will surely be much greater than our estimated values.

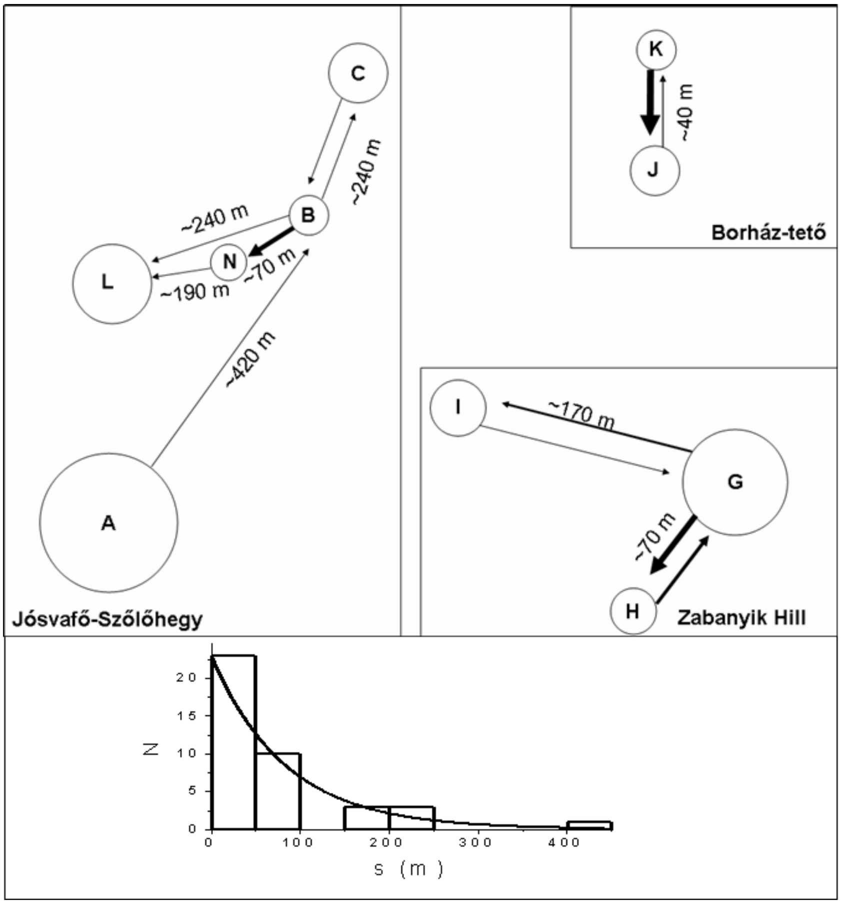

Figure 2: The observed movement events. The size of the circles is commensurate with the number of butterflies, and the thickness of the lines with the frequency of the movement events. The distances are the measured distances between the middles of the sample quadrats.

Discussion

The closely related *Melitaea cinxia* Linnaeus, 1758 can survive for 12–13 days in cloudy cool weather (Hanski et al. 2006). We get the same results in an air-conditioned laboratory environment, using overwintered *M. phoebe* and *M. telona* caterpillars collected from the sample sites in spring. These butterflies hatched in the laboratory, and were fed on *Spiraea* sp. flowers with a honey and water mixture as a supplement, and were able to survive more than 14 days at stable temperatures of 22 C°. During the field work, the warm, very dry weather probably reduced the butterflies' life expectancy. Because the intervals between samplings were usually 2 days (maximally 4 days), we think that the low level of recaptures cannot be explained simply by a high level of mortality caused by the dry weather. Rather by the mobility of butterflies – because all the sample sites are in large areas with suitable vegetation for *M. telona,* this species can easily move out from the sample sites. From our results we can see that the butterflies can move between patches several hundred metres apart. This ability could be very important for the long term survival of populations, especially in habitats which are subjected to deliberate partial or complete burning in spring. These fires can be extremely dangerous for *M. telona* – they could possibly kill all the individuals in a patch, because the species feeds, overwinters, and pupates in the litter at ground level. However if there are patches where the species can survive within flying distance, then recolonization is possible and the species will not necessarily become extinct.

An important question for the conservation of *M. telona* is its relationship with *M. phoebe*. In our survey, *M. phoebe* occurred at all the sample sites. Moreover, in some quadrats, greater numbers of *M. phoebe* were recorded than of *M. telona*. These were the sites with degraded vegetation. *M. phoebe* has the ability to develop on *Cirsium pannonicum* but interestingly *M. phoebe* tends to be found in lower numbers where that plant is abundant. We have information from studies of strong and stable populations over several decades. Almost certainly *M. phoebe* has always occurred in these places or nearby, but cannot displace *M. telona*. We think the main condition for the long-term survival of *M. telona* is the high density of the larval food plant.

An additional exciting question is the possibility of hybridisation. Enzyme electrophoresis has not shown any evidence of hybridisation between these two species (Pecsenye et al. 2007), but this does not mean that they do not mate. It is possible that the hybrids are sterile or have a lower viability (or perhaps they do not hatch from eggs). It might be that there is so-called ‘reproductive interference’ between these two species (Gröning and Hochkirch 2008) and that this could explain the extinction of *M. telona* from some of its former known localities and the continued occurrence *M phoebe* in these places. Further work is needed to clear up this question.

Our survey shows there is a strong connection between the target species observed numbers and the density of *Cirsium pannonicum* and *Dianthus pontederae*. In spite of the presence of many other flowering plants like *Campanula sibirica*, *Cytisus procumbens*, *Genista tinctoria*, *Helianthemum ovatum*, *Inula ensifolia*, *Lotus corniculatus*, *Onobrychis arenaria*, *Polygala major* and so on, feeding was recorded on mostly (96%) *Dianthus pontederae*.

Many plants like *Jurinea mollis*, *Centaurea scabiosa* or *Cirsium pannonicum* were flowering later and mostly in very low numbers because the extremely dry spring. We have recorded butterflies feeding on these plants several times in the last few years. So it could be a mistake to conclude from our findings this year that this species is a nectar source specialist – an impression supported by the fact that *M. phoebe* individuals were also only recorded on *D. pontederae*, when we know that this species also lives in habitats where pink flowers do not occur (for example several weedy associations). In order to get more complete information about the nectar sources, we should have to repeat the survey in a year with more average weather.

The situation with the larval food plant is totally different. The target species, *M. telona*, really is a specialist as we mentioned in the introduction. The species occurs only where *C. pannonicum* also occurs. One very interesting site is “I”, where the nectar source has a significant density ($6.75/m^2$) but the marked butterflies’ numbers were much lower than we expected (Table 2). The larval food plant also had a very low density here ($0.35/m^2$). If we delete this sample site from the dataset, we get a much stronger correlation ($p<0.01$, $r=0.892$) between nectar source and larval food plant, so this data is very valuable in our few samples because it indicates the secondary importance of the nectar source.

In conclusion, contrary to our expectations, *M. telona* has a greater mobility than we would have expected from its insular distribution and its specialisation (in nature) on one larval food plant. Future surveys will have to take this into consideration. If we want to increase the number of recaptures to get more accurate information about the population size or the dispersion ability, we must achieve a better coverage of the habitat with sample sites. It is better to choose habitats where there is a high density of food plants, not just because we observed the largest number of *M. telona* in these places but because we also observed the lowest number of *M. phoebe*.

Acknowledgement

Without the following people we would not have been able to carry out this survey. Wendy Astill, Karen Aylward, Tim Baker, Jonathan Bradley, Jess Chappell, Richard and Ann Collier, Sam Hyde-Roberts, Stephanie Rogers, Linda Meadows, Richard Muirhead, David Norfolk, and Tim Thomas all of whom worked very hard in the field. Thanks to Kathy Henderson the coordinator of the European Conservation Action Network, Attila Huber, Roland Farkas, and Sándor Boldogh, the members of Aggtelek National Park who helped us to organize the work. The survey was aided by the European Conservation Action Network, supported by the Leonardo da Vinci fund of the EU Lifelong Learning Programme.

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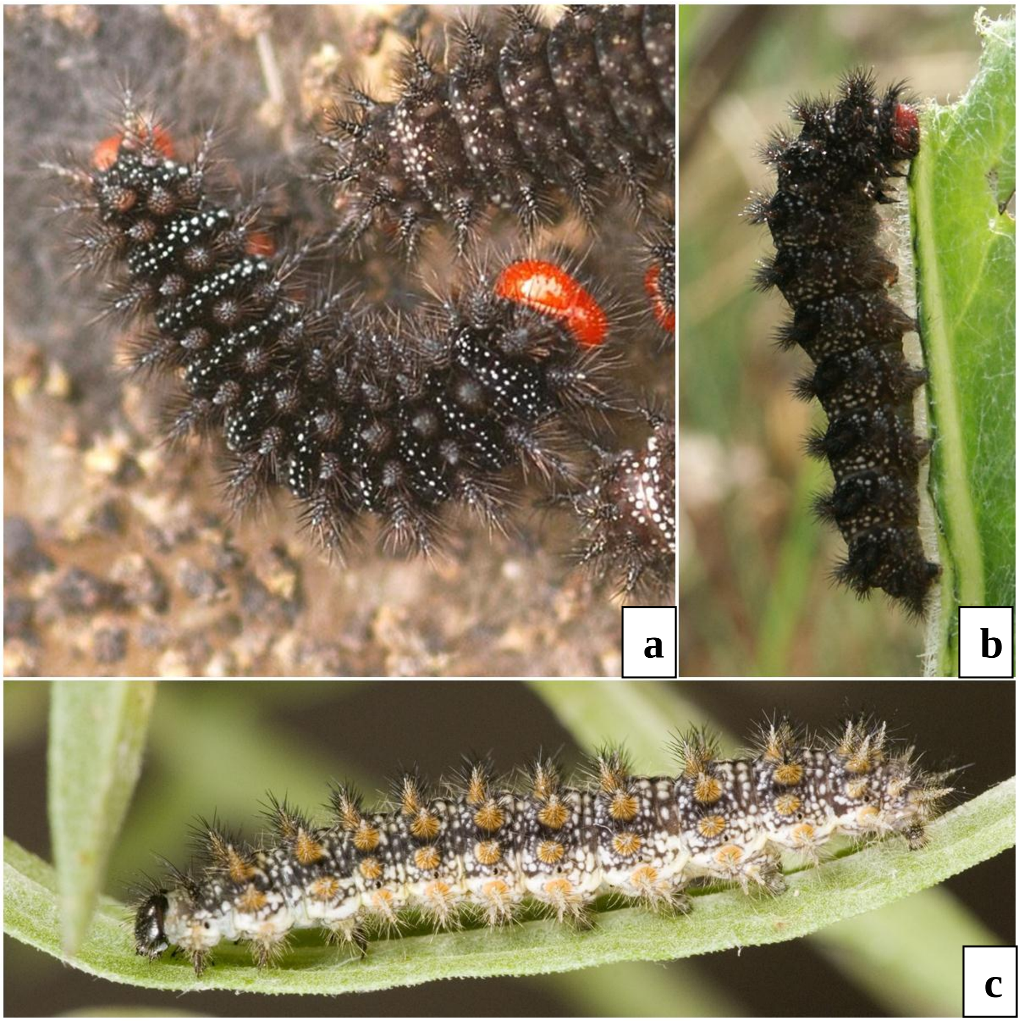
Függelék – Supplement

1. ábra – Figure 1. Tipikus *Melitaea ornata kovacsi* élőhely az Aggteleki-karszton. Typical habitat of *Melitaea ornata kovacsi* in the Aggtelek Karst.

2. ábra – Figure 2. A *Melitaea ornata kovacsi* különböző fejlődési stádiumai. The different developmental stages of *Melitaea ornata kovacsi*.



3. ábra – Figure 3. Hernyók a Kárpát-medencéből. *Melitaea cinxia* (a) vörös állábakkal és fejkapszulával. *M. ornata kovacsi* (b) vörös fejkapszulával (aestivatio után) és fekete állábakkal. *M. phoebe* (c) fekete fejkapszulával. Fotó: a, b: Varga Zoltán c: Mizsei Edvárd.

Caterpillars from the Carpathian Basin. *Melitaea cinxia* (a) with red prolegs and headcapsule. *M. ornata kovacsi* (b) with red headcapsule (after aestivation) and black prolegs. *M. phoebe* (c) with black headcapsule. Photo: a, b: Varga Zoltán c: Mizsei Edvárd.