Insect Conservation and Diversity (2014) 7, 41-54

Evolutionary units of *Coraebus elatus* (Coleoptera: Buprestidae) in central and eastern Europe – implications for origin and conservation

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Abstract. 1. The distribution of steppe-like habitats and, consequently, species dependent on these habitats in Eurasia is currently highly fragmented beyond the zone of continental climate, as a result of unfavourable climatic conditions and anthropogenic transformation of the environment. This patchy distribution may pose a threat for persistence of steppic species, especially in central Europe. To develop conservation strategies, it is essential to collect information on genetic structure of the species occupying this kind of habitats.

2. We investigated the genetic structure and diversity of central and eastern *Coraebus elatus* (F.) populations using sequences of mtDNA and an anonymous fragment of the nuclear genome. Both markers exhibited similar pattern, indicating the presence of four or five highly differentiated evolutionary units (2.1–3.2% sequence divergence in mtDNA and 0.7-2.1% in the nuclear marker) encompassing populations from the Caucasus, the Azov Sea coasts, central Europe with the Balkans (with further substructuring) and probably western Europe. These clusters should be considered evolutionary significant units for the conservation biology of this species, and may form the basis for a future taxonomic revision.

3. Pattern of *C. elatus* diversity suggests that this species presently occupies not only continental 'warm-stage' refugia formerly described in Pontic and Pannonian areas but also cryptic steppic 'warm-stage' refugia in north-central Europe.

4. In line with other studies on steppic beetles, our data strongly suggest that such species are strongly structured genetically, with very limited genetic variation within populations, which may have very serious consequences for their persistence in the future.

Key words. Buprestid, conservation genetics, isolation, phylogeography, steppe.

Introduction

Correspondence: Łukasz Kajtoch, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Science, Cracow, Poland. E-mail: kajtoch@isez. pan.krakow.pl The distribution of species in Eurasia has evolved as a result of historical range changes caused by climatic and environmental alterations as well as by the recent environmental impact of anthropogenic transformations. The origin and population structure of many species have been reconstructed on the basis of paleontological, chorological

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or genetic data. Most genetic studies have concentrated on species inhabiting temperate or boreal zones (reviewed in Taberlet et al., 1998; Hewitt, 1999; Avise, 2000; Hickerson et al., 2010). In contrast, the history and distribution of species that inhabit the continental zone (e.g. steppes) have been scarcely studied using genetic methods. During glaciations, steppe-tundra environments covered large areas between ice sheets in the north, and in the mountains or forest zones in the south (Nehring, 1890; Willis & van Andel, 2004). During inter-glacials (e.g. in the Holocene), these environments have been contracting and shifting: tundra to the north and to higher altitudes and steppes to the south-eastern and south-western areas of Europe and central Asia. The origin of these habitats remains unclear: while some scientists consider them relicts of steppe-tundra environments, others claim that they arose after man-made deforestation just a few thousand years ago (Ratyńska & Waldon, 2010). Unlike changes in the distribution of temperate and boreal environments (especially those associated with woods and freshwaters). steppe-like habitats (e.g. xerothermic grasslands) have regressed in the Holocene and are currently limited to their refugial areas. 'Warm-stage' refugium is the term proposed for areas where species adapted to cold climate (boreo-montane and arctic-alpine) and/or continental (dry) conditions (e.g. steppes) survive during inter-glacials (Schmitt, 2007; Ashcroft, 2010). Such species were more broadly distributed during glaciations (over large areas of steppe-tundra across Eurasia and other glaciated regions). Steppes and their extrazonal analogues fit the concept of continental/oceanic longitudinal gradient (Stewart et al., 2010) which describe the history of, for example, continental species and habitats. In the Old World, the main present-time 'warm-stage' refugium for steppic species spans parts of central Asia and eastern Europe. Other important 'warm-stage' refugium proposed by Stewart et al. (2010) for steppic species is in Pannonian Basin. Additionally, the Iberian Peninsula should be considered 'warm-stage' refugium (Ribera & Blasco-Zumeta, 1998; Piñero et al., 2011). Many isolated xerothermic grasslands of central and western Europe and the Balkans should be considered cryptic 'warm-stage' refugias for steppe-like species (by analogy to cryptic northern refugia for temperate species during glaciations).

The regression of steppe-like habitats, intensified by the anthropogenic fragmentation and isolation of such areas, has serious consequences for the persistence of steppic species. Currently, steppe-like habitats, and especially xerothermic grasslands in Europe, consist of partially or fully isolated patches limited to the areas unfavourable for agriculture or forest plantations. As fragmentation of natural habitat is generally considered a major threat to many species (for instance, by leading to reduced genetic diversity – e.g. Frankham *et al.*, 2002), the evaluation of the extent of isolation of the existing populations and their genetic diversity are of major concern in assessing the risk of local extinction of any threatened taxa. To develop wide-scale conservation strategies and plan man-

agement actions, it is essential to know the genetic structure of species throughout their distribution ranges (Avise et al., 1987; Moritz, 1994; Haig, 1998). Genetic analysis has become an important tool in many studies of threatened or endangered species (Moritz, 1994; Haig, 1998). Genetic markers can be used to determine whether differentiated units, indicating some extent of evolutionary independence exist below the species level (Moritz, 1994; Knapen et al., 2003). The implication of this finding is that simply conserving a species without regard to its population structure may fail to conserve the full spectrum of diversity the species encompasses. Consequently, it becomes critical to understand the nature and extent of genetic structuring of the population structure of species. For this purpose, conservation units like 'evolutionary significant units' (ESUs) (Ryder, 1986; Waples, 1991; Moritz, 1994) and 'management units' (MUs) (Moritz, 1994) were established.

Among steppic organisms the most numerous, both in terms of the number of species and the number of individuals, are insects. The only examples of phylogeographic analyses of steppic or semi-steppic insects in Europe are studies on butterflies (Lepidoptera) (e.g. Bereczki et al., 2005; Wahlberg & Saccheri, 2007; Rutkowski et al., 2009) and flies (e.g. Hall et al., 2009). There are also a few studies on species from saline environments (among others steppe-like) of Iberia: dung beetles (Diogo et al., 1999) or orthopterans (Ortego et al., 2009). On the other hand, little remains known about the history and genetic structure of steppic beetles from Pannonian, Pontic and Asiatic steppes. These problems have only been investigated in a few studies on steppic weevils (Kajtoch et al., 2009; Kajtoch, 2012) and leaf-beetles (Kajtoch et al., 2013; Kubisz et al., 2012a). There are many other species whose genetic diversity should be investigated, especially those whose populations are highly structured geographically. One of these species is Coraebus elatus (Fabricius, 1787). This species is quite abundant and characteristic for steppic beetle assemblages of Eurasia, but its distribution is not continuous, so it should constitute a good model species for studies on the genetic structure and gene flow of steppic Coleoptera.

This study assesses the genetic structure of C. elatus populations over a substantial part of its range in central, south-eastern and eastern Europe and the Caucasus. Mitochondrial (cytochrome oxidase I gene) and nuclear (anonymous genomic fragment) markers were used in phylogenetic and population genetic frameworks for the characterisation of the history of C. elatus and for the identification of evolutionary units. Their delineation will be important for conservation biology and management of C. elatus, particularly in those parts of the range where this species is rare and threatened (e.g. in central Europe - Schwier & Neumann, 2004; Farkač et al., 2005). Together with data from other studies on genetics of steppic beetles, this work may significantly contribute to the conservation genetics of entire steppic assemblages.

Materials and methods

Studied organism

Coraebus elatus is a member of the subfamily Agrilinae, tribe Coraebini. The genus Coraebus Gory and Laporte, 1839 comprises numerous (about 225) species with a centre of distribution in the Oriental region (Bilý, 2002) and occurs also in the Nearctic and Palearctic regions (Kubáň, 1995, 2006; Bellamy, 2011). The species has ponto-mediterranean-siberic distribution type. It inhabits Europe (mostly its southern, central and eastern parts), western Asia (up to Iran and the Altai Mountains) and north Africa. Its range is not continuous, but rather consists of partially or fully isolated regional populations. Such a patchy distribution is a result of its habitat preferences, which are limited mostly to steppes and similar dry grasslands (e.g. xerothermic grasslands and sandy turfs) (Kelch, 1846; Burakowski et al., 1985; Schwier & Neumann, 2004; Gutowski, 2006). Besides these habitats, its populations can be found on dry rocky mountain slopes up to 2000-2400 m a.s.l. (Niehuis, 1991; Bilý, 2002) as well as in entirely different habitats - in marshes and wet meadows (in central-eastern Poland and Belarus -Gutowski, unpubl. data; Bercio & Folwaczny, 1979; formerly also in southern Poland - Szymczakowski, 1960). Coraebus elatus is oligophagous, with its host plants belonging mainly to Rosaceae and Asteraceae, but in particular areas it feeds only on one or two plant species. Throughout most of its range it can be found between May and August, and is univoltine. The species is winged; however, its dispersal abilities are probably limited. No studies were performed on its mobility but observations indicate that in natural habitats adults may disperse outside natal places (e.g. Falcoz, 1922; Gutowski J., unpubl. data), but in strawberry fields (where C. elatus is considered pest) dispersal was limited (e.g. Lekič & Mihajlovič, 1969; Kovanci et al., 2005). This suggests that the mobility of C. elatus is depended mostly on accessibility of food plants and suitable habitats.

The imago of this species is 4.5-8 mm in length (Théry, 1942; Schaefer, 1949). According to Szymczakowski (1960), 'marshland' specimens from wet meadows are larger than those from dry grasslands, which may indicate their separate taxonomic status, or perhaps only ecological differences. There are only two described subspecies of C. elatus: the widespread C. e. elatus and C. e. repletus Abeille de Perrin, 1893 inhabiting the Taurus Mountains in Turkey (Abeille de Perrin, 1893; Kubáň, 2006) and Egypt (Alfieri, 1976). These two subspecies differ in size and shape (C. e. repletus is generally larger, being shorter and wider) and sculpture (C. e. repletus has denser spots), but these differences are rather minor. The subspecies status of C. e. repletus was based on the morphology alone. It is possible that also other evolutionary units exist within the wide range of C. elatus (e.g. 'marshland' populations). As there are many criteria used to recognise taxonomic units (especially of the species level), the most

practical strategy has been to apply a diverse kind of evidence (e.g. morphology, ecology, biogeography, genetics and others) to support the recognition of species (e.g. De Queiroz, 2007; Padial *et al.*, 2010; Tobias *et al.*, 2010). Nevertheless, the revision of the taxonomic status of evolutionary units within *C. elatus* is beyond scope of the present work.

Sampling

Specimens were collected during several field trips between 2009 and 2012 in central and eastern part of the species range. Additionally, some specimens from museum collections were used (from the Forestry Research Institute in Białowieża and the Institute of Systematics and Evolution of Animals, PAS), mostly 5-25 years old, but one specimen from the vicinity of Kraków in southern Poland, where the species is now extinct, was 80 year old (Kubisz D., unpubl. data). As this work focused on central and eastern populations of C. elatus, only a single specimen from France (provided kindly by Dr Christian Cocquempot) was added to analyses. Because in central Europe, this beetle is not common and local populations tend to be small, only a limited number of specimens could be collected in some localities. A full list of collected beetles is presented in Table 1 and localities are shown in Fig. 1. Subspecies identification revealed that most of the collected jewel beetles were C. e. elatus, and only specimens from the Caucasus (Azerbaijan and Georgia populations) were assigned the subspecies C. e. repletus on the basis of morphology. Additionally, single specimens of Coraebus rubi (Linnaeus, 1767) and Meliboeus graminis (Panzer, 1799) were collected in the field, and Meliboeus violaceus (Kiesenwetter, 1857), whose sequence was downloaded from GeneBank (FN298878), were used as outgroups. Specimens were first preserved in 99% ethanol, and afterwards stored at -22 °C.

Laboratory procedure

Whole insect bodies were used for DNA extraction using Nucleospin Tissue kit (Macherey-Nagel, Düren, Germany). Amplification of fragments of a widely used animal barcode - mitochondrial Cytochrome Oxidase I (COI) gene was performed using primers from Simon et al. (1994). Our attempts to amplify nuclear markers frequently used in phylogenetic and phylogeographic studies of beetles (e.g. rDNA internal transcribed spacers and the elongation factor 1-a gene) failed. Instead, we designed primers for amplification of a nuclear marker on the basis of DNA sequence of one of 45 randomly cloned nuclear DNA fragments (Bog C3), for which we were able to design primers working for all populations (but not for the outgroup taxa). Other markers failed to amplify in all populations were invariable or contained several indels which made their sequencing impossible for all individuals without

Country	Locality	Geographic group	Population symbol	Population no.	No. of specimens	Date	Collector
Belarus	Dikoe Marshes	Baltic	Belarus	1	5	2010	J.M. Gutowski
Poland	Biebrza Marshes	Baltic	Biebrza	2	5	2009-2011	J.M. Gutowski
Poland	Polesie Marshes	Baltic	Polesie	3	5	2011	J.M. Gutowski
Poland	Nida Valley	Carpathians	Nida	4	5	2010-2011	Ł. Kajtoch et al.
Poland	Kielce Upland	Carpathians	Kielce	5	3	2010-2011	D. Kubisz et al.
Poland	Krakow vicinity	Carpathians	Krakow	6	1	1932	S. Stobiecki
Poland	Lower Vistula Valley	Baltic	Vistula	7	4	2010-2011	J.M. Gutowski
Poland	Notec Valley	Baltic	Notec	8	2	2010	D. Kubisz et al.
Poland	Lower Oder Valley	Baltic	Oder	9	5	2010	M.A. Mazur et al.
Poland	Lower Silesia	Carpathians	Silesia	10	1	1984	L. Borowiec
Slovakia	Podunajská Lowland	Carpathians	Slovakia	11	3	1996, 2011	M. Miłkowski,
							M. Holecová et al.
Croatia	Krk Island	Balkans	Croatia	12	1	1989	D. Kubisz
Greece	Macedonia	Balkans	Greece	13	1	1987	E. Baraniak
Bulgaria	Black Sea coast	Balkans	Bulgaria	14	3	1986, 1994, 2005	S. Kapuściński,
							T. Mokrzycki, P. Górski
Turkey	Trace	Balkans	Turkey	15	1	1987	E. Baraniak
Ukraine	Middle Dniester Valley	Carpathians	Dniester	16	6	2010	D. Kubisz et al.
Ukraine	Crimea	Azov	Crimea	17	3	2009	M. Kunysz
Russia	Lower Don Valley	Azov	Don	18	1	2010	A. Nadachowski
Russia	Krasnodarskiy Region	Azov	Krasnodar	19	5	2009	B. Korotyaev
Azerbaijan	Mugan	Caucasus	Azerbaijan	20	8	2010	R. Królik
Georgia	Dedopliskaro	Caucasus	Georgia	21	2	2011	R. Plewa
France	Alpes Maritimes	West Europe	France	22	1	2009	Ch. Cocquempot
Hungary	Lénárddaróc vicinity	Carpathians	Hungary	23	2	2012	D. Kubisz et al.

Table 1. Localities and sampling dates of populations of Coreabus elatus with their symbols and number of specimens per population.



Fig. 1. Localisation of sampling sites of *Coraebus elatus* populations and their allocation to mitochondrial clades [black squares – Central-European + Balkans clade (M1); black stars – Kujawy clade (M2); black diamonds – West-European clade (M3); black dots – Azov clade (M4); black triangles – Caucasus clade (M5)]; and nuclear clades [rectangle – Central-European clade (N1); hexahedron – Balkans clade (N2); diamond – West-European clade (N3); circle – Azov clade (N4); triangle – Caucasus clade (N5)].

costly cloning. A BLAST search found no GenBank sequences matching the newly developed marker, so it is probably a non-coding nuclear sequence. We failed to amplify either COI or Bog_C3 markers for DNA from the older museum specimens. To solve this problem, internal primers were designed using Primer3 software (Rozen & Skaletsky, 2000). Even with these primers, we failed to amplify sequences from about half of the museum specimens, so only some of them could be used for analysis. The sequences of all primers used in this study are presented in Table 2.

PCR conditions for all markers were as in Kajtoch *et al.* (2009), Kajtoch (2012) and Kubisz *et al.* (2012b). After purification [NucleoSpin Extract II (Macherey-Nagel)], the PCR products were sequenced using the BigDye Terminator v.3.1. Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) and ran on an ABI 3100 Automated Capillary DNA Sequencer. All newly obtained sequences were deposited in GenBank (Accession nos.: JQ303259 for COI of *C. rubi*, JQ303260 for COI of *M. graminis*, JQ303261–JQ303299 for COI of *C. elatus*, and JQ303300–JQ303310 for Bog_C3 of *C. elatus*).

Data analysis

The sequences were checked by eye using BioEdit v.7.0.5.2 (Hall, 1999). All analyses were performed separately for COI and Bog C3 markers.

Table 2. Primers of used amplification and sequencing of Coraebus elatus COI and Bog C3 markers. In italic – internal primers.

Marker	Primer &name	Sequence
COI	C1-J-2183	CAACATTTATTTTGATTTTTTGG
	TL2-N-3014	ТССААТССАСТААТСТСССАТАТ ТА
	Ce_CO1_R1	TGTCATGAAGGACAATATCA
	Ce_CO1_F1	GAGTAATTCTAGCTAACTCATCAA
	Ce_CO1_R2	GAAATTAATGATCCAATRG
	Ce_CO1_F2	CYATTGGATCATTAATTTC
Bog_C3	Ce_C3_F	TACAAAATATACTGCAATCTTTC AT
	Ce_C3_R	AATTTAAGGAGCCAAAGTTTAT
	Ce_C3_F1	TTACCGCCAACGGATGAT
	Ce_C3_R2	AACCAGCCACCCATCATC

Phylogeny

The Akaike Information Criterion in MrModeltest 2.3 (Nylander, 2004) in conjunction with PAUP* (Swofford, 2002) were used to determine the best-fitting nucleotide substitution model (Kimura, 1980). Two methods for phylogeny reconstruction were used - Bayesian inference (BI) and maximum parsimony (MP). BI was run using MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Huelsenbeck et al., 2001) with one cold and three heated Markov chains for 3 000 000 generations and trees were sampled every 100th generation (according to Hall, 2007). Two simulations were run simultaneously. Convergence of Bayesian analyses was assessed using Tracer v. 1.5.0 (Rambaut & Drummond, 2003-2009) and appropriate number of sampled trees (60 000 generations) were discarded as 'burn-in', and the remainder used to reconstruct the majority rule consensus tree. MP was computed using PAUP* 4.0b10. For all MP analyses, heuristic search with tree bisection-reconnection (TBR) branch swapping and random addition sequences, Max-Trees = 500 were conducted with 500 random addition replicates. Node support was assessed with the bootstrap technique using 5000 pseudoreplicates and TBR branch swapping. COI tree was rooted with the Meliboeus sequence, Bog C3 tree was rooted in midpoint as amplification of this marker failed in all outgroup taxa. All trees were visualised with TreeView 1.6.6 (Page, 1996), and as topologies of Bayesian and MP trees were similar only one tree per marker were presented with Posterior Probabilities (PP) and Bootstrap Supports (BS) presented on tree branches. Mean net genetic distances among clades were calculated using MEGA5 (Tamura et al., 2011) under Kimura 2-parameter model (K2P), standard errors (SE) were obtained by bootstrap procedure (1000 replicates). Additionally, haplotype networks were constructed using the Median-Joining network method (Bandelt et al., 1999) in the Network 4.6.1.0. software (http:// www.fluxus-engineering.com/).

Population genetics

Standard genetic indices such as haplotype diversity (h), nucleotide diversity (π) and number of private haplotypes (Np) for populations were computed with DnaSP v.5 (Librado & Rozas, 2009). Pairwise FST among five geographic groups of populations (see below B-grouping for AMOVA, except B6 for which only one sample was available) were calculated using Arlequin 3.5 (Excoffier & Lischer, 2010). The Mantel test (Mantel, 1967) was performed in Arlequin to check if the differentiation among subpopulations fits an isolation by distance model (IBD) (Slatkin, 1993), using pairwise FST values and straight-line geographic distances. To test hierarchical geographical structuring of populations, an AMOVA was conducted in Arlequin. Populations were clustered according to their geographic location in two ways: (i) in two groups: A1 - European group – almost all populations from Europe (populations nos. 1-16 and 23) and A2 – Eastern group – populations from around the Azov Sea and the Caucasus (17-21); and alternatively (ii) in five groups: B1 – Baltic populations (located in lowlands south of the Baltic coast) (1-3, 7-9), B2 – Carpathian populations (located in uplands around the Carpathian Mountains) (4-6, 10, 11, 16, 23), B3 -Balkan populations (located in the Balkan Peninsula) (12-15), B4 - Azov populations (located around the Azov Sea) (17-19) and B5 - Caucasus populations (located in the Caucasus and its surroundings) (20, 21).

Results

No indels were observed in the 755 bp of COI or in the 441 bp of Bog_C3 markers. Standard genetic indices for *C. elatus* (for all the studied groups of populations and individual populations with a sample size = 3) are presented in Table 3.

Phylogenetic analysis

MrModeltest 2.3 identified the GTR + G + I model (proportion of invariable sites I = 0.61; gamma distribution shape parameter G = 1.48; $-\ln L = 3066.98$; AIC = 6153.96) and the HKY + I model for Bog_C3 (I = 0.94; $-\ln L = 731.46$; AIC = 1472.92) as the best nucleotide substitution models for MrBayes analyses of the COI and Bog_C3 markers respectively.

MP heuristic searches resulted in 1056 equally parsimonious COI trees [data for consensus tree: length = 429 steps; consistency index (CI) = 0.7040; retention index (RI) = 0.8396] based on 97 parsimony-informative characters, and one Bog_C3 tree (length = 19 steps; CI = 0.7368; RI = 0.8837] based on 12 parsimony-informative characters. MP and Bayesian methods resulted in similar topologies.

The COI trees (Fig. 2b) showed that *C. elatus* forms a monophyletic clade. The trees and network inferred from

Table 3. Standard genetic indices of genetic markers (COI, Bog_C3) calculated for studied *Coraebus elatus* groups of populations and single populations with sample size ≥ 3 . n – sample number; V – number of variable sites; S – number of segregating sites; H – haplotype number; h – haplotype diversity; π –nucleotide diversity; Np – number of private haplotypes.

		COI						Bog_C3							
Population	п	V	S	Н	$h \pm SD$	$\pi\pmSD$	Np	V	S	Н	$h \pm SD$	$\pi\pmSD$	Np		
All	73	94	67	40	0.965 (0.010)	0.020 (0.07)	_	15	13	12	0.841 (0.031)	0.008 (0.002)	_		
Central-east European	53	61	35	25	0.938 (0.018)	0.011 (0.005)	36	9	7	8	0.743 (0.053)	0.004 (0.002)	8		
Azov-Caucasus	19	44	35	14	0.965 (0.028)	0.020 (0.006)	35	5	5	3	0.632 (0.073	0.006 (0.002)	3		
Baltic	26	27	23	7	0.791 (0.053)	0.011 (0.002)	23	3	3	3	0.600 (0.074)	0.002 (0.001)	3		
Carpathians	21	27	8	14	0.943 (0.039)	0.006 (0.004)	8	4	4	4	0.695 (0.070)	0.003 (0.001)	4		
Balkans	6	20	2	3	0.600 (0.215)	0.009 (0.006)	2	2	0	2	0.333 (0.215)	0.002 (0.002)	2		
Azov	9	12	6	6	0.889 (0.091)	0.005 (0.003)	6	1	1	2	0.500 (0.128)	0.001 (0.001)	2		
Caucasus	10	27	18	8	0.956 (0.059)	0.012 (0.005)	18	0	0	1	0.000	0.000	1		
Belarus	5	3	1	3	0.800 (0.164)	0.002 (0.001)	1	0	0	1	0.000	0.000	0		
Biebrza	5	1	0	2	0.400 (0.237)	0.001 (0.001)	1	0	0	1	0.000	0.000	0		
Polesie	5	1	0	2	0.400 (0.237)	0.001 (0.001)	0	0	0	1	0.000	0.000	0		
Nida	5	4	0	4	0.900 (0.161)	0.002 (0.002)	3	0	0	1	0.000	0.000	0		
Vistula	4	0	0	1	0.000	0.000	0	0	0	1	0.000	0.000	0		
Oder	5	0	0	1	0.000	0.000	1	0	0	1	0.000	0.000	1		
Slovakia	3	5	0	3	1.000 (0.272)	0.004 (0.003)	3	0	0	1	0.000	0.000	1		
Bulgaria	3	0	0	1	0.000	0.000	0	0	0	1	0.000	0.000	0		
Dniester	6	14	2	5	0.933 (0.122)	0.007 (0.004)	5	0	0	1	0.000	0.000	1		
Crimea	3	0	0	1	0.000	0.000	1	0	0	1	0.000	0.000	1		
Krasnodar	5	7	4	4	0.900 (0.161)	0.005 (0.003)	4	0	0	1	0.000	0.000	1		
Azerbaijan	8	25	17	6	0.929 (0.084)	0.013 (0.006)	6	0	0	1	0.000	0.000	0		

mtDNA sequences (Figs 2a and 3a) showed that the C. elatus mtDNA forms three main clades, which have nonoverlapping geographic distributions: the central European clade including the Balkans, the eastern European clade including the Caucasus and the western European clade (represented only by a single sample from France); however, support for these clades is rather weak (only approx. 0.50-0.60 PP). These clades were divided into subclades containing populations from central Europe and the Balkans (M1; 0.71 PP); the Kujawy Lowland in northern Poland (M2; 1.00 PP); France (M3; single haplotype); the Azov Sea coast (M4; 0.86 PP) and the Caucasus Mountains with their surroundings (M5; 0.52 PP, but after exclusion of two haplotypes - 0.98 PP). Similarly, in the nuclear (Bog C3) tree and network (Figs 2b and 3b), five clades can be distinguished: central European (N1; PP below 0.5), Balkan (N2; 0.52 PP); western European (N3; single haplotype); Azov (N4; 0.87 PP), and Caucasus (N5; single haplotype).

The genetic distances between clades are presented for both markers in Table 4. Genetic distances between major clades were higher for COI (2.1–3.2%) than for Bog_C3 (0.7–2.1%). Inter-specific distances of COI were 12.8– 14.6% between *C. elatus* samples and *Coraebus rubi*, and approximately 20–24% between *Coraebus* and *Meliboeus*.

Population genetics

Populations grouped according to their geographic locations into two clusters (A1 and A2) seem to be differ-

entiated concordantly for both markers, but the magnitude of sequence differentiation is much higher for mtDNA. Genetic diversities, measured as regional haplotype diversities, varied in five geographic areas: Baltic, Carpathian, Balkan, Azov and Caucasus. In COI, Baltic region and Balkans are the least diverse. Considering within-population mtDNA variation, most diverse were populations from Slovakia, Ukraine (Dniester), the Caucasus (Azerbaijan), southern Russia (Krasnodar) and southern Poland (Nida). The lowest within-population variation was observed in northern Poland (the Vistula and the Oder), and also in Bulgaria, Hungary and Ukraine (Crimea). As far as lower overall variation permitted, the mtDNA pattern was corroborated by the nuclear marker: individual populations are generally monomorphic, but lowest regional diversity was found in Baltic region, Hungary, in the Balkans, and in the Caucasus.

It is worth noting that all of the above-mentioned groups of populations have their private haplotypes (both COI and Bog_C3), which suggests that they are highly isolated. Moreover, the high isolation of populations was supported by the high frequency of private haplotypes within particular populations. This is further corroborated by very high FST between populations within groups calculated for groups of populations, exceeding 0.25 for both markers (Table 5). The only exceptions were populations from the Baltic region and the Carpathians, which are highly differentiated in COI (FST = 0.24), but not in Bog_C3 (FST = 0.04). Considering pairwise comparisons between populations, low FST for mitochondrial and zero for nuclear marker were found between some populations



Fig. 2. Phylogenetic trees of *Coraebus elatus* haplotypes constructed using COI (a) and Bog_C3 (b) markers. Population names followed Table 1. Upper numbers indicate posterior probabilities of Bayesian inference, lower numbers – bootstrap values for maximum parsimony trees (shown only if above 0.50 and 50%, respectively). Mitochondrial clades: M1 – Central-European + Balkans; M2 – Kujawy; M3 – West-European; M4 – Azov; M5 – Caucasus; and nuclear DNA clades: N1 – Central-European; N2 – Balkans; N3 – West-European; N4 – Azov; N5 – Caucasus.

from the Baltic region (e.g. Biebrza-Polesie-Belarus) and the Carpathian area (e.g. Kielce-Nida) (Table 5). These populations, as well as some populations from the Balkans and the Caucasus, share regional haplotypes. Mantel tests showed low to moderate, significant correlations between geographic distances and genetic distances (FST) for both markers: COI (r = 0.2, P = 0.04) and Bog_C3 (r = 0.44, P < 0.01) (Fig. 4a,b).

The distinctiveness of populations, and particularly of their geographic groups (the two main groups: central European with the Balkans and eastern European with the Caucasus; or five groups: Baltic, Carpathian, Balkan, Azov, and Caucasus) was also corroborated by a high percentage of genetic variation among these groups according to AMOVA analyses (Table 6). In both groupings, the percentage of genetic variation among the groups is about 50–70%.

Discussion

Evolutionary units and their history

The patterns of genetic differentiation among C. elatus populations are quite similar for mitochondrial (COI) and nuclear (Bog C3) markers. All examined populations are grouped in a monophyletic clade and distances among subclades are low or moderate (up to 3% for COI and 2% for Bog C3). This and the lack of clear morphological differences in other characters between C. elatus populations, suggest that all the studied populations belong to single taxon (species), however, divided into subunits. A distinct nature of these units is partially confirmed by other characters like slight differences in morphology (Asian populations designated previously as subspecies C. e. repletus), geographic isolation of populations from different part of species range (mostly around the Black Sea) and ecological (habitat) preferences ('marshland' populations). Although these features have not been studied detailed in this work, no strong taxonomic conclusions can be drawn. Probably, more detailed studies on environmental niche divergence between genetically distant lineages (Kozak et al., 2008) could show additional support for their designation as separate taxonomic units within C. elatus, similarly like it has been done, for example, for Ochthebius glaber water beetle (Sánchez-Fernández et al., 2011).

General patterns of mitochondrial and nuclear differentiation in C. elatus populations are similar, although some minor differences between markers are observed. Two geographic regions show concordant differentiation in both markers: the Caucasus and the Azov Sea coast. It is also likely that a distinct clade inhabits western Europe; however, western part of the species range was only represented by a single specimen from France. Some discrepancies between markers were found in the central part of the C. elatus range. Two mitochondrial and two nuclear lineages are present there; however, their distributions do not overlap as almost all populations from this area belong to a single mtDNA clade, with the exception of two populations from Kujawy (north-central Poland), while in respect of the nuclear marker populations in this area are divided into central European and Balkan clades. The distinctiveness of C. e. repletus from Asia has been confirmed. On the other hand, there are only slight genetic indications that the ecologically distinct 'marshland' populations from the wet grasslands of Poland and Belarus could be considered a distinct



Fig. 3. Networks of *Coraebus elatus* haplotypes using COI (a) and Bog_C3 (b) markers. Numbers followed population numbers from Table 1. Mitochondrial clades: M1 – Central-European + Balkans; M2 – Kujawy; M3 – West-European; M4 – Azov; M5 – Caucasus; and nuclear DNA clades: N1 – Central-European; N2 – Balkans; N3 – West-European; N4 – Azov; N5 – Caucasus.

Table 4. Net mean genetic distances (%) among *Coraebus elatus* clades: mitochondrial (COI) and nuclear (Bog_C3). Distances are presented below diagonal, SE are presented above diagonal. Acronyms (M1–M5 and N1–N5) are the same than those used in Figs 1 and 2.

COI clades		M1	M2	M3	M4	M5
Central-European + Balkans	M1	-	0.005	0.005	0.005	0.006
Kujawy	M2	2.4	_	0.006	0.006	0.006
Azov	M3	2.8	3.1	_	0.005	0.006
Caucasus	M4	2.2	2.9	2.1	_	0.005
France	M5	2.8	3.2	2.1	2.1	_
Bog_C3 clades		N1	N2	N3	N4	N5
Central-European	N1	_	0.004	0.004	0.004	0.005
Balcans	N2	0.8	_	0.005	0.004	0.004
Azov	N3	1.0	0.7	_	0.006	0.007
Caucasus	N4	1.3	0.7	1.0	_	0.004
France	N5	1.1	1.4	1.8	2.1	_

genetic unit. Actually, these beetles may differ only in respect of their habitat preferences and their larger size may be caused by ecological factors. But, one example suggests that something more than mere ecological differences may be at play here. Highly similar mitochondrial haplotypes were found not only in the marshland populations of north-central Poland and Belarus but also in the 80-year-old specimen from the vicinity of Cracow in southern Poland (~300–400 km away in a straight line). It is interesting to note that also this population from Cracow (unfortunately now extinct) inhabited wet meadows. This phenomenon can be explained in three ways: intentional man-made translocation of beetles (rather implausible); natural migration in the past (theoretically possible); or a simultaneous persistence of two ecological forms of *C. elatus* in north-central Europe: one typical of steppe-like habitats and another one of wet grasslands. The mitochondrial similarities between marshland populations and the fact that they differ slightly from steppic populations in the same area suggest that these forms may be isolated, but this is currently too weak a premise for unit designation. On the other hand, significant genetic differences between populations from central Europe (including the Balkans) and the Azov Sea coast observed in both markers may be a strong indicator for designating these two groups of populations as evolutionary units.

The large-scale genetic structure of C. elatus may result from a fragmentation of the ancestral range and isolation in four or five areas which may have formed refugia of this species: the Caucasus, Crimea, the Balkans and/or central Europe and probably western Europe. This partially fits the major refugia identified in Europe - the Mediterranean and Asia Minor/Caucasus (e.g. Taberlet et al., 1998; Hewitt, 1999; Avise, 2000). Other refugia, located more to the north, were also postulated to exist in western and central Europe, for example, in the Pannonian Basin and in the Carpathians (Stewart & Lister, 2001; Adams, 2002; Schmitt, 2007), eastern Europe (Sommer & Nadachowski, 2006; Markova et al., 2009), and for some European species, also in Asia (Flanders et al., 2009). A similar pattern of genetic diversity is quite common in Eurasian insects (Rozas et al., 2003; Wahlberg & Saccheri, 2007; Maryanska-Nadachowska et al., 2012). It is worth to notice that for steppic insects there is substantial evidence for late Tertiary connection between Asianeastern European main steppe zone and steppes of Iberian Peninsula (Ribera & Blasco-Zumeta, 1998). Long-term survival and largely independent evolution of steppic insects in these two areas during Pleistocene glaciations

Table 5. The F_{ST} indices for COI (below diagonal) and Bog_C3 (above diagonal) for populations with sample size ≥ 3 (A) and geographically grouped populations of *Coraebus elatus* (B). Populations grouped as follows: Baltic (1, 2, 3, 7, 8, 9); Carpathians (4, 5, 6, 10, 11, 16); Balcans (12, 13, 14, 15); Azov (17, 18, 19); and Caucasus (20, 21). (in bold – P < 0.05)

А														
Population		1	2	3	4	5	6	7	8	9	10	11	12	13
Biebrza	1	_	0.00	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Polesie	2	0.00	-	0.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Belarus	3	0.10	-0.07	_	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dniester	4	0.42	0.37	0.30	_	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Kielce	5	0.95	0.94	0.82	0.40	_	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Nida	6	0.81	0.79	0.70	0.37	-0.13	_	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oder	7	0.96	0.96	0.86	0.53	1.00	0.87	_	1.00	1.00	1.00	1.00	1.00	1.00
Slovakia	8	0.72	0.70	0.56	0.24	0.72	0.63	0.81	_	1.00	1.00	1.00	1.00	1.00
Bulgaria	9	0.95	0.94	0.82	0.57	1.00	0.83	1.00	0.79	_	1.00	1.00	1.00	1.00
Vistula	10	0.99	0.99	0.96	0.85	1.00	0.96	1.00	0.94	1.00	_	1.00	1.00	1.00
Crimea	11	0.99	0.99	0.96	0.84	1.00	0.96	1.00	0.93	1.00	1.00	_	1.00	1.00
Krasnodar	12	0.92	0.92	0.90	0.82	0.91	0.90	0.93	0.87	0.91	0.92	0.51	_	1.00
Azerbaijan	13	0.74	0.73	0.71	0.65	0.70	0.72	0.73	0.66	0.72	0.74	0.65	0.67	_
В														
Region			Baltic		Carpa	athians		Balca	ans		Azov		Ca	ucasus
Baltic			0.00		0.04			0.79			0.82		0.8	38
Carpathians	arpathians 0.23			0.00			0.72			0.79			0.86	
Balcans	0.28			0.40			0.00			0.85			0.94	
Azov	0.71			0.83		0.80				0.00			0.95	
Caucasus	casus 0.63			0.73			0.69			0.69			00	

could lead to high level of endemism as demonstrated in south-eastern Spain and Anatolia (e.g. Mico *et al.*, 2008; Avgin & Emre, 2009; Piñero *et al.*, 2011).

The most surprising and difficult to explain observation is the distinctiveness of two populations from Kujawy (north-central Poland), which are located among other populations belonging to mitochondrial and nuclear central European clades. The Kujawy clade is highly distinct, with a mitochondrial genetic distance of about 3% mtDNA and approximately 1% nuclear DNA from the other clades. The Kujawy populations could not have persisted in situ for longer than 10 000-12 000 years (Lindner et al., 2006) or 17 000-18 000 years (Marks, 2002; Wysota et al., 2009), as earlier this area had been covered by an ice sheet. These populations are most similar in terms of their nuclear and mitochondrial DNA to the population from the Dniester valley (Ukraine), which suggests that the former had originated somewhere in eastern Europe. The most probable explanation for the presence of these distinct jewel beetles in Kujawy is their recent expansion from the east (probably along the Volhyn Upland migration route; Pawlowski, 1991) followed by settlement in a C. elatus -free area or by replacement of beetles from the central European evolutionary lineage. The presence of a unique and distinct population in Kujawy is not limited only to C. elatus. A similar pattern was found in a steppic weevil Centricnemus leucogrammus (Kajtoch et al., 2009; Kajtoch, 2012). This strongly suggests that relatively recently some steppic beetle populations colonised the Kujawy area from the east. It is difficult to explain why these lineages are absent in other areas of central Europe.

No fossil (from the Pleistocene or earlier times) remains of these beetles are known from the past, so strict dating for this species is impossible. Nevertheless, assuming the mutation rate to be approximately 2% per Myr, as calculated for the mtDNA of other beetles (Farrell, 2001; Ribera et al., 2001; Barraclough & Vogler, 2002), 2-3% of COI distances among C. elatus lineages (so per-lineage divergence 1-1.5%) suggest that they diverged approx. 0.5 -0.75 Ma, during the Middle Pleistocene (Donian/Ilynian/ Pokrovian stages according to the Russian Plain Stages or Cromerian complex stages according to the North-West European Stages) (Cohen & Gibbard, 2011). Pattern of C. elatus genetic diversity suggests that this species is presently divided into several evolutionary units whose distribution is limited to particular areas. Some of them (these from western Asia and eastern Europe) fit to main 'warmstage' refugia of continental species suggested by Stewart et al. (2010) in Pontic region. Distinct populations from the central Europe (mitochondrial clade extending from Hungary to the Baltic) could be connected to Pannonian 'warm-stage' refugia of steppic species proposed by Stewart et al. (2010). Other populations, especially north of Carpathians and in the Balkans, which also may be characterized as distinct evolutionary units (especially Kujawy



Fig. 4. The relationship between genetic (FST) and geographic distance (km) (Mantel tests) for populations of *Coraebus elatus* on the base of COI (a) and Bog_C3 (b) markers.

Table 6. Percentages of variation for three types of grouping methods obtained from AMOVA analysis of mitochondrial (COI) and nuclear (Bog_C3) markers for groups of *Coraebus elatus* populations. A – two main groups: European (populations no. 1–16 and 23) and eastern (Azov-Caucasus, population no. 17–21); B – 5 regional groups of populations: Baltic (1, 2, 3, 7, 8, 9); Carpathians (4, 5, 6, 10, 11, 16, 23); Balcans (12, 13, 14, 15); Azov (17, 18, 19); and Caucasus (20, 21). For all F_{SC} ; F_{ST} ; $F_{CT} P \ll 0.05$.

	COI		Bog_C3		
Source of variation	A	В	A	В	
Among groups Among populations within groups Within populations	52.0 36.2 11.8	54.2 30.8 15.0	56.6 43.4 0.0	70.1 29.0 0.0	

mtDNA clade and Balkan nuclear clade), strongly suggest that the steppic refugia described by Stewart *et al.* (2010) do not represent all possible refugial areas in Europe. Such cryptic 'warm-stage' refugia are probably located also in western Ukraine – southern Poland, northern Poland – Belarus, the Balkans and probably also in other areas in western Europe.

Population genetics

The highest mitochondrial diversity was found in populations from eastern regions (the Caucasus, the Azov Sea coast and the Dniester in Ukraine) and also in some central European populations (from Slovakia and southern Poland). The lowest mtDNA variation was found in the northernmost populations (from the Baltic region) but also central and southern populations (from the Balkans and Hungary, but samples available for these populations were very small). With respect to the nuclear marker, all populations appeared to be monomorphic, but moderate differentiation was found between most regions with the exception of the Balkans (without Croatia) and the Caucasus, which were surprisingly monomorphic. Female philopatry and only male dispersal is probably an insufficient explanation for this pattern. Some other factors may be involved in the discrepancies between nuclear and mitochondrial diversities, especially in the Caucasus.

The isolation of populations, especially between regions, is corroborated by a high number of private haplotypes, high values of FST indices, positive and high percentages of genetic variation found among these regional groups according to analyses of molecular variance, and significant correlations among genetic and geographic distances for both markers. On the other hand, sharing of mitochondrial haplotypes and the low values of FST indices indicate that gene flow occurs among adjacent populations, for example, between north-eastern Poland and Belarus, within north-central Poland, within southern Poland and within the Balkans. Also, the presence of the same nuclear haplotypes among populations in central Europe, within Balkans and within Caucasus suggests gene flows in these regions; however, due to the lower rates of evolution in the nuclear marker, this may also result from the retention of ancestral alleles.

The genetic distinctiveness of C. elatus populations and various levels of genetic diversity within regional groups of populations should be explained in the light of dispersal abilities of this species. C. elatus is winged; however, in stable environmental conditions (e.g. strawberry plantations) it appears sedentary. It can disperse in natural environments, where conditions are much more unpredictable and suitable patches are more fragmented. Its mobility is probably dependent mostly on availability of host plants and suitable environments. It seems that in areas further south (e.g. around Mediterranean and Black Seas) several distinct evolutionary units occur. These units may have evolved as a result of ancient isolation and low dispersal abilities. On the other hand, populations in central Europe appeared more recently and their relative genetic uniformity may result from recent common ancestry or present possibly migrations (gene flow). The Kujawy population may constitute the exception in this respect. It was showed that beetles living in more stable environmental conditions are worse flyers and have an increased phylogeographical structure than species occupying short-term geologically persistent habitats where they show higher dispersal abilities and increased gene flow among populations (e.g. Abellán et al., 2009; Arribas et al., 2012).

Highly similar patterns - genetic similarities of neighbouring populations and substantial differences between populations from different regions were also found for other steppic beetles from central-east Europe: weevils (Kajtoch, 2011; Kajtoch et al., 2012) and leaf-beetles (Kajtoch et al., 2013; Kubisz et al., 2012a), for steppic insects of Iberian Peninsula: the tiger beetle Cicindela deserticoloides (Diogo et al., 1999) and orthopteran Mioscirtus wagneri (Ortego et al., 2009) and for species distributed in these two steppe areas: butterfly Melitaea cinxia (Wahlberg & Saccheri, 2007) and fly Wohlfahrtia magnifica (Hall et al., 2009). This suggests that most steppic insects (including Coleoptera) are structured geographically and populations of a particular species are highly isolated from other regions, but migration is still possible within regions. This may have important implications for conservation and management strategies for steppic assemblages.

Conservation implications

The existence of reciprocally monophyletic groups within C. elatus is a valuable clue for the identification of evolutionary significant units. ESU status should be assigned to all the major regional groups, that is, populations from the Caucasus, the Azov Sea coast and central Europe (from the Baltic coast to the areas surrounding the Carpathian Mountains) with the Balkans. All these groups of populations are genetically distinct (however some of them, especially the Caucasus group, do not received substantial support in the phylogenetic analyses) and thus are genetically isolated from each other. On a more local scale populations from Kujawy in north-central Poland should be also designated as ESU. Very similar conservation units have been recently proposed for other steppic beetles (Kajtoch, 2012; Kajtoch et al., 2013; Kubisz et al., 2012a). Conservation planning and management strategies should be prepared for particular populations of C. elatus. This species is rare and endangered mainly in central European parts of its range, where it inhabits fragmented grasslands (xerothermic or wet), which are seriously threatened, mainly by anthropogenic pressure (intentional degradation through transformations into fields, forestation, or destruction). In southern and eastern Europe and Asia, this species seems to be still quite abundant, so no special protection is currently needed there. Special conservation actions should be undertaken in central Europe not only for this species, but for entire assemblages of steppe-like habitats. Under the present climatic and environmental conditions, additionally amplified by human activity, such habitats must be protected actively by grazing, bush-cutting, or even by periodically prescribed burning, which is considered a good method for keeping open lands, but is illegal, for example, in Poland. Additionally, for some species translocations of individuals or population reintroduction are probably necessary to sustain viable populations. Moreover, assessment of conservation priorities for steppic insects could be based on criteria proposed for, for example, water beetles in Spain (Abellán et al., 2005). Without active protection of grasslands (especially xerothermic areas and marshlands) in central Europe, the future of these habitats and their plant and animal assemblages remain highly uncertain.

Acknowledgements

We express our sincerest gratitude to M. A. Mazur, L. Borowiec, M. Miłkowski, M. Holecová, E. Baraniak, T. Mokrzycki, P. Górski, M. Kunysz, A. Nadachowski, B. Korotyaev, R. Królik, R. Plewa, P. Szwałko and Ch. Cocquempot, who helped us collect beetles or kindly provided museum specimens, Dorota Lachowska-Cierlik for help in data analyses; anonymous reviewers and the editor provided helpful comments. This study was supported by grant N N303 311137 from the Polish Ministry of Higher Education and Science (Kubisz D.).

References

- Abeille de Perrin, E. (1893) Nouveau supplément aux Buprestides d'Europe et circa. *Revue d'Entomologie*, **12**, 127–141.
- Abellán, P., Millán, A. & Ribera, I. (2009) Parallel habitat-driven differences in the phylogeographical structure of two independent lineages of Mediterranean saline water beetles. *Molecular Ecology*, 18, 3885–3902.
- Abellán, P., Sánchez-Fernández, D., Velasco, J. & Millán, A. (2005) Assessing conservation priorities for insects: status of water beetles in southeast Spain. *Biological Conservation*, **121**, 79–90.
- Adams, J. (2002) A quick background to the last ice age. *Global* Land Environment Since the Last Interglacial (ed. by J. Adams). < http://www.esd.ornl.gov/projects/qen/nerc.html > 23rd February 2012.
- Alfieri, A. (1976) The Coleoptera of Egypt. Memoires de la Societe Entomologique d'Egypte, 5, 1–361.
- Arribas, P., Velasco, J., Abellán, P., Sánchez-Fernández, D., Andújar, C., Calosi, P., Millán, A., Ribera, I. & Bilton, B.T. (2012) Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *Journal of Biogeography*, **39**, 984– 994.
- Ashcroft, M.B. (2010) Identifying refugia from climate change. Journal of Biogeography, 37, 1407–1413.
- Avgin, S.S. & Emre, I. (2009) A Taxonomic Review Of The Species Of Four Genera Of Pterostichinae (Coleoptera: Carabidae) From South-Central Turkey. *Journal of Entomological Science*, 44, 230–255.
- Avise, J.C. (2000) Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, Massachusetts.
- Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. & Saunders, N.C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Revue of Ecology and Systematics*, 18, 489–522.
- Bandelt, H.-J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology* and Evolution, 16, 37–48.
- Barraclough, T.G. & Vogler, A.P. (2002) Recent diversification rates in North American tiger beetles estimated from a dated mtDNA phylogenetic tree. *Molecular Biology and Evolution*, 19, 1706–1716.
- Bellamy, C.L. (2011) A Checklist of World Buprestoidea. < www. fond4beetles.com/Buprestidae/WorldCat/Classif/agrilinae.htm > 2nd December 2011.
- Bercio, H. & Folwaczny, B. (1979) Verzeichnis der Käfer Preußens. Verlag Parzeller et Co, Fulda, Germany.
- Bereczki, J., Pecsenye, K., Peregovits, L. & Varga, Z. (2005) Pattern of genetic differentiation in the Maculinea alcon species group (Lepidoptera, Lycaenidae) in Central Europe. *Journal of Zoological Systematics and Evolutionary Research*, 43, 157–165.
- Bilý, S. (2002) Summary of the bionomy of the Buprestid beetles of Central Europe (Coleoptera: Buprestidae). Acta Entomologica Musei Nationalis Pragae, Suppl., 10, 1–104.
- Burakowski, B., Mroczkowski, M. & Stefanska, J. (1985) Chrząszcze Coleoptera – Buprestoidea, Elateroidea i Cantharoidea. *Catalogus faunae Poloniae*, **23**, 1–401.
- Cohen, K.M. & Gibbard, P. (2011) Global Chronostratigraphical Correlation Table for the Last 2.7 million years. Subcommission on Quaternary Stratigraphy (International Commission on Stratigraphy), Cambridge, UK.

- De Queiroz, K. (2007) Species concepts and species delimitation. *Systematic Biology*, **56**, 879–886.
- Diogo, A.C., Vogler, A.P., Gimenez, A., Gallego, D. & Galian, D.J. (1999) Conservation genetics of Cicindela deserticoloides, an endangered tiger beetle endemic to southeastern Spain. *Journal of Insect Conservation*, **3**, 117–123.
- Excoffier, L. & Lischer, H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.
- Farkač, J., Král, D. & Škorpik, M. (eds.) (2005) Cervený seznam ohrožených druhu České republiky. Bezobratli [Red list of threatened species in the Czech Republic. Invertebrates]. Agentura ochrany přirody a krajiny ČR, Praha, Czech Republic.
- Farrell, B.D. (2001) Evolutionary assembly of the milkweed fauna: cytochrome oxidase I and the age of Tetraopes beetles. *Molecular Phylogenetics and Evolution*, 18, 467–478.
- Flanders, J., Jones, G., Benda, P., Dietz, C., Zhang, S., Li, G., Sharifi, M. & Rossiter, S.J. (2009) Phylogeography of the greater horseshoe bat, Rhinolophus ferrumequinum: contrasting results from mitochondrial and microsatellite data. *Molecular Ecology*, 18, 306–318.
- Frankham, R., Ballou, J.D. & Briscoe, D.A. (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge, England.
- Gutowski, J.M. (2006) Chrząszcze kózkowate i bogatkowate (Coleoptera: Cerambycidae, Buprestidae) rezerwatu lesnostepowego "Bielinek" nad Odrą oraz jego okolic [Longhorn and buprestidae beetles (Coleoptera: Cerambycidae, Buprestidae) of the forest-steppe reserve "Bielinek" and its surroundings]. *Parki narodowe i Rezerwaty Przyrody*, **25**, 31–64.
- Haig, S.M. (1998) Molecular contributions to conservation: molecular techniques in ecology. *Ecology*, **79**, 413–425.
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series (London)*, **41**, 95–98.
- Hall, B. (2007) Phylogenetic Trees Made Easy: A How-To Manual, 3rd Edition. Sinauer Associates Inc, Sunderland.
- Hall, M.J.R., Testa, J.M., Smith, L., Adams, Z.J.O., Khallaayoune, K., Sotiraki, S., Stefanakis, A., Farkas, R. & Ready, P.D. (2009) Molecular genetic analysis of populations of Wohlfahrt's wound myiasis fly, Wohlfahrtia magnifica, in outbreak populations from Greece and Morocco. *Medical and Veterinary Entomology*, 23, 72–79.
- Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of Linnean Society*, **68**, 87–112.
- Hickerson, M.J., Carstens, B.C., Cavender-Bares, J., Crandall, K.A., Graham, C.H., Johnson, J.B., Rissler, L., Victoriano, P.F. & Yoder, A.D. (2010) Phylogeography's past, present, and future: 10 years after Avise, 2000. *Molecular Phylogenetetics* and Evolution, 54, 291–301.
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.
- Huelsenbeck, J.P., Ronquist, F., Nielsen, R. & Bollback, J.P. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**, 2310–2314.
- Kajtoch, Ł. (2011) Conservation genetics of xerothermic beetles in Europe: the case of Centricnemus leucogrammus. *Journal of Insect Conservation*, 15, 787–797.
- Kajtoch, Ł., Korotyaev, B. & Lachowska-Cierlik, D. (2012) Genetic distinctness of parthenogenetic forms of European *Polydrusus* weevils of the subgenus *Scythodrusus*. *Insect Science*, **19**, 183–194.

- Kajtoch, Ł., Kubisz, D., Lachowska-Cierlik, D. & Mazur, M.A. (2013) Conservation genetics of endangered leaf-beetle Cheilotoma musciformis populations in Poland. *Journal of Insect Conservation*, **17**, 67–77.
- Kajtoch, Ł., Lachowska-Cierlik, D. & Mazur, M. (2009) Genetic diversity of xerothermic weevils Polydrusus inustus and Centricnemus leucogrammus (Coleoptera: Curculionidae) in central Europe. *European Journal of Entomology*, **106**, 325–334.
- Kelch, A. (1846) Grundlage zur Kenntniss der Käfer Oberschlesiens, insonders der Umgegend von Ratibor. Zu der öffentlichen Prüfung aller Classen des Königlichen Gymnasiums zu Ratibor den 4. und 7. April, und dem mit Entlassung der Abiturienten verbundenen Redeactus den 20, pp. 6–19. April laden ergebenst ein Director und Lehrer-Collegium, Ratibor, Silesia.
- Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120.
- Knapen, D., Knaepkens, G., Bervoets, L., Taylor, M.I., Eens, M. & Verheyen, E. (2003) Conservation units based on mitochondrial and nuclear DNA variation among European bullhead populations (Cottus gobio L., 1758) from Flanders, Belgium. *Conservation Genetics*, 4, 129–140.
- Kovanci, B., Akgul, H.C., Gencer, N.C. & Kowanci, O.B. (2005) Biology, distribution and seasonal occurrence of Coroebus elatus (F.): an emerging pest of strawberries in northwestern Turkey. *Polskie Pismo Entomologiczne*, **74**, 19–27.
- Kozak, K.H., Graham, C.H. & Wiens, J.J. (2008) Integrating GISbased environmental data into evolutionary biology. *Trends* in Ecology and Evolution, 23, 141–148.
- Kubáň, V. (1995) Palaearctic and Oriental Coraebini (Coleoptera: Buprestidae) Part. I. Entomological Problems, 26, 1–37.
- Kubáň, V. (2006) Subfamily Agrilinae Laporte, 1835 (without genus Agrilus). Catalogue of Palaearctic Coleoptera. Volume 3. Scarabaeoidea – Scirtoidea – Dascilloidea – Buprestoidea – Byrrhoidea (eds by I. Löbl and A. Smetana), pp. 403–421. Apollo Books, Stenstrup, Denmark.
- Kubisz, D., Kajtoch, Ł., Mazur, M.A., Lis, A. & Holecová, M. (2012a) Conservation genetics of highly isolated populations of xerothermic Crioceris quatuordecimpunctata (Coleoptera: Chrysomelidae). *Invertebrate Biology*, **131**, 333–344.
- Kubisz, D., Kajtoch, L., Mazur, M.A. & Rizun, V. (2012b) Molecular barcoding for central- eastern European Crioceris leaf-beetles (Coleoptera: Chrysomelidae). *Central European Journal of Biology*, 7, 69–76.
- Lekič, M. & Mihajlovič, L.J. (1969) Coraebus elatus F. (Buprestidae, Coleoptera) a strawberry pest in Serbia. *Journal Science Agricultural Research*, 22, 91–98.
- Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452.
- Lindner, L., Bogutsky, A., Gozhik, P., Marks, L., Lanczont, M. & Wojnatowicz, J. (2006) Correlation of Pleistocene deposits in the area between the Baltic and Black Sea, Central Europe. *Geological Quarterly*, **50**, 195–210.
- Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27, 209–220.
- Markova, A.K., Simakova, A.N. & Puzachenko, A.Y. (2009) Ecosystems of Eastern Europe at the time of maximum cooling of the Valdai glaciation (24-18 kyr BP) inferred from data on plant communities and mammal assemblages. *Quaternary International*, 201, 53–59.
- Marks, L. (2002) Last Glacial Maximum in Poland. *Quaternary Science Reviews*, 21, 103–110.

- Maryanska-Nadachowska, A., Kajtoch, L. & Lachowska-Cierlik, D. (2012) Genetic diversity of Philaenus spumarius and P. tesselatus (Hemiptera, Aphrophoridae): implications for evolution and taxonomy. *Systematic Entomology*, **37**, 55–64.
- Mico, E., Sanmartin, I. & Galante, E. (2008) Mediterranean diversification of the grass-feeding Anisopliina beetles (Scarabaeidae, Rutelinae, Anomalini) as inferred by bootstrap-averaged dispersal-vicariance analysis. *Journal of Biogeography*, 36, 546-560.
- Moritz, C. (1994) Defining "Evolutionarily Significant Units" for conservation. *Trends in Ecology and Evolution*, 9, 373–375.
- Nehring, A. (1890) Ueber Tundren und Steppen der Jetzt-und Vorzeit, F. Dümmler, Berlin, Germany.
- Niehuis, M. (1991) Beitrag zur Kenntnis der Coroebus-Arten des Nahen Ostens (Coleoptera: Buprestidae). Acta Coleopterologica, 7, 69–82.
- Nylander, J.A.A. (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Ortego, J., Bonal, R., Cordero, P.J. & Aparicio, J.M. (2009) Phylogeography of the Iberian populations of Mioscirtus wagneri (Orthoptera: Acrididae), a specialized grasshopper inhabiting highly fragmented hypersaline environments. *Biological Journal* of the Linnean Society, **97**, 623–633.
- Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, 7, 16.
- Page, R.D.M. (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*, **12**, 357–358.
- Pawłowski, J. (1991) Przemiany fauny od pleniglacjalu do czasów wspólczesnych. *Geografia Polski. Srodowisko Przyrodnicze* (ed. by L. Starkel), pp. 159–177. Wydawnictwo Naukowe PWN, Warszawa, Poland.
- Piñero, F.S., Tinaut, A., Aguirre-Segura, A., Miñano, J., Lencina, J.L., Ortiz-Sánchez, F.J. & Pérez-Lópe, F.J. (2011) Terrestrial arthropod fauna of arid areas of SE Spain: diversity, biogeography, and conservation. *Journal of Arid Environments*, 75, 1321–1332.
- Rambaut, A. & Drummond, A.J. (2003–2009) Tracer v1.4. Distributed by the Authors. < http://beast.bio.ed.ac.uk/Trace > 23rd February 2012.
- Ratyńska, H. & Waldon, B. (eds) (2010) Cieplolubne murawy w Polsce – stan zachowania i perspektywy ochrony. Wydawnictwo Uniwersytetu Kazimierza Wielkiego, Bydgoszcz, Poland.
- Ribera, I. & Blasco-Zumeta, J. (1998) Biogeographical links between steppe insects in the Monegros region (Aragon, NE Spain), the eastern Mediterranean, and central Asia. *Journal of Biogeography*, 25, 969–986.
- Ribera, I., Hernando, C. & Aguilera, P. (2001) Agabus alexandrae sp.n. from Morocco, with a molecular phylogeny of the Western Mediterranean species of the A. guttatus group (Coleoptera: Dytiscidae). *Insect Systematics and Evolution*, **32**, 253– 262.
- Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X. & Rozas, R. (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.
- Rozen, S. & Skaletsky, H.J. (2000) Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (ed. by S. Krawetz and S. Misener), pp. 365–386. Humana Press, Totowa, New Jersey.
- Rutkowski, R., Sielezniew, M. & Szostak, A. (2009) Contrasting levels of polymorphism in cross-amplified microsatellites in two
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endangered xerothermophilous, obligatorily myrmecophilous, butterflies of the genus Phengaris (Maculinea) (Lepidoptera: Lycaenidae). *European Journal of Entomology*, **106**, 457–469.

- Ryder, O.A. (1986) Species conservation and systematics: the dilemma of the subspecies. *Trends in Ecology and Evolution*, 1, 9–10.
- Sánchez-Fernández, D., Lobo, J.M., Abellán, P. & Millán, A. (2011) Environmental niche divergence between genetically distant lineages of an endangered water beetle. *Biological Journal* of the Linnean Society, **103**, 891–903.
- Schaefer, L. (1949) Les Buprestides de France. Miscellanea Entomologica, Supplement E, 1–511.
- Schmitt, T. (2007) Molecular biogeography of Europe: pleistocene cycles and postglacial trends. *Frontiers in Zoology*, 4, 1–13.
- Schwier, H.-J. & Neumann, V. (2004) Rote Liste der Prachtkäfer (Coleoptera: Buprestidae) des Landes Sachsen-Anhalt. Berichte des Landesamtes f
 ür Umweltschutz Sachsen-Anhalt, 39, 294–298.
- Simon, C., Frati, F., Bechenbach, A., Crespi, B., Liu, H. & Flock, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequence and compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87, 651–701.
- Slatkin, M. (1993) Isolation by distance in equilibrium and nonequilibrium populations. *Evolution*, 47, 264–279.
- Sommer, R.S. & Nadachowski, A. (2006) Glacial refugia of mammals in Europe: evidence from fossil records. *Mammal Review*, 36, 251–265.
- Stewart, J.R. & Lister, A.M. (2001) Cryptic northern refugia and the origins of the modern biota. *Trends in Ecology and Evolution*, **16**, 608–613.
- Stewart, J.R., Lister, A.M., Barnes, I. & Dalén, L. (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological sciences*, 277, 661–671.
- Swofford, D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland.

- Szymczakowski, W. (1960) Materiały do poznania kserotermofilnej fauny chrząszczy Wyżyny Małopolskiej [Matherials for the study of xerothermophilous beetle fauna of Malopolska Upland]. *Polskie Pismo Entomologiczne*, **30**, 173–242.
- Taberlet, P., Fumagalli, L., Wust-Saucy, A.-G. & Cossons, J.-F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, 7, 453–464.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
- Théry, A. (1942) Faune de France / 41 Coléoptères buprestides. Lechevalier, Paris, France.
- Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C. & Collar, N.J. (2010) Quantitative criteria for species delimitation. *Ibis*, **152**, 724–746.
- Wahlberg, N. & Saccheri, I. (2007) The effects of Pleistocene glaciations on the phylogeography of Melitaea cinxia (Lepidoptera: Nymphalidae). *European Journal of Entomology*, **104**, 675–684.
- Waples, R.S. (1991) Pacific salmon, Oncorhynchus spp., and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service, Marine Fisheries Review, 53, 11–22.
- Willis, K.J. & van Andel, T.A. (2004) Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Reviews*, 23, 2369–2387.
- Wysota, W., Molewski, P. & Sokolowski, R.J. (2009) Record of the Vistula Ice Lobe advances in the Late Weichselian glacial sequence in north-central Poland. *Quaternary International*, 207, 26–41.

Accepted 1 February 2013 First published online 26 March 2013

Editor: Calvin Dytham Associate editor: Ignacio Ribera