UDK 630 * 152 + 135 (*Castor fiber* L.) (001)

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GENETIC EVIDENCE ON THE ORIGIN OF THE CURRENT BEAVER (*Castor fiber*) POPULATION IN LOWER AUSTRIA

GENETIČKI DOKAZI O PORIJEKLU DANAŠNJE POPULACIJE EUROPSKOG DABRA (*Castor fiber*) U DONJOJ AUSTRIJI

Matthias KROPF[1], Gerald HÖLZLER[2], Rosemarie PARZ-GOLLNER[2]

Summary

Beside European beavers (*Castor fiber*) from Poland, Belarus and Sweden also individuals of *C. canadensis* have been released to the Austrian Danube watershed east of Vienna during the years 1976 and 1990. Based on reintroduction activities the Lower Austrian beaver population developed well to a current size of more than 3000 individuals. However, it remains ambiguous, which of the originally released animals account for this success. Here, we present mitochondrial (mt) DNA data to answer the questions whether *C. canadensis* is still traceable in Lower Austria and which intraspecific lineages of *C. fiber* are currently present?

Our Maximum Likelihood (ML) analysis based on 81 newly generated DNA sequences from the mitochondrial control region plus 30 reference beaver samples from the literature, revealed no evidence for the existence of *C. canadensis* in Lower Austria. With respect to *C. fiber*, three different intraspecific lineages (mainly corresponding to different subspecies) were identified. While the majority of samples represent either Scandinavian (subsp. *fiber*) or Central European beavers (likely *C. f. albicus x belorussicus/orientoeuropaeus*), one sample of *C. f. pohlei* was rather genetically distinct and unexpected from the documented reintroduction history. In addition, possible hybridisation among intraspecific beaver lineages is discussed, although the maternally inherited mtDNA analysed here did not give the ultimate answer to this question.

KEY WORDS: *Castor fiber*, Danube, Lower Austria, mitochondrial control region, reintroduction

Introduction

Uvod

The European beaver (*Castor fiber* L., 1758) was exterminated in the 1860s in Austria (Sieber & Bauer 2001). In Lower Austria the last beaver was shot 1863 in the Danube floodplain area east of Vienna near Fischamend. During the years 1976 to 1990, European beavers from Poland, Belarus and Sweden (Sieber & Bauer 2001), probably representing different subspecies (see Ducroz et al. 2005; Durka et al. 2005; Horn et al. 2011), have been released into the Austrian Danube watershed east of Vienna. Furthermore, also few individuals of *C. canadensis* have been introduced during that time. While the number of released *C. fiber* individuals was higher than 40, based on the available sources (i.e. Lutschinger 1988; Sieber 1999; Sieber & Bauer 2001) the number of *C. canadensis* individuals was close to twelve. None of the data published so far gives evidence of *C. ca-*

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[1] Dr. Matthias Kropff, Institute of Integrative Nature Conservation Research, matthias.kropf@boku.ac.at (corresponding author)

[2] Mag. Gerald Hölzler, Ass. Prof. Rosemarie Parz-Gollner, Institute of Wildlife Biology and Game Management, gerald.hoelzler@boku.ac.at, rosemarie.parz-gollner@boku.ac.at, University of Natural Resources and Life Sciences, Gregor-Mendel-Str. 33, A-1180 Vienna, Austria

nadensis presence in the recently expanding beaver population (e.g. Halley & Rosell 2002; Kautenburger & Sander 2008). It therefore remains ambiguous, which of the originally released animals account for the successful resettlement. Although based only on seven individual samples, a recent study using Randomly Amplified Polymorphic DNA (RAPD) variation rejected the existence of *C. canadensis* in Austria, but also indicated comparatively high heterogeneity with some genetically remarkably distinct individual samples (Kautenburger & Sander 2008).

In 2011 the total population size of beavers in the province Lower Austria was estimated to be about 3300 individuals (Parz-Gollner & Hölzler 2012). As a consequence of the increasing density as well as the ongoing beaver spread resulting in new settlements over the recent years, increasing conflicts with human land use have led to the implementation of a beaver management in Lower Austria. This federal state-wide beaver management basically follows a stepwise action plan, including prevention and repellent measures. Since the winter 2006/07 derogations given by the respective state administration authorities allow also trapping and killing of beavers under controlled conditions at locally restricted sites. Weight, sex, and morphometric data as well as organic material for DNA analysis were collected from all beavers killed so far under the stepwise action plan and stored deep-frozen for further research. Here, we present first genetic data on parts of these tissue sampling. By mtDNA sequence data analysis of the non-coding control region (D-loop; cf. Sbisà et al. 1997) we tried to answer the following questions:

1) Can the occurrence of *C. canadensis* within the current Lower Austrian beaver population be ruled out?
2) Can we identify different intraspecific lineages of the beaver often treated on the subspecific level representing both, the Western and the Eastern European *C. fiber* group?
3) Are there signs of hybridisation among intraspecific lineages of *C. fiber*?

Material and Methods
Materijal i metode

Sample collection – Prikupljanje uzoraka

Tissue and hair samples were collected during the years 2007 and 2011 based on the stepwise management plan as described above. In addition to the trapped animals (in some cases several family members per site) also road kill victims were included in our analysis. We selected individual tissue samples (i.e. muscle tissue) from different years covering most of the present distribution range of beavers in Lower Austria. Within areas of higher population density, causing more human land use conflicts in the cultural landscape area, also more individuals were trapped at specific sites and subsequently analysed. Altogether our data

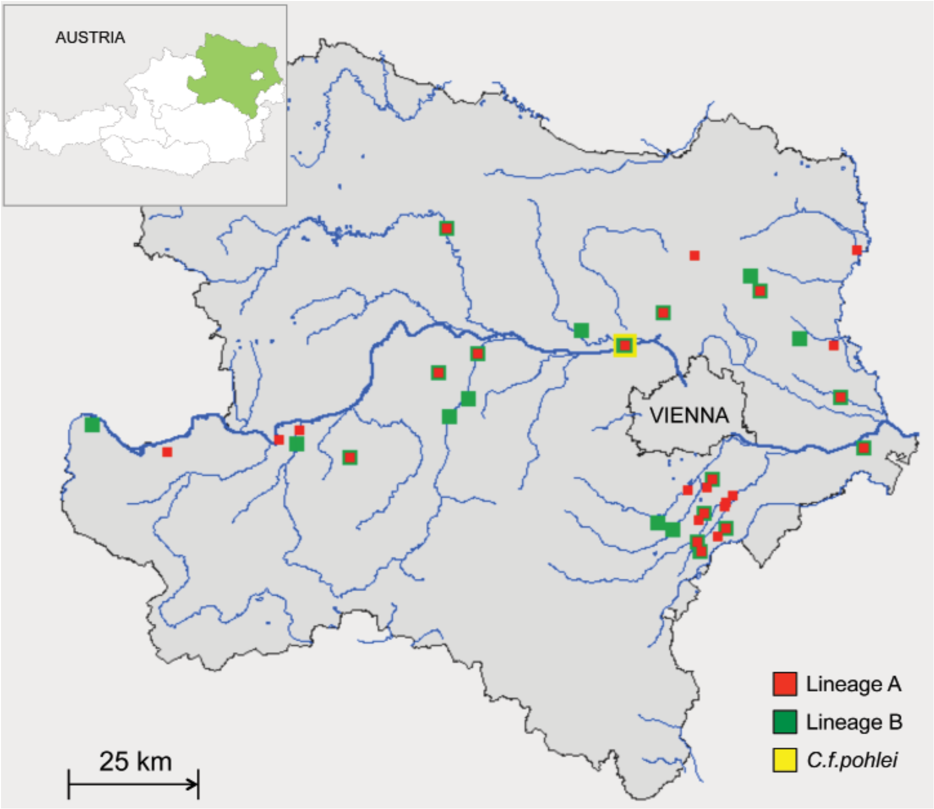

Figure 1. Localities of beaver samples in Lower Austria used in the present study. Colours indicate group memberships according to our genetic analysis (see Fig. 2). Overall, 81 individual samples collected on 40 localities were analysed. Note that some of the sample locations are spatially close and therefore not all are visible in detail on the present map scale. The inset indicates the position of Lower Austria within Austria.

Slika 1. Raspored uzorkovanih jedinki dabrova u Donjoj Austriji analiziranih provedenim istraživanjem. Različite boje označavaju grupiranje uzoraka na temelju provedene genetičke analize (vidi Sliku 2). Sveukupno, 81 individualni uzorak tkiva izuzet na 40 lokacija bio je predmetom ovog istraživanja. Zbog mjerila prikaza neki su prostorni podaci toliko blizu da je broj i pozicija lokaliteta istraživanja na slici pojednostavljen. Umetak ilustrira prostornu poziciju savezne pokrajine Donje Austrije.

set covers 81 DNA-samples (42 ♂♂, 38 ♀♀ and 1 unsexed individual) widely distributed over 40 localities (Fig. 1) of the Danube watershed including northern and southern tributaries in Lower Austria.

DNA analysis – DNA analiza

Total genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. The mtDNA control region (D-loop) was amplified using the universal primers DL-H16340 (5'-CCTGAAGTAGGAACCAGATG-3') and Thr-L15926 (5'-CAATTCCCCGGTCTTGTAAACC-3') and the protocol published by Vilà et al. (1999). This mtDNA region was chosen as it is known for its comparatively high, even intraspecific variability (cf. Sbisà et al. 1997) and the availability of reference sequences (see below). The cycle sequencing was processed by LGC Genomics GmbH (Berlin, Germany). Reference beaver D-loop sequences were obtained from the European Nucleotide Archive at the European Molecular Biology Laboratory (EMBL), originally generated by Ducroz et al. (2005: accession numbers AY623632–43), Durka et al. (2005: DQ088700–03), Fasanella et al. (unpubl. data: EU476079, GQ228450, JN655158–59), Lizarralde et al. (2008: AY787822–27, AY968083), and Horn et al. (2010: JF264886–88). We also deposited our new D-loop sequence variants at EMBL, under the accession numbers HF674455–58.

The 81 newly generated mtDNA sequences as well as the above 30 reference sequences were aligned using BioEdit 7.0.5.3 (Hall 1999) and restricted to a 455 bp alignment available for all 111 individuals. The hierarchical likelihood ratio test as implemented in Modeltest 3.06 (Posada & Crandall 1998) was used to detect the best evolutionary model based on our set of sequence data. The HKY85+Γ model found (see also Ducroz et al. 2005) was then run in PAUP* 4.0 (Swofford 2000) using the respective parameters in effect to generate a maximum likelihood (ML; Felsenstein 1981) tree reconstruction: $\pi_A = 0.3425$, $\pi_C = 0.2547$, $\pi_G = 0.1528$, $\pi_T = 0.25$; $I = 0$; $\Gamma = 0.1381$. We also generated neighbor-joining trees (NJ; Saitou & Nei 1987) based on HKY85 genetic distances and respective NJ bootstrap support values (Felsenstein 1985) running 10,000 replicates.

Results

Rezultati

Within our Lower Austrian beaver sampling of 81 individuals investigated so far, we found no evidence for the occurrence of *C. canadensis* as all D-loop sequences fell into the well-defined *C. fiber* cluster (Fig. 2; 100% bootstrap support). However, with respect to *C. fiber* our individual samples split into three unequal groups: one beaver from the Danube floodplains west of Vienna was identified representing the Eastern European beaver group, being most likely the West-Siberian subspecies *C. f. pohlei* (cf. Ducroz et al. 2005). The remaining 80 individuals split into two major lineages for which single reference mtDNA sequences could be identified (Fig. 2): the lineage "**A**" corresponds to a *C. f. albicus x C. f. belorussicus/orientoeuropaeus* sequence from Eastern Germany (locality "Halbe", published by Horn et al. 2010) without any DNA sequence variation within this group (41 sequences), and the lineage "**B**" (Fig. 2; 93% bootstrap support) to a beaver from Norway ("Bo i Telemark", published by Durka et al. 2005 as *C. f. fiber*). Within lineage "**B**" altogether 35 D-loop sequences were identical to the reference sequence, but additionally four sequences were identified all showing a joint single nucleotide mutation (i.e. EDFiba 03–04, Po000015, StP00001; Fig. 2). It has to be pointed out that often multiple samples from the same beaver territory were grouped inside both lineages "**A**" and "**B**" (e.g. BadDA, BaPira, Col, Woelbl etc.; Fig. 2). At least in four cases sexually mature individuals have been identified to form genetically mixed pairs representing both major mtDNA lineages. All the females from these mixed pairs already showed status of gestation. In other cases adult males identified showed a different genetic lineage compared to their assumed offspring (i.e. yearlings) belonging to the same family trapped within the same territory during the same season.

Discussion

Rasprava

Origin of the current Lower Austrian beaver population – Podrijetlo današnje populacije dabra u Donjoj Austriji

The three mtDNA groups found in our Lower Austrian beaver samples basically confirmed a variety of origins of the introduced beavers as documented by Sieber & Bauer (2001), i.e. *C. f. fiber* from Scandinavia (Norway and/or Sweden) and *C. f. albicus x C. f. belorussicus/orientoeuropaeus*, probably from Poland and Belarus. However, the current occurrence of *C. canadensis* was not confirmed (see also Halley & Rosell 2002; Kautenburger & Sander 2008), but instead, one beaver individual likely representing subspecies *C. f. pohlei* was detected. The latter result was surprising as from the literature there was no indication of a West-Siberian origin of reintroduced beavers in Austria. Although actually relict and reintroduced populations of beavers are growing together all over Central Europe, given their successful expansion (Halley & Rosell 2002, 2003; Dewas et al. 2012), it anyhow seems unlikely that beavers from the far West-Siberia entered Austria independently during this process. Presumably, documentation of the actual origin of reintroduced beaver individuals might have been imprecise, in a way that, for instance, reintroduced beavers from Belarus (cf. Sieber & Bauer 2001) need not to reflect solely autochthonous Belorussian material. In this

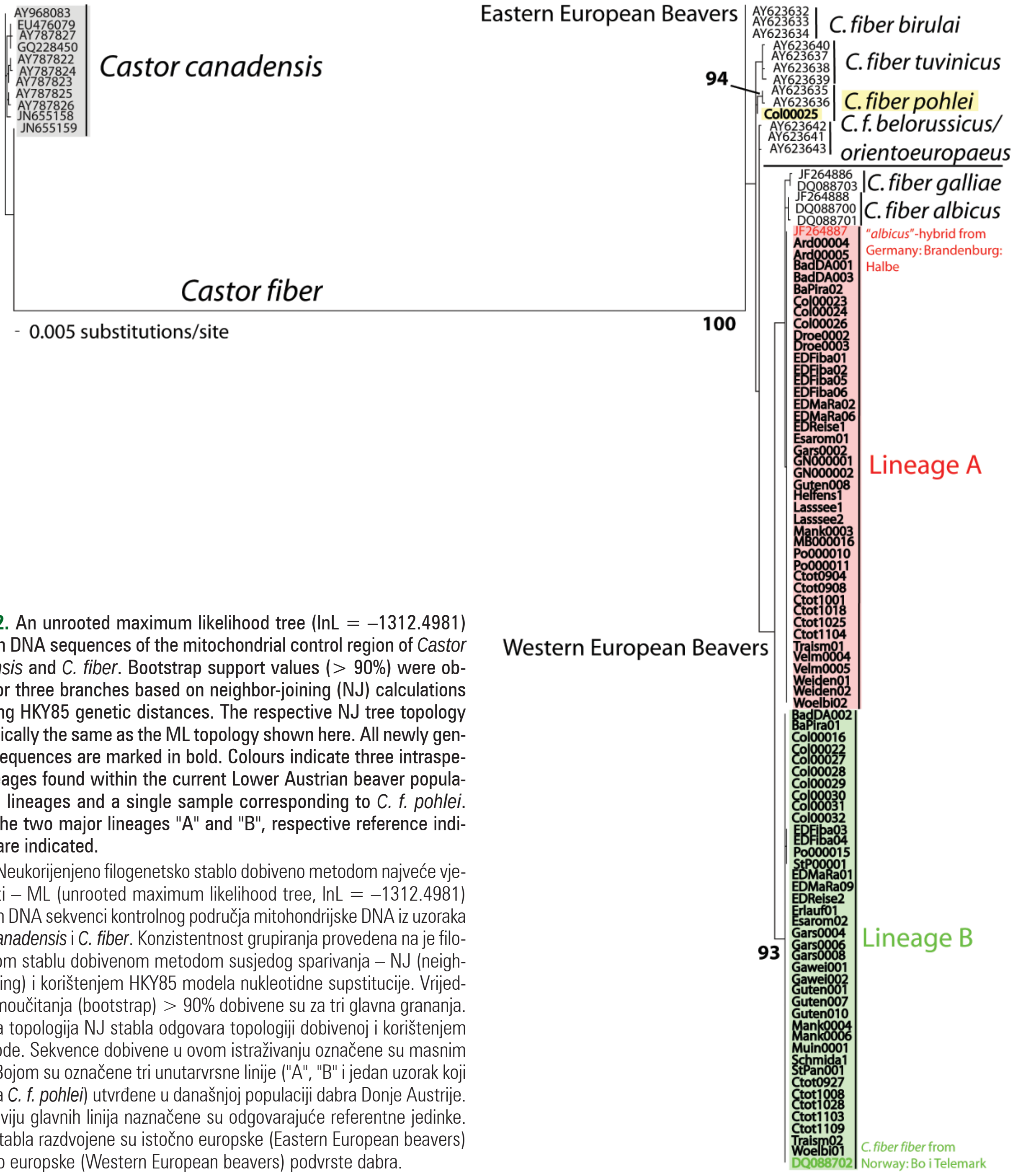

Figure 2. An unrooted maximum likelihood tree (lnL = –1312.4981) based on DNA sequences of the mitochondrial control region of *Castor canadensis* and *C. fiber*. Bootstrap support values (> 90%) were obtained for three branches based on neighbor-joining (NJ) calculations also using HKY85 genetic distances. The respective NJ tree topology was basically the same as the ML topology shown here. All newly generated sequences are marked in bold. Colours indicate three intraspecific lineages found within the current Lower Austrian beaver population, i.e. lineages and a single sample corresponding to *C. f. pohlei*. Within the two major lineages "A" and "B", respective reference individuals are indicated.

Slika 2. Neukorijenjeno filogenetsko stablo dobiveno metodom najveće vjerojatnosti – ML (unrooted maximum likelihood tree, lnL = –1312.4981) temeljem DNA sekvenci kontrolnog područja mitohondrijske DNA iz uzoraka *Castor canadensis* i *C. fiber*. Konzistentnost grupiranja provedena na je filogenetskom stablu dobivenom metodom susjedog sparivanja – NJ (neighbour joining) i korištenjem HKY85 modela nukleotidne supstitucije. Vrijednosti samoučitanja (bootstrap) > 90% dobivene su za tri glavna grananja. Dobivena topologija NJ stabla odgovara topologiji dobivenoj i korištenjem ML metode. Sekvence dobivene u ovom istraživanju označene su masnim tiskom. Bojom su označene tri unutarvrsne linije ("A", "B" i jedan uzorak koji odgovara *C. f. pohlei*) utvrđene u današnjoj populaciji dabra Donje Austrije. Unutar dviju glavnih linija naznačene su odgovarajuće referentne jedinke. Unutar stabla razdvojene su istočno europske (Eastern European beavers) i zapadno europske (Western European beavers) podvrste dabra.

sense, the true origin of beavers might have especially been masked when animals were taken from zoological gardens and/or captive breedings in Eastern Europe.

With respect to mating in the current beaver population of Lower Austria, it is obvious that no regional structure of different reintroduced subspecific groups exist, but in fact at most of the sampled localities both major mtDNA lineages could be identified (Fig. 1). Therefore, pairings between these lineages are highly likely, and proven in our study by at least four genetically mixed beaver pairs trapped in the status of gestation.

Indication of intraspecific hybridisation within the Lower Austrian beaver population – Naznake unutarvrsnog križanja unutar populacije dabra u Donjoj Austriji

Beside the differing origin of the introduced beavers in Lower Austria, there is also indication of intraspecific hybridisation within our beaver sample set. The assumption of hybridisation is not only indicated by the co-occurrence of the two major mtDNA lineages at the same site or within the same beaver territory, but is further supported by the fact that dark-coated individuals occur within both major mtDNA lineages ("**A**" and "**B**") observed. Moreover, one rather bright-coated individual was detected belonging to the Eastern European beaver group (*C. f. pohlei*). Following the argumentation line by Horn et al. (2010), we are interpreting the nuclear black-coat character typical for the Eastern European beaver group (cf. Durka et al. 2005) found in both major Western European beaver mtDNA lineages in Lower Austria as a strong indication for the assumed intraspecific hybridisation: to observe a female black-coated beaver characterised by, for instance, subsp. *fiber* mtDNA (i.e. representing a Western European beaver), the nuclear encoded coat colour should originate from the paternal line (i.e. representing an Eastern European beaver) following at least two reproduction events. In addition, also Kautenburger & Sander (2008) have speculated about possible hybridisation in their Austrian beaver sampling to explain some aberrant RAPD variation analysed.

However, direct evidence of such intraspecific hybridisation cannot be deduced from the solely maternally inherited mtDNA data set alone. Therefore, further molecular markers, especially representing the nuclear genome, need to be analysed (cf. Horn et al. 2010). This will represent the next step in the genetic part of the beaver management in Lower Austria. In addition, we are also interested in assessing family size and local patterns of beaver activity ranges and dispersal routes. Therefore, we will also use microsatellite markers (see Frosch et al. 2011) within our Lower Austrian beaver sampling in the future.

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Sažetak

Pored europskog dabra (*Castor fiber*) podrijetlom iz Poljske, Bjelorusije i Švedske, u razdoblju od 1976. do 1990. godine u porječje Dunava istočno od Beča u Republici Austriji unešene su i jedinke kanadskog dabra (*C. canadensis*). Zahvaljujući naporima oko reintrodukcije populacija dabra dobro se razvijala i danas se procjenjuje na više od 3000 jedinki. Postavlja se pitanje koje su od svojedobno unešenih jedinki (linija) "zaslužne" za ovaj uspjeh. U radu se prikazuje analiza mitohondrijske DNA (mtDNA) prikupljena iz uzoraka tkiva i dlake dabrova odstrijeljenih u razdoblju 2007–2011. Istraživanjem se željelo odgovoriti na pitanja o današnjoj zastupljenosti unutarvrsnih linija kojima su pripadale jedinke kod reintrodukcije te pitanje detekcije prisutnost gena udaljene vrste *C. canadensis*. Analiza metodom najveće vjerojatnosti (ML) bazirana na 81 generiranih DNA sekvenci mitohondrijskog kontrolnog područja u sprezi s 30 referentnih uzoraka dabra iz literaturnih izvora nije potvrdila prisutnost vrste *C. canadensis* u Donjoj Austriji. Što se pak europskog dabra (*C. fiber*) tiče, utvrđena je prisutnost tri unutarvrsne linije, uglavnom korespondirajuće sa tri različite podvrste. Dok je najveći broj uzoraka odgovarao skandinavskoj (ssp. *fiber*) ili srednjoeuropskoj podvrsti (vrlo vjerojatno *C. f. albicus x belorussicus/orientoeuropaeus)*, jedan je uzorak genetički odstupao (definiran kao *C. f. Pohlei)* i pojavio se neočekivano s obzirom na poznate podatke o izvršenoj reintrodukciji. U radu se također razmatraju mogući aspekti križanja između prisutnih unutarvrsnih linija, mada mtDNA analiza nije uspjela u potpunosti odgovoriti na ova pitanja.

In addition, possible hybridisation among intraspecific beaver lineages is discussed, although the maternally inherited mtDNA analysed here did not give the ultimate answer to this question

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KLJUČNE RIJEČI: Dunav, Donja Austrija, mtDNA, reintrodukcija