

Haplotype Diversity of Brown Trout (*Salmo trutta* L.) Populations from Una River Drainage Area in Bosnia and Herzegovina: Implications for Conservation and Fishery Management

Dubravka Škraba^{1*}, Azra Bećiraj², Irma Šarić², Irma Ićanović², Aida Džaferović², Marina Piria³, Radoslav Dekić⁴, Ana Tošić¹, Vera Nikolić¹ and Predrag Simonović¹

¹Faculty of Biology, University of Belgrade, 16 Studentski trg., 11000 Belgrade, Serbia

²Biotechnical Faculty, University of Bihać, Luke Marjanovica bb, 77000 Bihać, Bosnia and Herzegovina

³Department of Fisheries, Beekeeping, Game Management and Special Zoology, Faculty of Agriculture, University of Zagreb, 25 Svetošimunska Cesta, 10000 Zagreb, Croatia

⁴Faculty of Natural Sciences and Mathematics, University of Banja Luka, 2 Mladena Stojanovića, 78000 Banja Luka, Bosnia and Herzegovina

Abstract: Data about the mitochondrial DNA haplotypes of brown trout (*Salmo trutta* L.) in the area of the Danube River Basin in Bosnia and Herzegovina have not been hitherto published. Brown trout from the drainage area of the Una River was assessed for the first time using the Control Region of the mitochondrial DNA. Anal fin clips of 43 brown trout specimens were sampled from four sites in the headwater section and in two tributaries joining the Una River in the middle section. In total, three CR haplotypes were recorded: Da2 and Da22 from the Danubian and At1 from the Atlantic brown trout lineage. Da22 haplotype was inferred the native one in the Una River drainage area owing to its most frequent and most upstream dispersal in the headwater section. The other two haplotypes were found with the native one only in one or two tributaries joining the Una River in the middle section. They are considered alien and were most likely introduced by stocking with hatchery reared brown trout fingerlings. Occurrence of several trout hatcheries in the area poses strong risk of introduction of alien haplotypes into the headwater section and consequential breakdown in local native brown trout.

Key Words: mtDNA, Danubian lineage, alien haplotypes

Introduction

Brown trout (*Salmo trutta* L.) is a frequently studied species, especially in molecular studies using mitochondrial DNA (mtDNA) control region (CR) as a main marker (BERNATCHEZ 2001; CORTEY & GARCÍA-MARÍN 2002; DELLING 2003; DUFTNER *et al.* 2003; HORVATH *et al.* 2014). Mitochondrial DNA CR is often used in combination with other molecular markers (e.g., cytochrome *b*, some nuclear genes and microsatellites) in resolving phylogeographic (BERNATCHEZ 2001; CORTEY *et al.* 2002, 2004; ZHANG

& HEWITT 2003) as well as conservational, management and stocking problems (APOSTOLIDIS *et al.* 2008; VERA *et al.* 2010b; KOHOUT 2012; KOHOUT *et al.* 2013). Based on mtDNA data, BERNATCHEZ *et al.* (1992) and BERNATCHEZ (2001) recognised five major groups within the brown trout complex: Atlantic (AT), Danubian (DA), Mediterranean (ME), *marmoratus* (MA) and Adriatic (AD) lineages. In addition, the new mtDNA lineage in the Duero River Basin (DU) has been recently proposed (SUÁREZ *et*

*Corresponding author: dubravka@bio.bg.ac.rs

al. 2001; CORTEY & GARCÍA-MARÍN 2002). It is endemic to this river basin and probably diverged from other Atlantic populations during the Pleistocene (VERA *et al.* 2010a).

The Balkan Peninsula is considered a hotspot in the evolution of many European species (Conservation International 2004). This is also reflected in the numerous nominal trout taxa (e.g., *Salmo obtusirostris* HECKEL, 1851, *S. taleri* KARAMAN, 1932, *S. farioides*, *S. labrax* PALLAS, 1814 and *S. macedonicus* KARAMAN, 1924) that have been described for the region by early studies. The status of the majority of these nominal trout taxa is still uncertain. The faunal distinctness of this area resulted both from geotectonic events that separated the Mediterranean and Pontian drainages during the Tertiary, and from climatic events occurring in the beginning of the Quaternary that made the Balkan Peninsula a refuge area during that period (HEWITT 1999). Although brown trout is considered to be a component of the northern fish fauna, populations from non-glaciated refuge areas have persisted much longer than those in recently re-colonised northern areas after the glacial retreat, about 13,000 years BC (FERGUSON 1989).

In order to clarify the phylogeography of brown trout in the Balkan region, variability of external morphological (HECKEL & KNER 1858; KARAMAN 1927, 1932, 1933, 1938; KARAKOUSISET *et al.* 1991; DELLING 2002, 2003; SIMONOVIĆ *et al.* 2007) and various molecular characters have been studied. Studies on brown trout stocks in the Danubian lineage employing molecular markers are hitherto numerous (DUFTNER *et al.* 2003; JUG *et al.* 2004; SUŠNIK *et al.* 2005; MARIĆ *et al.* 2006; HASHEMZADEH *et al.* 2012; KOHOUT *et al.* 2013; TOŠIĆ *et al.* 2014). They all revealed a great number of mtDNA haplotypes. The most distinct ones in the Western Balkans area are Da*Vr and Da*Dž (MARIĆ *et al.* 2006), as they differ from other Danubian haplotypes by two to five mutations (Marić *et al.* 2006). However, relationships reconstructed between brown trout stocks employing either mtDNA or several informative microsatellite markers did not lead yet to any sound taxonomic inference. Hence, the still unresolved taxonomy (KOTTELAT 1997) of brown trout stocks in the Danube River Basin remains their prominent feature.

In the Danube River Basin of the Western Balkans and their adjacent regions, SNOJ (2004) reports seven CR mtDNA haplotypes in brown trout of the Danubian and one haplotype of the Atlantic lineage in Slovenia. Further, MRDAK (2011) reports only two (Da1 and Da2) widely dispersed CR mtDNA haplotypes and the same Atlantic hap-

lotype for brown trout in Montenegro. A high level of haplotype polymorphism with eight different CR mtDNA haplotypes has been found in the drainage area of the Danube River of Serbia. Four of them, i.e. Da*V1, Da*Dž, Da*Vr and Da23c, were novel for the Danubian lineage (MARIĆ *et al.* 2006; TOŠIĆ *et al.* 2014). JADAN (2007) reported Da2 haplotype for the upper section of the Gacka River in Western Croatia, in the drainage area of the Adriatic Sea Basin. In addition to these Danubian haplotypes, throughout Europe the introgression of brown trout of the Atcs1 haplotype is evident (SNOJ 2004; MRDAK 2011; MARIĆ *et al.* 2012; SIMONOVIĆ *et al.* 2014). Their hatchery origin in the Western Balkans area was clearly proved by MARIĆ *et al.* (2010).

Data for brown trout stocks in streams of the Danube River basin in the Bosnia and Herzegovina are not hitherto available. Brown trout is the prime target fly fishing species. The main fishery management measure that hitherto compensated the fishing pressure on brown trout and their taking out has been stocking. It has been badly controlled, as proved by the introduction and translocation of many non-native brown trout strains and haplotypes into the streams attractive for fishing. The purpose of this study is to present for the first time the variation in brown trout CR mtDNA that occur in the Una River drainage area as the westernmost part of the Danube River Basin in Bosnia and Herzegovina. This study would provide the starting point for greater conservation efforts of the fisheries management with the wild brown trout stocks in the area.

Materials and Methods

Studies of brown trout stock in the upper and middle sections of the Una River (a tributary of the Sava River) and its tributaries were accomplished at five sites (Fig. 1). The upper part of the Una River comprises the section close to Martin Brod with tributaries (Krka River) and is isolated from the downstream section by the Great and Štrbac Waterfalls which are 54 and 24 m high, respectively. That river section approximately matches the northern boundary of the Una National Park. The downstream section of the Una River is further fragmented by Kostela Waterfalls situated about seven kilometres downstream of the city of Bihać, with approximately the same height as the Štrbac Waterfall.

Brown trout sampling was conducted in the early autumn of 2012 and 2013, by electrofishing, using either an engine-powered electrofishing gear Suzuki-Bosch™ (220 V DC, $I = 6$ A max) or battery-powered portable electrofishing gear AquaTech™ IG200/1®

(380 / 600 V DC, $I = 15A$ max). Anal fin clip samples of brown trout collected from 43 brown trout were analysed: 28 samples from the Krka River, four samples from the Una River at Martin Brod, four samples from the Una River at Loskun, three samples from the Krušnica River and four samples from the Svetinja River at Bosanska Krupa (Fig. 1). After the fin clips were taken, fish was released into the water.

Total DNA was extracted from the tissue samples with the size of approximately 4 mm² preserved in 96% ethanol, using the High Salt Extraction technique (MILLER *et al.* 1988). Amplification of the CR was carried out using primers 28RIBa (5'-CACCTTAACTCCCAAAGCTAAG-3'; SNOJ *et al.* 2000) and HN20 (5'-GTGTTATGCTTTAGT-TAAGC-3'; BERNATCHEZ & DANZMANN 1993) under the following conditions: initial denaturation (95°C, 5 min) followed by 30 cycles of strand denaturation (94°C, 45 s), primer annealing (52°C, 45 s) and DNA extension (72°C, 2 min; the last extension prolonged to 5 min) in the programmable MultiGene[®] Thermo Cycler TC9600-G-230V[™] (Labnet International, Inc.[®]). Each PCR reaction within volume of 30 µl contained 10 µM of each

primer (Thermo Scientific[®]), 10 mM dNTP, 10X PCR buffer with MgCl₂ (Kapa Biosystems[®]), 1U of *Taq* polymerase (Kapa Biosystems[®] and Thermo Scientific[®]) and 100 ng (i.e. 1 µl) of genomic DNA. Amplified DNA fragments were run on a 1 % agarose gel using AppliChem[®] SYBR Green[™] for visualisation. Samples with PCR products were sequenced and purified at MACROGEN[®] Europe. Sequencing reactions were performed in a DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD) using the ABI BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), following the protocols supplied by the manufacturer by single-pass sequencing on each template using forward (28RIBa) primer that revealed the complete CR sequence. Sequences were aligned using program ClustalX2 with those from the GenBank (LARKIN *et al.* 2007). Haplotype diversity in all localities was calculated using Arlequin 3.5 software package (EXCOFFIER 2015).

Results

As a result of sequencing of each individual for a CR in a length of 1080 bp we detected three CR mtDNA



Fig. 1. Circles denote sampling sites from the Una River drainage area in Bosnia and Herzegovina (1 - Krka River, 2 -Una River at Martin Brod, 3 -Una River at Loskun, 4 -Krušnica River, 5 -Svetinja River). Squares denote hatcheries (1 - Martin Brod hatchery, 2 - Klokot hatchery, 3 - Krušnica hatchery). Rectangles denote waterfalls (A - Martin Brod waterfall, B - Štrbac waterfall, C - Kostela waterfall)

Table 1. Haplotype distribution for each sampling point according to Figure 1

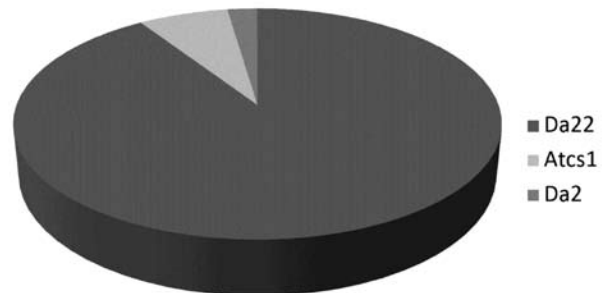
| Haplotype | Krka River | Una River at Martin Brod | Una River at Loskun | Krušnica River | Svetinja River |
|-----------|------------|--------------------------|---------------------|----------------|----------------|
| Da22 | 28 | 4 | 4 | 2 | 1 |
| Da2 | - | - | - | - | 1 |
| Atcs1 | - | - | - | 1 | 2 |

haplotypes in total: two CR mtDNA haplotypes from the Danubian lineage (Da2 and Da22) and one from the Atlantic lineage (Atcs1; Table 1). The overall haplotype diversity of brown trout in the Una River was $H = 0.0303$ (Fig. 2). In the uppermost section, all 28 brown trout specimens from the Krka River, four of them from the upper Una River at Martin Brod and four from the Una River at the downstream locality Loskun, were fixed for the Da22 haplotype. In the downstream section of the Una River, in the tributary Svetinja River one brown trout was of the Da22 haplotype (25%), one of the Da2 haplotype (25%) and two brown trouts of the Atcs1 haplotype (50%), whereas in the Krušnica River two brown trout were of the Da22 (67%), and one of the Atcs1 (33%) haplotypes. Haplotype diversity of brown trout in the Svetinja River was $H = 0.5208$ and in the Krušnica River $H = 0.2963$, whereas the pooled haplotype diversity for both downstream localities was $H = 0.4373$.

Discussion

Considering its dispersal and frequency of occurrence, Da22 haplotype is the most likely aboriginal haplotype in the drainage area of the Una River, as it is in the Lohnbach and Daglesbach streams in Austria (DUFTNER *et al.* 2003). This discovery of Da22 as a native CR mtDNA haplotype is the first one for the Balkans, as well as for the area southern of the Alps. The impassable natural obstacles that the Štrbac, Martinbrod and Kostela Waterfalls present to the upstream spreading of brown trout favour prevention of upstream spreading of other haplotypes that are recorded in tributaries of the downstream section of the Una River.

The occurrence of Atcs1 haplotype in the lower section of the drainage area of the Una River implies its introduction there. Atcs1 haplotype was hitherto recorded throughout the Western Balkans (MARIĆ *et al.* 2006; JADAN 2007; MRDAK 2011; SIMONOVIĆ *et al.* 2015). Its domestication for the hatchery rearing has long history but it also reveals apparent adverse effects of the strong introgression into the wild brown trout stocks in the streams where Atcs1 haplotype was stocked. SIMONOVIĆ *et al.* (2014, 2015) revealed

**Fig. 2.** Participation of brown trout CR mtDNA haplotypes in the Una River drainage area

its very strong invasive potential in trout streams in the area. That is a clear and unequivocal warning for the risk Atcs1 haplotype poses to the wild brown trout stocks.

Occurrence of the Da2 haplotype in trout streams joining the Una River in its middle section also implies its introduction into the wild trout stock by stocking. This haplotype was first reported by BERNATCHEZ *et al.* (1992). MARIĆ *et al.* (2006) recorded it from several localities in streams of the Danube River drainage area of Serbia together with the Da1 haplotype, as well as with the drainage-specific haplotype Da23c (TOŠIĆ *et al.* 2014), exclusive for the drainage area of the Crni Timok River (Danube River drainage area, Eastern Serbia) in streams known to be subject to stocking. MRDAK (2011) reported its occurrence in 18% of the brown trout specimens from the Tara River in Montenegro, together with brown trout of the Da1 and At1 haplotypes. He also reported the Da2 haplotype occurring alone in brown trout from the Vrijeka River in the Fatničko karst field, Herzegovina. JADAN *et al.* (2007) reported brown trout of the Da2 haplotype in the Gacka River in the Lika karst field, Western Croatia, where it occurred together with the stocked brown trout of the Atlantic lineage. The remarkably lower frequency of occurrence of brown trout of the Da2 haplotype as compared to that of Da1 in streams of Serbia suggests that brown trout specimens of Da2 haplotype were introduced there by stocking as demonstrated by SIMONOVIĆ *et al.* (2015). However, JADAN (pers. comm.) considers the status of brown trout of the Da2 haplotype indigenous.

In the study area there are hatcheries and trout farms: one each on the upper stretch of the Una River at Martin Brod, as well as on the Klokot and Krušnica Rivers that join the Una River in its middle section (Fig. 1). Those hatcheries mainly spawn and rear rainbow trout *Oncorhynchus mykiss*, but also brown trout and grayling *Thymallus thymallus* for stocking purposes. Considering that the biggest fish farm at the city of Martin Brod is in the area of the still wild brown trout stock, ultimate precautions are necessary to avoid introduction of brown trout of any non-indigenous haplotype there. In addition, it seems that brown trout of the Da22 haplotype have already been domesticated in hatcheries and reared for stocking purposes. MARIĆ *et al.* (2006) recorded brown trout of the Da22 haplotype in the Rosomačka River, while SIMONOVIĆ *et al.* (2015) recorded them in the Jerma River (Južna Morava River drainage area), both of them certainly being subject to stock-

ing since 2008 and likely even before. To the best of our knowledge, currently none of the streams in the Western Balkans holds that brown trout haplotype as a native one.

The preliminary findings reveal the introgression (75% in total) of two alien strains of brown trout into the stocks of the Svetinja River, and of brown trout of Atlantic lineage (33%) in the brown trout stock of the Krušnica River, which confirms the high invasion potential of these haplotypes as demonstrated by SIMONOVIĆ *et al.* (2015) and the risk they pose to the wild brown trout stock.

Acknowledgements: The study was supported by Grants 05-39-5614-1/14 of the Federal Ministry of Education and Science of Bosnia and Herzegovina and 173025 of the Ministry of Education, Science and Technology of the Republic of Serbia. Željko Mirković and Đuro Mirković provided full logistics for the fieldwork.

References

- APOSTOLIDIS, A. P., MADEIRA, M. J., HANSEN, M. M. & MACHORDOM, A. 2008. Genetic structure and demographic history of brown trout (*Salmo trutta* L.) populations from the southern Balkans. – *Freshwater Biology*, **53**: 1555–1566. doi:10.1111/j.1365-2427.2008.01988.x
- BERNATCHEZ, L. 2001. The Evolutionary History of Brown Trout (*Salmo trutta* L.) Inferred From Phylogeographic, Nested Clade, and Mismatch Analyses of Mitochondrial DNA Variation. – *Evolution*, **55**: 351–379.
- BERNATCHEZ, L. & DANZMANN, R.G. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchell). – *Molecular Biology Evolution*, **10**: 1002–1014.
- BERNATCHEZ, L., GUYOMARD, R. & BONHOMME, F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. – *Molecular Ecology*, **1**: 161–173.
- CORTEY, M. & GARCIA-MARIN, J. L. 2002. Evidence for phylogeographically informative sequence variation in the mitochondrial control region of Atlantic brown trout. – *Journal of Fish Biology*, **60**: 1058–1063. doi:10.1006/jfbi.2002.1910
- CORTEY, M., PLA, C. & GARCIA-MARIN, J. L. 2004. Historical biogeography of Mediterranean trout. – *Molecular Phylogenetic Evolution*, **33**: 831–844.
- DELLING, B. 2002. Morphological distinction of the marble trout, *Salmo marmoratus*, in comparison to marbled *Salmo trutta* from River Otra, Norway. – *Cybius*, **26**: 283–300.
- DELLING, B. 2003. Species diversity and phylogeny of *Salmo* with emphasis on southern trouts (Teleostei, Salmonidae). PhD Thesis. Stockholm: Stockholm University, Department of Zoology, 145 p.
- DUFTNER, N., WEISS, S., MEDGYESY, N. & STURMBAUER, C. 2003. Enhanced phylogeographic information about Austrian brown trout populations derived from complete mitochondrial control region sequences. – *Journal of Fish Biology*, **62**: 427–435.
- FERGUSON, A. 1989. Genetic differences among brown trout, *Salmo trutta* stocks and their importance for the conservation and management of the species. – *Freshwater Biology*, **21**: 35–46.
- HASHEMZADEH SEGHERLOO, I., FARAHMAND, H., ABDOLI, A., BERNATCHEZ, L., PRIMMER, C.R., SWATDIPONG, A., KARAMI, M. & KHALILI, B. 2012. Phylogenetic status of brown trout *Salmo trutta* populations in five rivers from the southern Caspian Sea and two inland lake basins, Iran: a morphogenetic approach. – *Journal of Fish Biology*, **81**: 1479–1500.
- HECKEL, J., & KNER, R. 1858. Die Süsswasserfische des Osterreich Monarchie. Leipzig: Verlag Von Wilhelm Engelmann, pp. 173–125.
- HEWITT, G. M. 1993. Post-glacial recolonization of European Biota. – *Biological Journal of the Linnean Society*, **68**: 87–112.
- HORVÁTH, A., HOITSY, G., KOVÁCS, B., SIPOS, D. K., ÓSZ, A., BOGATAJ, K. & URBÁNYI, B. 2014. The effect of domestication on a brown trout (*Salmo trutta* m. *fario*) broodstock in Hungary. – *Aquaculture International*, **22**: 5–11.
- JADAN, M., ČOŽ-RAKOVAC, R., TOPIĆ POPOVIĆ, N. & STRUNJAK-PEROVIĆ, I. 2007. Presence of unexpected phylogenetic lineages of brown trout *Salmo trutta* L. in Gacka River, Croatia. – *Aquaculture Research*, **38**: 1682–1685.
- JUG, T., DOVČ, P., POHAR, J. & SNOJ, A. 2004. RAPD analysis as a tool for discriminating marble trout from hybrids (marble trout×brown trout) in the zones of hybridization. – *Journal of Animal Breeding and Genetics*, **121**: 156–162.
- KARAKOUSIS, Y., TRIANTAPHYLIDIS, C. & ECONOMIDIS, P. S. 1991. Morphological variability among seven populations of brown trout, *Salmo trutta* L., in Greece. – *Journal of Fish Biology*, **38**: 807–817.
- KARAMAN, S. 1927. Les salmonides des Balkans. – *Bulletin de la Société Scientifique de Skoplje*, **2**: 258–268.

- KARAMAN, S. 1932. Novi prilozi poznavanju naših salmonida. [New contributions to the knowledge on our salmonids]. – *Ribarski list Sarajevo*, **9**, **10**: 1-3.
- KARAMAN, S. 1933. Prilozi ihtiologiji Crne Gore [Contributions to the ichthyology of Montenegro]. – *Ribarski list Sarajevo*, **8**: 104-106.
- KARAMAN, S. 1938. Beitrag zur Kenntnis der Süßwasserfische Jugoslaviens. *Bulletin de la Société Scientifique de Skoplje*, **18**: 131-139.
- KOHOUT, J. 2012. Effects of stocking on the genetic structure of brown trout, *Salmo trutta*, in Central Europe inferred from mitochondrial and nuclear DNA markers. *Turkish Journal of Fisheries and Aquatic Sciences*, **19**: 252-263. doi: 10.1111/j.1365-2400.2011.00828.x
- KOHOUT, J., ŠEDIVÁ, A., APOSTOLOU, A., STEFANOV, T., MARIĆ, S., GAFFAROGLU, M. & ŠLECHTA, V. 2013. Genetic diversity and phylogenetic origin of brown trout *Salmo trutta* populations in eastern Balkans. – *Biologia*, **68**: 1229-1237. doi: 10.2478/s11756-013-0271-6
- KOTTELAT, M. 1997. European freshwater fishes: an heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematics and comments on nomenclature and conservation. – *Bio-logia*, **52** (Suppl. 5): 1-271.
- MARIĆ, S., SUŠNIK, S., SIMONOVIĆ, P. & SNOJ, A. 2006. Phylogeographic study of brown trout from Serbia, based on mitochondrial DNA control region analysis. – *Genetics Selection Evolution*, **38**: 411-430. doi:10.1186/1297-9686-38-4-411
- MARIĆ, S., SIMONOVIĆ, P. & RAZPET, A. 2010. Genetic characterization of broodstock brown trout from Bled fish-farm, Slovenia. – *Periodicum Biologorum*, **112**: 145-148.
- MARIĆ, S., NIKOLIĆ, V., TOŠIĆ, A. & SIMONOVIĆ, P. 2012. Record of the brown trout *Salmo trutta* L., 1758 in the main riverbed of the Serbian part of the Danube River. – *Journal of Applied Ichthyology*, **28**: 135-137.
- MILLER, S. A., DYKES, D. D. & POLESKY, H. F. 1988. A simple salting out procedure from human nucleated cells. *Nucleic Acids Research*, **16**: 1215.
- MRDAK, D. 2011. Pastrmke (*Salmo* L., 1758) rijeka Crne Gore – diverzitet, taksonomski status i filogenetski odnosi [Trout (*Salmo* L., 1758) in rivers of Montenegro – diversity, taxonomic status and phylogenetic relationships]. PhD Thesis. Belgrade: University of Belgrade, Faculty of Biology, 152 pp.
- SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2015. Arlequin Ver. 3.5.2.2. Computational and Molecular Population Genetics Lab, CMPG, Institute of Ecology and Evolution, University of Berne, Swiss Institute of Bioinformatics. <http://cmpg.unibe.ch/software/arlequin>
- SIMONOVIĆ, P., MARIĆ, S. & NIKOLIĆ, V. 2007. Trout *Salmo* spp. complex in Serbia and adjacent regions of western Balkans: reconstruction of evolutionary history from external morphology. – *Journal of Fish Biology*, **70** (Supplement C): 359-380. doi:10.1111/j.1095-8649.2007.01516.x
- SIMONOVIĆ, P., TOŠIĆ, A., ŠKRABA, D., MRDAK, D., GRUJIĆ, S. & NIKOLIĆ, V. 2014. Effects of stocking with brood fish to manage resident stream dwelling brown trout *Salmo cf. trutta* L. stock. – *Journal of Fisheries Sciences*, **8**: 139-152.
- VIDOVIĆ, Z., TOŠIĆ, A., ŠKRABA, D., ČANAK-ATLAGIĆ, J. & NIKOLIĆ, V. 2015. Risks to stocks of native trout of the genus *Salmo* (Actinopterygii: Salmoniformes: Salmonidae) of Serbia and management for their recovery. – *Acta Ichthyologica et Piscatoria*, **45** (2): 161-173, DOI: 10.3750/AIP2015.45.2.06
- SNOJ, A. 2004. Filogeografska struktura postrvi v Sloveniji. – *Ribič*, **10**: 239-243.
- SNOJ, A., JUG, T., MELKIĆ, E., SUŠNIK, S., POHAR, J., DOVČ, P. & BUDIHNA, N. 2000. Mitochondrial and microsatellite DNA analysis of marble trout in Slovenia. – *Journal of Fish Biology*, (Quaderni ETP) **29**: 5-11.
- SUÁREZ, J., BAUTISTA, J. M., ALMODÓVAR, A. & MACHORDOM, A. 2001. Evolution of mitochondrial control region in Palaearctic brown trout (*Salmo trutta*) populations: the biogeographical role of the Iberian Peninsula. – *Heredity*, **87**: 198-206. doi:10.1046/j.1365-2540.2001.00905.x
- SUŠNIK, S., SCHÖFFMANN, J. & WEISS, S. 2005. Genetic verification of native brown trout from the Persian Gulf (Catak Cay River, Tigris basin). – *Journal of Fish Biology*, **67**: 879-884. doi:10.1016/j.ympcv.2006.08.021
- TOŠIĆ, A., ŠKRABA, D., NIKOLIĆ, V., MRDAK, D. & SIMONOVIĆ, P. 2014. New mitochondrial DNA haplotype of brown trout *Salmo trutta* L. from Crni Timok drainage area in Serbia. – *Turkish Journal of Fisheries and Aquatic Sciences*, **14**: 37-42. doi: 10.4194/1303-2712-v14_1_05
- VERA, M., CORTEY, M., SANZ, N. & GARCIA-MARIN, J. L. 2010a. Maintenance of an endemic lineage of brown trout (*Salmo trutta*) within the Duero river basin. – *Journal of Zoological Systematics and Evolutionary Research*, **48**: 181-187. doi: 10.1111/j.1439-0469.2009.00547.x
- VERA, M., SANZ, N., HANSEN, M. M., ALMODÓVAR, A. & GARCIA-MARIN, J. L. 2010b. Population and family structure of brown trout, *Salmo trutta*, in a Mediterranean stream. – *Marine and Freshwater Research*, **61**: 676 – 685. doi: 10.1071/MF09098 1323-1650/10/060676
- ZHANG, D. X. & HEWITT, G. M. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. – *Molecular Ecology*, **12**: 563-584. doi: 10.1046/j.1365-294X.2003.01773.x

Received: 24.02.2016
Accepted: 10.08.2016