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Mitochondrial DNA control region diversity in Common Terns *Sterna hirundo* from Slovenia and Croatia

Raznolikost kontrolne regije mitohondrijske DNK pri navadni čigri *Sterna hirundo* iz Slovenije in Hrvaške

Ida Svetličić[1], Jelena Kralj[2], Miloš Martinović[2], Davorin Tome[3], Tilen Basle[4], Luka Božić[4], Iztok Škornik[5], Luka Jurinović[6], Ana Galov[1*]

[1] University of Zagreb, Faculty of Science, Department of Biology, Rooseveltov trg 6, Zagreb, Croatia, e-mail: ida.svetlicic@biol.pmf.hr, anagalov@biol.pmf.hr
[2] Institute of Ornithology, Croatian Academy of Sciences and Arts, Gundulićeva 24, Zagreb, Croatia, e-mail: jkralj@hazu.hr, martinovic@hazu.hr
[3] National Institute of Biology, Večna pot 111, Ljubljana, Slovenia, e-mail: davorin.tome@nib.si
[4] DOPPS – BirdLife Slovenia, Tržaška cesta 2, Ljubljana, Slovenia, e-mail: tilen.basle@dopps.si, luka.bozic@dopps.si
[5] Sečovlje Salina Nature Park, SOLINE Pridelava Soli d.o.o., Seča 115, Portorož, Slovenia, e-mail: iztok.skornik@soline.si
[6] Croatian Veterinary Institute, Poultry Centre, Heinzelova 55, Zagreb, Croatia, e-mail: luka.jurinovic@gmail.com
[*] corresponding author

63 Common Tern *Sterna hirundo* samples from Croatia and Slovenia were analysed with respect to their genetic diversity and differentiation. Samples originated from two freshwater populations (areas of the rivers Sava and Drava) and one coastal population (Sečovlje Salina). The molecular marker of choice was 709 bp long fragment of the mitochondrial control region, the fastest-evolving part of the mitochondrial genome. 21 haplotypes with 12 polymorphic sites were identified. Overall haplotype diversity was substantial and estimated at 0.8599, while the overall nucleotide diversity was low and estimated at 0.0025. Diversity indices were highest for the Drava population, followed by the Sava and the lowest for the Sečovlje population. Overall genetic structure was significantly low (Fst=0.0377) and attributed to the differences in haplotype frequencies between the populations. The high level of genetic diversity found in continental populations illustrates the importance of their habitats as reservoirs of genetic diversity and calls for their further protection and management.

Key words: mtDNA, freshwater colonies, population genetics, Laridae, seabird
Ključne besede: mtDNA, celinske kolonije, populacijska genetika, Laridae, morske ptice

1. Introduction

Common Tern *Sterna hirundo* is a colonial seabird of the family Laridae that breeds both on freshwater and marine habitats of temperate and subarctic regions of Europe, Asia and North America. It is a long-distance migrator, wintering in tropical and subtropical coastal regions. Despite being listed as a species of least concern by the IUCN, the global population trend is

mostly unknown on account of variation in trends between different populations (Birdlife International 2018). Freshwater colonies are located mostly on river and lake islands. They are under a strong negative influence of anthropogenic activities, mainly river regulation, as well as under natural threats (e.g. competition with gulls and mammalian predation), which has led to a significant global reduction of the natural freshwater breeding sites. Freshwater populations of the Common Tern are now almost entirely dependent on artificial sites, specifically rafts and gravel islands (Becker & Ludwigs 2004). Freshwater breeding sites in Slovenia and Croatia can be found along the river Sava where the most important breeding site is Rakitje (Martinović *et al.* 2019) and in the river Drava area where the main colony inhabits the Ptuj reservoir. Many small coastal colonies are located along the Adriatic Sea, while one of the largest is located in Sečovlje Salina Nature Park in the Northern Adriatic (Denac *et al.* 2019).

Despite ongoing research, dynamics of both freshwater and coastal populations in Croatia and Slovenia are still relatively unclear. High breeding site fidelity, a well-known characteristic of the Common Tern (Austin 1949) and many other seabird species (Palestis 2014, Coulson 2016), can favour population differentiation by restricting gene flow (Friesen *et al.* 2007). Therefore, population substructuring in long-distance migratory seabirds is seemingly driven by behaviour rather than by physical land barriers (Friesen *et al.* 2007, Szczys *et al.* 2017A).

Genetic studies on wild animal populations are mostly performed using neutral genetic markers such as mitochondrial DNA control region and microsatellites, which are suitable for studies of genetic diversity, genetic structure and demographic processes of populations. Genetic diversity estimation constitutes the basis of many contemporary conservation efforts because it is crucial for the evolutionary potential of species and therefore one of the keys for its long-term survival (Van Dyke 2008). It can thus serve as a measure of the species capacity for future adaptation to environmental challenges.

The control region is the fastest evolving part of the mitochondrial genome, traditionally used as a marker in population genetic and phylogeographic studies. One of the reasons for the ubiquity of the mitochondrial DNA (mtDNA) control region genetic analysis is its exceptionally high polymorphism coupled with conserved arrangement of neighbour genes, which allows for consistent amplification by polymerase chain reaction with many primers. Extreme polymorphism aids in identification of interpopulation as well as intrapopulation lineages. Furthermore, an important property of mtDNA is the uniparental inheritance which results in a lack of recombination. Individual lineages can thus be traced through time in a more straightforward manner than with nuclear markers. Moreover, mtDNA is more sensitive to demographic changes such as bottlenecks and population expansion due to its haploid nature (Freeland *et al.* 2011). Despite downsides to mtDNA analyses (Hurst & Jiggins 2005), mitochondrial DNA has remained the marker of choice in many population and phylogeographic studies.

One of the first population genetic studies of Common Terns was undertaken on populations from North America, using isoelectric focusing of blood proteins. High levels of gene flow were suggested among four colonies as no genetic differentiation was found (Burson III 1990). It was followed by an investigation of Lithuanian colonies using allozymes where some genetic differentiation was reported (Sruoga *et al.* 2002). Further investigations were performed using nuclear genetic markers, specifically microsatellite loci, where significant differentiation among sampled locations was found in Lithuania (Sruoga *et al.* 2006) and in the North Atlantic region (Szczys *et al.* 2012). A later study by Szczys *et al.* (2017B) on cytochrome B revealed hierarchical metapopulation structure with the presence of small- and large-scale connectivity between metapopulations.

To our knowledge, there are no published population genetic studies of Common Tern inferred from an analysis of the mitochondrial DNA control region marker. Thus, in this paper we provide the first assessment of mtDNA control region diversity of Common Terns in general. The goal of this study was to analyse a 709 bp long fragment of the mitochondrial control region in two continental, freshwater populations of Common Terns from Slovenia and Croatia and in a coastal population from Slovenia (Sečovlje Salina). Considering the local and global threats affecting freshwater tern

habitats, this study aimed to quantify the levels of genetic diversity in populations of Common Terns from the three geographic areas as well as to detect a possible phylogeographic structure. Furthermore, the intention was to gain insight into possible expansions or bottlenecks in their population history.

2. Methods

We caught 63 Common Terns during their breeding period in the spring and summer of 2017 (6 individuals), 2018 (54 individuals) and 2019 (3 individuals). Sampling took place in gravel pits near the river Sava (30 individuals), the river Drava (22 individuals from Ptuj accumulation and 2 individuals further downstream) and in Sečovlje Salina (9 individuals) (Figure 1). Around 50 µL of blood was taken from the brachial vein and stored either in EDTA coated tubes on ice or on bloodstain storage cards (Nucleocard, Machery Nagel), while feathers were taken from some individuals and kept in paper envelopes at room temperature.

DNA was extracted using DNeasy Blood and Tissue kit (Quiagen, Hilden Germany) following the manufacturer's protocol. To amplify ~800 bp long fragment of the control region, we designed primers SH-mtCR4F (AACACCCATCCAACTCGGAA) and SH-mtCR4R (AATTTCACTGTCGTTGACGTGT) using the mitogenome sequence from GeneBank (Yang *et al.* 2017). PCR conditions were as follows: 3 min denaturation at 95°C, followed by 40 cycles of 30s at 95°C, 45s of annealing at 52°C, and 45s of elongation at 72°C. Final elongation period was 10 min at 72°C. Upon PCR amplification, the samples were sent for purification and sequencing to Macrogen Europe, Amsterdam, the Netherlands. Sequences were aligned, inspected and adjusted to the length of 709 bp using Applied Biosystems SeqScape software. Three samples from the river Sava area were excluded from further analysis as their electropherograms showed unresolved peak duplications.

Mega X (Kumar *et al.* 2018) was used to determine the best nucleotide substitution model according to the Bayesian Information Criterion (BIC). The selected model with the lowest BIC score was Kimura 2-parameter with Gamma distributed rates (Nei & Kimura 2000).Arlequin (Excoffier & Lischer 2010) was used to determine the number of haplotypes (H), assign haplotypes to the individuals, determine the number of variable positions, nucleotide composition, and for calculation of haplotype diversity (Hd) – the probability that two randomly chosen sequences are different. The mean number of pairwise differences and nucleotide diversity (π) – the probability that the two homologous nucleotides differentiate – were calculated with Arlequin as well. AMOVA (Analysis of Molecular Variance) was conducted in order to investigate spatial genetic structuring by analyzing covariance components utilizing information on haplotype frequencies and nucleotide distance between haplotypes (Excoffier *et al.* 1992), as implemented in Arlequin. Population groups were defined as ''Sava'', ''Drava'' and ''Sečovlje''. Pairwise FST and ΦST values for each group were calculated in Arlequin as well. Significance was tested using 10,000 non-parametric permutation procedures.

DnaSp (Rozas *et al.* 2017) was used to calculate Fu's Fs (Fu 1997) and Tajima's D (Tajima 1989) neutrality tests in order to detect past demographic changes. Fu's Fs is based on the deviation of haplotype frequencies in comparison with expectation under population stability, while Tajima's D calculates the difference between Theta estimation from the number of polymorphic sites and the Theta estimation from the average number of pairwise differences. Tajima's D value departs from neutrality to negative values in cases of demographic expansion or purifying selection. The significance of both Fu's Fs and Tajima's D was tested using a coalescent tool with 10,000 replicates.

PopART (Population Analysis with Reticulate Trees) (Leigh & Bryant 2015) was used for calculating and visualizating the median-joining haplotype network. Network methods are generally more fitting than tree algorithms in studies of intraspecific genetic diversity as they allow for the presence of ancestral haplotypes in a sample (Bandelt *et al.* 1999, Zachos *et al.* 2010).

3. Results

Analysis of the 709 bp fragment of mtDNA control region in 60 Common Tern individuals revealed 21 haplotypes (GenBank accession no. MN337406 - MN337426) with 12 segregating sites (Table 1). All of the observed substitutions were transitions,

while no deletions or insertions were observed. The most common haplotypes were Stehi01 and Stehi03 found in all groups of samples. Stehi03 was prevalent, being found in 35% of samples (Table 2). Low-frequency haplotypes were numerous as 12 haplotypes were found in only one individual each (six unique haplotypes from the Drava, five from the Sava and one from Sečovlje Salina). Seven haplotypes were shared between samples from the Sava and Drava (Stehi01, Stehi02, Stehi03, Stehi04, Stehi05, Stehi07 and Stehi12), while only two haplotypes (Stehi01 and Stehi03) were shared among all three populations. Haplotypes differed by one to four base substitutions (Figure 2), while mean number of pairwise differences in haplotype sequences of different groups ranged from 0.7044 to 2.1085 (Table 3).

Overall haplotype diversity was estimated at 0.8599, and nucleotide diversity at 0.0025. All diversity indices were highest for the river Drava population. Haplotype diversity for the river Drava was estimated at 0.9239, for the river Sava at 0.8946, while it was the lowest for Sečovlje Salina (0.4167). Nucleotide diversity estimates were found to be in the same order, as well as the mean number of pairwise distances (the highest values for the Drava, followed by the Sava, and the lowest values found in Sečovlje Salina) (Table 3).

Table 1: Segregating sites (S) on the mtDNA control region fragment (709 bp) of *S. hirundo*

Tabela 1: Ločitvena mesta (S) na mtDNA fragmentu kontrolne regije (709 bp) pri navadni čigri

	S1 (5)	**S2 (18)**	**S3 (42)**	**S4 (75)**	**S5 (92)**	**S6 (95)**	**S7 (98)**	**S8 (129)**	**S9 (269)**	**S10 (282)**	**S11 (438)**	**S12 (473)**
Stehi01	A	T	C	A	C	C	A	G	G	A	A	C
Stehi02	G	T	C	A	C	T	A	G	G	A	A	C
Stehi03	A	T	C	A	C	T	A	G	G	A	A	C
Stehi04	A	T	C	A	C	C	A	A	G	A	A	C
Stehi05	G	T	C	A	C	C	A	G	G	A	A	C
Stehi06	A	T	C	A	T	T	A	G	G	A	A	C
Stehi07	A	T	C	A	C	T	A	G	G	A	G	C
Stehi08	A	C	C	A	C	T	A	A	G	A	A	C
Stehi09	A	T	C	A	C	C	A	G	A	A	A	C
Stehi10	G	T	C	A	C	T	A	G	G	A	A	T
Stehi11	A	T	C	A	C	T	A	G	G	A	A	T
Stehi12	A	T	C	A	C	T	A	A	G	A	A	C
Stehi13	A	T	C	G	T	T	A	G	G	A	A	C
Stehi14	A	T	C	A	C	C	A	G	G	A	A	C
Stehi15	A	T	T	A	C	T	A	A	G	A	A	C
Stehi16	G	T	C	A	C	T	A	A	G	A	A	C
Stehi17	A	T	C	A	T	C	A	A	G	A	A	C
Stehi18	A	T	C	A	C	T	G	A	G	A	A	C
Stehi19	A	T	C	A	C	T	A	A	G	G	A	C
Stehi20	G	T	C	A	T	T	A	A	G	A	A	C
Stehi21	A	T	T	A	T	T	A	G	G	A	A	C

Table 2: Distribution and frequency (%) of 21 *S. hirundo* mtDNA control region haplotypes

Tabela 2: Razporeditev in frekvenca (%) 21 haplotipov mtDNA kontrolne regije pri navadni čigri

Haplotype haplotip	Sava (N=27)	Drava (N=24)	Sečovlje Salina (N=9)	Total (N=60)
Stehi01	3 (5.00)	2 (3.33)	1 (1.67)	6 (10.00)
Stehi02	3 (5.00)	2 (3.33)	0	4 (6.67)
Stehi03	8 (13.33)	7 (10.00)	6 (11.67)	21 (35.00)
Stehi04	2 (3.33)	1 (1.67)	0	3 (5.00)
Stehi05	1 (1.67)	3 (5.00)	0	4 (6.67)
Stehi06	2 (3.33)	0	0	2 (3.33)
Stehi07	2 (3.33)	1 (1.67)	0	3 (5.00)
Stehi08	1 (1.67)	0	0	1 (1.67)
Stehi09	1 (1.67)	0	0	1 (1.67)
Stehi10	1 (1.67)	0	0	1 (1.67)
Stehi11	1 (1.67)	0	0	1 (1.67)
Stehi12	1 (1.67)	2 (3.33)	0	3 (5.00)
Stehi13	0	1 (1.67)	0	1 (1.67)
Stehi14	0	1 (1.67)	0	1 (1.67)
Stehi15	0	1 (1.67)	0	1 (1.67)
Stehi16	0	1 (1.67)	0	1 (1.67)
Stehi17	0	1 (1.67)	0	1 (1.67)
Stehi18	0	0	1 (1.67)	1 (1.67)
Stehi19	1 (1.67)	0	0	1 (1.67)
Stehi20	0	2 (3.33)	0	1 (1.67)
Stehi21	0	2 (3.33)	0	1 (1.67)

Table 3: Genetic diversity indices and neutrality test results for 709 bp fragment of mtDNA control region in *S. hirundo*. Statistically significant values ($p < 0.02$ for Fs, $p < 0.05$ for D) are marked in bold. N – number of individuals, H – number of haplotypes, Hd – haplotype diversity, π – nucleotide diversity, k – mean number of pairwise differences, S – segregating sites, D – Tajima's D, Fs – Fu's Fs.

Table 3: Indeks genetske raznovrstnosti in test nevtralnosti za 709 bp dolge fragmente mtDNA kontrolne regije pri navadni čigri. Statistično značilne vrednosti ($p < 0.02$ za Fs, $p < 0.05$ za D) so v krepkem tisku. N – število osebkov, H – število haplotipov, Hd –haplotipska raznovrstnost, π – nukleotidska ranovrstnost, k – povprečno število parnih razlik, S – predeli ločevanja, D – Tajima's D, Fs – Fu's Fs.

Population populacija	N	H	Hd	Π	k	S	D	Fs
Sava	27	13	0.8946	0.0025	1.7920	9	-0.8998	**-8.8476**
Drava	24	14	0.9239	0.0030	2.1085	6	0.1367	**-10.2569**
Sečovlje Salina	9	3	0.4167	0.0010	0.7044	3	**-1.5130**	-0.3802
Total	60	21	0.8599	0.0025	1.7721	12	-1.0263	**-17.8652**

Table 4: Pairwise φST (above the diagonal) and FST values (below the diagonal) for the continental groups of *S. hirundo* populations. Statistically significant values ($p < 0.05$) are marked in bold.

Tabela 4: Parne φST (nad diagonalo) in FST vrednosti (pod diagonalo) za populacije navadne čigre. Statistično značilne vrednosti so v krepkem tekstu.

	Sava	Drava	Sečovlje Salina
Sava	-	-0.00561	-0.01439
Drava	-0.01230	-	0.05137
Sečovlje Salina	**0.09631**	**0.11969**	-

At least one neutrality test for each group of samples suggested historical expansion. Large negative and significant values of Fu's Fs were detected in sample sets from the Drava and the Sava and in the overall data set. Tajima's D was significant exclusively in the samples from Sečovlje Salina, where Fu's Fs test was not significant (Table 3).

Haplotypes were dispersed without evident spatial association (Figure 2). Within population measure inferred by AMOVA accounted for 98.81% of variance. Global ΦST based on molecular distance was low (0.0041) and insignificant, as well as pairwise ΦST values. However, global fixation index FST based on F-statistics (haplotype frequencies) was low (0.0377) but significant ($p = 0.0389$), as well as the pairwise FST values that were estimated at 0.09631 and 0.11969 when Sečovlje Salina was compared against the Sava and the Drava respectively (Table 4).

4. Discussion

In this paper we report results of the first assessment of Common Tern mtDNA control region diversity. The sample set consisted of individuals breeding in Slovenia and Croatia, with the majority of samples originating from continental, freshwater colonies.

During the conduction of this study, double electropherogram peaks were noticed in three samples (data not shown), indicating possible

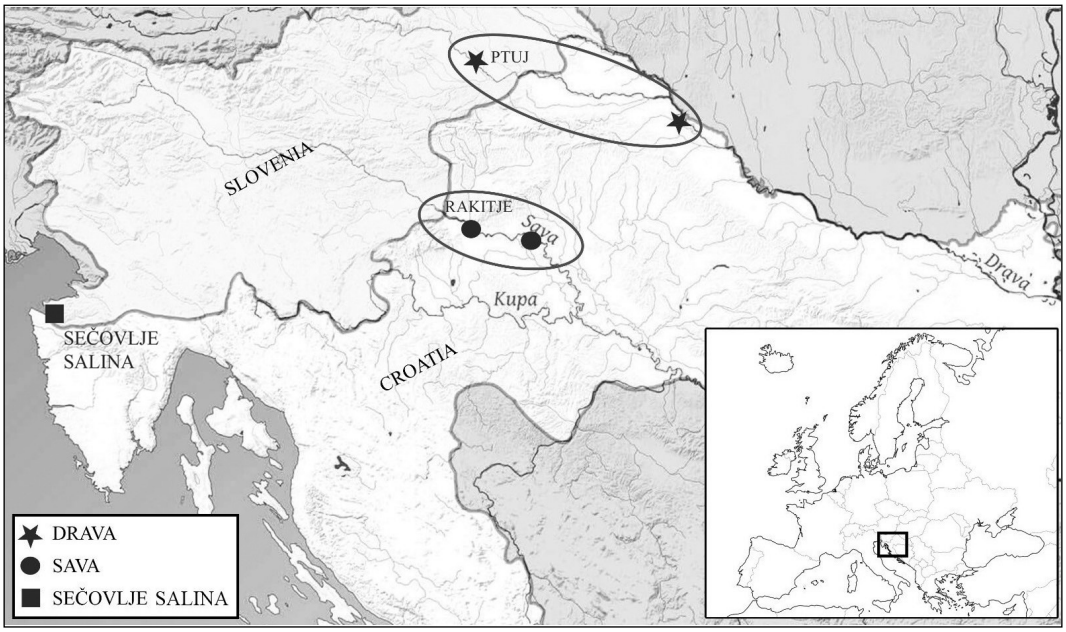

Figure 1: Sampling locations for Common Tern colonies in Croatia and Slovenia

Slika 1: Mesta vzorčenja kolonij navadne čigre na Hrvaškem in v Sloveniji

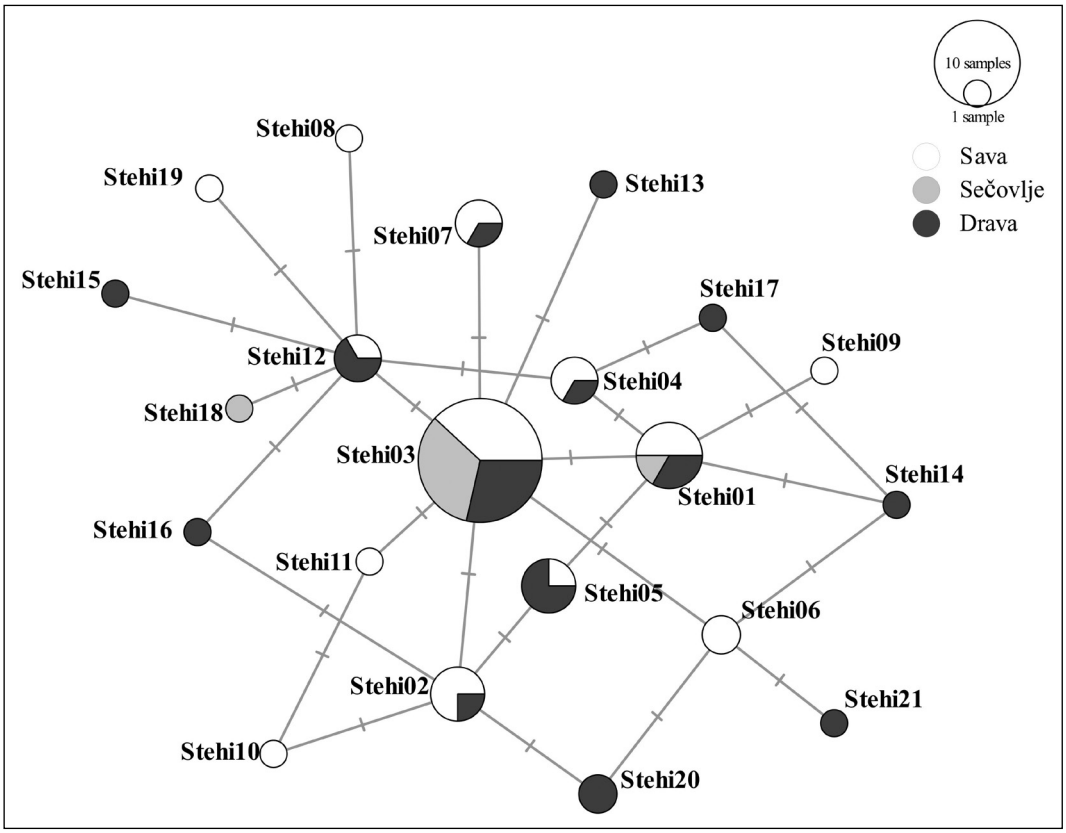

Figure 2: Median-joining haplotype network generated by the program Pop Art based on the mtDNA control region haplotypes of *S. hirundo*. Size of the circles is proportional to the frequency of the haplotype. Different shades represent sample groups Sava, Drava and Sečovlje Salina. The number of hatch marks indicates the number of mutational differences between haplotypes.

Slika 2: Haplotipska mreža mtDNA kontrolnih regij navadne čigre, narejena s programom Pop Art. Velikost kroga je proporcionalna frekvenci haplotipa. Različne sivine ponazarjajo skupine s Save, Drave in iz Sečoveljskih solin. Število oznak ponazarja število mutacijskih razlik med haplotipi.

control region duplication, and therefore those samples were excluded from further analysis. Ambiguities in the control region are regularly reported in seabird species (Friesen *et al.* 2007, Skujina *et al.* 2016) and for that reason, cytochrome B is usually the preferred choice of mitochondrial marker for population genetic studies in Terns (Faria *et al.* 2010, Miller *et al.* 2013, Boutillier *et al.* 2014; Szczys *et al.* 2017A, Szczys *et al.* 2017B). However, the control region is much more variable and is therefore more suitable for studies of genetic diversity at the population level. In our study, possible duplication of the control region occurred in low frequency (4.8%) and thus would have had a minor impact on the results. Further research will enable a more precise estimation of mtDNA control region duplication prevalence in Common Tern populations and should aim to resolve them.

Our analyses of samples of Common Terns from two freshwater habitats – rivers Sava and Drava, and from the coastal colony of Sečovlje Salina, revealed a substantial level of haplotype diversity as a large number of haplotypes was detected, specifically 21. This is particularly notable as only 60 samples were analysed in this study. It

is somewhat higher than 18 haplotypes found in 89 individuals of Sooty Tern (*Sterna fuscata*) from Eastern Australia and the Coral Sea (Peck & Congdon 2004), whereas it is comparable to 67 mtDNA control region haplotypes found in 188 individuals of Least Tern *Sternula antillarum* from North America (Draheim *et al.* 2010).

Haplotype diversity value of 0.8599 found in this study is substantially high and comparable to the Hd values found in Least Terns with Hd values ranging from 0.76 to 0.96 (Draheim *et al.* 2010). It is also similar to Hd values of cytochrome B mitochondrial marker found in Gull-billed Terns *Gelochelidon nilotica* (Hd values ranged from 0.843 to 0.908) (Miller *et al.* 2013). Moreover, haplotype diversity found in this study is higher than Hd values of *cytochrome-b* sequence found for Common Tern populations from northeastern United States and southern Canada, which ranged from 0.21 to 0.77 (Szczys *et al.* 2017B) and those reported for Eurasian Black Tern *Chlidonias niger* populations, which ranged from 0.33 to 0.82 (Szczys *et al.* 2017a). Higher Hd values found in this study come as no surprise as they are expected for more variable markers, such as the control region.

In contrast with high haplotype diversity values, nucleotide diversity values are very low, both in the overall sample (0.0025) as well as in the individual groups (0.0010–0.0030) (Table 3). They are lower than Π values previously reported for Least Terns (ranging from 0.0010 to 0.0069) (Draheim *et al.* 2010), but still higher than those reported for Sooty Terns (Peck & Congdon 2004), where the Π values ranged from 0.0007 to 0.0016. Mean number of pairwise differences between sequences was also low, reflecting small number of mutations between different haplotypes.

Samples from the colonies on the rivers Sava and Drava share more haplotypes (seven) than either of those sets shares with samples from Sečovlje Salina (two out of three haplotypes found in Sečovlje Salina are shared among all three groups and the third haplotype is not shared with any other population) (Table 2). One explanation can be insufficient sampling in Sečovlje Salina, as only nine individuals were sampled there. On the other hand, the finding could be explained by potentially higher movements of individuals between freshwater habitats of the Sava and Drava which have more similar ecological conditions than between any freshwater habitat and Sečovlje Salina, as it represents a dissimilar coastal habitat. Geographical distance could also be a reason for the observed pattern; the Sava and Drava are much closer to each other than the coastal colony of Sečovlje Salina and dispersal rates are expected to be higher in adjacent colonies. Similar results were obtained with tests of genetic differentiation, as pairwise FST values were statistically significant only for coastal-freshwater population pairs. Admittedly, genetic differentiation was weak (0.11969 for Sečovlje Salina – Drava and 0.09631 for Sečovlje Salina – Sava) and observed only in measures that utilize solely F–statistic based on haplotype frequencies, namely global and pairwise FST (Table 4). Again, possible differentiation between the coastal colony of Sečovlje Salina and both freshwater colonies could be due to contrasting ecological factors of freshwater and coastal habitats or to small sample size of the Sečovlje Salina population. However, measures dependent on nucleotide distance (global and pairwise ΦST) did not show differentiation between either any pair of population groups, or in the overall data set (Table 4). Very weak population differentiation, or its absence, observed using mitochondrial cytochrome B markers, was previously noted for Common Terns (Szczys *et al.* 2017b) and Black Terns (Szczys *et al.* 2017a), while those studies demonstrated some level of differentiation using nuclear markers. The opposite pattern was also documented, as Miller *et al.* (2013) detected significant genetic structure by mtDNA analysis of Gull-billed Terns, but did not demonstrate differentiation using microsatellites. Since the results might be highly dependent on the choice of marker, further research on nuclear markers such as microsatellites could offer additional insights into population structure. In general, mitochondrial markers reveal female ancestry while nuclear markers reflect biparentally inherited diversity. Moreover, mitochondrial DNA reflects historical processes, while microsatellites are more informative of contemporary population genetics processes. Therefore, the research on Common Terns that breed in Croatia and Slovenia should be expanded to microsatellite analysis which could offer broader insight into the demographic structure and the genetic variability of the populations.

The finding of numerous low frequency and private haplotypes (Table 2) indicates the populations are not homogenous. High haplotype diversity, low nucleotide diversity, and numerous low frequency haplotypes that differ by a small number of mutations can be explained as a signature of population expansion or indication of recent origin (Peck & Congdon 2004; Faria *et al*. 2010). Neutrality tests of Fu's Fs indicate possible population expansion in the overall sample set and individually in the samples from the Sava and Drava areas (Table 3). Fu's Fs test has a great statistical power to detect excess of low frequency alleles expected in the expanding population (Ramos-Onsins & Rozas, 2002). Tajima's D implied possible expansion for the Sečovlje Salina group as well. These findings might reflect expansion from glacial refugia during the last ice age (Oomen *et al* 2011, Szczys 2017 b) and they are consistent with other published data on terns (Peck & Congdon 2004, Faria *et al*. 2010, Szczys *et al*. 2017 a, Szczys *et al*. 2017 b).

Our findings on mtDNA control region diversity indicate that genetic diversity of Common Tern populations from Slovenia and Croatia is substantially high. Samples from the river Drava exhibited the highest values of haplotype and nucleotide diversity, closely followed by samples from the river Sava area, which illustrates the importance of these habitats as reservoirs of genetic diversity and specifically underscores the value of maintaining artificial sites for the long-term conservation of Common Tern populations in freshwater habitats.

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5. Povzetek

Avtorji prispevka so analizirali genetsko raznolikost in diferenciacijo 63 primerkov navadne čigre *Sterna hirundo* iz Slovenije in Hrvaške. Vzorce so pridobili iz dveh sladkovodnih (območji rek Save in Drave) in ene obalne populacije (Sečoveljske soline). Izbrali so 709 baznih parov dolg molekularni marker, fragment mitohondrijske kontrolne regije, ki je del mitohondrijskega genoma, izpostavljen najhitrejšim evolucijskim spremembam. Odkrili so 21 haplotipov z 12 polimorfičnimi mesti. Skupna haplotipska raznolikost je bila visoka (0,8599), medtem ko je bila nukleotidska raznolikost nizka (0,0025). Indeksi diverzitete so bili najvišji pri dravski, nato pri savski in najnižji pri sečoveljski populaciji. Skupna genetska strukturiranost, pripisana razlikam v pogostosti haplotipov med populacijami, je bila nizka (Fst=0,0377) in statistično značilna. Visoka genetska pestrost v celinskih populacijah navadne čigre prikazuje vrednost njihovih habitatov kot rezervoarjev genetske pestrosti in opozarja na pomembnost nadaljnjega ohranjanja in upravljanja takih habitatov.

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