NORTH-WESTERN JOURNAL OF ZOOLOGY 9 (2): 365-373
Article No.: 131604

http://biozoojournals.3x.ro/nwjz/index.html

Phylogenetic patterns of a nightingale population in a contact zone of *Luscinia megarhynchos* and *L. luscinia* in Hungary

Zoltán ÁCS[1] and Dávid KOVÁTS[2,*]

1. Vénic Nature Conservation Foundation, H-8400 Ajka, Tölgyfa u. 3., Hungary
2. Department of Evolutionary Zoology and Human Biology, University of Debrecen, H-4010 Debrecen, Egyetem tér 1., Hungary.
*Corresponding author, D. Kovats, E-mail: david.kovats@gmail.com

Received: 11. January 2012 / Accepted: 11. May 2013 / Available online: 04. June 2013 / Printed: December 2013

Abstract. Phylogenetic patterns of two closely related nightingale species the *Luscinia megarhynchos* and the *L. luscinia* were investigated in north-eastern part of Hungary. The mitochondrial cytochrome-*c* oxidase subunit I (COI) gene amplified by polymerase chain reaction (PCR) and sequenced directly to set up their phylogenetic relationships. In eastern Hungary (Szatmár-Beregi Landscape Protected Area, Vámosatya, Bockerek forest 48°11'N, 22°23'E), a new haplotype of *L. luscinia* is discovered. Based on our molecular result, however, no individual of *L. luscinia* was found in the Upper-Tisza Region (especially between Tiszabercel and Tiszatelek), where its old breeding stands formerly occurred. It suggests its population might extinct from this region, or partially shifted to undisturbed territories, probably due to ecological impoverishment of floodplain forest associations. Furthermore, it appears that range expansion of *L. megarhynchos* increased in those of fragmented habitats.

Key words: distribution, new haplotype, habitat requirements, habitat loss, interspecific competition, climate change.

Introduction

Because much of Europe was greatly affected by the ice age, this argument would apply to most hybrid zones in central and northern part of Europe, and its surrounding temperate regions (Hewitt 1999).

Overlapping zones are commonly found in regions where environmental conditions of native habitat of both sister species meet (Barton & Hewitt 1985, Harrison 1993). These areas often overlap with the spatial structure of vicariant phylogeographical patterns (Taberlet *et al.* 1998, Hewitt 2004). Concerning the European passerines, for example, breeding ranges of mostly or partly allopatric congener species like the *Parus caeruleus/P. cyaneus, P. palustris/P. montanus* or *Ficedula albicollis/F. hypoleuca* can partially overlap, thus, they often hybridize with each other (Martin 1990, Newton 2003). However, in many cases hybrids are less fit than parental forms because they lack the complete gene complexes of the parental that make them well adapted to environments either side of the hybrid zone. As a consequence, in case of hybridization, the reduced fertility of hybrids can be observed (Sætre et al. 2003, Sánchez-Guillén et al. 2011). However, there are some examples in which the hybrids are fitter than their parents (Barton & Hewitt 1985). Furthermore, where closely related taxa do not co-occur, reproductive interference may be a more likely cause than resource competition in many cases (Hochkirch et al. 2007, Gröning & Hochkirch 2008).

In Europe, two sister species of nightingales the common nightingale *Luscinia megarhynchos* Brehm, 1931 and the thrush nightingale *L. luscinia*, Linneaus, 1758 interbreed continuously in a narrow contact zone (Sorjonen 1986, Reifová et al. 2011). The *L. megarhynchos* which is widely distributed from south-western Europe via the Middle East to Central Asia and North Africa, is a frequent breeding and migratory species in the Carpathian basin (Cramp 1988). The *L. luscinia* is monotypic, widespread in temperate Asia (mostly in western Siberia) and north-eastern Europe (Cramp 1988). The north-eastern portion of Hungary (specifically between Tiszabercel and Tiszatelek in the Upper-Tisza Region) is situated in the south-western edge of its distribution area (Moreau 1972, Sorjonen 1986) (Figure 1, 2b) (Haraszthy 1998).

Supposedly, these two congener species have diverged by geographical isolation during the climatic oscillations of the early Pleistocene (Hewitt 2000, 2004) about 1.8 Mya (Storchová et al. 2010) and came into secondary contact in a narrow hybrid zone from northern Germany across Poland via Hungary towards to the Black Sea (Hagemeijer & Blair 1994, Reifová et al. 2011, Sorjonen 1986). Both species show interspecific responses to het-

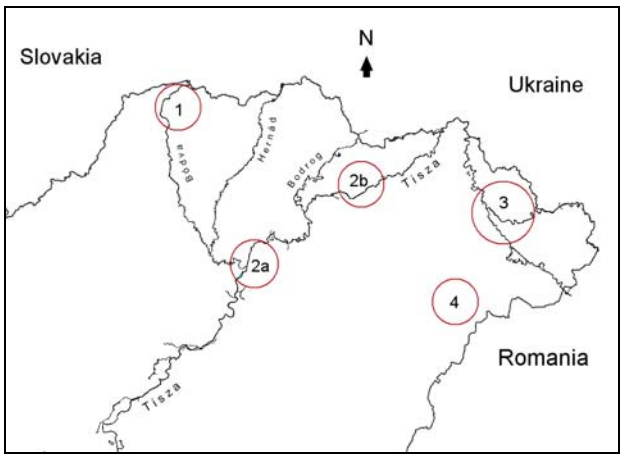

Figure 1. Map of the study areas in Hungary (1: river Bódva, 2: Upper-Tisza Region (2a: Kesznyéten LPA, 2b: Tiszabercel-Tiszatelek, 3: Szatmár-Beregi LPA, 4: Bátorliget)

erospecific song in playback experiments (Lille 1988, Sorjonen 1980) and strong habitat preferences for deciduous woodlands where bushes or undergrowth are commonly found (Sorjonen 1986). Despite the overall morphology is relatively similar, the two nightingale species can clearly be distinguished by morphological characteristics (Kverek et al. 2008, Svensson 1992) and plumage coloration (Cramp 1988, Svensson 1992).

Although the taxonomy of the nightingale species is relatively consistent (Cramp 1988, Svensson 1992), no long-term studies have been published showing the phylogenetic relationships in relation to their local populations in the Carpathian basin, specifically in the Upper-Tisza Region. Supposedly, the estimated number of singing thrush nightingale was approximately 30 in 1968-1975, 8-12 in 1979-1982 and only four in 1986 in this region (Schmidt 1986). In conclusion, the range of the species has contracted markedly over the last few decades (Haraszthy 1998). However, no explanation emerges why the breeding population of *L. luscinia* has continuously decreased, while the range expansion of *L. megarhynchos* increased (Haraszthy 1998). Schmidt hypothesized a partial hybridization between the two species, due to hard reduction of ancient floodplain forests (Schmidt 1986). However, distributional pattern of the two nightingale species have not been investigated over the past twenty years.

Thus, our aim in this paper is (1) to specify the current range of *L. luscinia* and *L. megarhynchos* in their contact zone in Hungary. (2), we assumed that remaining breeding populations of *L. luscinia* probably fragmented and partially shifted to undisturbed territories.

Materials and methods

Study area and data collecting

The region of north-eastern Hungary holds important wetland complexes and ecological corridors for several species (e.g. Ködöböcz et al. 2006, Magura et al. 2008) with different habitat types. Thus, our field work was carried out in five study sites as river Bódva (48°27′N, 20°43′E) (1), on the floodplain areas of the Upper-Tisza Region [(Kesznyéten Landscape Protected Area (LPA) 48°01′N, 21°06′E (2a) and between Tiszabercel and Tiszatelek 48°10′N, 21°42′E (2b)], the Szatmár-Beregi LPA (Bockerek-forest, near Vámosatya, 48°11′N, 22°23′E) (3) and in Bátorliget (47°45′N, 22°26′E) (4) (Fig. 1). The climate is predominantly continental with a mean annual temperature of 9.5–10 °C, and with a mean annual precipitation of 550–700 mm. The vegetation are dominated by soft-wood riparian forests (*Salicetum albae-fragilis*) consisting of *Salix alba, S. fragilis, Populus alba* and *P. nigra* and different sections of associated small oxbow lakes and backwaters with *Alnus glutinosa, S. alba*, *P. alba* and *Sambucus nigra*. Individuals were mist-netted from early May to mid June between 2006 and 2010 and in each study sites using Ecotone® mist-nets with tape luring. In the study areas, all birds were caught within the breeding season, except individuals with the GenBank accession numbers of JQ740231, JQ740233 and JQ740246 which were migrants. Birds were measured and ringed with individually numbered aluminium rings by a licensed ringer (DK) for further analyses. After ringing and measurements, birds were immediately realized alive into the wild.

DNA extraction-preparation and sequencing

In total, 30-40 µl blood was collected from the brachial vein of each individual nightingale. All blood samples were deposited in 1.5-2 cm^3 microtest tubes in ethanol and deep-frozen at -20 °C. For purification Qiagen Blood & Tissue DNA kit (Cat. No. 69504) was used. The dry blood samples were suspended in 1× PBS to provide a sample suitable for the *Qiagen DNA* purification kit. The

final elution step was performed using 150 µl purified H_2O at pH 7. For PCR amplification of the 5′ region of COI the primers described by Kerr et al. (2009) were used. Each reaction was done in 25 µl as follows: 10x PCR puffer (Fermentas) 2.5 µl, Mg_2Cl (25mM) 1.2 µl, dNTP (2.5 mM) 2 µl, primers BirdF1 and COIbirdR2 (10 pmol/µl) 0.6 µl, Taq polymerase (Fermentas) 0.25 µl (1 U), DNA template 5 ng. The PCR procedure was: denaturation at 94°C for 2 min. and 40 cycles of 94°C for 1 min., 54-62°C for 50 sec. and at 72°C for 2 min. The final elongation was at 72°C for 5 minutes. In each case negative controls were prepared without the addition of template DNA. The success of the PCR reactions was confirmed on 1.5% agarose gels (GIBCO). COI fragments were purified using the SAP-Exol method or excised from the gels and purified using the Qiagen Gel Extraction kit and sequenced by the laboratory of *Macrogen* in Amsterdam using the *BigDye* cyclic sequencing (Applied Biosystems). In each case, DNA was sequenced from both sides using the above-mentioned primers. The electropherograms from the DNA sequencer were analysed using the *Bioedit* software. Base positions were confirmed manually and corrected if necessary.

Phylogenetic analysis

For each forward and reverse sequence consensus sequences were generated using ClustalX v1.83. Sequences from previous studies (Aliabadian et al. 2007, Kevin et al. 2009, Kerr et al. 2007, Schindel et al. 2011, Yoo et al. 2006) were downloaded from GenBank and compared with our data. All 98 available COI sequences of the genus *Luscinia* were used in the analyses. Intra- and interspecies COI genetic distances were calculated on the basis of uncorrected *p*-distances using the MEGA5 software program (Tamura et al. 2011). The closely related *Luscinia* species, three *Ficedula* species and *Monticola gularis* were used as "outgroups". Maximum Parsimony, Neighbor-Joining Maximum Likelihood and Bayesian phylogenetic analyses were conducted using MEGA5 (Tamura et al. 2011) and MrBayes 3.2.1 (Ronquist & Huelsenbeck 2003). The new COI sequences of nightingale birds collected from Hungary were deposited in GenBank. Scientific names, sample codes, GenBank accession numbers and locations are given in Table 1.

Results

We analysed a 663 bp long part of the COI gene in 98 individuals belonging to 11 species. In principle, the phylogenetic analysis of COI suggested that the investigated taxons include 3 distinct species groups: the *Luscinia luscinia-megarhynchos-svecica* species group is closer to the *Ficedula albicollis-hypoleuca-semitorquata* group than to the *Luscinia brunnea-calliope-cyane-sibilans* group (Fig. 2). Furthermore, haplotypes of *L. megarhynchos* assume the existence of the species with relatively homogeneous genetics (Fig. 2). The interspecific genetic distance between the haplotypes of *L. luscinia* and *L. megarhynchos* was 5.5%, while the intraspecific variability was 0.0020 in the *L. luscinia* and lower, 0.0016 in the *L. megarhynchos*.

The primary finding of our analyses was a distinguishable population of *L. luscinia* collected from the eastern portion of Hungary (Bockerek-forest, GenBank accession number JQ740221, Fig. 2).

Second, we did not find the haplotype of *L. luscinia* neither in the Upper-Tisza Region nor in the river Bódva. It suggests it probably does not breed between Tiszabercel and Tiszatelek, but in slightly southern and more eastern territories. The new haplotype of *L. luscinia* of the Bockerek-forest implies that it may be a paraphyletic clade because its COI haplotype are well nested within a clade formed by haplotypes that correspond to populations to *L. luscina*.

Discussion

Overlapping zone of *L. luscinia* and *L. megarhynchos*

Although the frequency of hybridization between the two nightingale species is not fully known yet in the Carpathian basin, we emphasize that *L. luscinia* may potentially be out competed with *L. megarhynchos* in the whole study area, due to their coexistence position and habitat competition. Based on our field observations, the stability of suitable habitats was significantly decreased partly due to human disturbance in the last decades. Therefore, a marked fragmentation of the *L. luscinia* population started, while the range expansion of *L. megarhynchos* increased. However, further information is needed whether the non-overlapping haplotype of *L. luscinia* has a continuous or rather a fragmented distribution in eastern portion of Hungary and its surroundings.

Decrease and/or local extinction of the thrush nightingale

Approximately 30 years ago, both nightingale species occurred in similar densities in the Upper-Tisza Region. In sympatry, i.e. along the river Tisza, both nightingale species can use very similar habitats (Schmidt 1986). The proximity of water is a very common feature of the habitats of the *L. luscinia* (Cramp 1988, Sorjonen 1980). It prefers wet low-laying habitats of large expanded gallery forest, while *L. megarhynchos*, however, often breed

Table 1. Samples and identification data with GenBank accession number (COI) of examined individuals and specimens of museum collections.

Species	Specimen label	GenBank accession No. (COI)	Locality
Luscinia luscinia	UWBM49179	GQ482129	Moskov, RU
L. luscinia	UWBM49577	GQ482128	Moskov, RU
L. luscinia	UWBM49411	GQ482130	Sverdlovska, RU
L. luscinia	UWBM49514	GQ482131	Sverdlovska, RU
L. luscinia	UWBM74235	GQ482132	Kirov, RU
L. luscinia	UWBM59669	GQ482133	Smolensk, RU
L. luscinia	ZMMU 59a	GQ482134	Kaliningrad, RU
L. luscinia	NRM20026317	DQ683476	Malmon, SW
L. luscinia	BISE-Aves392	GU571964	Orebro, SW
L. luscinia	NHMO-BC40	GU571473	Telemark, NO
L. luscinia	NHMO-BC39	GU571474	Telemark, NO
L. luscinia	AE80017FL6	JQ740221	Vámosatya, E-HU
L. luscinia	AE36813FL16	JQ740231	Tiszadob, NE-HU
L. luscinia	AE49320FL18	JQ740233	Tiszadob, NE-HU
L. luscinia	AE26575FL33	JQ740246	Szalonna, N-HU
L. megarhynchos	UWBM64638	GQ482135	Krasnodar, RU
L. megarhynchos	UWBM46491	GQ482136	Alma-Ata, KA
L. megarhynchos	UWBM61111	GQ482137	Krasnodar, RU
L. megarhynchos	MIUT200359	DQ683477	Bazangan, IR
L. megarhynchos	USNM: Drov. 3745	JQ175292	MC
L. megarhynchos	USNM: Drov. 3733	JQ175293	MC
L. megarhynchos	AE36868FL1	JQ740216	Perkupa, N-HU
L. megarhynchos	AE36874FL2	JQ740217	Tornanádaska, N-HU
L. megarhynchos	AE36876FL3	JQ740218	Tornanádaska, N-HU
L. megarhynchos	AE36879FL4	JQ740219	Szögliget, N-HU
L. megarhynchos	AE36881FL5	JQ740220	Szögliget, N-HU
L. megarhynchos	AE80019FL7	JQ740222	Tarpa, E-HU
L. megarhynchos	AE80023FL8	JQ740223	Tivadar, E-HU
L. megarhynchos	AE80027FL9	JQ740224	Fehérgyarmat, E-HU
L. megarhynchos	AE80035FL10	JQ740225	Bátorliget, E-HU
L. megarhynchos	AE80128FL11	JQ740226	Tiszabercel, NE-HU
L. megarhynchos	AE80014FL12	JQ740227	Tiszalúc, NE-HU
L. megarhynchos	AE36900FL13	JQ740228	Tiszalúc, NE-HU
L. megarhynchos	AE36899FL14	JQ740229	Tiszalúc, NE-HU
L. megarhynchos	AE36898FL15	JQ740230	Tiszalúc, NE-HU
L. megarhynchos	AE80127FL19	JQ740234	Tiszabercel, NE-HU
L. megarhynchos	N115813FL20	JQ740235	Tiszalúc, NE-HU
L. megarhynchos	AE36889FL22	JQ740237	Tiszalúc, NE-HU
L. megarhynchos	AE36892FL23	JQ740238	Tiszalúc, NE-HU
L. megarhynchos	AE36896FL24	JQ740239	Tiszalúc, NE-HU
L. megarhynchos	AE44802FL25	JQ740240	Tiszabercel, NE-HU
L. megarhynchos	AE80018FL28	JQ740242	Vámosatya, E-HU
L. megarhynchos	AE36781FL29	JQ740243	Perkupa, N-HU
L. megarhynchos	AE36814FL30	JQ740244	Tiszadob, NE-HU
L. megarhynchos	AE80022FL32	JQ740245	Tarpa, E-HU
L. megarhynchos	AE80024FL34	JQ740247	Tivadar, E-HU
L. megarhynchos	AE36870FL35	JQ740248	Perkupa, N-HU
L. brunnea	USNM 620607	JQ175290	Chin, MM
L. brunnea	USNM 620595	JQ175291	Chin, MM
L. calliope	UWBM 44150	GQ482112	Kamchatka, RU
L. calliope	UWBM 51743	GQ482113	Krasnoyarsk, RU
L. calliope	UWBM 47214	GQ482114	Khabarovsk, RU
L. calliope	UWBM 52532	GQ482115	Magadansk, RU
L. calliope	UWBM 73298	GQ482116	Irkutsk, RU
L. calliope	UWBM 59869	GQ482117	Dornod, MO
L. calliope	ZMMU RYA 1681	GQ482118	Sopochnoe lake, RU
L. calliope	MMU RYA 1682	GQ482119	Sopochnoe lake, RU
L. calliope	ZMMU RYA 1680	GQ482120	Sopochnoe lake, RU
L. calliope	ZMMU RYA 1658	GQ482121	Sopochnoe lake, RU
L. cyane	UWBM 47130	GQ482122	Khabarovsk, RU

Table 1. (continued)

Species	Specimen label	GenBank accession No. (COI)	Locality
L. cyane	UWBM 74757	GQ482123	Primorskiy Kray, RU
L. cyane	UWBM 52522	GQ482124	Magadansk, RU
L. cyane	UWBM 51739	GQ482125	Krasnoyarsk, RU
L. cyane	UWBM 46940	GQ482126	Sakhalinsk, RU
L. cyane	UWBM 59709	GQ482127	Dornod, MO
L. sibilans	UWBM 47493	GQ482138	Sakhalin, RU
L. sibilans	UWBM 44562	GQ482139	Kamchatka, RU
L. sibilans	UWBM 78240	GQ482140	Irkuts, RU
L. sibilans	UWBM 47106	GQ482141	Khabarovsk, RU
L. sibilans	KRIBB338	EF515794	KR
L. svecica	UWBM 74242	GQ482142	Kirov, RU
L. svecica	UWBM 59422	GQ482143	Labytnangi, RU
L. svecica	UWBM 49697	GQ482144	Murmansk, RU
L. svecica	UWBM 75800	GQ482145	Tyva, RU
L. svecica	UWBM 44132	GQ482146	Chukotskiy Avtonomnaya, RU
L. svecica	ZMMU RYA 1926	GQ482147	Tormanskoe swamp, RU
L. svecica	ZMMU RYA 1927	GQ482148	Tormanskoe swamp, RU
L. svecica	UWBM 67624	DQ433776	Tyva, RU
L. svecica	UWBM 44078	DQ433777	Kamchatka, RU
L. svecica	NHMO-BC477	GU571475	Oppland, NO
L. svecica	NHMO-BC478	GU571476	Oppland, NO
L. svecica	USNM 608996	DQ433005	Lappland, SW
L. svecica	BISE-Aves310	GU571965	Norrbotten, SW
L. svecica	BISE-Aves157	GU571966	Norrbotten, SW
Ficedula albicollis	UWBM 49299	GQ481892	Kursk, RU
F. albicollis	UWBM 49388	GQ481893	Kursk, RU
F. albicollis	UWBM 49425	GQ481894	Kursk, RU
F. hypoleuca	UWBM 49352	GQ481896	Kursk, RU
F. hypoleuca	ZMMU 10a	GQ481897	Kaliningrad, RU
F. hypoleuca	UWBM 49395	GQ481898	Kursk, RU
F. hypoleuca	UWBM 49648	GQ481899	RU
F. hypoleuca	UWBM 61029	GQ481901	RU
F. hypoleuca	NHMO-BC494	GU571395	Oslo, NO
F. hypoleuca	NHMO-BC493	GU571396	Oslo, NO
F. semitorquata	UWBM 61130	GQ481913	Krasnodar, RU
F. semitorquata	UWBM 61175	GQ481914	Akhmetovska, RU
F. semitorquata	UWBM 64706	GQ481915	Krasnaya Polyana, RU
Monticola gularis	UWBM59864	GQ482168	New-Barag, MO

Abbreviations: KA: Kazakhstan, KR: South Korea, MC: Macedonia, MM: Myanmar, MO: Mongolia, E-HU: Eastern Hungary, NE-HU: North-eastern Hungary, N-HU: Northern Hungary, IR: Iran, RU: Russia, SW: Sweden, NO: Norway, KRIBB: Korea Research Institute of Bioscience and Biotechnology, UWBM: Burke Museum of Natural History and Culture (University of Washington), NHMO: Oslo Museum of Natural History, NRM: Swedish Museum of Natural History, USNM: United States National Museum, ZMMU: Zoological Museum of Moskow; specimen label started with „AE" or „N" are numbered aluminum rings (individuals were immediately released after marking and measurement).

further away from rivers and exhibit a decreased sensitivity in habitat use, which increases its dispersion abilities towards drier areas (e.g. in nettled embankment).

In relation to habitat requirements of the *L. luscinia*, thickets and undergrowth seem to be more important inside territories, because they provide suitable cover for the species, which breeds on the ground and as well as for fledglings before capable of fly (Sorjonen 1980). Taking into consideration the fact that only the surrounding of the Upper-Tisza Region (specifically between Tiszabercel and Tiszatelek, see Fig. 1, study area No. 2b) was known as a potentially breeding population of *L. luscinia*, and where extension and high degree of deforestation was particularly high in the past (Schmidt 1986, Haraszthy 1998). Based on our repeated field experiences, woodlands have been intensively exploited by humans along Upper-Tisza and, respectively river Bódva, during the last ten years. Consequently, the quality of undergrowth is currently not suitable for the *L. luscinia*, because riparian forest associations have already been roughly altered. This may be a possible explanation of the dramatic population decline of the *L. luscinia*, simultaneously with a general ex-

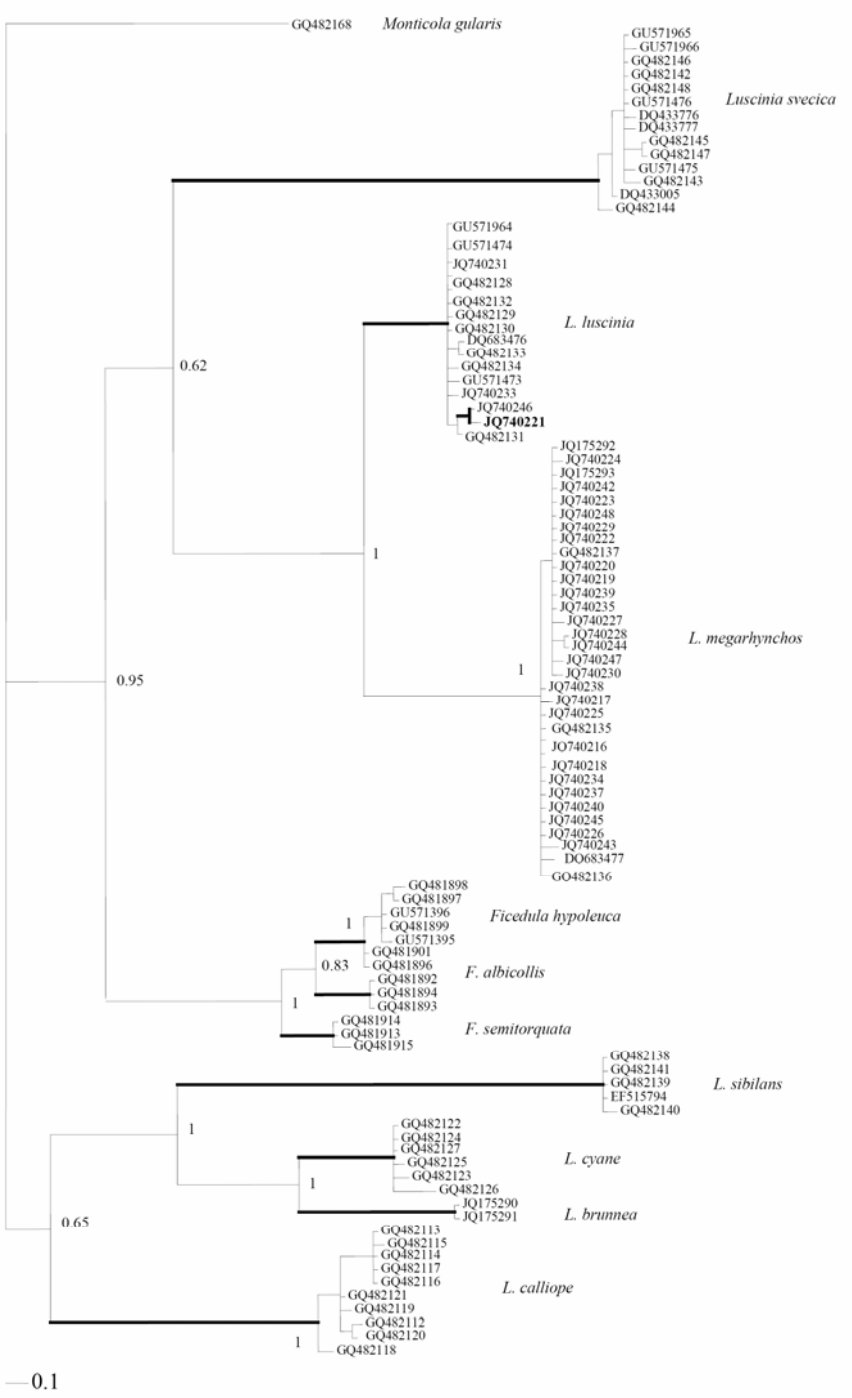

Figure 2. Phylogenetic classification of the nightingale population included newly discovered haplotype of *L. luscinia* (JQ740221) with respect to other genus of *Luscinia* and *Ficedula* and outgroup *Monticola gularis*, based on cytochrome *c* oxidase I gene (COI) sequences (663 bp) set up by MrBayes algorithm. Phylogenetic branches corresponding to species are marked by thick lines. Only bifurcations above a posterior probability of 50% are shown. GenBank accession numbers and species identification are given to the right of each phylogenetic clade.

pansion of the *L. megarhynchos*.

Competition and/or introgression and possible consequences

Intraspecific competition may force subordinate individuals to accept poorer quality habitats, but the highest settling densities are nonetheless expected in the best habitats. In contrast this statement, interspecific competition may result in either the exclusion of a species from its preferred habitat or in each species becoming a specialist on its preferred habitat type (Lawlor & Maynard Smith 1976). Interspecific interactions are known to influence patterns of spatial occupancy in a wide range of passerines (e.g. Saether 1983, Sorjonen 1986). However, where closely related taxa do not co-occur, reproductive interference may be a more likely cause than resource competition in many cases (Hochkirch et al. 2007, Gröning & Hochkirch 2008).

It is well known that *L. luscinia* and *L. megarhynchos* have similar habitat requirements (Cramp 1988) and both species show interspecific competition (Sorjonen 1986). However, the *L. megarhynchos* is known as a less effective competitor than *L. luscinia* partly due to its slightly smaller body size (Cramp 1988) and habitat shifts or lesser ability to match its neighbours (Sorjonen 1986). For example, in some areas in southern part of Russia where formerly the *L. megarhynchos* was the only nightingale species, later the *L. luscinia* occurred in higher portion (Sorjonen 1986). The reason of lower competitiveness of the *L. megarhynchos* has partly been revealed. For instance, this species usually shows significant shift in the relative bill size between the range of sympatry and allopatry, but this morphological trait does not differ between these ranges in the *L. luscinia* (Reifová et al. 2011). The size of bill also determines what kind of prey is available for the species to catch in various composition of habitats (Schoener 1965, 1974). This suggests that habitat selection is partly defined by the available size and type of food with respect to habitat competition.

In sympatry, habitats of the *L. megarhynchos* usually occur in drier areas, while *L. luscinia* is more frequent in wetter territories due to shifting by their interspecific competition (Sorjonen 1986, Ranoszek 2001). Because *L. luscinia* protects its territory stronger it is also capable of driving the weaker competitor *L. megarhynchos* out of its habitats. Further, habitat fragmentation, where a habitat is separated into fragments that lack effect ecological connectivity, reducing the viability of some of the territorial species like the *L. luscinia*.

Based on this phenomenon, the contact zone of the two species in north-eastern Hungary may probably start to shift slightly southwards but eastern direction. It is also possible that there is a so-called reproductive interference between the two taxa (Gröning & Hochkirch 2008). This explains that *L. luscinia* has already been extinct from its previous habitats partly due to its fundamental resources. However, because relatively poor sample was collected from eastern Hungary (specifically in the Bockerek-forest), more field investigations of the local population are needed in order to specify how competition among individuals of the two species influences their distribution.

Phylogenetic connections

Despite the avian diversity of the western Palearctic is relatively depauperate (Cramp 1988, Sibley & Monroe 1990), molecular techniques often help to recognize overlooked populations (Knox et al. 2002). DNA barcoding employs sequences from a short standardized gene region to identify species (e.g. Hebert et al. 2003) or hardly distinguishable populations of closely related species (e.g. Kevin et al. 2009).

Until recently, *L. luscinia* has not been detected in the Bockerek-forest, although it had a strong breeding stand in the near-lying Lónyai-forest in the 1970-80's (Varga & Aradi pers. obs.). The new clade of the species is paraphyletic and nested within the *L. luscinia* cluster as the sister line to the northern (Russian) groups of the species. Further, the subgeneric separation of the Eastern Palearctic *L. cyane-calliope* species group is strongly supported by our analysis of COI fragments, however, later surveys combining larger taxonomical spectrum of congener species and genetic data can be recommended to confirm its current position.

Outlook in connection with the climate change

Climate change plays a major role in all aspects of ecology. There is no doubt that climate plays a major role in limiting terrestrial species' ranges (Parmesan 1996). Observations of range shifts in parallel with climate change have been particularly rich in northern European countries, where observational records for many several species of trees, butterflies or birds date back to the mid-1700s (Andrewartha & Birch 1954).

Presently, the climate change is one of the most important problems. In the last hundred

years, the warming of Earth increased by 0.5 °C (IPCC 2001). Environmental changes are already affecting the dynamics of bird populations. This occurs because local weather and regional climate patterns have a strong influence on bird behavior in both breeding and non-breeding seasons (Thomas & Lennon 1999, Seather et al. 2004, Crick & Sparks 2006). For example, many long-term migrant passerines have shifted their timing of migration in the last decades (Crick & Sparks 2006): some species arrive earlier while others significantly later to their breeding stand (Zalakevicius & Zalakeviciute 2001). However, climate change affects not only the timing of migration, but also other behavioral and physiological traits, such as large-scale distribution factors (Crick 2004, Seather et al. 2004), phenology (Lehikoinen et al. 2004), the timing of breeding (Crick et al. 1997, Forchhammer et al. 1998), or the pattern of moulting (Hedenström et al. 2007). On the other hand, evolutionary adaptations to warmer conditions have occurred in the interiors of species' ranges, and resource use and dispersal have evolved rapidly at expanding range margins. Observed genetic shifts modulate local effects of climate change, but there is little evidence that they will mitigate negative effects at the species level (Parmesan 2006). Due to climate change, the proportion of bird communities will change by shifting to new areas (Williamson 1975, Lundberg & Edholm 1982, Berthold 1990). As a result, the type and abundance of species upon which birds depend (e.g. food sources such fruits, insects, as well as nesting materials) may decline and interspecific interactions intensify. The disrupted ecological populations mean the nightingales may also face new competitors, predators, prey and parasites to which they are not adapted, and that "optimal" habitats for the species may disappear. As old ecosystems disappear or replaced by new ones, the consequences are unknown and largely unpredictable.

Based on our results, however, we indicate the extinction of *L. luscinia* in the Upper-Tisza valley, to highlight the importance of preserving of the remaining unfragmented forests in eastern Hungary.

Ackowledgements. We especially thank Helga Urbán, Beatrix Ferencz, Eszter Zsófia Kovács, and Imre Mihalik who assisted in the field. Permits for field work were obtained from the National Inspectorate of Environment, Nature and Water. We would also like to thank to Prof. Dr. Zoltán Varga for his valuable suggestions and comments on the earlier form of the manuscript and Dr. Zsolt Végvári who kindly revised the language of the paper. Our work was funded by the Vénic Nature Conservation Foundation (Ajka, Hungary). This article was prepared in a PhD program of „Biodiversity" of University of Debrecen Faculty of Science.

References

Aliabadian, M., Kaboli, M., Prodon, R., Nijman, V., Vences, M. (2007): Phylogeny of Palearctic whetears (genus Oenanthe) – congruence. Molecular Phylogenetics and Evolution 42(3): 665-675.

Andrewartha, H.G., Birch, L.C. (1954): The Distribution and Abundance of Animals. Chicago, IL: Univ. Chicago Press.

Avise, J.C. (1994): Molecular markers, natural history and evolution. Chapman & Hall, New York, 511 pp.

Barton, N.H., Hewitt, M.G. (1985): Analysis of hybrid zones. Annual Review of Ecology and Systematics 16: 113–148.

Berthold, P. (1990): Patterns of avian migration in light of current global 'greenhouse' effects: a central European perspective. Acta Congressus Internationalis Ornithologici 20: 780-786.

Cramp, S. (1988): The Birds of the Western Palearctic. Vol. 5, Oxford University Press, Oxford.

Crick, H.Q.P. (2004): The impact of climate change on birds. Ibis 146: 48-56.

Crick, H.Q.P., Sparks T.H. (2006): Changes in the phenology of breeding and migration in relation to global climate change. Acta Zoologica Sinica 52: 154-157.

Crick, H.Q.P., Dudley, C., Glue, D.E. (1997): UK birds are laying eggs earlier. Nature 399: 423-424.

Forschhammer, M.C., Post, E., Stenseth, N.C. (2002): North Atlantic Oscillation timing of long- and short-distance migration. Journal of Animal Ecology 71: 1002-1014.

Gienapp, R., Leimu, R., Merilä, J. (2007): Responses to climate change in avian migration time – microevolution versus phenotypic plasticity. Climate Research 35: 25-35.

Gröning, J., Hochkirch, A. (2008): Reproductive interference between animal species. The Quaterly Review of Biology 89(3): 257-282.

Hagemeijer, W.J.M., Blair, M.J. (1997): EBCC Atlas of European Breeding Birds. London.

Haraszthy, L. (1998): Birds of Hungary. Mezőgazda Kiadó, Budapest. [in Hungarian]

Hebert, P.D., Stoeckle. .N.M.Y., Zemlak, T.S., Francis, C.M. (2004): Identifications of birds through DNA barcodes. PloS Biology 2: 1657-1663.

Hedenström, A., Barta, Z., Helm, B., Houston, A.I., McNemara, J.M., Jonzen, N. (2007): Migration speed and scheduling of annual events by migrating birds in relation to climate change. Climate Research 35(1-2): 79-91.

Hewitt, G.M. (1999): Post-glacial recolonization of European Biota. Biological Journal of the Linnean Society 68: 87–112.

Hewitt, G.M. (2000): The genetic legacy of the Quaternary ice ages. Nature 405: 9074-913.

Hewitt, G.M. (2004): Genetic consequences of climatic oscillation in the Quaternary. Philosophical Transactions of the Royal Society B 359: 183-195.

Hochkirch, A., Gröning, J., Bücker, A. (2007): Sympatry with the devil: reproductive interference could hamper species coexistence. Journal of Animal Ecology 76(4): 633-642.

Hogg, I.D., Hebert, P.D.N. (2004): Biological identifications of springtails (Hexapoda: Collembola) from the Canadian arctic using mitochondrial DNA barcodes. Canadian Journal of Zoology 82: 749-754.

Illera, J.C., Richardson, D.S., Helm, B., Atienza, J.C., Emerson, B.C. (2008): Phylogenetic relationships, biogeography and speciation in the avian genus *Saxicola*. Molecular Phylogenetics and Evolution 48: 1145-1154.

IPCC (International Panel of Climate Change) (2001): Climate Change 2001: The scientific basis. pp. 21-83. In: Albritton, D.L., Meira Filho, L.G. (eds), Third assessment Report of Working Group I. Cambridge University Press, Cambridge.

Kerr, K.C.R., Stoeckle, M.Y., Dove, C.J., Weigt, L.A., Francis, C.M., Hebert, P.D. (2007): Comprehensive DNA barcode coverage of North American birds. Molecular Ecology Notes 7: 535-543.

Kerr, K.C.R., Lijmaer, D.A., Barreira, A.S., Hebert, P.D.N., Tubaro, P.L. (2009): Probing evolutionary patterns in Neotropical birds through DNA barcodes. PLoS ONE 4: 6.

Kevin, C.R.K., Sharon, M.B., Mikhail, V.K., Yaroslav A.R., Eugeny, A.K., Paul, D.N.H. (2009): Filling the gap – COI barcode resolution in eastern Palearctic birds. Frontiers in Zoology 6: 29.

Knox, A.G., Collinson, M., Helbig, A.J., Parkin, D.T., Sangster G. (2002): Taxonomic recommendations for British birds. Ibis 144: 707-710.

Ködöböcz, V., Juhász, P., Kiss, B., Müller, Z. (2006): Faunistical results of the Coleoptera investigations carried out in the frames of the ecological survey of the surface waters of Hungary (ECOSURV) in 2005. Folia Historico-Naturalia Musei Matraensis 30: 349-355.

Kverek, P., Storchová, R., Reif, J., Nachman, W.M. (2008): Occurrence of a hybrid between the Common Nightingale (*Luscinia megarhynchos*) and the Thrush Nightingale (*Luscinia luscinia*) in the Czech Republic confirmed by genetic analyses. Sylvia 44: 17-26.

Lawlor, L.R., Maynard Smith, J. (1976): The coevolution and stability of competing species. American Naturalist 110: 79-99.

Lehikoinen, E., Sparks, T.H., Zalakevicius, M. (2004): Arrival and departure dates. Advances in Ecological Research 35: 1-31.

Li, W., Zhang, Y. (2004): Subspecific taxonomy of *Ficedula parva* based on sequences of mitochondrial cytochrome b gene. Zoological Research 25: 127-131.

Lille, R. (1988): Species-specific song and mixed singing of Nightingale and Thrush Nightingale (*Luscinia megarhynchos, L. luscinia*). Journal of Ornithology 129: 133-159.

Lundberg, A., Edholm, M. (1982): Earlier and later arrivals of migrants in central Sweden. British Birds 75: 583-585.

Magura, T., Lövei, G.L., Tóthmérész, B. (2008): Time-consistent rearrangement of carabid beetle assemblages by an urbanisation gradient in Hungary. Acta Oecologica 34: 233–243.

Martin, J.L. (1990): The *Parus caeruleus* complex revisited. Ardea 79: 429-438.

Moreau, R.E. (1972): The Palearctic-African bird migration systems. Academic Press (New York) 384 pp.

Moritz, C., Cicero, C. (2004): DNA Barcoding: promises and pitfalls. Public Library of Science. PloS Biology 2: 354.

Newton, I. (2003): The speciation and biogeography of birds. Academic Press, London, 668 pp.

Parmesan, C. (1996): Climate and species' range. Nature 382: 765–766.

Parmesan, C. (2001): Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and Systematics 37: 637-669.

Ranoszek, E. (2001): Occurrence and habitat preferences in breeding season of the Thrush Nightingale *Luscinia luscinia* and the Nightingale *Luscinia megarhynchos* in the Barycz river valley. Ptaki Slaska 13: 19–30.

Reifová, R., Reif, J., Antczak, M., Nachman, M.W. (2011): Ecological character displacement in the face of gene flow: Evidence from two species of nightingales. BMC Evolutionary Biology 11: 138.

Ronquist, F., Huelsenbeck, J.P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Sánchez-Guillén, R.A., Wellenreuther, M., Cordero-Rivera, A., Hansson, B. (2011): Introgression and rapid species turnover in sympatric damselflies. BMC Evolutionary Biology 11: 210.

Schindel, D.E., Stockle, M.Y., Milensky, C., Trizna, M., Schmidt, B., Gebhard, C., Graves, G. (2011): Project description: DNA barcodes of bird species in the national museum of natural history, smithsonian institution, USA. Zookeys 152: 87-92.

Schmidt, E (1986): The Nightingale and the Thrush Nightingale. MME, Budapest. [in Hungarian]

Schoener, T.W. (1965): The evolution of bill size differences among congeneric species of birds. Evolution 19: 189-213.

Schoener, T.W. (1974): Resource in ecological communities. Science 185: 27-29.

Sætre, G.P., Borge, T., Lindroos, K., Haavie, J., Sheldon, B.C., Primmer, C., Syvänen, A.C. (2003): Sex chromosome evolution and speciation in *Ficedula* flycatchers. Proceeding of the Royal Society London B 270: 53–59.

Saether, B.E. (1983): Habitat selection, foraging niches and horizontal spacing of willow warbler *Phylloscopus trochilus* and chaffchaff *Phylloscopus collybita* in an area of sympatry. Ibis 125: 24-32.

Seather, B.E., Sutherland, W.J., Engen, S. (2004): Climate influences on avian population dynamics. pp. 185-210. In: Moller, A., Fiedler, W. Berthold P. (eds.). 2004: Birds and climate change. Elsevier Ltd., Amsterdam.

Seehausen, O. (2006): Conservation: losing biodiversity by reverse speciation. Current Biology 16: R334-R337.

Sibley, C.G., Monroe, B.L. (1990): Distribution and Taxonomy of Birds of the World. Yale University Press, New Haven and London.

Sorjonen, J. (1980): Selection of breeding habitat by the Thrush Nightingale *Luscinia luscinia* and its position in bird communities. Ornis Scandinavica 11: 125-134.

Sorjonen, J. (1986): Mixed singing and interspecific territoriality – consequences of secondary contact of two ecologically and morphologically similar nightingale species in Europe. Ornis Scandinavica 17: 53-67.

Storchová, R., Reif, J., Nachman, W.M. (2010): Female heterogamety and speciation: reduced introgression of the Z chromosome between two species of nightingales. Evolution 64(2): 456-471.

Svensson, L. (1992): Identification Guide to European Passerines. 4th ed. Stockholm.

Taberlet, P., Fumagalli, L., Wust-Saucy, A-G., Cosson, 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 Analyses using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28: 2731-2739.

Tavares, E., Baker, A. (2008): Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. Evolutionary Biology 8: 81.

Thomas, C.D., Lennon, J.J. (1999): Birds extend their range northwards. Nature 399: 213.

Voous, K.H. (1970): Atlas of European birds. Nelson, London.

Wiens, J.J., Pyron, R.A., Moen, D.S. (2011): Phylogenetic origins of local-scale diversity patterns and the causes of Amazonian megadiversity. Ecology Letters 14: 643-652.

Williamson, K. (1975): Birds and climate change. Bird Study 22: 143-164.

Yom-Tov, Y., Yom-Tov, S., Wright, J., Du Feu, T., Du Feu, R. (2006): Recent changes in body weight and wing length among some British passerine birds. Oikos 112: 91-101.

Yoo, H.S., Eah, J.Y., Kim, J.S., Kim, Y.J., Min, M.S., Paek, W.K., Lee, H., Kim, C.B. (2006): DNA barcoding Korean birds. Molecules and Cells 22(3): 323-327.

Zalakevicius, M., Zalakeviciute, R. (2001): Global climate change impact on birds: a review of research in Lithuania. Folia Zoologica 50: 1-17.