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Source: Zoological Science, 34(3):185-200.
Published By: Zoological Society of Japan
https://doi.org/10.2108/zs160119
URL: http://www.bioone.org/doi/full/10.2108/zs160119

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ZOOLOGICAL SCIENCE **34**: 185–200 (2017)

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Deep Phylogeographic Breaks in Magpie *Pica pica* Across the Holarctic: Concordance with Bioacoustics and Phenotypes

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We examined sequences of the mitochondrial control region in magpies (*Pica pica*) from the entire distribution range and found deep genetic splits into four major lineages: (1) group West (Europe-Siberia), (2) group East (southern Far East), (3) *P. p. mauritanica* (North Africa), and (4) *P. p. hudsonia* (North America). These lineages show a geographic pattern corresponding to known subspecies or subspecies groups. Genetic variation within the widely-distributed group West is low and neutrality tests supported a recent expansion scenario. The haplotypes from Kamchatka, representing a separated sublineage with clear affinity to the European-Siberian group, are almost identical, implying a recent bottleneck. Group East contained two subclades without clear geographic pattern, presumably due to admixing of populations that had diverged in Pleistocene refuges. The homogeneity of the Kyushu population supports historical reports of introduction of the species from Korea. In contrast, the high variation in the recently established Hokkaido population may reflect an ongoing invasion from several populations of the Far Eastern mainland. Bioacoustic data based on chatter call differentiate groups of subspecies and reflect phylogeographic patterns, i.e., mitochondrial lineages. Furthermore, we report the fast spreading of *P. p. jankowskii* towards the west along the upper Amur River, and a slower shifting of *P. p. leucoptera* in the opposite direction thus yielding a new contact zone. Overall, our data support a scenario of divergence in geographic isolation, but the ongoing expansion of distribution ranges may lead to major changes in phylogeographic patterns.

Key words: magpie, mitochondrial DNA, control region, phylogeography, Palearctic, range shift, bioacoustics

INTRODUCTION

Current patterns of species ranges are generally assumed to be the result of extensive climatic and environmental changes in the late Pleistocene and Holocene. Modern phylogeography commonly deals with hypotheses on Pleistocene refugia and processes of range expansion based on the present distribution of genetic lineages (Avise and Walker, 1998; Avise, 2000; Hewitt, 2000). Although phylogeographic reconstructions frequently take into account possible ancient shifts of distribution ranges, the effect of recent range changes on the distribution of genetic lineages, and thus the impact of these changes on phylogeographic scenarios, are mostly unknown and probably underestimated. Such recent shifts may be partly due to direct or indirect anthropogenic influences.

The Eurasian magpie *Pica pica* (Linnaeus, 1758) serves as a good example. This widely distributed mostly Holarctic species occurs from Morocco and Spain to western North America with several isolated locations (Fig. 1). It inhabits semi-open, semi-arid habitats and is often found in anthropogenic landscapes. In recent decades, this species has experienced increasing density and distributional shifts (Choe, 2000; Konstantinov et al., 2004; Madge, 2016a). According to historic reports, it was introduced from Korea to Kyushu, Japan, 400 years ago. Furthermore, it appeared in southern Hokkaido about 30 years ago, and successfully

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doi:10.2108/zs160119

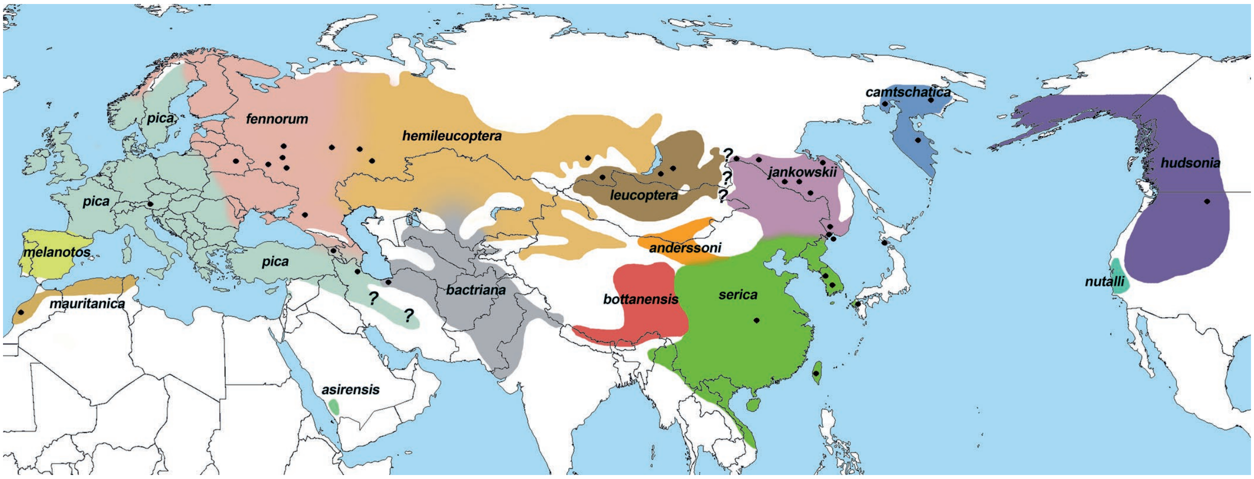

Fig. 1. Distribution of subspecies of *Pica pica* and collecting locations.

established mainly in Tomakomai city. On the mainland in the Russian Far East population numbers are currently increasing drastically as this bird now occupies urban areas and cities (Babenko, 2000; Nazarov, 2004; Fefelov, 2014; Nazarenko et al., 2016).

Importantly, in Eurasia, the main distribution range is split into three isolated parts. *Pica pica camtschatica* is found in the easternmost part of the continent on the Kamchatka Peninsula and the adjacent mainland. The western Palearctic part of the range extends from Spain to Transbaikalia, and the eastern part from eastern Siberia to southeastern China and Japan. There may have been a connection between *P. p. jankowskii* and *P. p. leucoptera* in east Mongolia (Tugarinov, 1932), but with a gap in southern Siberia – Transbaikalia (Stegmann, 1932; Rustamov, 1954; Flint et al., 1968; Stepanyan, 2003). Nonetheless, this gap is apparently shrinking year by year, and the lack of conclusive published data on the current state of this gap explains why recent distribution maps and checklists report a continuous range in this region (Goodwin, 1986; Madge and Burn, 1999; Zhang et al., 2012; Madge, 2016a; http://www.ijon.de/elster/unterart.html).

Depending on the author, *P. pica* comprises a varying number of subspecies (e.g., nine: Dickinson and Christidis, 2014; ten: Madge, 2016a; eleven: Dickinson, 2003; thirteen: Madge and Burn, 1999). At least seven additional subspecies have been described previously, but these are no longer supported (Madge, 2016a). While the North American yellow-billed magpie *P. nutalli* (Audubon, 1837), which is characterized by a yellow bill, a very narrow tail and much smaller size, is generally accepted, the status of the other Nearctic taxon, the black-billed magpie *P. p. hudsonia* (Sabine, 1823), is controversial. Some authors treat it as a distinct species, for example Dickinson and Christidis (2014) and Madge (2016a). Since these two taxa are very closely related, at least as suggested by mitochondrial DNA sequences, and their position in the phylogenetic tree renders *P. pica* paraphyletic (Lee et al., 2003), their species status remains disputable. Here, we followed the recommendation of Lee et al. (2003) by treating both taxa provisionally as subspecies and thus we accept altogether 15 subspecies within *P. pica* (Fig. 1).

In Eurasia, we distinguish two major groups of subspecies: *pica* and *serica*. The group of green-winged magpies ("*pica* group") is distributed from the British Islands and the Atlantic coast to south Siberia and Mongolia. There are clines from south-west to north-east in the total size and amount of white feather coloration (Snow and Perrins, 1998; Ebels, 2003). Nonetheless, six subspecies can be reasonably recognized within this group: *P. p. melanotos* A.E. Brehm, 1857; *P. p. pica* (Linnaeus, 1758), *P. p. fennorum* Lönnberg, 1927; *P. p. hemileucoptera* Stegmann, 1928; *P. p. bactriana* Bonaparte, 1850; and *P. p. leucoptera* Gould, 1862. The other subspecies group ("*serica* group") comprises the blue-winged forms *P. p. serica* Gould, 1845, *P. p. jankowskii* Stegmann, 1928 and *P. p. anderssoni* Lönnberg, 1923, but not *P. p. bottanensis* Delessert, 1840 (see Taxonomic notes in the Discussion section below). This group is distributed from Laos, Vietnam, Taiwan and Korea to the Amur River basin (Russian Far East) in the North. It can be also distinguished by its smaller overall size and a smaller white portion in the primaries.

Besides *pica* and *serica* groups*,* several additional isolated forms exist. *Pica pica camtschatica* Stejneger, 1884 from the Kamchatka Peninsula and the adjacent mainland is morphologically similar to the western group. These birds are greenish in secondaries and wing covers, and have large white portions on primaries (Lobkov, 1999; Ebels, 2003). This form is strictly isolated by a large gap in east Yakutia and the Okhotsk sea coast. *Pica pica bottanensis* Delessert, 1840 from the northern Himalayas (Qinghai and Sichuan in China) and *P. p. anderssoni* Lönnberg, 1923 from Alashan and the adjacent Gobi are both semi-isolated from *P. p. serica. Pica pica asirensis* Bates, 1936 from the Arabian Peninsula and *P. p. mauritanica* Malherbe, 1845 from North Africa are geographically completely isolated and morphologically distinct forms. Beyond the abovementioned differing opinions on subspecies status, species status has been proposed for the taxa *P. p. bottanensis* and *P. p. asirensis* (Goodwin, 1986).

Subspecies differentiation and genetic diversity within *P. pica* were first investigated by restriction fragment polymorphism of mitochondrial DNA by Zink et al. (1995). This revealed a particularly high level of differentiation for *P. p.*

camtschatica and *P. p. hudsonia*. For the magpie of the Korean Peninsula, a clear subdivision into western and eastern population groups separated by mountains was discovered by RAPD-analysis (Eo et al., 2002). Moreover, the Kyushu population, which was reportedly introduced from Korea, appeared in the eastern group, albeit somewhat separated from the Korean populations in that clade (Op. cit.). Later, analyses of the mitochondrial genes for *16s rRNA*, *tRNA-Leu* and *ND1* (*NADH dehydrogenase subunit 1*) revealed a phylogenetic affinity of Kamchatka's subspecies to the European nominate form, and their relationship to both American species *P. hudsonia* and *P. nutalli* (Lee et al., 2003). In contrast, the Far Eastern *P. p. serica* samples from Korea proved to be highly distant, splitting from a basal node in the tree. That study (Lee et al., 2003), however, was based on only 13 samples representing five *Pica* taxa, and most subspecies were omitted entirely. Despite limited data, Lee et al. (2003) hypothesized that the ancestral forms of magpies and other corvids had spread from Southeast Asia, preceded by a radiation in Southeast Asia of initial lineages that had arrived from the Australian region (Sibley and Ahlquist, 1990; Ericson et al., 2002, 2005). Spreading to the north up to Kamchatka and further across the Bering land bridge, populations might eventually have colonized North America, giving rise to the two extant North American magpie taxa. Somewhat later, the species could have expanded its distribution range from the Kamchatka population far to the west, ultimately all over the Western Palearctic (Lee et al., 2003). A different scenario has been proposed based on an analysis of partial DNA sequences of the *cytochrome b* (*cytb*) gene and the mitochondrial control region (CR) of six and eight Eurasian subspecies, respectively (Kryukov et al., 2004; Haring et al., 2007). Those studies showed a clear division between two clades separated by quite high genetic distances (e.g., *cyt b*: 5.1%, CR: 6.6% average p distances; Kryukov et al., 2004). The first consisted of *P. p. serica* and *P. p. jankowskii* ("eastern subclade" in Haring et al., 2007), and the second of *P. p. pica*, *P. p. melanotos*, *P. p. bactriana*, *P. p. hemileucoptera*, *P. p. leucoptera*, and *P. p. camtschatica* ("western subclade" in Haring et al., 2007). The origin of these two clades was explained by separate glacial refugia. Later, three mitochondrial and two nuclear marker sequences were used to assess diversity, phylogeographic structure and demography of the magpie populations in China and Iberia (Zhang et al., 2012). Besides the confirmation of the deep differentiation between European and Far Eastern clades, the authors of that study proposed that *P. pica* demonstrated less genetic structure within East China due to more intensive gene flow compared with the other magpie species, *Cyanopica cyanus*, which has different habitat preferences, social structure and mobility. Nonetheless, the phylogeographic interpretation of those results is still preliminary, as the data set is rather incomplete. Indeed, 11 East Chinese populations and just one North Chinese population were compared with one from Spain, and no samples from the wide area between those regions (namely China and West Europe) were included (Zhang et al., 2012). The abovementioned investigations analyzed samples from several regions in varying numbers. Yet, the distribution range of *P. pica* is huge and it is, therefore, not surprising that no comprehensive analysis covering the whole range has been accomplished so far. The isolated taxa *P. p. mauritanica*, *P. p. asirensis* as well as *P. p. bottanensis* and *P. p. anderssoni* have never been included in any phylogenetic analysis.

Behavioral characters and especially mating displays are considered to be important isolating mechanisms (Mayr, 1958; Panov, 1989). For birds, features of sound communication have been regarded as additional extremely useful complex traits in taxonomy and phylogeny (Alström and Ranft, 2003). Several aspects of geographic variation in acoustic signals have been explored (e.g., Hamao, 2013). The difference in the calls between western and eastern forms of magpies was briefly discussed by Ebels (2003) when the first spectrogram of *P. p. serica* chattering was published. The difference in alarm call between *P. p. hudsonia* and European magpies was described by Enggist-Dublin and Birkhead (1992), but neither comparisons with Far Eastern subspecies nor a complete comparative analysis of calls of subspecies have been carried out.

The present study was designed to obtain more information on patterns of genetic variation of *P. pica*, particularly in its eastern distribution, i.e., with special emphasis on populations from the Russian Far East, Japan, Korea and Taiwan. Additionally, we include, for the first time, *P. p. mauritanica* in our genetic analyses. While our former studies are based on only partial sequences of the CR, we now amplified the complete CR to better resolve the relationships among populations. Moreover, we compiled own and published bioacoustic data on the chatter calls of *P. pica* and tested correspondence with genetic results. On the basis of this data together with recent reports on the current range shifts and spreading of the species, we discuss possible scenarios of population expansion and demographic history.

MATERIALS AND METHODS

Sampling

Samples of ten subspecies of *P. pica* representing the majority of Holarctic subspecies were analyzed: *P. p. fennorum*, *P. p. pica*, *P. p. bactriana*, *P. p. hemileucoptera*, *P. p. leucoptera*, *P. p. camtschatica*, *P. p. jankowskii*, *P. p. serica*, *P. p. mauritanica*, and *P. p. hudsonia.* Tissue, feather and blood samples were stored in 80% or 100% ethanol and Queen's Buffer. We captured and sampled 53 individuals in the field. Additional 56 individual samples were provided by six institutions, so we sequenced the mitochondrial CR of 109 individuals in total (Table 1). We also included two sequences from GenBank: one (accession number HQ915867) which presumably belongs to *P. p. serica* from China (according to the reference data in GenBank), and the other one of *Nucifraga columbiana* (GenBank NC_022839, positions 15628–16905) which was used as an outgroup sequence. Sequences determined in the present study are deposited in GenBank under accession numbers LT671303–LT671411.

Laboratory methods

To amplify the complete mitochondrial CR, primers binding in the adjacent genes (*tRNA-Glu*, *tRNA-Phe*) were used: CR-Cor16+ (5′-GTTCCTGCTTGGACCCTCCCCAAG-3′) and Phe-Cor– (5′-TTGACATCTTCAGTGTCATGC-3′). The resulting PCR fragment has a length of 1466 bp in the reference sequence of *P. pica* in GenBank (HQ915867). For sequencing, in addition to the two amplification primers, we employed the internal primers CR-Cor+ (5′-ACCCTTCAAGTGCGTAGCAG-3′) and CR-Cor15– (5′-GATGTACACGTCAAGAGGAAG-3′). In some cases, when the complete CR fragment could not be obtained, it was amplified in two sections

Table 1. List of specimens with collection numbers, geographic origins and GenBank accession numbers.

Taxon					Accession number
	Lab-code	Tiss. No.	Museum No.	Geographic origin	
Pica pica pica					
	9pic (Ppicpic9)	–	N/A	Austria, Gars/Kamp	LT671303
	1218pic*	AK-1218	EAK134[1]	Iran, N. Elburs mountain range, Valasht Lake	LT671304
	8pic*	–	N/A	Armenia	LT671305
Pica pica fennorum					
	0112fen (Ppicbac6)	AK-0112	R-115831[1]	S.-W. Russia, N. Caucasus, Kislovodsk city	LT671306
	0223fen (Ppicbac5)	AK-0223	KIL021[1]	Russia, Ivanovo oblast Il’insky district	LT671307
	0224fen	AK-0224	N/A	Russia, Kirov oblast, Darovsky district	LT671308
	0225fen	AK-0225	N/A	Russia, Kirov oblast, Darovsky district	LT671309
	0113fen (Ppicpic4)	AK-0113	R-116993[1]	Russia, Smolensk oblast, Vyazemsky district	LT671310
	0378fen (Ppicpic5)	AK-0378	LVE65[1]	Russia, Smolensk oblast, Vyazemsky district	LT671311
	0379fen	AK-0379	LVE63[1]	Russia, Smolensk oblast, Vyazemsky district	LT671312
	0380fen	AK-0380	LVE64[1]	Russia, Smolensk oblast, Vyazemsky district	LT671313
	0434fen	AK-0434	LVE132[1]	Russia, Smolensk oblast, Vyazemsky district	LT671314
	0520fen	AK-0520	SKY177[1]	Russia, Smolensk oblast, Vyazemsky district	LT671315
	0756fen	AK-0756	NIA152[1]	Russia, Smolensk oblast, Vyazemsky district	LT671316
	0757fen	AK-0757	NIA158[1]	Russia, Smolensk oblast, Vyazemsky district	LT671317
	0762fen	AK-0762	NIA182[1]	Russia, Kaluga oblast, Balabanovsky district	LT671318
	1214fen	AK-1214	NIA224[1]	Russia, Moscow oblast	LT671319
	1215fen	AK-1215	NIA223[1]	Russia, Moscow oblast	LT671320
	1905fen	AK-1905	TMA130[1]	Russia, Moscow oblast, Odintsovo district	LT671321
	1970fen	AK-1970	GVV124[1]	Belarus Republic, Minsk city	LT671322
	0168fen**(Ppicbac4)	AK-0168	N/A	Russia, Kirov oblast, Zuevsky district	LT671323
	0169fen**	AK-0169	N/A	Russia, Kirov oblast	LT671324
	0170fen**	AK-0170	R-119840[1]	Russia, Kirov oblast, Malmyzhsky district	LT671325
	1903fen**	AK-1903	MVK199[1]	Russia, Kirov oblast	LT671326
	1904fen**	AK-1904	MVK265[1]	Russia, Kirov oblast	LT671327
Pica pica hemileucoptera					
	1217hem	AK-1217	CBH964[1]	Russia, Sverdlovsk oblast, Irbitsky district	LT671328
	1907hem	AK-1907	CBH2565[1]	Russia, Chelyabinsk oblast, Sosnovsky district	LT671329
	1902hem	AK-1902	CBH2563[1]	Russia, Chelyabinsk oblast, Uisky district	LT671330
Pica pica bactriana					
	1219bac	AK-1219	EAK149[1]	Iran, Caspian sea, Miankale peninsula	LT671331
Pica pica leucoptera					
	0135leu	AK-0135	R-116766[1]	Russia, Tyva Republik, Mugur-Aksy settl.	LT671332
	0136leu (Ppichem1)	AK-0136	R-116767[1]	Russia, Tyva Republik, Mugur-Aksy settl.	LT671333
	0137leu	AK-0137	N166[1]	Russia, Tyva Republik, Mugur-Aksy settl.	LT671334
	0138leu (Ppichem2)	AK-0138	N280[1]	Russia, Tyva Republik, Mugur-Aksy settl.	LT671335
	1leu (Ppicleu1)	–	OLML12/251[2]	Russia, Buryatia Republik, Ulan-Ude city	LT671336
	3leu (Ppicleu3)	–	N/A	Russia, Buryatia Rep., Kurumkansky dist.	LT671337
	4leu (Ppicleu4)	–	N/A	Russia, Buryatia Republik, Ulan-Ude city	LT671338
Pica pica camtschatica					
	0736cam (Ppiccam1)	AK-0736	N/A	Russia, Chukotka peninsula, Anadyr river	LT671339
	2046cam	AK-2046	N/A	Russia, Kamchatka peninsula, Kamchatka river	LT671340
	2256cam	AK-2256	N/A	Russia, Kamchatka peninsula, Kamchatka river	LT671341
	2257cam	AK-2257	N/A	Russia, Kamchatka peninsula, Kamchatka river	LT671342
	2258cam	AK-2258	N/A	Russia, Kamchatka peninsula, Kamchatka river	LT671343
	2259cam	AK-2259	N/A	Russia, Kamchatka peninsula, Kamchatka river	LT671344
	2260cam	AK-2260	N/A	Russia, Kamchatka peninsula, Kamchatka rriver	LT671345

2261cam	AK-2261	N/A	Russia, Kamchatka krai, Penzhina river	LT671346
2262cam	AK-2262	N/A	Russia, Kamchatka krai, Penzhina river	LT671347
2263cam	AK-2263	N/A	Russia, Kamchatka krai, Penzhina river	LT671348
2264cam	AK-2264	N/A	Russia, Kamchatka krai, Penzhina river	LT671349
2265cam	AK-2265	N/A	Russia, Kamchatka krai, Penzhina river	LT671350
2266cam	AK-2266	N/A	Russia, Kamchatka krai, Penzhina river	LT671351
2267cam	AK-2267	N/A	Russia, Kamchatka krai, Penzhina river	LT671352
2268cam	AK-2268	N/A	Russia, Kamchatka krai, Penzhina river	LT671353
2269cam	AK-2269	N/A	Russia, Kamchatka krai, Penzhina river	LT671354
Pica pica jankowskii				
1495jan	AK-1495	IBSS38/09	Russia, Jewish oblast, Birofel'd vil.	LT671355
1842jan	AK-1842	N/A	Russia, Amur oblast, Novo-Bureinks settl.	LT671356
1879jan	AK-1879	N/A	Russia, Amur oblast, Skovorodino settl.	LT671357
1896jan	AK-1896	N/A	Russia, Amur oblast, Belogorsk town	LT671358
1897jan	AK-1897	N/A	Russia, Amur oblast, Belogorsk town	LT671359
0281jan (Ppicjan2)	AK-0281	N/A	Russia, Khabarovsk krai, Solnechny settl.	LT671360
1900jan	AK-1900	CBH1284[1]	Russia, Primorsky krai, Spassk town	LT671361
1901jan	AK-1901	CBH321[1]	Russia, Primorsky krai, Reineke Island	LT671362
1220jan	AK-1220	АСФ1284[1]	Russia, Primorsky krai, Spassk town	LT671363
0277jan	AK-0277	N/A	Russia, Primorsky krai, Vladivostok city	LT671364
1368jan	AK-1368	N/A	Russia, Primorsky krai, Vladivostok city	LT671365
0738jan	AK-0738	N/A	Russia, Primorsky krai, Vladivostok city	LT671366
0712jan (Ppicjan1)	AK-0712	N/A	Russia, Primorsky krai, Nadezhdinsk vil.	LT671367
2115jan	AK-2115	N/A	Russia, Primorsky krai, Ussurijsk city	LT671368
2116jan	AK-2116	N/A	Russia, Primorsky krai, Ussurijsk city	LT671369
2143jan	AK-2143	N/A	Russia, Primorsky krai, Ussurijsk city	LT671370
2144jan	AK-2144	N/A	Russia, Primorsky krai, Ussurijsk city	LT671371
2145jan	AK-2145	N/A	Russia, Primorsky krai, Ussurijsk city	LT671372
2146jan	AK-2146	N/A	Russia, Primorsky krai, Ussurijsk city	LT671373
2147jan	AK-2147	N/A	Russia, Primorsky krai, Ussurijsk city	LT671374
2205jan	AK-2205	N/A	Russia, Primorsky krai, Ussurijsk city	LT671375
2206jan	AK-2206	N/A	Russia, Primorsky krai, Ussurijsk city	LT671376
2270jan	AK-2270	N/A	Russia, Primorsky krai, Tavrichanka town	LT671377
2271jan	AK-2271	N/A	Russia, Primorsky krai, Tavrichanka town	LT671378
0343jan	AK-0343	CBH47[2]	Russia, Primorsky krai, Gaivoron vil.	LT671379
0344jan	AK-0344	АСФ54[2]	Russia, Primorsky krai, Gaivoron vil.	LT671380
0345jan (Ppicjan3)	AK-0345	EAK041[2]	Russia, Primorsky krai, Gaivoron vil.	LT671381
Pica pica serica				
0418ser (Ppicser3)	AK-0418	cgrb250[3]	South Korea, Daedeongri, Whayang-myoen Socho	LT671382
0420ser	AK-0420	cgrb0192[3]	South Korea, Suncheon city	LT671383
0422ser	AK-0422	cgrb0226[3]	South Korea, Seoul Grand park, Gwacheon city	LT671384
0423ser (Ppicser1)	AK-0423	cgrb0244[3]	South Korea, Chuncheon city, Gangwon prov.	LT671385
0424ser	AK-0424	cgrb0245[3]	South Korea, Chuncheon city, Gangwon prov.	LT671386
1167ser	AK-1167	TESRI-509[4]	Taiwan	LT671387
1168ser	AK-1168	TESRI-599[4]	Taiwan	LT671388
1serKy	–	N/A	Japan, Kyushu Isl., Fukuoka, Asakura-gun	LT671389
2serKy	–	N/A	Japan, Kyushu Isl., Saga, Kratsu-shi, Hamatama-cho	LT671390
3serKy	–	NSMT-52070[5]	Japan, Kyushu Isl., Saga, Ogi-shi, Ashikari-cho	LT671391
4serKy	–	N/A	Japan, Kyushu Isl., Saga, Saga-shi, Suehiro	LT671392
5serKy	–	N/A	Japan, Kyushu Isl., Saga, Morotomi-cho, Tokutomi	LT671393
6serKy	–	NSMT-50657[5]	Japan, Kyushu Isl., Saga, Higashi-Yamashiro-cho	LT671394
7serKy	–	N/A	Japan, Kyushu Isl., Saga, Kanzaki-shi, Tamitigari	LT671395

8serKy	–	N/A	Japan, Kyushu Isl., Fukuoka, Yame-shi, Moto-mura	LT671396
9serKy	–	N/A	Japan, Kyushu Isl., Fukuoka, Kurume-shi, Zendouji	LT671397
10serKy	–	NSMT-51583[5]	Japan, Kyushu Isl., Fukuoka, Kashima-shi, Mori	LT671398
11serKy	–	NSMT-51746[5]	Japan, Kyushu Isl., Fukuoka, Ogoori-shi, Oho	LT671399
12serKy	–	NSMT-52194[5]	Japan, Kyushu Isl., Fukuoka, Kurume-shi, Araki-cho	LT671400
13serHo	–	YIO-65198[6]	Japan, Hokkaido Isl., Tomakomai-shi, Uenae	LT671401
14serHo	–	YIO-65206[6]	Japan, Hokkaido Isl., Tomakomai-shi, Numanohata	LT671402
15serHo	–	NSMT-52193[5]	Japan, Hokkaido Isl., Tomakomai-shi, Sumikawa-cho	LT671403
16serHo	–	NSMT-52205[5]	Japan, Hokkaido Isl., Tomakomai-shi, Takuyu-nishi	LT671404
17serHo	–	N/A	Japan, Hokkaido Isl., Tomakomai-shi, Shirakaba-cho	LT671405
18serHo	–	N/A	Japan, Hokkaido Isl., Tomakomai-shi, Shirakaba-cho	LT671406
19serHo	–	N/A	Japan, Hokkaido Isl., Tomakomai-shi, Yufutsu	LT671407
20serHo	–	N/A	Japan, Hokkaido Isl., Tomakomai-shi, Yufutsu	LT671408
21serHo	–	N/A	Japan, Hokkaido Isl., Tomakomai-shi, Yufutsu	LT671409
HQ915867	–	N/A	China (sequence downloaded from GenBank)	HQ915867
Pica pica hudsonia				
0514hud	AK-0514	SKY004[1]	USA, N. Dakota	LT671410
Pica pica mauritanica				
1908mau	AK-1908	TMA250[1]	W. Morocco, Agadir	LT671411

Note – Labcode: Besides labcodes of samples used in the present study, the labcodes for those specimens included in Haring et al. (2007) are listed in parentheses. Tiss. No. = collection number in the tissue collection of the Institute of Biology and Soil Science, Vladivostok, Russia. Museum No. = collection numbers in various museums: 1 – Zoological Museum Moscow State University, Russia; 2 – Oberösterreichisches Landesmuseum, Linz, Austria; 3 – Conservation Genome Resource Bank for Korean Wildlife, South Korea; 4 – Endemic Species Research Institute, Taiwan; 5 – National Museum of Nature and Science, Tokyo, Japan; 6 – Yamashina Institute for Ornithology, Japan. N/A = no voucher sample available or sample obtained from living bird. * presumably hybrids of *Pica pica pica* x *P. p. fennorum*, ** *P. p. fennorum* x *P. p. hemileucoptera*.

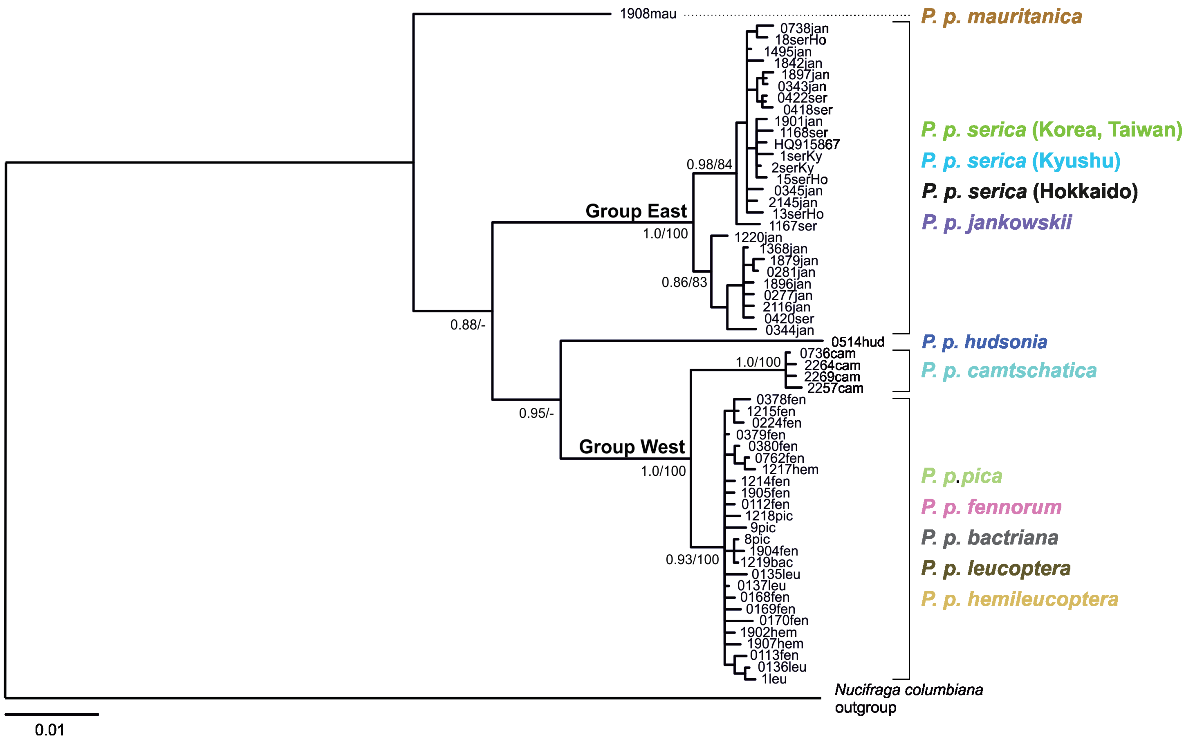

Fig. 2. BI tree for haplotypes of *Pica pica* mitochondrial control region sequences. Numbers at the branches indicate Bayesian posterior probability values as well as bootstrap values of the ML analysis. Distribution of subspecies in the tree can be traced by the labcodes containing the first three letters of subspecies. Ho = Hokkaido, Ky = Kyushu. For information on shared haplotypes see Supplementary Figure S1.

with the primer combinations CR-Cor16+/CR-Cor15– (primers designed for the present investigation) and CR-Cor+/Phe-Cor– (primers published first in Kryukov et al., 2004).

Molecular genetic analyses were performed in three labs: (1) IBSS (Institute of Biology and Soil Science, Russian Academy of Sciences; Vladivostok, Russia), (2) NSMT (National Museum of Nature and Science; Tsukuba, Japan), and (3) NHMW (Natural History Museum Vienna; Vienna, Austria). Details of PCR and sequencing conditions are presented in the Supplementary Text S1 online.

Data analysis

The sequences were assembled using the Staden 1.53 software package (Bonfield et al., 1995). The nucleotide sequences were aligned using the ClustalW program proposed in MEGA 6 (Tamura et al., 2013), and the alignments were edited manually in BioEdit ver. 6.0.7.1 (Hall, 1999). The resulting alignment was trimmed at the ends yielding a final length of 1326 bp (or 1318 bp without the outgroup sequence of *Nucifraga columbiana,* which was not used for network calculation). Phylogenetic trees were calculated with the Maximum Likelihood (ML) or Bayesian inference (BI) algorithm. ML was performed using MEGA 6, with the HKY+G substitution model determined as the optimal model under the BIC criterion. MEGA 6 was also used to calculate average p distances between and within populations. Bootstrap support was assessed with 1000 replicates (Felsenstein, 1985). BI analysis was carried out with MrBayes v.3.2.2 (Huelsenbeck et al., 2001; Ronquist et al., 2012). Applying the respective model parameters, the analyses were run for 6×10^6 generations each (two runs each with four chains, one of which was heated), sampling every hundredth tree. The first 25% of the trees were discarded as burnin and a 50% majority rule consensus tree was calculated from the remaining trees. Polymorphism parameters were computed with DnaSP ver. 5.10.01 (Librado and Rozas, 2009), and the same program was used to estimate neutrality tests such as Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) to construct mismatch distribution graphs (Rogers, 1995; Harpending, 1994) to analyze past demographic history. A Median Joining (MJ) network (Bandelt et al., 1999) was constructed with Network 4.612 (available at www.fluxus-engineering.com). To reduce the number of cycles in the network, we down-weighted nine rapidly mutating positions revealed by the software Network as recommended in the manual.

Bioacoustics

Recordings of the chatter call in several populations of Eurasian magpie and black-billed magpie (122 individuals from 14 subspecies, Supplementary Table S1 online) were obtained in the field by the authors (VA, AK and EL recorded 36 individuals from 7 subspecies), by other wildlife sound recordists, by online sound libraries such as www.xeno-canto.org, and from published commercial recordings (Peyton, 1999; Veprintsev et al., 2007). There is only one available record of *P. p. mauritanica* (Lachmann, www.xeno-canto.org/90790), so we could only illustrate it by a sonogram (Fig. 6) but did not include it into the analysis. Two recordings potentially belonging to *P. p. anderssoni* were analyzed together with the *P. p. serica* group because we did not find any differences in their chatters.

All digital audio recordings were transformed into mono wave files; the sampling frequency was set to 44.1 kHz with 16-bit using Adobe Audition 3.0. Sonograms were created in Syrinx version 2.6h (Burt, 2006). We used only those recordings of chattering that were appropriate for this analysis. Specifically, in our preliminary analysis, we used long recordings of the same bird (*P. p. fennorum*, Kholm, Novgorod Oblast, Supplementary Table S1 online) in different conditions and stimulation levels. This revealed that chattering series longer than six elements were more stable in sequence than shorter ones, whereby speed decreased toward the end of a longer series. We therefore discarded all shorter series and only used

Table 2. Mean distances between and within groups/lineages in %.

	P.p.W (+cam)	P.p.W	P.p.cam	P.p.E	P.p.mau	P.p.hud
P.p.W (+cam)	*0.8*	–	–	4.4	4.7	4.5
P.p.W	–	*0.2*	1.4	4.4	5.1	4.6
P.p.cam	–	1.6	*0.1*	5.2	5.0	5.3
P.p.E	5.1	4.9	5.5	*0.6*	5.1	5.6
P.p.mau	5.1	5.2	5.0	5.4	–	6.1
P.p.hud	4.9	4.8	5.4	5.9	6.1	–
outgroup	13.8	13.8	13.8	13.2	13.3	13.1

Note: p distances below diagonal, net distances above diagonal. Within group distances are given in italics. P.p.W(+cam): group West + *P. p. camtschatica*; P.p.W: group West; P.p.cam: *P. p. camtschatica*; P.p.E: group East; P.p.mau: *P. p. mauritanica;* P.p.hud: *P. p. hudsonia.*

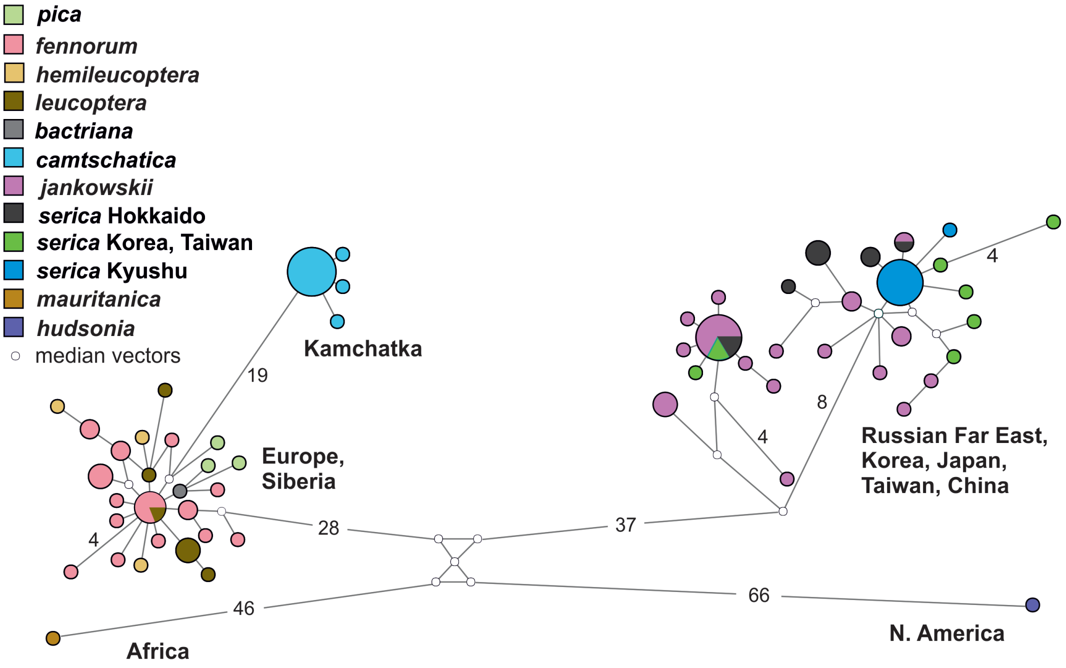

Fig. 3. Phylogenetic network constructed from complete mitochondrial Control Region with Median Joining algorithm. Size of circles corresponds to sample size. Numbers of mutations (> 4) are shown.

chatterings with six or more elements. Next, we measured the intervals between the first and the sixth elements in chattering and calculated the speed of chattering (number of elements per second). Each sample included only one or a part of the chattering call (series of six elements) produced by the same individual. We used ANOVA to test whether the set of samples from one subspecies differed significantly from those of other subspecies. In those cases where differences were found, we performed further analyses. Samples greatly varied in size, and the assumption of homogeneity of variances was violated. For the cases of unequal variances, the Games-Howell test was used (Games and Howell, 1976; Sokal and Rohlf, 2012) to examine differences in chattering speed between magpie populations.

Table 3. DNA diversity indices and neutrality tests.

Subspecies/ population	N	S	k	Pi ± SD	h	Hd ± SD	D	Fs
fennorum	22	20	2.918	0.00223±0.00028	14	0.948±0.029	–1.740 $P > 0.05$	–7.78*
leucoptera	7	7	2.762	0.00211±0.00061	5	0.857±0.137	–0.173 $P > 0.10$	–0.871
camtschatica	16	4	0.500	0.00038±0.00019	4	0.350±0.148	–1.831 $P < 0.05$*	–1.790
jankowskii	27	32	8.519	0.00653±0.00054	16	0.906±0.046	0.096 $P > 0.10$	–2.129
serica Kyushu	12	2	0.333	0.00026±0.00021	2	0.167±0.134	–1.451 $P > 0.10$	0.432
serica Hokkaido	9	17	7.444	0.00570±0.00128	5	0.861±0.087	0.926 $P > 0.10$	2.21
serica Korea,Taiwan	8	21	9.357	0.00718±0.0010	7	0.964±0.077	0.811 $P > 0.10$	–0.584
serica all	29	25	5.507	0.00422±0.00084	13	0.840±0.059	–0.483 $P >0.10$	–1.403

N: sample size; S: number of polymorphic sites; k: average number of nucleotide differences; Pi: nucleotide diversity; SD: standard deviation; h: number of haplotypes; Hd: haplotype diversity; D: Tajima's test statistics and its significance P; Fs: Fu's test statistics, * = significance at $P < 0.05$.

RESULTS

Sequence variation and phylogenetic reconstruction

The data set of complete CR sequences of *Pica pica* (110 sequences, 1318 bp) contained 167 (12.7%) variable sites, 100 of which were parsimony informative (gaps were not treated as the fifth character state). At the other 67 positions, singleton substitutions were observed. Some of the indels in the alignment were also phylogenetically informative; e.g., indels at pos. 1110–1114 and 1134–1136 unambiguously discriminate sequences of *P. p. jankowskii* and *P. p. serica* from all the others. Figure 2 shows the BI tree calculated from the alignment of haplotypes including the outgroup. (To show the positions of all sequences, the respective BI tree is provided in Supplementary Figure S1 online). It displays the previously reported intraspecies phylogenetic structure concerning a major division into two highly supported clades (designated "western" and "eastern" subclades in Haring et al., 2007). The western one (hereafter "group West") includes the European and Siberian subspecies: *P. p. pica, P. p. fennorum, P. p. bactriana, P. p. hemileucoptera* and *P. p. leucoptera* as well as *P. p. camtschatica*, which appears here as a distinct subclade. The eastern one comprises two Far Eastern subspecies (hereafter "group East"): *P. p. serica* and *P. p. jankowskii.* This latter clade is further subdivided into two subclades but without any geo-

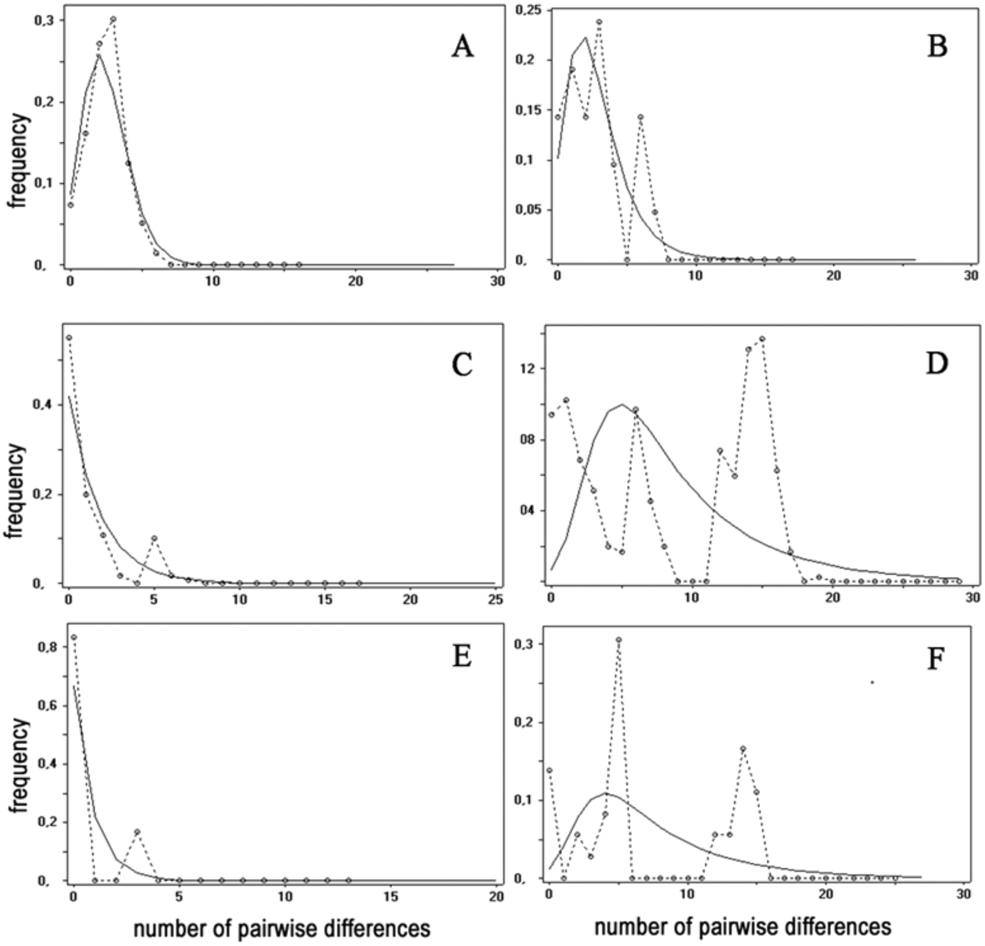

Fig. 4. Mismatch distribution of nucleotide differences between sequences within populations: A – *P. p. fennorum*, B – *P. p. leucoptera*, C – *P. p. camtschatica*, D– *P. p. jankowskii*, E – *P. p. serica* Kyushu Isl., F – *P. p. serica* Hokkaido Isl. Solid lines, expected distribution (under expectation of population growth); dashed lines, observed distributions.

graphic pattern (see below). The North American *P. p. hudsonia* forms a distinct lineage, which is the sister group of clade West. Moreover, *P. p. mauritanica* represents a highly divergent haplotype splitting at the basal node of this tree (Fig. 2). Yet, considering low branch support, the branching order of the four main lineages remains unresolved.

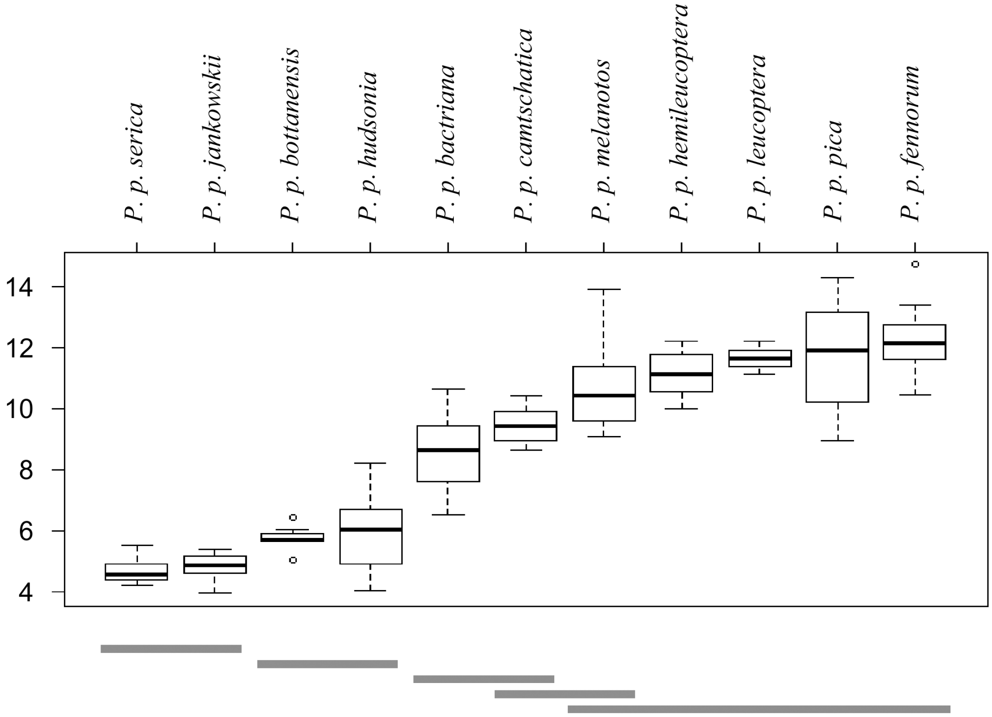

Fig. 5. Box plot diagram of the chatter calls speed of various magpie taxa. Y axis, elements per second: median, quantiles 25%, 75%, range and outliers. Horizontal lines below present combined samples without significant difference ($P < 0.05$).

The phylogenetic network illustrates the geographic distribution of haplotypes (Fig. 3). *Pica pica hudsonia* and *P. p. mauritanica* (two single sequences) are remote from the central unobserved haplotypes (median vectors) being separated by 66 and 46 steps, respectively. One major group represents the European and Siberian subspecies *P. p. pica*, *P. p. fennorum*, *P. p. bactriana, P. p. hemileucoptera* and *P. p. leucoptera* (i.e., clade West). *Pica pica camtschatica* is separated by at least 20 substitutions from that haplogroup. Within *P. p. camtschatica*, the haplotypes (sampled at two localities) differ only weakly from each other. Group East is separated from group West by at least 66 substitutions, including indels. Overall, this group is characterized by high nucleotide and haplotype diversity and is subdivided into two subgroups. Nonetheless, no clear geographic pattern is evident. With the exception of the homogeneous Kyushu population of *P. p. serica*, all populations (from the Far East mainland, Hokkaido and Taiwan) are distributed in both subgroups with no clustering within one of the two subgroups. Notably, even highly diverged haplotypes were found at the same location. Thus, three Korean samples belong to the smaller subgroup and two to the bigger one, with 18 to 19 mutations separating them.

Table 2 shows sequence divergence within and between the various genetic groups (clades/subclades in Fig. 2, and haplogroups in Fig. 3). Four lineages are more or less equidistant from each other: group West, group East, *P. p. hudsonia*, and *P. p. mauritanica* (Table 2). This is reflected in low support values at the node combining group East + group West + *P. p. hudsonia* (Fig. 2, Supplementary Figure S1). For comparison, the distances for group West were calculated in two ways: (1) including *P. p. camtschatica* (i.e., P.p.W(+cam)), (2) P. p.West (P.p.W) separately from *P. p. camtschatica* (P.p.cam). When considering *P. p. camtschatica* as part of group West, the within group mean distances are similar to those within group East.

Table 4. Mean values of the chatter call speed (elements per second) of various taxa of magpies.

Taxon	mean	sd	var	n
P. p. serica	4.68	0.45	0.20	7
P. p. jankowskii	4.84	0.40	0.16	12
P. p. bottanensis	5.73	0.42	0.17	7
P. p. hudsonia	5.98	1.20	1.44	17
P. p. bactriana	8.43	1.32	1.75	13
P. p. camtschatica	9.44	0.66	0.44	7
P. p. melanotos	10.66	1.39	1.92	14
P. p. hemileucoptera	11.13	0.84	0.70	7
P. p. leucoptera	11.64	0.42	0.18	6
P. p. pica	11.76	1.60	2.55	17
P. p. fennorum	12.05	1.10	1.20	14

Subspecies and population demography

Population parameters were calculated for *P. p. fennorum*, *P. p. leucoptera*, *P. p. camtschatica*, *P. p. jankowskii*, and *P. p. serica.* The isolated populations of *P. p. serica* (Kyushu and Hokkaido) were treated separately. Diversity parameters and the results of neutrality tests are summarized in Table 3. Because the values obtained for Tajima's D cannot exclude population expansion, we calculated the mismatch distribution of nucleotide substitutions (Fig. 4).

The only Tajima's D value significantly different from 0 ($P < 0.05$) was obtained for *P. p. camtschatica*. The negative D value in the neutrality test, together with small Fs values in *P. p. camtschatica* and *P. p. serica* from Kyushu, correspond to a left-side unimodal mismatch distribution pattern (Table 3, Fig. 4). This pattern reflects the low population diversity and suggests population growth after a bottleneck or founder effect. For *P. p. leucoptera*, *P. p. fennorum* and *P. p. serica* from Kyushu, Tajima's D was negative but not significantly different from 0; for *P. p. serica* of Korea and the Hokkaido population (according to the literature *P. p. serica*) it was positive. The Fs test yielded significant (negative) values for *P. p. fennorum* only, implying population growth in the more recent past (left-side unimodal mismatch curve in Fig. 4). For *P. p. jankowskii* and the Hokkaido populations, the graphs were polymodal and thus do not indicate population growth.

Bioacoustics – differentiation in chatter calls

We analyzed chattering calls for the 11 forms of magpie treated here as subspecies (Table 4). There is a pronounced difference between the European-Siberian subspecies group (*pica*, *melanotos*, *fennorum*, *leucoptera*, *hemileucoptera*, *bactriana*, *camtschatica*) and the southeastern subspecies (*serica*, *jankowskii*). The speeds of chatter calls of these two groups differ considerably without overlap. The mean values of the speed in the former group are 1.7–2.7 times higher than those in the latter (Table 4). *Pica pica bottanensis* and *P. p. hudsonia* represent a third, intermediate group (Fig. 5).

The bioacoustic results also show significant differences in the speed of chattering among the European-Siberian subspecies (group West). *Pica pica fennorum*, *P. p. pica*, *P. p. leucoptera*, and *P. p. hemileucoptera* are very similar, while the geographically isolated *P. p. camtschatica* and the somewhat isolated *P. p. bactriana* both have much slower chattering. *Pica pica melanotos* is positioned between these subgroups (Fig. 5)*. Pica pica hudsonia*, whose CR sequence is closer to group West, is more similar in its chattering speed to *P. p. serica* and *P. p. jankowskii*, which are subspecies of group East. The latter two show no significant difference in chattering speed, while the Himalayan form *P. p. bottanensis* (whose genetic association is still unknown) has significantly faster calls and differs by this parameter from *P. p. serica* and *P. p. jankowskii* but not from the American black-billed magpie *P. p. hudsonia*. Despite the similar mean chattering speed of these forms, however, they differ in the variability of this parameter (Table 4). The structure of the signals also differs between taxa and groups of taxa (Fig. 6). All European-Siberian magpies including *P. p. camtschatica* have quite simple and similar structures of sound signals. Our data clearly demonstrate that the well-known "magpie chattering" is characteristic for the European-Siberian subspecies including *P. p. melanotos*, *P. p. hemileucoptera*, *P. p. fennorum*, *P. p. pica*, *P. p. bactriana*, *P. p. leucoptera*, and *P. p. camtschatica.* By contrast, in the Far Eastern subspecies group as well as in *P. p. hudsonia* and *P. p. nutalli*, the sound units of chattering have complex structures and are characteristic for each form (Fig. 6). The single sonogram available of *P. p. mauritanica* also has a very peculiar signal shape, different from all the other taxa. The call speed of *P. p. mauritanica* may thus be even a bit faster than that of the European-Siberian subspecies (group West) (13.9 elements per second). Unfortunately, this single sample could not be included in the ANOVA.

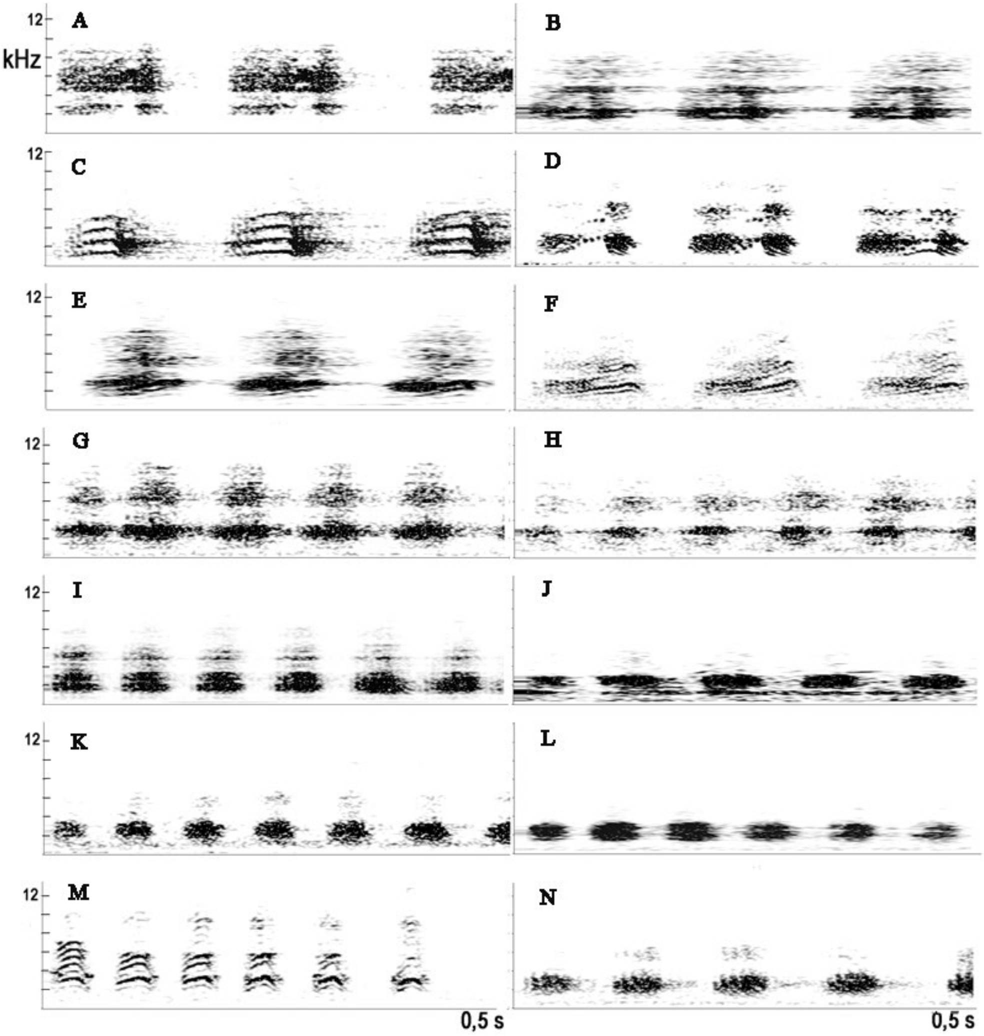

Fig. 6. Sonograms of the chatter calls. A – *P. p. serica*, B – *P. p. jankowskii*, C – *P. p. bottaneneis*, D – *P. p. anderssoni*, E – *P. p. hudsonia*, F – *P. p. nutalli*, G – *P. p. melanotos*, H – *P. p. hemileucoptera*, I – *P. p. leucoptera*, J – *P. p. bactriana*, K – *P. p. pica*, L – *P. p. fennorum*, M – *P. p. mauritanica*, N – *P. p. camtschatica.*

DISCUSSION

Deep splits of mitochondrial lineages and bioacoustic differentiation

Based on mitochondrial CR sequences of representative material from ten subspecies of *Pica pica* we demonstrate deep genetic divergences within the species. In addition to the groups "East" and "West" already known from earlier studies (Haring et al., 2007), *P. p. mauritanica* represents another distinct lineage highly differentiated from the other lineages (4.7–5.1% net distances and 5.0–5.4% mean p distances, respectively, Table 2). Likewise, *P. p. hudsonia* represents a separate lineage which is the highly supported sister group of the Western clade. Although these results are preliminary, because only one sample was available for each of *P. p. mauritanica* and *P. p. hudsonia*, the sequence divergences indicate that these four lineages clearly correspond to groups of subspecies or geographically isolated subspecies that have evolved in the remote past.

Our bioacoustic analysis, although it did not cover all taxa, provides the first broad overview of geographic variation in the chattering of *P. pica*. The results, agreeing with the genetic data, disclosed the distinctness of the subspecies of the southern Far East from the remaining ones in terms of chatter call speed (Fig. 5, Table 4). Likewise, the homogeneity of chatter call speed seen in the members of the European-Siberian subspecies (except of the slightly different *P. p. camtschatica* and *P. p. bactriana*) is in good accord with those from the genetic analysis. Though just a single chattering record is available for *P. p. mauritanica*, its sonogram pattern is found to be peculiar, differing from all other taxa of magpies investigated, while its chattering speed is similar to group West (Fig. 6). *Pica pica hudsonia* and *P. p. nutalli* are similar to each other in their vocalization (see sonograms, Fig. 6), which is in accord with the few genetic data available so far (Lee at al., 2003; as well as unpublished data from GenBank). Interestingly, they are more similar to the subspecies of the southern Far East (group East), which is in contrast to the genetic affiliation of *P. p. hudsonia* as revealed by the mitochondrial sequences. *Pica pica hudsonia* has two kinds of calls: basic chatter and staccato chatter (Buitron, 1983), both varying in length and intensity but having the same interval between calls (Trost, 1999). *Pica pica bottanensis*, in turn, uses only one type of chattering. Of course, the available bioacoustic data were not expected to sufficiently reconstruct the phylogenetic relationships of *P. pica;* still, the sonograms largely reflect the genetic groups revealed by the mitochondrial marker sequence. In summary, the good agreement of sonograms and divergent mitochondrial lineages underlines the importance of these characters for taxonomic questions. As soon as more comprehensive bioacoustic data are gathered, they may be also used in phylogenetic reconstructions.

A phylogenetic subdivision into two genetic clades was reported for several Palearctic bird species, including corvid genera, such as *Cyanopica* (Fok et al., 2002; Kryukov et al., 2004), *Corvus* (Kryukov and Suzuki, 2000), and *Pica* (Haring et al., 2007). Alternating episodes of shrinking and expansion of distribution ranges during the Pleistocene climatic oscillations may have played a major role in the divergence of lineages. Haring et al. (2007) assumed that similar habitat requirements of these taxa shaped their phylogeographic pattern (i.e., two distinct groups), since the number and location of glacial refugia is highly dependent on such specific ecological factors. Interestingly, a high genetic variation and differentiation of populations from the southern Far East was reported for several species with wide Palearctic ranges, including birds, mammals, fishes and amphibians (Kryukov, 2010). This may indicate that this region represented a major biodiversity refuge throughout the Pleistocene.

Diversification of *Pica pica*

The phylogenetic relationships among the four lineages are not clearly resolved with this genetic marker and thus the order of splits is still unclear. Given the very similar distances between the lineages, it might turn out that this is a hard polytomy, i.e., they separated more or less at the same time. Future analyses based on additional (nuclear and mitochondrial) sequences will address this issue.

Nazarenko (1982) hypothesized the diversification of *P. pica* in the Holocene in connection with ancient agricultural regions, e.g., in South China and Mesopotamia. Yet, the deep divergences reported here between the two major mitochondrial clades (West and East) and the other two lineages detected clearly disprove the assumption of an anthropogenic factor behind the initial diversification within *P. pica* which must have started much earlier.

Although a molecular clock for the splits within *Pica* cannot be reasonably calibrated, an approximate temporal assignment can be made based on two facts: (1) the divergence rates of *cytb* and the CR were shown to be in the same range for *P. pica* (Kryukov et al., 2004), and (2) the divergence rate for *cytb* of approximately 2.1% per million years (± 0.1%, 95% confidence interval) was maintained over a 12-million-year interval and across a wide range of avian orders (Weir and Schluter, 2008). Based on these rates, the split between the main lineages within *Pica pica* (5.2–5.7%) can be estimated to have occurred in the late Pliocene. Accordingly, the spread of open habitats (grasslands or savannas) during the Pliocene climate change could have favored the spread of the ancestral *P. pica*, while the divergence of the main lineages could be connected with the Pliocene-Pleistocene climate transition.

Phylogeographic patterns, Pleistocene refugia and recent migration scenarios of *Pica pica*

Clearly, the current distribution of mitochondrial haplotypes must be interpreted in terms of population dynamics and range shifts. The existence of two haplogroups within group East (comprising the subspecies *P. p. jankowskii* and *P. p. serica*), as shown by our investigations, may indicate two glacial refuge areas in which populations or subspecies diverged. Two (or three) Pleistocene refuges, e.g., in the Korean Peninsula and in the South Russian Far East and Manchuria, were proposed for several other taxa, e.g., for chipmunk (Lee et al., 2008), raccoon dog (Kim et al., 2013), and field mice (Serizawa et al., 2002). The high intrapopulation haplotype and nucleotide variation (except the Kyushu population) and the pattern of haplotype distribution does not contradict divergence within two refugial areas, but rather indicates recent admixture of two formerly geographically separated haplogroups (Fig. 3). A similar result was reported for the Eastern Carrion Crow *Corvus corone orientalis* and the Collared Crow *Corvus pectoralis*. The latter species was embedded within the *C. c. orientals* clade (Haring et al., 2012). This might be explained by mtDNA capture by *C. c. orientalis* from *C. pectoralis* after admixture of formerly separated populations.

Under the latter assumption, migration and population mixture in the Holocene led to the present high intrapopulation diversity observed for the analyzed mitochondrial marker. Zhang et al. (2012) did not find any genetic structure when comparing populations of *P. p. serica* in East China using mtDNA (*ND2* and CR) and two nuclear genes. The authors explain this by recolonization of East China from a single refuge and current abundant gene flow among populations. Unfortunately, the findings of the two studies cannot be combined in a meaningful way, as no locality details are available for the sequences of Zhang et al. (2012) in GenBank. Furthermore, there is no overlap between sampling areas, and *P. p. jankowskii* was not included in that study. The geographically adjacent subspecies *P. p. anderssoni* and *P. p. bottanensis* remain to be investigated genetically to determine whether they also represent distinct haplogroups. Judging by the speed of chatter calls, they fit well with *P. p. serica* and *P. p. jankowskii*, albeit the frequency patterns are slightly different.

The two isolated populations of Kyushu and Hokkaido are positioned within clade East. Our comparison of these two populations pointed to very dissimilar demographic histories. It was well documented that the Kyushu population was established by introduction of the species from Korea in the late 16th century (Eguchi and Kubo, 1992). Only several pairs were introduced (Eguchi, 2016). That population was protected and had a restricted range for a long time, but over the last 30 years began expanding into the northern part of the island (Eguchi, 2016). The nucleotide and haplotype diversity in Kyushu is very low: only two haplotypes were found (Fig. 3, Table 3). The graph of mismatch distribution has a left-side peak (Fig. 4) implying population growth. In summary, the genetic findings agree with historical documentation, i.e., a founder effect in the recent past.

In contrast, the Hokkaido population yielded unexpected results. Considering that the founders of the present population are thought to have appeared in Hokkaido in the 1980s (Horimoto, 2004; Kurosawa and Horimoto, 2015), one would expect a quite homogeneous population structure also due to the founder effect. Surprisingly, nucleotide and haplotype diversity were quite high (almost as high as in *P. p. jankowskii*), which suggests colonization by a larger number of genetically different founders (Fig. 3). Positive values of both Tajima's D and Fu's Fs tests, as well as the mismatch distribution (Table 3, Fig. 4), do not support an assumption of population growth, but also do not contradict the assumption of a stable population; they merely reflect admixture of different haplotypes. Taking into account the high mtDNA diversity of magpies in Hokkaido, it was probably colonized by many founders of both *P. p. serica* and/or *P. p. jankowskii*, and this process may still be going on. Yet, lacking any data on the morphology of the Hokkaido population, this assumption is so far based on genetic data only.

After the first observation of a single magpie in Muroran, South-West Hokkaido, in 1984, breeding has been documented since 1993 (Horimoto, 2004). Details of this colonization are still ambiguous. The local magpie population near Tomakomai port increased to ca 200 pairs during the last 10 years (Hasegawa et al., unpublished). The only plausible route for colonizing Hokkaido is across the Japanese Sea, because there are no records of nesting magpies for Sakhalin Island or for Honshu (only rare visitors were observed there). As *P. pica* is a not strong flier, it might have crossed the sea on ships. In Zarubino port in South Primorye, magpies use depots and ships in the port for roosting (O. Burkovsky, personal communication). Occasionally, birds stay on board when a ship departs, e.g. at night. In the 1980–90s, the shipping traffic between several ports in the South Russian Far East and Hokkaido was high, potentially leading to the invasion of Japan by magpies. Ten magpies are reported to have flown out from the inside of a Russian cargo vessel at a port in Niigata prefecture (western coast of Japan). For about 10 years they inhabited the locality and then disappeared (Washizawa, 2008). A similar ship-assisted scenario was also proposed for magpie transportation from Europe to the eastern coast of North America (Ebels, 2003). Such “hitchhiking” on ships is known for many birds, for example, the Indian crow *Corvus splendens*, which has established populations in the Middle East and Africa, reaching even Europe, America, and Australia (Ryall, 2016). In summary, based on the high mtDNA diversity of magpies in Hokkaido, not a few founder individuals have to be assumed, and the colonization might still be ongoing.

An interesting question is the extent to which the representatives of the West and East groups come into contact on the continent. Reports on the occurrences of *P. pica* in its eastern Palearctic range—the earliest ones date back to the 19th century—show a continuous expansion of distribution ranges, mostly in connection with human settlements. Stegmann (1932) stated that there were no magpies at all along the lower Shilka and upper Amur rivers, in Yakutia and the Khabarovsk territory, down to the mouth of the Amur in the south. The two subspecies were isolated by a gap of over 600 km in southern Siberia even 60 years ago (Rustamov, 1954; Stepanyan, 2003), but the gap has been decreasing year by year. This dynamic expansion of marginal populations of *P. p. leucoptera* to the east and *P. p. jankowskii* to the west in southern Siberia continues today (Fig. 7; detailed description by Goroshko, in prep.). In eastern Mongolia (Tugarinov, 1932) and northern China (Cheng, 1987) they might already have been in contact in the early 20th century, or even earlier. This is supported by study skins of *P. p. jankowskii* from eastern Mongolia kept in the museum of the Zoological Institute of St. Petersburg (Russia). Nonetheless, comprehensive data on the recent situation is lacking, and we are currently investigating this issue. Parapatry or population admixture may occur in the basins of the lower Kerulen and Halkin-Gol Rivers and, somewhat less likely, along Argun' and Shilka Rivers (Fig. 7). Despite very low population densities, however, ongoing sporadic contact appears to be taking place. Preliminary field surveys indicate that there might be some hybridization. Yet, frequent observations of unfertilized eggs in this area suggest that reproductive barriers are separating the two taxa (unpublished data). Notably, our observations suggest that *P. p. jankowskii* is spreading to the west along the Amur

Fig. 7. Dynamics of the ranges of *P. p. leucoptera* and *P. p. jankowskii* in the upper Amur basin within the administrative regions Zabaykalsky Krai and Amur Oblast (including distribution of non-breeding birds).

River basin much faster than *P. p. leucoptera* is spreading in the opposite direction.

The observed high mobility and expansion capacity of *P. pica* has probably also influenced the distribution and phylogeographic patterns in the past. The low nucleotide variation and high haplotype diversity in the subspecies *P. p. fennorum*, *P. p. pica*, *P. p. bactriana*, *P. p. hemileucoptera*, and *P. p. leucoptera* (forming the western main clade) can be explained by assuming a single refuge and, according to the mismatch distribution pattern, a fast post glacial population expansion. This has already been proposed by Haring et al. (2007). In that study, the distant position of *P. p. camtschatica* was not observed, which was due to a much shorter and more conserved analyzed section of the CR. Based on the network presented here, a separate isolated glacial refuge for *P. p. camtschatica* is plausible.

Given the phylogenetic affinity of group West (including the Kamchatka lineage) to its sister group *P. p. hudsonia* (Fig. 2), past expansions from Chukotka to Alaska and further into North America can be assumed. Such an early expansion could have led to a split of the Nearctic lineage (*P. p. hudsonia* and *P. p. nutalli).* The divergence of *P. p. camtschatica*, however, has started later: it might have settled in Kamchatka before the last glaciation or even during the Pleistocene. In the Last Glacial Maximum, more than 40% of the Kamchatka territory was covered by ice; trees and bushes existed most probably in the Central Kamchatka depression (Melekescev et al., 1974), where magpie could have survived in appropriate conditions. The post-glacial spread along the Peninsula to the north and to adjacent continental parts might have been related to human activities (the oldest reported upper-Paleolithic settlement in Kamchatka, Ushkovskaya, is dated as 10.6–14.3 thousand years ago; Dikov, 1979). Unlike magpies of many other cities throughout the species' range, *P. p. camtschatica* does not nest within the cities Petropavlovsk-Kamchatsky and Elisovo, except in parks. It prefers city suburbs, country sites, and forest lines along roads (Konstantinov et al., 2004). This may imply a type of initial stage of adaptation of a low density population to anthropogenic landscapes. This is common in other parts of the range where adaptation was accompanied by strongly increasing population size, e.g., in Vladivostok city after the 1970s (Nazarov, 2004). The low genetic diversity within the Kamchatka lineage (Table 3, Figs. 3, 4) implies a single invasion by a few founder individuals, and/or a severe bottleneck. Such a scenario is common for Kamchatka birds due to the island-like geographic status. The colonization of Kamchatka by new invaders is currently ongoing, as has been shown for several migrating bird species (Lobkov, 2016). Thus, it remains to be investigated whether the haplotype diversity in *P. p. camtschatica* has increased over the past years.

Taxonomic notes

To what extent do the available data agree with subspecific differentiation? The western subspecies *P. p. fennorum*, *P. p. pica*, *P. p. bactriana*, *P. p. hemileucoptera*, and *P. p. leucoptera* (as well as *P. p. melanotos*, according to Haring et al., 2007) are almost the same in the mitochondrial CR. This is in accordance with their low morphological differentiation and slight differences which we observed in their sonogram spectrums. In contrast, the isolated North African *P. p. mauritanica* exhibits high genetic divergence from all other subspecies. Ebels (2003) considered it as distinct species based on its short wings and small overall size, rather long tail, absence of white coloration on the rump, and some blue skin below the eye. However, these are features it shares to some extent with *P. p. melanotos* samples from Spain (Madge and Burn, 1999). In any case, based on the single specimen analyzed genetically so far, any discussion on species rank of *mauritanica* is premature.

Kamchatka magpies, *P. p. camtschatica*, represent a distinct lineage in the mitochondrial network. They are also distinct based on their shorter tail, wider white coloration on primaries and rump, and more pronounced greenish color of secondaries and wing covers. Both European and Kamchatka's magpies belong to the group of subspecies with green wings, in contrast to the blue wings in southeastern subspecies. Depigmentation of plumage is a general characteristic feature of the subspecies endemic to Kamchatka which are mainly resident forest birds (Lobkov, 1999). A closer relationship of Kamchatka magpies to the western group is supported by their calls, which resemble those of European rather than southeastern magpies (Figs. 5, 6). This Kamchatka form should be studied in more detail, and the question of the taxonomic rank of magpies inhabiting Kamchatka remains to be answered.

The genetic marker used in this study does not differentiate between *P. p. serica* and *P. p. jankowskii*: the two subspecies are intermixed among the two mitochondrial subclades. Nevertheless, consistent morphological differences as well as dissimilarity in the frequency spectra of their chatter calls support their subspecific status. Magpies inhabiting the Korean Peninsula, most of south-eastern China, Myanmar, Vietnam, Laos and Taiwan and partly Japan are very similar; they differ from the northern *P. p. jankowskii* by a shorter wing and tail, but longer bill and tarsus (48.7 mm in males *jankowskii* in average and 50.2 mm in *serica*). These morphological differences clearly remain stable despite the presumed gene flow due to range expansions after the Pleistocene. Thus, we do not support uniting *P. p. serica* and *P. p. jankowskii* as has been suggested by other authors (Vaurie, 1959; Goodwin, 1986; Dickinson and Christidis, 2014) at this stage. Thorough comparative studies of nuclear markers will be necessary to test the coherence of these taxa.

The Kyushu population is traditionally designated *P. p. serica* (Ornithological Society of Japan, 2012) which is in accordance with mitochondrial data and the above-mentioned historical records. However, we cannot conclude whether the Hokkaido population is *P. p. serica*, *P. p. jankowskii* or admixed. The finding that the Hokkaido population most probably originated from founders of both subspecies highlights the limitation of the subspecies category for such populations.

Certain other subspecies that have not been included in the genetic analyses are also somewhat differentiated in their chatter calls, which is particularly obvious in sonogram shapes of, e.g., *P. p. bottaneneis* and *P. p. anderssoni*. *Pica pica bottanensis* is of special interest, because apparently it does not belong to the *serica* group: it has a much larger body size and some distinct features of the chattering call described above. Yet, its intergradation with *serica* in east Qinghai and west Sichuan supposed by Vaurie (1959) was not confirmed by Cheng (1987) who reported a prolonged but parapatric contact zone. Moreover, the absence of any intermediates in our recently surveyed museum collections suggests that the two taxa do not interbreed. Observations of the current situation in the contact zone *serica*/*bottanensis* as well as genetic data are needed to make any further considerations about the status of *bottanensis*. We were unable to include vocalizations of *P. p. asirensis*, the isolated Arabian subspecies, whose coloration is the darkest of all and whose bill is large. Also its distinctive vocal difference briefly described by Yahya and Salamah (1996) suggests treating this form as a separate species (Madge, 2016b).

Altogether, in our view, there are good arguments for keeping (at least) the subspecific status of all subspecies analysed so far based on morphological, bioacoustic and/or genetic differentiation. Regarding taxonomic rank, the position of *P. p. hudsonia* as a sister group to the western clade (including *P. p. camtschatica*) is well supported and is in accordance with Lee at al. (2003). As noted earlier, treating it as a distinct species as suggested by several authors (e.g., Dickinson and Christidis, 2014; Madge, 2016a) would render *P. pica* paraphyletic; the same is true for *P. p. nutalli* (*P. nuttalli* in the above publications), which is the sister group of *P. p. hudsonia* (Lee at al., 2003).

Splitting *Pica pica* into several species would eliminate this paraphyly and could thus be a formally suitable solution. Indeed, there are four allopatric lineages that proved to be distinct in morphology, mtDNA and bioacoustics: clade West, clade East, *mauritanica*, and *hudsonia*. First hints on reproductive isolation between the two clades (West/East) can be considered as a further argument in the frame of the biological species concept. Thus, dividing *Pica* into the species *P. pica*, *P. serica*, *P. mauritanica*, and *P. hudsonia* would appear as a sound taxonomic solution. *Pica pica* and *P. serica*, in turn, would be subdivided into several subspecies. For *P. pica,* these comprise at least the subspecies *pica*, *melanotos*, *fennorum*, *bactriana*, *hemileucoptera*, *leucoptera* and *camtschatica*, and for *P. serica*, the subspecies *serica* and *jankowskii*. Nevertheless, we hesitate to formally propose these taxonomic changes at this stage, before the assignment or relationships of the remaining Palearctic subspecies is investigated. Concerning *nutalli*, all genetic data published so far do not distinguish it from *hudsonia*. Therefore, it should provisionally be classified as a subspecies. Importantly, obtaining a comprehensive picture of *P. pica* taxonomy will require detailed investigations in potential hybrid zones. In particular, the poorly studied taxa *P. p. mauritanica*, *P. p. bottanensis*, *P. p. asirensis*, and *P. p. anderssoni* remain to be thoroughly investigated genetically by applying mitochondrial as well as nuclear markers.

ACKNOWLEDGMENTS

We are grateful to the following colleagues for providing tissue samples: S. Akulinkin, K. Eguchi, E. Fedotov, P. Fisenko, M. Fujioka, A. Gamauf, D. Gluhov, G. Gorchakov, V. Grichik, V. Il'ashenko, D. Karpov, J.T. Kim, Y.J. Kim, H. Murayama, H. Park, F.C. Rohwer, M. Romanov, F. Sadeghi, M. Sementsova, A. Shestakov, V. Sotnikov, N. Teufelbauer, A. Tsvetkov, S. Weigl, C.-te Yao. We are thankful to the staff of the following institutions for providing samples: Zoological Museum of Moscow State University, Russia; Institute of Biology and Soil Science, Vladivostok, Russia; Conservation Genome Resource Bank for Korean Wildlife, South Korea; Endemic Species Research Institute, Taiwan; National Museum of Nature and Science, Tsukuba, Japan; Yamashina Institute for Ornithology, Japan; Oberösterreichisches Landesmuseum, Linz, Austria. We are grateful to Sang-im Lee for informing us about measurements of *P. p. serica*. We also thank Wilhelm Pinsker for critical comments on the manuscript. The work was supported by the Russian Foundation for Basic Research, project #16-04-01304. The work of S. Mori was supported by JSPS KAKENHI, project #JP26440232. The work of Ya. Red'kin was supported by Russian Science Foundation, project #14-50-00029.

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

YR, EL, SM, AK and EH collected samples, LS, EH and SM performed PCR and sequencing, VA and AK made sound records and VA developed the bioacoustic data, YR and OG contributed to biogeographic interpretation and drew the maps, AK and EH conducted the molecular phylogenetic analyses and wrote the paper. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online (URL: http://www.bioone.org/doi/suppl/10.2108/zs160119).

Supplementary Text S1. Details of PCR and sequencing conditions.

Supplementary Figure S1. BI tree of all mitochondrial CR sequences.

Supplementary Table S1. Geographic forms, recording localities, recordists and sources of sounds reviewed.

REFERENCES

Alström P, Ranft R (2003) The use of sounds in avian systematics and the importance of bird sound archives. Bull British Ornith Club 123A: 114–135

Avise JC (2000) Phylogeography. The history and formation of species. Harvard Univ Press, Boston, MA

Avise JC, Walker D (1998) Pleistocene phylogeographic effects on avian populations and speciation process. Proc R Soc Lond B 265: 457–463

Babenko VG (2000) Birds of low Amur River basin. Prometey, Moscow (in Russian)

Bandelt H-J, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16: 37–48

Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Research 24: 4992–4999

Buitron D (1983) Variability in the responses of Black-billed Magpies to natural predators. Behaviour 87: 209–236

Burt J (2006) Syrinx, Version 2.6h. University of Washington, Seattle, USA. http://SyrinxPC.com/. Accessed November 2015

Cheng Tso-hsin (1987) A synopsis of the Avifauna of China. Beijing

Choe JC (2000) A long-term phylogeographic study of magpies (genus *Pica*): behavior, ecology and evolution. Ecosystem Research and Sustainable Management. Proc. Intern. LTER Joint Seminar between Korea and China. Kookmin Univ, Seoul, pp 279–293

Dickinson EC (ed) (2003) The Howard & Moore complete checklist of the birds of the World. 3rd ed, C Helm, London

Dickinson EC, Christidis L (eds) (2014) The Howard & Moore complete checklist of the birds of the World. 4th ed, vol 2. Aves Press, Eastbourne, UK

Dikov NN (1979) Ancient cultures of North-East Asia (Asia in junction with America in ancient times. Nauka, Moscow (in Russian)

Ebels EB (2003) Trends in systematics. Speciation in *Pica* magpies. Dutch Birding 25: 103–116

Eguchi K (2016) The Eurasian Magpie. Jpn J Ornithol 65: 5–30

Eguchi K, Kubo H (1992) The Origin of the Magpie *Pica pica sericea* in Japan – an investigation of ristorical records. J Yamashina Inst Ornithol 24: 32–39 (in Japanese with English abstract)

Enggist-Dublin P, Birkhead TR (1992) Differences in the calls of European and North American Black-billed Magpies and the Yellow-billed Magpie. Bioacoustics 4: 185–194

Eo SH, Hyun JO, Lee W-S, Choi TB, Rhim S-J, Eguchi K (2002) Effects of topography on dispersal of black-billed magpie *Pica pica sericea* revealed by population genetic analysis. J Ethol 20: 43–47

Ericson PGP, Christidis L, Cooper A, Irestedt M, Jackson J, Johansson US, Norman LA (2002) A Gondwanan origin of the passerine birds supported by DNA sequences of the endemic New Zealand wrens. Proc R Soc B 269: 435–441

Ericson PGP, Jansen A-L, Johansson US, Ekman J (2005) Intergeneric relationships of crows, jays, magpies and allied groups (Aves, Corvidae) based on nucleotide sequence data. J Avian Biology 36: 222–234

Fefelov IV (2014) Nesting of common magpie in settlements in Irkutsk Province. Amurian Zool J 6: 447–449 (in Russian)

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791

Flint VE, Beme RL, Kostin YuV, Kuznetsov AA (1968) Birds of the USSR. Mysl', Moscow (in Russian)

Fok KW, Wade CM, Parkin DT (2002) Inferring the phylogeny of disjunct populations of the azure-winged magpie *Cyanopica cyanus* from mitochondrial control region sequences. Proc Royal Soc Lond B, Biol Sci 269: 1671–1679

Fu Y-X (1997) Statistical test of neutrality against population growth, hitchhiking and background selection. Genetics 147: 915–925

Games PA, Howell JF (1976) Pairwise multiple comparison procedures with unequal N's and/or variances: a Monte Carlo study. J Educational Statistics 1: 113–125

Goodwin D (1986) Crows of the world. Seattle, WA

Hamao S (2013) Acoustic structure of songs in island populations of the Japanese bush warbler, *Cettia diphone*, in relation to sexual selection. J Ethology 31: 9–15

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuclear Acids Symp 41: 95–98

Haring E, Gamauf A, Kryukov A (2007) Phylogeographic patterns in widespread corvid birds. Mol Phylogenet Evol 45: 840–862

Haring E, Däubl B, Pinsker W, Kryukov A, Gamauf A (2012) Genetic divergences and intraspecific variation in corvids of the genus *Corvus* (Aves: Passeriformes: Corvidae) – a first survey based on museum specimens. J Zool Syst Evol Research 50: 230–246

Harpending HC (1994) Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Human Biol 66: 591–600

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405: 907–913

Horimoto T (2004) Records of magpie *Pica pica* in Iburi district, South-western Hokkaido. J Yamashina Inst Ornithol 36: 87–90 (in Japanese with English abstract)

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314

Kim S-I, Park S-K, Lee H, Oshida T, Kimura J, Kim Y-J, et al. (2013) Phylogeography of Korean raccoon dogs: implications of peripheral isolation of a forest mammal in East Asia. J Zoology 290: 225–235

Konstantinov VM, Rodimtsev AS, Ponomarev VA, Klimov SM, Margolin VA, Lebedev IG (2004) Magpie (*Pica pica* L.) by anthropogenic landscapes of Palearctic (problems of sinantropization and urbanization). Prometey Press, Moscow (in Russian)

Kryukov AP (2010) Comparative phylogeographic patterns of several vertebrates in the East Palearctic. Moscow Univ Biol Sci Bull 65: 184–186

Kryukov AP, Suzuki H (2000) Phylogeography of carrion, hooded and jungle crows (Aves, Corvidae) inferred from partial sequencing of the mitochondrial *cytochrome b* gene. Russian J Genetics 36: 922–929

Kryukov A, Iwasa MA, Kakizawa R, Suzuki H, Pinsker W, Haring E (2004) Synchronic east-west divergence in azure-winged magpies (*Cyanopica cyanus*) and magpies (*Pica pica*). J Zool Syst Evol Research 42: 342–351

Kurosawa R, Horimoto T (2015) Changes of distribution of the Eurasian magpie *Pica pica* in the Iburi region of Southern Hokkaido. J Yamashina Inst Ornithol 46: 83–88 (in Japanese with English abstract)

Lee MY, Lissovsky AA, Park SK, Obolenskaya EV, Dokuchaev NE, Zhang YP, et al. (2008) Mitochondrial cytochrome *b* sequence variation and population structure of Siberian chipmunk (*Tamias sibiricus*) in Northeastern Asia and population subdivision in South Korea. Mol Cells 26: 566–575

Lee S, Parr CS, Hwang Y, Mindell DP, Choe JC (2003) Phylogeny of magpies (genus *Pica*) inferred from mtDNA data. Mol Phylogenet Evol 29: 250–257

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452

Lobkov EG (1999) Kamchatka as a local center of formation in birds. In “Bird Biology and Protection in Kamchatka, Is 1” Dialog-MSU Press, Moscow, pp 5–23 (in Russian)

Lobkov EG (2016) Birds – recent invaders to Kamchatka: the population’s fate. Russ J Ornithol 25: 1281–1294 (in Russian)

Madge S (2016a) Common Magpie (*Pica pica*). In “Handbook of the Birds of the World Alive” Ed by del Hoyo J, Elliott A, Sargatal J, Christie DA, de Juana E. Lynx Edicions, Barcelona (retrieved from http://www.hbw.com/node/60753 on 21 September 2016)

Madge S (2016b) Arabian Magpie (*Pica asirensis*). In “Handbook of the Birds of the World Alive” Ed by del Hoyo J, Elliott A, Sargatal J, Christie DA, de Juana E. Lynx Edicions, Barcelona (retrieved from http://www.hbw.com/node/60753 on 21 September 2016)

Madge S, Burn H (1999) Crows and Jays: A Guide to the Crows, Jays and Magpies of the World. C Helm, London

Mayr E (1958) Behavior and systematics. In “Behavior and Evolution”. Ed by A Roe, GA Simpson, Yale Univ Press, New Haven, pp 341–362

Melekescev IV, Braitseva OA, Erlikh EN, Shantser AE, Chnelebaeva AI, Lupikina EG, et al. (1974) Kamchatka, Kurils and Komandor Islands. History of landscape development in Siberia and Far East. Nauka Press, Moscow (in Russian)

Nazarenko AA (1982) On faunistic cycles (extinction – expansion – extinction…) with special reference to the East Palearctic dendrophilous avifauna. J Obshei Biologii 43: 823–835 (in Russian)

Nazarenko AA, Gamova TV, Nechaev VA, Surmach SG, Kurdyukov AB (2016) Handbook on the Birds of Southwest Ussuriland: Current Taxonomy, Species Status and Population Trends. National Institute of Biological Resources, Inchon, Korea

Nazarov YuN (2004) Birds of Vladivostok and suburbs. Far Eastern Univ Press, Vladivostok (in Russian)

Ornithological Society of Japan (2012) Checklist of Japanese Birds. 7th revised ed. Ornithological Society of Japan, Sanda (in Japanese)

Panov EN (1989) Natural hybridisation and ethological isolation in birds. Nauka, Moscow (in Russian)

Peyton LJ (1999) Bird Songs of Alaska. CD. Library of Natural Sounds: Cornell Laboratory of Ornithology

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. Evolution 49: 608–615

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. (2012) MRBAYES 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Syst Biol 61: 539–542

Rustamov AK (1954) Family Corvidae. In “Birds of Soviet Union, vol 5” Ed by Dementiev GP, Gladkov NA. Sovetskaya Nauka, Moscow, pp 13–104 (in Russian)

Ryall C (2016) Further records and updated of ranges expansion of House Crow *Corvus splendens*. Bull B O C 136: 39–45

Serizawa K, Suzuki H, Iwasa M, Tsuchiya K, Pavlenko MV, Kartavtseva IV, et al. (2002) A spatial aspect of mitochondrial DNA genealogy in *Apodemus peninsulae* from East Asia. Biochem Genet 40: 149–161

Sibley CG, Ahlquist JE (1990) Phylogeny and Classification of Birds. Yale University Press, New Haven, CT

Snow DW, Perrins CM (1998) The Birds of Western Palearctic: Passerines. Oxford Univ Press, Oxford

Sokal RR, Rohlf FJ (2012) Biometry: the principles and practice of statistics in biological research, 4th ed. WH Freeman and Co, New York

Stegmann BK (1932) Corvid Birds. Birds of the USSR, 6. USSR Acad Sci Press, Leningrad (in Russian)

Stepanyan LS (2003) Conspectus of the ornithological fauna of Russia and adjacent territories (within the borders of the USSR as a historic region). Academkniga, Moscow (in Russian)

Tajima F (1989) Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 123: 585–595

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molec Biol Evol 30: 2725–2729

Trost ChH (1999) Black-billed Magpie (*Pica hudsonia*). The Birds of North America Online. Ed by A Poole. Cornell Lab of Ornithology, Ithaca. Retrieved from the Birds of North America

Tugarinov AYa (1932) Birds of East Mongolia on observations of the expedition of 1928. Acad Sci Press, Leningrad (in Russian)

Vaurie C (1959) The Birds of the Palearctic Fauna. Order Passeriformes. XIII. London

Veprintsev BN, Veprintseva OD, Ryabitsev VK, Dmitrenok MG, Bukreev SA, Gashkov SI (2007) Voices of birds of the Russia. Voice guide. 1: European Russia, Ural and Western Siberia. Ural University Press, Ekaterinburg (in Russian). MP3-CD

Yahya HSA, Salamah M (1996) The Asir magpie: results of recent field surveys. Phoenix 13: 13–14

Zhang R, Song G, Qu Y, Alstrom P, Ramos R, Xing X, et al. (2012) Comparative phylogeography of two widespread magpies: importance of habitat preference and breeding behavior on genetic structure in China. Mol Phylogenet Evol 65: 562–572

Zink RM, Rohwer S, Andreev AV, Dittmann DL (1995) Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97: 639–649

Washizawa S (2008) Kasasagi no hansyoku no rekishi. In Yukiguni Joetsu no tori wo mitsumete. Ed M Nakamura. Niigata nippo jigyousya, Niigata (in Japanese)

Weir JT, Schluter D (2008) Calibrating the avian molecular clocks. Mol Ecol 17: 2321–2328

(Received July 24, 2016 / Accepted December 25, 2016)