Complete mitochondrial genomes of five subspecies of the Eurasian magpie *Pica pica*, obtained with Oxford Nanopore MinION, and their interpretation regarding intraspecific taxonomy

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ABSTRACT

The complete mitochondrial (mt) genomes of five subspecies of the Eurasian (Common) magpie *Pica pica* were determined for the first time. Lengths of the circular genomes comprise 13 protein-coding genes, two rRNA genes (for 125 rRNA and 16S rRNA), 22 tRNA genes, and the non-coding control region (CR). Gene content and lengths of the genomes (16,936–16,945 bp) are similar to typical vertebrate mt genomes. The subspecies studied differs by several single substitutions and indels, especially in the CR. The phylogenetic tree based on complete mt genomes shows a deep divergence of the two groups of subspecies which supports the proposed division into two distinct species: *P. pica* and *P. serica*.

The assembled genomes are 16,936–16,945 bp in length and each contains 37 genes comprising 13 protein-coding genes, two rRNA genes (for 125 rRNA and 16S rRNA), and 22 tRNA genes. The control region (CR) is located between rRNA-Glu and tRNA-Phe genes and has a length of 1351–1362 bp. The H- and L-strands encode 28 and nine genes, respectively. The gene arrangement is the same in all genomes studied. GC content of the new genomes is 43.3–43.5%. Proportions of A, T, C, and G in subspecies have the following ranges (in %): 31–31.1, 25.4–29.2, 29.2–29.5, and 14.0–14.1, respectively.

For illustrating phylogenetic relations of the complete mt genomes obtained, we constructed a maximum-likelihood (ML) tree (Figure 1) using MEGA X (Kumar et al. 2018).

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average. In general, this is consistent with the previous results obtained using individual genes: cytB and CR (Kryukov et al. 2004, 2017; Haring et al. 2007); cytB, ND2, and two nuclear introns (Song et al. 2018). This degree of divergence supports classifying the two clades as separate species.

The subspecies name of HQ915867 is not mentioned in its GenBank entry. Its near identity to jankowskii suggests that it could belong to jankowskii or a closely related subspecies, such as serica or anderssoni. Kryukov et al. (2017) showed close affinity of serica and jankowskii, while Song et al. (2018) showed near identity of serica and anderssoni, and suggested grouping them under Pica serica species. Based on our complete mt genome data, we support classifying Pica serica as a species, distinct from P. pica (the western clade). Pica serica species may include jankowskii, serica, and anderssoni subspecies.

Continuation of genomic studies is necessary for clarifying taxonomy of the remaining subspecies.

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No potential conflicts of interest were reported by the author(s).

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Data availability statement
The data that support the findings of this study are openly available in the National Center for Biotechnology Information database (NCBI/GenBank) at https://www.ncbi.nlm.nih.gov/, reference numbers MT792352, MT792353, MT792354, MT792355, and MT792356.

References
Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo Metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319.
Haring E, Gamauf A, Kryukov A. 2007. Phylogeographic patterns in widespread corvid birds. Mol Phylogenet Evol. 45(3):840–862.
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 Res. 42(4):342–351.
Kryukov AP, Spiridonova LN, Mori S, Arkhipov VY, Redkin YA, Goroshko OA, Lobkov EG, Haring E. 2017. Deep phylogeographic breaks in magpie Pica pica across the Holarctic: concordance with bioacoustics and phenotypes. Zool Sci. 34(3):185–200.
Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35(6):1547–1549.
Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics. 30(22):3276–3278.
Madge S, Burn H. 1999. Crows and Jays: a guide to the Crows, Jays and Magpies of the world. London: C Helm.
Song G, Zhang R, Alström P, Irestedt M, Cai T, Qu Y, Ericson PGP, Fjeldså J, Lei F. 2018. Complete taxon sampling of the avian genus Pica (Magpies) reveals ancient relictual populations and synchronous Late-Pleistocene demographic expansion across the Northern Hemisphere. Avian Biol. 49:e01612.