ISSN 1022-7954, Russian Journal of Genetics, 2021, Vol. 57, No. 5, pp. 568–581. © Pleiades Publishing, Inc., 2021.
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ANIMAL GENETICS

Genetic Properties and Evolution of Asian Honey Bee *Apis cerana ussuriensis* from Primorsky Krai, Russia

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Received June 16, 2020; revised September 4, 2020; accepted October 9, 2020

Abstract—*Apis cerana ussuriensis* Ilyasov et al., 2019 is the northernmost subspecies of the Asian honey bee *A. cerana* Fabricius, 1793, common in the forests of Primorsky krai and Khabarovsk krai as far as 47°54′ N. Genetic studies of this subspecies are of great interest for science and apiculture, since all its adaptive traits were formed under the influence of the natural environment without human interference. We sequenced and annotated the complete mitochondrial DNA (mtDNA) sequences of bees of subspecies *Apis cerana ussuriensis* Ilyasov et al., 2019 (Genbank accession number AP018450) from Primorsky krai and *Apis cerana koreana* Ilyasov et al., 2019 from South Korea, as well as six exons of the nuclear DNA (nDNA) vitellogenin *VG E2–E7* gene of bee subspecies *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica* Radoszkowski, 1887, *A. c. cerana*, and *A. c. indica* Fabricius, 1798. Cluster analysis of the mtDNA and the nDNA *VG* gene sequences showed the division of bees into two groups, the southern subspecies *A. c. indica* and the northern subspecies *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, and *A. c. cerana*. On the basis of the genetic divergence, we showed that subspecies *A. c. ussuriensis* was genetically closer to subspecies *A. c. japonica*, *A. c. koreana*, and *A. c. cerana* than to subspecies *A. c. indica*. Values of genetic divergence (0.80%–8.00%) and Jukes–Cantor genetic distance (0.005–0.100) for mtDNA and nDNA *VG* gene between subspecies *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, *A. c. cerana*, and *A. c. indica* are within the range of intraspecific differences between insect subspecies. The estimated time of the emergence of the *A. cerana* subspecies is from two to one million years ago.

Keywords: *Apis cerana*, *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, *A. c. cerana*, *A. c. indica*, mitochondrial genome, subspecies, divergence, evolution, Primorsky krai, vitellogenin, mtDNA, nDNA

DOI: 10.1134/S1022795421050033

INTRODUCTION

The Asian honey bee *Apis cerana* Fabricius, 1793 is the second most important for humans after the honey bee *Apis mellifera* Linnaeus, 1758. Until recently, their natural distribution ranges did not overlap, being limited to Europe and Africa for *A. mellifera* and Asia for *A. cerana*. Both bee species are adapted to a wide range of climatic zones, from cold temperate to hot equatorial [1]. In Asian countries, *A. cerana* is bred in apiaries; in Russia, it is only found in the wild in the Far Eastern forests in the Khabarovsk krai and Primorsky krai as far as 47°54′ N and is included in the Red Book [2, 3]. V.N. Kuznetsov [3] found wild *A. cerana* families living in the hollows of 80 trees and suggested that more than 1000 families of the Asian honey bee inhabit the forests of the Primorsky krai and Khabarovsk krai as far as 47°54′ N.

The Asian honey bee *A. cerana* is a well-known important pollinator of agricultural crops in Asia and the producer of honey, wax, royal jelly, and bee pollen [4]; the scale of its commercial use is comparable to the honey bee *A. mellifera* and it possesses high potential for genetic improvement using selective breeding on the basis of molecular markers. Recently, the numbers of *A. cerana* have sharply decreased in Asian countries because of the spread of the sacbrood virus (SBV) and of the mass import of *A. mellifera* [5–7].

According to the published data, there are more than 20 subspecies within *A. cerana*, most of which do not have clear taxonomic diagnoses [8–19]. Preserva-

tion of the gene pool of local *A. cerana* subspecies is possible with the application of marker-mediated identification and selection. Molecular genetic studies provide the means for the development of basic strategies for the conservation of *A. cerana*. Mitochondrial DNA (mtDNA) markers are effective tools in the study of evolution and inter- and intraspecific phylogenetic relationships of honey bees [18, 20–25].

In this study, we sequenced and annotated the complete mitochondrial DNA (mtDNA) sequences of the bees of subspecies *Apis cerana ussuriensis* Ilyasov et al., 2019 (GenBank/DDBJ AP018450) (ZooBank 06874b0a-029b-40e2-b4a8-1a20f7692ed3) from the Primorsky krai and *Apis cerana koreana* Ilyasov et al., 2019 (GenBank/DDBJ AP018431) (ZooBank 290E12BA-FC5F-4907-AE50-EF0FA8DC8D9C) from South Korea, as well as six exons of the vitellogenin *VG E2–E7* nuclear DNA (nDNA) gene of bee subspecies *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, *A. c. cerana*, and *A. c. indica*. We assessed the phylogenetic relationships of *A. c. ussuriensis* with other subspecies from South Korea, China, Japan, Taiwan, and Indonesia on the basis of the complete mtDNA and the nDNA *VG* gene.

MATERIALS AND METHODS

Ten adult worker bees were selected from each of the following families: *A. c. ussuriensis* Ilyasov et al., 2019 from a hollow in the forest in the vicinity of Vladivostok, Primorsky krai, Russia (43°11′ N 132°55′ E); *A. c. koreana* Ilyasov et al., 2019 from the Gokseong-gun apiary, Gokseong-eup, Hakjung-ri, Jeollanam-do, South Korea (35°24′ N, 127°27′ E); from the Sangju-si apiary, Gyeongsangbuk-do, South Korea (36°42′ N, 128°18′ E); from the Sancheong-gun apiary, Gyeongsangnam-do, South Korea (35°36′ N., 128°88′ E); *A. c. japonica* from the Kitahiroshima apiary, Hokkaido, Japan (42°95′ N, 141°53′ E); *A. c. indica* from Taichung Apiary, Taiwan (24°04' N, 120°73′ E). The species identification of *A. cerana* bees was performed morphometrically according to V.N. Kuznetsov [3].

Total DNA was extracted from the thoracic muscle tissue of each bee using the Wizard Genomic DNA Purification Kit (PROMEGA, Madison, WI, United States) according to the manufacturer's recommendations. DNA samples were stored at –20°C until further use. PCR of exons 2–7 of the vitellogenin gene (*VG*) was conducted in a Applied Biosystems Veriti HID 96-well thermal cycler on the basis of the already developed primers [26] with a TaKaRa PCR kit (100 μL PCR × 100 reactions) (TAKARA BIO INC., Shiga, Japan) according to the manufacturer's instructions. All PCR products were purified using the QIAquick PCR Purification Kit (250) (QIAGEN, Hilden, Germany) following the manufacturer's instructions.

One worker from each bee colony was used for mtDNA sequencing using the NextSeq 500/550 High Output Kit v. 2 (75 cycles) (ILLUMINA, United States) and paired-end reads (2 × 150 bp), following the manufacturer's instructions, with an Illumina Next Seq 500 sequencer (ILLUMINA, United States) at Kyoto Sangyo University (Kyoto, Japan). Genomic libraries were prepared using a Nextera DNA library preparation kit (ILLUMINA, United States) according to the manufacturer's instructions. Genomes were assembled on the basis of 1 662 000 reads with a 75x mean coverage using Geneious R9 (BIOMATTERS, New Zealand). The genome was annotated using MITOS (Germany) [27] and tRNAscan-SE (CA, USA) [28]. One worker from each bee colony was used for sequencing exons of the nDNA vitellogenin *VG* gene from both ends using the Sanger method [29] with the ABI PRISM BigDye Terminator v3.1 kit on an ABI 3130 sequencer (Applied Biosystems, Foster City, CA, United States) at Incheon National University (Incheon, South Korea).

The complete mtDNA nucleotide sequences were deposited into the GenBank/DDBJ databases under accession numbers AP018431 for *A. c. koreana* (15925 bp) (ZooBank 290E12BA-FC5F-4907-AE50-EF0-FA8DC8D9C) (Gokseong-gun, Jeollanam-do, South Korea) and AP018450 for *A. c. ussuriensis* (15 919 bp) (ZooBank 06874b0a-029b-40e2-b4a8-1a20f7692ed3) (Primorsky krai, Russia). The nucleotide sequences of exons 2–7 of the *VG* gene were deposited under the accessions numbers MH755745, MH755780, MH755815, MH755850, MH755885, and MH755920 (4125 bp) and MH755746, MH755781, MH755816, MH755851, MH755886, and MH755921 (4125 bp) for two specimens of *A. c. ussuriensis* (Primorskiy krai, Russia); accession numbers MH755735, MH755770, MH755805, MH755840, MH755875, and MH755910 for *A. c. koreana* (Sancheong, Gyeongsangnam-do, South Korea); MH755741, MH755776, MH755811, MH755846, MH755881, and MH755916 (4125 bp) and MH755742, MH755777, MH755812, MH755847, MH755882, and MH755917 (4125 bp) for *A. c. japonica* (Kitahiroshima, Hokkaido, Japan); and MH755747, MH755782, MH755817, MH755852, MH755887, and MH755922 (4128 bp) and MH755748, MH755783, MH755818, MH755853, MH755888, and MH755923 (4128 bp) for *A. c. indica* (Taichung, Taiwan).

Comparative analysis of total mtDNA was performed using the following sequences from Genbank: *A. c. japonica* AP017314 (15 917 bp) (Kyoto, Japan), *A. c. japonica* AP017941 (15 778 bp) (Amami, Japan), *A. c. cerana* AP017983 (15 460 bp) (Jiangsu, China), *A. c. cerana* KM244704 (15 712 bp) (Yunnan, China), *A. c. indica* AP017984 (15 376 bp) (Taipei, Taiwan), *A. c. indica* AP018149 (15884 bp) (Sabah, Borneo, Malaysia), *A. c. koreana* AP018431 (15 925 bp) (Jeollanam-do, South Korea), *A. c. koreana* KX908206 (15904 bp) (Chungcheongbukdo, South Korea), and

A. m. ligustica NC 001566 (16 324 bp) (outgroup, United States). Comparative analysis of the *VG* nDNA gene was performed using the following sequences from Genbank: *A. c. cerana* KT725235 (4125 bp) (Yunnan, China), *A. c. cerana* ApisCC1.0 (4125 bp, fragment 781683–788069 from the genomic sequence KZ288206) (Yunnan, China), *A. m. mellifera* JN557295, JN557387, JN557201, JN557573, JN557481, and JN557109 (4074 bp) (outgroup, isolate M2261, Warsaw, Poland).

Divergence of nucleotide sequences and genetic Jukes–Cantor distance [30], Tamura–Nei distance [31], and *p*-distance [32] were calculated using Unipro UGENE 1.28 (UNIPRO, Russia) and CLC Genomics Workbench 11 (CLCbio, Denmark). Phylogenetic analysis based on the DNA sequences was performed using MEGA7 [33] and Statistica 8.0 (StatSoft, Inc., Tulsa, OK, United States) and JMP14 (SAS Institute Inc., North Carolina, United States). Phylogenetic trees were constructed using the neighbor joining method [34] based on Jukes–Cantor distances with 1000 bootstrap replications and the Reltime [35] method for estimating the branch lengths. A physical map of the complete mitochondrial genome was constructed using CLC Genomics Workbench 11 (CLCbio, Denmark) and Artemis 17.0.1 (The Sanger Institute, Hinxton, Cambridge, UK).

RESULTS

The mitochondrial genome of *A. c. ussuriensis* (Primorsky krai, Russia) contains 42% A, 42% T, 6% G, and 10% C nucleotides, is AT-enriched by 84%, and contains the highest frequencies of dinucleotides AA (19%), AT (18%), TT (18%), and TA (16%) and the lowest frequencies of dinucleotides GG (1%), GC (1%), CG (1%), and CC (2%), which is typical of the majority of Hymenoptera [25, 36, 37]. The average GC content in mtDNA of *A. c. ussuriensis* is 16%, and the maximum level does not exceed 40%. The value of genetic diversity and variability is in direct proportion to the GC composition: the higher the GC content, the higher the genetic diversity and gene variability. The GC content in mtDNA below 40% is considered low [38] (Table 1).

MtDNA of *A. c. ussuriensis* (15 919 bp) is slightly shorter than mtDNA of *A. mellifera* (16 343 bp) and *Drosophila yakuba* (16 019 bp). The sum of all intergenic noncoding regions of *A. c. ussuriensis* mtDNA (1252 bp) is also slightly shorter than that of *A. mellifera* (1639 bp) and *D. yakuba* (1262 bp) [36].

The mitochondrial genome of *A. c. ussuriensis* contains 30 protein-coding genes (CDS), 22 transport RNA (tRNA) genes, two genes of the ribosomal RNA (rRNA) subunits (*16S rRNA* and *12S rRNA*), and four noncoding intergenic regions (*NC1–NC4*). The synteny of *A. c. ussuriensis* mtDNA is similar to the mtDNA synteny in most Hymenoptera with slight differences [25, 36]. The majority of the genes (*ATP6*, *ATP8*, *COX1*, *COX2*, *COX3*, *CYTB*, *ND2*, *ND3*, *ND6*, *tRNA-Ala*, *tRNA-Asn*, *tRNA-Asp*, *tRNA-Gln*, *tRNA-Glu*, *tRNA-Gly*, *tRNA-Ile*, *tRNA-Leu*(UUR), *tRNA-Lys*, *tRNA-Met*, *tRNA-Ser*(AGN), *tRNA-Ser*(UCN), *tRNA-Thr*, and *tRNA-Trp*) are located on the light mtDNA strand, with the exception of four subunit genes (*ND1*, *ND4*, *ND4L*, and *ND5*), two rRNA genes (*12S* and *16S rRNA*), and eight tRNA genes (*tRNA-Arg*, *tRNA-Cys*, *tRNA-His*, *tRNA-Leu*(CUN), *tRNA-Phe*, *tRNA-Pro*, *tRNA-Tyr,* and *tRNA-Val*), which are located on the heavy mtDNA strand (Table 2, Fig. 1).

Protein-coding genes *ND3*, *ND4L*, *ND5*, *COX1*, *ND6*, *COX2*, *ND1*, and *ND2* have the ATT start codon; genes *COX3*, *ATP6*, and *CYTB* have the ATG start codon; gene *ND4* has the ATA start codon, and gene *ATP8* has the ATC start codon. All protein-coding mtDNA genes of *A. c. ussuriensis* have the common TAA stop codon. Some mtDNA genes (*tRNA-Gln* and *tRNA-Ala* (4 bp), *ND2* and *tRNA-Cys* (1 bp), *COX1* and *tRNA-Leu(UUR*) (5 bp), *COX2* and *tRNA-Asp* (1 bp), and *ATP8* and *ATP6* (19 bp)) of *A. c. ussuriensis* typically have an overlap, which is possibly inherited from the prokaryotic genome with a polycistronic transcription type (Table 2).

The length of all protein-coding mtDNA genes of *A. c. ussuriensis* is 11 058 bp and they encode 3686 amino acids. The rRNA genes of *A. c. ussuriensis* mtDNA have a total size of 2116 bp (*12S rRNA* is 787 bp and *16S rRNA* is 1329 bp). The length of tRNA genes of *A. c. ussuriensis* mtDNA ranges from 60 bp (*tRNA-Ser*(AGN)) to 78 bp (tRNA-Pro).

The mtDNA of *A. c. ussuriensis* contains four noncoding intergenic regions (*NC1–NC4*), which is typical of all subspecies of *A. cerana* [39]. The noncoding intergenic region *NC1* (228 bp) is located between the *tRNA-Met* and *tRNA-Gln* genes, *NC2* (89 bp) is between the *tRNA-Leu* (TAA) and *COX2* genes, *NC3* (68 bp) is between the *COX3* and *tRNA-Gly* genes, and *NC4* (51 bp) is between the *tRNA-Pro* and *ND6* genes. All noncoding intergenic and AT-rich regions in *A. c. ussuriensis* amount to less than 8% of the total mtDNA size. Noncoding intergenic and AT-rich regions are regulatory and contain repetitive motifs. The AATTAATT motif was found in mtDNA of *A. c. ussuriensis* 48 times; the AATAAATT motif, 74 times; and the TACTTA motif, a likely binding site for the mitochondrial transcription terminator (mtTERM), 8 times [40].

Noncoding intergenic region *NC2* in *A. c. ussuriensis* was identical to the haplotype *Japan01* (sequences KP064995 and AP018431) and was not identical to any of the ten published haplotypes (*ACNC101–ACNC110*) [18, 19, 39]; it was designated as haplotype *ACNC111* (Fig. 2). The *ACNC111* haplotype is the most similar to the *ACNC101* haplotype (sequences KP064870 and KP064972) and differs in the 31insT

Table 1. Characteristics of nucleotide composition of complete mtDNA in *A. c. ussuriensis* from Primorsky krai, Russia

Nucleotides	Number (frequency)
GC composition	2540 (0.16)
AT composition	13373 (0.84)
Adenine A	6729 (0.42)
Cytosine C	1542 (0.10)
Guanine G	998 (0.06)
Thymine T	6644 (0.42)
Dinucleotide AA	2973 (0.19)
Dinucleotide AC	475 (0.03)
Dinucleotide AG	358 (0.02)
Dinucleotide AT	2921 (0.18)
Dinucleotide CA	675 (0.04)
Dinucleotide CC	239 (0.02)
Dinucleotide CG	70 (0.01)
Dinucleotide CT	558 (0.04)
Dinucleotide GA	471 (0.03)
Dinucleotide GC	116 (0.01)
Dinucleotide GG	136 (0.01)
Dinucleotide GT	275 (0.02)
Dinucleotide TA	2609 (0.16)
Dinucleotide TC	712 (0.05)
Dinucleotide TG	432 (0.03)
Dinucleotide TT	2886 (0.18)
Nucleotide A in position 1/2/3	240 (0.47)/128 (0.25)/249 (0.48)
Nucleotide C in position 1/2/3	37 (0.07)/70 (0.14)/22 (0.04)
Nucleotide G in position 1/2/3	50 (0.1)/19 (0.04)/11 (0.02)
Nucleotide T in position 1/2/3	188 (0.37)/298 (0.58)/233 (0.45)
Size, bp	15919
Strain weight, kDa	4904.98

insertion relative to the beginning of the *NC1* sequence (Fig. 2).

Comparative analysis of the complete mtDNA sequence made it possible to calculate transitions and transversions between bees in different populations of *A. cerana*. Similar to the genomes of most organisms, transitions in *A. cerana* are more frequent than transversions. Transitions and transversions between the *A. cerana* bees in different populations were calculated for the total mtDNA, as well as only for the protein-coding mtDNA genes. This makes it possible to assess the contribution of the coding and noncoding parts of the mitochondrial genome to the genetic variability. Notably, the role of the noncoding part of mtDNA in maintaining genetic variability is greater than that of the coding part (Table 3).

The divergence (%) between *A. c. japonica* and *A. c. cerana* was 2.85 and 0.80 based on mtDNA and the *VG* nDNA gene, respectively; between *A. c. japonica* and *A. c. indica*, 5.26 and 0.85; between *A. c. japonica* and *A. c. koreana*, 1.33 and 0.80; between *A. c. cerana* and *A. c. indica*, 4.95 and 0.80; between *A. c. cerana* and *A. c. koreana*, 2.85 and 0.80; between *A. c. koreana* and *A. c. indica*, 5.45 and 0.88; between *A. c. ussuriensis* and *A. c. cerana*, 2.55 and 1.25; between *A. c. ussuriensis* and *A. c. japonica*, 1.10 and 0.93%; between *A. c. ussuriensis* and *A. c. koreana*, 1.30 and 0.90; between *A. c. ussuriensis* and *A. c. indica*, 5.15 and 1.50; between *A. c. ussuriensis* and *A. mellifera*, 21.05 and 9.05; between *A. c. koreana* and *A. mellifera*, 21.70 and 8.75; between *A. c. cerana* and *A. mellifera*, 21.70 and 8.70; between *A. c. japonica* and *A. mellifera*, 20.45 and 8.70; and between *A. c. indica* and *A. mellifera*, 21.35 and 8.75 (Tables 4 and 5).

On the basis of the cluster analysis of the complete sequences of mtDNA and of the nDNA *VG* gene, we constructed dendrograms for all the *A. cerana* speci-

Table 2. Annotation of complete mtDNA of *A. c. ussuriensis* from Primorsky krai, Russia

No.	Gene	Start position	Stop position	Size, bp
1	*tRNA-Ser*(AGN)	1	60	60
2	*tRNA-Glu*	64	129	66
3	*tRNA-Met*	164	229	66
4	*tRNA-Gln*	462	527	66
5	*tRNA-Ala*	524	589	66
6	*tRNA-Ile*	608	673	66
7	*ND2*	674	1669	996
8	**tRNA-Cys*	1669	1734	66
9	**tRNA-Tyr*	1740	1808	69
10	*tRNA-Trp*	1825	1893	69
11	*COX1*	1894	3459	1566
12	*tRNA-Leu*(UUR)	3455	3524	70
13	*COX2*	3614	4294	681
14	*tRNA-Asp*	4294	4361	68
15	*tRNA-Lys*	4368	4439	72
16	*ATP8*	4446	4607	162
17	*ATP6*	4589	5266	678
18	*COX3*	5284	6063	780
19	*tRNA-Gly*	6136	6202	67
20	*ND3*	6203	6556	354
21	**tRNA-Arg*	6577	6645	69
22	*tRNA-Asn*	6665	6732	68
23	**tRNA-Phe*	6751	6821	71
24	**ND5*	6828	8495	1668
25	**tRNA-His*	8496	8561	66
26	**ND4*	8579	9910	1332
27	**ND4L*	9913	10176	264
28	*tRNA-Thr*	10200	10266	67
29	**tRNA-Pro*	10282	10359	78
30	*ND6*	10411	10923	513
31	*CYTB*	10936	12084	1149
32	*tRNA-Ser*(UCN)	12108	12174	67
33	**ND1*	12187	13101	915
34	**tRNA-Leu*(CUN)	13102	13170	69
35	**16S rRNA*	13171	14499	1329
36	**tRNA-Val*	14500	14566	67
37	**12S rRNA*	14567	15353	787
	Complete mtDNA	1	15919	15919

* Gene transcribing from the heavy chain of mtDNA.

mens showing the phylogenetic relationships of *A. cerana* bees from different populations (Fig. 3). Specimens of *A. m. mellifera*, which were located separately from *A. cerana*, were used as an outgroup. Specimens of *A. c. ussuriensis*, *A. c. cerana*, *A. c. japonica*, and *A. c. indica*, with the exception of *A. c. koreana*, were clustered on both dendrograms separately from each other. On the dendrogram based on mtDNA,

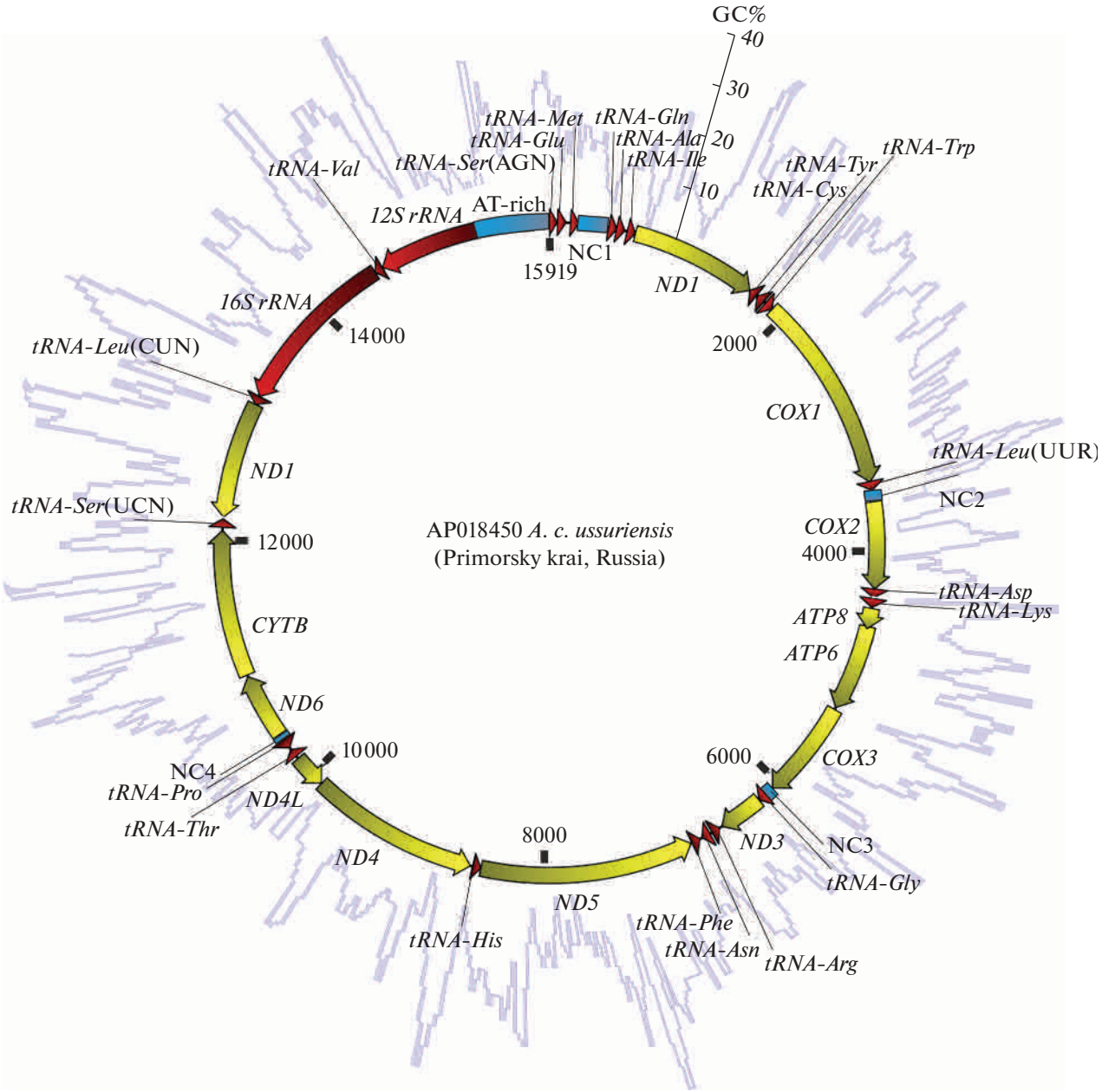

Fig. 1. Circular physical map of complete mtDNA of *Apis c. ussuriensis* from Primorsky krai, Russia.

A. c. koreana was divided between the *A. c. japonica* and *A. c. ussuriensis* groups, which may reflect the historical process of migration and gene flow.

DISCUSSION

The content of AT nucleotides in the complete mtDNA sequence of *A. c. ussuriensis* was 84% similar to other insects: *A. c. cerana*, 84%; *A. c. koreana*, 84.1%; *A. mellifera*, 84.9%; *Bombus hypocrita*, 85.3%; *B. ignitus*, 86.8%; *Cephus cinctus*, 82%; *Enicospilus* sp., 85.2%; *Melipona bicolor*, 86.7%; *Polistes humilis*, 84.7%; and *Spathius agrili*, 84% [18, 25].

Phylogenetic analysis of the noncoding intergenic region *NC1* sequence identified nine haplotypes of *A. cerana* (*ACNC101*, *ACNC102*, *ACNC103*, *ACNC104*, *ACNC105*, *ACNC106*, *ACNC107*, *ACNC108*, and *ACNC109*), subdivided into two groups: A and B [39]. Haplotype *ACNC110* was found in *A. c. koreana* [18]. In the present study, we found in *A. c. ussuriensis* a new haplotype *ACNC111*, which differed from *ACNC110* in the 31insT insertion relative to the beginning of the *NC1* sequence. The phylogenetic analysis of the second noncoding intergenic *NC2* region sequence identified six *A. cerana* haplotypes (*Japan1*, *Nepal1*, *ThaiS1*, *BurmaN1*, *BurmaN2*, and *BurmaN3*), subdi-

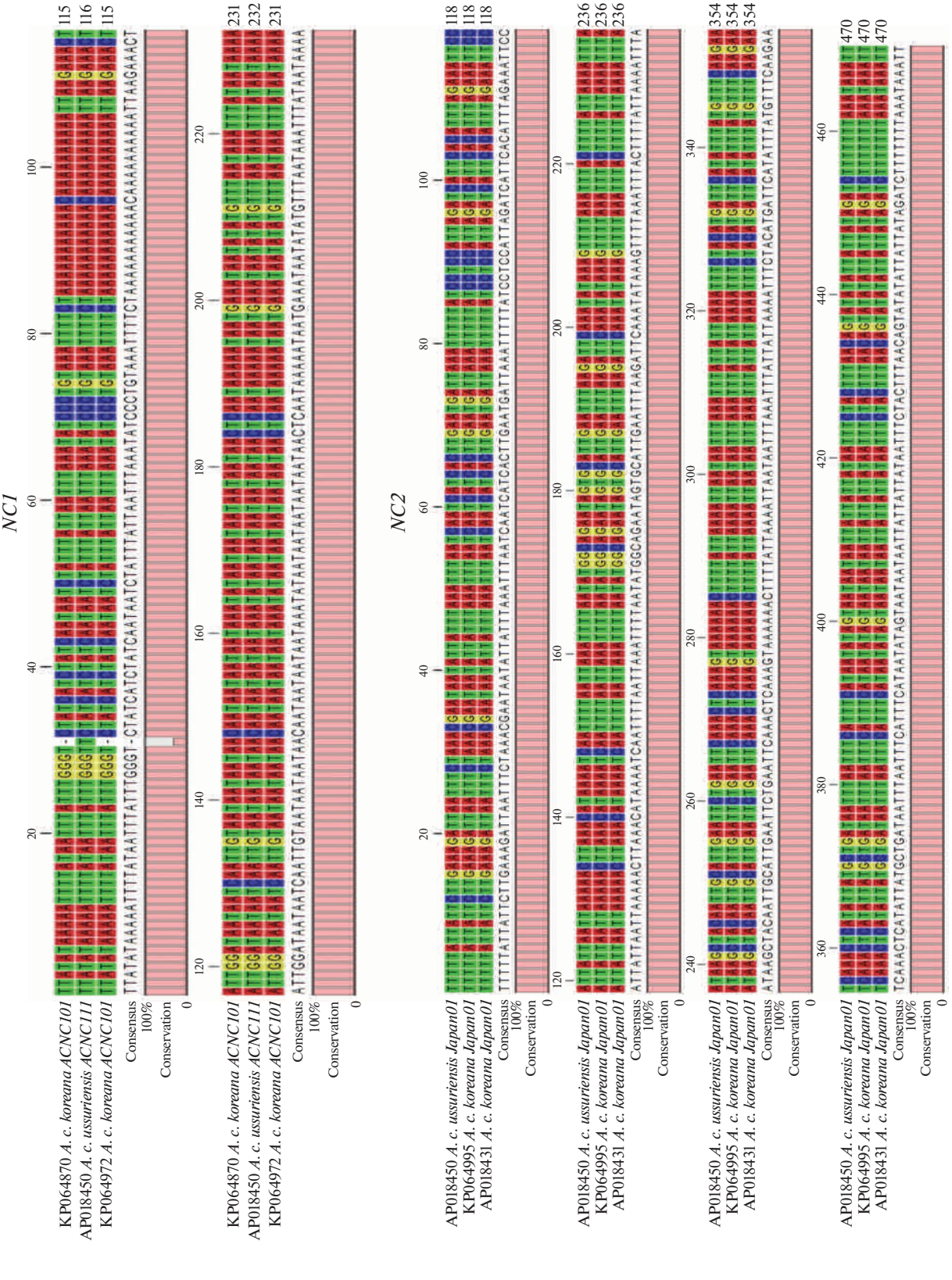

Fig. 2. Alignment of sequences of two noncoding intergenic regions *NC1* and *NC2* of *Apis c. ussuriensis* from Primorsky krai, Russia, with haplotypes *ACNC101* and *Japan01*.

Table 3. Transitions and transversions between complete mtDNA sequences of representatives of different *A. cerana* populations

A. cerana representatives		AP018450, *A. c. ussuriensis*, Primorsky krai, Russia	KM244704, *A. c. cerana*, Yunnan, China	AP017983, *A. c. cerana*, Jiangsu, China	AP017314, *A. c. japonica*, Kyoto, Japan	AP017941, *A. c. japonica*, Amami, Japan	KX908206, *A. c. koreana* Chungcheongbuk-do, Korea	AP018431, *A. c. koreana*, Jeollanam-do, Korea	AP017984, *A. c. indica* Taipei, Taiwan	AP018149, *A. c. indica* Sabah, Borneo, Malaysia	NC 001566, *A. m. ligustica*, United States
		Transversions in complete mtDNA / protein-coding mtDNA genes									
AP018450, *A. c. ussuriensis*, Primorsky krai, Russia	Transitions in complete mtDNA/ protein-coding mtDNA genes		29/12	26/15	15/5	14/5	18/8	42/10	99/65	308/138	1506/957
KM244704, *A. c. cerana*, Yunnan, China		56/41		9/3	17/9	17/9	21/12	53/18	88/59	274/130	1438/950
AP017983, *A. c. cerana*, Jiangsu, China		64/52	26/19		14/12	14/12	18/15	50/21	85/58	225/131	1367/951
AP017314, *A. c. japonica*, Kyoto, Japan		29/21	60/49	70/60		2/1	9/5	43/13	89/62	311/135	1492/953
AP017941, *A. c. japonica*, Amami, Japan		30/23	61/51	71/62	9/8		6/5	45/13	89/62	285/135	1452/952
KX908206, *A. c. koreana*, Chungcheongbuk-do, Korea		38/25	73/54	80/63	30/18	31/20		48/16	92/65	316/136	1498/955
AP018431, *A. c. koreana*, Jeollanam-do, Korea		40/24	63/48	73/57	45/33	45/35	39/23		122/71	317/142	1510/959
AP017984, *A. c. indica*, Taipei, Taiwan		237/197	251/209	253/214	244/206	242/204	253/207	245/204		228/139	1320/947
AP018149, *A. c. indica*, Sabah, Borneo, Malaysia		339/272	337/273	334/277	338/278	342/280	355/281	337/274	345/294		1492/931
NC 001566, *A. m. ligustica*, United States		715/550	713/554	704/556	718/557	710/554	725/558	709/546	691/543	694/539	

Table 4. Genetic distances (above diagonal) and genetic differences (below diagonal) between complete mtDNA sequences of *A. cerana* samples

A. cerana representatives		AP018450, *A. c. ussuriensis*, Primorsky krai, Russia	KM244704, *A. c. cerana*, Yunnan, China	AP017983, *A. c. cerana*, Jiangsu, China	AP017314, *A. c. japonica*, Kyoto, Japan	AP017941, *A. c. japonica*, Amami, Japan	KX908206, *A. c. koreana*, Chungcheongbuk-do, Korea	AP018431, *A. c. koreana*, Jeollanam-do, Korea	AP017984, *A. c. indica* Taipei, Taiwan	AP018149, *A. c. indica* Sabah, Borneo, Malaysia	NC 001566, *A. m. ligustica*, United States
		Jukes–Cantor / Tamura–Nei / *p*-distances									
AP018450, *A. c. ussuriensis*, Primorsky krai, Russia	Divergence, % / number of nucleotide substitutions/amino acid substitutions		0.006/ 0.002/ 0.002	0.006/ 0.002/ 0.002	0.005/ 0.001/ 0.001	0.005/ 0.001/ 0.001	0.005/ 0.001/ 0.001	0.005/ 0.002/ 0.002	0.022/ 0.006/ 0.006	0.042/ 0.013/ 0.013	0.159/ 0.097/ 0.088
KM244704, *A. c. cerana*, Yunnan, China		1.8/ 85/ 13		0.002/ 0.001/ 0.001	0.005/ 0.001/ 0.001	0.005/ 0.001/ 0.001	0.006/ 0.001/ 0.001	0.007/ 0.003/ 0.003	0.022/ 0.005/ 0.005	0.040/ 0.013/ 0.013	0.155/ 0.096/ 0.087
AP017983, *A. c. cerana*, Jiangsu, China		3.3/ 90/ 16	1.5/ 35/ 5		0.005/ 0.001/ 0.001	0.006/ 0.001/ 0.001	0.006/ 0.001/ 0.001	0.008/ 0.003/ 0.003	0.022/ 0.005/ 0.005	0.037/ 0.013/ 0.013	0.152/ 0.096/ 0.087
AP017314, *A. c. japonica*, Kyoto, Japan		0.9/ 44/ 7	2.8/ 77/ 13	3.5/ 84/ 16		0.002/ 0.001/ 0.001	0.003/ 0.001/ 0.001	0.003/ 0.002/ 0.002	0.022/ 0.005/ 0.005	0.042/ 0.014/ 0.014	0.158/ 0.096/ 0.087
AP017941, *A. c. japonica*, Amami, Japan		1.2/ 44/ 8	2.2/ 78/ 14	2.9/ 85/ 17	0.7/ 11/ 3		0.005/ 0.001/ 0.001	0.006/ 0.002/ 0.002	0.022/ 0.005/ 0.005	0.041/ 0.014/ 0.014	0.156/ 0.096/ 0.087
KX908206, *A. c. koreana*, Chungcheongbuk-do, Korea		1.1/ 56/ 13	1.9/ 94/ 19	3.4/ 98/ 22	0.9/ 39/ 12	1.2/ 37/ 13		0.003/ 0.003/ 0.003	0.023/ 0.006/ 0.006	0.044/ 0.014/ 0.014	0.159/ 0.096/ 0.087
AP018431, *A. c. koreana*, Jeollanam-do, Korea		1.5/ 82/ 12	2.4/ 116/ 19	3.9/ 123/ 22	1.3/ 88/ 15	1.9/ 90/ 16	1.1/ 87/ 17		0.024/ 0.007/ 0.008	0.043/ 0.014/ 0.014	0.159/ 0.097/ 0.088
AP017984, *A. c. indica*, Taipei, Taiwan		5.5/ 336/ 46	4.5/ 339/ 48	3.1/ 338/ 47	5.7/ 333/ 45	5.12/ 331/ 44	5.6/ 345/ 51	6.1/ 367/ 52		0.038/ 0.014/ 0.014	0.148/ 0.095/ 0.087
AP018149, *A. c. indica*, Sabah, Borneo, Malaysia		4.8/ 647/ 89	5.5/ 611/ 88	6.7/ 559/ 85	4.9/ 649/ 87	5.3/ 627/ 90	4.9/ 671/ 93	5.2/ 654/ 95	6.9/ 573/ 93		0.156/ 0.095/ 0.087
NC 001566, *A. m. ligustica*, United States		20.6/ 2221/ 585	21.2/ 2151/ 585	22.2/ 2071/ 583	20.5/ 2210/ 581	20.9/ 2162/ 580	20.5/ 2223/ 588	20.6/ 2219/ 587	22.2/ 2011/ 584	20.5/ 2186/ 590	

vided into two groups: mainland Asia and Sundaland [41]. On the basis of the nucleotide sequence of *NC2*, *A. c. ussuriensis* belongs to the mainland Asia haplotype *Japan1*. It is likely that the presence of the *Japan1* haplotype in all populations of *A. cerana* in Asia indicates the common origin of the entire population of *A. cerana* and its subsequent distribution over Asia. Thus, on the basis of the noncoding intergenic *NC1* and *NC2* regions, *A. c. ussuriensis* belongs to the continental Asian group *A. cerana* and is different from the populations of *A. c. cerana*, *A. c. koreana*, and *A. c. japonica*. The genetic differences of *A. c. ussuriensis* are presumably the result of natural selection and adaptive evolution of *A. cerana* in the sharply continental climate of Primorsky krai.

The local properties of the mutation process can be described not only by the probability of single nucleotide mutations per site but also by the ratio of transitions (tr) to transversions (tv). The tr/tv ratio is considered a basic property of the mutation process and is

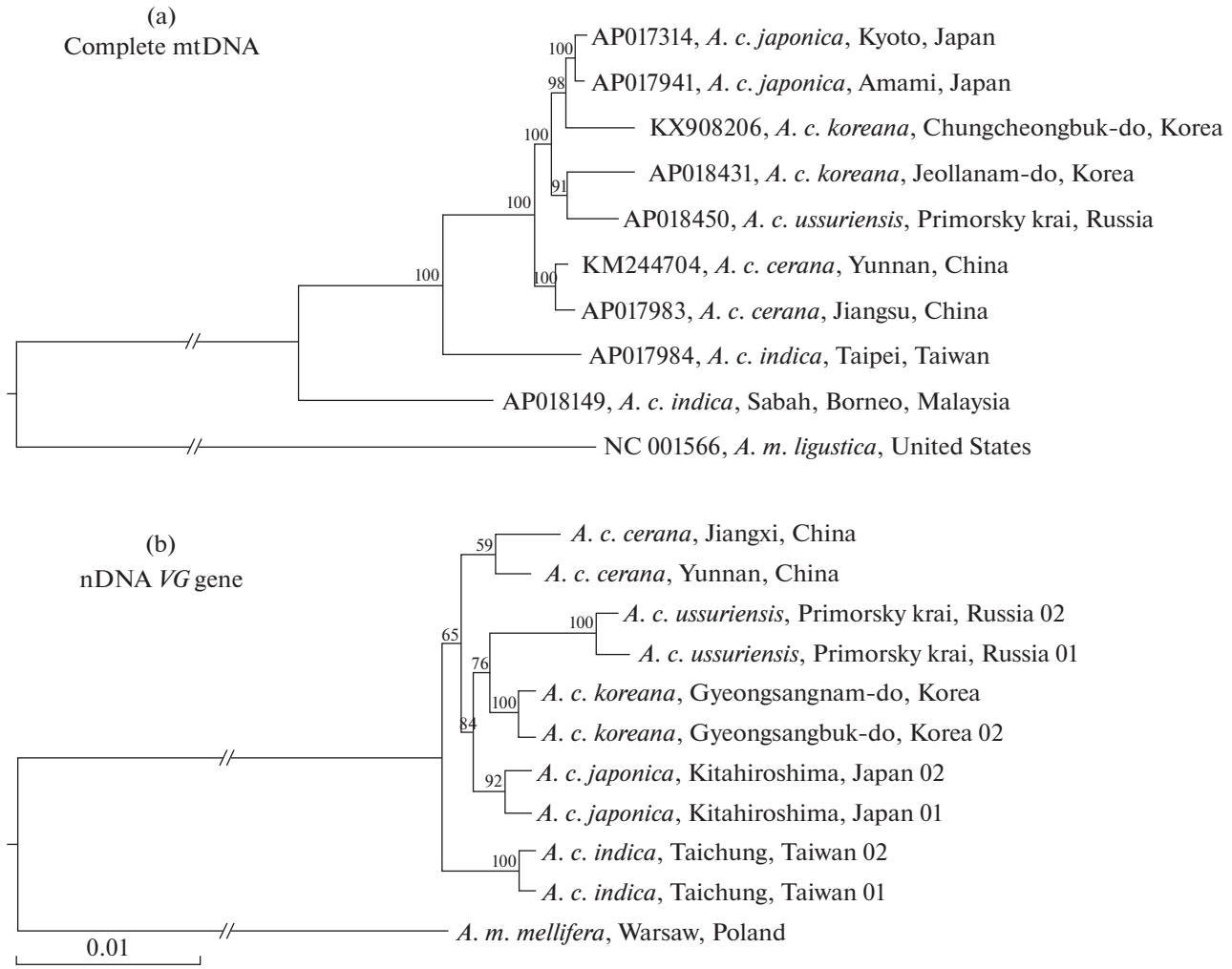

Fig. 3. Phylogenetic relationships between representatives of different *A. cerana* populations based on cluster analysis by the neighbor-joining method and Jukes–Cantor genetic distances. (a) On the basis of the complete mtDNA sequence; (b) on the basis of the sequence of nDNA *VG* gene.

a widely used one-parameter characteristic of the mutation spectrum. For the majority of known eukaryotes, the normal ratio is tr/tv > 1, while tr/tv < 1 indicates the increased frequency of single nucleotide mutations, insertions, or deletions (indels) or the decreased efficiency of DNA repair. The variability of the tr/tv ratio in the genome may indicate a local change in the mutational mechanism during adaptation to changing environmental conditions [42–44].

The ratio of transitions to transversions in total mtDNA was 2.46 between *A. c. ussuriensis* and *A. c. cerana*, 2.14 between *A.* c. *ussuriensis* and *A. c. japonica*, 2.11 between *A. c. ussuriensis* and *A. c. koreana*, 2.39 between *A. c. ussuriensis* and *A. c. indica*, and 0.47 between *A. c. ussuriensis* and *A. m. ligustica* (Table 3), which is similar to the 2.06 tr/tv ratio for mtDNA between *Drosophila melanogaster* and *D. yakuba* [45]. The smallest value of the tr/tv ratio between *A. cerana* and *A. mellifera* of 0.47 is indicative of a change in the mutational mechanism during adaptation to changing environmental conditions as a result of divergence and allopatric speciation.

The Jukes–Cantor genetic distance, Tamura–Nei distance, and *p*-distance for total mtDNA and the nDNA *VG* gene showed that their absolute values do not differ very much and can be successfully used in cluster analysis. For each distance, a similar clustering pattern can be obtained. It was revealed that *A. c. ussuriensis* subspecies was genetically closer to *A. c. japonica* (the divergence was 1.10% for mtDNA and 0.93% for *VG* nDNA gene; the Jukes–Cantor distances were 0.005 for mtDNA and 0.009 for *VG* nDNA gene) and *A. c. koreana* (the divergence was 1.30% for mtDNA and 0.90% for the *VG* nDNA gene; the Jukes–Cantor distances were 0.005 for mtDNA and 0.012 for the *VG* nDNA gene), *A. c. cerana* (the divergence was 2.55% for mtDNA and 1.25% for *VG* nDNA gene; the Jukes–Cantor distances were 0.006 for mtDNA and 0.012 for

Table 5. Genetic distances (above diagonal) and genetic differences (below diagonal) between sequences of *VG* gene in nDNA of *A. cerana* samples

A. cerana representatives		*A. c. ussuriensis*, Primorsky krai, Russia 01	*A. c. ussuriensis*, Primorsky krai, Russia 02	*A. c. cerana*, Jiangxi, China	*A. c. cerana*, Yunnan, China	*A. c. japonica*, Kitahiroshima, Japan 01	*A. c. japonica*, Kitahiroshima, Japan 02	*A. c. koreana*, Gyeongsangbuk-do, South Korea	*A. c. koreana*, Gyeongsangnam-do, South Korea	*A. c. indica*, Taichung, Taiwan 01	*A. c. indica*, Taichung, Taiwan 02	*A. m. mellifera*, Warsaw, Poland
		Jukes–Cantor / Tamura–Nei / *p*-distances										
A. c. ussuriensis, Primorsky krai, Russia 01	Divergence, %/number of nucleotide substitutions/amino acid substitutions		0.002/ 0.003/ 0.001	0.013/ 0.013/ 0.005	0.013/ 0.013/ 0.005	0.008/ 0.008/ 0.004	0.009/ 0.009/ 0.004	0.010/ 0.010/ 0.005	0.010/ 0.011/ 0.005	0.014/ 0.014/ 0.005	0.014/ 0.014/ 0.005	0.081/ 0.082/ 0.026
A. c. ussuriensis, Primorsky krai, Russia 02		0.3/ 12/ 6		0.012/ 0.012/ 0.004	0.012/ 0.012/ 0.005	0.010/ 0.010/ 0.004	0.010/ 0.010/ 0.005	0.008/ 0.008/ 0.004	0.008/ 0.008/ 0.004	0.013/ 0.012/ 0.004	0.013/ 0.013/ 0.004	0.081/ 0.082/ 0.026
A. c. cerana, Jiangxi, China		1.3/ 52/ 22	1.2/ 48/ 22		0.007/ 0.007/ 0.001	0.007/ 0.007/ 0.001	0.006/ 0.006/ 0.001	0.007/ 0.007/ 0.001	0.007/ 0.007/ 0.001	0.009/ 0.009/ 0.001	0.009/ 0.010/ 0.001	0.077/ 0.078/ 0.022
A. c. cerana, Yunnan, China		1.3/ 53/ 26	1.2/ 49/ 24	0.8/ 27/ 6		0.008/ 0.008/ 0.002	0.006/ 0.006/ 0.001	0.007/ 0.006/ 0.001	0.007/ 0.007/ 0.001	0.008/ 0.008/ 0.001	0.008/ 0.008/ 0.001	0.077/ 0.078/ 0.022
A. c. japonica, Kitahiroshima, Japan 01		0.8/ 32/ 20	1.0/ 40/ 22	0.8/ 30/ 6	0.8/ 31/ 10		0.002/ 0.002/ 0.001	0.007/ 0.007/ 0.002	0.007/ 0.007/ 0.002	0.009/ 0.009/ 0.002	0.008/ 0.008/ 0.002	0.076/ 0.077/ 0.023
A. c. japonica, Kitahiroshima, Japan 02		0.9/ 37/ 20	1.0/ 41/ 22	0.7/ 25/ 4	0.7/ 26/ 8	0.2/ 7/ 2		0.006/ 0.006/ 0.001	0.005/ 0.005/ 0.001	0.008/ 0.008/ 0.001	0.007/ 0.008/ 0.001	0.075/ 0.076/ 0.022
A. c. koreana, Gyeongsangbuk-do, South Korea		1.0/ 42/ 24	0.8/ 32/ 18	0.8/ 30/ 6	0.8/ 27/ 8	0.8/ 28/ 8	0.7/ 25/ 6		0.002/ 0.002/ 0.001	0.008/ 0.008/ 0.001	0.008/ 0.008/ 0.001	0.077/ 0.078/ 0.022
A. c. koreana, Gyeongsangnam-do, South Korea		1.0/ 43/ 25	0.8/ 31/ 19	0.8/ 27/ 7	0.8/ 28/ 9	0.8/ 27/ 9	0.7/ 24/ 7	0.2/ 7/ 1		0.008/ 0.008/ 0.001	0.009/ 0.009/ 0.001	0.078/ 0.079/ 0.022
A. c. indica, Taichung, Taiwan 01		1.4/ 56/ 24	1.4/ 54/ 24	0.9/ 36/ 8	0.8/ 31/ 10	0.9/ 36/ 8	0.8/ 31/ 6	0.8/ 32/ 8	0.9/ 33/ 9		0.001/ 0.001/ 0.001	0.077/ 0.078/ 0.022
A. c. indica, Taichung, Taiwan 02		1.4/ 56/ 23	1.4/ 54/ 23	1.0/ 38/ 9	0.8/ 31/ 11	0.9/ 34/ 9	0.8/ 29/ 7	0.9/ 34/ 9	0.9/ 35/ 10	0.1/ 4/ 1		0.076/ 0.077/ 0.022
A. m. mellifera, Warsaw, Poland		9.0/ 311/ 157	9.1/ 312/ 158	8.7/ 296/ 143	8.7/ 297/ 145	8.7/ 295/ 146	8.6/ 292/ 144	8.7/ 298/ 147	8.8/ 300/ 148	8.8/ 297/ 145	8.7/ 295/ 146	

VG nDNA gene) than to *A. c. indica* (divergence was 5.15% for mtDNA and 1.50% for the *VG* nDNA gene; Jukes–Cantor distances were 0.032 for mtDNA and 0.013 for the *VG* nDNA gene) (Tables 4 and 5). Thus, on the basis of the genetic distances and divergence, the subspecies are subdivided into two groups: North and South Asia.

Phylogenetic trees constructed from the complete mtDNA and the *VG* nDNA gene showed similarities by clustering into two groups: South and North Asia (Fig. 3). Specimens of *A. mellifera* are located separately and function as an outgroup. All *A. cerana* subspecies, with the exception of *A. c. koreana*, are grouped into separate clusters based on the complete mtDNA and the *VG* nDNA gene, which indicates that the paternal and maternal components of the genome were geographically distributed together. This is possible during natural migration, as well as when the beekeeper moves bee colonies. In the case of *A. c. koreana*, specimens of this subspecies were grouped on the

basis of mtDNA both with *A. c. japonica* and with *A. c. ussuriensis*, which may be due to the flow of genes along the maternal line from these subspecies to the *A. c. koreana* population, the distribution range of which is geographically located between them. This can be a result of the import of *A. c. japonica* and *A. c. ussuriensis* queen bees to South Korea, where they were later bred with the local *A. c. ussuriensis* drones.

As we know, the range of genetic divergence of 0.80–8.00% and the Jukes–Cantor genetic distance of 0.005–0.100 correspond to the range of intraspecific differences in insects [19, 25, 46, 47]. Values of genetic divergence and genetic distance based on mtDNA and the *VG* nDNA gene between *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, *A. c. cerana*, and *A. c. indica* are within the range of intraspecific differences between insect subspecies.

Since the divergence of mtDNA proceeds at a rate of 2.3% per 1 Ma [48, 49], we can calculate that the age of isolation and subsequent divergence of *A. c. ussuriensis* is probably 0.50 Ma (1.10%) from *A. c. japonica*, 0.56 Ma (1.30%) from *A. c. koreana*, 1.1 Ma (2.55%) from *A. c. cerana*, and 2.2 Ma (5.15%) from *A. c. indica* (Tables 4 and 5). The probable time of the emergence of *A. cerana* subspecies is approximately 2–1 Ma.

Thus, *Apis cerana ussuriensis* Ilyasov et al., 2019 is the least studied of all subspecies owing to the fact that it is rare and only occurs in the wild in the forests of Primorsky krai and Khabarovsk krai as far as 47°54′ N. Genetic studies of this subspecies are of great interest for science, since this subspecies was subjected to natural selection and adaptive evolution, where all the features of these bees would have been formed under the critical conditions of North Asia. We sequenced and annotated the complete mtDNA sequences of the subspecies *A. c. ussuriensis* (AP018450) and *A. c. koreana* (AP018431) and six exons of the vitellogenin *VG* nDNA gene of subspecies *A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, *A. c. cerana*, and *A. c. indica*. The mtDNA sequence is 15 919 bp in length, contains 84% AT and 16% GC, and includes 22 tRNA genes, 13 protein-coding genes, two rRNA genes (*16S rRNA* and *12S rRNA*), one AT-rich region, and four noncoding intergenic regions *NC 1–4*. All protein-coding genes begin with the ATT and ATG codons, with the exception of the *ATP8* gene with the ATC start codon, and end with the TAA and TAG stop codons. We show that, on the basis of the *NC1* region, *A. c. koreana* belongs to the *ACNC110* haplotype and *A. c. ussuriensis* belongs to the *ACNC111* haplotype, while on the basis of the *NC2* region, both subspecies belong to the *Japan1* haplotype. Cluster analysis of the mtDNA and the *VG* nDNA gene sequences showed the division of bees into two groups: the southern subspecies (*A. c. indica*) and the northern subspecies (*A. c. ussuriensis*, *A. c. koreana*, *A. c. japonica*, and *A. c. cerana*). We show that subspecies *A. c. ussuriensis* is genetically more similar to *A. c. japonica*, *A. c. koreana*, and *A. c. cerana* than to *A. c. indica*. The values of genetic divergence (0.80%–8.00%) and Jukes–Cantor genetic distance (0.005–0.100) for the mtDNA and *VG* nDNA gene between subspecies *A. c. ussuriensis* Ilyasov et al., 2019, *A. c. koreana* Ilyasov et al., 2019, *A. c. japonica* Radoszkowski, 1887, *A. c. cerana* Fabricius, 1793, and *A. c. indica* Fabricius, 1798 are within the intraspecific differences between subspecies.

ACKNOWLEDGMENTS

We are grateful to Dr. Hisashi Okuyama for the assistance with sequencing.

FUNDING

The present study was supported by the government assignment (registration no. 01201350736) (IR), by the Russian Foundation for Basic Research (no. 19-54-70002 e-Asia_t) (NA)), and by the postdoctoral research programs of Incheon National University 2017–2019 (IR).

COMPLIANCE WITH ETHICAL STANDARDS

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Ruttner, F., *Breeding Techniques and Selection for Breeding of the Honeybee*, Derby, UK: British Isles Bee Breeders Association, 1988.
2. Proshchalykin, M.Yu., Novomodnyi, E.V., Bezborodov, V.G., and Koshkin, E.S., The first modern findings of a wax bee *Apis cerana* Fabricius, 1793 (Hymenoptera, Apidae) in Khabarovsk Krai, *Evras. Entomol. Zh.*, 2014, vol. 13, no. 3, pp. 295—298.
3. Kuznetsov, V.N., *Kitaiskaya voskovaya pchela Apis cerana cerana F. (Hymenoptera, Apidae) na Dal'nem Vostoke Rossii* (Chinese Wax Bee, *Apis cerana cerana* F. (Hymenoptera, Apidae) in the Russian Far East), Moscow: KMK, 2005.
4. Behura, S.K., Analysis of nuclear copies of mitochondrial sequences in honeybee (*Apis mellifera*) genome, *Mol. Biol. Evol.*, 2007, vol. 24, no. 7, pp. 1492—1505. https://doi.org/10.1093/molbev/msm068
5. Choi, Y.S., Lee, M.Y., Hong, I.P., et al., Occurrence of sacbrood virus in Korean apiaries from *Apis cerana* 1 (Hymenoptera: Apidae), *J. Apic.*, 2010, vol. 25, no. 3, pp. 187—191.
6. Koetz, A.H., Ecology, behaviour and control of *Apis cerana* with a focus on relevance to the Australian incursion, *Insects*, 2013, vol. 4, no. 4, pp. 558—592. https://doi.org/10.3390/insects4040558

7. Vung, N., Lee, M.-L., Lee, M.-Y., et al., Breeding and selection for resistance to sacbrood virus for *Apis cerana, J. Apic.*, 2017, vol. 32, pp. 345—352. https://doi.org/10.17519/apiculture.2017.11.32.4.345
8. Pesenko, Yu.A., Lelei, A.S., Radchenko, V.G., and Filatkin, G.N., Chinese wax bee *Apis cerana cerana* F. (Hymenoptera, Apidae) in the Soviet Far East, *Entomol. Obozr.*, 1989, vol. 68, no. 3, pp. 527—548.
9. Zhuang, D., New subspecies of *Apis cerana* (in Chinese), *Southwest China J. Agric. Sci.*, 1989, vol. 2, pp. 61—65.
10. Zhen-Ming, J., Yang, G., Huang, S., et al., *The advancement of beekeeping science and technology in China, Honeybees in Mountain Agriculture*, Verma, L.R., Ed., New Delhi: Oxford and IBH Publ., 1992.
11. Diniz-Filho, J.A.F., Malapsina, O., and Pignata, M.I.B., Geographic variation in *Apis cerana indica* F.: a spatial autocorrelation analysis of morphometric patterns, *J. Apic. Res.*, 1993, vol. 32, pp. 65—72. https://doi.org/10.1080/00218839.1993.11101289
12. Engel, M.S., The taxonomy of recent and fossil honey bees (Hymenoptera, Apidae, *Apis*), *J. Hymenoptera Res.*, 1999, vol. 8, no. 2, pp. 165—196. https://doi.org/10.1007/978-1-4614-4960-718
13. Sugawara, M., Feral colonies of Japanese honey bees, *Apis cerana japonica* and their life history: 2. Natural nests and swarming, *Mitsubachi Kagaku (Honeybee Sci.)*, 2000, vol. 21, no. 1, pp. 35—39.
14. Hepburn, H.R., Smith, D.R., Radloff, S.E., and Otis, G.W., Infraspecific categories of *Apis cerana*: morphometric, allozymal and mtDNA diversity, *Apidologie*, 2001, vol. 32, no. 1, pp. 3—23. https://doi.org/10.1051/apido:2001108
15. Takahashi, J. and Yoshida, T., The origin of Japanese honey bee *Apis cerana japonica* inferred from mitochondrial DNA, *Mitsubachi Kagaku (Honeybee Sci.)*, 2003, vol. 24, no. 2, pp. 71—76.
16. Radloff, S.E., Hepburn, C., Hepburn, R.H., et al., Population structure and classification of *Apis cerana, Apidologie*, 2010, vol. 41, no. 6, pp. 589—601. https://doi.org/10.1051/apido/2010008
17. Takahashi, J., Wakamiya, T., Kiyoshi, T., et al., The complete mitochondrial genome of the Japanese honeybee, *Apis cerana japonica* (Insecta: Hymenoptera: Apidae), *Mitochondrial DNA, Part B*, 2016, vol. 1, no. 1, pp. 156—157. https://doi.org/10.1080/23802359.2016.1144108
18. Ilyasov, R.A., Park, J., Takahashi, J., and Kwon, H.W., Phylogenetic uniqueness of honeybee *Apis cerana* from the Korean peninsula inferred from the mitochondrial, nuclear, and morphological data, *J. Apic. Sci.*, 2018, vol. 62, no. 2, pp. 189—214. https://doi.org/10.2478/Jas-2018-0018
19. Ilyasov, R.A., Han, G.Y., Lee, M.L., et al., Phylogenetic relationships of Russian Far-East *Apis cerana* with other north Asian populations, *J. Apic. Sci.*, 2019, vol. 63, no. 2, pp. 297—322. https://doi.org/10.2478/JAS-2019-0024
20. Cornuet, J.-M., Garnery, L., and Solignac, M., Putative origin and function of the intergenic region between COI and COII of *Apis mellifera* L. mitochondrial DNA, *Genetics*, 1991, vol. 128, no. 2, pp. 393—403.
21. Garnery, L., Cornuet, J.-M., and Solignac, M., Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis, *Mol. Ecol.*, 1992, vol. 1, no. 3, pp. 145—154. https://doi.org/10.1111/j.1365-294x.1992.tb00170.x
22. Garnery, L., Mosshine, E.H., Oldroyd, B.P., and Cornuet, J.-M., Mitochondrial DNA variation in Moroccan and Spanish honey bee populations, *Mol. Ecol.*, 1995, vol. 4, pp. 465—472. https://doi.org/10.1111/j.1365-294X.1995.tb00240.x
23. Arias, M.C. and Sheppard, W.S., Molecular phylogenetics of honey bee subspecies (*Apis mellifera* L.) inferred from mitochondrial DNA sequence, *Mol. Phylogenet. Evol.*, 1996, vol. 5, no. 3, pp. 557—566. https://doi.org/10.1006/mpev.1996.0050
24. Songrarn, O., Sittipraneed, S., and Klinbunga, S., Mitochondrial DNA diversity and genetic differentiation of the honeybee (*Apis cerana*) in Thailand, *Biochem. Genet.*, 2006, vol. 44, nos. 5—6, pp. 256—269. https://doi.org/10.1007/s10528-006-9030-5
25. Tan, H.W., Liu, G.H., Dong, X., et al., The complete mitochondrial genome of the Asiatic cavity-nesting honeybee *Apis cerana* (Hymenoptera: Apidae), *PLoS One*, 2011, vol. 6, no. 8. e23008. https://doi.org/10.1371/journal.pone.0023008
26. Kent, C.F., Issa, A., Bunting, A.C., and Zayed, A., Adaptive evolution of a key gene affecting queen and worker traits in the honey bee, *Apis mellifera, Mol. Ecol.*, 2011, vol. 20, no. 24, pp. 5226—5235. https://doi.org/10.1111/j.1365-294X.2011.05299.x
27. Bernt, M., Donath, A., Jühling, F., et al., MITOS: improved de novo metazoan mitochondrial genome annotation, *Mol. Phylogenet. Evol.*, 2013, vol. 69, no. 2, pp. 313—319. https://doi.org/10.1016/j.ympev.2012.08.023
28. Lowe, T.M. and Eddy, S.R., tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence, *Nucleic Acids. Res.*, 1997, vol. 25, no. 5, pp. 955—964. https://doi.org/10.1093/nar/25.5.0955
29. Sanger, F., Nicklen, S., and Coulson, A.R., DNA sequencing with chain-terminating inhibitors, *Proc. Natl. Acad. Sci. U.S.A.*, 1977, vol. 74, no. 12, pp. 5463—5467. https://doi.org/10.1073/pnas.74.12.5463
30. Jukes, T.H. and Cantor, C.R., *Evolution of protein molecules, Mammalian Protein Metabolism*, Munro, H.N., Ed., New York: Acad. Press, 1969, pp. 21—132.
31. Tamura, K., Battistuzzi, F.U., Billing-Ross, P., et al., Estimating divergence times in large molecular phylogenies, *Proc. Natl. Acad. Sci. U.S.A.*, 2012, vol. 109, no. 47, pp. 19333—19338. https://doi.org/10.1073/pnas.1213199109
32. Nei, M. and Kumar, S., *Molecular Evolution and Phylogenetics*, New York: Oxford Univ. Press, 2000.
33. Kumar, S., Stecher, G., and Tamura, K., MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, *Mol. Biol. Evol.*, 2016, vol. 33, no. 7, pp. 1870—1874. https://doi.org/10.1093/molbev/msw054
34. Saitou, N. and Nei, M., The neighbor-joining method: a new method for reconstructing phylogenetic trees,

Mol. Biol. Evol., 1987, vol. 4, no. 4, pp. 406—425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

35. Tamura, K. and Nei, M., Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, *Mol. Biol. Evol.*, 1993, vol. 10, no. 3, pp. 512—526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
36. Crozier, R.H. and Crozier, Y.C., The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization, *Genetics*, 1993, vol. 133, no. 1, pp. 97—117. https://doi.org/10.1111/j.1365-2583.1993.tb00131.x
37. Okuyama, H., Tingek, S., and Takahashi, J., The complete mitochondrial genome of the cavity-nesting honeybee, *Apis cerana* (Insecta: Hymenoptera: Apidae) from Borneo, *Mitochondrial DNA, Part B*, 2017, vol. 2, no. 2, pp. 475—476. https://doi.org/10.1080/23802359.2017.1361344
38. Kent, C.F., Minaei, S., Harpur, B.A., and Zayed, A., Recombination is associated with the evolution of genome structure and worker behavior in honey bees, *Proc. Natl. Acad. Sci. U.S.A.*, 2012, vol. 109, no. 44, pp. 18012—18017. https://doi.org/10.1073/pnas.1208094109
39. Lee, J.Y., Wang, A.R., Choi, Y.S., et al., Mitochondrial DNA variations in Korean *Apis cerana* (Hymenoptera: Apidae) and development of another potential marker, *Apidologie*, 2016, vol. 47, no. 1, pp. 123—134. https://doi.org/10.1007/s13592-015-0381-y
40. Tan, Y.D., Wan, C.L., Zhu, Y.F., et al., An amplified fragment length polymorphism map of the silkworm, *Genetics*, 2001, vol. 157, no. 3, pp. 1277—1284.
41. Smith, C.R., Smith, C.D., Robertson, H.M., et al., Draft genome of the red harvester ant *Pogonomyrmex barbatus, Proc. Natl. Acad. Sci. U.S.A.*, 2011, vol. 108, no. 14, pp. 5667—5672. https://doi.org/10.1073/pnas.1007901108
42. Tian, D., Wang, Q., Zhang, P., et al., Single-nucleotide mutation rate increases close to insertions/deletions in eukaryotes, *Nature*, 2008, vol. 455, no. 7209, pp. 105—108. https://doi.org/10.1038/nature07175
43. McDonald, M.J., Wang, W.C., Huang, H.D., and Leu, J.Y., Clusters of nucleotide substitutions and insertion/deletion mutations are associated with repeat sequences, *PLoS Biol.*, 2011, vol. 9, no. 6. e1000622. https://doi.org/10.1371/journal.pbio.1000622
44. Koren, A., Polak, P., Nemesh, J., et al., Differential relationship of DNA replication timing to different forms of human mutation and variation, *Am. J. Hum. Genet.*, 2012, vol. 91, no. 6, pp. 1033—1040. https://doi.org/10.1016/j.ajhg.2012.10.018
45. Seplyarskiy, V.B., Kharchenko, P., Kondrashov, A.S., and Bazykin, G.A., Heterogeneity of the transition/transversion ratio in *Drosophila* and Hominidae genomes, *Mol. Biol. Evol.*, 2012, vol. 29, no. 8, pp. 1943—1955. https://doi.org/10.1093/molbev/mss071
46. Han, T., Lee, W., Lee, S., et al., Reassessment of species diversity of the subfamily Denticollinae (Coleoptera: Elateridae) through DNA barcoding, *PLoS One*, 2016, vol. 11, no. 2. e0148602. https://doi.org/10.1371/journal.pone.0148602
47. Eimanifar, A., Kimball, R.T., Braun, E.L., et al., The complete mitochondrial genome of the Egyptian honey bee, *Apis mellifera lamarckii* (Insecta: Hymenoptera: Apidae), *Mitochondrial DNA, Part B*, 2017, vol. 2, no. 1, pp. 270—272. https://doi.org/10.1080/23802359.2017.1325343
48. DeSalle, R., Freedman, T., Prager, E.M., and Wilson, A.C., Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila, J. Mol. Evol.*, 1987, vol. 26, nos. 1—2, pp. 157—164. https://doi.org/10.1007/BF02111289
49. Johns, G.C. and Avise, J.C., A comparative summary of genetic distances in the vertebrates form the mitochondrial cytochrome b gene, *Mol. Biol. Evol.*, 1998, vol. 15, pp. 1481—1490. https://doi.org/10.1093/oxfordjournals.molbev.a025875

Translated by A. A. Lisenkova

SPELL: 1. sacbrood