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Evidence of Northward Expansion of a Southern Carnivore: Genetic Identification of the Golden Jackal *Canis aureus* L. from the Central Non-Black Earth Region and the Dynamics of Its Range in the Historical Period

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Abstract—The results of molecular genetic identification of two individuals of the golden jackal, *Canis aureus* Linnaeus 1758 (Carnivora, Mammalia), hunted in Vladimir oblast of Russia are presented. The initial morphological diagnosis was confirmed by both the maternal (sequenced fragment of the mtDNA cytochrome *b* gene) and paternal (fragment analysis of amplicons of the *ZfY* gene localized on the Y chromosome) lineages. Both individuals were identified as golden jackals, the sequences of which correspond to the European—Middle Eastern lineage of *C. aureus*. The data presented confirm new records of the golden jackal outside the stable part of the historical range of the species—in the northern part of the front of its dispersal in the Central Non-Black Earth Region. These facts are analyzed in the context of the expansion of the golden jackal range to the central and northern parts of the Russian Plain.

Keywords: golden jackal, range expansion, genetic identification, mtDNA, Y-chromosome markers, hybridization, introgression

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INTRODUCTION

In modern times, many Palearctic vertebrate species, including some predatory mammals, usually considered to be elements of more southern ecosystems, have shown northward expansion into habitats not typical for them. The process of range expansion or shift is rightly considered as a consequence of ecosystem restructuring due to global warming (Williams and Blois, 2018). Milder winters in the boreal—Arctic zone and the increasing capacity of ecotopes due to the abundance of prey contribute to such invasions in the meridional direction. The golden (also known as common) jackal *Canis aureus* Linnaeus, 1758 as a representative of the family Canidae (order Carnivora, class Mammalia) belongs to the phylogenetic lineage of the wolf (*Canis lupus* Linnaeus, 1758, subtribe Canina, tribe Canini). The golden jackal occupies an extreme position in the genus of wolves and dogs (*Canis*), being the most divergent representative of the genus in its modern composition from the wolf—domestic dog pair. The golden jackal is a medium-sized predator, significantly smaller than a wolf, and accordingly, its diet consists largely of small vertebrates and invertebrates, as well as carrion and plant food. Unlike the wolf, the golden jackal does not hunt in large packs. The historical range of the golden jackal, which is more thermophilic than the wolf, occupied the territories of Eurasia from Southern Europe through the entire desert, semi-desert, and steppe zone of Eurasia to the east to Indochina. However, in recent decades, the range of the jackal has expanded significantly and climate niche modeling confirms the real ecological prospects of the species (Cunze and Klimpel, 2022). In a number of regions, the jackal recolonizes localities where it was exterminated in previous periods of time and expands its range to higher latitudes. Representatives of the species have begun to be noted in Western, Central, and Northern Europe up to the Baltics and Scandinavia, as well as on the Russian Plain. The latest findings in Moscow (Blokhina et al., 2018) and Arkhangelsk (Rykov et al., 2022) oblasts have been confirmed using molecular genetic identification methods and indicate processes of settlement on the territory of the Russian Federation similar to those observed in Central Europe and Eastern Europe outside of Russia.

Due to the ecological significance of the golden jackal as an element of ecosystems, namely the negative impact on the populations of birds, rodents, lagomorphs, small ungulates, and some predators (for example, the introduced raccoon) that it exterminates, monitoring the jackal invasion into boreal forest zone biotopes in Europe has become an important part of national (Dziech et al., 2023) and international (Hatlauf et al., 2021) environmental protection measures and initiatives. As a result of the interaction of climatic and anthropogenic factors, the restructuring of communities with the participation of predatory mammals often includes the appearance of the jackal in the local theriofauna. At the same time, the range of habitats suitable for it significantly expands, while the ecological niche of some autochthonous top predators narrows. A similar phenomenon has been described for the lynx *Lynx lynx,* a species that has the status of near extinction in many European countries. The lynx overlaps with the jackal in ecological preferences and experiences from the expansion of *C. aureus* an additional negative effect (Serva et al., 2023). In addition, the jackal, as a species that is fairly tolerant of humans, is exploring synanthropic habitats. Thus, in a densely populated region of Italy, the naturalization of the jackal with the formation of a breeding group has been noted (Travain et al., 2024).

The dispersal of invasive species can be recorded using telemetry—GPS and GSM trackers (Lanszki et al., 2018). However, not only a successful complementary method, but also the most effective method for mass application of monitoring biological invasions is molecular genetic analysis, which allows one not only to identify the species, but also to identify the sex, kinship of individuals, and in some cases their belonging to a particular donor population, as well as the levels of genetic diversity of pioneering groups. This communication is aimed to DNA identification of two individuals of the golden jackal from the Central Non-Black Earth Region, as well as to the analysis of the dynamics of the species range in Europe, including the European part of Russia.

MATERIALS AND METHODS

Biological material. Two individuals belonging to the genus *Canis*, morphologically corresponding to the description of the golden jackal, were obtained by hunters in Vladimir oblast (the eastern part of the Central Non-Chernozem zone of Russia) and transferred to the local hunting department, and then to the Federal Research Center for the Development of Hunting. The first individual was hunted on June 24, 2024, in the Kovrovskii district; the second, on October 20, 2024, in the Melenkovskii district of Vladimir oblast. Both representatives of the genus *Canis* were young adult males. Small fragments of tongue muscle tissue were taken from the frozen whole carcasses and stored in screw-type tubes with 96% ethanol until DNA extraction.

DNA analysis. Total DNA was isolated using DiaTom DNAPrep kits (Isogen Laboratories Ltd., Moscow); the lysis time was set to three hours. Ethanol-fixed tissue samples from four individuals of the golden jackal, harvested in the Azov district of Rostov oblast in January 2022 (collected by V.V. Stakheev) and used by us earlier to identify putative hybrid canids of the Voronezh Reserve and Dagestan (Kazimirov et al., 2024), were used as reference samples.

Identification was carried out using uniparental markers. Since both individuals were morphologically unambiguously identified as males, there was no need to use additional nuclear markers. Fragment of mitochondrial cytochrome *b* gene (cyt *b*) marked inheritance through the maternal line. For PCR amplification, a GeneExplorer GE-96G thermocyler (Bioer Technology Co LTD, China) and universal oligonucleotide primers MVZ04 and MVZ05 (Smith and Patton, 1991) were used. The protocol (Kheydorova et al., 2018) was modified: we used GenPak PCR Core PCR kits (Isogen Laboratories Ltd., Russia), as well as a modified PCR regime: initial denaturation at 95°C for 5 min; 35 cycles: denaturation at 95°C for 15 s, annealing at 45°C for 1 min, elongation at 72°C for 1 min; and final elongation at 72°C for 5 min. Sequencing of the amplified mtDNA fragments in both directions using primers MVZ04 and MVZ05 was carried out on the basis of the services of the Evrogen (Moscow, Russia).

The cytochrome *b* gene sequences of the golden jackal from various parts of its range were downloaded from NCBI GenBank for comparative analysis of the nucleotide sequences. Sequences were loaded using the Entrez package for the R environment (R Core Team, 2022) using the queries "term = 'Canis aureus[ORGN] AND cytb[Gene]'" and "term = 'Canis aureus[ORGN] AND cyb[Gene].'" The downloaded sequences were aligned with our obtained sequences using Geneious 9.1.8. For the further construction of the tree, identical sequences representing golden jackals from the same region were removed from the analysis to improve its clarity.

The haplotype tree using the maximum likelihood (ML) method was constructed using the IQTree service (Trifinopoulos et al., 2016; Kalyaanamoorthy et al., 2017; Minh et al., 2020) based on the TPM2 + I + F nucleotide substitution model, selected as optimal according to the Bayesian criterion (BIC). Branch node support was calculated using the UltraFast Bootstrap method for 1000 replications (Hoang et al., 2017). As outgroups, we used sequences from the grey wolf (*Canis lupus*)—both downloaded from GenBank and obtained in our previous study (Kazimirov et al., 2024)—as well as from the wild boar (*Sus scrofa*) and brown long-eared bat (*Plecotus auritus*). The tree was visualized graphically in the R environment (R Core Team, 2022) using the ggtree package (Yu et al., 2017, 2018; Yu, 2020, 2022).

To determine the species affiliation along the paternal line, a set of primers (YintF2, YintR, and Yint2-335) was used, developed specifically for the unambiguous detection of the presence or absence of an insertion in the zinc finger gene on the Y chromosome (*ZfY*) by the presence of one or two amplified fragments (Galov et al., 2014). In dogs and wolves, which have this insertion, two amplified fragments are observed on electrophoresis, while in jackals a single fragment is being detected. We used GenPak PCR Core PCR kits (Isogen Laboratories Ltd., Russia), as well as a modified PCR mode: initial denaturation at 95°C for 5 min; 35 cycles: denaturation at 95°C for 15 s, annealing at 58°C for 20 s, elongation at 72°C for 1 min, and final elongation at 72°C for 5 min. For fragment analysis of the PCR results, electrophoresis in horizontal blocks of agarose gel was used using a Tris-acetate-EDTAbuffer system and a 1 kb size standard ladder manufactured by Sibenzym Ltd. (Novosibirsk, Russia). Visualization was performed by staining the fragments with ethidium bromide and capturing images using a gel documentation system.

To reconstruct the dynamics of the range of the golden jackal, published monographs, articles, and dissertations were used, as well as data from the Mammals of Russia portal (*Mlekopitayushchie Rossii,* rusmam.ru) (Lissovsky et al., 2018) from the request for a map of *Canis aureus* records.

RESULTS

mtDNA. Identical cytochrome *b* gene sequences (449 nucleotides in length) were obtained for the two specimens studied. The sequences have been deposited in NCBI GenBank under accession numbers PQ723763 and PQ726764. A BLAST search against the GenBank database revealed maximum similarity with the reference mitochondrial genome of *C. aureus* (Table 1). The sequences differed by a single nucleotide substitution outside the cytochrome *b* gene (99.77% identity). Complete sequence identity was also observed in overlapping regions with sequences we previously obtained (Kazimirov et al., 2024) for jackals from Rostov oblast (accession numbers PP166661–PP166664). Thus, the mitochondrial data confirm the maternal lineage of the studied specimens as *C. aureus* (Table 1).

A 320-nucleotide alignment was obtained corresponding to positions 14249–14566 of the jackal reference mitochondrial genome, accession number NC067757.1. Based on the results of alignment and construction of the ML tree (Fig. 1), there was a correspondence between the obtained sequences and the sequences obtained from golden jackals in Serbia, Israel, Egypt, Turkey, and Russia (the above-mentioned jackals from the Rostov region). Jackals from India and Afghanistan formed a more distant group (which, however, included a jackal from Bulgaria).

***Y*-chromosome.** Fragment amplification of *ZfY* with internal primers YintF2, YintR, and Yint2-335 showed the identity of the spectrum, single-band and matching in length with previously studied golden jackal samples and corresponding to the size described in the literature for the golden jackal, and differing from those typical for the grey wolf and domestic dog (Fig. 2).

Table 1. Results of BLAST analysis of 449 bp sequences. Ten best matches from search results

Sequence number	BLAST grade, %	Species
NC067757	99.9	*C. aureus*
MZ433379	99.8	*C. aureus*
KT448274	99.7	*C. aureus*
ON986207	98.3	*C. aureus*
NC027956	98.0	*C. anthus = C. lupaster*
MZ433368	98.0	*C. lupaster*
KT448273	98.0	*C. aureus*
KT448272	98.0	*C. aureus*
KT378607	98.0	*C. anthus = C. lupaster*
KT378606	98.0	*C. anthus = C. lupaster*

The BLAST grade corresponds to a weighted score of identity based on the fragment length, number of substitutions, and E-value (the probability of random sequence matches). The ten best matches are shown.

DISCUSSION

DNA analysis of the two individuals killed by hunters, morphologically corresponding to the description of the golden jackal, showed that they belong to the species *C. aureus* by the maternal (sequence of the mitochondrial cytochrome gene *b*) and paternal (Y-chromosome markers) lines. Genetic identification helps build evidence of the spread of *C. aureus*, in this case from a new habitat in the northeastern part of the Non-Black Earth Zone of Russia. In this context, it is interesting to trace the dynamics of the range of the golden jackal in Europe, and, in more detail, in Russia and the former Soviet Union.

In southern Europe, the originally larger historical range of the golden jackal had been significantly reduced by the 1960s as a result of the loss of suitable habitats and the use of poisoned bait (Spassov, 1989). After the introduction of protective measures in 1962, the species gradually began to restore its lost range from the few places where it persisted (Peloponnese, Dalmatian coast, Aegean Macedonia, and Strandzha National Park on the border of Bulgaria and Turkey). Thus, having recolonized Bulgaria (Spassov, 1989), Romania, and Serbia (Kryštufek et al., 1997), the jackal moved further to the northwest, north, and northeast in the 1980s and 1990s and was recorded in Italy, Austria, Croatia, Hungary, and Slovakia (Arnold et al., 2012).

The jackal has demonstrated further northward expansion in Central, Eastern, and Northern Europe

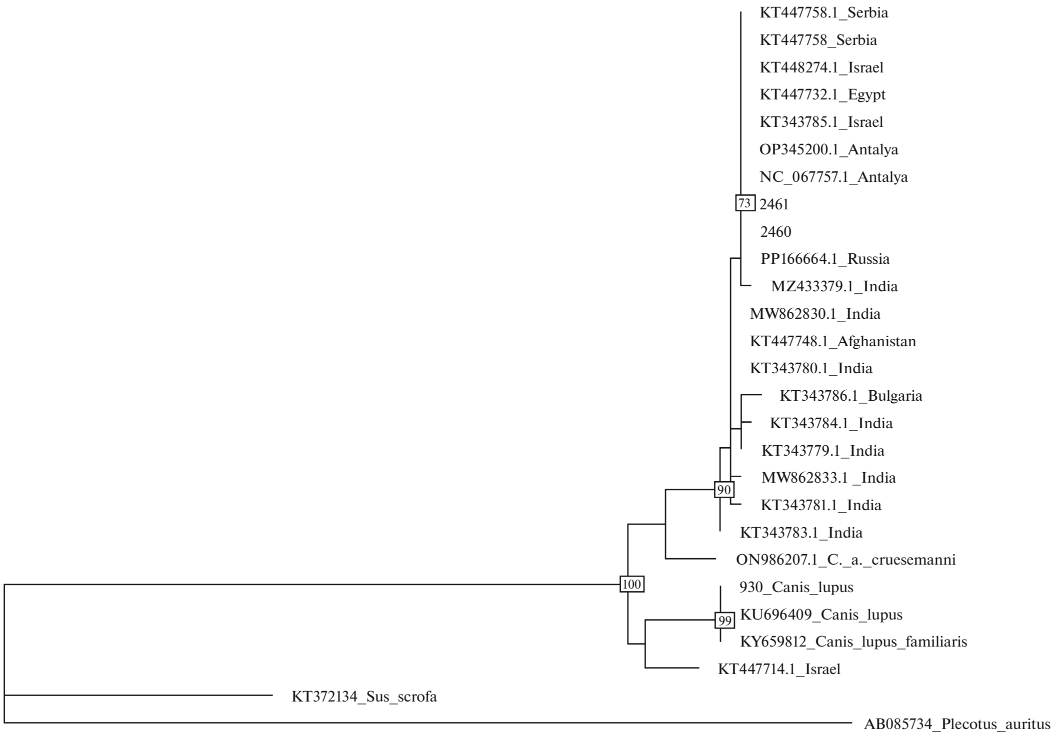

Fig. 1. ML tree of haplotypes of aligned sequences of a 320 bp fragment of cytochrome *b* of identified specimens (2460 and 2461) and golden jackal sequences from GenBank.

from the end of the 20th century to the present day. The extreme points of sporadic distribution of the jackal in Western Europe include regions as remote from the main range as the Spanish Pyrenees (Bogdanowicz et al., 2024), northwestern and southern France, and the Netherlands (https://www.lcie.org/Large-carnivores/Golden-jackal). The distribution limit has almost everywhere reached territories located near the coasts of the North Sea and the Baltic Sea: in Denmark (Trolle, 2015), Poland (Kowalczyk et al., 2015, 2020), Estonia (Männil and Ranc, 2022), Lithuania (Paulauskas et al., 2018), and Finland (Honkala and Nummi, 2019; Kojola et al., 2023; Bogdanowicz et al., 2024). Finally, the jackal has recently been recorded in Norway (Sørensen and Lindsø, 2021; Bogdanowicz et al., 2024).

Moreover, not only are findings beyond the previous distribution boundaries recorded, but so is reproduction of the species in new areas. In the 21st century, the golden jackal continues to naturalize and integrate into ecosystems in the countries of Southern, Central, and Eastern Europe. Reproduction has been noted since 2008 in Austria (Herzig-Straschil, 2008), and in 2022 in the densely populated Tuscan Apennines in Italy (Bacci and Lunghi, 2022). In 2021–2022, jackal reproduction was recorded in Germany (Böcker et al., 2023). Similar processes are observed in Eastern Europe, for example, in Hungary and the Czech Republic (Jirků et al., 2018). In Poland in 2020, for the first time (Lanszki et al., 2018), jackal breeding was recorded at latitude 53.7° N, in a habitat located in the north of the country, approximately 500 km north of a previously recorded similar case in the Czech Republic. The most active, stable breeding population of the golden jackal in Europe is the Estonian subpopulation (Männil and Ranc, 2022), which apparently serves as a secondary dispersal center for Northern and Eastern Europe.

For the fauna of Belarus, the golden jackal is a fairly new species; records have been mentioned in the literature since 2011 and geographically they cover mainly the southern and southwestern parts of the country, up to the Kobrin–Soligorsk–Bobruisk line (Grichik et al., 2018). For three of the six individuals encountered, population genetic analysis was performed based on sequences of the mtDNA control region, showing 98% identity with jackals previously deposited in the database and significantly higher divergence estimates with wolves and dogs, as well as coyotes, red foxes, Arctic foxes, and raccoon dogs. In the study of individuals of the genus *Canis* with a dubious species diagnosis from the territory of Belarus (Kheydorova et al., 2018), two of the three individuals analyzed for the cytochrome gene *b* turned out to be wolves according to this marker (inherited through the

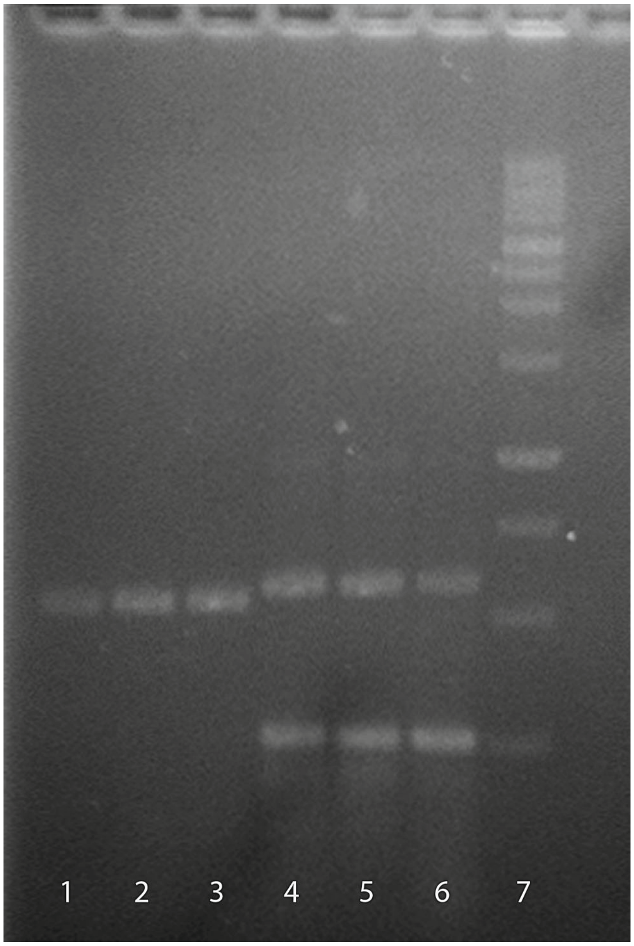

Fig. 2. Electropherogram of gene amplification products *ZfY,* localized on the Y chromosome. Track numbers: 1, 2, studied individuals with the jackal phenotype; 3, golden jackal (Rostov oblast); 4, 5, domestic dog; 6, grey wolf; 7, molecular marker of 1 kb fragment lengths.

maternal line), and one sample showed compliance with the species diagnosis of a golden jackal.

The golden jackal entered the territory of the Ukrainian SSR starting in the middle of the 20th century, but its location in the Severskii Donets River basin (Luhansk oblast of the Ukrainian SSR) is documented only from the words of local residents (Zagorodnyuk, 2014). Based on the map provided and the data analysis based on it (Zagorodnyuk, 2014), three migration routes can be traced to Transcarpathia and the Carpathian region from Romania and Hungary (Pannonia) and, possibly, Slovakia. In addition, the jackal spread from Romania and Moldova to the Northern Black Sea region through the lower reaches of the Danube and Dniester rivers, and this route continues further north, to Polesia, and probably even further north, to Belarus. Finally, since the end of the 20th century, the jackal has also penetrated into Crimea and the Azov Sea region from the east, from the Caucasus and Kuban, probably through the lower reaches of the Don River (but possibly also through the Kerch Strait on the ice). As of 2018, the northernmost location was noted to be a find in the Zhytomyr region (Zagorodnyuk, 2014).

All this together with the proven increasing pressure from the jackal on local populations and ecosystems is causing concern among scientific and environmental organizations (Trouwborst et al., 2015; Ranc et al., 2018; Hatlauf et al., 2021). The importance of monitoring canid populations, which may include hybrid individuals, is emphasized.

In Russia, the golden jackal, as a species with an expanding range and relatively prosperous, does not have a special conservation status, but at the same time, systematic regulation of its numbers is not carried out either within the stable part of the range or in new regions. In this regard, it is interesting to trace how the range of this species has expanded over the past century and a half.

Dynamics of the range of the jackal in the Russian Empire, Soviet Union, and post-Soviet Russia in the 20th–21st centuries. Since the end of the 19th century, researchers have noted not only that the golden jackal in Russia and the Soviet Union is a southern species, but also that there is extreme dynamism of its distribution boundaries. In addition to the Caucasus and Transcaucasia, the jackal used to inhabit the southeastern part of Turkmenistan, the foothills of Tajikistan, along the Amu Darya River, and in the middle reaches of the Syr Darya River and the lower reaches of the Zarafshon River (Novikov, 1956). The Caucasian–Transcaucasian population was the most studied by the beginning of the 20th century. K.A. Satunin (1915) noted that its relatively stable northern border on the Caspian is the mouth of the Terek. A.K. Tembotov (1972) described the distribution of the species in the Caucasus in approximately the same way for the middle of the 20th century. "It is distributed from the southeastern part of the North Caucasus to Mozdok, where it prefers to live on the plains and low mountains at an altitude of up to 1000 m above sea level." At the same time, constant fluctuations in the range in the Caucasus with a description of the western and eastern routes by which the jackal bypassed the Greater Caucasus Range were cited by many authors. The most informative review of this issue can be found in Yu.N. Bakeev (1978). From 1950 to the mid-1960s in the Caucasus and Ciscaucasia, significant volumes of jackal skin procurement took place only in Krasnodar krai and the Chechen–Ingush and Dagestan ASSRs (Autonomous Soviet Socialist Republics). However, since the late 1960s, jackals have also been hunted in Kabardino-Balkaria, North Ossetia, and Stavropol krai, and sporadic encounters with the predator have been recorded in Rostov oblast since the 1960s. In the same work, the author provides a map showing jackal visits to the territory of the European part of the Soviet Union to the north beyond the natural range known at the end of the 1970s. In addition to repeated cases of jackal hunting in the Ciscaucasia, the map also shows localities much further north than the line formed by the lower reaches of the Don River, the Volgodonsk Canal, and the lower Volga River. This more southern point in the interfluve of the Severskii Donets River and the Don is geographically close to locality "A" in the Luhansk region of the Ukrainian SSR, discussed in the paper (Bakeev, 1978). Two more points in the upper reaches of the Don and three points in the interfluve of the Don and Volga are located within the Black Earth Zone. At the same time, the data in the Table 7 in the same work by Yu.N. Bakeev (1978) testify that already in the middle of the 20th century, jackal visits were recorded in the eastern Non-Black Earth Region in Gorky (now Nizhny Novgorod) oblast, Tatarstan, Chuvashia, Bashkiria, and even Perm oblast. These cases, however, were rare and were probably to some extent connected with the weakening of human economic activity during the Great Patriotic War (1941–1945).

The expansion of the golden jackal in the meridional direction has continued over the past two decades. In addition to the Caucasus and Kuban, where the jackal is continuously increasing in number and occupying new areas (Yarovenko et al., 2014; Yarovenko, 2022), the species, apparently settling along the Don valley, has advanced into Voronezh and Tambov oblasts of the Chernozem region and, apparently, further into the Serebryanoprudskii district of Moscow oblast (portal Mammals of Russia, rusmam.ru) (Lissovsky et al., 2018). Another route can be traced along the Volga River through the most stable golden jackal grouping in Saratov oblast, then to the northeast to Mordovia, Chuvashia, and Tatarstan. It is likely that from this route there may also be an eastern branch of the migration route towards Orenburg; however, independent resettlement of the jackal there through Kazakhstan should also be considered. In general, the expansion of the Central Asian and Kazakh jackal populations to the north, if it takes place, is either not as pronounced as in Europe or less thoroughly documented for the past decade. The jackal penetrated into southern Kazakhstan and spread along the Ili River in the 1980s (Musabekov, 2014). Currently, there is data on the species records in most regions of Kazakhstan (Kyzylorda, Almaty, Atyrau, Zhambyl, Mangistau, Turkestan, and West Kazakhstan) (https://www.inform.kz/ru/volki-i-shakaly-otsutstvuyut-na-territorii-sko_a3942837), but corresponding information is absent from the North Kazakhstan region.

Encounters of golden jackals in the wild are regularly recorded on the website Mammals of Russia (Lissovsky et al., 2018). We analyzed the possible routes by which the species could have spread beyond its main range (Fig. 3). In the locality west of Moscow, the dispersal could have proceeded from the southwest through Bryansk oblast, where the jackal penetrates from the Ukrainian and Belarusian Polesie. Finds in Pskov and Leningrad oblasts could also be explained by dispersal both through the Polesie corridor and through the Baltics from a donor population with a center in Estonia. For Pskov oblast, a detailed study of the craniology and the mtDNA control region confirmed the conclusion about the identification of the hunted individual as a golden jackal (at least on the maternal line), its belonging to the European–Asia Minor macropopulation of the species, and its dispersal to the north through one of the Eastern European corridors (Korablev et al., 2024). The jackal has also been noted in Tver oblast, according to survey data from the Federal Research Center for the Development of Hunting (Federal State Budgetary Institution

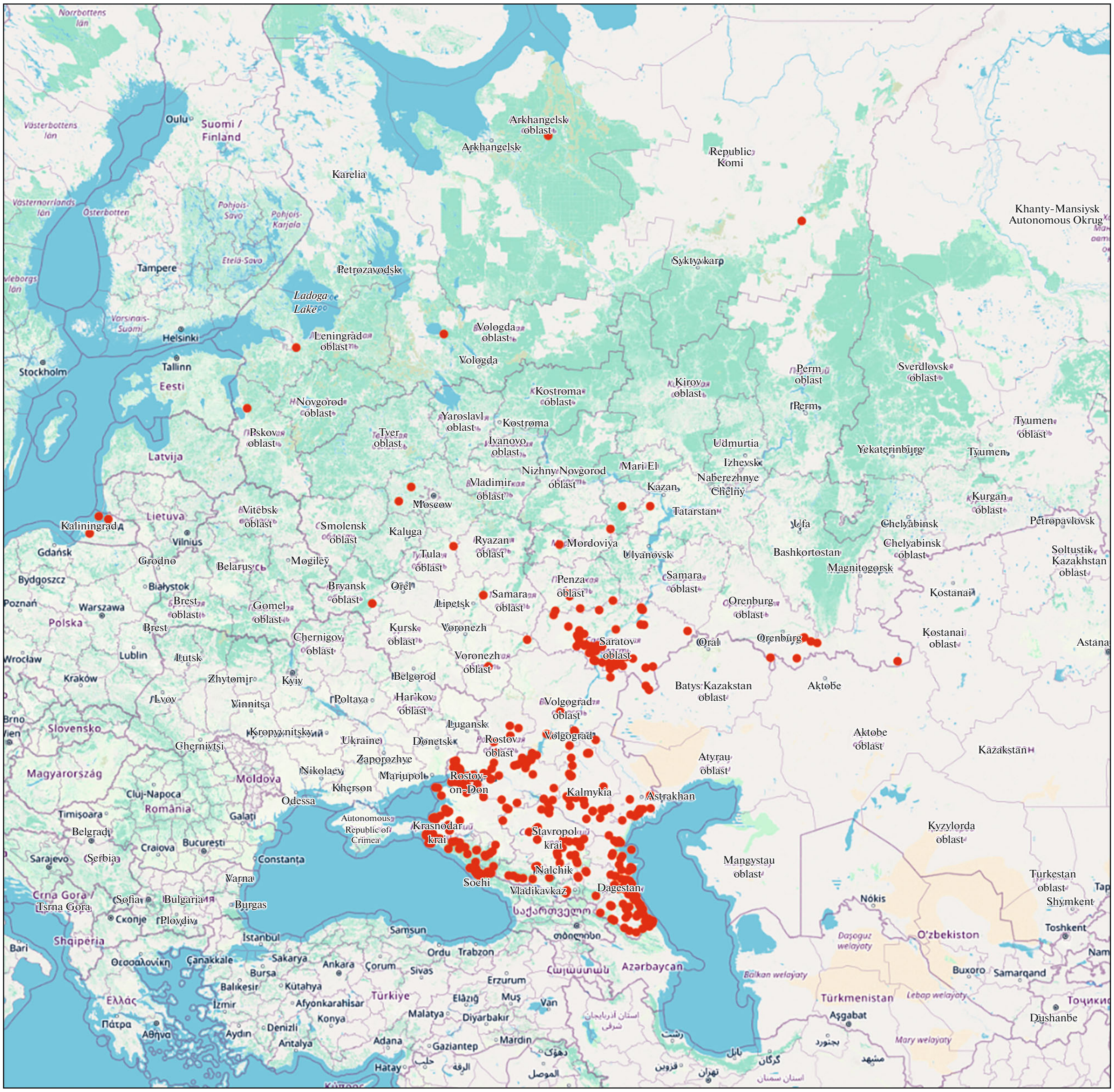

Fig. 3. Map of golden jackal records in Russia (according to the portal Mammals of Russia, rusmam.ru).

‘Okhota’). The jackal rarely ventured further north; encounters were recorded in Vologda and Arkhangelsk oblasts (rusmam.ru) (Lissovsky et al., 2018). The latest case from the Pinezhskii district is described in detail, providing the results of not only morphological but also molecular genetic analysis of the sequences of the mtDNA control region (Rykov et al., 2022), which allowed the specimen to be identified as a golden jackal. Finally, the most northeastern place where the jackal was found, according to the website rusmam.ru (Lissovsky et al., 2018), was linked to a photograph of an individual hit by a car in the Troitse-Pechorskii district of the Komi Republic; however, it is not documented in much detail and, given the significant distance from other finds, requires verification.

Previously published results of genetic identification of the jackal in Europe using sequencing of the mtDNA control region made it possible to establish the relative homogeneity of the maternal lineage of the species and the absence of mitochondrial lines of the Indian subspecies; this also applied to the individual obtained in Arkhangelsk oblast (Rykov et al., 2022). The cytochrome *b* sequences studied in this work and comparative analysis also allow us to classify the jackals we studied as a donor population of the European subspecies *C. aureus moreoticus* or a closely related type subspecies of the Persian (Asian) jackal *C. aureus aureus*, and not to the populations of the southern Middle East, Hindustan, or Indochina (Fig. 1), which is quite logical based on geographical considerations. Taking into account similar conclusions from the results of the study of finds in Pskov oblast (Korablev et al., 2024) and our data on Vladimir oblast, the belonging of the settling jackals to these two subspecies (which some experts consider to be one) is quite obvious.

An even more detailed molecular genetic study of the origins of three male golden jackals from Spain, Finland, and Norway, based on population assignment technologies, used both autosomal microsatellites and the mtDNA control region (Bogdanowicz et al., 2024) showed that the first two individuals originate from Pannonia or the Balkans, and the Norwegian one from the Caucasus or from a secondary center of settlement in the Baltics.

Hybridization and identification of hybrids. In recent decades, due to the shift in the boundaries of habitats and ecological niches, a process of intensification of interspecific gene flow has been observed in many groups of animals and plants. Of particular interest is the possibility of using molecular methods to identify hybrids between wild and domesticated animal species, including predatory mammals, and, in particular, representatives of the genus *Canis*. The golden jackal can potentially form hybrids with another wild species of the genus *Canis,* the wolf; however, numerous attempts to identify similar hybrids in Europe have not yielded significant results. Apparently, such cases, if possible in principle, occur extremely rarely. Our genetic studies using the same molecular markers as in this work on the enigmatic canids of the Voronezh Reserve and Daghestan did not clearly reveal golden jackal blood in them; that is, they were not hybrids *C. lupus*/*C. familiaris* × *C. aureus* of the first generation (Kazimirov et al., 2024), while maintaining the possibility that they could be backcrosses, tracing their lineage back to distant events of primary interspecies contact. However, wild canids across Europe consistently show evidence of both wolf–dog hybrids (Stronen et al., 2022; Dziech et al., 2013) and jackal–dog hybrids (Galov et al., 2014; Ninausz et al., 2023). The latter often exhibit melanistic or merle coat patterns atypical for golden jackals, while light-colored or white individuals may carry corresponding mutations yet still be identified as pure jackals (Ninausz et al., 2023). Different canid species pairs demonstrate varying hybridization potential (Gopalakrishnan et al., 2018). Dogs, having undergone millennia of domestication with their genomes shaped by artificial selection, clearly hybridize more readily with other *Canis* species than wild canids do. A remarkable recent case involved hybridization between a pampas fox (*Lycalopex gymnocercus*) and a domestic dog in Brazil (Szynwelski et al., 2023). A roadkill specimen displaying unusual phenotypic characteristics was genetically confirmed as a hybrid between these two canid lineages that had diverged during the Miocene. Mitochondrial DNA identified the mother of the female as a pampas fox, while nuclear markers revealed a domestic dog father, with the hybrid showing an intermediate chromosome number ($2n = 76$) between the parental species. The role of free-ranging (feral) domestic dogs in hybrid population establishment and genetic introgression among wolves, jackals, and dogs warrants more rigorous investigation.

CONCLUSIONS

Thus, in this report we present data on a new discovery of the golden jackal outside the stable part of its historical range—in the northern part of the known frontier of settlement in the Central Non-Black Earth Region. We support the initial species diagnosis with molecular genetic data on the maternal and paternal lines, excluding for these individuals their possible hybrid nature (at least they are not descendants of the first generations of hybridization). We analyze these findings in the context of the clear fact of the expansion of the golden jackal's range, a process that occurred in the past as a sporadically observed phenomenon, but that is now acquiring a more intense character, with the formation of breeding populations and secondary centers of settlement, as well as migration routes along large rivers. However, now the expansion of the species to higher latitudes has a certain ecological–evolutionary perspective, since it is moderated by the high density of the species in southern habitats, which causes migrations of a certain part of the population in the northerly direction. In addition, the observed expansion of the range is associated with a general trend towards a milder climate on the Russian Plain, facilitating the naturalization of the species, if not in the extreme conditions of the tundra and forest–tundra, then at least in the zone of mixed boreal forests and the southern taiga. The process of expansion of the jackal's range and the impact of the species on boreal ecosystems should be included in national environmental monitoring programs using molecular genetic analysis and remote tracking as effective tools and integrated with international tools for analysis and decision-making in this area.

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

No ethical standards were violated in the treatment of animals; no animals were obtained specifically for this study. All samples were taken from legally harvested individuals. All applicable international, national, and/or institutional guidelines for the use of animals were followed.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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