

1-1-2016

Genetic characterization of the raccoon dog (*Nyctereutes procyonoides*), an alien species in the Baltic region

ALGIMANTAS PAULAUSKAS

LORETA GRICIUVIENE

JANA RADZIJEVSKAJA

VACLOVAS GEDMINAS

Follow this and additional works at: <https://journals.tubitak.gov.tr/zoology>



Part of the [Zooology Commons](#)

Recommended Citation

PAULAUSKAS, ALGIMANTAS; GRICIUVIENE, LORETA; RADZIJEVSKAJA, JANA; and GEDMINAS, VACLOVAS (2016) "Genetic characterization of the raccoon dog (*Nyctereutes procyonoides*), an alien species in the Baltic region," *Turkish Journal of Zoology*. Vol. 40: No. 6, Article 11. <https://doi.org/10.3906/zoo-1502-34>

Available at: <https://journals.tubitak.gov.tr/zoology/vol40/iss6/11>

This Article is brought to you for free and open access by TÜBİTAK Academic Journals. It has been accepted for inclusion in Turkish Journal of Zoology by an authorized editor of TÜBİTAK Academic Journals. For more information, please contact academic.publications@tubitak.gov.tr.

Genetic characterization of the raccoon dog (*Nyctereutes procyonoides*), an alien species in the Baltic region

Algimantas PAULASKAS^{1*}, Loreta GRICIUVIENĖ¹, Jana RADZIJEVSKAJA¹, Vaclovas GEDMINAS²

¹Department of Biology, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania

²Kaunas Tadas Ivanauskas Museum of Zoology, Kaunas, Lithuania

Received: 20.02.2015 • Accepted/Published Online: 07.07.2015 • Final Version: 06.12.2016

Abstract: The raccoon dog *Nyctereutes procyonoides* is a newly established alien species in Europe that spread rapidly into many European countries. They were introduced into Latvia, Estonia, and Belarus. Lithuania has been colonized by immigrants from neighboring countries (Belarus and Latvia) and since 1960 the species has occupied the whole country. The data on genetic diversity of the raccoon dog are rather limited, and not all parts of the Baltic region have been covered by previous analyses. In order to compare the level of genetic diversity and describe phylogeographic patterns throughout the raccoon dog distribution in the Palearctic region, a mitochondrial DNA control region was used, and the current 22 sequences were combined with the sequences from earlier data (n = 32). The analysis of a 540-bp fragment of the mtDNA control region in raccoon dogs inhabiting Lithuania revealed 9 haplotypes with 19 variable positions. The phylogenetic relationships among the haplotypes demonstrated the presence of two haplogroups. The patterns of molecular genetic variation in raccoon dogs from Lithuania obtained in the present study indicated higher genetic diversity of these animals as compared with those from West Europe, but lower genetic polymorphism as compared with raccoon dogs introduced to the European part of Russia.

Key words: Invasive species, *Nyctereutes procyonoides*, mitochondrial DNA, control region, Baltic region

1. Introduction

The raccoon dog *Nyctereutes procyonoides* (Gray, 1834) is a newly established alien species that has had a long and complicated history of introduction and immigration within Europe. The historical distribution of this species was in the Far East, from northern Indochina to the southeast corner of Russia, and also in Mongolia and in the Japanese Archipelago. The native range covers East Asia, the Amur-Ussuri region in Russia, northern Vietnam, Korea, China, and Japan (Lavrov, 1971; Kauhala and Saeki, 2004). Currently six subspecies of *N. procyonoides* are recognized: *N. p. ussuriensis* inhabits Russia, northeastern China, and Eurasia; *N. p. procyonoides* inhabits Vietnam and southern China; *N. p. albus* and *N. p. viverrinus* inhabit Japan; *N. p. orestes* inhabits China; and *N. p. koreensis* inhabits the Korean Peninsula (Hong et al., 2013).

The introduction of raccoon dogs as fur-bearing animals into Siberia and the European part of the former Soviet Union started in 1929 and continued until 1955 (Kauhala and Saeki, 2004; Ansorge et al., 2009). One part of these animals was introduced to Europe from breeding farms and another part from reacclimatized

sites after World War II. The majority of captive bred raccoon dogs of the subspecies *N. p. ussuriensis* (about 9000–10,000 animals) were released in several places during the 1940s and 1950s (Lavrov, 1971; Kauhala and Saeki, 2004; Ansorge et al., 2009) (Figure 1a). After active introductions and acclimatization in the European part of Russia, the raccoon dog has dispersed into new areas without active human support and has spread rapidly into many European countries (Bobrov et al., 2008; Ansorge et al., 2009; Drygala et al., 2010; Kauhala and Kowalczyk, 2011).

Today *N. p. ussuriensis* is abundant throughout Finland, Sweden, Estonia, Latvia, Lithuania, Belarus, western Russia, Poland, Ukraine, and Germany, and it has also been reported in the Czech Republic, Slovakia, Hungary, Bulgaria, Moldova, Romania, Serbia, France, Switzerland, and Italy (Long, 2003; Genovesi, 2009; Kauhala and Kowalczyk, 2011). Although the precise origin of the specific introduced populations is unknown, it seems that they have descended from populations inhabiting the easternmost part of the former Soviet Union (Ansorge et al., 2009; Pitra et al., 2010; Korabiev et al., 2011). In

* Correspondence: a.paulaskas@gmf.vdu.lt

Lithuania raccoon dogs have been noticed since 1948 (Prūsaitė et al., 1988; Balčiauskas, 1996). The first animals were observed in the eastern part of the country. Showing great plasticity in adaptation to various environmental and climatic conditions, raccoon dogs spread into different areas of Lithuania and by 1960 had colonized the whole country (Prūsaitė et al., 1988). After this expansion, raccoon dogs prospered until the 1980s. Then they were affected by an intense hunting pressure and also by rabies. Since 1970 the species has been declared as invasive, and hunting of these animals has been permitted throughout the year.

The use of genetic tools in the investigation of invasive species has increased over the past two decades. Mitochondrial DNA (mtDNA) is highly variable and can render information on historical patterns of population demography, admixture, biogeography, and speciation (Avice, 2000; Ballard and Whitlock, 2004). In recent studies (Pitra et al., 2010; Korablev et al., 2011), polymorphism of the mtDNA control region has been examined in order to estimate the genetic diversity and understand the phylogeography of the invasive raccoon dogs. The distribution of genetic variation within and among introduced populations of the raccoon dog in Russia, Finland, and Germany (Pitra et al., 2010; Korablev et al., 2011) was investigated. It was considered that raccoon dogs from introduced sites in northwestern European Russia had spread over the Baltic region through Poland to West Europe (Korablev et al., 2011). Genetic analysis showed a close relationship between matrilineages of German and Finnish populations and confirmed that

raccoon dogs had colonized Germany from Finland along the Baltic Sea coastline (Pitra et al., 2010). However, the data on genetic variability and population structure of the invasive raccoon dogs in colonized areas in the Baltic region are still scarce, and nothing is known about the genetic diversity of raccoon dogs inhabiting Lithuania.

The goal of this study was to assess the phylogeographic structure of raccoon dogs from the Baltic region based on mtDNA control region sequences and to compare results with other studies conducted in the Palearctic region.

2. Materials and methods

2.1. Sample collection and DNA isolation

Muscle samples from 21 raccoon dogs legally harvested by hunters were collected from different locations in Lithuania. One specimen of raccoon dog was found road-killed in northern Latvia (Jelgavkrasti) (Figure 1b). The geographic distance between each pair of sampling sites was 30–50 km. Total genomic DNA was extracted from tissue samples using a Genomic DNA purification kit (Thermo Scientific, Lithuania) according to the protocol of the manufacturer.

2.2. PCR and sequencing

Amplification of a 565-bp fragment of the mtDNA control region was performed using the newly designed primers Rac-1F 5'-TCG TGC ATT AAT GGC TTG C-3' and Rac-1R 5'-CCA TTG ACT GAA TAG CAC CTT G-3'. PCR was performed in 25-μL volumes including: 1X PCR buffer (Thermo Scientific), 2 mM MgCl₂, 0.4 μM of each primer, 2 mM dNTPs, 2 U of Taq DNA polymerase, and

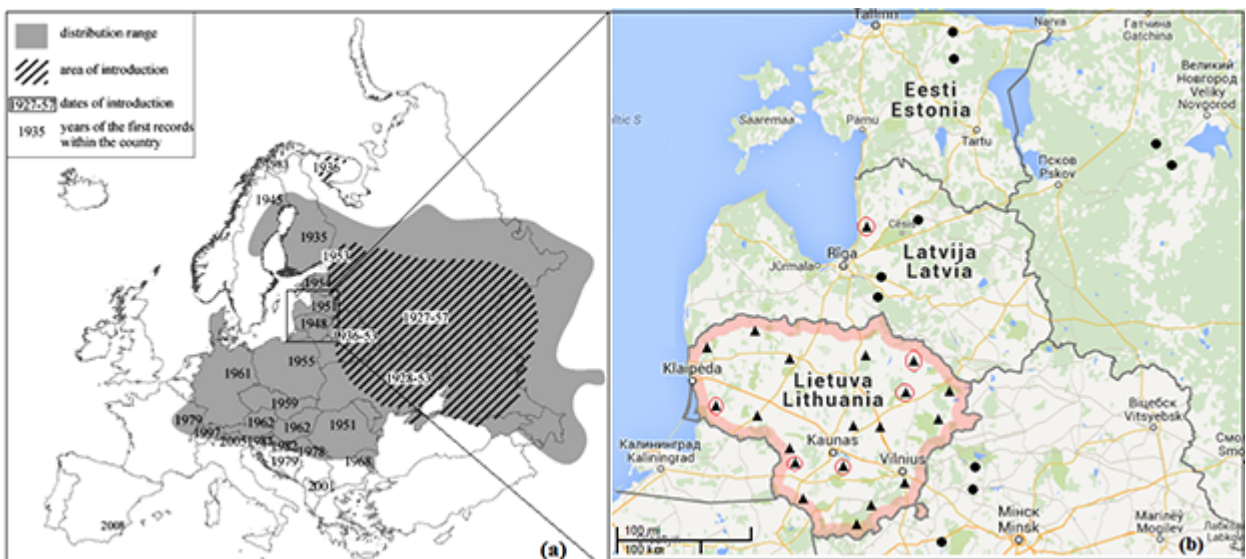


Figure 1. A map showing: (a) the distribution of raccoon dog *N. procyonoides* in Europe (Kauhala and Kowalczyk, 2011), and (b) the sampling localities of the present study (triangles). (b) Introduction sites are marked by circles (according to Bobrov et al., 2008). The distribution of mtDNA haplotypes belonging to haplogroup II are marked in outlined triangles.

100 ng of template DNA. PCR reactions were carried out in an Eppendorf Mastercycler gradient, 5331 (Germany). Cycling parameters were an initial denaturation step at 95 °C for 5 min, followed by 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min. This cycle was repeated 35 times, followed by 7 min of extension at 72 °C. The PCR products were visualized on 1.5% agarose gel stained with ethidium bromide. The positive bands of the expected size range were excised from the gel. DNA was extracted using the GeneJET Gel Extraction Kit (Thermo Scientific). Amplicons were then sequenced in both directions on an automated sequencer, ABI 3130xl (Applied Biosystems, USA).

2.3. Data analysis

Electropherograms were checked by eye for poor base calls and sequence quality and then sequences were edited and aligned using the ClustalW (Thompson et al., 1994) algorithm in MEGA v.5.05 (Tamura et al., 2011).

Phylogenetic relationships among control region haplotypes were reconstructed using the neighbor-joining (NJ) (Saitou and Nei, 1987), maximum likelihood (ML) (Felsenstein, 1981), and maximum parsimony (MP) (Nei and Kumar, 2000) methods as implemented in MEGA5 software (Tamura et al., 2011), as well as the Bayesian inference (BI) approach (Huelsenbeck et al., 2001) using the program Mr.Bayes v.3.2.1 (Ronquist and Huelsenbeck, 2003). The gaps were treated as missing (Bayesian and network analyses), or as the fifth character state.

The most appropriate model of nucleotide substitution for our data was determined according to the Bayesian information criterion (BIC) using the program jModelTest2 (Guindon and Gascuel, 2003; Darriba et al., 2012). The model selected was the HKY (Hasegawa et al., 1985) + G (Yang, 1994) substitution model [$-\ln L = 1113.68$ (BIC); base frequencies set to A = 0.2823, C = 0.2543, G = 0.1697, T = 0.2938; AC = 0.3320, AG = 6.4693, AT = 0.5250, CG = 0.3304, and CT = 6.1042, GT = 1.0000; Ti/tv ratio = 4.3386; (a) = 0.0211, invariable sites (pinv) = 0]. This model was implemented in ML and Bayesian analyses. The NJ tree was generated using the Kimura 2-parameter model (Kimura, 1981). The significance of NJ, MP, and ML trees was tested using 1000 bootstrap replicates for each (Felsenstein, 1985). For the BI analysis, four independent runs of Markov chain Monte Carlo were launched for 1.0×10^6 generations and sampled every 100 generations. The first 25% of samples were discarded as burn-in, and the remaining saved samples were used to estimate posterior probabilities (PP) of each bipartition. Bayesian tree diagrams were obtained with a tree figure drawing tool, FigTree v1.3.1 (Rambaut, 2009).

A BLAST search was used to confirm *N. procyonoides* mtDNA. All sequences were deposited in the GenBank database under the accession numbers KC344215–

KC344235 (Lithuania-LT) and KC509604 (Latvia-LV). We compared mtDNA sequences acquired in the present study with previously published GenBank homologous sequences under the accession numbers FJ888513–FJ888521 (originated from Finland-FI, Germany-DE, Hungary-HU) (Pitra et al., 2010); JF809822–JF809848 (Russia-RU, upper Volga basin: Vol-Vologda oblast, Nel-Nelidovo raion, And-Andreapol raion, Tor-Toropets raion, Ol-Olenino raion, Ud-Udomlya raion) (Korablev et al., 2011); EU642411, EU642412, EU642415, EU642417, EU642425, EU642426, EU642430, EU642436, EU642440, EU642445, and EU642449 (South Korea-SKR); EU642416, EU642417, EU642451, and EU642452 (Russian Far East-RU); and EU642443 and GU256221 (China-CN) (Park et al., unpublished data; Chen et al., unpublished data). For the outgroup, we used a sequence of *N. p. viverrinus*, accession number D83614 (Japan-JP) (Okumura et al., 1996).

To address the phylogenetic and phylogeographic relationships among the haplotypes, a median-joining network was constructed using NETWORK 4.6.1.1 (Bandelt et al., 1999).

3. Results and discussion

The analysis of 22 sequences ($n = 21$) of a 540-bp fragment of the mtDNA control region in the raccoon dogs inhabiting Lithuania ($n = 21$) and Latvia ($n = 1$) revealed 9 haplotypes with 19 variable positions, including 15 transitions, 3 transversions, and one deletion (Table 1).

Haplotype diversity (h) among the 22 samples was estimated to be 0.881 ± 0.045 , and nucleotide diversity (π) was 0.01351 ± 0.00251 . The observed relatively high level of haplotype diversity and low level of nucleotide diversity could indicate rapid population growth. In such a case new haplotypes have accumulated without large sequence differences (Avise, 2000). Similarly, high haplotype diversity and low nucleotide diversity values have been reported in other canine species such as the gray wolf (*Canis lupus* L) (Gomerčič et al., 2010), coyotes (*Canis latrans*) (Bozarth et al., 2011), and red foxes (*Vulpes vulpes* L.) (Edwards et al., 2012).

Haplotype H_1 was the most frequent and was found in 6 out of 20 different collection sites, followed by H_2 (3 sites) and H_9 (3 sites) (Table 1). Haplotypes H_3, H_6, and H_8 were specific for respective sampling locations. Haplotype H_7 was obtained from two sites in Latvia and Lithuania. Haplotype H_5, detected in Lithuania, is unique, and differs from the most similar sequences in GenBank by one nucleotide.

The phylogenetic relationships among the haplotypes of *N. procyonoides* in Lithuania demonstrated the presence of two haplogroups (clades) (Figures 2–5). The first haplogroup (I) consisted of 6 haplotypes ($n = 16$), while

Table 1. Variable sites observed among mitochondrial control region sequences (540 bp) of *N. procyonoides* from Lithuania and other countries. Dot indicates the same base; dash indicates the base deletion.

Haplogroups	Haplotypes	Accession number	Variable sites																Other countries			
			3	7	7	7	8	8	9	9	1	1	1	2	2	2	3	4		4	4	4
			8	6	7	8	1	9	0	7	0	5	9	1	6	8	8	3		4	6	8
										1	7	7	6	4	7	5	5	2	9	5		
I	H_1	KC344215																				HU
		KC344217																				RU
		KC344226	T	A	T	C	G	G	A	C	T	C	T	C	T	T	G	T	T	A	G	
		KC344224																				
		KC344229																				
		KC344220																				
	H_2	KC344218																				HU
		KC344225	.	.	C	T	C	FI
		KC344230																				DE
	H_3																					RU
		KC344219	.	.	C	T	DE
	H_4	KC344235																				FI
		KC344231	.	.	C	T	C	-	RU
		KC344228																				
	H_5	KC344227	-	
		KC344233																				
	H_6	KC344232	-	.	.	.	A	FI
																					DE	
II	H_7	KC344221	C	G	.	T	A	A	G	.	.	T	C	A	C	A	.	.	C	G	A	RU
		KC509604																				LV
																						FI
	H_8		C	G	.	T	A	A	G	.	.	T	C	A	C	A	.	A	C	G	A	DE
		KC344222																				RU
																						CN
																						FI
		KC344234																				
	H_9	KC344216	C	G	.	T	A	A	G	.	.	T	C	A	C	A	-	A	C	G	A	DE
		KC344223																				RU

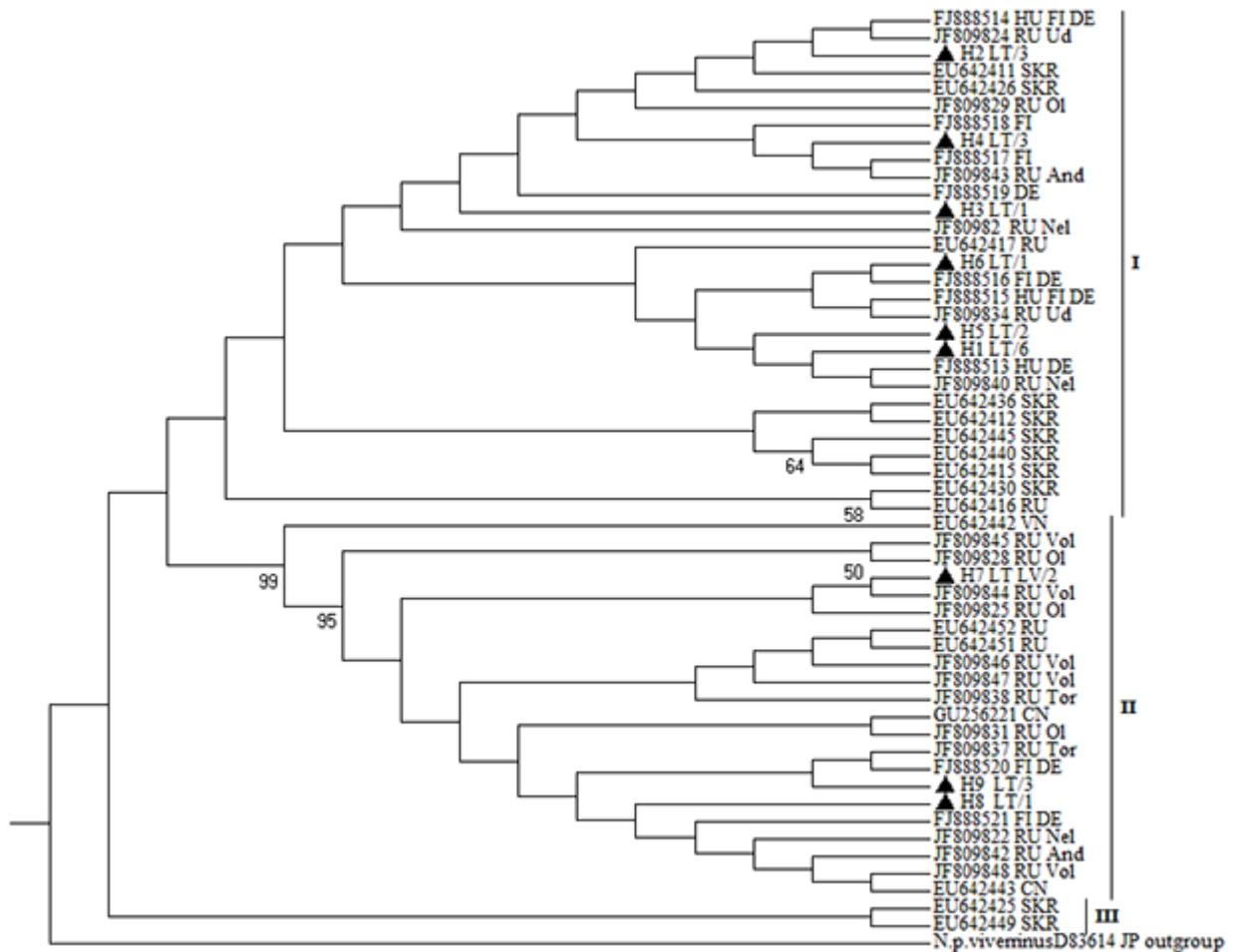


Figure 2. A maximum likelihood tree constructed from haplotypes of *N. procyonoides*, based on the mtDNA control region. Numbers above the branches show the bootstrap values. Lithuanian haplotypes were marked with solid triangles. I, II, and III indicate the number of the haplogroup.

the second (II) consisted of only 3 haplotypes ($n = 6$). Haplogroup II had lower nucleotide diversity and fewer divergent haplotypes than haplogroup I. The sample size, number of haplotypes, values of nucleotide diversity, and haplotype diversity within each haplogroup in Lithuania and other regions are presented in Table 2.

Although haplotypes and haplogroups were not related with any spatial geographic structure of the populations, haplotypes attributed to haplogroup II were detected only in raccoon dogs hunted in sites located near rivers in the lowlands, whereas animals with haplotypes belonging to haplogroup I were found across the whole country (Figure 1b).

In order to compare the level of genetic diversity and describe phylogeographic patterns throughout the raccoon dog's distribution in Eurasia, we combined our data ($n = 22$) with sequences available from earlier studies ($n = 32$) for an overall sample size of 54.

The phylogenetic relationships among raccoon dogs from different parts of the modern species distribution range were reconstructed using comparative analysis of sequences from Lithuania and Latvia and the GenBank sequences of *N. procyonoides* from Finland, Germany, Hungary, Russia, China, South Korea, and Vietnam (Figures 2–5). The NJ, ML, MP, and BI trees showed similar topologies and had similar branching structures (Figure 2–5). In NJ, MP, and BI trees, the mtDNA control region haplotypes were clearly separated into two groups: haplogroup I and haplogroup II. However, the ML tree (Figure 2) consisted of three haplogroups, and haplogroup III contained the South Korean haplotypes. In the NJ tree (Figure 3), haplogroup I was the same as that of the MP tree (Figure 4). The Bayesian tree (Figure 5) exhibited two haplogroups supported by higher PP values (95%–100%) than the bootstrap values (12%–99%) of the ML, MP, and NJ trees (Figures 2–4).

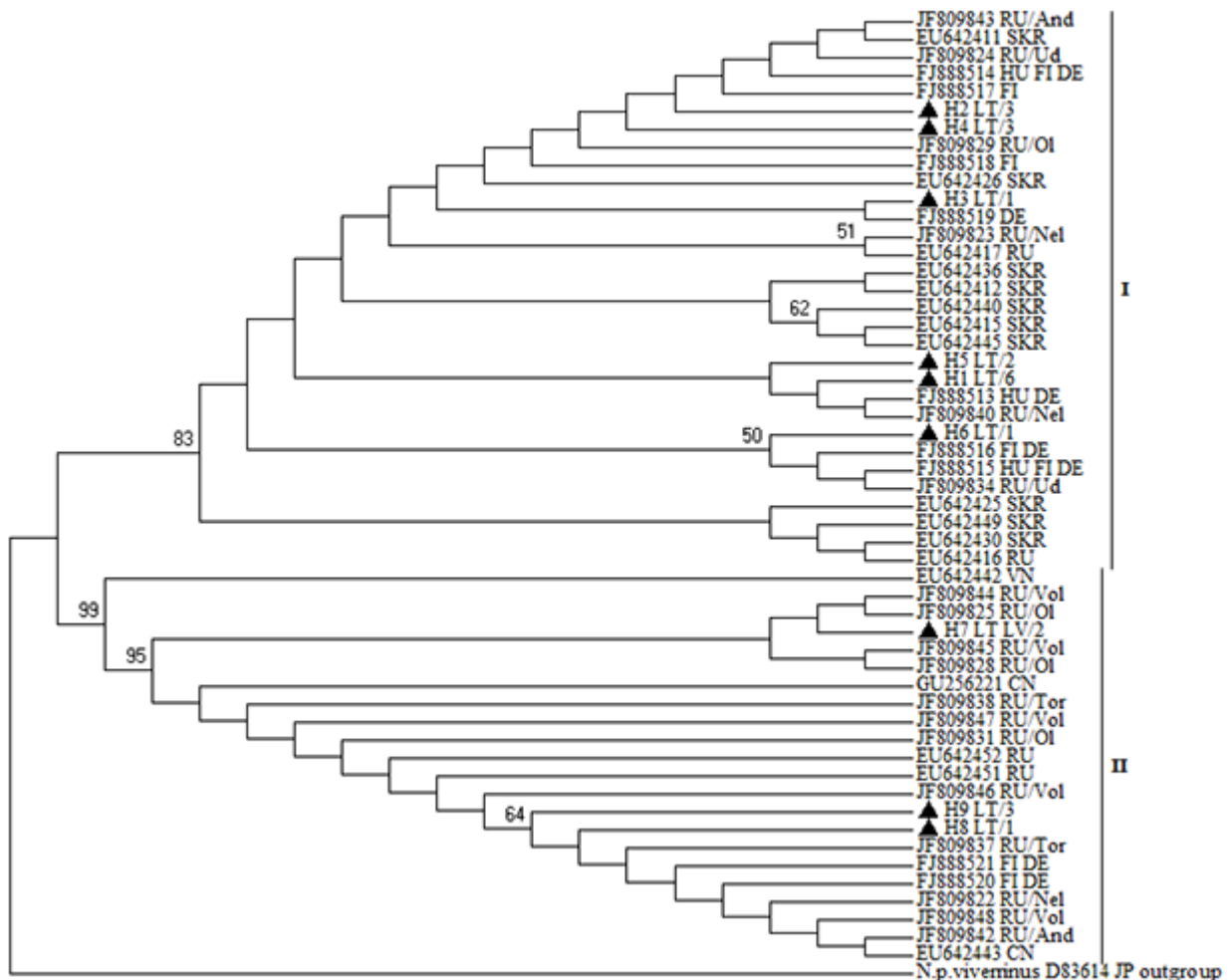


Figure 3. A neighbor-joining tree constructed from haplotypes of *N. procyonoides*, based on the complete sequences of the mitochondrial control region. Numbers above the branches show the bootstrap values. Lithuanian haplotypes were marked with solid triangles. I and II indicate the number of the haplogroup.

All haplotypes detected in Lithuania, except for one (haplotype H_5) (Table 1), were identical to those obtained in Finland (H_2, H_4, H_6, H_8, H_9), Germany (H_1, H_2, H_3, H_6, H_8, H_9), Hungary (H_1, H_2), Russia (upper Volga basin) (H_1, H_2, H_4, H_7, H_8, H_9), and China (H_8). Raccoon dogs from China and Vietnam both clustered in haplogroup II. The sequence of *N. procyonoides* from Vietnam was distinct and separated from other sequences of this haplogroup (Figures 2–5). The geographically adjacent populations clearly show some degree of genetic divergence and similarities of certain mtDNA haplotypes (Figure 6).

Combined analyses of our sequences and others deposited previously in GenBank revealed 3.1% sequence divergence between the two haplogroups. The mean distance between sequences within haplogroup I was

higher and equaled 0.6%, as compared with 0.4 % in haplogroup II.

The geographical structure of the raccoon dogs in Europe was supported by the median-joining network, which confirmed a clear bipartition of raccoon dog haplotypes in Europe: there is a visible separation into two main haplogroups (Figure 7). The haplogroups are separated by 11 mutation steps.

The primary separation of *N. procyonoides* into two clades is not clear and is still being discussed. The climate changes during the mid-Pleistocene in continental Siberia might have led to genetic differentiation within species (Prokopenko et al., 2002; Pitra et al., 2010). It was suggested that the diversification of clades occurred long before the recurrent introductions in Europe and is the result of environmentally isolating mechanisms (Pitra et al., 2010).

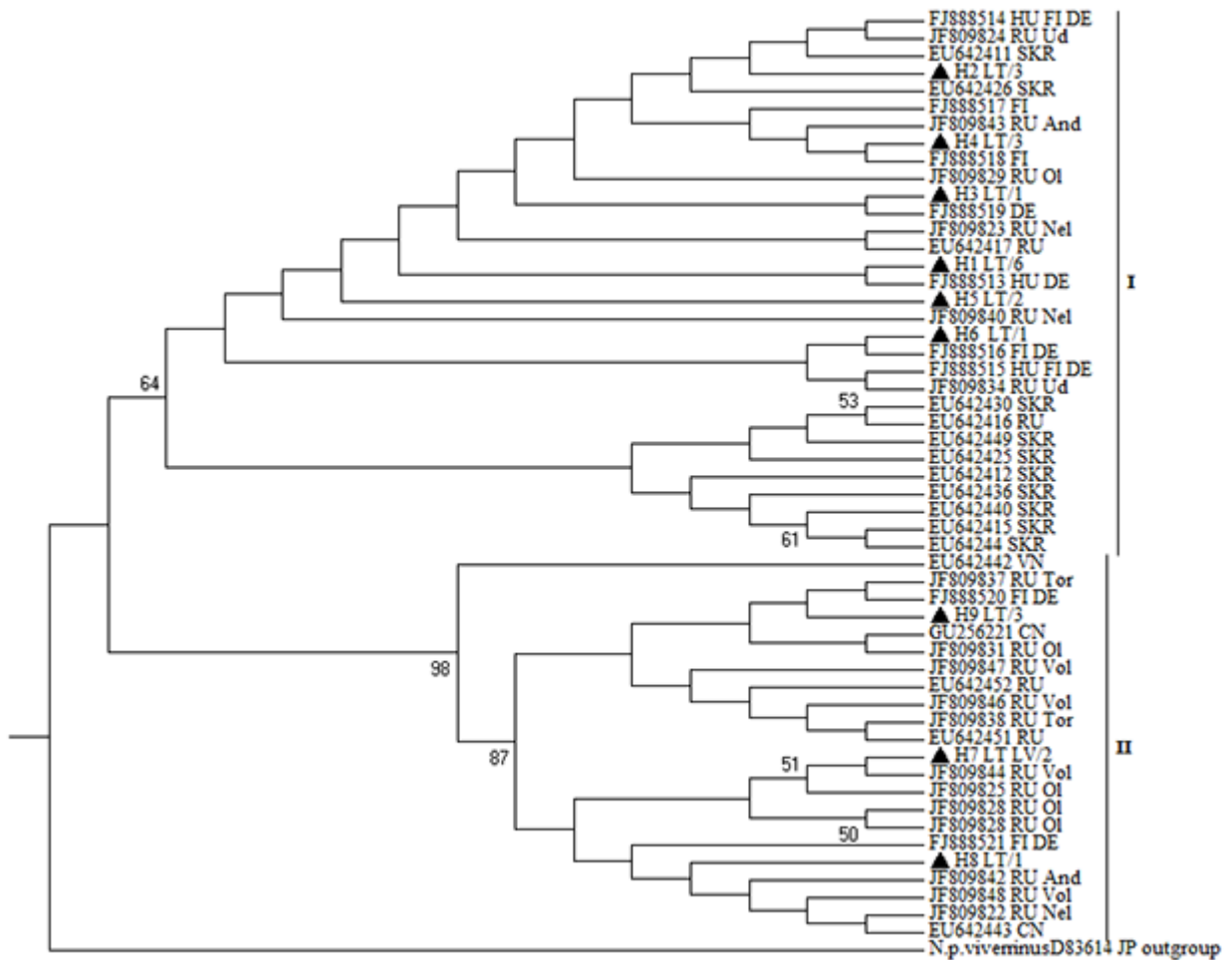


Figure 4. A maximum parsimony tree constructed from haplotypes of *N. procyonoides*, based on the mtDNA control region. Numbers above the branches show the bootstrap values. Lithuanian haplotypes were marked with solid triangles. I and II indicate the number of the haplogroup.

The existence of two clades in the introduced population is associated with the introduction of raccoon dogs from two spatially isolated autochthonous populations (Korablev et al., 2011). It seems that repeated translocation events in the early period of introduction included individuals of already differentiated mtDNA lineages (Pitra et al., 2010).

A similar distribution of molecular genetic variation in two haplogroups was observed in *N. procyonoides* from Lithuania and northern and western Europe (Pitra et al., 2010). On the contrary, in Russia, Korablev et al. (2011) obtained higher genetic diversity in haplogroup II (defined as "haplogroup I" in Korablev et al., 2011). The patterns of molecular genetic variation in raccoon dogs from Lithuania obtained in the present study indicate higher genetic diversity of these animals as compared with those from northern and western Europe (Pitra et al., 2010), but lower genetic polymorphism as compared with raccoon dogs

from South Korea (autochthonous population; haplogroup I) and from Russia (introduced population; Korablev et al., 2011) (Table 2). Higher levels of genetic diversity observed in the introduced populations in Russia could be a result of the introduction of a large group of animals from different sites (Korablev et al., 2011). The lower genetic diversity in Lithuania could be a result of immigrants from the neighboring countries of Belarus and Latvia. Raccoon dogs were introduced in Latvia in 1947 and in Estonia in the 1950s from reacclimatized sites in Russia. In Belarus, the species was introduced between 1936 and 1956 from breeding farms and reacclimatized sites in Russia (Long, 2003). The most abundant haplotype (H₁) occurring in Lithuania was also found in Russia and Hungary, while it was not detected in other investigated European countries (Table 1). This suggests an invasion from the introduced population, possibly through Belarus. In Latvia about

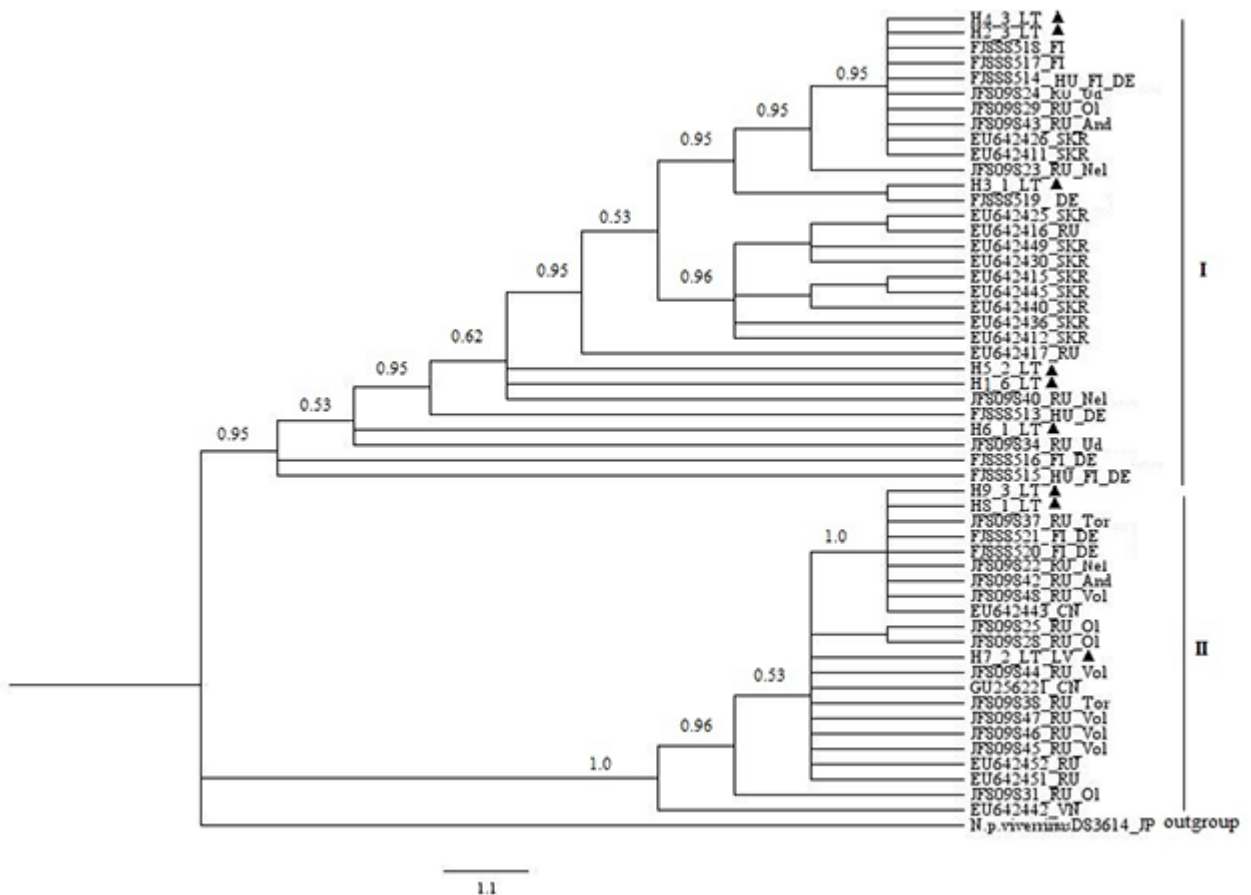


Figure 5. A Bayesian phylogenetic tree constructed from haplotypes of *N. procyonoides*, based on the complete sequences of the mitochondrial control region. Numbers above the branches show the Bayesian posterior probabilities. Lithuanian haplotypes were marked with solid triangles. I and II indicate the number of the haplogroup.

1000 raccoon dogs were reported to have been observed or hunted during 1951. In 1960 about 4210 hunted raccoon dogs were reported. Although raccoon dogs were found all over Estonia in the 1950s (Lavrov, 1971), their numbers remained low because of the presence of numerous wolves and lynx populations, the natural enemies of raccoon dogs in Estonia. In contrast, the number of wolves and lynx decreased in 1940–1950 in Lithuania, which allowed the rapid spread of raccoon dogs in the country. This species colonized Lithuania in about 10 years (Balčiauskas, 1996). Multiple introductions (in different years and from different locations) and a rapid population growth during the first decade of invasion, as well as admixture and subsequent intraspecific hybridization of invasive populations, could explain the high genetic diversity of the Lithuanian raccoon dog population observed in the present study.

Currently the hunting of raccoon dogs in Lithuania is permitted throughout the year. The density of the

Lithuanian population varied in different time periods (according to data of the Ministry of the Environment of the Republic of Lithuania, <http://www.am.lt>), and according to monitoring data between 1960 and 1970 it reached from 3000 to 14000 in that 10-year period. Hunting statistics showed that more than 5000 raccoon dogs were hunted in 2002, 4000 in 2003, 3439 in 2004–2005, 2818 in 2006–2007, 5554 in 2008–2009, 10290 in 2010–2011, and 4791 in 2011–2012. Regulating populations by hunting could affect the dispersal behavior and the population structure of this species.

Our results indicate that a high variation of donor autochthonous populations, as well as repeated introductions, may have rendered invaders with high adaptive potential. Multiple immigration events could prevent genetic bottlenecks and generate genetic diversity through genetic exchanges. This could have allowed raccoon dogs to evolve in response to the changing climate,

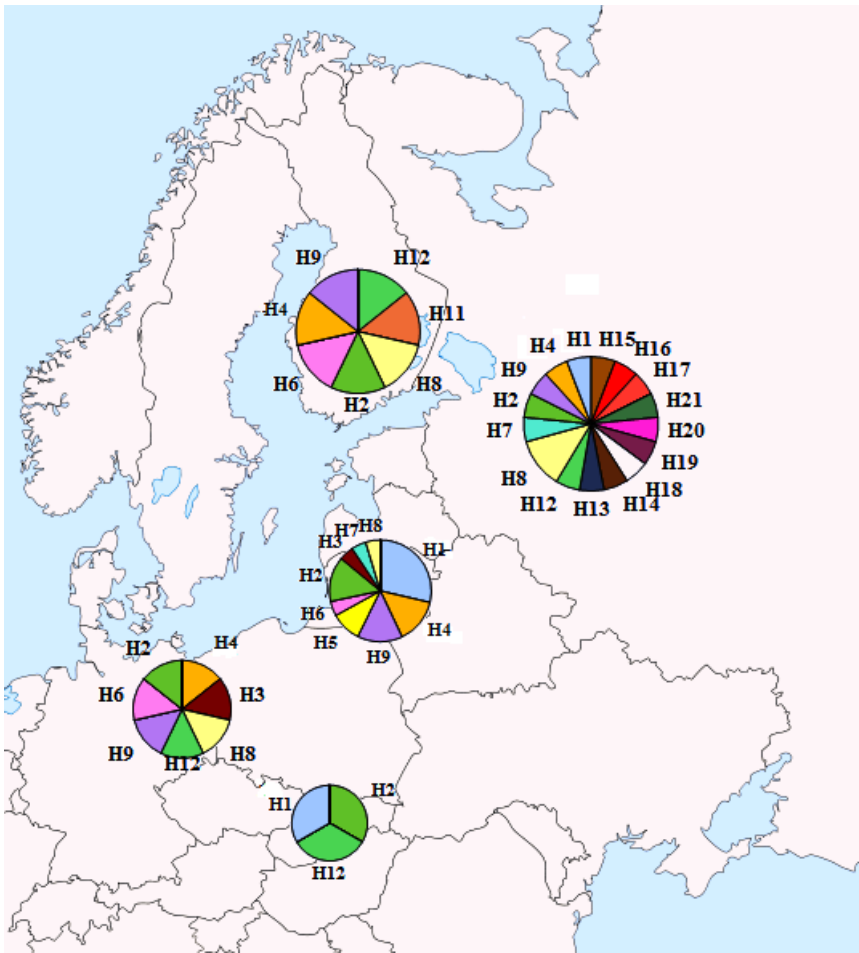


Figure 6. A geographical distribution of the mtDNA control region haplotypes in Europe. Pie charts show the proportions of haplotypes.

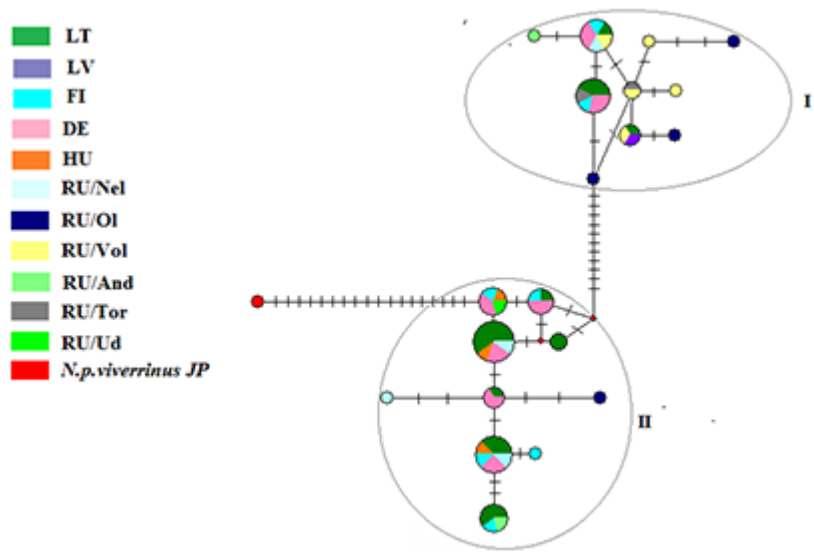


Figure 7. A median joining network based on mtDNA sequences of *N. procyonoides*. The circles represent haplotypes, with size proportional to relative frequencies. The network branches linking the cycles indicate one mutation step; two or more mutations are represented by slashes crossed with the network branches.

Table 2. Diversity indices for the mitochondrial control region of *N. procyonoides* from different regions.

Location	Haplogroup	n	s	η	n_h	π (\pm SD)	h (\pm SD)	Number of pairwise differences
Baltic region (this study)	I	16	5	5	6	0.00403 \pm 0.00041	0.817 \pm 0.068	2.18 \pm 2.31
	II	6	3	3	3	0.00309 \pm 0.00075	0.733 \pm 0.155	1.67 \pm 0.81
North and West Europe (Pitra et al., 2010)	I	65	6	6	7	0.00354	0.753 \pm 0.001	2.12 \pm 2.88
	II	8	1	1	2	0.00095	0.571 \pm 0.010	0.57 \pm 0.25
Russia, Upper Volga basin (Korablev et al., 2011)	I	21	10	9	12	0.0040 \pm 0.0026	0.970 \pm 0.044	2.37 \pm 1.38
	II	9	9	10	6	0.0056 \pm 0.0038	1.000 \pm 1.00	3.35 \pm 2.00
South Korea (GenBank data)	I	10	9	9	8	0.00514 \pm 0.0010	0.956 \pm 0.06	2.64 \pm 2.33

and could allow them to have an increasing impact on native communities and ecosystems in the future (Robert et al., 2003; Lavergne and Molofsky, 2007).

Acknowledgment

This study was supported by the Research Council of Lithuania (grant no. LEK-14/2012).

References

- Ansorge H, Ranyuk M, Kauhala K, Kowalczyk R, Stier N (2009). Raccoon dog *Nyctereutes procyonoides* populations in the area of origin and in colonised regions-the epigenetic variability of an immigrant. *Ann Zool Fenn* 46: 51-62.
- Avice JC (2000). *Phylogeography: The History and Formation of Species*. 1st ed. Cambridge, MA, USA: Harvard University Press.
- Balciauskas L (1996). Lithuanian mammal fauna review. *Hystrix* 8: 9-15.
- Ballard JW, Whitlock MC (2004). The incomplete history of mitochondria. *Mol Ecol* 13: 729-744.
- Bandelt HJ, Forster P, Rohl A (1999). Median joining networks for inferring intra specific phylogenies. *Mol Biol Evol* 16: 37-48.
- Bobrov VV, Varshavskii AA, Khlyap LA (2008). Raccoon dog *Nyctereutes procyonoides* (Gray, 1834). In: Drebuadze JJ, Neronov VM, editors. *Alien Mammals in the Ecosystems of Russia*. 1st ed. Moscow, Russia: KMK Scientific Press Ltd, pp. 111-117 (in Russian).
- Bozarth CA, Hailer F, Rockwood LL, Edwards CW, Maldonado JE (2011). Coyote colonization of northern Virginia and admixture with Great Lakes wolves. *J Mammal* 92: 1070-1080.
- Darriba D, Taboada GL, Doallo R, Posada D (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772.
- Drygala F, Zoller H, Stier N, Roth M (2010). Dispersal of the raccoon dog (*Nyctereutes procyonoides*) in newly invaded area in central Europe. *Wildl Biol* 16: 150-161.
- Edwards CJ, Soulsbury CD, Statham MJ, Ho SYW, Wall D, Dolf G, Iossa G, Baker PJ, Harris S, Sacks BN et al. (2012). Temporal genetic variation of the red fox, *Vulpes vulpes*, across western Europe and the British Isles. *Quat Sci Rev* 57: 95-104.
- Felsenstein J (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17: 368-376.
- Felsenstein J (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- Genovesi P, Bacher S, Kobelt M, Pascal M, Scalera R (2009). Alien mammals of Europe. In: Drake JA, editor. *Handbook of Alien Species in Europe. Invading Nature: Springer Series in Invasion Ecology*. 1st ed. Dordrecht, the Netherlands: Springer, pp. 119-128.
- Gomerčić T, Sindičić M, Galov A, Arbanasić H, Kusak J, Kocijan I, Gomerčić MD, Huber Đ (2010). High genetic variability of Croatian grey wolf (*Canis lupus* L.) population as revealed by mitochondrial DNA control region sequences. *Zool Stud* 49: 816-823.
- Guindon S, Gascuel O (2003). A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Syst Biol* 52: 696-704.
- Hasegawa M, Kishino H, Yano T (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* 22: 160-174.
- Hong YJ, Kim KS, Lee H, Min MS (2013). Population genetic study of the raccoon dog (*Nyctereutes procyonoides*) in South Korea using newly developed 12 microsatellite markers. *Genes Genet Syst* 88: 69-76.

- Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.
- Kauhala K, Kowalczyk R (2011). Invasion of the raccoon dog *Nyctereutes procyonoides* in Europe: history of colonization, features behind its success, and threats to native fauna. *Curr Zool* 57: 584-598.
- Kauhala K, Saeki M (2004). Finnish and Japanese raccoon dogs: on the road to speciation? In: Macdonald DW, Sillero-Zubiri C, editors. *Biology and Conservation of Wild Canids*. 1st ed. Oxford, UK: Oxford University Press, pp. 217-226.
- Kimura M (1981). Estimation of evolutionary distances between homologous nucleotide sequences. *P Natl Acad Sci* 78: 454-458.
- Korablev NP, Korablev MP, Rozhnov VV, Korablev PN (2011). Polymorphism of the mitochondrial DNA control region in the population of raccoon dog (*Nyctereutes procyonoides* Gray, 1834) introduced into the Upper Volga basin. *Russ J Genet* 47: 1378-1385.
- Lavergne S, Molofsky J (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *P Natl Acad Sci* 104: 3883-3888.
- Lavrov NP (1971). Results of raccoon dog introductions in different parts of the Soviet Union. *Trudy Kafedry Biologii MGZPI* 29: 101-160.
- Long J (2003). *Introduced Mammals of the World: Their History, Distribution and Influence*. 1st ed. Melbourne, Australia: CSIRO Publishing.
- Nei M, Kumar S (2000). *Molecular Evolution and Phylogenetics*. 2nd ed. Oxford, UK: Oxford University Press.
- Okumura N, Ishiguro N, Nakano M, Matsui A, Sahara M (1996). Intra- and interbreed genetic variations of mitochondrial DNA major non-coding regions in Japanese native dog breeds (*Canis familiaris*). *Anim Genet* 27: 397-405.
- Pitra C, Schwarz S, Fickel J (2010). Going west-invasion genetics of the alien raccoon dog *Nyctereutes procyonoides* in Europe. *Eur J Wild Res* 56: 117-129.
- Prokopenko AA, Williams DE, Kuzmin MI, Karabanov EB, Khursevich GK, Peck JA (2002). Muted climate variations in continental Siberia during the mid-Pleistocene epoch. *Nature* 418: 65-68.
- Prūsaitė J, Mažeikytė R, Pauža D, Paužienė N, Baleišis R, Juškaitis R (1988). *Fauna of Lithuania*. 1st ed. Vilnius, Lithuania: Mokslas Publishers (in Lithuanian).
- Rambaut A (2009). FigTree: Tree Figure Drawing Tool, Version 1.3.1. Edinburgh, UK: Institute of Evolutionary Biology, University of Edinburgh.
- Robert A, Couvet D, Sarrazin F (2003). Bottlenecks in large populations: the effect of immigration on population viability. *Evol Ecol* 17: 213-231.
- Ronquist F, Huelsenbeck JP (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406-425.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011). MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731-2739.
- Thompson JD, Higgins DG, Gibson TJ (1994). Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673-4680.
- Yang Z (1994). Estimating the pattern of nucleotide substitution. *J Mol Evol* 39: 105-111.