INVESTIGATION OF GENETIC VARIABILITY IN THE EUROPEAN EEL (*ANGUILLA ANGUILLA* (L.)) IN LITHUANIA USING MICROSATELLITE DNA MARKERS

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Abstract. To find out if native and introduced European eel (*Anguilla anguilla*) populations differ from one another genetically, we used five microsatellite markers and compared naturally recruited and introduced European eel populations at four different locations. Samples of naturally recruited eels were taken from the Baltic Sea and the Curonian Lagoon, while introduced eels were sampled from Lakes Dringis and Siesartis. Results of the study do not support the hypothesis on panmixia in the European eel, since low but significant genetic differentiation ($F_{ST} = 0.0238, p = 0.0148$) was found to exist among introduced eel groups. Significant genetic differentiation ($F_{ST} = 0.0228, p = 0.0281$) among the Baltic Sea, the Curonian Lagoon, Lake Dringis and Lake Siesartis populations was ascertained too.

Key words: *A. anguilla*, European eel, DNA, microsatellites, polymorphism, genetic variability, panmixia, temporal variation

INTRODUCTION

The genus *Anguilla* comprises 15 species (Tzeng 2004; Watanabe *et al.* 2005) and belongs to the order Anguilliformes (Jing & Li 1999; Virbickas 2000). A complex life cycle is characteristic of *Anguilla* sp. (Ellerby *et al.* 2001b; Sasai *et al.* 2001; Tseng *et al.* 2003; Dannewitz *et al.* 2005), since these fish species have a few morphological forms, inhabit a different environment depending on their life stage or age (Poole & Reynolds 1998; Tzeng *et al.* 1999; Tsukamoto & Arai 2001; Powles & Warlen 2002) and migrate over long distances (Virbickas 2000; Katoh & Kobayashi 2001; Okamura *et al.* 2002; Limburg *et al.* 2003; Bardonnnet & Riera 2005) to their spawning grounds. No one knows exactly where the European eel's (*Anguilla anguilla*) spawning ground is (Lokman & Young 2000; Ringuet *et al.* 2002). The smallest larvae found in the Sargasso Sea, south of Bermuda, suggest that spawning of the *A. anguilla* as well as that of the American eel (*Anguilla rostrata*) occurs nearby (Wirth & Bernatchez 2001, 2003; Mank & Avise 2003; Versonnen *et al.* 2004; Dannewitz *et al.* 2005; Palstra *et al.* 2006). Meanwhile, the Japanese eel's (*Anguilla japonica*) spawning ground appears to be in the west of the Mariana Islands in the North Pacific (Katoh & Kobayashi 2001; Tsukamoto & Arai 2001; Ringuet *et al.* 2002; Tseng *et al.* 2003; Tsukamoto 2006). Species of the Anguillidae family, especially the *A. anguilla*

(Virbickas 2000; Hansen *et al.* 2001; Tzchori *et al.* 2004), the American eel and the Japanese eel (Katoh & Kobayashi 2001) are of great commercial interest (Jing & Li 1999; Tsukamoto & Arai 2001; Ringuet *et al.* 2002; Altun *et al.* 2005). Consequently, a decrease in European (Limburg *et al.* 2003; Dannewitz *et al.* 2005), American (Wirth & Bernatchez 2003) and Japanese eel (Tseng *et al.* 2003) catches during the past decades in Europe, America and Asia, respectively, has inspired many studies on physiological (Ellerby *et al.* 2001a, b), ecological (Poole & Reynolds 1998; Tzeng *et al.* 1999; Okamura *et al.* 2002; Bardonnnet & Riera 2005; Palstra *et al.* 2006) and genetic (Jing & Li 1999; Wirth & Bernatchez 2001, 2003; Mank & Avise 2003; Tseng *et al.* 2003; Dannewitz *et al.* 2005) aspects of eel biology and an increased concern over the eel restocking strategy (Ringuet *et al.* 2002). As an outcome of various studies, it has become clear that the decrease in stocks of all the three eel species during the past decades could have been caused by a number of factors: abiotic (Ringuet *et al.* 2002; Wirth & Bernatchez 2003), biotic (Kirk *et al.* 2002; Genç *et al.* 2005), anthropogenic, including intense stock exploitation, habitat loss (Katoh & Kobayashi 2001; Limburg *et al.* 2003; Palstra *et al.* 2006), and maybe genetic factors (Wirth & Bernatchez 2001; Dannewitz *et al.* 2005; Laffaille *et al.* 2005). Despite various hypotheses the essential cause of eel stock decline remains indeterminate.

It is worth mentioning that up till now no *A. anguilla* studies seem to have been carried out to find out if naturally recruited *A. anguilla* differ genetically from the introduced *A. anguilla* group within a particular region of the range. However, if true genetic differentiation in *A. anguilla* populations exists, eel restocking can have a negative effect on *A. anguilla* populations (Dannewitz *et al.* 2005). Therefore further investigations into *A. anguilla* population genetics are necessary.

The Baltic Sea and the Curonian Lagoon are the most important water bodies for commercial eel fishery in Lithuania. However, during the 1955–1975 period *A. anguilla* landings in the Curonian Lagoon declined from 200–480 t per year to 27–87 t per year and did not increase during the past decades (Virbickas 2000). The decline in eel stocks and great commercial interest in Lithuania have boosted importation of glass eels from England and France (Shiao *et al.* 2006) and their release into Lithuanian water bodies, mostly lakes. Recent findings based on the *A. anguilla* otolith microchemistry analysis suggest that eel fishery in the Curonian Lagoon mainly depends on naturally recruited eel catches (Shiao *et al.* 2006), since restocked eels accounted for only 20% of eels in the Curonian Lagoon and 2% in the Lithuanian coastal zone of the Baltic Sea. Therefore, Lithuanian fishery in the coastal waters and the Curonian Lagoon depends on natural recruitment primarily, while restocking is still not very essential. Furthermore, besides other eel restocking problems, such as spread of parasites or infections (Madsen *et al.* 2000; Hansen *et al.* 2001; Marco-Noales *et al.* 2001; Kirk *et al.* 2002; Shin 2004), eel restocking can modify genetic variability and even reduce reproductive success if genetic differentiation in *A. anguilla* populations exists.

This study presents results of the initial investigation into the *A. anguilla* population in Lithuania using

microsatellite DNA markers. The study aimed at finding out if naturally recruited eels sampled in the Baltic Sea and the Curonian Lagoon differ from introduced eels sampled in Lake Dringis and Lake Siesartis genetically. Overall genetic differentiation among sampled eel populations from all the studied water bodies was evaluated too.

MATERIAL AND METHODS

Sampling

During the 2004–2006 period 60 European eels, of which 21 were silver eels and 39 yellow eels, were sampled from the Baltic Sea, the Curonian Lagoon, Lakes Dringis and Siesartis (Fig. 1). 24, 8, 8 and 20 eels were taken from these locations, respectively. Samples from the Baltic Sea and the Curonian Lagoon

Figure 1. Sampling locations of European eel indicated as black circles represent Baltic Sea (1), Curonian Lagoon (2), Lake Dringis (3) and Lake Siesartis (4) respectively.

Table 1. Characteristics of primers (F-forward and R-reverse primers).

Primer	Sequences of primer pairs (5'-3')	T _m , °C	Species of origin	Loci
AF237898	F: TTGGAAGGTCATGGACGTG R: CTAATAAATGTCTGGGTAGGC	73.8 61.7	<i>Anguilla rostrata</i>	Aro121
AF237899	F: CCAGATACCTTGACAACGGC R: TCAAGAGCTTCCTGACCCTC	69.7 69	<i>Anguilla rostrata</i>	Aro063
AF237900	F: GAAAACAATCGGGTACCACAG R: ACAGTCAGTCACAATGAGCC	68.1 66.3	<i>Anguilla anguilla</i>	Ang101
AF237901	F: GATCTGTGGAGAGATGTTGG R: AGTAGCATGCCTAGAACTGG	65.5 63.8	<i>Anguilla anguilla</i>	Ang151
AF237902	F: CCTGTGAATCCAACAGGTGG R: GGATAATGCGGCAGAGTTCCC	71 73.5	<i>Anguilla anguilla</i>	Ang114

predominantly represented the naturally recruited eel population, while fish from the lakes were eels introduced from Western Europe.

Genotyping and data analysis

Genetic variation was estimated at 5 microsatellite loci: *Aro121*, *Aro063*, *Ang101*, *Ang151* and *Ang114* corresponding to the same set of primers used by Wirth and Bernatchez (2001). For that purpose, oligonucleotides AF237898, AF237899, AF237900, AF237901 and AF237902 synthesized by biometers.net GmbH (Table 1) were used respectively.

DNA was extracted from the frozen- or ethanol-preserved muscle tissue using universal and rapid salt-extraction of genomic DNA for PCR-based analysis (Aljanabi & Martinez 1997). PCR was performed using the Eppendorf Mastercycler gradient PCR machine. PCR volume was 25 µl and consisted of: 5 µl of genomic DNA, the concentration of which was 50 µg/µl, 0.75 µl 1 U Taq Polymerase (MBI Fermentas), 2.5 µl PCR buffer, 2.5 µl MgCl₂, the concentration of which was 2.5 mM, 2.5 µl 2 mM dNTP, 0.1 µM forward and reverse primers and water. Since different pairs of primers required different annealing temperatures, four PCR programmes (Table 2) were used. Procedure for the amplification of the loci *Aro121* and *Ang151* was as follows: initial denaturation for 3 min at 94°C, every cycle denaturation for 1 min at 94°C, first cycle annealing for 1 min at 53°C, every cycle annealing (until cycle 13) for 1 min adding +1°C for the next cycle, from cycle 13 to 30 annealing for 1 min at 65°C, every cycle elongation for 1 min at 72°C, ending with a 5 min final elongation step at 72°C. PCR procedures for the amplification of the loci *Aro063*, *Ang101* and *Ang114* were the same as those applied for the loci *Aro121* and *Ang151*. However, the annealing temperature for the amplification of the locus *Ang101* was stable, annealing temperatures for the amplification of other loci differing. After amplification each PCR product was mixed with 4 µl of loading dye and 2 µl of sacharose and then loaded in 12% polyacrylamide gel. Electrophoresis was

carried out in the MAXI vertical dual plate electrophoresis unit, 20 × 20 cm, for 4–5 hours at 200 V following staining with ethidium bromide solution for 15 min. Then gel was examined using the Herolab firm MiniDoc™ system. The results were registered and documented.

Relative allele sizes at all loci were determined using the TotalLab v1. 10 programme (Amersham Biosciences). Allele frequencies, heterozygosity, HWE, F statistics and genetic distance were calculated using the TFPGA v1.3 programme.

RESULTS

This study examined five microsatellite loci and established that relative allele sizes varied from 109 to 228 bp among all loci (Table 3). In total, 35 alleles were scored at 5 microsatellite loci, of which 8 were recorded at *Aro121*, 9 at *Aro063*, 4 at *Ang101*, 5 at *Ang151* and 9 at *Ang114*. The largest and the smallest allele number was 31 and 22 in the Baltic Sea and Lake Dringis populations, respectively. Two specific alleles were detected in the Curonian Lagoon population and one in each of the remaining populations.

The observed and expected mean heterozygosity values calculated for all sampled populations are presented in Table 4.

A significant deviation from HWE at two different loci was detected in each investigated population, except the Curonian Lagoon population. The latter had only one significant deviation from HWE at the locus *Aro121*. Such two loci with deviations from HWE were characteristic of the group of naturally recruited eels and three loci were detected in the group of introduced *A. anguilla*.

On the basis of the F_{IS} value (0.3084), which reflects the degree of inbreeding in a population, we drew the conclusion that non-random mating is characteristic of the *A. anguilla* population in Lithuania represented by all individuals sampled in the sea and lakes in the course of this investigation (Table 5). This F_{IS} value can also reflect

Table 2. PCR conditions for microsatellite assays.

Loci	Initial denaturation		Cycle denaturation		1 cycle annealing		12 cycle annealing		30 cycle annealing		Cycle elongation		Final elongation	
	°C	Min	°C	Min	°C	Min	°C	Min	°C	Min	°C	Min	°C	Min
<i>Aro121</i>	94	3	94	1	53	1	64	1	65	1	72	1	72	5
<i>Aro063</i>	94	3	94	1	57	1	68	1	69	1	72	1	72	5
<i>Ang101</i>	94	3	94	1	67	1	67	1	67	1	72	1	72	5
<i>Ang151</i>	94	3	94	1	53	1	64	1	65	1	72	1	72	5
<i>Ang114</i>	94	3	94	1	60	1	71	1	72	1	72	1	72	5

Table 3. Allele frequencies at microsatellite loci in different sampled European eel populations.

Locus	Alleles	Allele frequencies			
		Baltic Sea	Curonian Lagoon	Lake Dringis	Lake Siesartis
<i>Aro121</i>	1	0.0833	0.0000	0.0000	0.1250
	2	0.1250	0.0625	0.0625	0.2500
	3	0.1875	0.3125	0.1250	0.1000
	4	0.1250	0.1875	0.1875	0.1750
	5	0.1875	0.1250	0.2500	0.1000
	6	0.1875	0.0625	0.1875	0.1750
	7	0.0833	0.1875	0.1250	0.0750
	8	0.0208	0.0625	0.0625	0.0000
<i>Aro063</i>	1	0.0000	0.0625	0.0000	0.0000
	2	0.0208	0.0625	0.0000	0.0250
	3	0.0208	0.0625	0.0000	0.0250
	4	0.0000	0.0000	0.1875	0.0000
	5	0.1875	0.3125	0.3750	0.3500
	6	0.6042	0.3750	0.2500	0.4000
	7	0.1458	0.0000	0.1875	0.1750
	8	0.0208	0.0000	0.0000	0.0250
	9	0.0000	0.1250	0.0000	0.0000
<i>Ang101</i>	1	0.2500	0.3750	0.3125	0.1750
	2	0.4167	0.4375	0.2500	0.2250
	3	0.2917	0.1875	0.4375	0.4250
	4	0.0417	0.0000	0.0000	0.1750
<i>Ang151</i>	1	0.0208	0.0000	0.0000	0.0250
	2	0.1458	0.0625	0.1875	0.0000
	3	0.5208	0.3125	0.3750	0.2250
	4	0.2917	0.5625	0.3750	0.7000
	5	0.0208	0.0625	0.0625	0.0500
<i>Ang114</i>	1	0.0208	0.1250	0.1250	0.0000
	2	0.1250	0.0625	0.0625	0.0250
	3	0.0417	0.0625	0.1250	0.0250
	4	0.2917	0.1250	0.6875	0.3750
	5	0.1875	0.1875	0.0000	0.2000
	6	0.2500	0.2500	0.0000	0.2250
	7	0.0625	0.1875	0.0000	0.1250
	8	0.0000	0.0000	0.0000	0.0250
	9	0.0208	0.0000	0.0000	0.0000

Table 4. Observed (H_O) and expected (H_E) heterozygosity values in sampled European eel populations.

Loci	H_E				H_O			
	Baltic Sea	Curonian Lagoon	Lake Dringis	Lake Siesartis	Baltic Sea	Curonian Lagoon	Lake Dringis	Lake Siesartis
<i>Aro121</i>	0.8670	0.8583	0.8833	0.8564	0.6250	0.5000	0.5000	0.5500
<i>Aro063</i>	0.5895	0.7833	0.7750	0.7026	0.3333	0.6250	0.5000	0.4000
<i>Ang101</i>	0.6915	0.6750	0.6917	0.7256	0.5000	0.3750	0.2500	0.4000
<i>Ang151</i>	0.6348	0.6167	0.7250	0.4679	0.2917	0.5000	0.8750	0.4500
<i>Ang114</i>	0.8121	0.8833	0.5250	0.7705	0.7083	0.8750	0.5000	0.6000
Mean heterozygosity	0.7190	0.7633	0.7200	0.7046	0.4917	0.5750	0.5250	0.4800

A. anguilla restocking nuances. Glass eels migrate from spawning sites in the Sargasso Sea to Europe in shoals, consisting of presumably akin individuals, and accumulate in river entries. There they are caught by fishermen and are later translocated upstream, into other river basins or even other countries within great distances.

Overall genetic differentiation among the sampled eel populations from all the studied water bodies was evaluated too. Since the F_{ST} value at all loci was 0.0228 and the p value was 0.0281, it seems that there is small but significant genetic differentiation among the four examined populations (Table 5). Similarly, small but significant genetic differentiation was detected within the naturally recruited and introduced *A. anguilla* groups. Since the F_{ST} value calculated for the naturally recruited and introduced *A. anguilla* groups was 0.000 and 0.0238, respectively, no genetic differentiation is likely to exist in the group of naturally recruited *A. anguilla*. However, there is small but significant genetic differentiation within introduced fish groups. The lack of genetic differentiation within the group of naturally recruited *A. anguilla* suggests that the Baltic Sea and the Curonian Lagoon samples could belong to a genetically uniform population.

One of the reasons determining a rather high calculated value of the inbreeding coefficient F_{IS} (0.3084) in the Lithuanian eel population, which is represented by the individuals caught in the Baltic Sea, the Curonian Lagoon, as well as Lakes Siesartis and Dringis, can be a decline in heterozygosity. The latter phenomenon can be explained by mating specificity and a non-random distribution of individuals at spawning sites that have an effect on the formation of rather homogeneous shoals of glass eels,

consisting of individuals of similar genotypes. It might be suggested that the majority of naturally recruited eels reaching the Lithuanian coastal waters yearly belong to such a genetically uniform shoal. This particular model of spatial distribution of this species might be one of the main reasons causing the high F_{IS} value.

Significant differentiation between most pairs of these populations was confirmed using the Raimond and Rousset test. Samples from the Curonian Lagoon and the Baltic Sea formed the only significantly non-differentiated ($p = 0.1120 > 0.05$) pair among the investigated samples (Table 6). This fact means that they represent a single population consisting of presumably naturally recruited eels.

It is known that the introduction of glass eels into Lakes Dringis and Siesartis was performed repeatedly over several years. Presumably, they were introduced from different countries or at least from different locations within the country. As a result, eel populations are likely to have different genetic pools in the lakes under study (p value was $0.0008 < 0.05$, Raimond and Rousset test). The dendrogram based on standard Nei genetic distances (Fig. 2) indicates that the Curonian Lagoon and the Baltic Sea samples form one cluster which also reflects the closest relations between these groups of *A. anguilla*. What is more, these groups consisting of naturally recruited eels could be considered as subpopulations of a single population. The dendrogram, as well as the Raimond & Rousset test confirms our suggestion that glass eels introduced into Lake Dringis and Lake Siesartis were transported from different locations or countries in different time periods and, as a result, they exhibit higher genetic distances.

Table 5. Evaluation of inbreeding (F_{IS}) and genetic differentiation (F_{ST}) within naturally recruited and introduced European eel populations using F statistics.

Population	F_{IS}	F_{ST}
Naturally recruited	0.3046	0.0000
Introduced	0.3128	0.0238
Overall	0.3084	0.0228

Table 6. Significance of genetic differentiation between pairs of populations (Raimond & Rousset test).

Population	Curonian Lagoon	Baltic Sea	Lake Dringis
Curonian Lagoon	-		
Baltic Sea	0.1120	-	
Lake Dringis	0.0243*	0.0153*	-
Lake Siesartis	0.0140*	0.0022*	0.0008*

* – significant differentiation

Figure 2. Dendrogram based on Nei (1972) genetic distances. Numbers 1, 2, 3 and 4 indicate the Curonian Lagoon, Lake Dringis, the Baltic Sea and Lake Siesartis populations, respectively.

DISCUSSION

During this study the genetic structure of the European eel populations sampled in the Baltic Sea, the Curonian Lagoon, Lake Dringis and Lake Siesartis was examined using microsatellite assay. The percentage of introduced eels among samples taken from the Baltic Sea and the Curonian Lagoon was determined using otolith microchemistry analysis by a group of Taiwanese and Lithuanian researchers (Shiao *et al.* 2006). It was ascertained that introduced eels constitute 2% and 20% of eel populations in the Baltic Sea and the Curonian Lagoon, respectively, whereas individuals collected from the lakes were introduced totally. Although there is no reliable information regarding the countries from which glass eels were introduced into Lake Dringis and Lake Siesartis, it is known that the restocking of Lithuanian inland waters with glass eels from England and France was performed on a regular basis over several decades (Shiao *et al.* 2006). Furthermore, there are reliable data indicating that all eels, which inhabit Lake Baluošai were introduced (Shiao *et al.* 2006). Eels inhabiting Lakes Dringis and Siesartis are likely to have been introduced in a similar way.

Results of the study suggest that sampled eels from two lakes might be considered as representatives of different populations. However, the Baltic Sea and the Curonian Lagoon samples could be ranged as subpopulations of a single population. As it was suggested earlier, the significant genetic differentiation between the populations of the two lakes might be caused by the following factors: different age of individuals (which means that glass eels were caught and translocated into two lakes in different years); different restocking time and different sites from which glass eels were collected. It was demonstrated that naturally recruited

A. anguilla differed genetically from the introduced ones. Thus, there is small but significant genetic differentiation in the *A. anguilla* population consisting of the groups of naturally recruited and introduced *A. anguilla*. Due to some methodical and sampling differences it should not be stressed that the calculated F_{ST} values were higher than those of other studies (Wirth & Bernatchez 2001; Dannewitz *et al.* 2005). However, previous studies did not examine genetic differentiation between the groups of naturally recruited and introduced *A. anguilla*. Hence, the comparison of the F_{ST} value with the results of alternative studies is complicated.

Wirth and Bernatchez (2001) found genetic differentiation within the *A. anguilla* stock, which could be determined applying the isolation by distance (IBD) pattern in *A. anguilla*. However, some other studies proposed contradictory explanations, stating that there was no genetic differentiation determined by the IBD pattern between different *A. anguilla* populations, whereas the presence of the temporal genetic structure in the *A. anguilla* was determined (Dannewitz *et al.* 2005).

The purpose of the *A. anguilla* restocking is to increase its resources in a particular country (Ringuet *et al.* 2002; Wickström 2005). However, some countries take a different approach to the *A. anguilla* restocking strategy (Limburg *et al.* 2003; Sjöberg *et al.* 2005). For example, in Sweden eels are released not only into inland waters but even into the sea itself. Therefore, the purpose of fish restocking is not only to enhance local fishery but also to sustain *A. anguilla* population by means of stock protection (Sjöberg *et al.* 2005; Wickström 2005). As *A. anguilla* otolith microchemistry studies describing spawning migrations of the silver eel are controversial (Limburg *et al.* 2003; Sjöberg *et al.* 2005; Wickström 2005), the question as to whether introduced *A. anguilla* reach the Sargasso Sea or lose their way somewhere in the Baltic Sea still remains unanswered. Furthermore, the effect of crossbreeding of restocked and naturally recruited eels on this species in the Sargasso Sea could be examined too. If Wirth and Bernatchez's (2001) hypothesis concerning the different timing of reaching the Sargasso Sea by eels from different geographical regions of their distribution range is correct, it is plausible that the *A. anguilla* introduced into a particular country could reach the Sargasso Sea at the same time as the naturally recruited individuals.

Our findings on the existence of genetic differentiation among eels could be in agreement with the conclusions drawn by Dannewitz *et al.* (2005) due to the existence of temporal genetic variation, which means that samples consisting of individuals of similar age

have similar genetic structures. As there are different populations with different age structures in Lake Dringis and Lake Siesartis (Ložys, unpubl.), it is plausible that our F_{ST} values reflect temporal genetic variation in the studied populations too.

To sum up, the initial investigations into genetic variability and genetic structure of the *A. anguilla* population representing naturally recruited and introduced individuals do not support the hypothesis on the existence of complete panmixia in *A. anguilla* species. Consequently, we think that the restocking strategy of this fish should be viewed with precaution. More investigations must be carried out to find out whether introduced *A. anguilla* are able to reach the Sargasso Sea and if there is genetic differentiation caused by the isolation by distance in this fish species.

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LIETUVOS EUROPINIŲ UNGURIŲ (*ANGUILLA ANGUILLA* (L.)) VIDURŪŠINĖS GENETINĖS ĮVAIROVĖS TYRIMAS NAUDOJANT MIKROSATELITINIUS DNR ŽYMENIS

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SANTRAUKA

Šio darbo metu naudojant 5 mikrosatelitinius DNR žymenis buvo lyginamos vietinės ir introdukuotos Europinių ungurių (*Anguilla anguilla*) populiacijos. Mėginiai, reprezentuojantys vietinės ungurių populiacijas surinkti Baltijos jūroje ir Kuršių mariose, o reprezentuojantys introdukuotas ungurių populiacijas – Dringio ir Siesarties ežeruose. Tyrimo rezultatai neparemia panmiksijos hipotezės *A. anguilla* rūšyje, kadangi, tiek introdukuotų upinių ungurių grupės ($F_{ST} = 0,0238$, $p = 0,0148$), tiek ir lyginant visas tirtas imtis ($F_{ST} = 0,0228$, $p = 0,0281$), buvo gautos mažą, bet patikimą genetinę populiacijų diferenciaciją atspindinčios reikšmės.

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