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Invasion of the European River Lamprey *Lampetra fluviatilis* in the Upper Volga

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Supplementary materials 1

Taxonomic status of adult lampreys and their position in the Petromyzontidae

To estimate the taxonomic status of the adult lampreys Key to Lamprey Adults of the World [1] was used.

In either event the area discussed may be inhabited by the representatives of any species of the genera *Caspiomyzon*, *Lethenteron*, *Eudontomyzon*, or *Lampetra* the key starting from 5a were used with some restrictions (6; 9; 15-18; 23; 27; 37 were excluded from the analysis as non-variative, unsearchable, noninformative or unexplored). Each key thesis and antithesis was broken down into simple statements. For descriptive characteristics, the break point were a coma, a semicolon, or a conjunction in the thesis / antithesis, i.e.

5	5b	5b.1	Supraoral lamina with two teeth;
5	5b	5b.2.1	either unicuspid
5	5b	5b2.2	or bicuspid;
5	5b	5b.3	separated by a wide bridge,
5	5b	5b.3.1	which bears cusps
5	5b	5b.3.2	or does not bear cusps

For the measured features, a step of 0.1% was used, since the measurements in the Key are given with an accuracy of tenths, i.e.

26a.2.1.1	Disc Length/Total Length, 4,7%
26a.2.1.2	Disc Length/Total Length, 4,8%
26a.2.1.3	Disc Length/Total Length, 4,9%
26a.2.1.4	Disc Length/Total Length, 5,0%
26a.2.1.5	Disc Length/Total Length, 5,1%
26a.2.1.6	Disc Length/Total Length, 5,2%
26a.2.1.7	Disc Length/Total Length, 5,3%
26a.2.1.8	Disc Length/Total Length, 5,4%
26a.2.1.9	Disc Length/Total Length, 5,5%

For the meristic characters, a step of 1 was used, i.e.

11b.1.1	Trunk myomers, 64
11b.1.2	Trunk myomers, 65
11b.1.3	Trunk myomers, 66
11b.1.4	Trunk myomers, 67
11b.1.5	Trunk myomers, 68
11b.1.6	Trunk myomers, 69
11b.1.7	Trunk myomers, 70
11b.1.8	Trunk myomers, 71
11b.1.9	Trunk myomers, 72
11b.1.10	Trunk myomers, 73
11b.1.11	Trunk myomers, 74

There were obtained 263 variants of all kinds of key attributes, 123 variants played role in estimation of diversity within *Lampetra*, as in [1]: 10–14, 19–22, 26, 28, 29b, 30–32, 34.

The samples were analyzed en bloc. The keys and descriptions given in [1] provide limits, so in the analysis of the features we believed that the minimum and maximum values are valid (coef. 1), the values that are within the limits were considered as equiprobable (may or may not be) (coef. 0.5); values outside the limits were taken as impossible (coef. 0). Thus, the entire population was analyzed in a ternary system.

Samples used in the analysis. Mediterranean Sea Basin, Portugal [2]: **1.** *Lampetra lusitanica*, n = 38; **2.** *Lampetra auremensis*, n = 31; **3.** *Lampetra planeri*, Ribeiras do Oeste, n = 31; **4.** *Lampetra planeri*, Lis, n = 27; **5.** *Lampetra alavariensis*, n = 36. Baltic Sea Basin, Russia: **6.** *Lampetra fluviatilis* resident, Serebristaya River, n = 23 [3]; **7.** *Lampetra fluviatilis* resident, Chernaya River, ZISP-36153, n = 6; **8.** *Lampetra fluviatilis* resident Plyussa River, ZISP-23439, n = 5; **13.** *Lampetra fluviatilis* resident, Izhora River, ZISP-23157, n = 10; **14.** *Lampetra fluviatilis* lake form (Lake Ladoga), Volkhov River, ZISP-46186, n = 18; **15.** *Lampetra fluviatilis* praecox, Neva, ZISP-29185, n = 20; **16.** *Lampetra fluviatilis* lake form, Lososinka (Lake Onega) [4]; **17.** *Lampetra fluviatilis* lake form, Lake Onega, ZISP-43038, n = 80; **18.** *Lampetra fluviatilis* resident, Oshta, ZISP-25635, n = 6; **19.** *Lampetra fluviatilis* anadromous, Nazia, 2015, n = 2; **20.** *Lampetra fluviatilis* lake form, Povenetskaya Bay (Lake Onega), ZISP-43038, n = 17; **21.** *Lampetra fluviatilis* lake form (Lake Ladoga) [5]; **22.** *Lampetra fluviatilis* praecox, Neva, 2010, n = 3; **23.** *Lampetra fluviatilis* anadromous, Neva, 2010, n = 19; **24.** *Lampetra fluviatilis* anadromous, Neva, 2010, n = 44; **25.** *Lampetra fluviatilis* anadromous, Vistula, 2010, n = 4. Caspian Sea Basin, Russia: **9.** *Lampetra fluviatilis* resident, Bolshaya Sestra, 2014, n = 14; **10.** *Lampetra fluviatilis* resident, Kamenka, 2018, n = 5; **11.** *Lampetra fluviatilis* resident, Vysochinsky, 2019, n = 30; **12.** *Lampetra fluviatilis* resident, Saragozha, 2019, n = 7.

Euclidian distances were measured for each pair of samples (Table S1) and the maximal likelihood tree was built (Figure S1), mds-scaling was performed both for genus *Lampetra* compared to the other genera of lamprey and for samples within the genus.

Table S1. Results of analysis of the Euclidian distances for each pair of samples.

	C. wagneri	*E. mariae*	*L. fluviatilis*	*L. planeri*	*L. camtschaticum*	11	12	10	9	21	19	17	25	23	24	15	22	20	16	14	13	8	7	18	6	5	4	3	2	1
C. wagneri	0.0	7.45	7.86	7.6	10.4	9.22	8.3	8.38	8.4	7.8	7.7	7.8	7.3	7.4	7.5	7.2	7.2	8.1	7.3	7.5	8.0	7.7	8.2	7.4	8.2	7.9	7.6	7.3	7.4	7.5
E. mariae		0.00	7.63	8.0	9.2	8.22	8.6	8.11	7.8	8.4	8.5	7.9	8.0	7.7	7.7	7.7	8.4	8.3	7.6	7.5	8.3	8.0	8.5	8.0	8.9	7.8	7.4	8.2	8.0	8.1
L. fluviatilis			0.00	4.4	9.8	5.50	5.7	5.66	5.4	5.4	5.4	5.0	4.7	4.1	4.9	4.8	4.9	4.7	4.6	4.4	4.7	5.3	5.3	5.1	5.8	5.2	5.1	5.7	5.1	5.2
L. planeri				0.0	10.2	6.26	5.4	5.48	5.3	5.6	5.2	5.1	5.3	5.4	5.8	5.2	4.9	5.2	4.7	5.0	2.2	4.9	5.1	4.7	5.4	5.2	4.8	5.4	5.4	5.4
L. camtschaticum					0.0	9.76	11.1	9.33	10.1	10.7	11.1	10.4	11.1	10.4	10.8	10.5	10.9	10.2	10.1	10.1	10.5	10.9	10.6	10.9	10.2	11.0	10.7	11.4	11.5	11.5
11						0.00	6.3	5.94	5.3	6.4	7.0	6.0	7.0	6.2	6.9	6.1	7.1	5.4	5.8	5.6	6.0	6.0	5.3	5.8	5.7	6.7	6.3	7.3	7.0	7.3
12							0.0	6.22	5.8	6.3	5.8	5.8	5.8	5.8	5.7	6.0	5.7	5.8	5.7	5.9	4.9	5.2	5.4	5.3	5.6	5.8	5.2	5.7	5.3	5.6
10								0.00	5.1	6.6	6.6	6.3	6.5	6.2	6.3	6.2	6.5	6.2	6.0	6.1	5.6	5.9	5.8	6.0	5.4	6.2	5.7	6.1	6.4	6.5
9									0.0	5.9	6.0	5.0	5.9	5.6	5.7	5.5	6.0	5.1	4.9	5.1	4.9	4.6	4.3	4.7	4.9	5.8	5.1	5.5	5.8	6.0
21										0.0	4.6	4.2	5.3	4.6	4.6	3.5	3.9	4.2	4.0	4.1	5.5	5.7	5.7	4.4	6.2	6.5	6.0	6.4	6.2	6.4
19											0.0	4.4	4.7	4.8	5.0	4.1	3.9	4.3	4.3	4.6	4.9	4.7	5.0	4.3	5.7	5.8	5.3	5.6	5.8	5.9
17												0.0	5.0	4.5	4.8	3.7	4.5	4.1	1.9	3.1	4.9	4.5	5.0	4.2	5.8	6.2	5.6	6.0	5.9	6.3
25													0.0	3.8	3.2	4.0	4.2	4.7	4.5	4.1	5.4	4.8	5.8	4.8	6.3	5.7	5.2	5.1	5.0	4.9
23														0.0	3.7	3.7	3.8	4.9	4.0	3.7	5.6	5.6	5.7	4.6	6.6	5.8	5.2	5.5	5.1	5.4
24															0.0	4.1	4.0	4.9	4.4	4.0	5.8	5.5	5.8	4.9	6.3	5.5	5.2	5.4	4.8	5.0
15																0.0	3.8	3.8	3.2	3.1	5.2	4.9	5.4	3.7	6.1	5.9	5.5	5.7	5.7	6.0
22																	0.0	4.6	4.0	4.3	5.1	5.4	5.1	4.3	6.0	5.7	5.3	5.3	5.1	5.1
20																		0.0	3.9	3.7	4.9	4.5	4.9	4.3	5.4	6.1	5.5	6.3	6.4	6.2
16																			0.0	2.5	4.8	4.3	4.9	4.0	5.6	5.9	5.4	5.9	5.7	6.0
14																				0.0	5.1	4.8	5.4	4.3	6.0	6.2	5.5	5.9	5.7	5.9
13																					0.0	4.5	4.6	4.4	5.1	5.2	4.6	5.3	5.3	5.5
8																						0.0	4.2	4.5	4.5	5.5	4.9	5.4	5.7	5.7
7																							0.0	4.2	4.6	5.5	5.1	5.5	5.6	5.7
18																								0.0	5.5	5.7	4.8	5.3	5.1	5.5
6																									0.0	5.7	5.6	5.9	6.2	6.1
5																										0.0	3.4	4.1	3.9	4.3
4																											0.0	3.4	3.7	3.9
3																												0.0	2.8	3.3
2																													0.0	2.9
1																														0.0

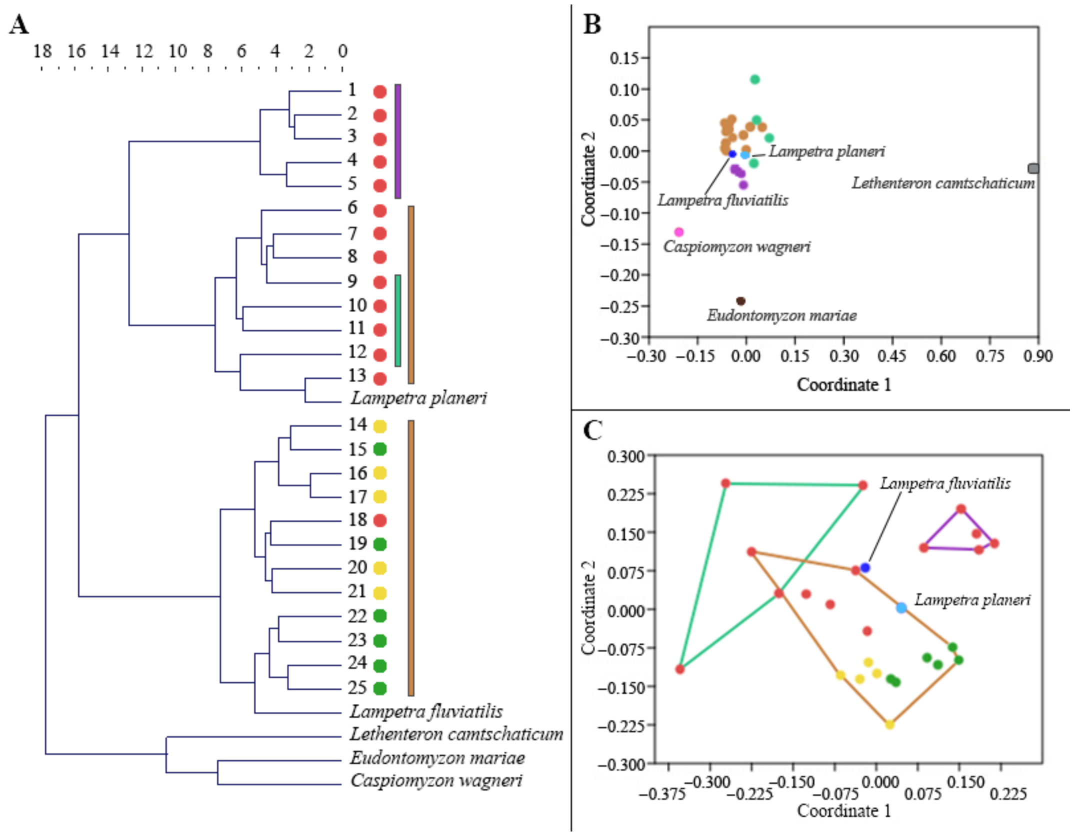

Figure S1. Analyses of lampreys diversity based on their key features. Note: **A.** Tree showing the results of the key features analysis of lamprey species (binominals) and samples (numbers). Euclidian distances are on the top of the tree. Sample numbers as above; tree branches with the species binomials are results, achieved following the keys [1]; red dots are for samples of resident form, yellow dots for lake form, and green dots are for anadromous form of lamprey. Samples from the Mediterranean Sea Basin (purple line), Baltic Sea Basin (brown line), and Caspian Sea Basin (green line). **B.** Positioning of samples and species in space (MDS-scaling) **C.** Positioning of samples within genus *Lampetra* in space (MDS-scaling).

Supplement 2: Genetic Status of Animals

To examine the genetic differentiation and relationship of the specimens from the Upper Volga with anadromous and freshwater European river lamprey (*Lampetra fluviatilis* and *Lampetra planeri* complex), we sequenced 1173 base pairs from gene Cyt-b segment of the mtDNA. Because of the fact that it is nearly impossible to reliably discriminate the larvae of both forms using morphological characters, we used adult freshwater (resident, nonparasitic) individuals from the Kamenka River (n = 2), tributary of Luga; anadromous (marine parasitic) individuals from Chernaya (n = 1) and Neva (n = 1); potamodromous (lake parasitic) from the Lake Ilmen (n = 1), and the Says River (n = 2) from the Baltic Basin to compare them to appropriately preserved (in alcohol) ammocoetes, which habitats are relatively distant from the Volga-Baltic watershed boundary, the Bolshaya Sestra (n = 1) and Yakhroma (n = 1) rivers (Table 1 in the paper). Additionally, we sequenced the same number of base pairs of larvae from the Kobona River (n = 2). DNA extraction process, primers, and other protocols we used are described in [6].

Comparative analysis. Examination of the obtained haplotype to known Cyt-b haplotypes of various European *Lampetra*, *Caspiomyzon wagneri* (GenBank: GQ206152.1), *Lethenteron camtschaticum* (GenBank: KX691482.1) and *Eudontomyzon mariae* (GenBank: AM051061.1) was performed with EMBnet-Server [7].

Online search of similar haplotypes was performed by Program: FASTA. Version 36.3.8h Aug, 2019. Input Parameters: Sequence type dna; Matrix none; Match/mismatch scores +5/−4; Gap open −14; Gap extend −4; Display of multiple high-scoring alignments (HSPs) false; Expectation upper limit 10.0; Expectation lower limit 0.0; Nucleotide strand both; Histogram false; Scores 50, Alignments 50; Score report format default; Statistical estimates 1; Annotation Features false; Database range START-END; Sequence range START-END; Filter none; Database em_all; KTUP 6.

Possible paths of the haplotype. To determine the breadth of distribution of the haplotype in the area, we used our own and published data [8–11]. Initially, points reflecting the ancestral and descendant haplotypes were plotted on the map, thereafter these points were connected by lines indicating the possible paths of movement/migration and/or dispersal of these haplotypes.

Results of genetic status estimation. The Cyt-b haplotype (Table S2) examination to the *C. wagneri* haplotype showed 85.3% identity (1133 bp overlap); to the L. *camtschaticum* haplotype, 93.2% (1173 bp overlap); to the *E. mariae*, 95.8% (1172 bp overlap).

The Cyt-b haplotype (Table S2) of the lamprey from Bolshaya Sestra (12) and Yakhroma (14) rivers differ from the most abundant known haplotype of the *Lampetra fluviatilis-planeri* complex on the area with the only replacement (Cyt-b125 g > a), which is 99.9% identity (99.9% similar).

Furthermore, in the open databases there are at least 50 sequences of *Lampetra fluviatilis*, *L. planeri* or *Lampetra* sp., demonstrating close relationship to the found in the Upper Volga area haplotype (Table S3). Sequences designated as *L. fluviatilis* (n = 22) showed identity of 99.5 to 99.9%; *L. planeri* (n = 21) as high as 99.4 to 99.9%, and *Lampetra* sp. (n = 7), 99.3 to 99.8%.

For two other known samples from the Volga River system, i.e., KP135462 and KP135463 from the Linda River, published by [11] we found no replacements compared to our data for the 1164-bp overlapping parts of the Cyt-b (bolded lines in Table S3).

Table S2. Cyt-b (1173 bp) of the lamprey from Bolshaya Sestra and Yakhroma rivers.

atgtcccacccaccaaccattcttcgaaaaactcacccactcctatcacttggtaa-
tagcatgttagttgaccttccttctcctgctaatatctcagcctgatgaaattttggctcacttttaagcctatgtttaatcttacaaattattacaggactaattcttgcgatacactat
accgccaacactgaactagccttctcttcagttatacacatttgtcgtgatgttaacaac-
ggatggcttatacgaaatcttcatgccaatggggcctctatattctttatttgtatttatgctcacatcgggcgagggatctactacggctcttatttatataaagaaacatgaaac
gttggagtcatcttatttgcattaactgcagctactgctttcgttggttatgtactac-
catgagggcaaatatccttctgaggggcaaccgttattacaaatttaatttcagcagtaccttatgtaggagatgatattgtagtatgattatgaggcggcttctcagtatcaaac
gccacattaacccggtttttacattccattttattttaccatttatcctagcagcaataac-
tataatccatattatattcctccatcaaacaggatctagtaacccccctaggaattaactctaatttggataaaattcaatttcacccatacttctctttcaaagacatttttggctttgtta
ttttacttggggttctttttataatttctcttttagccccaaatgcactaggtgagccaga-
caattttatttatgccaaccctcttagtaccccacctcacattaagccagaatgatacttcctgttcgcctatgcgattctacgctctattcctaataaattaggtggggtcatagcttt
agcagcagccatcataatcctcctggttatcccctttacccacacctctaaacaac-
gaggcattcaatttcgtccgctcgcccaagttacattttggattctgattgctgatctagcgctactcacttgactagggggagaaccagctgaacacccatttattttaataacac
aaattgcatcaacagtgtactttataatttttattttaatctttccaattctaggtcgcctagaaaacaagttaattctgctatcaaagaacaccggtaaattcaac

Table S3. Results for the Cyt-b haplotype of the lamprey from Bolshaya Sestra (12) and Yakhroma (14) rivers comparation to the deposited in the open sources data.

DB:ID	Species	Length	Score (Bits)	Identities (%)	Positives (%)	Source
EM_OV:KJ684748.1	*L. fluviatilis*	1191	1233.2	99.90	99.90	[2]
EM_OV:KJ684746.1	*L. fluviatilis*	1191	1233.2	99.90	99.90	[2]
EM_OV:AJ966337.1	*L. planeri*	2001	1232.7	99.9	99.9	[3]
EM_OV:AJ937929.1	*L. fluviatilis*	2101	1232.6	99.90	99.90	[4]
EM_OV:AJ937934.1	*L. planeri*	2101	1232.6	99.9	99.9	[4]
EM_OV:KJ684744.1	*L. fluviatilis*	1191	1231.3	99.80	99.80	[2]

EM_OV:KJ684747.1	*L. fluviatilis*	1191	1231.3	99.80	99.80	[2]
EM_OV:KJ684745.1	*L. fluviatilis*	1191	1231.3	99.80	99.80	[2]
EM_OV:AJ966335.1	*L. planeri*	2001	1230.8	99.8	99.8	[3]
EM_OV:AJ937941.1	*L. planeri*	2101	1230.7	99.8	99.8	[4]
EM_OV:AJ937926.1	*L. fluviatilis*	2101	1230.7	99.80	99.80	[4]
EM_OV:AJ937945.1	*L. planeri*	2101	1230.7	99.8	99.8	[4]
EM_OV:AJ937936.1	*L. fluviatilis*	2101	1230.7	99.80	99.80	[4]
EM_OV:AJ937928.1	*L. fluviatilis*	2101	1230.7	99.80	99.80	[4]
EM_OV:FN641832.2	*Lampetra* sp.	2102	1230.7	99.8	99.8	[5]
EM_OV:FN641829.2	*Lampetra* sp.	2102	1230.7	99.8	99.8	[5]
EM_OV:AJ966334.1	*L. planeri*	2001	1228.9	99.7	99.7	[3]
EM_OV:AJ966336.1	*L. planeri*	2001	1228.9	99.7	99.7	[3]
EM_OV:AJ937942.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937951.1	*L. planeri*	2101	1228.8	99.7	99.7	[4]
EM_OV:AJ937924.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937930.1	*L. planeri*	2101	1228.8	99.7	99.7	[4]
EM_OV:AJ937935.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937937.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937954.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937939.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:AJ937927.1	*L. fluviatilis*	2101	1228.8	99.70	99.70	[4]
EM_OV:FN641831.2	*Lampetra* sp.	2102	1228.8	99.7	99.7	[4]
EM_OV:FN641830.2	*Lampetra* sp.	2102	1228.8	99.7	99.7	[5]
EM_OV:AJ937944.1	*L. fluviatilis*	1173	1226.3	99.60	99.6	[4]
EM_OV:AJ937931.1	*L. planeri*	2101	1227.0	99.7	99.7	[4]
EM_OV:AJ937952.1	*L. planeri*	2101	1227.0	99.7	99.7	[4]
EM_OV:AJ937925.1	*L. fluviatilis*	2101	1227.0	99.70	99.70	[4]
EM_OV:AJ937938.1	*L. fluviatilis*	2101	1227.0	99.70	99.70	[4]
EM_OV:AJ937940.1	*L. fluviatilis*	2101	1227.0	99.70	99.70	[4]
EM_OV:AJ937922.1	*L. planeri*	2101	1227.0	99.7	99.7	[4]
EM_OV:AJ937932.1	*L. planeri*	2101	1227.0	99.7	99.7	[4]
EM_OV:AJ937933.1	*L. planeri*	2101	1225.1	99.6	99.6	[4]
EM_OV:AJ937943.1	*L. fluviatilis*	2101	1225.1	99.60	99.6	[4]
EM_OV:KP135462.1	***L. planeri***	**1164**	**1223.8**	**99.9**	**99.9**	**[7]**
EM_OV:KP135463.1	***L. planeri***	**1164**	**1223.8**	**99.9**	**99.9**	**[7]**
EM_OV:AJ937953.1	*L. planeri*	2101	1223.2	99.5	99.5	[4]
EM_OV:AJ937921.1	*L. fluviatilis*	2101	1223.2	99.50	99.5	[4]
EM_OV:AJ937950.1	*L. planeri*	2101	1223.2	99.5	99.5	[4]
EM_OV:AJ937923.1	*L. planeri*	2101	1223.2	99.5	99.5	[4]
EM_OV:FN641833.2	*Lampetra* sp.	2102	1223.2	99.5	99.5	[5]
EM_OV:FN641825.2	*Lampetra* sp.	2102	1223.2	99.5	99.5	[5]
EM_OV:AJ937946.1	*L. planeri*	2101	1221.3	99.4	99.4	[4]
EM_OV:FN641828.2	*L. planeri*	2102	1221.3	99.4	99.4	[5]
EM_OV:FN641834.2	*Lampetra* sp.	2102	1219.4	99.3	99.3	[5]

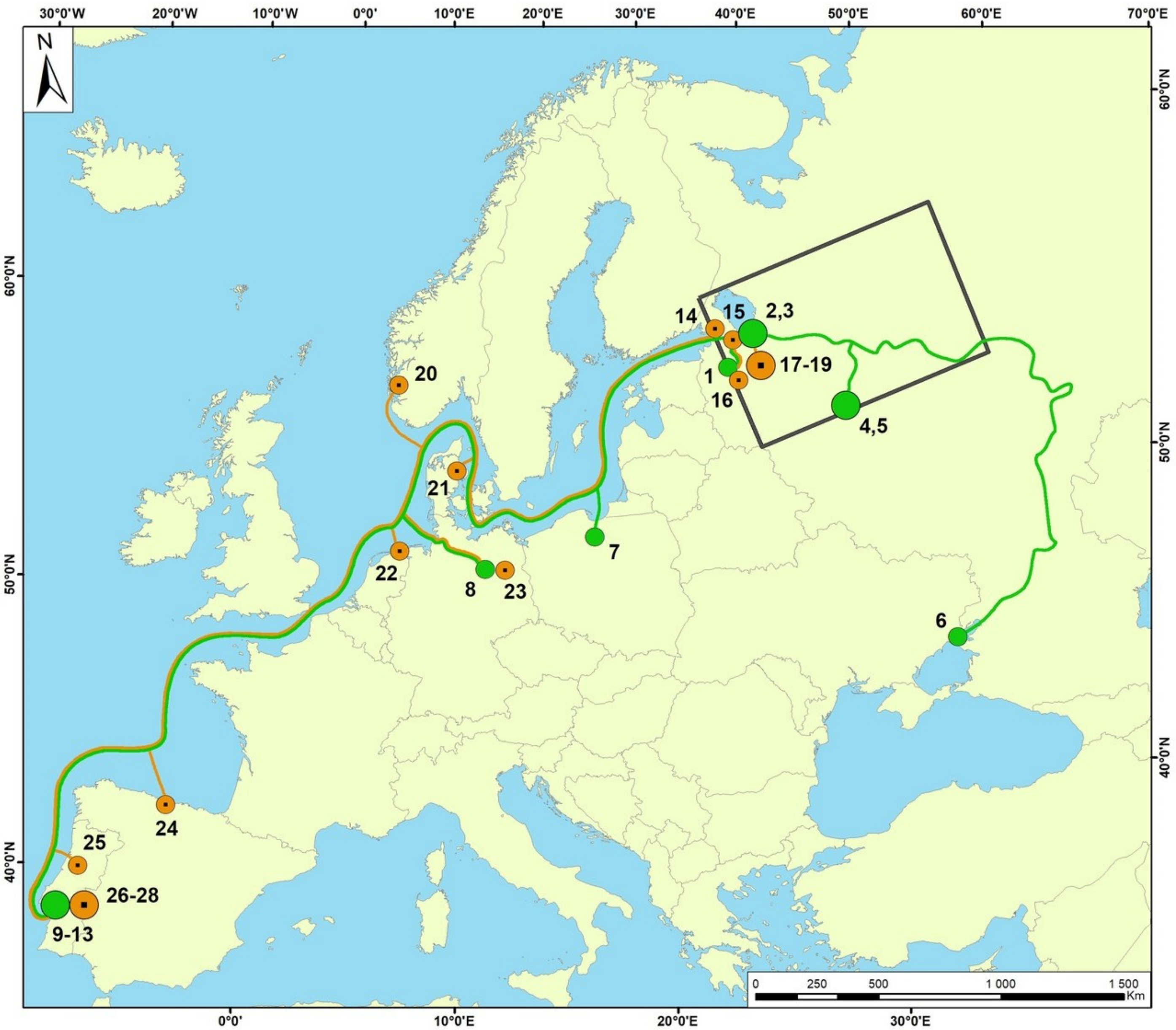

Figure S2: Known distribution of the ancestor and descendant haplotypes on the range of the *Lampetra fluviatilis-planeri* complex and their possible migration paths.

Descendent haplotype (green dots):

Kamenka, tributary of Luga (1); Kobona (2); Syas (3); Bolshaya Sestra (4); Yakhroma (5); Sea of Azov (6) [2]; Vistula (7) [4]; Elbe (8) [4]; Ponta d'Erva (9) [5]; Canha (10) [5]; Tagus (11) [4]; Erra (12) [5]; Sorraia (13) [4]

Ancestor haplotype (orange dots):

Chernaya (14); Neva (15); Kamenka, tributary of Luga (16); Lake Ilmen (17); Kobona (18); Syas (19) [2]; Vikedalselva (20) [4]; Gudenaa (21) [4]; Wadden Sea (22) [4]; Elbe (23) [4]; Deva (24) [5]; Criz (25) [5]; Sorraia (26) [4]; Canha (27) [5]; Tejo (28) [4]

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