S7 characterization of Western European pikes Esox spp. (Actinopterygii, Esociformes)

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by

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Abstract. – The comparison of a 1635 bp fragment of the first intron of the S7 ribosomal protein coding gene, a commonly used phylogenetic marker, for specimens from the three European pike species *Esox aquitanicus*, *Esox cisalpinus* and *Esox lucius* highlights diagnostic sites and indels constituting molecular synapomorphies. Both the sequence alignment and the phylogenetic tree discriminate these three species, even with a short sequence fragment. Three *Esox lucius* haplogroups can be separated. These haplogroups might correspond to the evolutionary lineages highlighted by previous mitochondrial studies. Finally this study confirms hybridization between *Esox aquitanicus* and *Esox lucius*, but also the absence of geographical structure between *Esox lucius* haplogroups in France following restocking from East European piscicultures. The S7 marker is excellent for molecular identification, and could be used for environmental DNA.

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Résumé. – Caractérisation du marqueur S7 des brochets *Esox* spp. (Actinopterygii, Esociformes) ouest-européens.

Cette étude compare un fragment de 1635 pb du premier intron du gène de la protéine codante ribosomale S7 d'individus des trois espèces de brochets européens *Esox aquitanicus*, *Esox cisalpinus* et *Esox lucius*. L'alignement des séquences et l'arbre phylogénétique obtenu permettent la discrimination des trois espèces par des sites et des indels diagnostiques constituant des synapomorphies moléculaires. Trois haplogroupes d'*Esox lucius* ont également été mis en évidence, qui pourraient correspondre aux des lignées évolutives déjà mises en évidence par des études mitochondriales précédentes. Enfin, cette étude a permis de confirmer l'hybridation entre *Esox aquitanicus* et *Esox lucius*, mais aussi l'absence de structuration géographique en France entre les haplogroupes d'*Esox lucius*, due aux repeuplements à partir de piscicultures d'Europe de l'Est. Ainsi le marqueur S7 est excellent pour l'identification moléculaire, y compris pour l'ADN environnemental.

Key words
Esocidae
Esox aquitanicus
Esox cisalpinus
Esox lucius
First intron of the S7 ribosomal protein coding gene
Indel diagnostic

Pikes *Esox* spp. (Actinopterygii, Esociformes) constitute an emblematic fish group in Western Europe because of its high socioeconomic interest for recreational and commercial fishing (Raat, 1988; Mann, 1996). It is also a valuable model in ecology and evolutionary biology (Forsman *et al*., 2015). Scientists and fisheries managers considered European pikes well characterized with a single species, *Esox lucius* Linnaeus, 1758 (*e.g*. Raat, 1988). Its aquaculture was well developed (Billard, 1983), providing juveniles to restock waterbodies including in France (Keith *et al*., 2011).

During the last decade, the description of two new pike species changed the taxonomy of this genus: *Esox cisalpinus* Bianco & Delmastro, 2011 (synonym *Esox flaviae* Lucentini *et al*., 2011; see Bianco, 2014) from Northern and Central Italy, and *Esox aquitanicus* Denys *et al*., 2014 endemic from South-West of France (Bianco and Delmastro, 2011; Lucentini *et al*., 2011; Denys *et al*., 2014). These three morphologically diagnosable species are also well delimited with mitochondrial (Nicod *et al*., 2004; Lucentini *et al*., 2011; Denys *et al*., 2014; Gandolfi *et al*., 2016; 2017) and nuclear markers (Launey *et al*., 2006; Lucentini *et al*., 2006, 2010; Denys *et al*., 2014; Gandolfi *et al*., 2017). While mitochondrial data is divergent enough to provide discrimination on short sequences, they only reflect the maternal lineage; nuclear markers are often less variable. Moreover, *E. lucius* has already been introduced in the native areas of the two other species, and can hybridize with them (Lucentini *et al*., 2011; Denys *et al*., 2014; Gandolfi *et al*., 2017). With this

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pressing conservation issue in mind, a nuclear marker variable enough to identify easily the three pike species and their hybrids is needed. Denys *et al*. (2014) used the nuclear Pleiomorphic adenoma gene-like 2 marker (Plagl2), with three diagnostic sites on 531 bp distinguishing *E. aquitanicus* from *E. lucius*. Other common nuclear markers like the rhodopsin retrogen (Rh) or the Recombination activating gene 1 (RAG1) are even less variable (Appendix 1, 2). Microsatellites and AFLP methods are often used, but not very practical for identification purposes (Launey *et al*., 2006; Lucentini *et al*., 2006, 2010; Gandolfi *et al*., 2017).

The first intron of the S7 ribosomal protein coding gene (S7), and especially its deletions and insertion variability within and between species, is a very useful marker for species delineation in cyprinids (Mendel *et al*., 2008; Zaccara *et al*., 2014; Stierandová *et al*., 2016), cottids (Marešová *et al*., 2012) and mormyrids (Lavoué, 2016). This marker is therefore also valuable for detecting hybridizations (Marešová *et al*., 2012; Zaccara *et al*., 2014; Perea *et al*., 2016). It had not been often used in the past, as sequences for heterozygous specimens have different lengths because of the insertions and deletions (indels). These render Sanger chromatograms illegible after the length difference region. However, Next Generation Sequencing techniques (NGS) overcome this problem by assembling independently both alleles for heterozygous specimens (*e.g*. Medvedev *et al*., 2009).

This study focuses on a sequence fragment of 1635 bp of the S7 intron for the three pike species of Western Europe to determine whether part of it can be an efficient short marker for molecular identification.

MATERIAL AND METHODS

Thirty specimens were included in this study. Twenty-six came from France (see sampling details and morphological

Table I. – Sampling with S7 haplotypes affiliation and GenBank Accession numbers (in progress). *: identifications were made by Denys *et al*. (2014), §: specimens with the North American mitochondrial haplotype (see Denys *et al*., 2014), $: holotype of *Esox aquitanicus* Denys *et al*., 2014 (MNHN 2013-1246).

Country	Basin (Stream)	Town	ID	Taxa	S7 Haplotype	GenBank Accession Number
Canada				*Esox lucius*	H4	AZJR00000000
France	Charente (Antenne)	Le Seure	EM17880	*Esox lucius**	H1/H4	MH976775
France	Charente	Saint-Saviol	BRO25	*Esox lucius**§	H1/H4	MH976772
France	Charente (Lien)	Condac	BRO505	*Esox lucius**	H2/H8	MH976765
France	Dordogne	Cénac-et-Saint-Julien	BRO22	*Esox cf. lucius**	H1/H3	MH976776
France	Dordogne (Isle)	Trélissac	BRO24	*Esox lucius**	H4	MH976791
France	Dordogne (Isle)	Saint-Médard-de-Guizière	BRO453	*Esox lucius**	H1/H3	MH976778
France	Dordogne (Isle)	Saint-Médard-de-Guizière	BRO455	*Esox lucius**	H1	MH976784
France	Dordogne (Isle)	Saint-Médard-de-Guizière	BRO457	*Esox lucius**	H1	MH976783
France	Loire (Sèvre Nantaise)	Saint-Malo-du-Bois	BRO1	*Esox lucius**§	H1/H4	MH976768
France	Meuse	Han-sur-Meuse	BRO6	*Esox lucius**	H1/H4	MH976770
France	Rhône (Clauge)	La Loye	BRO428	*Esox lucius**	H3/H4	MH976793
France	Rhône (Clauge)	La Loye	BRO430	*Esox lucius**	H1/H3	MH976777
France	Seine (Blaise)	Saint-Ange-et-Torçay	BRO525	*Esox lucius**	H1/H4	MH976774
France	Seine	Serein	BRO464	*Esox lucius**	H4	MH976792
France	Seine	Serein	BRO466	*Esox lucius**	H2/H4	MH976767
France	Seine (Superbe)	Pleurs	BRO10	*Esox lucius**	H1/H4	MH976771
France	Seine (Superbe)	Pleurs	BRO9	*Esox lucius**	H4	MH976790
France	Somme (Canal de la Maye)	Favières	BRO2	*Esox lucius**	H1/H4	MH976769
France	Adour	Estirac	BRO462	*Esox lucius*§ x *aquitanicus**	H1/H4	MH976773
France	Eyre	Mios	BRO23	*Esox aquitanicus* x *lucius**	H8	MH976785
France	Adour (Estampon)	Saint-Gor	BRO531 $	*Esox aquitanicus**	H8	MH976787
France	Adour (Geloux)	Garein	BRO534	*Esox aquitanicus**	H8	MH976788
France	Charente (Lien)	Condac	BRO509	*Esox cf. aquitanicus**	H2/H8	MH976766
France	Eyre	Bélin-Béliet	BRO443	*Esox aquitanicus**	H8	MH976786
France	Eyre	Bélin-Béliet	BRO445	*Esox aquitanicus**	H1/H8	MH976784
France	Eyre	Bélin-Béliet	BRO536	*Esox aquitanicus**	H8	MH976789
Italy	Po (Bealera Riana)	Carmagnola	Ecis1	*Esox cisalpinus*	H7	MH976779
Italy	Po (Bealera Riana)	Carmagnola	Ecis2	*Esox cisalpinus*	H5/H6	MH976780
Italy	Po (Bealera Bassa)	Cercenasco	Ecis3	*Esox cisalpinus*	H6/H7	MH976781

identifications in Denys *et al*., 2014). All were previously identified also with the cytochrome oxidase subunit 1 (COI) and the nuclear Plagl2 markers (Denys *et al*., 2014). Seventeen *E. lucius*, five *E. aquitanicus* and four hybrids (2 F1 and 2 Fn+1) are included in our sampling (Table 1), as well as three specimens of *Esox cisalpinus* from the Po basin in Italy. Finally, we included the S7 sequence from a Canadian specimen for whom the complete genome is available on GenBank AZJR00000000 (Rondeau *et al*., 2014) (Tab. I). New primers were defined in Geneious v 9.0.5 (Kearse *et al*., 2012) to flank a large fragment using this sequence as reference.

DNA extraction was as described in Denys *et al*. (2014). DNA amplification was performed by PCR in a final 20 µl volume containing 5% DMSO, 1 µl of BSA at 5 µg/ml (Bovine Serum Albumin), 1 µl of dNTP 6.6 mmol/L, 0.15 µl of Qiagen Taq DNA polymerase, using 2 µl of the buffer provided by the manufacturer, and 0.4 µl of each of the two primers at 10 pmol/L S7Fex1bro 5'-CCA CAT YTT CAA AGA TGG CTG CC-3' and S7R1710bro 5'-CAT GAG GCA GCC TAG ACA GAG T-3'; 1.5 µl of DNA extract was added. After denaturation for 2 min, the long PCR was run for 60 cycles of (30 s, 94°C; 50 s, 55°C; 3 min, 72°C) on an Eppendorf Mastercycler Epgradient. NGS sequencing was performed using two-level multiplexing protocols following Hinsinger *et al*. (2015) on the Ion Torrent PGM of the MNHN SSM platform. The reads were assembled following Hahn *et al*. (2013) with Geneious v. 9.0.5 using the GenBank sequence as a reference. The quality control and coverage (from 21.6 to 662.5 in average) were checked. All new sequences with their voucher information were deposited into GenBank (Tab. I).

For heterozygous sequences of S7, reads from the contig generated by the Geneious software were first assembled through a *de novo* assembly. Then, the reads from each allele were separated and assembled in haplotypes. All assemblies were checked manually.

Alignments were performed using ClustalW (Thompson *et al*., 1994) and rechecked by eye.

The best-fitting model for phylogenetic analysis was assessed using jModeltest 2 (Darriba *et al*., 2012). The selected HKY model (Hasegawa *et al*., 1985) was used for Bayesian inference (MrBayes 3.2, Ronquist *et al*., 2012). Two analyses were run with 10 million generations and sampling every 200 generations; 10% of trees were eliminated as burnin after checking for convergence.

RESULTS

Coverage for the thirty sequences ranged from 9 to 1275 (average: 146 to 447). There are 8 distinct haplotypes. 18 of the 30 sequences are heterozygous, and the separation in two alleles yielded a total of 48 sequences. The 48 sequences and 8 haplotypes assembled in 5 haplogroups in the phylogenetic tree. These groups are supported in the alignment by shared nucleotides and indels (Fig. 1; Tab. II).

The first haplogroup (*Esox lucius* A) contains only one haplotype (H1) represented by 14 sequences including 11 from specimens identified as *Esox lucius* (Fig. 1) and caught from Adour, Charente, Dordogne, Eyre, Loire, Meuse, Rhone, Seine and Somme basins. It is supported by 6 diagnostic sites: C (*vs*. T) in positions 127 and 1595, T (*vs*. A) in position 433, G (*vs*. C) in position 1180 and A (*vs*. G) in positions 1289 and 1475 (Table II). The second haplogroup (*Esox lucius* B) contains a single haplotype (H2) represented here by 3 sequences including two specimens identified as *Esox lucius* and caught from Charente and Seine basins. It is supported by 4 diagnostic sites: A (*vs*. C) in position 131, A (*vs*. G) in position 700, G (*vs*. T) in position 1605, and an indel in position 1658 to 1674. The third haplogroup (*Esox lucius* C) includes two haplotypes H3 and H4. It is represented by 17 sequences, 15 of which are from specimens identified as *Esox lucius* and caught from Adour, Charente, Dordogne, Loire, Meuse, Rhone, Seine and Somme basins as well as Canada. It is supported by 4 diagnostic sites: T (*vs*. C) in position 421, an indel in position 707 to 721, and A (*vs*. T) in positions 970 and 1156. The fourth haplogroup corresponds to *Esox cisalpinus* specimens and includes the three haplotypes H5 to H7 (5 sequences from Italian *Esox cisalpinus* specimens). It is supported by 5 diagnostic sites: T (*vs*. C) in positions 293, 653 and 1256, A (*vs*. C) in position 372 and A (*vs*. C) in position 1367. The haplogroups *Esox lucius* C and *Esox cisalpinus* share an indel in position 882-883 and a T (*vs*. C) in position 884. Finally the fifth and last haplogroup corresponds to *Esox aquitanicus*. It includes a single haplotype H8 represented by 8 sequences, 5 corresponding to specimens identified as *Esox aquitanicus* and including the sequence of the holotype BRO531 (MNHN 2013-1246). These specimens came from Adour, Charente, and Eyre basins. H8 is supported by 2 diagnostic sites: an indel in position 815 to 842, and T (*vs*. C) in position 885.

The three pike species are well separated. Heterozygous specimens within *Esox lucius* have the haplotypes H1/H3 (3 specimens from Dordogne and Rhone basins), H1/H4 (8 specimens from Adour, Charente, Loire, Meuse, Seine and Somme basins), H2/H4 (1 specimen from the Seine catchment), and H3/H4 (1 specimen from the Rhone catchment) (Table I). Among *Esox cisalpinus*, 2 specimens are heterozygous sharing the haplotypes H5/H6 and H6/H7. Finally, three hybrids *Esox aquitanicus* x *Esox lucius* share respectively the haplotypes H1/H8 and H2/H8 (BRO445, BRO505 and BRO509), whereas BRO445 and BRO505 were not identified as hybrids by Denys *et al*. (2014).

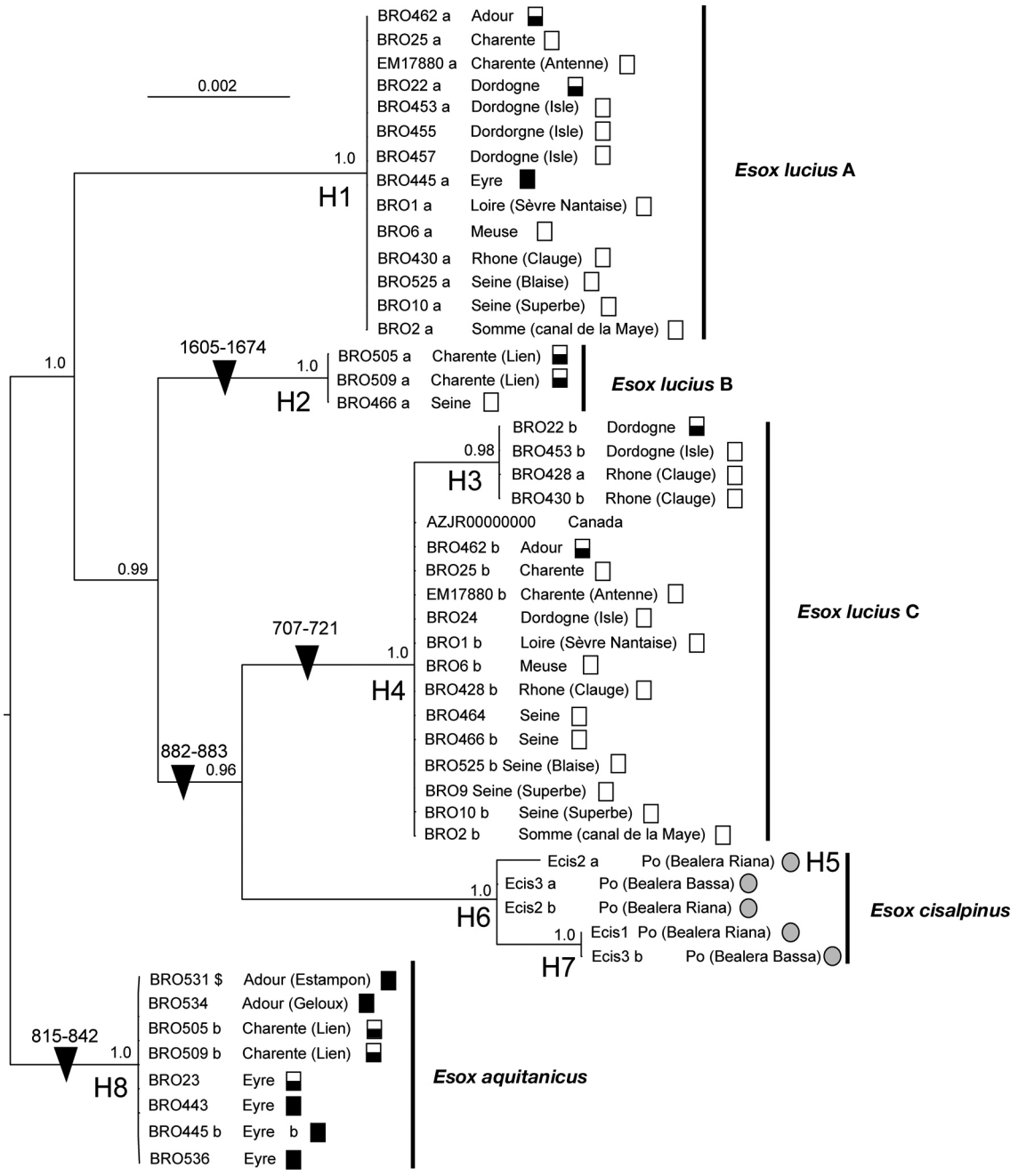

Figure 1. – Bayesian tree of the 1635 bp of the first intron of the S7 ribosomal protein coding gene (S7) for the 48 sequences separated by alleles of *Esox* spp. Numbers on the nodes represent posterior probabilities. Diagnostic indels and their location in the alignment are marked by a black triangle and position in the sequence above. Vouchers identifications are symbolized by black and white squares representing respectively *E. aquitanicus* and *E. lucius* (see Denys *et al*., 2014), and gray dots designate *E. cisalpinus*. $: indicates the holotype of *Esox aquitanicus* Denys *et al*., 2014 (MNHN 2013-1246).

DISCUSSION

The analysis of 1635 bp of the S7 intron discriminates clearly the three pike species *E. lucius*, *E. cisalpinus* and *E. aquitanicus*, with both diagnostic bases and indels (Fig. 1; Tab. II). It allows the identification of possible hybrids. Previously, the best nuclear markers for this identification were more complex approaches like microsatellites and AFLP methods (Launey *et al*., 2006; Lucentini *et al*., 2006, 2010; Gandolfi *et al*., 2017). The S7 intron sequence is more variable, and provides more diagnostic sites than Plagl2, Rh and RAG1 (Denys *et al*., 2014; Appendix 1, 2).

Table II. – Diagnostic sites highlighting mutations (in bold) and indels for the 8 haplotypes of *Esox* spp. on the S7 marker.

	127	131	171	272	293	372	421	433	653	700	707-721	815-842	882-883
H1	**C**	C	C	T	C	C	C	**T**	C	G	–	–	–
H2	T	**A**	.	.	.	.	.	A	.	**A**	–	–	–
H3	T	.	.	.	.	.	**T**	A	.	.	15 bp indel	–	2 bp indel
H4	T	.	.	.	.	.	**T**	A	.	.	15 bp indel	–	2 bp indel
H5	T	.	.	**A**	**T**	**A**	.	A	**T**	.	–	–	2 bp indel
H6	T	.	.	.	**T**	**A**	.	A	**T**	.	–	–	2 bp indel
H7	T	.	.	.	**T**	**A**	.	A	**T**	.	–	–	2 bp indel
H8	T	.	**T**	.	.	.	.	A	.	.	–	28 bp indel	–

	884	885	970	1092	1099	1156	1180	1256	1289	1319	1367	1475	1595	1605	1658-1674
H1	C	C	T	**G**	C	T	**G**	C	**A**	G	G	**A**	**C**	G	–
H2	.	.	.	A	.	.	C	.	G	.	.	G	T	**T**	17 bp indel
H3	**T**	.	**A**	A	**T**	**A**	C	.	G	.	.	G	T	.	–
H4	**T**	.	**A**	A	.	**A**	C	.	G	.	.	G	T	.	–
H5	**T**	.	.	A	.	.	C	**T**	G	.	**A**	G	T	.	–
H6	**T**	.	.	A	.	.	C	**T**	G	.	**A**	G	T	.	–
H7	**T**	.	.	A	.	.	C	**T**	G	**A**	**A**	G	T	.	–
H8	.	**T**	.	.	.	.	C	.	G	.	.	G	T	.	–

This nuclear marker also distinguishes clearly 3 haplogroups of *E. lucius* (Fig. 1; Tab. II). Biogeographic events during the Pleistocene structured the Northern pike in three lineages: a circumpolar lineage (Eastern Europe, Asia and North America), a southern lineage linked to the Danube drainage, and a northern lineage (Skog *et al*., 2014). French waterbodies have been restocked several times, often from hatcheries of central Europe (Poland, Czech Republic and Hungary) (Grès, 1994; Launey *et al*., 2006). Pike restocking from several origins led to introgression in native populations (Launey *et al*., 2006) and a mosaic of genotypes in Western Europe (Maes *et al*., 2003; Nicod *et al*., 2004; Lucentini *et al*., 2011; Denys *et al*., 2014; Skog *et al*., 2014; Gandolfi *et al*., 2017).

Each haplogroup observed could correspond to a lineage defined by Skog *et al*. (2014). The haplogroups *Esox lucius* A and C are the most represented as they occur in the main drainages of our sampling. The presence of the Canadian homozygous sequence (H4) in the *Esox lucius* C haplogroup might indicate that this group corresponds to the circumpolar lineage. The haplogroup *Esox lucius* A might corresponds to the Northern lineage. According to the COI marker, three specimens (BRO1, BRO25 and BRO462) had the North American mitochondrial haplotype (Denys *et al*., 2014), corresponding to the circumpolar lineage. However, they are all heterozygous H1/H4 for the S7 marker, despite distinct origin locations. More specimens from native populations in Eastern Europe to North America and Danube drainage are needed to confirm this.

While the relationship between haplotypes and *E. lucius* lineages needs a larger study, the S7 marker is an efficient tool to identify pike species. It might also discriminate some of the *E. lucius* lineages. It allowed us to detect hybrids, including some which were not detected by Denys *et al*. (2014) with only mitochondrial and one nuclear markers (Plagl2). The use of several nuclear markers is important for hybrid detection, especially for non-first generation hybrids where not all loci are still heterozygous (*e.g*. Wang *et al*., 2017). Moreover, we propose here some short fragments of the marker which flank parts of sequence including diagnostic sites and indels to identify *E. cisalpinus* (642 bp: S7F963 5' TTG TAG CCA TGG CAA CTG GT 3'; S7R1605 5' ATG GGT ATC GTT TTT AGC CCA 3') or *E. aquitanicus* (210 to 238 bp: S7F632 5' AAA AAT ACA GCG AGT CTC ACC 3' S7R891 5' AGG ATC CAC CAA TAG GAG AAC 3'). S7 could be also a good nuclear marker for environmental DNA (eDNA), as the use of a combination of mitochondrial and nuclear DNA markers increases the efficiency of species detection (*e.g*. Taberlet *et al*., 2012), although the often-used ribosomal markers are not variable enough for this purpose. This new variable molecular marker is useful for the determination of pike species and provides conservation data to managers.

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Appendix 1. – Rhodopsin retrogen sequences (822 pb) of 16 French pikes *Esox* spp. with location, specimen identification, GenBank Accession numbers (in progress) and mutation sites. Identifications were made by Denys *et al*. (2014). PCR and sequencing protocols are given by Lautredou *et al*. (2012).

Basin (Stream)	Town	ID	GenBank Accession Number	Identification by Denys *et al*. (2014)	425	734
Eyre	Bélin-Béliet	BRO443	MH976721	*Esox aquitanicus*	T	C
Eyre (Grande Leyre)	Sabres	BRO538	MH976722	*Esox aquitanicus*	T	C
Eyre (Grande Leyre)	Sabres	BRO541	MH976723	*Esox aquitanicus*	T	C
Adour	Estirac	BRO462	MH976724	*Esox lucius* x *Esox aquitanicus*	K	C
Eyre	Bélin-Béliet	BRO536	MH976725	*Esox aquitanicus*	K	C
Eyre	Bélin-Béliet	BRO445	MH976726	*Esox aquitanicus*	K	M
Charente (Sonsonnette)	Saint-Front	BRO433	MH976727	*Esox aquitanicus*	G	M
Rhône	Massigneu-de-Rives	BRO529	MH976728	*Esox lucius*	G	A
Garonne	Verdun-sur-Garonne	BRO20	MH976729	*Esox lucius*	G	C
Rhône	Breignier	BRO27	MH976730	*Esox lucius*	G	C
Rhône	Breignier	BRO28	MH976731	*Esox lucius*	G	C
Rhône	Breignier	BRO13	MH976732	*Esox lucius*	G	M
Rhône (Clauge)	La Loye	BRO431	MH976733	*Esox lucius*	G	M
Bourget Lake		BRO5	MH976734	*Esox lucius*	G	M
Somme (Canal de la Maye)	Favières	BRO2	MH976735	*Esox lucius*	G	M
Meuse	Han-sur-Meuse	BRO8	MH976736	*Esox lucius*	G	M

Appendix 2. – RAG1 sequences (1552 pb) of 28 pikes *Esox* spp. with location, specimen identification, GenBank Accession numbers and mutation sites. *: identifications were made by Denys *et al*. (2014), $: holotype of *Esox aquitanicus* Denys *et al*., 2014 (MNHN 2013-1246). Amplification after Chen *et al*. (2003) and sequencing follows Hinsinger *et al*. (2015).

Basin (Stream)	Town	ID	GenBank Accession Number	Identification	278	707	875	1424
Adour (Estampon)	Saint-Gor	BRO531 $	MH976737	*Esox aquitanicus*	R	G	G	A
Adour (Geloux)	Garein	BRO534	MH976738	*Esox aquitanicus*	A	G	G	A
Eyre (Grande Leyre)	Sabres	BRO541	MH976739	*Esox aquitanicus*	A	G	G	A
Eyre	Bélin-Béliet	BRO443	MH976740	*Esox aquitanicus*	A	G	G	A
Eyre	Bélin-Béliet	BRO536	MH976741	*Esox aquitanicus*	A	G	G	A
Eyre (Grande Leyre)	Sabres	BRO538	MH976742	*Esox aquitanicus*	A	G	G	A
Po (Bealera Riana)	Carmagnola	Ecis2	MH976743	*Esox cisalpinus*	G	A	G	C
Po (Bealera Bassa)	Cercenasco	Ecis3	MH976744	*Esox cisalpinus*	G	A	G	C
Provincial Fish hatchery	Carmagnola	Ecis5	MH976745	*Esox cisalpinus*	G	A	G	C
Rhône	Breignier	BRO13	MH976746	*Esox lucius*	G	G	G	C
Charente (Sonsonnette)	Saint-Front	BRO433	MH976747	*Esox aquitanicus*	G	G	G	C
Charente (Antenne)	Le Seure	EM17879	MH976748	*Esox lucius*	G	G	G	C
Somme (Canal de la Maye)	Favières	BRO2	MH976749	*Esox lucius*	G	G	G	C
Seine (Superbe)	Pleurs	BRO9	MH976750	*Esox lucius*	G	G	G	C
Dordogne	Cénac-et-Saint-Julien	BRO19	MH976751	*Esox lucius*	G	G	G	C
Charente (Lien)	Condac	BRO509	MH976752	*Esox cf. aquitanicus**	G	G	G	C
Bourget Lake		BRO5	MH976753	*Esox lucius*	G	G	G	C
Rhône	Massigneu-de-Rives	BRO529	MH976754	*Esox lucius*	G	G	G	C
Rhône (Clauge)	La Loye	BRO430	MH976755	*Esox lucius*	G	G	G	C
Rhône (Clauge)	La Loye	BRO428	MH976756	*Esox lucius*	G	G	G	C
Dordogne	Cénac-et-Saint-Julien	BRO22	MH976757	*Esox cf. lucius**	G	G	G	C
Dordogne (Isle)	Saint-Médard-de-Guizière	BRO453	MH976758	*Esox lucius**	G	G	G	C
Rhône (Clauge)	La Loye	BRO427	MH976759	*Esox lucius*	G	G	G	C
Geneva Lake		BRO30	MH976760	*Esox lucius*	G	G	G	C
Seine (Blaise)	Saint-Ange-et-Torçay	BRO525	MH976761	*Esox lucius**	G	G	G	C
Seine (Serein)	Pontigny	BRO464	MH976762	*Esox lucius**	G	G	G	C
Loire (Boivre)	Béruges	BRO502	MH976763	*Esox lucius**	G	G	G	C
Loire (Sèvre Nantaise)	Saint-Malo-du-Bois	BRO1	MH976764	*Esox lucius**	G	G	R	C