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First record of natural hybridization and introgression between pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*)

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A case of natural hybridization between pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*) was confirmed based on the intermediate morphological, anatomical and genetic characteristics of the hybrid. Microsatellite analyses ruled out the possibility that the focal individual was of either pure species, and indicated it was a *S. lucioperca* backcross.

Introduction

Hybridization is a common phenomenon in freshwater fish, and particularly among closely related species (Keck & Near 2009). However, gene flow between species via introgression is far less common due to pre- and post-zygotic isolation mechanisms (Scribner *et al*. 2000). Yet, hybridization and introgression does sometimes occur, and modern genetic tools have facilitated the detection of hybrids and backcrosses, often challenging the traditional perception of rigid species boundaries (Scribner *et al*. 2000). Pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*) are two divergent and distinct species which have overlapping distribution areas across Europe, but to the best of our knowledge, their hybridization has not been verified in nature (Craig 1987, Lappalainen *et al*. 2003). Here, we provide the first record of natural hybridization and introgression between the pikeperch and perch.

Material and methods

The putative hybrid was captured with a lure by a recreational fisherman from the Kokemäenjoki delta in western Finland (ca. 61°34´N, 21°36´E) on 2 Oct. 2009. This river has a long industrial and municipality sewage history. The specimen was frozen by the fisherman, and submitted to the University of Helsinki laboratory for genetic and morphological analysis.

In addition to a genetic sample from the putative hybrid, muscle tissue (a piece of filet) samples from 20 *P. fluviatilis* and 20 *S. lucioperca*

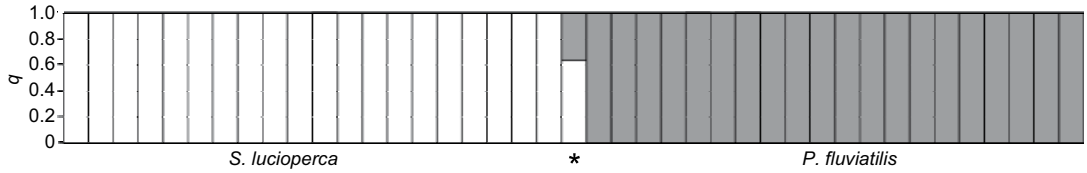

Fig. 1. Genetic assignment (*q*) of the *S. lucioperca* backcross (marked with a star) to reference species (white = *S. lucioperca*, grey = *P. fluviatilis*) using STRUCTURE. Each vertical column represents one individual.

individuals captured by the same fisherman at the same location were obtained. Tissue samples were stored in ethanol and total DNA was extracted using the Chelex method (Walsh *et al*. 1991). Nuclear genetic variation was assessed using nine microsatellite loci (*PUMF6*, *PUMF9*, *PUMF22*, *PUMF23*, *PUMF27*, *PUMF63*, *PUMF65*, *PUMF69m* and *PUMF71*; (GenBank accession numbers: NCBI PUIDs 10552749–10552757; T. Shikano unpubl. data). PCR-amplifications were conducted in a total volume of 10 μl: 2 pmol of each primer, 1× Phusion® Flash High-Fidelity PCR Master Mix (F-548L, Finnzymes), and approximately 20 ng of DNA. The cycling profile used for all loci was: 98 °C for 1 min, followed by 32 cycles of 98 °C for 1 sec, 60 °C for 15 sec and 72 °C for 20 sec, and a final extension at 72 °C for 1 min. The PCR-products were diluted 1:100 with Milli-Q water and mixed with Et-ROX 400 size standard (GE-Healthcare, Life Sciences). The samples were then analysed using a MegaBace 1000 capillary sequencer, and genotypes were scored using the Fragment Profiler 1.2 software (GE Healthcare, Life Sciences).

The microsatellite data were examined for deviations from the Hardy-Weinberg equilibrium (tests for each locus in each population), and for the linkage disequilibrium, using GenePop on the web (Raymond & Rousset 1995). The data were then analysed using NewHybrids ver. 1.1 to estimate the posterior probability that each individual falls into each of the six pre-defined hybrid categories: pure *S. lucioperca*, pure *P. fluviatilis*, F1, F2, *S. lucioperca* backcross, *P. fluviatilis* backcross (Anderson & Thompson 2002). For the NewHybrids analysis, we used 10 000 sweeps for burnin, and 50 000 sweeps for calculating Monte Carlo averages. STRUCTURE ver. 2.3.2 was then used to visualize the data and to calculate the proportion of the hybrid's ancestry that was attributable to each parental species (Pritchard *et al*. 2000). STRUCTURE analyses were run assuming two clusters (K = 2) and using 10 000 burnin runs and 50 000 MCMC repetitions.

To determine the maternal parent of the putative hybrid, we sequenced 1123 base pairs of the cytochrome *b* gene from the putative hybrid and five individuals of each parental species. For this, we used the primers L14724F and H15918R (Song *et al*. 1998). PCRs were performed in a total volume of 50 μl: 0.5 μM of each primer, 1× Phire® reaction buffer mix (Finnzymes, Espoo), 200 μM of each dNTP (Finnzymes, Espoo), 1 μl of Phire® Hot Start DNA polymerase (Finnzymes, Espoo) and approximately 20 ng of DNA. The cycling profile was: 98 °C for 30 s, followed by 34 cycles of 98 °C for 10 sec, 54 °C for 10 sec and 72 °C for 30 sec, and a final extension at 72 °C for 1 min. The PCR products were purified and sequenced in both directions (using the same primers) on an ABI3700 capillary sequencer (Macrogen, Korea). Sequences were checked and aligned using the program Geneious ver. 5.1 (Drummond *et al*. 2010). The similarity of the putative hybrid sequence to that of the two parental species was examined by eye. Sequences were deposited in GenBank (accession numbers: GU936789–GU936791).

A photo of the left flank of the fish was taken, and the number of fin rays and lateral line scales were counted. Meristic counts were compared with the data from the literature (Craig 1987). The shapes of the operculum bones, scales and gonads were compared with those of both parental species (*P. fluviatilis* and *S. lucioperca*). Unfortunately, intact *S. lucioperca* and *P. fluviatilis* specimens from the same location were not available, thus roughly equal-sized reference fish captured from a lake in southern Finland are presented in the photographs. Both scale

and operculum samples were used for aging, and growth was back-calculated from the scale sample using the Fraser-Lee procedure (Bagenal & Tesch 1978) with the intercept value of 44 mm (Vinni *et al*. 2009). A visual inspection was carried out to verify the sex of the specimen, as well as to compare the gonadal structure with that of the parental species.

Results

The microsatellite loci showed some interesting characteristics — loci *PUMF9* and *PUMF71* would not amplify any *P. fluviatilis* samples, nor the putative hybrid, but amplified well in *S. lucioperca* (Appendix, though heterozygote deficiency was noted for both loci, *PUMF9*: $p = 0.0001$, and *PUMF71*: $p = 0.026$). These loci provided no information for the further analyses so there were excluded, but these results do suggest that the focal fish was not a pure *S. lucioperca*. Additionally, *PUMF63* showed slight heterozygote deficiency in *P. fluviatilis* ($p = 0.021$) and was fixed in *S. lucioperca*. No consistent evidence of the linkage disequilibrium was detected. The results of the NewHybrids analysis were very clear, indicating that all *P. fluviatilis* and *S. lucioperca* individuals were assigned to their own pure species with $p > 0.999$. The putative hybrid individual was assigned as follows: pure *S. lucioperca* $p = 0$, pure *P. fluviatilis* $p = 0$, F1 $p = 0$, F2 $p = 0.208$, *S. lucioperca*-backcross $p = 0.791$, *P. fluviatilis*-backcross $p = 0$, indicating strongly that the putative hybrid was an *S. lucioperca* backcross. The STRUCTURE results indicated clear grouping according to species (*viz. S. lucioperca* or *P. fluviatilis*) (Fig. 1). Assignment strength was high for pure species ($p \geq 0.992$), but the hybrid individual was an admixture of the pure species: the proportion of its ancestry attributable to *P. fluviatilis* was 0.368, and that to *S. lucioperca* 0.632, providing support for the NewHybrids analysis (Fig. 1).

We did not find evidence for mtDNA introgression in pure species; all five *P. fluviatilis* sequences were identical to each other, as were all five *S. lucioperca* sequences to each other. The sequence divergence between the two species was 14.6% (164 polymorphic bases). The analyses of mtDNA indicated that the *S. lucioperca* backcross sequence was identical to that of *S. lucioperca*.

Morphologically the *S. lucioperca* backcross (total length 250 mm, mass 136.5 g) was intermediate between *S. lucioperca* and *P. fluviatilis*, especially with respect to the shapes of the head, fins and scales (Fig. 2). Coloration of the *S. lucioperca* backcross caudal, anal, pelvic, and dorsal fins was more similar to that of *P. fluviatilis* than *S. lucioperca*. The number of vertical bands on the flanks of the *S. lucioperca* backcross was close to that of *S. lucioperca*, and the length of bands was between that of *S. lucioperca* and *P. fluviatilis*. The *S. lucioperca* backcross had very closely situated first and second dorsal fins, with a higher number of spiny fin rays than either of the pure species (Fig. 2 and Table 1). The number of fin rays on the second dorsal fin, and the anal fin, as well as the number of scales, were intermediate between the pure species (Table 1). The *S. lucioperca* backcross had very distinct gonads that were fused from double (*S. lucioperca* character) to single (*P. fluviatilis* character) at the midsection (Fig. 2b). The shape of scales and operculum bones of the *S. lucioperca* backcross were also intermediate to the pure species (Fig. 2c and d). The *S. lucioperca* backcross was an immature female (3+ years old) with back-calculated total length

Table 1. Range of meristic counts for *S. lucioperca* and *P. fluviatilis* from literature (Craig 1987) and the values for the *S. lucioperca* backcross described in this study.

Meristic count	*S. lucioperca*	*S. lucioperca* backcross	*P. fluviatilis*
Number of spines in the first dorsal fin	13–17	18	12–13
Number of fin rays in the second dorsal fin	20–30	18	14–18
Number of fin rays in the anal fin	13–16	12	9–11
Number of scales along the lateral line	80–97	70	56–77

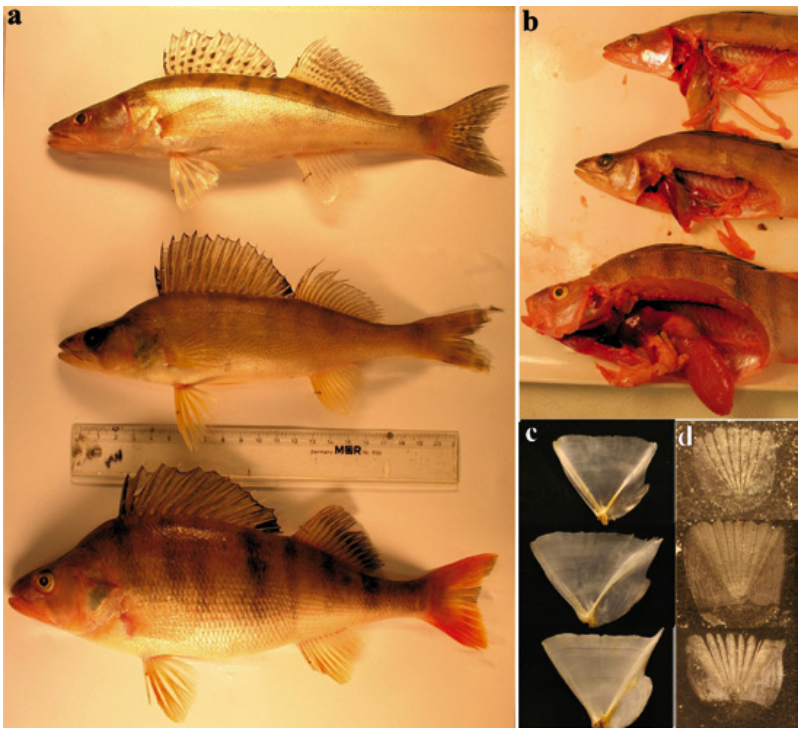

Fig. 2. Morphological characteristics of the *S. lucioperca* backcross (middle) and representative individuals of *S. lucioperca* (top) and *P. fluviatilis* (bottom) females in respect to (**a**) general appearance, (**b**) overview of gonads, (**c**) operculum and (**d**) scale.

at ages 1, 2 and 3 years of 79, 135 and 170 mm, respectively. Such maturation and growth pattern is clearly in-between typical *S. lucioperca* and *P. fluviatilis* from this latitude (Lappalainen *et al.* 2003, Keskinen & Marjomäki 2003, Tammi *et al.* 2004, Heibo *et al.* 2005).

Discussion

The evidence outlined above indicates that the putative hybrid was indeed an *S. lucioperca* backcross. Genetic results did not indicate signs of introgression in pure *P. fluviatilis* and *S. lucioperca* samples, and the general rarity of hybrids between *P. fluviatilis* and *S. lucioperca* suggest that the two species are reproductively well isolated. Since the focal specimen was a backcross and not a first generation hybrid, mtDNA results were not informative with respect to its' maternal origin. Namely, several possible mating scenarios are possible. Initially there could have been spawning between a female *S. lucioperca* and a male *P. fluviatilis* to produce an F1 hybrid. Subsequently there could either have been spawning between a female F1 with a male *S. lucioperca*, or between a male F1 with a female *S. lucioperca*. Yet another scenario, involves spawning between a male *S. lucioperca* and a female *P. fluviatilis* which could have produced a male F1 that spawned with a female *S. lucioperca*. In all of these cases, the focal specimen would carry the mtDNA of *S. lucioperca*.

As hybrids and backcrosses may occur between the two study species, it would be worthwhile to conduct additional sampling at the study site in order to quantify the amount of gene flow between them. Here, conclusive temporal and spatial sampling using less selective fishing gear would give representative size ranges of both species, and thus may give further insights into hybridization and introgression. Evidence from closely related species suggests that hybridization is rare; in an artificial crossing of walleye (*Sander vitreus*) and yellow perch (*Perca flavescens*), the offspring died either before hatching, or soon after induced hatching, and had vertebral deformities (Wiggins *et al.* 1983). However, hybridization and introgression between different species of darters (Percidae,

Etheostomatinae) is even quite frequent (Keck & Near 2009). Natural hybridization can also occur between close relatives within the genus *Sander* (Billington *et al*. 1988, Müller *et al*. 2010), and between *P. fluviatilis* and *Gymnocephalus cernuus* (Kammerer 1907). Despite the fact that *S. lucioperca* and *P. fluviatilis* are important species in Finnish fisheries, there is only one previous – but genetically unconfirmed — report of hybridization between these species (Peippo 1962). This comes from a very turbid bay of the Baltic Sea in southern Finland which had been subject to heavy pollution by municipality sewage for decades at the time of capture (Peippo 1962). Sewage increases algae-induced turbidity and may contain hormones, both of which are factors known to promote hybridization (cf. Seehausen *et al*. 1997, Scott & Sloman 2004). Regardless of the ultimate cause of the hybridization and introgression events, the case documented here appears to be the first verified natural *S. lucioperca* backcross between these species.

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Appendix. Raw genotyping data (allele sizes) for 20 *Perca fluviatilis* (A1–A20), 20 *Sander lucioperca* (K1–K20), and the putitive hybrid (H) at nine microsatellite loci (T. Shikano unpubl. data). Missing data indicates failure of PCR amplification.

	PUMF6		*PUMF22*		*PUMF69m*		*PUMF63*		*PUMF9*		*PUMF71*		*PUMF27*		*PUMF23*		*PUMF65*	
A1	334	338	264	266	327	339	299	299	–	–	–	–	307	307	279	279	350	350
A2	321	334	264	264	319	343	293	299	–	–	–	–	307	307	279	279	350	350
A3	321	334	264	264	319	343	293	299	–	–	–	–	307	307	279	279	350	350
A4	336	342	264	264	291	311	297	297	–	–	–	–	307	310	279	279	336	350
A5	334	338	264	264	291	327	297	297	–	–	–	–	307	307	279	279	350	350
A6	334	353	264	264	315	341	293	297	–	–	–	–	307	310	279	279	336	350
A7	342	353	264	264	337	341	297	297	–	–	–	–	307	307	279	279	350	352
A8	336	336	264	264	317	343	297	297	–	–	–	–	307	307	279	279	336	354
A9	336	342	264	264	311	347	293	299	–	–	–	–	307	307	279	279	350	354
A10	321	338	264	264	317	337	299	299	–	–	–	–	307	307	279	279	348	350
A11	342	353	264	264	285	291	299	299	–	–	–	–	307	307	279	279	336	350
A12	334	353	264	266	291	325	297	297	–	–	–	–	307	307	279	279	350	350
A13	342	359	264	264	325	343	287	299	–	–	–	–	307	310	279	279	350	354
A14	321	338	264	264	319	337	299	299	–	–	–	–	307	307	279	279	348	350
A15	334	338	264	264	317	323	299	299	–	–	–	–	307	307	279	279	350	350
A16	334	342	264	264	291	313	297	297	–	–	–	–	307	307	279	279	350	350
A17	338	340	264	264	293	343	293	299	–	–	–	–	307	310	279	279	354	354
A18	334	357	264	264	335	337	299	299	–	–	–	–	307	307	279	279	336	352
A19	338	353	264	264	339	341	297	297	–	–	–	–	307	307	279	279	–	–
A20	334	340	264	264	317	345	297	297	–	–	–	–	307	307	279	279	336	336
H	**340**	**341**	**264**	**281**	**294**	**294**	**265**	**299**	**–**	**–**	**–**	**–**	**307**	**317**	**279**	**281**	**323**	**323**
K1	335	339	265	265	294	294	265	265	353	353	295	315	314	317	281	281	323	323
K2	335	355	265	265	294	294	265	265	355	355	305	315	314	314	281	281	323	323
K3	339	355	265	265	288	294	265	265	343	353	295	295	314	317	281	281	323	323
K4	335	355	265	281	288	294	265	265	343	353	305	313	314	314	281	281	323	323
K5	335	355	265	265	294	294	265	265	357	357	295	305	314	314	281	281	323	323
K6	335	339	265	265	294	294	265	265	353	353	313	313	317	317	281	281	323	323
K7	335	355	265	265	288	294	265	265	345	353	315	315	314	314	281	281	323	323
K8	341	341	265	281	288	294	265	265	343	355	305	313	314	314	281	281	323	323
K9	335	355	265	265	288	294	265	265	351	351	315	315	314	314	281	281	323	323
K10	335	355	265	265	288	288	265	265	353	353	305	313	314	317	281	281	323	323
K11	341	341	265	281	288	294	265	265	357	357	305	315	314	314	281	281	323	323
K12	335	339	265	265	288	294	265	265	355	355	313	313	317	317	281	281	323	323
K13	339	355	265	265	290	294	265	265	345	353	295	305	314	317	281	281	323	323
K14	335	355	265	265	288	294	265	265	345	353	315	315	314	314	281	281	323	323
K15	335	355	265	265	294	294	265	265	355	355	305	315	314	314	281	281	323	323
K16	335	341	265	281	288	294	265	265	355	355	303	315	314	314	281	281	323	323
K17	343	355	281	281	288	288	265	265	345	353	303	303	314	317	281	281	323	323
K18	335	355	265	265	294	294	265	265	345	353	305	313	314	314	281	281	323	323
K19	343	355	265	265	294	294	265	265	343	353	295	305	317	317	281	281	323	323
K20	335	355	265	265	294	294	265	265	345	353	303	313	314	314	281	281	323	323