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MORPHOLOGICAL CHARACTERISTICS OF HYBRID PIKEPERCH (*SANDER LUCIOPERCA* ♀ × *SANDER VOLGENSIS* ♂) (OSTEICHTHYES, PERCIDAE)

SPECZIÁR, A.[1], BERCSÉNYI, M.[2] and MÜLLER, T.[3*]

[1]*Balaton Limnological Research Institute of the Hungarian Academy of Sciences*
H-8237 Tihany, Klebelsberg K. u. 3, Hungary
[2]*Pannon University, Georgikon Faculty of Agriculture*
H-8360 Keszthely, Deák F. u. 16, Hungary
[3]*Szent István University, Faculty of Agricultural and Environmental Sciences*
H-2103 Gödöllő, Páter K. u. 1, Hungary, E-mail: Muller.Tamas@mkk.szie.hu

Sander lucioperca (LINNAEUS, 1758) and *S. volgensis* (GMELIN, 1788) have been successfully hybridized under laboratory conditions with the common fertilization procedure, resulting fertile hybrids as viable as the parent species are. Although natural hybrids have not been reported yet, failing an effective postzygotic reproductive barrier, natural hybridization can also not be excluded. In order to facilitate the recognition of hybrid *Sander* we studied morphological characteristics of *S. lucioperca* and *S. volgensis* compared with their laboratory bred F_1 hybrids. There are some evident external features, which are generally used to distinguish the parent species, such as the presence of canine teeth in *S. lucioperca*, the position of the end of maxilla related to centre of eye and the different body pattern of the two species. However, in case of hybridization, these characters cannot be used for identification since even F_1 hybrids cover the full transitional scale between the parent species. Out of the 38 morphometric and 10 meristic characters examined, only the number of perforated scales in lateral line did not overlap among the three genotypes. We found, however, that *S. lucioperca*, *S. volgensis* and their F_1 hybrids can clearly be separated based on multivariate analysis of meristic and morphometric characters.

Keywords: hybrid, morphology, pikeperch, *Sander lucioperca*, *Sander volgensis*

INTRODUCTION

Many fish species are known to hybridize in the nature (SCHWARTZ 1981, SCRIBNER *et al.* 2000, BETTLES *et al.* 2005, KOZFKAY *et al.* 2007). Hybridization events may be facilitated by human activities, such as the modification of natural habitats and species introductions (FITZMAURICE 1984, SCRIBNER *et al.* 2000). Different kinds of fish hybrids are also generally used in aquaculture in order to benefit from their phenotypic or genetic advantages compared to those of their parent species. Inter-specific hybrids are produced for aquaculture to increase growth rate, combine desirable traits of two species, to reduce unwanted reproduction through production of sterile or monosex stocks, to take the advantages in sexual dimorphism, to increase harvestability and to increase environmental tolerance

(BARTLEY *et al.* 2000). Introduction or eventual escape of bred hybrid fish can affect living conditions of natural fish populations, and if they are fertile, genetic differences between the natural populations of the parent species can be compromised as well (FISS *et al.* 1997).

Hybridization among some species of the genus *Sander* also occurs. Thanks to its better growth and lower sensitivity to environmental conditions, the saugeye *S. vitreus* (MITCHILL, 1818) ♀ × *S. canadensis* (GRIFFITH & SMITH, 1834) ♂ has widely been used in North American aquaculture and stocked for angling purposes into natural waters. Saugeye may also engender under natural conditions and since they are fertile, they reproduce with both parent species as well as with each other (VAN ZEE *et al.* 1996, FISS *et al.* 1997, WHITE *et al.* 2005).

The two sympatric European freshwater *Sander* species, the *S. lucioperca* (LINNAEUS, 1758) and *S. volgensis* (GMELIN, 1788), which diverged about 1.8 million years ago (FABER & STEPIEN 1998), have not been found to hybridize in the nature yet. However, according to MÜLLER *et al.* (2004, 2006*c*) crossing of the two species can be induced easily in the laboratory with the common propagation technique applied also in the breeding of the parent species. Moreover, since fertilization and hatching rate in hybridization, as well as the survival and growth of hybrid larvae and fingerlings are similar to those of the parent species, it seems that there are neither morphological nor biochemical barriers against natural hybridization. Consequently, possibly only some prezygotic barriers may block natural hybridization of the two species, such as differences in their reproductive behaviour (BALON *et al.* 1977). However, time and habitat factors, as well as some behavioural factors which secure the reproductive isolation of the two species may vanish under particular conditions, e.g., in case of habitat alteration (SHERIDAN 1995, EVANS *et al.* 1998, SCRIBNER *et al.* 2000). On the other hand, since hybrids of *S. lucioperca* and *S. volgensis* were unknown before, there is no guarantee that we could recognize them in nature where molecular methods are not available. Hybrid specimens of closely related species are not always easy to identify based on external morphological features, while genetic studies are too expensive and time consuming to be applied extensively in faunistical and ecological studies. When hybrids are especially rare, practically the only possibility for a successful genetic validation of natural hybridization is if we have morphological keys enabling the detection of probable hybrid specimens. Detection of hybrid individuals is also of special importance when parent stocks are collected for experimental purposes or breeding (BILLINGTON *et al.* 1997).

The aim of our study was thus to facilitate the detection of hybridization between the two European freshwater *Sander* species. Under controlled laboratory conditions we separately bred *S. lucioperca*, *S. volgensis* and their F_1 hybrid stocks

originating from genetically proved pure-blood parent stocks. Based on these three known genotypes we performed a detailed morphological analysis to facilitate the detection of hybridization both in natural and artificial stocks.

MATERIAL AND METHODS

For morphological analyses we used laboratory reared 6 month old specimens of *S. lucioperca* (standard body length, SL = 68.9–96.7 mm), *S. volgensis* (SL = 56.7–71.0 mm) and their F_1 hybrids (SL = 67.2–83.7 mm). *S. lucioperca* parent stock originated from a commercial fish farm Aranyponty Ltd. (Sáregres-Rétimajor, Hungary) and the *S. volgensis* parents were caught from Lake Balaton. Parent stocks intended for laboratory breeding were acclimatized for one month. Spawning of females of *S. lucioperca* was induced by gradual increase of temperature in the tanks from 5 to 14°C for 8 days and by hormonal treatment with 250 International Unit (IU) human chorion gonadotropin (hCG) and 6 mg carp pituitary per fish on day 4 and 500 IU hCG per fish on day 5 (MÜLLER *et al.* 2006*c*). Females of *S. volgensis* were injected with a single dose of 6 mg per kg of body weight of dry carp pituitary extract 68 hours before stripping. Males of both *Sander* species were injected with a single dose of 4 mg per kg of body weight of dry carp pituitary extract 24 hours before milt stripping. Hybrid *Sander* was obtained by fertilizing eggs of *S. lucioperca* by *S. volgensis* milt. Juveniles of the three genotypes were reared in laboratory separately, but under the same conditions.

Fish selected for morphological analyses were killed by immersion in 0.1% solution of MS 222, then were preserved and stored in 4% formalin until morphological analyses. Morphological comparison of the parent species and their F_1 hybrid were done according to 38 morphometric (Fig. 1, Table 1) and 10 meristic (Table 1) characters on 15 specimens per genotype. Measurements were done by a digital calliper with an accuracy of 0.05 mm. Mouth gape width (GW) was measured by a height scaled copper cone. The tip of the cone was inserted into the mouth cavity of the fish and pushed posteriorly until the jaws reached their maximum aperture angle without deformation.

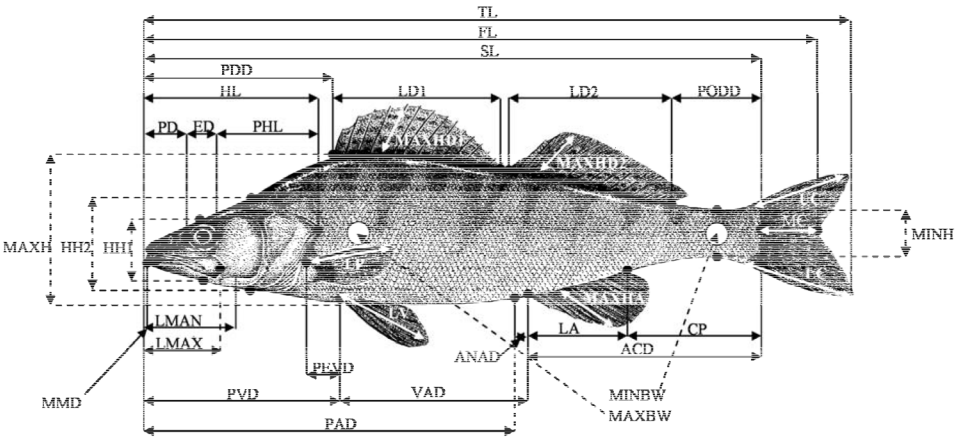

Fig. 1. Morphometric characters used to differentiate *Sander lucioperca* and *S. volgensis* and their hybrid. Each measurement was taken as the shortest (direct) distance between two corresponding reference points. Full names of analysed characters are given in Table 1

Table 1. Statistical parameters of meristic and relative morphometric characters (in % of *SL, HL* or *ED*) in *Sander lucioperca*, *S. volgensis* and their F_1 hybrid (n = 15 for each). Mean values of genotypes were compared pair-wise with t-test after a priori F-tests, except for the MMD where non-parametric Mann-Whitney U test was applied. Genotypes are statistically different for a given character at $P < 0.05$ if their values do not share any superscript letter.

			S. lucioperca	F_1 hybrid	*S. volgensis*
			mean±SD (range)		
Related characters in % of SL					
1.	TL	total length	118.0±2.38 (113.3–122.2)^a	119.2±1.26(116.9–121.3)^a	121.4±2.02 (117.5–125.1)^b
2.	FL	fork length	111.8±1.74 (109.3–114.8)^a	113.0±1.01(111.6–114.7)^b	112.8±1.56 (110.3–115.5)^{ab}
3.	PAD	preanal distance	60.3±1.53 (57.1–62.7)^a	58.9±0.85 (57.5–60.4)^b	55.1±0.88 (54.0–56.8)^c
6.	PVD	preventral distance	33.1±0.42 (32.0–33.6)^a	32.1±0.77 (30.8–33.7)^b	32.3±1.11 (30.8–34.9)^b
7.	PEVD	distance between pectoral and ventral fins	3.1±0.64 (1.2–3.8)^a	3.6±0.56 (2.7–4.6)^b	2.8±1.47 (0.0–5.7)^{ab}
8.	VAD	distance between ventral and anal fins	30.2±1.66 (27.3–33.5)^a	31.0±1.25 (29.3–32.8)^a	28.2±1.39 (25.4–30.2)^b
9.	ANAD	distance between anus and anal fin	3.0±1.18 (0.7–5.0)^a	4.2±1.28 (2.8–7.8)^b	5.4±0.83 (4.1–7.5)^c
10.	LP	length of pectoral fins	16.4±0.87 (15.2–17.7)^a	18.5±0.62 (17.5–19.5)^b	18.3±1.60 (14.5–20.8)^b
11.	LV	length of ventral fins	16.5±1.01 (15.3–18.4)^a	17.6±0.74 (16.4–19.3)^b	19.5±1.12 (17.7–21.9)^c
12.	LD1	length of first dorsal fin basis	22.4±1.05 (20.5–23.9)^a	22.7±1.38 (20.6–24.4)^a	20.8±1.16 (18.8–22.9)^b
13.	LD2	length of second dorsal fin basis	22.8±1.33 (21.0–24.9)^a	26.4±0.95 (24.3–28.3)^b	25.8±2.83 (17.4–30.2)^b
14.	LA	length of anal fin basis	11.2±1.07 (9.3–13.3)^a	12.8±1.56 (10.1–15.0)^b	11.8±1.23 (10.0–14.0)^a
15.	CP	length of caudal peduncle	25.5±1.62 (23.7–29.2)^a	24.0±1.07 (21.9–26.0)^b	27.8±1.49 (25.4–29.9)^c
16.	PODD	postdorsal distance	17.7±1.85 (14.1–21.0)^a	15.1±1.00 (13.7–17.6)^b	17.1±2.61 (13.3–25.0)^a
17.	UC	length of upper part of caudal fin	21.7±1.32 (19.8–24.2)^a	22.5±1.11 (20.4–23.9)^a	24.8±1.56 (22.2–28.7)^b
18.	MC	length of median part of caudal fin	11.8±1.74 (9.3–14.8)^a	13.0±1.01 (11.6–14.7)^b	12.8±1.56 (10.3–15.5)^{ab}
19.	LC	length of lower part of caudal fin	20.8±1.81 (18.1–23.6)^a	21.3±1.35 (19.4–23.6)^a	24.4±1.24 (22.1–26.2)^b
20.	HL	head length	30.2±1.05 (28.2–32.1)^a	28.3±0.65 (27.0–29.8)^b	29.7±0.95 (27.2–30.9)^a
21.	MAXH	maximum body height	19.5±1.19 (17.5–21.6)^{ab}	19.3±0.59 (18.3–20.3)^a	20.2±0.70 (19.1–21.3)^b
22.	MINH	minimum body height	8.5±0.27 (8.1–8.9)^a	8.9±0.25 (8.5–9.5)^b	9.1±0.32 (8.6–9.8)^b
23.	MAXHD1	maximum height of first dorsal fin	12.5±1.35 (10.3–15.1)^a	11.5±0.86 (10.1–13.3)^b	16.2±1.02 (13.4–17.3)^c
24.	MAXHD2	maximum height of second dorsal fin	14.9±0.99 (13.0–16.1)^a	15.3±0.90 (14.1–17.1)^a	16.4±1.09 (14.3–18.0)^b
25.	MAXHA	maximum height of anal fin	16.1±0.88 (14.7–17.9)^a	16.4±0.90 (15.0–18.1)^a	18.4±1.00 (16.2–20.3)^b
26.	MAXBW	maximum body width	12.8±1.20 (11.2–14.6)^a	13.2±0.56 (12.2–14.0)^a	12.8±0.95 (11.5–15.4)^a

Table 1 (continued)

		S. lucioperca	F_1 hybrid mean±SD (range)	*S. volgensis*
27. MINBW	body width at minimum caudal peduncle height	5.7±0.31 (5.2–6.3)[a]	6.0±0.24 (5.5–6.4)[b]	5.8±0.52 (4.9–6.6)[ab]
28. GW	gape width	13.7±0.75 (12.2–15.0)[a]	12.6±0.64 (11.5–13.5)[b]	13.4±1.08(11.1–15.0)[a]
Related characters in % of HL				
29. PD	preorbital distance	27.2±1.47 (24.7–29.4)[a]	27.4±1.16 (25.5–29.8)[a]	25.2±1.16 (23.7–27.7)[b]
30. PHL	postorbital head length	48.5±1.66 (46.2–51.1)[a]	44.0±2.44 (41.2–49.2)[b]	42.2±2.48 (37.9–46.1)[b]
31. ED	eye diameter	24.3±1.28 (20.7–25.7)[a]	28.6±2.23 (23.4–31.0)[b]	32.6±1.90 (30.1–36.3)[c]
32. LMAX	length of maxilla	45.2±2.47 (40.8–50.7)[a]	43.7±1.83 (39.8–47.8)[b]	40.6±1.11 (39.1–42.6)[c]
33. LMAN	length of mandible	60.4±2.58 (56.5–64.8)[a]	60.3±2.03 (55.8–63.3)[a]	56.9±2.74 (53.4–63.6)[b]
34. ID	interorbital distance	15.1±1.15 (12.9–17.2)[a]	16.8±0.89 (15.9–19.5)[b]	14.2±0.97 (12.7–16.2)[c]
35. HH1	orbital height of head	36.7±2.14 (33.7–41.3)[a]	40.6±1.36 (38.4–44.0)[b]	40.0±1.84 (37.2–44.9)[b]
36. HH2	occipital height of head	51.2±2.39 (45.6–55.2)[a]	54.8±1.65 (52.0–58.6)[b]	54.1±2.77 (50.8–60.4)[b]
37. MMD	distance between the end of maxilla and the end of mandible[1]	–2.9±0.56 (–3.7–1.9)[a]	1.5±3.74 (–3.7–6.4)[b]	–0.7±1.21 (–2.3–2.9)[c]
Related character in % of ED				
38. EMD	distance between the anterior verge of the eye and the end of the maxilla	74.3±10.11 (47.7–93.9)[a]	56.9±5.93 (48.2–67.3)[b]	47.6±5.83 (38.3–59.9)[c]
Meristic characters				
39. SPBR	number of gill rakers on first left gill arch	12.1±0.83 (11–14)[a]	12.6±0.74 (11–14)[a]	14.7±0.49 (14–15)[b]
40. LL	number of perforated scales in lateral line	89.1±2.31 (86–93)[a]	77.1±2.69 (73–81)[b]	69.6±1.92 (66–72)[c]
41. D1R	number of rays in first dorsal fin	13.5±0.64 (12–14)[a]	14.0±0.00 (14)[b]	12.5±0.52 (12–13)[c]
42. D2SR	number of spiny rays in second dorsal fin	1.3±0.49 (1–2)[a]	1.4±0.51 (1–2)[a]	1.7±0.59 (1–3)[a]
43. D2BR	number of branched rays in second dorsal fin	21.0±0.38 (20–22)[a]	22.5±0.52 (22–23)[b]	21.5±0.92 (20–23)[a]
44. PR	number of rays in pectoral fins	15.2±1.01 (14–17)[a]	17.1±0.70 (16–18)[b]	15.7±0.70 (14–17)[a]
45. VSR	number of spiny rays in ventral fins	1.0±0.00 (1)[a]	1.0±0.00 (1)[a]	1.0±0.00 (1)[a]
46. VBR	number of branched rays in ventral fins	5.0±0.00 (5)[a]	5.0±0.00 (5)[a]	5.0±0.00 (5)[a]
47. ASR	number of spiny rays in anal fin	2.0±0.00 (2)[a]	2.0±0.00 (2)[a]	2.0±0.00 (2)[a]
48. ABR	number of branched rays in anal fin	10.8±0.68 (10–12)[a]	11.3±0.80 (10–13)[a]	9.3±0.62 (9–11)[b]

Statistical analyses included both univariate and multivariate methods. Differences in relative morphometric and raw meristic characters between *S. lucioperca*, *S. volgensis* and their F_1 hybrids were first tested pair-wise by Student's t-test for each character, except the non-normally distributed distance between the anterior ends of the maxilla and mandible (MMD) values which were compared with Mann-Whitney U test. Morphometric characters numbered 1–28 were expressed in percentages of the standard body length (SL), those numbered 29–37 in percentages of the head length (HL) and the character numbered 38 in percent of eye diameter (ED).

Multivariate data analyses included principal component analysis (PCA) and discriminant function analysis (DFA). These analyses were based on size adjusted and log transformed raw data. Since no significant correlations were observed between meristic characters and *SL* of samples, meristic characters were just log transformed. However, significant correlations were found between raw morphometric measurements and *SL*, except for MMD. Moreover, in accordance with differences in growth rates of the parental species, *SL* also varied significantly among the three genotypes (ANOVA for *SL*, $F_{2,42} = 69.4$, $P < 0.001$). In order to account for the allometric effect in the morphometric data, raw data were size adjusted using the method of SENAR *et al.* (1994) and ELLIOTT *et al.* (1995):

$$\log y_i' = \log y_i - b \times (\log SL_i - \log SL_M)$$

where y_i' is the size-adjusted value of variable y for fish i, SL_i is the standard length of fish i, SL_M is the mean standard length for all fish combined (including all *S. lucioperca*, *S. volgensis* and F_1 hybrids), and b is the regression coefficient of log y on log *SL* using all specimens of a given genotype. For multivariate analyses log *y'* values were used. We ensured that the size adjusted variables were not themselves correlated with the *SL*. Since morphometric characters were size adjusted, *SL* was left out from multivariate analyses, as well as three meristic characters, numbers of spiny rays in ventral fins (VSR), number of branched rays in ventral fins (VBR) and number of spiny rays in anal fin (ASR) which did not have any variance among specimens.

Standard (centred) PC analyses were conducted separately on transformed morphometric and meristic characters to evaluate whether genotypes could be separated without any a priori loading on sample categorization and to investigate which characters are most important in sample discrimination.

Following the PCA, a linear DFA using a forward stepwise method based on the Mahalanobis distance was conducted on log transformed size-adjusted morphometric and log transformed meristic data to establish the relative significance of those characters in distinguishing among the parental species and their hybrids (PIETSCH & ORR 2006, LATTIG *et al.* 2007). The resultant discriminant functions were used to assign individuals into samples. The classification success rate was evaluated on the basis of percent of correctly classified individuals. The relative importance of morphometric and meristic characters in discriminating genotypes were assessed using the F-to-remove statistic. The graphical representation for the distinction between the two species and their hybrids was performed by a canonical analysis, and 95% confidence ellipses around the group centroids were used to visualize relationships between genotypes. All statistical analyses were performed in Statistica 6.0 (StatSoft, Inc.).

RESULTS

Variation ranges (min-max) of relative values of morphometric and absolute values of meristic characters in *S. lucioperca*, *S. volgensis* and their F_1 hybrids with corresponding statistical parameters (means and standard deviations) and the re-

sults of the pair wise t-tests are presented in Table 1. Out of the 48 analyzed variables, the means of 31 differed significantly between *S. lucioperca* and *S. volgensis*. Hybrids were intermediate in morphology compared to their parents in only 17 of these characters. Means of the hybrid exceeded the values of either parent species in five characters, while in six characters mean values of the hybrid were lower than in either parent species. However, in other characters observed values overlapped considerably among the genotypes. The only character showing absolutely no overlap among the three genotypes was the number of perforated scales on the lateral line (LL) being 86–93 in *S. lucioperca*, 66–72 in *S. volgensis* and 73–81 in *S. lucioperca* × *S. volgensis*. Eye diameter (ED) ranges related to head length overlapped only slightly among genotypes as they were 20.7–25.7% in *S. lucioperca*, 31.1–36.6% in *S. volgensis* and 23.4–31.0% in *S. lucioperca* × *S. volgensis* (Table 1). The most characteristic morphological consequence of the hybridization was the deformed mandible which was observed in half of the hybrids. The deformed mandible sticks out visibly and its tip curves upwards (Fig. 2). Parent species do not possess such features. Other half of the hybrids showed intermediate signs in this characteristic (see also MMD values in Table 1).

According to the PCA parent and hybrid genotypes separate clearly both by morphometric and meristic characters (Fig. 3). In PCA on meristic characters, PC1 accounted for 40.4% of the total variance and was correlated most positively with number of gill rakers on the left gill arch (SPBR) and most negatively with LL, number of rays in first dorsal fin (D1R) and number of branched rays in anal fin (ABR). PC2 in this analysis accounted for 24.8% of the total variance and was correlated positively with LL and negatively with number of branched rays in second dorsal fin (D2RB) and number of rays in pectoral fin (PR) (Fig. 4). PC1 separated *S. volgensis* from *S. lucioperca* and their hybrids, while the latter two genotypes separated only along PC2 (Fig. 3).

In the PCA of morphometric characters, PC1 and PC2 accounted for 29.7% and 18.7% of the total variance, respectively. PC1 correlated most positively with some characters measured on the head, i.e. length of mandible (LMAN), interorbital distance (ID) and preorbital distance (PD), and on the anterior part of the body, i.e. distance between pectoral and ventral fins (PEVD) and preventral distance (PVD), as well as the preanal distance (PAD). Negative correlations with PC1 were found in some fin related characters, i.e. maximum height of dorsal fin (MAXHD1), length of ventral fin (LV), length of second dorsal fin basis (LD2) and length of upper part of caudal fin (UC), and with the ED. The greatest positive contribution to the variance along the morphometric PC2 was loaded by length of caudal peduncle (CP) and the greatest negative contribution loaded by length of median part of the caudal fin (MC), fork length (FL), maximum body width

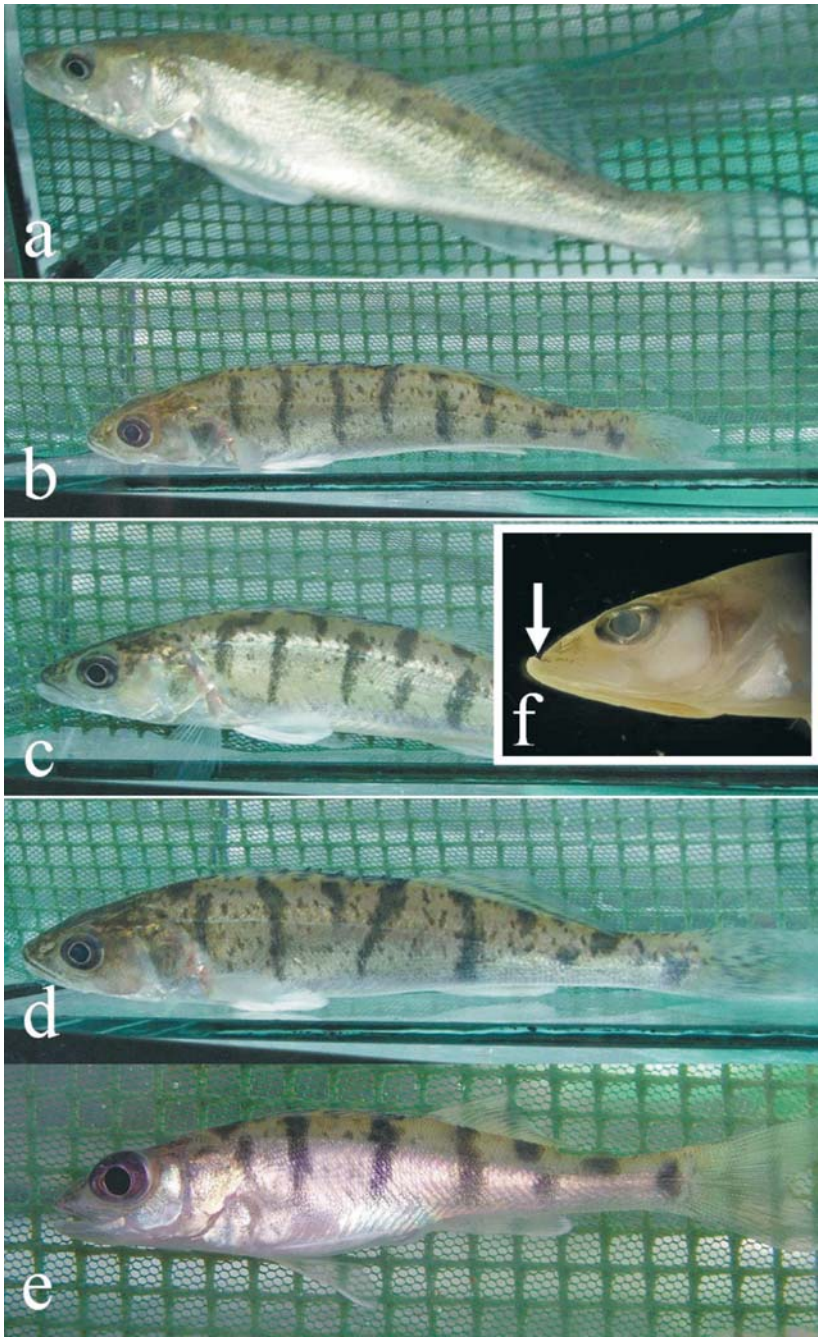

Fig. 2. a – *S. lucioperca*; b-d – *S. lucioperca* × *S. volgensis* F_1 hybrids; e – *S. volgensis*; f – hybrid with deformed mandible

(MAXBW), distance between ventral and anal fins (VAD), and length of pectoral fin (LP) (Fig. 4). Similarly to results on meristic characters, in PCA on morphometric characters PC1 separated *S. volgensis*, while PC2 separated *S. lucioperca* and hybrids (Fig. 3).

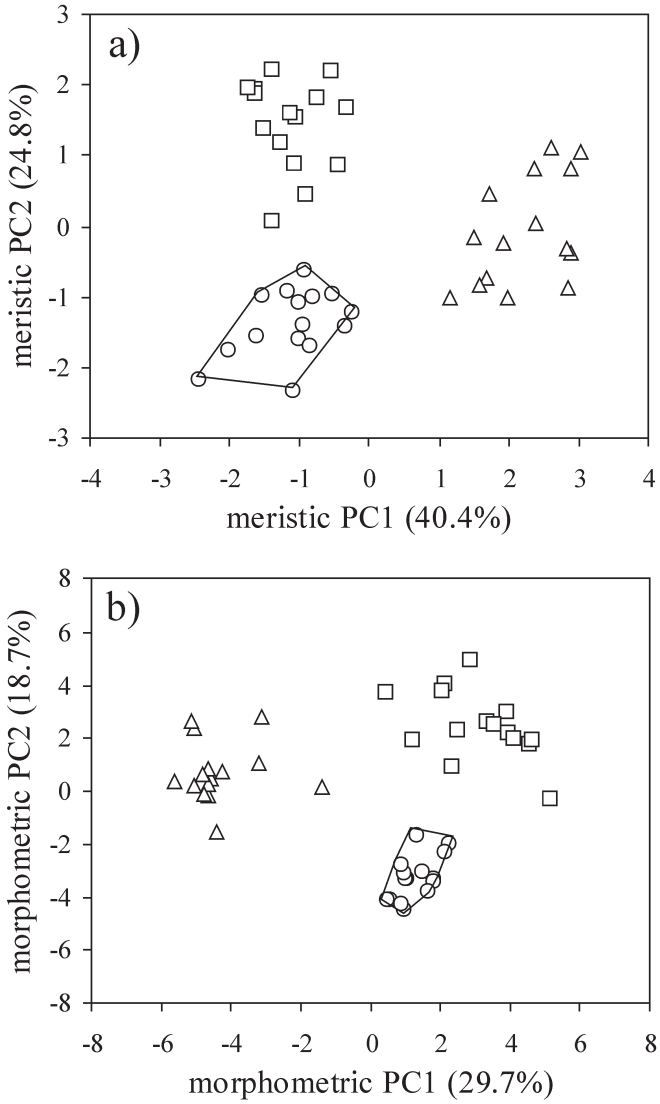

Fig. 3. Plots of scores of the first and second principal components (PC) for meristic (a) and morphometric characters (b) of *S. lucioperca* (square), *S. volgensis* (triangle) and their F_1 hybrid (circle). Variance proportions represented by each PC are indicated

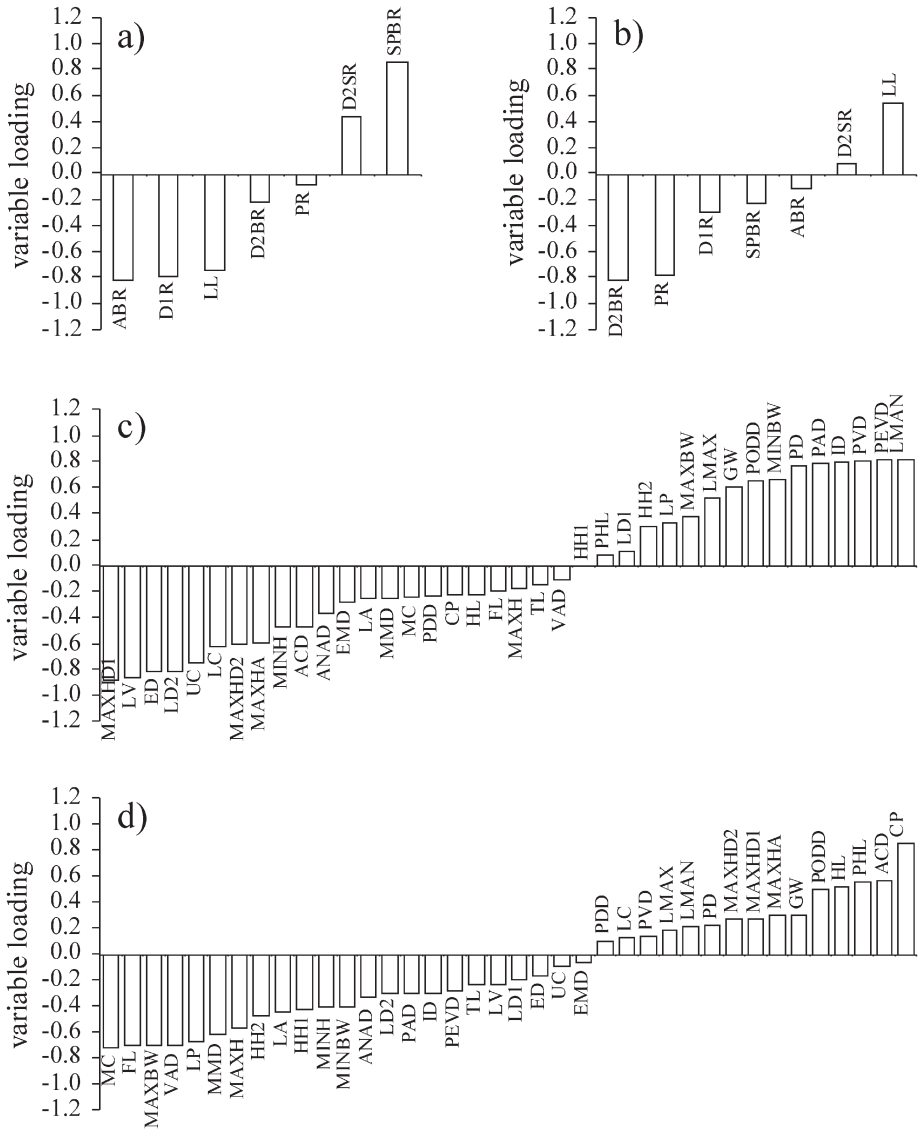

Fig. 4. Variable loadings of principal component (PC) analyses of meristic and morphometric characters of *S. lucioperca* (square), *S. volgensis* (triangle) and their F_1 hybrid (circle). a – meristic PC1; b – meristic PC2; c – morphometric PC1; morphometric PC2. Full names of analysed characters are given in Table 1

Table 2. Morphological characters selected by forward stepwise discriminant analysis to separate *S. lucioperca*, *S. volgensis* and their F_1 hybrid (Wilks' lambda: 0.003, $F_{16,70}$=76.16, P<0.001). Full names of characters are given in Table 1.

	Wilks' lambda	Partial lambda	F-remove	P
LL	0.011	0.274	46.31	<0.001
MAXHD1	0.004	0.686	8.01	0.001
CP	0.005	0.650	9.41	0.001
PR	0.005	0.591	12.11	<0.001
MINH	0.004	0.761	5.49	0.008
SPBR	0.004	0.688	7.92	0.001
D1R	0.004	0.744	6.03	0.006
PD	0.004	0.810	4.12	0.025

The eight characters selected by the discriminant analysis using the forward stepwise method, presented significant discriminatory power (Table 2). Based on these characters all individuals were classified correctly. Fig. 5 shows three distinguishable clusters, each one corresponding to a distinct genotype without any overlap among their 95% confidence ellipses.

Regarding pigmentation (stripe pattern), hybrid specimens showed continuous transitions between parent phenotypes (Fig. 2).

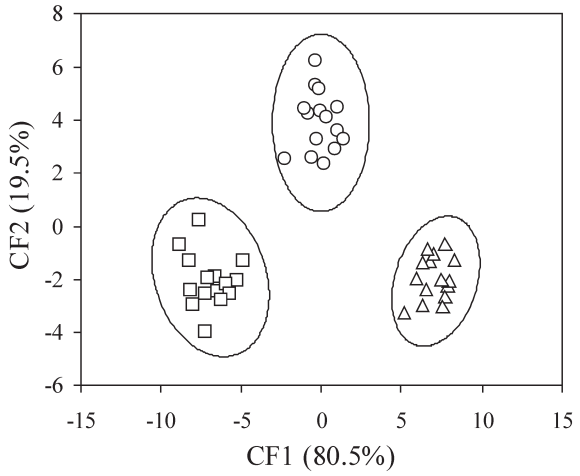

Fig. 5. Canonical analysis based on the eight most important morphological characters (Table 2) selected by the forward stepwise discriminant function analysis for *S. lucioperca* (square), *S. volgensis* (triangle) and their F_1 hybrid (circle). 95% confidence ellipses are drawn around group centroids, and variance proportions represented by each canonical function (CF) are indicated

DISCUSSION

S. lucioperca and *S. volgensis* are commonly distinguished by some evident external characters such as the presence of canine teeth in *S. lucioperca,* the position of the end of maxilla related to centre of the eye and the different body pattern of the two species (BĂNĂRESCU 1964, BERINKEY 1966). However, according to our observations, in case of hybridization these characteristics can not be used as even F_1 hybrids cover the full transitional scale between the parent species. We found, however, that meristic and morphometric characters enable to separate *S. lucioperca*, *S. volgensis* and their F_1 hybrids without any overlap in clusters of their scores along PC1 and PC2 in both PC analyses. Moreover, genotypes were classified with 100% success by the DFA. Results are thus very useful in providing a cheap and quick identification tool for searching for putative hybrids in field samples, which could also assist in selecting specimens for genetic and other molecular studies.

Univariate analyses showed that all morphological characters, except one, overlapped at least between two, but mostly among all three genotypes. The only morphological character that did not show any overlap among genotypes was the number of perforated scales in the lateral line. Contrary to this, literature data suggest that even the parent species may show some overlap in this character, as scale number was found to vary from 79 to 105 in *S lucioperca* (BĂNĂRESCU 1964, BERINKEY 1966, CHITRAVADIVELU & OLIVA 1973, KRPO-ĆYETKOVIĆ & STAMENKOVIĆ 1996) and from 70 to 83 in *S. volgensis* (BĂNĂRESCU 1964, BERINKEY 1966, NOVITSKY & ZHUKOV 2000, SCHERBUKHA & DJACHUK 2000). However, since we examined only 15 specimens in each group, thence our data may not cover the whole meristic ranges of genotypes. On the other hand, present counts on the numbers of spiny and soft rays in dorsal, anal, pelvic, and ventral fins coincide with those described by other authors for the two parent species. PCA proved that *S. lucioperca*, *S. volgensis* and their F_1 hybrids clearly separate even on the basis of meristic characters.

Morphometric characters also enabled a clear separation among genotypes. Results of the PCA suggest that together with some other morphometric characters, fin morphology has a special importance in discriminating genotypes. DFA also proved the high discriminatory power of some fin related characters.

Hybrid specimens separated from *S. volgensis* along the first PC1 in both meristic and morphometric PC analyses, but from *S. lucioperca* only along PC2, suggesting that hybrids are more similar to *S. lucioperca* (♀) than to *S. volgensis* (♂). Similarly to some other case studies (SEILER & KEELEY 2007), morphological relationships found in *S. lucioperca* × *S. volgensis* hybrid suggest that a consid-

erable maternal effect might predominate in the present hybridization. Further attribute of this F_1 hybrid is that for several relative morphological characters (in 11 out of the 48 studied) the mean values of the hybrid dropped outside the ranges observed in the parental species. However, unfortunately the hybrid vigor did not express in some economically profitable features, such as an improved growth rate or lower oxygen demand (MÜLLER *et al.* 2006*a,b*).

In fish, morphometric characters are generally size-dependent and they change according to allometric growth (e.g., ELLIOTT *et al.* 1995, RINCON 2000). Size dependent variations thus should be considered in comparative morphometrics. In the present study, although the size ranges of the studied genotypes were quite narrow and not considerably different, a significant allometric effect still could be observed and therefore data were adjusted accordingly. However, since allometric relationships can not be extrapolated, our results on fingerlings cannot be automatically applied for adult size-groups. Further limit of using morphometric keys is that body proportions may considerably vary among habitats (KRPO-ĆETKOVIĆ & STAMENKOVIĆ 1996), likely including laboratory stocks too. For example, eye diameter was found to vary from 10.0% to 26.0% of head length in *S. lucioperca* and from 17.8 to 24.0% in *S. volgensis* for specimens within the size range of 52–850 mm SL and from different habitats (BĂNĂRESCU 1964, BERINKEY 1966, CHITRAVADIVELU & OLIVA 1973, KRPO-ĆETKOVIĆ & STAMENKOVIĆ 1996, NOVITSKY & ZHUKOV 2000, SCHERBUKHA & DJACHUK 2000). These values, especially those for *S. volgensis*, are much lower than in the present study on fingerlings. Variances in the eye diameter (and other morphometric characters) are probably common consequences of differences in fish size and habitat. In contrary, e.g., the present data on the length of maxilla coincide with the range described in the literature for both species (e.g., BĂNĂRESCU 1964, BERINKEY 1966, CHITRAVADIVELU & OLIVA 1973, KRPO-ĆETKOVIĆ & STAMENKOVIĆ 1996, NOVITSKY & ZHUKOV 2000, SCHERBUKHA & DJACHUK 2000). However, since previous studies were based on much larger individuals (in standard length) than our, it would be unpractical to compare all literature and present results on morphometric measurements in detail.

Since crossing the two *Sander* species in laboratory is as easy as to breed the parent species, and the hybrid offspring is as viable and develops as well as that of the parental species (MÜLLER *et al.* 2004, 2006*c*), postzygotic reproductive barriers seem to be unlikely. However, natural hybridization between *S. lucioperca* and *S. volgensis* still has not been detected yet. It can be assumed thus that natural hybridization of the two *Sander* species is mainly blocked by prezygotic barriers, most probably by differences in their reproductive behaviour (BALON *et al.* 1977) and spawning times (SPECZIÁR & BÍRÓ 2002). Leastways, there are several examples on that prezygotic reproductive barrier between sympatric species may fail

(FISS *et al.* 1997, BETTLES *et al.* 2005, KOZFKAY *et al.* 2007), and thus the possibility of natural hybridization between *S. lucioperca* and *S. volgensis* can not be excluded, either. Up to now the detection of possibly occurring natural hybrids was retarded by the lack of knowledge on their morphology. In our study we found that beside the complex morphological analysis, e.g., the deformed, protruding and curved mandible occurring in half of the *S. lucioperca* × *S. volgensis* F_1 hybrids may also be considered as a good indicator of hybridization. However, it is also important that according to MÜLLER *et al.* (2006a) F_1 hybrids are fertile. Thus, in case of population interbreeding we can expect full transitions in most morphological characters. This assumption is supported by the example of the two North American *Sander* species which by now have mixed populations in several natural habitats (HEARN 1986, FISS *et al.* 1997, WHITE *et al.* 2005). Consequently, further investigations are needed to examine morphological characteristics of F_x hybrids, genetic integrity of sympatric *S. lucioperca* and *S. volgensis* stocks, and stability of the prezygotic reproductive barrier between the two species.

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