



DISCRIMINATION OF MORPHOLOGICAL TRAITS BETWEEN ATLANTIC AND DANUBIAN BROWN TROUT LINEAGES AND THEIR HYBRIDS

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ABSTRACT

Brown trout of non-native lineages (e.g. Atlantic) have been stocked into Croatian streams and rivers primarily to meet angling demand, resulting in the presence of admixed populations at many locations. The clear, easily distinguishable morphological differences between the brown trout of the introduced Atlantic (AT) and indigenous Danubian (DA) lineages, as well as their hybrids (HY), were unknown. Therefore, the aim of this study was to identify classical morphometric and meristic traits that differentiate them. Brown trout were collected by electrofishing in ten streams from three mountain areas (Gorski Kotar, Mt. Žumberak and Mt. Papuk) during 2017 and 2018. Additionally, 10 specimens belonging to the AT lineage were obtained from a fish farm in continental Croatia. In total, 90 fish specimens were analysed: 17, 22 and 51 specimens of AT, DA and HY, respectively. The results revealed the most prominent differences in the head features of the AT compared to the DA lineage and their HY. Discriminant analysis demonstrated that eye diameter and postorbital length were significantly smaller, whereas interorbital width and head height were significantly larger in the AT lineage. These findings suggest the need for a detailed head morphometry analysis to identify distinctive morphological traits that differentiate brown trout lineages and detect their hybrids.

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INTRODUCTION

Black Sea salmon *Salmo labrax* Pallas, 1814, which also includes a resident, stream-dwelling brown trout, is a native species in the rivers and streams of the Danube River Basin in Croatia (Simonović et al., 2017). Over the past 150 years, extensive translocations and introductions of the Atlantic lineage (AT) of brown trout *Salmo trutta* L., 1758 between watercourses and watersheds have been conducted in Croatian streams and rivers primarily to meet angling demand (Pofuk et al., 2017). This practice, involving the use of fish farm stock, has led to the establishment of admixed populations at many sites (Jadan et al., 2014). Ongoing introductions and cross-breeding between the introduced AT and native Danubian (DA) lineages have resulted in hybrid (HY) brown trout populations (Kanjuh et al., 2020; Škraba Jurlina et al., 2020; Kanjuh et al., 2024).

The morphological differentiation of *Salmo* stocks is very difficult because of their phenotypic plasticity (Simonović et al., 2005; Simonović et al., 2007). It is particularly difficult to distinguish their HY because they more often resemble one or the other parent species in one or more characters and are less often intermediate in phenotype (Wilkins et al., 1994). Brown trout populations from different basins can be distinguished by their external body morphology, with the difference in morphometric traits at the head being the most pronounced (Simonović et al., 2007). The geometric morphometrics approach has recently been used to analyse the shape of the AT, DA and HY specimens, which revealed significant differences between them, but the origin of this variation remains unclear (Špelić et al., 2021).

Due to the high stocking pressure with the introduced AT lineages in Croatian streams and rivers, hybrid stocks are the most abundant in Croatian rivers (Kanjuh et al., 2024). While molecular methods for identifying native, non-native and hybrid *Salmo* stocks are highly reliable, their practical application remains limited. For effective conservation measures, clear morphological differences between brown trout lineages and their HY are required for immediate *in situ* identification. Therefore, the aim of this study was to employ an analysis of classical morphometric and meristic brown trout characters to find those that can provide reliable discrimination between brown trout of the AT and DA lineages, and their HY.

MATERIALS AND METHODS

Brown trout specimens were collected by electrofishing from 10 streams in three mountain areas, Gorski Kotar, Mt. Žumberak and Mt. Papuk, during 2017 and 2018 (Figure 1). Additionally, 10 specimens belonging to the AT were obtained at the fish farm in continental Croatia. In total, 90 fish specimens were analysed: 17, 22 and

51 specimens of AT, DA and HY, respectively (Figure 2). Data on the specimen affiliation to haplotype were taken according to Kanjuh et al. (2020). Measurements of 28 morphometric (Figure 3) and counts of six meristic characters (Appendix 1) were taken for each specimen.

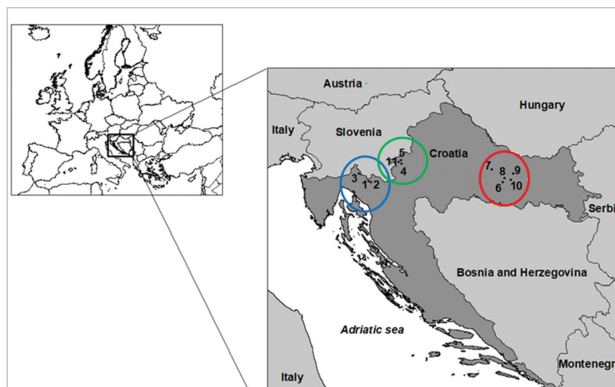


Fig 1. Map of sampling sites: Gorski kotar (blue circle; 1 – Mala lešnica, 2 – Bresni potok, 3 – Curak), Žumberak (green circle; 4 – Kupčina, 5 – Slapnica, 11 – fish farm), Papuk (red circle; 6 – Orljava, 7 – Toplica, 8 – Brzaja, 9 – Jankovac, 10 – Veličanka)

Prior to analysis, all morphometric measurements were standardized following the modified method of Reist (1985) to minimize any variation resulting from allometric growth. Specifically, a value of 1 was added to each measurement, and the square root of each value was calculated to avoid negative results.

The transformation equation was as follows:

$$e = \sqrt{[\log Y - b(\log X - \log X_L)] + 1}$$

where

e is the standardized measurement;

Y is the character length;

b is the slope of $\log Y$ against $\log X$ plot for each population;

X is the standard length of the specimen;

X_L is the mean standard length.

According to Reist (1985), this transformation best reflects shape variation among groups independently of size factors. The total and standard length of each specimen were excluded from the final analysis.

Meristic counts and standardized morphometric measurements were assessed for outliers using box-and-whisker plots and tested for normality using the Shapiro-Wilk test. Meristic data were analysed with the non-parametric Kruskal-Wallis test, while morphometric data were evaluated using MANOVA, Wilks' Lambda multivariate test and Tukey's HSD post hoc test. Discriminant analysis was conducted to identify the variables that best differentiate the tested groups. All analyses were performed using the SPSS 16 software package.

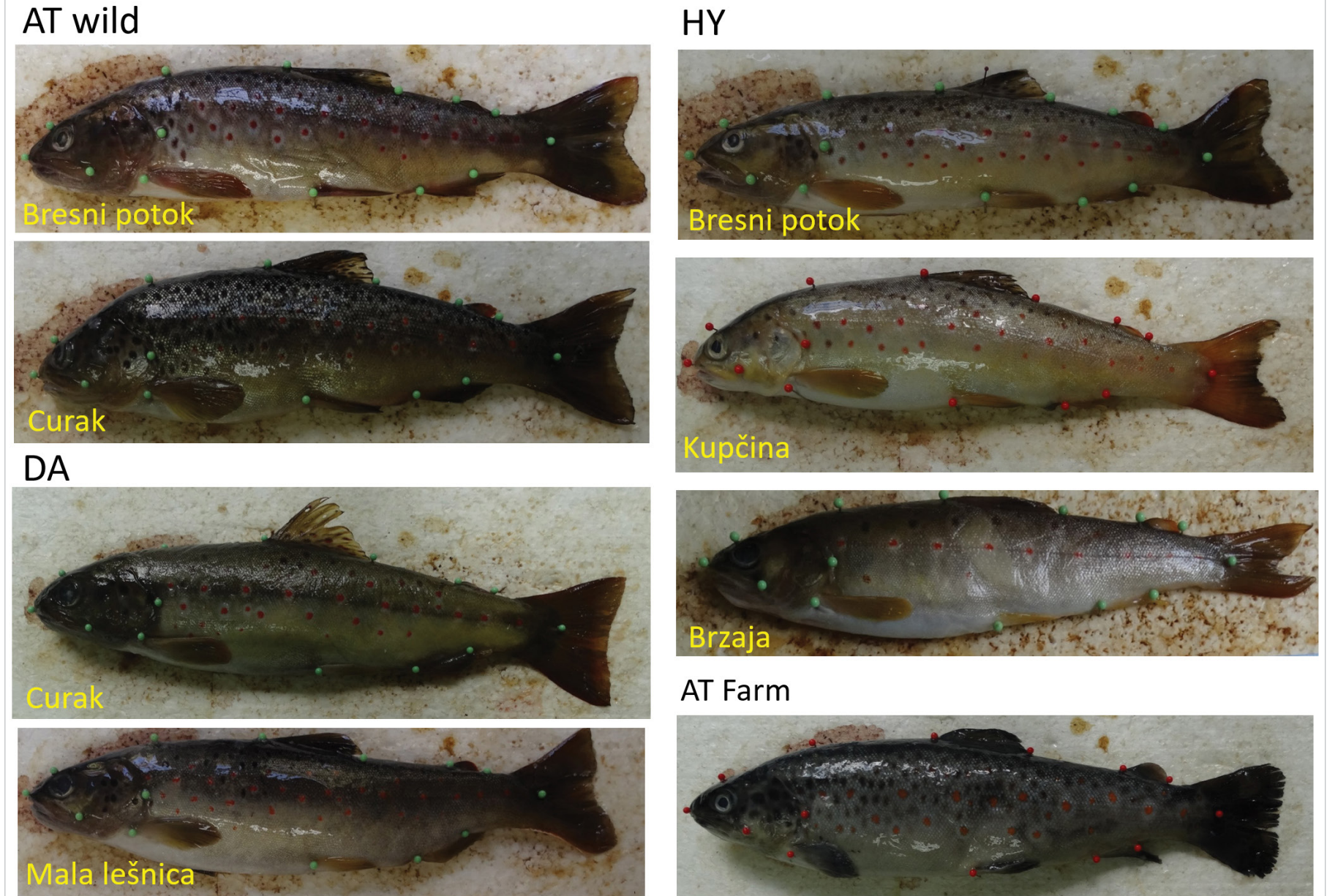


Fig 2. Analysed wild and farmed brown trout specimens: AT – Atlantic lineage (wild and farmed); DA – Danube lineage (wild); HY – Hybrids (wild)

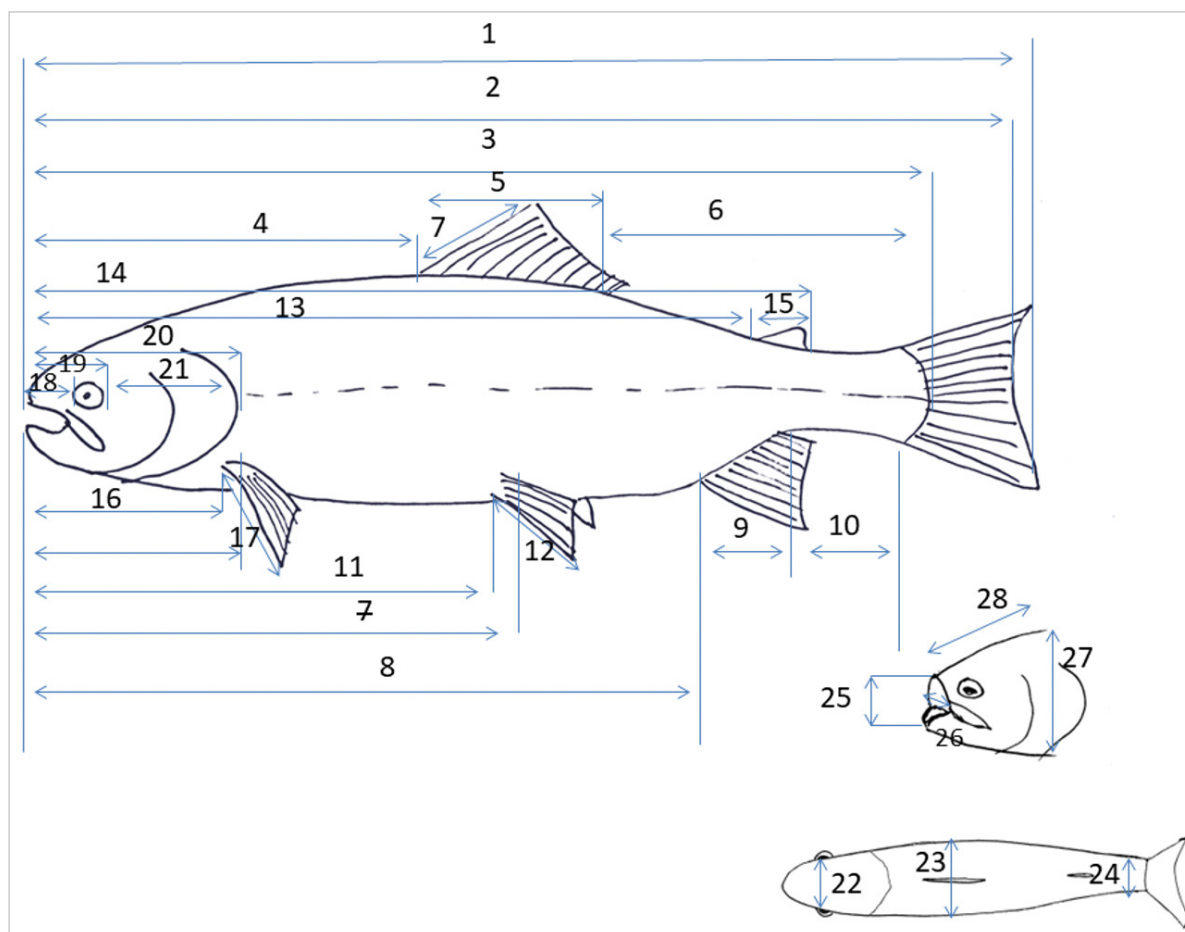


Fig 3. Morphometric measurements: 1 total length; 2 fork length; 3 standard length; 4 predorsal length; 5 length of base of dorsal fin; 6 postdorsal length; 7 length of dorsal fin; 8 preanal length (distance); 9 anal fin base length; 10 length of caudal peduncle; 11 prepelvic distance; 12 length of pelvic fin; 13 preadipose length; 14 post-adipose length; 15 adipose fin base length; 16 prepectoral distance; 17 pectoral fin length; 18 snout length; 19 eye diameter; 20 head length; 21 postorbital length; 22 interorbital width; 23 body height; 24 depth of caudal peduncle; 25 mouth width; 26 mouth height; 27 head height; 28 dorsal head length

RESULTS

Through the exploration of meristic data, 18 specimens (4 AT, 4 DA, 10 HY) identified as extreme outliers were removed prior to analysis. The Shapiro-Wilk test indicated that the meristic data were not normally distributed ($P < 0.05$). The Kruskal-Wallis test revealed no significant differences ($P > 0.05$) in meristic measurements among AT, DA or hybrid specimens (Table 1).

Furthermore, outliers were detected in the standardized morphometric data for predorsal length (PREDL), anal fin base length (AFBL), adipose fin base length (ADFBF) and preanal length (PRLD). These measurements also exhibited a non-normal distribution ($P < 0.01$) and were excluded from further analysis to prevent distortion of group means and variances, and to avoid misclassification and biased results. The Box's Test of Equality of Covariance Matrices for the dependent morphometric variables indicated equality across groups ($P > 0.001$). The results of MANOVA indicated significant differences between lineages and their hybrids in morphometric measurements ($P < 0.01$) (Table 2).

By Tukey's post hoc test, strong significant differences were found in length of base of dorsal fin (LBDF), head height (HEADH), snout length (SLE), interorbital width (INTERORW), mouth height (MOUTH) between AT and DA lineages and their hybrids ($p < 0.01$), but not between DA and HY ($P > 0.05$) (Appendix 2). Also, significant differences were found in other head morphometric traits, such as dorsal head length (DORHL), head length (HEADL) or mouth width (MOUTW) ($P < 0.05$).

Discriminant analysis was performed on nine variables of head morphometry (Table 1), correctly classifying 62.2% of the original grouped cases and 60.0% of the cross-validated grouped cases (Table 3). The results indicated significantly higher HEADH and INTERORW and smaller eye diameter (EDIAM) and postorbital length (POSTORL) in the AT lineage compared to DA and their hybrids (Table 4; Appendix 3). Canonical Discriminant Function analysis showed clear separation of AT lineage in the first two canonical functions in comparison with the other two groups (Figure 4).

Table 1. Standardized data of morphometric measurements and abbreviations
(AT – Atlantic lineage; DA – Danube lineage; HY – Hybrids; n = number of analysed specimens; \bar{x} = mean; sd = standard deviation)

Morphometric character	Abbreviation	AT, n = 17				DA, n = 22				HY, n = 51			
		\bar{x}	sd	min	max	\bar{x}	sd	min	max	\bar{x}	sd	min	max
Body and fin measurements													
Predorsal length	PREDL	2.05	0.14	1.82	2.28	1.86	0.04	1.76	1.91	1.85	0.04	1.73	1.91
Length of base of dorsal fin	LBDF	1.50	0.12	1.23	1.68	1.33	0.23	0.83	1.64	1.20	0.22	0.72	1.74
Postdorsal length	PDL	2.03	0.15	1.79	2.28	1.82	0.25	1.33	2.21	1.69	0.23	1.25	2.31
Length of dorsal fin	LDF	1.51	0.08	1.37	1.63	1.48	0.14	1.14	1.67	1.39	0.14	1.09	1.72
Preanal length (distance)	PRLD	1.59	0.16	1.43	2.10	1.59	0.14	1.38	1.93	1.67	0.14	1.34	1.97
Anal fin base length	AFBL	0.65	0.20	0.36	1.28	0.60	0.17	0.24	0.82	0.49	0.17	0.01	0.82
Length of caudal penducle	LCP	1.65	0.16	1.41	1.96	1.44	0.26	0.87	1.87	1.29	0.26	0.89	1.97
Prepelvic distance	PREPD	2.04	0.08	1.90	2.15	1.93	0.14	1.67	2.14	1.86	0.13	1.57	2.20
Length of pelvic fin	LPF	1.50	0.10	1.28	1.64	1.33	0.20	0.95	1.69	1.22	0.20	0.83	1.72
Preadipose length	PREADL	2.30	0.13	2.06	2.51	2.11	0.22	1.69	2.46	2.00	0.21	1.56	2.53
Adipose fin base length	ADFBL	0.89	0.17	0.58	1.14	0.63	0.20	0.27	0.94	0.53	0.23	0.04	1.06
Post-adipose length	POSTAL	1.63	0.17	1.37	1.90	1.41	0.27	0.82	1.83	1.25	0.25	0.85	1.95
Prepectoral distance	PREPL	1.71	0.13	1.47	1.93	1.55	0.21	1.14	1.86	1.43	0.20	1.02	1.95
Pectoral fin length	PFINL	1.45	0.16	1.04	1.63	1.46	0.16	1.16	1.74	1.37	0.15	1.08	1.79
Body height	BH	1.79	0.14	1.52	2.01	1.58	0.22	1.16	1.93	1.47	0.22	0.97	2.03
Depth of caudal peduncle	DCAUP	1.38	0.15	1.06	1.58	1.18	0.24	0.73	1.55	1.05	0.24	0.55	1.61
Head morphometry													
Head height	HEADH	1.64	0.13	1.41	1.87	1.45	0.20	1.04	1.76	1.34	0.19	0.89	1.85
Dorsal head length	DORHL	1.63	0.12	1.38	1.80	1.46	0.20	1.09	1.76	1.36	0.19	0.94	1.84
Snout length	SLE	1.27	0.14	1.02	1.47	1.05	0.24	0.62	1.44	0.93	0.23	0.49	1.53
Eye diameter	EDIAM	1.03	0.06	0.89	1.12	0.95	0.15	0.65	1.14	0.86	0.14	0.61	1.21
Postorbital length	POSTORL	1.50	0.13	1.26	1.73	1.36	0.22	0.96	1.71	1.23	0.20	0.79	1.79
Head length	HEADL	1.79	0.11	1.57	1.98	1.63	0.21	1.27	1.96	1.52	0.20	1.11	2.05
Interorbital width	INTERORW	1.35	0.13	1.12	1.54	1.10	0.27	0.60	1.56	0.98	0.23	0.54	1.60
Mouth width	MOUTW	1.39	0.11	1.15	1.55	1.20	0.23	0.77	1.58	1.07	0.24	0.61	1.72
Mouth height	MOUTH	1.56	0.13	1.32	1.80	1.37	0.18	1.03	1.63	1.27	0.18	0.88	1.75

Table 2. Kruskal-Wallis test on meristic traits between Atlantic and Danubian lineages and their hybrids

Character	Chi-Square	df	P - level
Spines in dorsal fin	0.03	1	0.863
Soft rays in dorsal fin	0.054	1	0.816
Spines in pectoral fin	0.028	1	0.867
Soft rays in pectoral fin	0.953	1	0.329
Spines in ventral fin	2.741	1	0.098
Soft rays in ventral fin	0.639	1	0.424

Table 3. Discriminant analysis classification results with cross validation; AT – Atlantic lineage; DA – Danubian lineage; HY – Hybrids

Classification		Lineage	Predicted group membership			Total
			AT	DA	HY	
Original	Count	AT	11	0	6	17
		DA	2	3	17	22
		HY	1	8	42	51
	%	AT	64.7	0	35.3	100
		DA	9.1	13.6	77.3	100
		HY	2.0	15.7	82.4	100
Cross-validated ^a	Count	AT	11	0	6	17
		DA	2	3	17	22
		HY	1	10	40	51
	%	AT	64.7	0	35.3	100
		DA	9.1	13.6	77.3	100
		HY	2.0	19.6	78.4	100

Table 4. Fisher's linear Discriminant Classification Function coefficients of head morphometric characters of AT – Atlantic lineage, DA – Danube lineage and HY – Hybrid (see Abbreviations in Table 1)

Character	Lineage		
	AT	DA	HY
HEADH	641	559	574
INTERORW	-705	-776	-760
EDIAM	423	477	460
POSTORL	140	252	224
(Constant)	-303	-310	-296

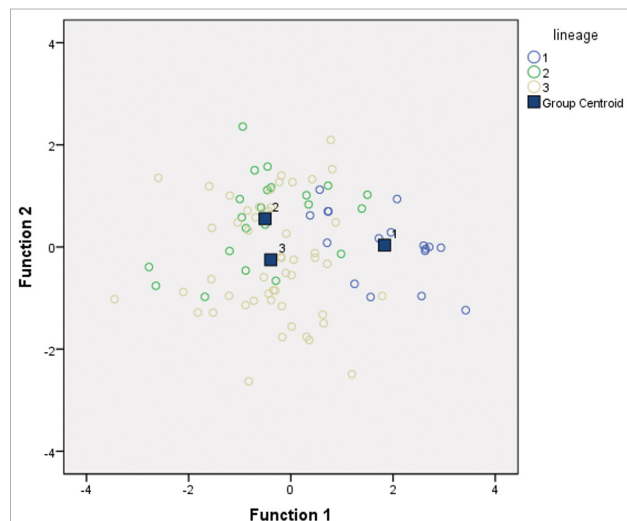


Fig 4. Canonical Discriminant Functions of head morphometric characters; 1 – Atlantic lineage; 2 – Danube lineage; 3 – Hybrids

DISCUSSION

In this study, no significant differences in meristic traits, including the number of spines and soft rays in the dorsal, pectoral and pelvic fins were detected among the three examined populations (AT, DA, HY). Similar results were reported in Romanian waters, where overlapping of meristic traits was observed between *S. labrax* and *S. trutta* (Latiu et al., 2020). However, the most notable differences in the Romanian study were found in the number of gill rakers (Latiu et al., 2020), a trait that was not examined in the present study.

The most distinctive morphological differences, which can differentiate the AT lineage from the DA and HY populations, were identified in head morphometry, particularly in HEADH, SLE, INTERORW, MOUTH, but also in longer LBDF. Similar findings were reported within five distinct drainage areas in the Western Balkans, where AT lineages could be distinguished by head characteristics, such as total head length, preocular length, lower jaw length, ventral head length and LBDF (Simonović et al., 2007). Furthermore, Simonović et al. (2007) identified five clusters of *Salmo* stocks, which in addition to clear morphological differences between the DA and AT lineages also exhibited distinct morphological variation within the DA lineage (specifically, stocks from the Mlava and Velika Morava River systems). These findings suggest that the Croatian DA lineage may represent a separate phenetic cluster; however, further research is needed to confirm this hypothesis.

In the present study, other morphometric traits, such as fin lengths, also indicated significant differences, particularly between AT and DA lineages. However, those differences may be the result of farming practices or sexual dimorphism (Lorenzoni et al., 2003). The specimens analysed in this study primarily consisted of females, preventing an assessment of morphological differences between sexes. However, the farming practice of AT specimens could bias the result, therefore, differences in fin lengths were not considered reliable.

In this study, separate analysis of head morphometry using discriminant analysis revealed that smaller EDIAM and POSTORL were also among the most prominent distinguishing features of the AT lineage. The same results were obtained using the same dataset with a geometric morphometrics approach, where HEADL and EDIAM emerged as the primary differentiating characteristics between the DA lineage and the introduced AT specimens (Špelić et al., 2021).

On the contrary, the HY population exhibited substantial overlap with the DA population in most of the analysed morphological traits, underscoring the complexity of distinguishing between these groups. Similarly, in the geometric morphometrics approach, the shape of the HY did not differ significantly from that of the pure DA populations (Špelić et al., 2021). The HY population

examined in this study hatched in the same habitat as the DA population and shares genetic material with the DA brown trout lineage (Kanjuh et al., 2020). In contrast, the pure AT specimens sampled from natural streams originate from fish farms due to stocking activities (Piria et al., 2020) with no significant differences between stocked and farmed individuals (Špelić et al., 2021). The HY specimens have probably inherited most of their traits from the pure DA population and have adapted to the conditions of wild habitat and natural flow regimes (Stelkens et al., 2012).

The findings of this study confirmed differences in head morphometry between the AT and DA lineages but not between their HY. However, these morphological traits have limited practical applicability, e.g. AT and HY to be recognized *in situ* by anglers/non-specialists. Therefore, the results of the classical morphometric approach suggest the need for a detailed head morphometry analysis to identify distinctive traits that may differentiate brown trout lineages and HY for recommending appropriate conservation measures to preserve native pure DA populations.

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RAZLIKOVANJE MORFOLOŠKIH ZNAČAJKI IZMEĐU ATLANTSKE I DUNAVSKE LINIJE POTOČNE PASTRVE I NJIHOVIH HIBRIDA

SAŽETAK

Unesena atlantska linija potočne pastrve porijeklom je u hrvatskim potocima i rijekama prvenstveno kako bi zadovoljila zahtjeve ribiča, što je rezultiralo prisutnošću miješanih populacija na mnogim lokacijama. Jasne morfološke razlike između potočne pastrve atlantske (AT) i dunavske (DA) linije, kao i njihovih hibrida (HY), još uvijek nisu poznate. Stoga je cilj ove studije bio analizirati klasična morfometrijska i meristička mjerenja za identifikaciju značajki koja mogu razlikovati AT, DA linije ili njihove HY. Potočna pastrva prikupljena je putem elektroribolova tijekom 2017. i 2018. godine iz 10 vodotoka koji obuhvaćaju tri planinska područja: Gorski kotar, Žumberak i Papuk. Dodatno, 10 jedinki AT linije prikupljeno je iz ribogojilišta u kontinentalnoj Hrvatskoj.

Ukupno je analizirano 90 primjeraka riba: 17 AT, 22 DA i 51 HY. Rezultati su pokazali najistaknutije razlike u značajkama glave kod AT linije u usporedbi s DA linijom i njihovim HY. Diskriminantna analiza morfometrijskih značajki glave je pokazala da su promjer oka i postorbitalna duljina, značajno manji, a interorbitalna širina i visina glave značajno veći kod AT linije. Ovi nalazi sugeriraju potrebu za detaljnom morfometrijom glave u svrhu identifikacije karakterističnih morfoloških značajki za razlikovanje linija potodne pastirke i njihovih hibrida.

Glavne riječi: salmonidi, unos, crnomorski slijev, hibridizacija

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APPENDIX

Appendix 1. Morphometric and meristic characters of brown trout expressed in % of the standard length (AT – Atlantic lineage; DA – Danube lineage; HY – Hybrids; n = number of the analysed specimens; $\bar{x} \pm sd$ = mean \pm standard deviation)

Morphometric character	AT n = 17	DA n = 22	HY n = 51
	$\bar{x} \pm sd$	$\bar{x} \pm sd$	$\bar{x} \pm sd$
Standard length	20.35 \pm 3.07	16.62 \pm 4.04	14.48 \pm 3.73
In % of standard length			
Total length	113.72 \pm 2.17	117.02 \pm 2.86	117.37 \pm 2.53
Fork length	109.62 \pm 0.94	110.42 \pm 2.34	110.27 \pm 4.27
Predorsal length	44.72 \pm 1.39	45.15 \pm 1.76	45.55 \pm 1.69
Length of base of dorsal fin	12.74 \pm 1.23	13.29 \pm 1.06	13.14 \pm 1.16
Postdorsal length	42.48 \pm 2.85	41.28 \pm 2.52	40.50 \pm 2.73
Length of dorsal fin	14.80 \pm 3.60	18.77 \pm 2.56	19.12 \pm 2.53
Preanal length (distance)	70.72 \pm 1.64	71.23 \pm 1.97	70.47 \pm 2.20
Anal fin base length	9.18 \pm 0.88	9.49 \pm 0.89	9.01 \pm 1.34
Length of caudal penducle	17.63 \pm 1.93	17.52 \pm 2.65	16.69 \pm 3.39
Prepelvic distance	51.89 \pm 1.60	53.04 \pm 2.34	53.35 \pm 2.53
Length of pelvic fin	13.04 \pm 1.27	13.48 \pm 0.86	13.43 \pm 1.32
Preadipose length	79.63 \pm 1.44	81.10 \pm 1.85	81.67 \pm 2.66
Adipose fin base length	3.22 \pm 0.65	2.77 \pm 0.73	2.88 \pm 0.90
Post-adipose length	16.68 \pm 1.56	16.35 \pm 2.28	15.23 \pm 2.25
Prepectoral distance	20.87 \pm 1.37	22.08 \pm 1.29	22.05 \pm 1.30
Pectoral fin length	13.34 \pm 4.95	18.14 \pm 1.37	18.18 \pm 1.44
Body height	24.67 \pm 1.60	23.90 \pm 1.16	24.20 \pm 1.65
Depth of caudal peduncle	9.48 \pm 0.63	9.50 \pm 0.47	9.40 \pm 1.05
Head length	24.93 \pm 2.78	26.81 \pm 1.41	26.66 \pm 1.25
In % of head length			
Head height	71.34 \pm 7.01	65.24 \pm 6.25	66.95 \pm 5.86
Dorsal head length	70.03 \pm 9.57	67.54 \pm 4.30	70.17 \pm 6.66
Snout length	29.87 \pm 3.83	26.20 \pm 1.80	26.38 \pm 1.98
Eye diameter	18.75 \pm 2.34	20.71 \pm 2.20	21.26 \pm 2.43
Postorbital length	51.37 \pm 3.58	53.09 \pm 1.97	52.36 \pm 2.41
Interorbital width	35.17 \pm 2.39	29.84 \pm 4.37	29.92 \pm 3.47
Mouth width	39.22 \pm 3.52	37.04 \pm 3.08	36.41 \pm 4.44
Mouth height	61.04 \pm 8.17	54.97 \pm 5.96	56.84 \pm 8.39
Meristic character			
Spines in dorsal fin	2 \pm 0.59	2 \pm 1.14	2 \pm 0.80
Soft rays in dorsal fin	10 \pm 1.21	10 \pm 0.96	9 \pm 1.02
Spines in pectoral fin	1 \pm 0.47	1 \pm 0.59	1 \pm 0.42
Soft rays in pectoral fin	11 \pm 1.01	11 \pm 1.00	11 \pm 1.02
Spines in ventral fin	1 \pm 0.80	1 \pm 0.55	1 \pm 0.67
Soft rays in ventral fin	8 \pm 0.95	8 \pm 0.86	7 \pm 0.82

Appendix 2. Results of MANOVA Tukey post hoc test. The error term is Mean Square (Error) = 0.006. *The mean difference is significant at the 0.05 level. AT – Atlantic lineage; DA – Danube lineage; HY – Hybrids; abbreviations are provided in Table 1.

Dependent Variable	(I) lineage	(J) lineage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LBDF	AT	DA	0.0766*	0.03094	0.040	0.0028	0.1503
		HY	0.1324*	0.02683	0.000	0.0684	0.1963
PDL	AT	DA	0.0793*	0.02738	0.013	0.0140	0.1446
		HY	0.1298*	0.02375	0.000	0.0732	0.1864
LDF	AT	DA	0.0154	0.01769	0.659	-0.0268	0.0576
		HY	0.0501*	0.01534	0.004	0.0135	0.0867
LCP	AT	DA	0.0879*	0.03368	0.028	0.0076	0.1683
		HY	0.1539*	0.02921	0.000	0.0843	0.2236
PREPD	AT	DA	0.0404*	0.01494	0.022	0.0048	0.0760
		HY	0.0668*	0.01295	0.000	0.0360	0.0977
LPF	AT	DA	0.0731*	0.02701	0.022	0.0087	0.1375
		HY	0.1251*	0.02342	0.000	0.0693	0.1809
PREADL	AT	DA	0.0630*	0.02296	0.020	0.0083	0.1178
		HY	0.1034*	0.01991	0.000	0.0559	0.1508
POSTAL	AT	DA	0.0931*	0.03395	0.020	0.0122	0.1741
		HY	0.1624*	0.02944	0.000	0.0922	0.2326
PREPL	AT	DA	0.0655*	0.02610	0.037	0.0032	0.1277
		HY	0.1131*	0.02264	0.000	0.0592	0.1671
PFINL	AT	DA	-0.0077	0.02145	0.932	-0.0588	0.0435
		HY	0.0309	0.01860	0.226	-0.0134	0.0753
BH	AT	DA	0.0826*	0.02788	0.011	0.0161	0.1491
		HY	0.1300*	0.02418	0.000	0.0724	0.1877
DCAUP	AT	DA	0.0914*	0.03490	0.028	0.0082	0.1746
		HY	0.1542*	0.03027	0.000	0.0820	0.2263
HEADH	AT	DA	0.0809*	0.02581	0.007	0.0193	0.1424
		HY	0.1256*	0.02238	0.000	0.0722	0.1789
DORHL	AT	DA	0.0680*	0.02461	0.019	0.0093	0.1266
		HY	0.1089*	0.02135	0.000	0.0580	0.1598
SLE	AT	DA	0.1096*	0.03569	0.008	0.0245	0.1947
		HY	0.1716*	0.03095	0.000	0.0978	0.2454
EDIAM	AT	DA	0.0441	0.02299	0.140	-0.0108	0.0989
		HY	0.0901*	0.01994	0.000	0.0425	0.1376
POSTORL	AT	DA	0.0623	0.02800	0.073	-0.0045	0.1291
		HY	0.1167*	0.02428	0.000	0.0588	0.1746
HEADL	AT	DA	0.0611*	0.02406	0.034	0.0038	0.1185
		HY	0.1073*	0.02087	0.000	0.0575	0.1570
INTERORW	AT	DA	0.1196*	0.03603	0.004	0.0337	0.2055
		HY	0.1797*	0.03125	0.000	0.1052	0.2543
MOUTW	AT	DA	0.0896*	0.03360	0.025	0.0094	0.1697
		HY	0.1536*	0.02914	0.000	0.0841	0.2230
MOUTH	AT	DA	0.0819*	0.02477	0.004	0.0228	0.1409
			0.1261*	0.02149	0.000	0.0749	0.1774

Appendix 3. Discriminant analysis results; (A) Wilks' Lambda; (B) Functions at Group Centroids, Unstandardized canonical discriminant functions evaluated at group means; AT – Atlantic lineage; DA – Danube lineage; HY – Hybrids

A

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.495	60.096	8	0.000
2	0.897	9.273	3	0.026

B

Lineage	Function	
	1	2
AT	1.833	0.036
DA	-0.505	0.553
HY	-0.393	-0.251