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Morphometric and meristic characteristics of the Amur sleeper (*Perccottus glenii*) from the Danube River drainage channel

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Abstract: In recent decades, the Amur sleeper (*Perccottus glenii)* is one of the most impressive east-to-west invaders of European inland waters, but there are insufficient data on its biology in the countries it has entered. Specimens of two sets of samples from November 2015 (n=25) and October 2016 (n=39) were caught in the Danube River channel near Veliko Gradište (Serbia) by electrofishing. Thirty morphometric and eight meristic characteristics of the collected fish were measured with the aim of describing the general body shape in more detail using the "point-to-point" method. This is the first attempt to obtain morphometric and meristic characteristics of Amur sleeper caught in Serbia. The results revealed a relatively low variability in the morphometric and meristic characters of the studied population. When compared to other studies, there was a great variability of the studied characteristics between geographically distant European populations that inhabit different water body types. Female fish had more robust bodies compared to males of the same length, while males had longer pectoral, anal and dorsal fins. Additionally, 2+ individuals had more robust heads and jaws, as well as longer anterior parts of the body compared to 1+ individuals of the same size.

Keywords: invasive fish; rotan; body shape; traditional morphometry; meristics

INTRODUCTION

The spread of invasive species in native ecosystems, the extent of which is currently alarming, has a negative effect on original biodiversity and the normal functioning of these ecosystems [1-3]. Invasive species are recognized as one of the greatest threats to the ecological and economic well-being of the planet [2,4,5]. Some features of the most successful invasive fish species (e.g. pumpkinseed *Lepomis gibbosus* (Linnaeus 1758), topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel 1846) and brown bullhead *Ameiurus nebulosus* (Lesueur 1819)) are related to the energetic trade-off between somatic growth (a small body and short life span), and reproduction strategy (early maturation, longer spawning period or multiple spawning events, parental care) [3-6].

The Amur (Chinese) sleeper or rotan, *Perccottus glenii* Dybowski, 1877 (Gobiiformes, Odontobutidae), is a freshwater fish that inhabits lakes, ponds, marshes and lentic waters with dense vegetation [7]. This species is native to the Russian far-east, northeastern China and the northern part of North Korea [7-9]. During the last couple of decades, the Amur sleeper became one of the most impressive east-to-west invaders of European inland waters [10] and it can currently be found in 15 European countries [11]. The first record of the Amur sleeper in Serbia was in 2001 [12], which was followed by different reports of its spread throughout the Danube River drainage system [13-16].

Certain characteristics of the Amur sleeper, such as its ability to effectively use trophic resources ranging from microorganisms to vertebrates [17], pro-

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longed reproductive period [18], territorial behavior [8], and the ability to escape competition and predation by inhabiting water bodies unsuitable for most other freshwater fish, are typical for highly invasive species [19]. The dispersal of this species is also facilitated by floods [17] and by its ability to tolerate low water oxygen concentrations and habitat degradation [6]. Species' invasions provide an opportunity to investigate the phenotypic plasticity of life-history traits among populations that are geographically distant and exposed to different environmental conditions [20,21]. Furthermore, within the introduced population, shifts in life-history traits may occur for a prolonged period after their arrival [20,22]. Reshetnikov [17] has shown that the Amur sleeper has a negative impact on macroinvertebrates, amphibians and fish in small aquatic ecosystems. The Amur sleeper has no commercial importance in Serbia, but it could serve as a vector for the introduction of more than 100 species of parasites [6].

Since there are insufficient data on the Amur sleeper in invaded countries, the aim of the current paper was to fill this gap by analyzing the morphometric and meristic features of Amur sleeper caught in the Serbian part of the River Danube.

MATERIALS AND METHODS

Fish sampling

Sexually mature specimens of the Amur sleeper (n=64) were caught in the Danube River channel near Veliko Gradište (44°45'3.9'' N, 21°28'55.74'' E, 1060 river km) with the electrofishing device Elemax SHX 2000 (Sawafuji, 220V, 8.5A). The specimens, divided into two samples, from November 2015 (n=25) and October 2016 (n=39), were stored in 95% ethanol immediately upon collection from their natural habitat.

Laboratory analysis

All morphometric measurements were taken as straight lines using an electronic digital caliper (the "point-to-point" method; ±0.01 mm), and variation ranges, mean values with standard variations and variation coefficients of these characters were analyzed. The body weight was measured using a digital scale (±0.1 g precision), sex was determined by macroscopic examination of the gonads, and the age was analyzed from the scales taken from the left flank at the level of the second dorsal fin. Standard length (SL, mm) and head length (HL, mm) were measured from the tip of the snout (upper jaw) to the posterior end of the hypural complex (localized by bending out the

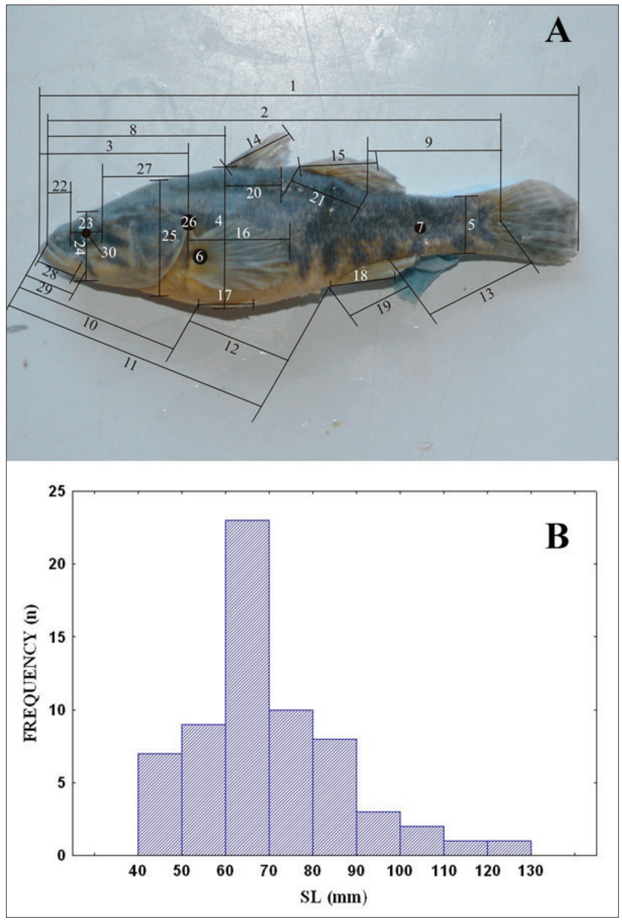

Fig. 1. Measurement diagram of the Amur sleeper (**A**). 1 - total length (TL); 2 – standard length (SL); 3 – head length (HL); 4 – maximum body depth; 5 – depth of caudal peduncle; 6 – body width at dorsal fin origin; 7 – width of caudal peduncle at anal fin insertion; 8 – predorsal length; 9 – postdorsal length; 10 – prepelvic length; 11 – preanal length; 12 – pelvic to anal fin origin distance; 13 – length of caudal peduncle; 14 – length of dorsal fin (D1); 15 – length of dorsal fin (D2); 16 – length of pectoral fin; 17 – length of pelvic fin; 18 – length of anal fin; 19 – length of base of anal fin; 20 – length of base of D1 fin; 21 – length of base of D2 fin; 22 – snout length; 23 – horizontal eye diameter; 24 – head depth at eye center; 25 – head depth at nape; 26 – head width at posterior margin of preopercle; 27 – postorbital length; 28 – upper jaw length; 29 – lower jaw length; 30 – inter orbital width, the least fleshy width. **B** – Frequency distribution of examined fish.

Table 1. Descriptive statistics of the analyzed morphometric characters: SD – standard deviation; CV – coefficient of variation.

Character	Range	Mean ± SD	CV
Total length (TL)	53.70-140.70	83.65±18.697	22.351
Standard length (SL)	43.80-120.70	70.40±16.374	23.257
in % of SL			
Head length (HL)	31.66-39.08	35.46±1.434	4.046
Maximum body depth	21.6-29.94	24.95±1.713	6.864
Depth of caudal peduncle	10.67-13.65	12.23±0.654	5.349
Body width at dorsal fin origin	15.42-26.04	18.24±1.696	9.302
Width of caudal peduncle at anal fin insertion	4.77-8.66	6.40±0.926	14.474
Predorsal length	37.55-47.75	42.46±1.793	4.222
Postdorsal length	20.63-34.41	28.13±1.808	6.427
Prepelvic length	31.86-38.38	35.40±1.477	4.171
Preanal length	57.52-65.39	61.24±1.868	3.050
Pelvic to anal-fin origin distance	22.95-31.16	27.14±1.839	6.774
Length of caudal peduncle	18.20-29.44	26.13±1.857	7.108
Length of dorsal fin (D1)	13.69-20.69	16.99±1.591	9.365
Length of dorsal fin (D2)	16.50-29.21	22.82±2.365	10.365
Length of pectoral fin	17.72-25.02	21.13±1.511	7.149
Length of pelvic fin	11.68-18.60	14.69±1.673	11.385
Length of anal fin	16.66-24.68	20.71±1.695	8.187
Length of base of anal fin	10.88-19.77	13.81±1.505	10.985
Length of base of D1 fin	8.36-13.80	11.44±1.183	10.341
Length of base of D2 fin	13.63-23.79	17.68±1.573	8.894
in % of HL			
Snout length	16.37-32.22	20.86±2.629	12.604
Horizontal eye diameter	12.07-21.39	14.98±1.815	12.121
Head depth at eye center	35.17-48.44	41.45±2.564	6.186
Head depth at nape	49.45-62.88	55.76±3.237	5.805
Head width at posterior margin of preopercle	44.92-62.01	51.23±3.220	6.286
Postorbital length	52.84-63.01	57.74±2.463	4.266
Upper jaw length	25.90-38.45	33.08±2.869	8.674
Lower jaw length	28.48-40.10	35.21±2.699	7.667
Interorbital width, the least fleshy width	13.00-23.43	17.72±2.057	11.605

caudal fin), and the posterior end, the most point of the opercular membrane, respectively.

Statistical analysis

All values are expressed as the mean (μ) ± standard deviation (SD). The coefficient of variation (CV, in %) was calculated using the following equation: $CV=SD\ \mu^{-1} \times 100$. Shapiro-Wilk's test and Levene's test were used to test the normality of the data distribution and homogeneity of variances, respectively. If the data sets lacked normality of distribution, comparisons of morphometric features of age groups/gender were performed using the Mann-Whitney *U*-test. If the data sets passed the normality and homoscedasticity assumptions, significant differences among groups were tested using the *t*-test. Significance for all conducted tests was considered at level of $P \le 0.05$. Statistica 7.0 Software (StatSoft, Inc., Tulsa, OK, USA) was used to perform all statistical analyses.

RESULTS

According to Hubbs and Lagler [23], 30 morphometric and 8 meristic characteristics (Fig. 1A) of the collected fish were measured with the aim of describing the general body shape in more detail. The SL values of the Amur sleeper varied between 40 mm and 130 mm, with most individuals (n=23) between 61 mm and 70 mm SL (Fig. 1B). The body weight ranged from 1.9 g to 40.0 g with an average weight of 8.27±7.09 g.

The Amur sleeper exhibited relatively low variability of morphometric characters (Table 1). The CV values were relatively low (CV<15%) for all measured characters. The lowest CV was recorded for preanal length (CV=3.05), and the highest CV was recorded for the width of the caudal peduncle at anal fin insertion (CV=14.47). All specimens had a longer lower jaw compared to the upper jaw, predorsal length compared to the postdorsal length, second dorsal fin (D2) compared to the first dorsal fin (D1), and base of the D2 compared to the base of the D1.

Table 2 contains data on fin composition in the analyzed specimens. The D1 was composed of unbranched rays with a low variation in number. All other fins were composed of unbranched and branched rays and the greatest variation in ray number was

Table 2. Fin composition of the examined fish: D1 – first dorsal; D2 – second dorsal; P – pectoral; V – ventral; A – anal; Roman numerals – unbranched rays; Arabic numbers – branched rays; n – number of fish.

D1		D2		P		V		A	
Formula	n	Formula	n	Formula	n	Formula	n	Formula	n
VI	2	I 9	7	I 13	7	I 4	2	I 8	4
VII	26	I 10	27	I 14	3	I 5	4	I 9	19
VIII	30	I 11	20	II 12	15	I 6	1	I 10	21
		I 12	1	II 13	22	II 3	47	I 11	12
		I 13	3	II 14	11	II 4	3	II 7	1
						II 5	1	II 9	1

Table 3. Meristic characters: total number of rays in first dorsal (D1), second dorsal (D2), pectoral (P), ventral (V), and anal (A) fin; number of scales in the lateral row (sq), circumference row (cf), and circumpenducular row (cp).

Number of individuals with the observed character													
Character	5	6	7	8	9	10	11	12	13	14	15	16	
D1		2	26	30									
D2						7	27	20	1	3			
P										22	25	11	
V	49	7	2										
A					5	19	22	12					
Number of individuals with the observed character													
Character	14	15	16	17	18	37	38	39	40	41	42	43	44
sq						1	10	23	15	6	2		
cf							1		7	7	22	12	8
cp	4	2	16	19	16								

recorded in the anal fin. According to the presented data, the fin formula was: D1 VI-VIII; D2 I + 9-13; P I-II + 12-14; V I-II + 3-6; A I-II + 7-11, where the Roman and Arabic numbers refer to unbranched and branched rays, respectively; P refers to pectoral, V to ventral, and A to the anal fin. The number of scales in the lateral row (sq) varied between 37 and 42, the number of scales in the circumference row (cf) ranged from 38 and 44, and the number of scales in the circumpenducular row (cp) varied between 14 and 18 (Table 3).

The sex ratio was near 1:1 (31 males to 33 females). According to Chilton and Beamish [24], three age groups were determined. Most of the specimens (n=38) belonged to the 1+ age class, followed by 2+ (n=25) and 3+ (n=1).

Statistical analyses revealed significant differences between the age groups (1+ and 2+) regarding standard length (SL) in % of TL; head length (HL), width of the caudal peduncle at the anal fin insertion, postdorsal length, prepelvic length, preanal length, the length of the pelvic fin, and the length of base of the anal fin in % of SL; snout length, horizontal eye diameter, interorbital width and upper and lower jaw length in % of HL (Table 4). Significant differences between sexes were found for SL in % of TL; maximum body depth, body width at the dorsal fin origin, width of the caudal peduncle at the anal fin insertion, the length of the dorsal (D1 and D2), pectoral, pelvic, anal fins in % of SL; horizontal eye diameter and head depth at the nape in % of HL (Table 4).

DISCUSSION

In our sample, the number of individuals declined with age, which was also noted [8]. The trophic and reproductive plasticity of the Amur sleeper prevented intraspecific competition and allowed the coexistence of individuals of multiple different sizes [17,18].

The length values from this study differed from those of the Amur sleeper in its native range: 72.0-106.0 mm [25] and 33.0-116.0 mm [26]; in addition, they differed from the length values recorded in Poland, 77.7-106.5 mm [27], Belarus, 82.2-131.0 mm [28], and Germany, 22.0-110.0 mm [29], but were within the range of populations from Russia, 48.0-155.0 mm [30] and 46.0-189.0 mm [31].

Considerable variations in life-history traits occurs in species with wide range distributions in response to local environmental conditions [32]. This variation often displays a geographical pattern [33], which is important to analyze when species' invasions far beyond their native regions are monitored [20,21,34,35]. Invasive success strongly depends on the plasticity of the life-history traits (i.e., high reproductive investment, early maturation, small body size) of the invaders [20,21]. Length growth and weight growth of the Amur sleeper in the non-native range can vary notably, depending on food supply [8]. Some authors have observed a distinct ecological dimor-

Table 4. Values of morphometric characters in two age classes and different sexes of Amur sleeper individuals from Veliko Gradište presented as the mean±SD.

	Age		Gender	
	1+	2+	Male	Female
in % of TL				
Standard length (SL)	83.44±1.70[b]	84.64±1.42[a]	83.40±1.29[b]	84.46±1.88[a]
in % of SL				
Head length (HL)	35.00±1.45[b]	36.03±1.08[a]	35.76±1.60	35.17±1.22
Maximum body depth	24.92±1.82	24.98±1.61	24.51±1.54[b]	25.37±1.78[a]
Depth of caudal peduncle	12.08±0.68	12.40±0.52	12.35±0.55	12.11±0.73
Body width at dorsal fin origin	18.15±1.88	18.37±1.44	17.56±1.12[b]	18.88±1.90[a]
Width of caudal peduncle at anal fin insertion	6.16±0.81[b]	6.67±0.92[a]	6.04±0.89[b]	6.73±0.84[a]
Predorsal length	42.15±1.71	42.90±1.89	42.60±2.06	42.32±1.52
Postdorsal length	28.39±1.74[a]	27.74±1.91[b]	28.17±1.49	28.09±2.08
Prepelvic length	34.87±1.48[b]	36.18±1.10[a]	35.39±1.70	35.42±1.26
Preanal length	60.43±1.75[b]	62.42±1.39[a]	61.10±2.04	61.37±1.71
Pelvic to anal-fin origin distance	26.93±1.98	27.42±1.62	26.94±1.66	27.33±2.00
Length of caudal peduncle	26.36±1.64	25.78±2.17	25.85±1.54	26.39±2.11
Length of dorsal fin (D1)	17.15±1.52	16.77±1.72	17.62±1.64[a]	16.39±1.31[b]
Length of dorsal fin (D2)	22.85±2.19	23.02±2.37	23.99±2.14[a]	21.71±2.03[b]
Length of pectoral fin	21.20±1.55	21.07±1.50	21.66±1.47[a]	20.64±1.39[b]
Length of pelvic fin	15.35±1.57[a]	13.76±1.33[b]	13.92±1.36[b]	15.41±1.63[a]
Length of anal fin	20.77±1.73	20.67±1.68	21.30±1.92[a]	20.15±1.24[b]
Length of base of anal fin	14.12±1.67[a]	13.42±1.09[b]	14.09±1.58	13.55±1.41
Length of base of D1 fin	11.30±1.21	11.69±1.13	11.56±1.43	11.33±0.90
Length of base of D2 fin	17.85±1.73	17.48±1.31	17.87±1.62	17.51±1.53
in % of HL				
Snout length	20.31±2.39[b]	21.51±2.72[a]	20.73±1.66	20.99±3.31
Horizontal eye diameter	15.59±1.91[a]	14.13±1.21[b]	14.44±1.28[b]	15.48±2.10[a]
Head depth at eye center	41.14±2.32	42.13±2.68	40.83±2.58	42.03±2.45
Head depth at nape	55.22±3.12	56.40±3.29	54.69±3.06[b]	56.77±3.11[a]
Head width at posterior margin of preopercle	50.71±2.55	51.88±3.98	50.43±3.09	51.98±3.20
Postorbital length	57.21±2.47	58.40±2.24	57.67±2.08	57.81±2.81
Upper jaw length	31.83±2.67[b]	34.88±2.11[a]	33.38±3.16	32.80±2.58
Lower jaw length	34.11±2.52[b]	36.81±2.14[a]	35.55±2.96	34.89±2.44
Interorbital width, the least fleshy width	17.26±1.80[b]	18.45±2.29[a]	17.77±2.43	17.68±1.67

[a,b] Values with different letters in the same row are significantly different (Mann-Whitney *U*-test, P≤0.05 or *t*-test, P≤0.05).

phism between populations of Amur sleeper in the European part of the former USSR (as a consequence of acclimatization), and even between populations within its natural range (due to the long isolation of waterbodies) [8]. Authors distinguished between two body forms, the "light" rapidly growing form with a stronger head, terminal mouth, more elongated body, second dorsal and anal fins shifted backwards, and a diet consisting mostly of fish, and the "dark" form, which feeds on aquatic invertebrates [8].

After comparing our results with those of Nowak et al. [27], we found that 15 out of 22 morphometric characteristics had a wider range of variation for the Amur sleeper from Veliko Gradište. Only two traits (head width at the posterior margin of the preopercle and interorbital width) from the samples caught in Krakow-Mydlniki ponds showed a wider range of variation than our samples, indicating that the Amur sleeper from Poland had a shorter postorbital length, but a wider head depth at the nape than in Serbia. Bearing this in mind, we believe that there is greater morphological plasticity of the Amur sleeper caught in Serbia than in Poland. It was shown [30] that 7 out of 11 morphometric characteristics had a wider range of variations in the population from Lake Krugloe of the Mordovo floodplain (Saratov Reservoir) than in the Amur sleeper from Veliko Gradište. The Amur sleeper from Serbia had a greater horizontal eye diameter, snout length, maximum body depth and length of the base of the second dorsal fin, which indicates that this population is more similar to the "light" form. In [28], the morphometric characters of the Amur sleeper from two sites in Belarus were compared. The first population was from the channel of the Pripyat River basin and the second from fishponds near Minsk. We compared eighteen morphometric characters of Amur sleeper from Belarusian populations with our findings. In contrast to our data, Amur sleeper individuals caught in Belarus had a narrower range of variation for the length of the caudal peduncle, pelvic to anal-fin origin distance, the length of base of the second fin, the length of base of the anal fin, a 2-fold smaller snout length, horizontal eye diameter and postorbital length, and 2-fold wider caudal peduncle at the anal fin insertion, on average. The Minsk population had seven more characteristics with a narrower range of variation compared to the population from Veliko Gradište. On the other hand, individuals from the channel of the Pripyat River basin had, on average, a larger head depth at the eye center, head depth at the nape, maximum body depth, predorsal length, postdorsal length, prepelvic length, length of the base of D1, length of pectoral fin, length of pelvic fin, but a smaller preanal length than individuals from our research.

Comparison of several meristic features of Amur sleeper in different types of waterbodies in the native and non-native (European Russia) ranges of *P. glenii* showed that the higher number of lake populations significantly differed in comparison to the native population from the Amur River. Considering the number of rays, the D1 fin and D2 fin values increased, while the anal fin (A) values decreased [31]. The means of the D1 rays in both native and non-native ranges were close in all waterbody types. Only the values of D2 and A rays in populations of the native range and quarries of the non-native range were significantly lower than in Amur sleeper populations introduced to lakes and ponds.

A study of the meristic characteristics of Amur sleeper from ponds in Krakow-Mydlniki showed that the number of rays in the D1 fin varied between seven and nine [27]. In two more studies, the number of rays in the D1 fins was 6-7 [30] and 6-9 [28]. We found one unbranched ray in the second dorsal fin, the same as described in [29], but in [28] individuals with two unbranched rays in the D2 fin were recorded. The number of branched rays in the second dorsal fin ranged from 10-12 for the ponds in Krakow-Mydlniki, and 10-14 for the Veliko Gradište populations, where in both cases almost half of the population had 11 branched rays in D2. Additionally, individuals caught in Lake Krugloe [30] and those from the channel of the Pripyat River basin [28] had 10-11 and 9-13 branched rays in D2, respectively. The Amur sleeper from this study had one or two branched rays in the anal fin, the same as individuals from the Pripyat River basin channel [28]. In our study, the number of branched rays in the anal fin varied from 9-12 as in [27], and individuals with 11 rays dominated in both populations, which was not the case in [28]. The same number of unbranched rays for the pectoral fin was observed when compared with the results reported in [28]. Moreover, most individuals from both populations had 15 and 5 branched rays in pectoral and ventral fins, respectively. The number of scales in the circumference differed between the Amur sleeper from Veliko Gradište and from ponds in Krakow-Mydlniki, as well as the number of scales in the circumpenducular row [27]. The number of scales in the lateral row in both populations ranged from 37-42. In the population from the Pripyat River basin channel [28], there were 36-41 scales in the lateral row.

According to our results, female fish had more robust bodies compared to males of the same length, while males had longer fins. This could be explained by the fact that males grew faster than females in the 1st and 2nd years of life, and since males provide parental care by guarding the eggs, they often take less food than females or even starve during the spawning season [3]; on the other hand, longer fins allow them to fan the clutch (ibid.). Additionally, 2+ individuals had more robust heads and jaws, as well as longer anterior parts of the body compared to 1+ individuals of the same size. This could be due to the carnivorous diet, since there are some indications that smaller individuals are omnivorous.

To conclude, there is considerable variability in the studied characteristics between geographically distant populations that inhabit different waterbody types throughout Europe. The differences between the morphometric and meristic characters of the Amur sleeper from different sites could be caused by the heterogeneity of the comparable material, the intraspecific variability, and differences in habitat conditions.

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