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Research Article

First report of the Chinese sleeper *Perccottus glenii* Dybowski, 1877 in the Drava River, Croatia

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Abstract

Chinese sleeper (*Perccottus glenii*) is one of the most successful invasive fish species, spreading rapidly to colonize a wide range of ecosystem types, from lentic oxbows to large rivers and freshwater lakes. Previously, only a single individual of *P. glenii* has been recorded from Croatian waters, in the Sava River catchment. This study reports the presence of additional record and the first population of *P. glenii* in the Croatian part of the Drava River. During a field survey, eight individuals (n = 8) of *P. glenii* were collected from a shallow and overgrown freshwater oxbow in the Drava River flood zone, within a NATURA 2000 protected site. Since several individuals were within the mature size class (i.e., larger than 55 mm standard length), the results emphasize the importance of early confirmation of the presence of this invasive species, and the need for comprehensive biological surveillance of the Drava River and similar floodplain habitats, especially downstream of this collection site. Two hypotheses (north and south) explaining the arrival of Chinese sleeper into the Croatian part of Drava River are proposed. Semi-closed oxbows, such as the present one, could represent a suitable ecosystem for detailed *in situ* experiments on *P. glenii* ecology and migration patterns.

Key words: invasive species, flood zone, NATURA 2000, oxbow

Introduction

Non-native species, especially invasive ones, pose a serious threat to biodiversity and can cause multiple adverse impacts on the newly inhabited ecosystems. The number of introduced non-native fish worldwide has rapidly increased in recent years, with the introduction rate doubling in the last 30 years (Gozlan et al. 2010; Cucherousset and Olden 2011; Toussaint et al. 2018). The negative impacts of non-native taxa on native, endemic or endangered ichthyofauna can be ecological, evolutionary or economic, including habitat degradation, competition for predation, food and space, hybridization, transmission of parasites and pathogens, and changes to food niches (Gozlan et al. 2010; Cucherousset and Olden 2011; Dulčić et al. 2017; Mihinjač et al. 2019).

The Chinese sleeper, *Perccottus glenii* Dybowski, 1877 (Gobiiformes, Odontobutidae) is a medium sized (max. total length ~ 25 cm; Baensch and Riehl 1991) freshwater invasive fish widely distributed throughout western Eurasia. It has been spreading westwards since the early 20th century (Elovenko 1981; Reshetnikov 2003, 2004). The native distribution range of *P. glenii* extends from the inland Far East of Russia (Berg 1949), northeast China (Zhu 1995) and North Korea (Mori 1936), including the Amur River drainage and surrounding adjoining tributaries (Reshetnikov 2010). However, over the past 50 years, this highly invasive fish species has proliferated throughout Europe, and is presently documented in numerous European countries (Reshetnikov and Ficetola 2011; Reshetnikov 2010, 2013; Nehring and Steinhof 2015; Kvach et al. 2016). The westernmost record of *P. glenii* in freshwater ecosystems in Europe to date is in the Upper Danube (Bavaria), Germany (Reshetnikov and Schliewen 2013; Nehring and Steinhof 2015), raising major scientific concerns over further western proliferation. Reshetnikov (2004, 2013) discussed the penetration patterns and historical centers of distribution of *P. glenii* outside of its natural range, emphasizing that localities from the Far East of Russia to more western regions should be considered the historical centers of distribution during the introduction period (1912–1971), and potential secondary invasive sources to several countries in Central Europe. Specifically, around the 1960s, *P. glenii* was introduced to western Ukraine (Lviv region) during cyprinid fish stocking. This was likely unintentional, but from there, it has spread widely westwards via well-established freshwater networks (Reshetnikov 2013). Likewise, Grabowska et al. (2020) suggested that, according to a population genetic structure analysis performed on the *cyt b* genetic marker, European populations of *P. glenii* consist of at least three distinct haplogroups (i.e., Baltic, East-European and Carpathian), which could indicate separate introduction events from different parts of the native range and subsequent multidirectional pathways to Central Europe. Other authors (Dmitriev 1971; Reshetnikov 2013; Grabowska et al. 2020; Kvach et al. 2021) have proposed two hypothetical ecological invasion pathways of freshwaters by *P. glenii*, either via an inland freshwater aquatic network (primary source), or from oligohaline waters such as coastal brackish ecosystems (secondary source), as suggested for the south-eastern Baltic Gulf of Finland. Reshetnikov (2003, 2013) further emphasized that anthropogenic activities, such as fish farming, fish stocking, and sport angling, play a prominent role in the introduction of *P. glenii* into isolated and geographically distant waterbodies.

Throughout its range, *P. glenii* occupies natural and artificial stagnate and lentic eutrophic backwaters (oxbows, floodplains, swamps and ponds) rich in aquatic vegetation with a silty substrate (Nikolskii 1956; Grabowska et al. 2011; Reshetnikov 2013; Rechulicz et al. 2015). *Perccottus glenii* is rarely caught from a main riverine channel or fast-flowing water currents, although it can temporarily inhabit such habitats, possibly during the invasion period via active or passive dispersal migration (Reshetnikov 2013). It is an opportunistic predatory fish, feeding voraciously on a wide variety of vertebrates (fish, tadpoles and newts) and small benthic or planktonic invertebrates (larvae of Insecta, Oligochaeta or Mollusca), meaning that it competes with the native fish species for the same food resources (Koščo 2003; Reshetnikov 2001, 2003). During the reproduction period, males are highly territorial and exhibit aggressive behavior towards male intruders, while females are attracted to the spawning ground (i.e. nest) by a suite of behavioral and acoustic cues (Horvatić et al. 2019). Evidently, *P. glenii* is capable of drastically affecting the trophic structures of freshwaters, perhaps leading to a decrease in abundance or even local extinction of certain native species. Due to its ecological characteristics and invasive nature, particularly its broad ecological tolerance, opportunistic generalist feeding strategy and aggressive behavior, *P. glenii* is considered the *perfect conqueror* (Ćaleta et al. 2010).

Reshetnikov (2013) discussed the possible migration routes of invasive *P. glenii* into European freshwaters, highlighting West Ukraine as the West-European center of distribution and a crucial spreading "hotspot" from which *P. glenii* migrated into many different European watersheds. The author suggested that between 2005 and 2013 *P. glenii* likely invaded the Dniester, Vistula, Dnieper, Bug, and Danube catchments from the West Ukraine, using rivers as natural passages to rapidly proliferate downstream from riverheads to river mouths, but also to slowly migrate upstream into the adjoining tributaries (Reshetnikov 2013). Other studies have highlighted two important natural corridors providing a potential gateway from colonized areas in Eastern and Central Europe into Western Europe: 1) northern corridor – waters of Poland and Germany, characterized by having many artificial inter-river connecting channels, and 2) southern corridor – Danube Basin including Croatia and Slovenia, and the watersheds of northern Italy and southern France (Leuven et al. 2009; Reshetnikov and Ficetola 2011). Based on bioclimatic analysis, the mountainous regions of Europe (Dinaric, Alpine and Carpathian Mountain ranges) constitute a barrier to the spread of *P. glenii* (Reshetnikov and Ficetola 2011). Furthermore, Reshetnikov (2013) highlighted the Transcarpathian region (in West Ukraine) as a source of downstream colonization of the Danube Basin by *P. glenii* since this region is considered a natural spring area of streams and rivers of the Danube Basin. From there, the expansion of *P. glenii* to the middle Tisza happened via three rivers: Uzh, Latorica and the upper Tisza (Reshetnikov 2013).

Chronologically, the first record of *P. glenii* in Balkan waters (Danube Basin) was from the Tisza River (Hungary) in 1997, after a southward expansion of its initial range in the West-Ukrainian subrange (Harka 1998; Reshetnikov 2013). In 2001, it was reported as present in Romania (Nalbant et al. 2004) and Serbia (Gergely and Tucakov 2003), in Bulgaria in 2005

Figure 1. Mature individual of *Perccottus glenii* (SL = 71 mm) collected from Drava River backwater in Croatia, April 2021. Photo by Sven Horvatić.

(Jurajda et al. 2006), and in the Sava River, Croatia in 2008 (Ćaleta et al. 2010). By 2010, *P. glenii* had already acclimatized throughout the Tisza River and proliferated further southward through the Danube Basin, likely facilitated by flooding events, to colonize the adjoining tributaries, such as the Sava River in Croatia (Ćaleta et al. 2010; Reshetnikov 2013). Until 2010, *P. glenii* also established stable populations in Lake Balaton, which is artificially connected with the Danube River (Eros et al. 2008; Harka et al. 2008; Antal et al. 2009).

Perccottus glenii is considered the most widespread (Reshetnikov 2010; Reshetnikov and Ficetola 2011) and highly successful invasive fish species in European freshwaters (Copp et al. 2005). It has been included on the European Union Concern List of invasive alien species (European Commission 2016), and was assessed as having a high risk of invasiveness for Croatian waters (Piria et al. 2016).

Since the first record of *P. glenii* in Croatia, when a single adult was caught by angler from a well-vegetated fishpond channel (Ćaleta et al. 2010), extensive ichthyological field surveys have been carried out in Croatian artificial (reservoirs, channels or irrigations waterways) and natural (lakes, ponds, rivers) freshwaters, but without positive confirmation of the presence of *P. glenii*. This study reports a new record of its presence, the first record in the Drava River and the first recorded population of *P. glenii* in Croatia (Figure 1). Individual body measurements (morphometric measurements and meristic characteristics) are reported here for the purposes of future comparison with possible neighbouring or other European populations. Possible migration routes of *P. glenii* to Croatian waters are discussed in light of the present finding.

Materials and methods

A shallow Drava River backwater (oxbow lake) (45°42′3,45″N; 18°26′37,17″E), situated near the City of Belišće, and with a total area of 2400 m^2 (0.24 ha)

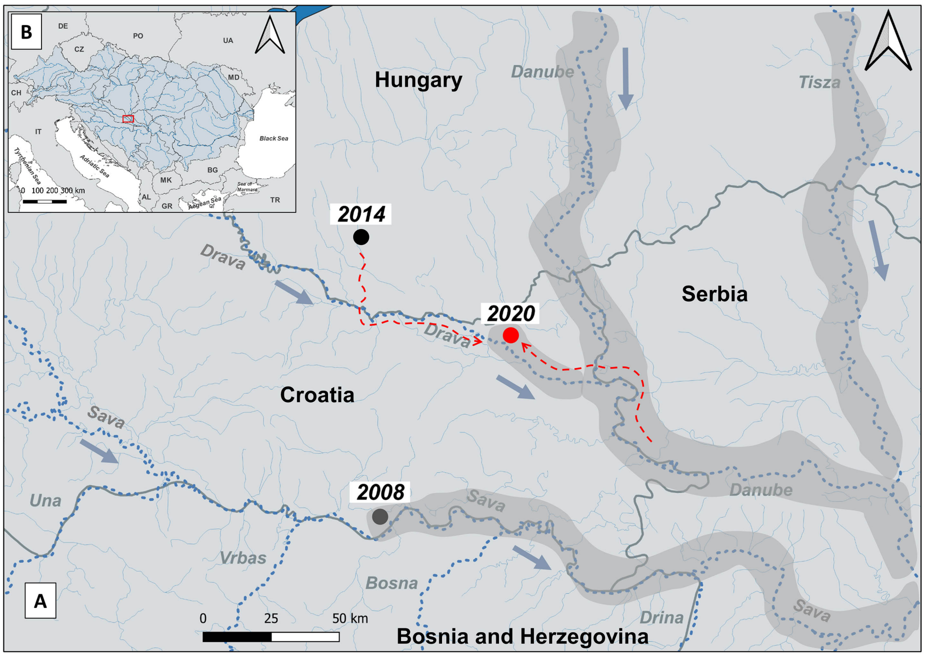

Figure 2. Distribution of *Perccottus glenii* in Croatia and adjoining freshwaters from the neighbouring countries. A) Black circles indicate the closest previous finding site of *P. glenii* to Drava River, while the red circle shows the present find site from Croatia. Grey shaded area highlights large freshwater drainages where *P. glenii* has been previously reported. Red broken lines present potential migration routes of *P. glenii* into the Croatian part of the Drava River, while grey solid lines indicate the country borders. B) The inset indicates the Danube River Basin with the water network (blue lines) and the collection site in Croatia (red square). Abbreviations correspond to the country names.

(Figures 2, 3) was sampled twice during field studies, on 30 July 2020 and 01 April 2021. The sampling was conducted by boat using electrofishing equipment with direct continuous current (Hans Grassl model EL64 IIGI, 7.5 kW) in combination with hand nets for sample collection. Individuals of *P. glenii* were caught by a hand-held anode from the oxbow, near the shore at a depth of around 0.5 meters. The oxbow, measuring 35 × 80 m, is enclosed on the north side by a river embankment and it is situated within the Drava flooding zone (approximately 500 m from the main river channel). The aquatic and riparian vegetation (predominately, common reed, *Phragmites australis* and yellow iris, *Iris pseudacorus*) is well-developed and dense, and the substrate is mud-silt (Figure 3). Physicochemical characteristics of the water were measured with a two-channeled HQ40D Portable Meter (Hach®, Colorado, USA). We measured water temperature (°C), oxygen saturation (in %), dissolved oxygen (mg O_2/L), conductivity (µS/cm), pH, total dissolved solids (TDS; mg/L), available from the portable HQ40D to characterize the waterbody from which *P. glenii* was sampled. The water transparency was measured by using Secchi disc. The sampling site and fish specimens were photographed with a Canon camera. All specimens were euthanized with an overdose of MS-222 and were preserved in absolute solution of ethyl alcohol for further laboratory analyses. The sampling site is part of the European ecological network NATURA 2000 (site code: HR2001308 Lower course of the Drava River) and

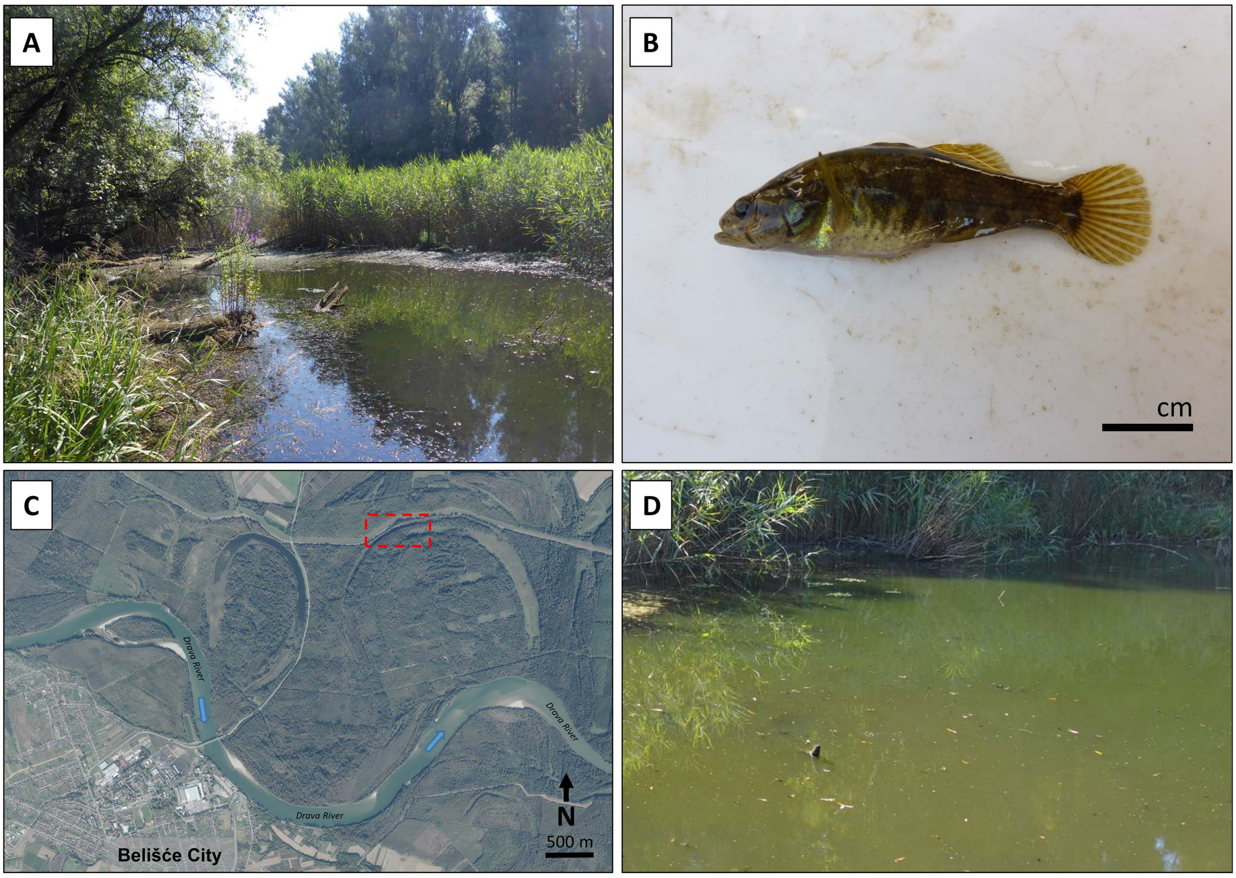

Figure 3. Invasive *Perccottus glenii* (Odontobutidae) collected from Drava River (Croatia) backwaters in July 2020. A) Sampling site from which four immature individuals of *P. glenii* were collected by electrofishing. B) Immature specimen of *P. glenii* (41 mm standard length) sampled immediately from the oxbow. C) Ortho-photo map of the collection site (red dashed square) of *P. glenii* population in the Drava River flooding zone (dark green). D) Water quality from the sampling site where *P. glenii* was caught. The green colour indicates the highly eutrophic condition of the shallow and overgrown oxbow. Photo by Sven Horvatić.

also lies within the Mura-Drava Regional Park protected area. Species determination was based on morphological descriptions and taxonomic keys (Miller 2003; Kottelat and Freyhof 2007). The standard length (SL, mm), total length (TL, mm) and weight (W, g) of each fish was measured in the field using a ruler (1 mm precision) and digital scale (0.1 g precision), respectively. In the laboratory, 30 morphometric measurements were recorded for each fish using digital callipers CD-15APX with precision of 0.01 mm (Mitutoyo, Japan). Meristic counts (eight traits) were taken from the left lateral side of the body, using a magnifying glass (Zeiss Stemi 2000-C). Morphometric and meristic measurements generally followed the description by Hubbs and Lagler (1947) and Nikolić et al. (2021). In order to group individuals according to the maturation size (immature < 55 mm SL; mature > 55 mm SL), we followed Miller (2003). One individual was deposited in the collection of the Rijeka Natural History Museum (Croatia) under catalogue number PMR VP4988.

Results

During the field surveys, eight individuals of *P. glenii* (n = 8, four in 2020 and four in 2021) were collected from the Drava River oxbow (Figure 1),

Table 1. Physicochemical characteristics of the sampled Drava River oxbow collected during 2020 and 2021.

Water parameters	July 2020	April 2021
Water temperature (°C)	29.0	17.0
Water transparency (m)	0.3	0.5
Oxygen saturation (%)	71.2	93.8
Dissolved oxygen (mg O_2/L)	5.4	8.9
pH	7.7	8.0
Conductivity (µS/cm)	308	355
Total dissolved solids (TDS) (mg/L)	168	209

about 60 kilometers upstream of the confluence of the Drava and Danube Rivers (Figure 2). This is the first finding of *P. glenii* from the Croatian part of the Drava River, since it was previously recorded in a channel of the Sava River (near Slavonski Brod), about 70 km southeast from the current site. There are no artificial connections between these sites and their corresponding watersheds, hence, they can be assumed to represent two independent *P. glenii* occurrences in Croatia.

On the collection site, the physicochemical characteristics of the water were measured in two occasions (July 2020 and April 2021), and the observed field conditions (algal bloom), water parameters (fluctuating water temperature, low oxygen level and low transparency; Table 1), suggest that sampled well-vegetated and shallow oxbow is a eutrophic waterbody, especially during the summer period when the water level drops significantly (Figure 3). These field surveys recorded a relatively highly diverse fish assemblage at this site: weatherfish *Misgurnus fossilis* (Linnaeus, 1758) (Cobitidae), European bitterling *Rhodeus amarus* (Bloch, 1782) (Acheilognathidae), common carp *Cyprinus carpio* Linnaeus, 1758, crucian carp *Carassius carassius* (Linnaeus, 1758), gibel carp *Carassius gibelio* (Bloch, 1782) (Cyprinidae), topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846) (Gobionidae), white bream *Blicca bjoerkna* Linnaeus, 1758, sunbleak *Leucaspius delineates* Heckel, 1843, asp *Leuciscus aspius* (Linnaeus, 1758), roach *Rutilus rutilus* (Linnaeus, 1758), rudd *Scardinius erythrophthalmus* (Linnaeus, 1758) (Leuciscidae), pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) (Centrarchidae), European perch *Perca fluviatilis* Linnaeus, 1758 (Percidae), black bullhead *Ameiurus melas* Rafinesque, 1820 (Ictaluridae), Northern pike *Esox lucius* Linnaeus, 1758 (Esocidae). The overall abundance of each recorded fish species is shown in Table 2. Briefly, the invasive and alien species (*C. gibelio*, *P. parva, L. gibbosus, A. melas and P. glenii*) make up nearly one-third of the recorded species and dominate the overall fish abundance with roughly 94% (Table 2). On the other hand, protected native fish species (*L. delineates*, *C. carassius* and *M. fossilis*) were very rare in the catch and accounted for barely 2% of the sample (Table 2). In total, native species (including protected taxa) accounted for only around 5% of the sampled individuals from the overall abundance (Table 2).

Table 2. Fish species captured from Drava River oxbow during the 2020 and 2021 field surveys, with the total number of sampled individuals per species indicated and their percentage from the overall fish abundance highlighted. Plus ($^+$) indicates the invasive species, while asterisk (*) indicates strictly protected.

Taxa	Total number	Percentage (%)
Carassius gibelio$^+$	1087	71.28
Pseudorasbora parva$^+$	237	15.54
Ameiurus melas$^+$	73	4.79
Lepomis gibbosus$^+$	35	2.30
Scardinius erythrophthalmus	21	1.38
*Misgurnus fossilis**	19	1.25
Rutilus rutilus	14	0.92
Blicca bjoerkna	11	0.72
Perccottus glenii$^+$	8	0.52
Perca fluviatilis	5	0.33
Esox lucius	5	0.33
*Leucaspius delineatus**	4	0.26
Aspius aspius	2	0.13
*Carassius carassiu**	2	0.13
Cyprinus carpio	1	0.07
Rhodeus amarus	1	0.07

Table 3. Capture date, maturation size groups, gender and main body characteristics of *P. glenii* individuals sampled from Drava River oxbow.

Individual	Capture date	Size group	Gender	Total length (TL; mm)	Standard length (SL; mm)	Body weight (W; g)
#1	01 April 2021	Mature	Female	75.88	63.13	9.40
#2	01 April 2021	Mature	Male	69.44	58.93	8.10
#3	01 April 2021	Mature	Female	85.70	69.40	10.10
#4	01 April 2021	Mature	Male	84.16	70.90	10.80
#5	30 July 2020	Immature	unknown	54.72	46.10	6.70
#6	30 July 2020	Immature	unknown	49.62	41.80	6.90
#7	30 July 2020	Immature	unknown	51.70	37.90	7.60
#8	30 July 2020	Immature	unknown	60.85	51.06	7.90

According to Miller (2003), several of the individuals collected here were immature (< 55 mm SL; summer 2020 sample), while others can be considered mature (> 55 mm SL; spring 2021 sample; Table 3). The morphometric and meristic characteristics of *P. glenii* from the Drava River oxbow are given in Table 4. Generally, for *P. glenii,* SL values ranged from 38 to 71 mm (mean ± standard deviation = 54.9 ± 12.5), TL varied between 49 and 86 mm (66.5 ± 14.4), and W ranged from 7 to 11 g (8.4 ± 1.5) (Table 4). All individuals had longer predorsal (PRL) than postdorsal (POL) area (37.3 ± 1.9% *versus* 25.2 ± 0.8%, respectively). The caudal peduncle was relatively thin in all individuals, making up around 13.9% of the SL. On the body, HL accounted for 33.3 ± 1.1% of SL (range 31.0–34.3). Body was higher (MBD: 29.5 ± 2.5%) than wider (BW: 21.6 ± 1.6%), while the lower jaw (LJL: 34.3 ± 3.5%) was longer than the upper jaw (UJL: 31.4 ± 6.3%) (Table 4). D1 was composed from 7.1 ± 0.8 branched rays, while D2, P, V and A consisted of a single unbranched ray and a variable number of branched rays (Table 4). In addition, the number of scales in the lateral line ranged 36.0–40.0 (38.3 ± 1.51), while in the transverse and circumpeduncular rows, the number of scales was lower (15.5 ± 1.3 and 9.5 ± 0.7 respectively).

Table 4. Body proportions of eight individuals (n = 8) of Chinese sleeper (*Perccottus glenii*) caught from Drava River oxbow (Croatia). The measurements and nomenclature were adapted from Hubbs and Lagler (1947) and Nikolić et al. (2021).

Morphometric variable	Acronym	*x*	SD	Range
Total length (mm)	TL	66.51	14.40	49.62–85.7
Standard length (mm)	SL	54.90	12.55	37.9–70.9
Body weight (g)	W	8.44	1.50	6.7–10.8
% of SL				
Head length	HL	33.31	1.14	31.04–34.34
Maximum body depth	MBD	29.53	2.57	25.6–32.86
Depth of caudal peduncle	CPD	12.72	0.92	11.24–13.96
Body width at dorsal fin origin	BW	21.68	1.69	19.38–23.7
Width of caudal peduncle at anal fin insertion	CPW	12.92	0.54	12.2–13.68
Predorsal length	PRL	37.38	1.90	34.45–40.06
Postdorsal length	POL	25.21	0.85	23.16–25.83
Prepelvic length	PRPL	35.89	3.82	29.9–40.9
Preanal length	PRAL	63.75	2.44	60.29–67.14
Pelvic to anal-fin origin distance	PAD	31.71	1.72	29.08–33.97
Length of caudal peduncle	CPL	41.07	2.19	38.77–44,5
Length of dorsal fin (D1)	D1L	17.49	0.67	16.15–18.18
Length of dorsal fin (D2)	D2L	20.25	2.40	16.75–23.13
Length of pectoral fin	PECL	18.66	4.36	11.72–24.12
Length of pelvic fin	PELL	11.83	1.59	9.33–13.82
Length of anal fin	AL	15.86	2.17	11.9–18.68
Length of base of anal fin	ABL	16.03	1.41	14.53–18.42
Length of base of D1 fin	D1BL	12.87	1.77	10.05–15.09
Length of base of D2 fin	D2BL	20.97	1.59	18.66–22.85
% of HL				
Snout length	SNL	16.86	0.59	16.1–17.71
Horizontal eye diameter	HED	20.67	1.16	19.23–22.06
Head depth at eye centre	HEDC	44.66	2.40	41.18–47.33
Head depth at nape	HDN	67.46	2.46	64.16–71.57
Head width at posterior margin of preopercle	HWPP	73.12	6.87	63.24–81.68
Postorbital length	POSTL	57.51	2.90	52.94–60.91
Upper jaw length	UJL	31.48	6.37	21.32–39.09
Lower jaw length	LJL	34.35	3.54	28.68–38.27
Interorbital width, the least fleshy width	IOW	11.09	4.07	4.41–16.05
Meristic character				
Total number of rays in first dorsal fin	D1	7.13	0.83	6–8
Total number of rays in second dorsal fin	D2	I11	1.07	I10–I13
Total number of rays in pectoral fin	P	I14.25	1.04	I13–I16
Total number of rays in ventral fin	V	I4.87	0.83	I4–I6
Total number of rays in anal fin	A	I10.25	0.71	I9–I11
Number of scales in the lateral row	*sl*	38.38	1.51	36–40
Number of scales in the transverse row	*st*	15.50	1.31	14–17
Number of scales in the circumpenducular row	*sc*	9.50	0.76	9–11

Discussion

The present study reports on the new occurrence of *P. glenii* from a shallow oxbow situated in the floodplain of the Drava River, and provides new data on the spread of this species in Croatian waters. This finding is the second known occurrence of *P. glenii* from Croatia (previously, a single individual of *P. glenii* was caught from the Sava River watershed) and in the scope of this study, it offers morphological details of the investigated population. More importantly, the oxbow is situated within a protected area and NATURA 2000 site, both characterized by the specific and endangered flora and fauna. In 2014, a single specimen of *P. glenii* was collected from the Keleti-Gyöngyös channel near Szigetvár (Hungary), which belongs to the Drava River watershed and is situated around 20 km aerial distance from the main river course (Takács et al. 2015), and about 62 km northwest from this site. This was the first record of *P. glenii* from the Drava River watershed, and was believed to have likely originated from a fishpond located upstream of the channel. Accordingly, the present study expands the current knowledge about the potential distribution range of *P. glenii* in the Drava River watershed.

Habitats similar to this oxbow, such as the freshwater (irrigation) channels, reservoirs, gravel pits and fishponds, have previously been proposed as suitable biotopes for *P. glenii* acclimatization and population establishment (Reshetnikov 2013). Our field observations suggest that this shallow oxbow, which is rich in aquatic vegetation with well-developed algal bloom, high (and fluctuating) water temperature and low visibility, could present a suitable habitat for the limnophilic *P. glenii*, and more importantly, could serve as a potential spawning ground for this invasive species. Small and shallow habitats, overgrown with dense aquatic vegetation and covered with ground material or debris are ideal spawning grounds for this litho/phytophilous species. It is noted that in *P. glenii,* spawning starts when the water temperature exceeds 15 °C, which stimulates females after spawning to start depositing eggs on the lower surface of submerged objects, such as plants, stones, roots, etc. (Miller 2003). Afterwards, the males provide parental care, aggressively guarding the nest and fanning the clutch with their pectoral fins (Bogutskaya and Naseka 2002; Miller 2003). Considering the above, this Drava River oxbow could serve as an ideal spawning ground for *P. glenii*.

Although limited (n = 8), our sample offers the opportunity to compare the morphometric measurements and meristic characters of this Croatian *P. glenii* population with the nearest neighbouring population from the Danube River channel near Veliko Gradište, Serbia (Nikolić et al. 2021). Specimens in Serbia were larger (both in TL and SL) in comparison with our specimens. In our case, the largest individuals attained a total body length (TL) of 87 mm, while the Serbian specimens achieved a TL of around 140 mm. Body depth (MBD) was greater (in % of SL) in our specimens compared to the Serbian specimens, possibly reflecting the habitat conditions, although our sample size could be too small to reveal an ecological pattern. In Croatia, *P. glenii* was sampled from a stagnant limnophilic waterbody, while in Serbia, this species inhabits the Danube River channel, where the moderate water current that leads to a more fusiform body form of Serbian specimens. However, additional laboratory and field data are necessary to support this hypothesis. Moreover, the width of the caudal peduncle at the anal fin insertion (CPW) was twice as large in Croatian than Serbian specimens (12% *versus* 6%, respectively).

Though the Croatian specimens achieved a shorter length, they had a larger caudal peduncle (CPL) compared to the Serbian specimens (41 *versus* 26 in % of SL). Finally, the snout from Croatian specimens was blunter, while the head was higher at the nape and wider at the posterior margin of preopercle in comparison with Serbian specimens (Table 2). Other morphometric traits (Table 4) were similar between the two populations, in term of % of SL, with only slight differences in the mean and range values. The meristic traits of Croatian specimens, such as number of unbranched and branched rays in the dorsal, pectoral, ventral and anal fins, were similar to Serbian specimens, with minor differences (Nikolić et al. 2021).

According to the sampled fish community, we can hypothesize that this oxbow (connected with the Drava River during floods) is a feeding ground for certain predatory fish (*L. aspius* and *E. lucius)*, and it serves as a breeding area for other limnophilic and phytophilous taxa (*R. rutilus, S. erythrophthalmus, C. carassius,* etc.). However, it is important to note that the majority of individuals from our sample were invasive and alien species, such as *C. gibelio*¸ *P. parva, L. gibbosus, A. melas* or *P. glenii* (> 90%), while the native species represented only up to 5% of collected individuals. It is known that alien (and invasive) fish species represent a great ecological stress and significant biological threat to the overall biodiversity, particularly to the native ichthyofauna and protected NATURA 2000 sites, such as this oxbow. It is hypothesized that *P. glenii* has a detrimental effect of the fish community and other animal groups (herpetofauna, insects, crustaceans, etc.). In most cases, *P. glenii* occurs in direct competition with native (and often protected) fish species. Several studies have indicated the adverse impacts of *P. glenii* on native fish fauna, especially *C. carassius*, *L. delineatus*, *R. amarus* or European mudminnow *Umbra krameri* Walbaum, 1792, mainly through egg and larvae predation or niche competition, resulting in the decline of *U. krameri* populations (Shlyapkin and Tikhonov 2001; Bogutskaya and Naseka 2002; Reshetnikov 2003; Reshetnikov and Petlina 2007; Koščo et al. 2008; Reshetnikov and Chibilev 2009; Grabowska et al. 2019). Some authors even suggest that the invasion of *P. glenii* could potentially hasten the extinction rate of the endangered *U. krameri*, specifically in small and isolated water bodies such as the sampled oxbow (Grabowska et al. 2019). However, in the most extreme scenarios, *P. glenii* can have an even greater negative impact on nature and related tropic structures, affecting the complete ecosystem and all of its components (fish, zoobenthos, primary producers), as suggested for the Ilgas Nature Reserve ecosystem in Latvia (Kutsokon et al. 2021). There, even though the fish community was never rich in species, *P. glenii* invaded the shallow marsh waterbodies and completely replaced *C. gibelio*, leaving behind a monospecific fish community represented by a single invasive fish (Kutsokon et al. 2021). In our situation, this is not yet the case, as the present oxbow (and its fish assemblage) was composed of 16 species, though the majority are alien species, including *P. glenii*. However, *P. glenii* was not as abundant (< 1 %) as other invasive species, such as *C. gibelio* or *P. parva*. From these observations, it can be assumed that *P. glenii* likely does not tolerate coexistence with other species, especially those with the similar life history traits (aggressive nest guarding and opportunistic feeding), such as *L. gibbosus* or *A. melas* (Kottelat and Freyhof 2007) or that it has not yet managed to establish a stable population. However, having in mind the case from Latvia, special emphasis should be given to the present finding, as the investigated oxbow lies within the NATURA 2000 protected area. In Croatia, and especially within the Mura-Drava Regional Park, populations of *C. carassius*, *L. delineatus* and *M. fossilis* are strictly protected species (Official Gazette of the Republic of Croatia 2016). Within the Mura-Drava protected area, the *U. krameri* forms several isolated populations in similar stagnant and well-vegetated waterbodies. We hypothesize that at higher densities, *P. glenii* could pose a serious threat to *U. krameri* populations in the Drava River watershed. Reshetnikov (2013) emphasized that after the initial introduction and establishment of a population within oxbows, downstream migrations during a flooding event and colonization of floodplain waters are evident throughout the invaded zone. *Perccottus glenii* is a poor swimmer and therefore inhabits the waterbodies which have weak currents or are overgrown with aquatic macrophytes (Bogutskaya and Naseka 2002). In addition, it is an invasive alien species that poses a significant concern in European Union because of its dramatic influence on the ecosystem, which is so significant that specific measures need to be implemented into the existing protocols with the purpose of its spatial eradication (European Community 2014).

The first sampling session in July 2020, when the *P. glenii* population was initially recorded from the oxbow, can be considered an early detection of this invasive alien species in the Drava River watershed. The second survey in April 2021 confirmed the presence of *P. glenii*. According to EU regulations (EU Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species), monitoring and surveillance of newly reported invasive species is imperative, and measures, such as specific target field survey and the inclusion of various stakeholders or sectors, should be taken to conduct rapid eradication to prevent their establishment and spread while the number of specimens is still limited, as in our case. Accordingly, monitoring and surveillance of the Drava River and adjoining floodplain habitats is recommended, especially downstream of the present collection site to confirm the possible presence of the additional populations within vulnerable and protected areas such as the Drava River flooding zone and the Mura-Drava Regional Park. Measures should also be implemented to eradicate all individuals of *P. glenii.* In addition, the cross-border cooperation is strongly recommended in this case, particularly with the Croatian neighboring countries from the same biogeographical region of the EU where the presence of the *P. glenii* has already been confirmed (such as Hungary and Serbia), with the mutual goal of the proper management and biomonitoring of this highly invasive fish species.

Recent studies have shown that rivers are common natural corridors for the dispersal of *P. glenii* (Harka et al. 2003; Hegediš et al. 2007; Reshetnikov and Petlina 2007; Reshetnikov and Chibilev 2009). Dynamics of spatio-temporal migrations of the West-Ukrainian subrange have confirmed this, where rivers such as Dniester, Western Bug, Vistula, Tisza, Siret, Prut and Danube have served as migration corridors and increased the invasive success of *P. glenii* in floodplain biotopes (Reshetnikov 2013). The exact origin of the *P. glenii* in the Drava River catchment in Croatia remains debatable. However, based on the previous findings, two hypotheses on the migration route can be proposed (Figure 2): North hypothesis – where individuals migrated southward from the Keleti-Gyöngyös channel (Hungary) and entered the Drava River directly via adjoining water channels and tributaries, or the South hypothesis – where individuals travelled upstream via the Danube River from Serbia and then entered the Drava River at its confluence. In the case of both hypotheses, we believe additional *P. glenii* findings can be expected downstream of the present record site, possibly within the protected Kopački Rit Nature Park. The South hypothesis could be supported by the first record of *P. glenii* in the Sava River, situated south of the present sampling site and connected with the Danube River. However, considering that the spread of *P. glenii* could be rapid from riverheads downstream, but slower when migrating upstream into adjoining waters (Reshetnikov 2013), this may support the North hypothesis, i.e., the present individuals stem from northern Hungarian location (Keleti-Gyöngyös channel); morphological or population genetic analysis could provide further insight. In terms of the size classes of collected individuals, our results are similar to those reported by Skorić et al. (2017), i.e., that the majority of *P. glenii* individuals collected from the Danube River channel, belonged to 50.0–60.0 mm size classes. Here several of the individuals collected were immature (< 55 mm SL; summer 2020 sample), while others can be considered mature (> 55 mm SL; spring 2021 sample) (Miller 2003).

In conclusion, it is reasonable to expect additional *P. glenii* individuals during future surveys within the Drava River flooding zone. As Nehring and Steinhof (2015) and Skorić et al. (2017) suggested, eradication or biocontrol of *P. glenii* populations may be possible if appropriate actions and measures are taken after first confirmation and early detection of this highly invasive species in the newly invaded habitat. While sustainable and practical solutions for management of *P. glenii* are still largely missing, good example of biological control measures in managing (eradication or suppression) the *P. glenii* population was proposed by Rakauskas et al. (2019). Authors carried out the biocontrol experiments by introducing the native piscivorous fishes (*Esox lucius* and *Perca fluviatilis*) into the invaded eutrophic lakes of eastern Lithuania, and the abundance of the *P. glenii* individuals was noted during the pre-stocking, transitional (stocking) and post-stocking events (Rakauskas et al. 2019). Four years of biocontrol implementation (i.e. repeated stocking of piscivorous taxa) led to a positive effect on the fish community observed in the progressive decline in *P. glenii* abundances, while the final outcome was the complete disappearance of usually abundant *P. glenii* from the investigated eutrophic lakes (except for Lake Cirkliškis where the population was strongly suppressed) (Rakauskas et al. 2019). These results suggested the effectiveness of piscivorous fish (such as *Esox lucius* and *Perca fluviatilis*) in controlling well-established *P. glenii* populations (Rakauskas et al. 2019). Therefore, detailed fish monitoring surveys are recommended in the coming years in floodplain areas such as the Drava River zone.

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Authors' contribution

S.H. and M.Ć. established the concept of this study; S.H., Z.M., R.K., D.Z. and M.Ć. defined the sample design and methodology; S.H., Z.M., R.K., D.Z. and M.Ć. performed the investigation and data collection; S.H., Z.M, P.M. and M.Ć. realized the data analysis and interpretation; D.Z. and M.Ć ensured the funding provision; S.H. and M.Ć. wrote the original draft; S.H., Z.M., R.K., D.Z., P.M., I.B., L.O., L.I. and M.Ć. performed writing – review and final editing.

Ethics and permits

All conducted field surveys involving the fish sampling were approved by the Croatian Ministry of Agriculture (permission no. 525-1311855-19-2).

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