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Parasitization of monkey goby, *Neogobius fluviatilis* (Pallas, 1814) (Actinopterygii: Gobiidae), at localities with different salinity levels

by

Yuriy Kvach[1,2,*], Markéta Ondračková[2], Teodora Trichkova[3], Oleksandra Drobiniak[1], Veniamin Zamorov[4], Pavel Jurajda[2]

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[1]*Institute of Marine Biology, National Academy of Sciences of Ukraine, Pushkinska 37, 65011 Odessa, Ukraine*

[2]*Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 60365 Brno, Czech Republic*

[3]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel Blvd., 1000 Sofia, Bulgaria*

[4]*Odessa I.I. Mechnikov National University, Department of Hydrobiology and General Ecology, Dvorianska Str. 2, 65082 Odessa, Ukraine*

* Corresponding author: *yuriy.kvach@gmail.com*

Abstract

Parasitism of monkey goby, *Neogobius fliviatilis*, was assessed at three brackish water localities with different salinity levels (Gulf of Odessa, Khadzibey Estuary, Lake Kytay) and one freshwater site along the Lower Danube River (Vidin). A total of 25 parasite taxa were identified, with minimum parasite richness recorded in the Khadzhibey Estuary (three species) and the maximum along the Lower Danube (11 species). Parasite richness in the mesohaline Gulf of Odessa and oligohaline Lake Kytay was lower, but still relatively high compared to the Khadzibey Estuary. Our study indicates that freshwater populations of monkey goby host richer and more abundant parasite communities than those inhabiting brackish waters with (more or less) stable salinity. Unstable abiotic conditions that probably affected the parasite's intermediate hosts contributed to the reduction in parasite species in the estuarine zone of the Danube.

Key words: Ponto-Caspian gobies, Danube River, Black Sea, human-impacted waterbodies, native range, parasites

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Introduction

The monkey goby, *Neogobius fluviatilis* (Pallas, 1814) (Actinopterygii: Gobiidae), is a Ponto-Caspian fish species with a natural range encompassing the coastal zones of the Black Sea and lower reaches of riverine drainages (Miller 1986). It is associated with a group of neo-limnetic species originating from brackish waters but has expanded its range into fresh waters (Kvach, Kutsokon 2017). The first record of its range expansion comes from 1914, when the species was found in the Middle Dnieper near Kiev (Beling 1914). In 1970, it was recorded in Lake Balaton in Hungary (Bíró 1971) and in 1977 along the Middle Danube in Serbia (Lenhardt et al. 2011). It is now considered an invasive species in a range of water bodies, including the lower Volga (Neilson, Stepien 2011), the Middle Danube River basin (Jurajda et al. 2005), the Vistula River basin (Kostrzewa et al. 2004), the Gulf of Gdańsk (Lejk et al. 2013) and the Rhine River basin (Borcherding et al. 2011).

The Danube basin forms part of the so-called Southern Aquatic Invasion Corridor, which connects the Black Sea with the North Sea (Panov et al. 2009). The original source of the Middle Danube monkey goby "population" is believed to be the Lower Danube and the delta system (Neilson, Stepien 2011). Other invasive gobiids in the Middle Danube, i.e. round goby, *Neogobius melanostomus* (Pallas, 1814) and bighead goby, *Ponticola kessleri* (Günther, 1861), with the same source of invasion, show high parasite and microsatellite diversity, probably resulting from multiple introductions (Ondračková et al. 2012). In comparison, the parasite community of monkey gobies from the Hron River (Middle Danube tributary), Slovakia (Ondračková et al. 2005) and the Danube River near Budapest, Hungary (Molnár 2006) consists primarily of local parasite species with limited abundance.

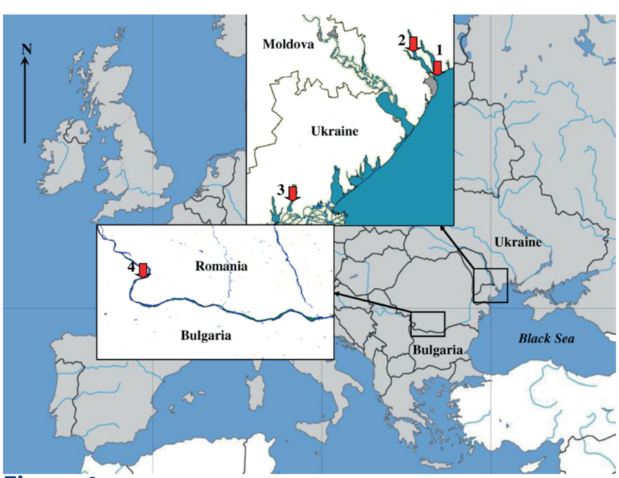

Figure 1

Map of the study area, with sampling localities marked by red arrows: 1) Gulf of Odessa, Black Sea, Ukraine; 2) Khadzibey Estuary, Ukraine; 3) Lake Kytay, Danube Delta, Ukraine; 4) Lower Danube, Vidin, Bulgaria

Ponto-Caspian gobiid parasite communities are strongly dependent on water salinity (Kvach 2004b,c). In riverine deltas and estuaries, for example, the parasite fauna consists of both marine and freshwater species (Kvach 2004c; Kvach et al. 2014), while neo-limnetic gobiids tend to be parasitized by local freshwater parasite fauna in freshwater biotopes (Ondračková et al. 2005; 2012; Kvach et al. 2014; Mierzejewska et al. 2014). In mesohaline and polyhaline lagoons, both marine and brackish water parasite species tend to form the core parasite fauna (Kvach 2004b; Krasnovyd et al. 2012), both showing an increased tendency to join the infracommunity (Kvach, Oğuz 2009).

The objective of the present study was to analyze the parasite community of monkey gobies along the Southern Invasion Corridor and adjacent waters within their native range.

Materials and methods

Fish were sampled at four localities differing in salinity (S): 1) the Gulf of Odessa, the Black Sea, Ukraine; 46.554167°N, 30.773720°E; April–October 2013, May 2014; N = 31; S = 10–17 PSU; brackish (salinity range data from Zaitsev 1992); 2) Khadzibey Estuary, Ukraine; 46.757105°N, 30.523497°E; June–July 2013; N = 37; S = 3–4 PSU; brackish (salinity range data from Kvach 2004a); 3) Lake Kytay, the Danube Delta, Ukraine; 45.596152°N, 29.215446°E; May 2010; N = 77; S = 1.6–6.5 PSU; brackish (salinity range data from Zamorov et al. 2014); and 4) the Lower Danube near the city of Vidin, Bulgaria; 43.976885°N, 22.872576°E; April, October 2006; N = 78; freshwater (Fig. 1). At the brackish water localities, salinity was measured at the time of sampling (data provided by the Hydrochemical Laboratory of the Institute of Marine Biology, NASU). The data set for the Lower Danube comprised data obtained from a previous study aimed at assessing the microsatellite and parasite diversity variation between native and non-native populations (Ondračková et al. 2012) and an additional sample from the same locality collected in a different season.

The fish were transported live in aerated containers to the laboratory, where they were sexed, measured for standard length (SL, mm; Table 1), then sacrificed and dissected within two days of capture

Table 1

Number of fish from different localities examined and their standard length (SL, mm)

	Gulf of Odessa	Khadzhibey Estuary	Lake Kytay	Lower Danube
Number	31	37	77	78
SL; mean ± SD	90 ± 27.5	58 ± 14.9	75 ± 20.7	66 ± 9.0
SL; min.–max	30–125	38–115	50–146	50–90

(Kvach et al. 2016). The fish were subjected to a full standardized parasitological dissection of fins, skin, gills, muscles and internal organs (Pritchard, Kruse 1982). Living unicellular parasites were studied using light microscopy. Myxozoan parasites were smeared on slides and mounted in gelatin gel as semi-permanent slides. Monogeneans were preserved in GAP (Glycerine-ammonium-picrate) and prepared as semi-permanent slides (Malmberg 1957). Digeneans, cestodes and nematodes were preserved in hot 4% formaldehyde (Cribb, Bray 2010), while digeneans and cestodes were then stained using iron acetic carmine, dehydrated in ethanol of increasing concentration and mounted in Canada balsam as permanent slides (Georgiev et al. 1986). Acanthocephalans were pressed between two slides and fixed in 70% ethanol. Preserved acanthocephalans and nematodes were mounted in glycerol as temporary slides for light microscopy. Glochidia and crustaceans were preserved in 4% formaldehyde and identified under a light microscope. All parasites were identified to the species level or to the highest possible taxa. Data for each species are presented as prevalence (P, %), mean intensity (MI) and mean abundance (A) (Bush et al. 1992). Infracommunity richness was assessed as the number of parasite species in each host, with mean infracommunity characterized as the mean number of parasite species per host (Zander 2004). The gradual scale of intensity proposed by Mierzejewska et al. (2012) was used for microparasites, i.e. S = sporadic, from 1 to < 10 individuals in the material examined; NN = not numerous, < 10 individuals in < 10% fields of vision; N = numerous, up to 20 individuals in > 50% fields of vision; VN = very numerous, > 20 individuals in > 50% fields of vision; and M = mass, dozens of individuals in each field of vision. The total number of xenomas was calculated for microsporidians.

Differences in fish SL, parasite abundance and infracommunity richness between the localities were tested by non-parametric Kruskal-Wallis ANOVA followed by post-hoc Mann-Whitney *U* pairwise tests. Bonferroni correction was applied for multiple testing and corrected *p*-values (Zar 1996). The Mann-Whitney *U* test was used for comparisons of parasite abundance/infracommunity richness between sexes. Associations between fish SL and parasite abundance were tested using Spearman correlation coefficients. No correlation tests were conducted for the Khadzibey Estuary due to the low parasite abundance. All statistical analyses were conducted using PAST software (Hammer et al. 2001).

Results

Fish from the Danube Delta and the lower reaches of the Danube differed significantly in SL (H = 34.9, $p < 0.001$). While fish from the Khadzhibey Estuary were significantly smaller than those from all other localities (all $p < 0.002$), fish from the Gulf of Odessa were significantly larger than those from Vidin ($p < 0.001$). Salinity levels at the brackish water localities ranged from S = 16 PSU in the Gulf of Odessa to S = 3 PSU in the Khadzibey Estuary and Lake Kytay.

A total of 25 parasite taxa were recorded (Table 2), with minimum parasite richness being recorded in the Khadzhibey Estuary (three species) and the maximum in the Lower Danube near Vidin (11 species). No species common to all sites were recorded. The monogenean *Gyrodactylus proterorhini* was found at three localities, while the nematode *Eustrongylides excisus* and microsporidian *Loma acerinae* were both found at two localities (Table 2).

The overall parasite abundance differed between localities (H = 105.5, $p < 0.001$), with significantly higher levels observed compared to the other freshwater sites on the Lower Danube (all $p < 0.001$). In addition, fish from Khadzibey had a significantly lower parasite abundance compared to those from Kytay ($p < 0.001$) and from the Gulf of Odessa ($p = 0.02$), parasite abundance at the Gulf of Odessa being comparable with that at Kytay. Differences in the infracommunity richness showed the same pattern (H = 119.2, $p < 0.001$), with the freshwater Lower Danube site exceeding all Danube Delta localities (all $p < 0.001$). Khadzibey had a significantly lower infracommunity richness than both Kytay ($p < 0.001$) and the Gulf of Odessa ($p = 0.02$), with no difference between Kytay and the Gulf of Odessa.

No difference in parasite infection was observed between male and female overall abundance, infracommunity richness or abundance of individual parasite species (all $p > 0.05$). Significant correlations with fish SL were observed for several parasite species.

Table 2

Parasite component communities of monkey goby (*Neogobius fluviatilis*) from four localities in its native range (n = number of fish studied, P = prevalence as %, MI = mean intensity with intensity range in brackets, A = mean abundance, S = sporadic infection, NN = not numerous infection, met = metacercariae, pl = plerocercoids, L3 = 3rd stage larvae, ca = cystacanths, gl = glochidia)

Parasite taxa	Gulf of Odessa (n = 31)			Khadzibey Estuary (n = 37)			Lake Kytay (n = 77)			Lower Danube (n = 78)		
	P	MI	A	P	MI	A	P	MI	A	P	MI	A
Ciliata												
Trichodina sp.	9.7	S										
Microspora												
Loma acerinae							7.8	4.3 (1–7)	0.3	61.5	124 (1–500)	76.1
Myxozoa												
Kudoa nova	19.4	NN										
Monogenea												
Gyrodactylus proterorhini	3.2	1.0 (1)	0.03	2.7	1.0 (1)	0.03				16.7	9.4 (1–48)	1.6
Cestoda												
Ligula pavlovskii pl							64.9	2.2 (1–10)	1.4			
Proteocephalus gobiorum							6.5	1.4 (1–3)	0.1			
Bothriocephalus scorpii pl	3.2	2.0 (2)	0.1									
Digenea												
Apophallus sp. met										28.2	2.4 (1–8)	0.7
Cryptocotyle concava met				2.7	3.0 (3)	0.1						
Diplostomum sp. met										20.5	1.2 (1–2)	0.2
Metagonimus sp. met										41.0	9.1 (1–51)	3.7
Nicolla skrjabini										25.6	16.7 (1–115)	4.3
Pygidiopsis genata met				5.4	1.0 (1)	0.1						
Nematoda												
Anguillicoloides crassus L3										2.6	1.0 (1)	0.03
Contracaecum rudolphii L3							1.3	1.0 (1)	0.01			
Dichelyne minutus	12.9	1.0 (1)	0.1									
Eustrongylides excisus L3							58.4	2.0 (1–7)	1.2	20.5	1.8 (1–5)	0.4
Hysterothylacium aduncum L3	3.2	1.0 (1)	0.03									
Raphidascaris acus L3							14.3	2.3 (1–7)	0.3			
Acanthocephala												
Pomphorhynchus laevis ca										51.3	3.9 (1–15)	2.0
Telosentis exiguus	6.4	2 (1–3)	0.1									
Crustacea												
Ergasilus sieboldi							2.6	1.5 (1–2)	0.04			
Mothocya epimerica	3.2	1.0 (1)	0.03									
Bivalvia												
Anodonta anatina gl										1.3	1.0 (1)	0.01
Pseudoanodonta complanata gl										16.7	3.6 (1–17)	0.6
Species richness	8			3			7			11		
Mean abundance ± SD	1.3 ± 2.8			0.2 ± 0.5			1.6 ± 2.1			13.5 ± 21.2		
Intensity of infection (min.–max)	1–12			1–3			1–9			1–121		

Trichodina sp. were negatively correlated with fish SL, infecting only smaller fish (r_s = −0.38, p = 0.03) in the Gulf of Odessa, while *L. acerinae* abundance increased with fish SL at both Lake Kytay (r_s = 0.36, p = 0.001) and the Lower Danube (r_s = 0.27, p = 0.016). The cestodes *Ligula pavlovskii* (r_s = 0.25, p = 0.027) and *Proteocephalus gobiorum* (r_s = 0.25, p = 0.030) and the nematode *E. excisus* (r_s = 0.29, p = 0.012) were positively correlated with fish SL in Lake Kytay. The digenean metacercariae *Apophallus* sp. (r_s = 0.29, p = 0.009) and *Metagonimus* sp. (r_s = 0.39, p < 0.001) and the acanthocephalan *Pomphorhynchus laevis* (r_s = 0.46, p = < 0.001) were positively correlated with fish SL along the Lower Danube.

Discussion

In this study, the richest and most abundant monkey goby parasite community was recorded at the freshwater locality in the Lower Danube, near the city of Vidin. High parasite diversity and abundance were also observed along this Danubian stretch for other Ponto-Caspian gobies, such as round goby, bighead goby and racer goby, *Babka gymnotrachelus* (Kessler, 1857) (Ondračková et al. 2006; 2012; Mühlegger et al. 2009; 2010; Francová et al. 2011). The high parasite species richness observed in the monkey goby appears to be common in freshwater localities, both in its native and invasive ranges. In the Vistula River, for example, where the species was first recorded in 2002 (Kostrzewa et al. 2004), and in river systems connecting the Vistula with the Dnieper, parasite species richness was always relatively high, with 14 species recorded throughout the Bugo-Narew sector (including the Zegrze Reservoir and the Lower Narew River), 16 species in the Włocławek Reservoir, 10 species along the Lower Vistula and 12 species in the Middle Dnieper (Kvach et al. 2014; Mierzejewska et al. 2014). Similarly, nine parasite species (though generally at low abundance) were recorded along the Lower Rhine by Ondračková et al. (2015), who expected a subsequent increase in species richness due to acquisition of local parasites.

The Lower Danube forms part of the Ponto-Caspian zoogeographic zone encompassing all lower reaches of rivers flowing into the Black Sea. The natural range of Ponto-Caspian gobiids up the Danube is limited by the Iron Gates Reservoir, located upstream of Vidin (Smirnov 1986). Three of the parasite species recorded in the Lower Danube, i.e. *G. proterorhini*, *Apophallus* sp. and *Nicolla skrjabini*, are related to this Ponto-Caspian group, and two trematodes have parthenogenetic stages that develop in the Ponto-Caspian gravel snail, *Lithoglyphus natocoides* (Pfeiffer, 1828) (Stenko 1976; Sándor et al. 2017). The high diversity of Ponto-Caspian neo-limnetic fauna in the Lower Danube means that the monkey goby is exposed to a larger number of parasite species compared to the Black Sea. As the monkey goby prefers waters with salinity lower than 13 PSU (Smirnov 1986), it is only found in small numbers in the Gulf of Odessa (Kvach 2015). On the other hand, the species can be found in relatively large numbers in estuarine zones and the lower reaches of rivers (neo-limnetic). Populations in mesohaline waters are probably dependent on migration from rivers, hence their parasite community tends to include freshwater parasites, even in typical Black Sea biotopes, though at low abundance. In the mesohaline Sukhyi Estuary, for example, only *Diplostomum* sp. were recorded (Krasnovyd et al. 2012). In the Gulf of Odessa, freshwater parasites were absent, probably due to the lack of river inflows at nearby sites.

Parasite community structure in fish from the Danube Delta showed clear differences compared to that from the freshwater site. In the Gulf of Odessa (mesohaline) and Lake Kytay, parasite richness was lower but still relatively high compared to the oligohaline Khadzibey Estuary (see Table 2). The parasite community from the Gulf of Odessa comprised mainly marine (*Kudoa nova*, *Bothriocephalus scorpii*, *Hysterothylacium aduncum*, *Telosentis exiguus*, *Mothocya epimerica*) and brackish water (*Dichelyne minutus*) species, absent at the other localities, while only fresh/brackish water parasites occurred in fish from the oligohaline Khadzibey Estuary and Lake Kytay.

In this study, an extremely low parasite load (three species with mean abundance < 0.2) was recorded in the Khadzibey Estuary, which corresponds with the results of the previous study (Kvach 2004a). Compared with the previous data, all parasite species observed in this study were new to the locality. Kvach (2004a) reported that changes in gobiid parasite fauna in the Khadzibey Estuary were directly due to the unstable salinity levels found within the estuary. In the present study, we found two brackish-water digenean species, *Cryptocotyle concava* and *Pygydiopsis genata*, as well as the fresh/brackish-water Ponto-Caspian monogenean *G. proterorhini* in the estuary, all at low abundance. Fish SL was the lowest from all localities, possibly contributing to the low parasite load found. Although Lake Kytay also exhibits unstable salinity, the parasite community was richer, comprising seven species with a mean abundance of 1.6. At the same locality, we also failed to record trematodes, probably due to the absence of mollusks, its intermediate host. A similar situation was described for the Khadzhibey Estuary in 2000–2001, when the absence of mollusks was also considered to be the reason for the absence of trematodes in the parasite community of gobiids (Kvach 2004a). Both the Khadzhibey Estuary and Lake Kytay are human-regulated water bodies, used as water reservoirs, and this may be one of the main reasons for the unstable salinity conditions and consequent absence and/or low abundance of mollusks, the digenean's intermediate host.

Apart from the cestode *L. pavlovskii* and the nematode *E. excisus* in Lake Kytay, and the microsporidian *L. acerinae* and the acanthocephalan *P. laevis* along the Lower Danube, most parasite species occurred at low prevalence (< 50%) and abundance in this study. *L. pavlovskii* is a typical parasite of lentic reservoirs with high levels of biogenic pollution (Kvach 2001; 2004; Yuryshynets et al. 2017), and is transmitted to the fish host by planktonic crustaceans. The presence of *E. excisus* larvae is common in the estuarine zones of large rivers, especially where cormorant colonies (the definitive host) are located (Morgun 2012) or at sites with a high density of *Tubifex* oligochaetes, the nematode's first intermediate host (Moravec 2013). The acanthocephalan *P. laevis* is one of the most common intestinal parasites of gobies, both in their native and non-native range (e.g. Ondračková et al. 2010, 2015; Francová et al. 2011; Mierzejewska et al. 2014), and is usually transmitted to gobies via ingestion of amphipod crustaceans, which act as intermediate hosts. All three species mentioned, together with the passively transmitted cestode *P. gobiorum*, increased in abundance with fish SL, most probably as a result of increased food intake in larger individuals.

Loma acerianae was originally described from the European ruffe, *Gymnocephalus cernuus* (L., 1758), but is also commonly found in gobiids and in the sand smelt, *Atherina boyeri* Risso, 1810, mainly from freshwater or desalinated regions (Ovcharenko et al. 2017). None of the microsporidian parasites have active stages outside their host cells and, as such, can only survive by living inside other cells (Franzen 2008). Microsporidians infecting fish have predominantly direct life cycles that involve horizontal transmission and severe pathology (Dunn, Smith 2001). Spores of *Loma* spp., released from xenomas, may be released directly into the external environment and become infectious only when ingested (Shaw, Kent 1999). Autoinfection as well as transmission between the same host species are common for *Loma* microsporidians, hence infestation of monkey goby with microsporidians is higher at the localities with denser populations.

Our study has shown that parasite communities of freshwater monkey goby populations are more abundant and species rich than populations inhabiting brackish waters with more or less stable salinity. The composition of the monkey goby's parasite community, which is similar to that of other sympatric Ponto-Caspian gobies, reflects that of the established parasite population in the freshwater part of the Danube (Ondračková et al. 2012). On the other hand, unstable abiotic conditions in the Danube estuarine zone probably contribute to the reduction in parasite species by creating unsuitable conditions for the parasite's intermediate hosts (Kvach 2004a).

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