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Parasites of non-native gobies in the Włocławek Reservoir on the lower Vistula River, first comprehensive study in Poland

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ABSTRACT

Key-words: *alien species, goby fish, parasite community, Vistula River*

A parasitological study of Ponto-Caspian gobies, including the monkey goby *Neogobius fluviatilis*, racer goby *Babka gymnotrachelus* and tubenose goby *Proterorhinus semilunaris*, carried out over four years in the lower Vistula River is described. These fish species represent one of the most impressive invasions of European inland waters, connected with the spontaneous, east-to-west intracontinental movement observed in the last two decades. The parasite community consisted of 24 taxa. Typical for racer goby were: *Trichodina domerguei*, *Diplostomum pseudospathaceum*, *Gyrodactylus proterorhini* and glochidia of unionids. The list of parasites typical for monkey goby is supplemented with: *Tylodelphys clavata* (met.) and *Eimeria* sp., while the glochidia were rarely detected in this fish host. *Holostephanus* spp., *Apatemon gracilis*, *Diplostomum gobiorum* and glochidia predominated in parasite fauna of tubenose goby. Unlike the other species tested, *P. semilunaris* was poorly infected with *T. domerguei* and *G. proterorhini*. Parasites commonly distributed through native fishes in the observed area prevailed in gobies. Species rare in natives were also numerously represented. In this way, alien fish reinforce populations of those parasites in invaded waters. Parasites dragged to the colonized area (*G. proterorhini*, *Holostephanus* spp., *A. gracilis* and *D. gobiorum*) complement the community. Larval stages were typical for parasite fauna of all studied gobies.

RÉSUMÉ

Parasites des gobies non indigènes dans le réservoir Włocławek sur la partie inférieure du fleuve Vistule, première étude exhaustive en Pologne

Mots-clés : *espèces*

L'étude parasitologique des gobies Ponto-Caspiens, comprenant le gobie fluviatile *Neogobius fluviatilis*, le gobie coureur *Babka gymnotrachelus* et le gobie demie-lune *Proterorhinus semilunaris* réalisée sur quatre ans dans le cours inférieur du

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fleuve Vistule est présentée. Ces espèces de poissons représentent l'une des invasions les plus impressionnantes dans les eaux intérieures européennes, avec un déplacement spontané intracontinental d'est en ouest observé dans les deux dernières décennies. La communauté des parasites est composée de 24 taxons. Typiquement pour le gobie coureur on trouve : *Trichodina domerguei*, *Diplostomum pseudospathaceum*, *Gyrodactylus proterorhini* et des glochidiums d'unios. La liste des parasites typiques du gobie fluviatile est complétée par : *Tylodelphys clavata* (met.) et *Eimeria sp*, tandis que les glochidiums étaient rarement détectés dans ce poisson – hôte. *Holostephanus spp., Apatemon gracilis, Diplostomum gobiorum* et les glochidiums prédominaient dans la faune parasitaire du gobie demie-lune. Contrairement aux autres espèces testées, *P. semilunaris* a été peu infecté par *T. domerguei* et *G. proterorhini*. Les parasites généralement rencontrés chez les poissons indigènes dans la zone observée sont présents dans les gobies. Les espèces rares dans les poissons indigènes sont également abondantes. De cette façon, les poissons exotiques renforcent les populations de ces parasites dans les eaux envahies. Les parasites apportés à la région colonisée (*G. proterorhini, Holostephanus spp., A. gracilis* et *D. gobiorum*) complémentent la communauté. Les stades larvaires étaient typiques de la faune de parasites de toutes les espèces de gobies étudiées.

INTRODUCTION

Initial reports on Ponto-Caspian gobies in Polish inland waters date back to 1995. They concern the racer goby *Babka gymnotrachelus* (formerly *Neogobius*, see Neilson and Stepien 2009) caught in the Bug River (the Vistula basin) (Kostrzewa and Grabowski, 2001). The monkey goby *Neogobius fluviatilis* joined the first goby fish in 1997 (Kostrzewa and Grabowski, 2003; Grabowska *et al.*, 2010). The tubenose goby *P. semilunaris* (formerly *P*. *marmoratus*) (Stepien and Tumeo, 2006) was caught for the first time in Poland in the lower Vistula River course in 2008 (Grabowska *et al.*, 2008). These species migrate to Poland from the Black Sea basin by the Dnieper River, *via* the Pripyat-Bug Canal to the Vistula, through the so-called central corridor of transEuropean migration of Ponto-Caspian hydrobionts (Semenchenko i in. 2011). A new potential source of these fish supporting populations in Poland is in Germany, due to the rapid expansion of Gobiids in the Rhine (Roche *et al.*, 2013). The importance of parasites in the host expansion and their impact on the final result of the competition between native and alien species (Torchin *et al.*, 2003; Torchin and Mitchell, 2004) as well as the lack of complete data on parasites of newly-introduced gobies in Poland prompted us to undertake a comprehensive study of these fish species in the lower Vistula.

MATERIAL AND METHODS

The Włocławek Reservoir is located in the lower flow of the Vistula River (in Central Poland) between the dam in Włocławek and a backwater near Wykowo (Fig. 1). The racer goby reached the Reservoir in 2000, the monkey goby in 2002 (Kostrzewa *el al.* 2004; Grabowska *et al.* 2010) and the tubenose goby joined them in 2008 (Grabowska *et al.* 2008). Gobies were sampled in the middle and upper parts of the reservoir: the racer goby was mainly caught with traps, distributed at the bottom along the cross-profile Dobiegniewo (52.626916N, 19.318943E) - Dobrzyń (52.626603N, 19.321003E), the monkey goby by electrofishing (IUP-12) or with nets in shallow ($<$1 m) and sandy coastal zones near the Dobiegniewo locality, and the tubenose goby by the same fishing gears as previous species, the majority of individuals in the shallow bay covered with macrophytes in the area of Wola Brwileńska (52.556357N, 19.557721E) (Fig. 1). Fish samples were collected seasonally (spring, summer and fall) from May 2006 to July 2010 (Fig. 2). A total of 318 fish were examined; all of them were measured, weighed and analyzed by necropsy (up to 6 hours after

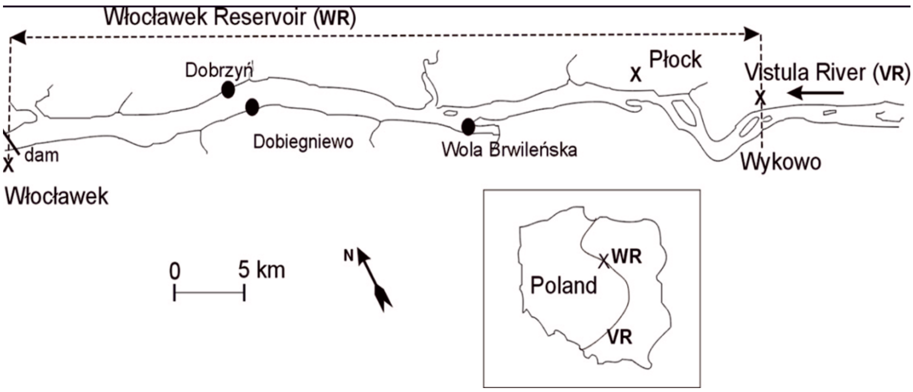

Figure 1
Sampling sites (•) of Gobiidae in the Włocławek Reservoir (WR) and a map of Poland, with the Vistula River (VR) position shown.

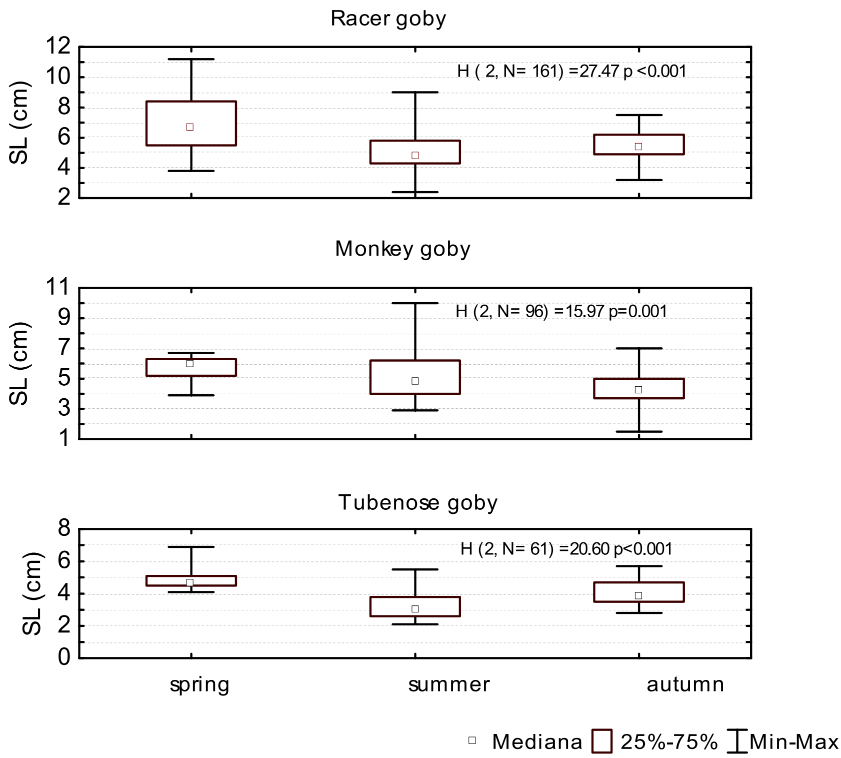

Figure 2
Comparison by the Kruskal-Wallis test of the fish length (SL) in seasons. Significant differences ($p < 0.05$) were detected in all studied species of gobies.

capture, retained alive in aerated tanks). The total length (TL) of racer, monkey and tubenose gobies ranged between 2.8–13, 2 cm, 2.0–11.5 cm and 2.5–8.1 cm, respectively (Fig. 2).

Standard methods of parasite detection were used (Lom 1958; Bylund *et al.*, 1979; Berland 1984; Georgiev *et al.*, 1986). Crash preparations were examined either with a compound light microscope (ECLIPSE E600W; Nikon) or a stereomicroscope (SMZ-UDIA STAND; Nikon). Prevalence (percentage of infected fish), intensity of infection (number of parasites per infected fish), mean values and ranges, and abundance (mean number of parasites per fish studied) (Złotorzycka *et al.*, 1998) were recorded for each parasite species. For uncountable parasites, a gradual scale of intensity (occurrence) was adopted: (1) sporadic, from 1 to <10 individuals in the examined material, (2) not numerous, <10 individuals in <10% fields of vision, (3) numerous, up to 20 individuals in >50% fields of vision, (4) very numerous,

>20 individuals in >50% fields of vision, and (5) mass, dozens of individuals in each field of vision (Mierzejewska *et al.*, 2012).
Parasites were divided into dominant, satellite and intermediate, according to Kennedy (1993), when the prevalence exceeds 20.0%, is in the range 10.0–19.9%, or is less than 10%, respectively.

STATISTICS

Differences in the infection level, as well as in the fish size (expressed by total length - TL, standard length – SL and Weight – W) in seasonal samples were examined with the Kruskal-Wallis test. Spearman's correlation coefficient was used to analyze the association between fish size and infection (intensity and abundance), as well as between infection with core species and the number of taxa in infracommunities. The level of significance accepted for all tests was $\alpha = 0.05$.

RESULTS

> *PARASITE COMMUNITY OF THE RACER GOBY*

Throughout the study period, at least 19 different parasites (excluding juvenile eyeflukes) were detected in the racer goby: 1 ciliate, 1 coccidian, 1 cestode, 1 monogenean, 9 digeneans (8 as metacercariae and *Bucephalus polymorphus* (Bucephalidae) both as metacercariae and as adults), 2 nematodes, and glochidia of 3 different species (Table I). The ciliate *Trichodina domerguei* (Ciliophora, Peritricha), the eyefluke *Diplostomum pseudospathaceum* (Diplostomidae), encysted larvae of *Holostephanus luehei* (Cyathocotylidae) and glochidia of Unionidae (three species considered together but with a clear predomination of *U. tumidus*) were ranked by core species with an overall prevalence of up to 31.7%, 37.9%, 19.9% and 21.7%, respectively (Table I). *Gyrodactylus proterorhini* (Gyrodactylidae), with a prevalence of 9.9%, was the intermediate species, while the remaining parasites were included in the satellite group (Table I). *Apatemon gracilis* (Strigeidae) and larvae of *Eustrongylides* spp. (Dioctophymatidae) in racer goby have been regularly detected since summer 2009.

> *PARASITE COMMUNITY OF THE MONKEY GOBY*

Parasitofauna of the monkey goby consisted of 16 different taxa: 4 protozoans, 1 cestode, 1 monogenean, 8 digeneans (7 as metacercariae and *Bucephalus polymorphus* as an adult), 1 nematode, and 1 species of glochidia (Table II). The ciliate *T. domerguei*, coccidia of the genus *Eimeria* sp. (Apicomplexa, Coccidiomorpha), the eyefluke *D. pseudospathaceum* and metacercariae of *H. luehei* were included in the core species group with an overall prevalence of up to 75.6%, 23.3%, 38.5% and 29.2%, respectively (Table II). *G. proterorhini* was the intermediate species (prevalence 10.5%); other parasites occurring rarely and in small numbers were included as satellite species (Table II). *A. gracilis* and larvae of *Eustrongylides* sp. were not detected in the monkey goby before 2008.

> *PARASITE COMMUNITY OF THE TUBENOSE GOBY*

In the tubenose goby, 17 different parasites (excluding juvenile eyeflukes) were noted, including 2 protozoans, 1 monogenean, 9 digeneans (8 as metacercariae), 1 nematode, 1 acanthocephalan and glochidia of 3 species (Table III). Metacercariae of *H. luehei*, *A. gracilis* and glochidia of *Unio tumidus* predominated in the community (core species) with an overall prevalence of 63.9%, 60.7% and 24.6%, respectively (Table III). The eyefluke *Diplostomum*

Table I

*Species diversity of parasites of racer goby in the Włocławek Reservoir (n = 161). P – prevalence [%], MI – mean intensity, A – abundance, (met.) – metacercariae, (gloch.) – glochidia, *species considered together due to uncertain identification in a few cases, †Juvenile forms.*

Parasites	*P* (%)	MI ± SD/Range	A	Location
Ciliophora				
1. *Trichodina domerguei* Wallengren, 1897	31.7	-/ S-VN	-	gills, skin
2. *Eimeria* sp.	1.2	-/S-N	-	intestine
Cestoda				
3. Caryophyllidae gen. sp.	0.6	1.0/1	0.01	intestine
Monogenea				
4. *Gyrodactylus proterorhini* Ergens, 1967	9.9	2.0 ± 1.4/1–6	0.20	skin, gills
Digenea				
5. *Bucephalus polymorphus* Baer, 1827 (met.)	1.9	4.7 ± 3.9/1–10	0.09	skin (encysted),
B. polymorphus				intestine
6. *Diplostomum paracaudum* (Iles, 1959) (met.)	6.3	11.8 ± 21.3/1–60*	4.5	lens
7. *Diplostomum pseudospathaceum* (Niewiadomska, 1984) (met.)	37.9			lens
8. *Diplostomum* sp. (met.)†	9.3	9.1 ± 14.9/1–52	0.9	lens
9. *Tylodelphys clavata* (Nordmann, 1832) (met.)	18.6	7.8 ± 9.7/1–37	1.4	
10. *Holostephanus luehei* Szidat, 1936 (met.)	19.9	3.6 ± 4.3 /1–22	0.7	muscles (encysted)
11. *H. cobitidis* Opravilova, 1968 (met.)				
12. *Ichthyocotylurus variegatus* (Creplin, 1825) (met.)	0.6	1.0/1	0.01	abdominal cavity (encysted)
13. *Apatemon gracilis* (Rudolphi, 1819) (met.)	1.2	1.0 ± 0.0/1–1	0.01	abdominal cavity (encysted)
14. *Echinochasmus spinosus* Odhner, 1911 (met.)	0.6	6.0/6	0.04	gills (encysted)
15. Digenea gen. sp. (met.)	0.6	1.0/1	0.01	gills (encysted)
Nematoda				
16. *Eustrongylides excisus* Jägerskiöld, 1909 (larvae)	3.1	1.0 /1–1*	0.03	mesentery, internal organs
17. *E. tubifex* (Nitzsch,1819) (larvae)				(encapsulated or free)
Mollusca				
18. *Anodonta anatina* (L.) (gloch.)				
19. *Unio pictorum* L. (gloch.)	21.7*	10.2± 16.7/1–94*	2.2	skin, gills, fins
20. *U. tumidus* (Philipsson) (gloch.)				

Table II
*Species diversity of parasites of monkey goby in the Włocławek Reservoir (n = 96). P – prevalence [%], MI – mean intensity, A – abundance, (met.) – metacercariae, (gloch.) – glochidia, *species considered together due to uncertain identification in a few cases.*

Parasites	*P* (%)	MI ± SD/Range	A	Location
Ciliophora				
1. *Chilodonella* sp.	1.0	-/L	-	gills,
2. *Trichodina domerguei* Wallengren, 1897	75.6	-/ S-M	-	gills, skin
3. *Eimeria* sp.	23.3	-/ S-M	-	intestine
4. *Apiosoma* sp.	3.1	-/NL-L	-	gills, fins
Cestoda				
5. Caryophyllidae gen. sp.	3.5	1.0 ± 0.0/1–1	0.03	intestine
Monogenea				
6. *Gyrodactylus proterorhini* Ergens, 1967	10.5	2.6 ± 2.6/1–9	0.27	gills, skin
Digenea				
7. *Bucephalus polymorphus* Baer, 1827	1.0	2.0/2	0.02	intenstine
8. *Diplostomum paracaudum* (Iles, 1959) (met.)	6.3	5.2 ± 7.5 /1–39*	2.00	lens
9. *Diplostomum pseudospathaceum* (Niewiadomska, 1984) (met.)	38.5			lens
10. *Tylodelphys clavata* (Nordmann, 1832) (met.)	18.8	5.3 ± 7.6/1–35	1.00	lens
11. *Holostephanus luehei* Szidat, 1936 (met.)	29.2	2.3 ± 2.3/1–13	0.7	muscles (encysted)
12. *Apatemon gracilis* (Rudolphi, 1819) (met.)	2.1	1.0 ± 0.0 /1–1	0.02	abdominal cavity (encysted)
13. *Echinochasmus spinosus* Odhner, 1911 (met.)	2.1	3.0 ± 2.8/1–5	0.06	gills (encysted)
14. Digenea gen. sp.	3.1	1.7 ± 0.6/1–2	0.05	skin, gills (encysted)
Nematoda				
15. *Eustrongylides* sp. (larvae)	2.1	1.0 ± 0.0 /1–1	0.02	mesentery (encapsulated)
Mollusca				
16. *Unio tumidus* Philipsson (gloch.)	1.0	5.0/5	0.05	gills

Table III
*Species diversity of parasites of tubenose goby in the Włocławek Reservoir (n = 61). P – prevalence [%], MI – mean intensity, A – abundance, (met.) – metacercariae, (gloch.) – glochidia, *species considered together due to uncertain identification in a few cases, †Juvenile forms.*

Parasites	*P* (%)	MI ± SD/Range	A	Location
Ciliophora				
1. *Trichodina domerguei* Wallengren, 1897	1.6	-/NL	-	skin
2. *Apiosoma* sp.	3.3	-/NL	-	gills, fins
Monogenea				
3. *Gyrodactylus proterorhini* Ergens, 1967	1.6	1.0/1	0.02	gills
Digenea				
4. *Diplostomum paracaudum* (Iles, 1959) (met.)	4.9	1.3 ± 0.6/1–2	0.07	lens
5. *Diplostomum gobiorum* Shigin, 1965 (met.)	18.0	3.5 ± 7.2/1–25	0.62	lens
6. *Diplostomum* sp. (met.)†	8.2	12 ± 11.8/3–30	1.0	lens
7. *Ichthyocotylurus variegatus* Odening 1969 (met.)	11.5	1.7 ± 6.4/1–4	0.20	abdominal cavity (encysted)
8. *Ichthyocotylurus erraticus* Odening 1969 (met.)	1.6	1.0/1	0.02	abdominal cavity (encysted)
9. *Holostephanus luehei* Szidat, 1936 (met.)	63.9	7.4 ± 6.4/1–27	4.7	muscles (encysted)
10. *Apatemon gracilis* (Rudolphi, 1819) (met.)	60.7	7.9 ± 11.5/1–50	4.8	abdominal cavity (encysted)
11. *Echinochasmus spinosus* Odhner, 1911 (met.)	6.6	2.5 ± 3.0/1–7	0.16	gills (encysted)
12. Digenea gen. sp.	1.6	1.0/1	0.01	intestine
13. Digenea gen. sp. (met.)	4.9	1.0 ± 0.0/1–1	0.05	muscles (encysted)
Nematoda				
14. *Eustrongylides* sp. (larvae)	8.2	1.6 ± 1.3/1–4	0.13	mezentery (encapsulated) abdominal cavity (free)
Acanthocephala				
15. *Pomphorhynchus laevis* (Müller, 1776)	0.2	2.0/2	0.03	intestine
Mollusca				
16. *Pseudoanodonta complanata* (Rossm) (gloch.)	4.9	1.0 ± 0.0/1–1	0.05	skin, fins
17. *Unio pictorum* L. (gloch.)	9.8	3.2 ± 2.6/1–7	0.30	gills
18. *U. tumidus* (Philipsson) (gloch.)	24.6	8.3 ± 16.7/1–66	2.03	gills, fins

gobiorum (Diplostomidae), with a prevalence of 18.0%, was classified as an intermediate species and the remaining parasites were satellite (Table III). *A. gracilis* and *D. gobiorum* were found for the first time during this study in samples of tubenose goby (in 2008).

> SEASONALITY

The fish examined in particular seasons differed significantly in size. A Kruskal-Wallis test for standard length (SL) confirmed such differences at $p \leqslant 0.001$ for all studied species of gobies (Fig. 2). Generally, the larger fish prevailed in spring, contrary to summer and/or fall samples (Fig. 2).

In racer goby, significant seasonal differences were noted in the abundance and/or intensity of infection with *T. domerguei*, *G. proterorhini*, *T. clavata*, *H. luehei* and glochidia of unionids. These parasites occurred most numerously in spring, except *H. luehei*, which was most abundant in summer (Table IV). The prevalence of these parasites varied seasonally from the lowest values in fall (respectively, for parasites: 13.9%, 0.0%, 10.3%, 7.6% and 1.3%) to the highest values in spring or summer (53.1%, 24.2%, 29.2%, 40.8% and 51.5%) (Table IV).

The prevalence of *Eimeria* sp., *T. domerguei*, *G. proterorhini*, *Diplostomum* spp. and *H. luehei* in monkey goby ranged in seasons between 2.6–38.5%, 60.5–84.2%, 5.3–15.8%, 21.1–57.9% and 0.0–36.8%, respectively (Table IV). Significant differences in the abundance or/and intensity of infection in seasons with those parasites were confirmed by a Kruskal-Wallis test; *Eimeria* and *H. luehei* appeared most numerously in fall and the remaining species in spring or summer (Table IV).

In tubenose goby, the metacercariae of *H. luehei* were most abundant in the fall and the glochidia of unionids were most abundant in the summer. The prevalence of both parasites ranged in seasons from 51.7% to 89.41% and from 0.0% to 61.5%, respectively (Table IV).

> INFECTIONS RELATED TO THE FISH SIZE

Infection with *G. proterorhini* as well as with glochidia positively correlated with the standard length of the racer goby (Spearman's correlation coefficient, respectively, for parasites: $R = 0.22$ and $R = 0.20$, both at $p < 0.05$). Infection with *T. domerguei*, *T. clavata* and *Diplostomum* spp. increased together with the standard length of monkey goby (respectively, for parasites: $R = 0.42$, $R = 0.27$ and $R = 0.41$, at $p < 0.05$) in contrast to an infection with *H. luehei*, which decreased with the fish body length ($R = -0.23$, $p < 0.05$). Infection of tubenose goby with *Apatemon gracilis* correlated positively with the fish size ($R = 0.37$, $p < 0.05$).

DISCUSSION

The parasite assemblage of Ponto-Caspian gobies in the Włocławek Reservoir was relatively highly diversified. In the three fish species under study, 24 taxa of parasites were identified, twice as many as in three species of gobies in the Danube (Ondračková *et al.*, 2005), for instance. Most likely it is an effect of extending the research period to cover several years as well as different seasons. Typical for racer goby in the Włocławek Reservoir were the ciliate *Trichodina domerguei*, the eyefluke *Diplostomum pseudospathaceum*, metacercariae of *Holostephanus luehei*, glochidia of unionids (dominants) and the monogenean *Gyrodactylus proterorhini* (intermediate species). In comparison with the racer goby, the list of typical parasites for monkey goby is supplemented with *Eimeria* sp. and *Tylodelphys clavata*, while glochidia (common in *N. gymnotrachelus*) were rare in this fish host. In the tubenose goby (contrary to racer and monkey gobies), *T. domerguei* and *G. proterorhini* were not commonly detected. Apart from *H. luehei* (met.) and glochidia, metacercariae of *Diplostomum gobiorum* and *Apatemon gracilis* should be regarded as typical parasites for this fish host in the studied place. The last two parasites were never detected in the Włocławek Reservoir before

Table IV

*Significant differences of the gobies infection with the parasites in seasons. AD – difference in the parasite abundance, ID – difference in the intensity of infection. P – prevalence [%], MI – mean intensity, n – number of the examined fish, * uncountable parasite, S-sporadic occurrence, NN – not-numerous, N-numerous, VN-very numerous and M-mass occurrences.*

The fish parasites	Kruskal-Wallis test AD or ID	Spring			Summer			Autumn		
		P = (%)/A	MI ± SD	Range	*P* (%)/A	MI ± SD	Range	*P* (%)/A	MI ± SD	Range
Racer goby		*n* = 33			*n* = 49			*n* = 79		
Trichodina domerguei	$H = 21.53$ $p < 0.001$ for AD $H = 10.73$ $p = 0.005$ for ID	42.4 / -*	-*	NN-VN	53.1/-*	-*	S-VN	13.9/-*	-*	S-VN
Gyrodactylus proterorhini	$H = 20.16$ $p < 0.001$ for AD	24.2/0.61	2.5 ± 1.77	1–6	8.2/0.10	1.25 ± 0.50	1–2	0.0/0.0	0.0	
Tylodelphys clavata	$H = 8.77$ $p = 0.013$ for AD	24.2/2.42	10.0 ± 11.74	1–37	29.2/2.13	7.29 ± 10.37	1–35	10.3/0.65	6.38 ± 6.70	1–19
Glochidia Unionidae	$H = 42.39$ $p < 0.001$ for AD	51.5/8.39	16.29 ± 22.71	1–94	34.7/1.92	5.53 ± 5.04	1–15	1.3/0.04	3.00 ± 0.00	0
Monkey goby		*n* = 19			*n* = 38			*n* = 39		
Trichodina domerguei	$H = 8.05$ $p = 0.018$ for AD $H = 10.15$ $p = 0.006$ for ID	84.2/-*	-*	NN-M	60.5/-*	-*	NN-M	66.7/-*	-*	S-VN
***Eimeria* sp.**	$H = 4.49$ $p < 0.001$ for AD	21.1/-*	-*	N-M	2.6/-*	-*	N	38.5/-*	-*	
Gyrodactylus proterorhini	$H = 6.264$ $p = 0.044$ for ID	15.8/0.37	2.33 ± 0.58	2–3	5.3/0.32	6.00 ± 4.24	3–9	10.3/0.13	1.25 ± 0.5	1–2
***Diplostomum* spp.**	$H = 13.24$ $p = 0.001$ for AD $H = 6.26$ $p = 0.044$ for ID	21.1 / 0.26	1.25 ± 6.50	1–2	57.9/2.95	5.09 ± 5.00	1–22	28.2/0.82	2.91 ± 1.55	1–5
Holostephanus luehei	$H = 9.39$ $p = 0.009$ for AD	0.0/0.00	0.0	0	36.8/0.58	1.57 ± 1.09	1–5	35.9/1.08	1.08	1–13
Tubenose goby		*n* = 13			*n* = 29			*n* = 19		
Holostephanus luehei	$H = 18.57$ $p < 0.001$ for AD $H = 12.96$ $p = 0.002$ for ID	61.5/2.38	3.88 ± 2.30	1–7	51.7/2.31	4.47 ± 3.65	1–16	89.47/10.11	11.29 ± 7.36	2–27
Glochidia Unionidae	$H = 14.39$ $p < 0.001$ for AD	61.5/2.00	3.25 ± 2.82	1–8	24.1/3.38	14.00 ± 12.62	1–66	0.0/0.0	0.0	0

their appearance in tubenose goby (before 2008). The above observations suggest two determinants which affect the parasite communities of goby fish in the studied area: 1) habitat preferences of the fish species. For example, the sparse occurrence of glochidia in monkey goby, a fish preferring sandy bottoms (Pinchuk *et al.*, 2003), contrary to both racer and tubenose gobies, which inhabit muddy and overgrown near-shore zones of the reservoir, rich in molluscs including different species of Unionidae, mainly: *Anodonta anatina, Unio tumidus* and *U. pictorum* (Jurkiewicz-Karnkowska and Żbikowski, 2004). 2) Co-existence with a new, closely related invader could be seen as the second determinant. It was clearly visible in the case of *Apatemon gracilis*. The parasite first infected the tubenose goby, then expanded to the other goby species which previously inhabited the colonized area and had been free of the parasite until 2008. Taking into account the seasonal differences in parasite occurrence (Table IV) and the dependence of infection on the fish size, it could be seen that the infection is generally highest in warmer seasons but the lowest infection with *Holostephanus* spp. in spring (Table IV) could be caused by the greater fish size in samples, due to negative correlation of the fish length with the parasite number (see results). During our study, carried out over several years and in different seasons, it was also found that the migrating Ponto-Caspian gobies are mainly infected with parasites commonly distributed through native fishes in the observed area. Ciliate *T. domerguei* infects native (own observation, unpublished) as well as alien fish species, at present (Table I–III) and, according to previous data from the Włocławek Reservoir (Waluga and Własow, 1988), was also abundant in natives in the 1970s. The larvae of the nematode *Eustrongylides* spp. were detected in gobies as well as in other fish species in the studied area; the Chinese sleeper (with prevalence >60% in warm seasons) (Mierzejewska *et al.*, 2012) and the perch (>50% own observations, unpublished). The tested gobies also became hosts for parasites that are less common in natives and, in this way, the newcomers reinforce populations of these parasites in the occupied area. Infection with metacercariae of the genus Holostephanus was not described in Polish fish before this study (except for *H. dubinini* in white bream *Blicca bjoerkna* in unpublished data) (Niewiadomska, 2003). According to our observations, encysted larvae of *Holostephanus* spp. have appeared in alien fish species very frequently and numerously. It should be stressed that this parasite was also very common in Chinese sleeper *Perccottus glenii* (Mierzejewska *et al.*, 2012), an invasive fish from eastern Asia, which has also spread spontaneously in the Włocławek Reservoir since the early 1990s (Kakareko 1999; Kostrzewa *et al.* 2004). Mature *Holostephanus* spp. in birds have been recorded in Poland and metacercariae of these genera were also expected in Poland due to their distribution in fish near the eastern border (Niewiadomska, 2003). Another example of reinforcing the parasite population by newly-introduced goby fish, as well as by the Chinese sleeper, is *Bucephalus polymorphus*. This example has just been described in detail (Kvach and Mierzejewska, 2011). The third parasite, *Apatemon gracilis*, is widely distributed in Polish fish but was not recorded in the Włocławek Reservoir in a previous study, conducted prior to the expansion of gobies (Waluga and Własow, 1988). In our study, metacercariae of *A. gracilis* appeared for the first time in great numbers (up to 50 per fish) on the internal organs of tubenose goby in 2008. Since this time, in subsequent samples, the parasite has been regularly reported in other gobies from the Włocławek Reservoir (Table I–III). New parasites brought to the colonized area were also detected in the studied fish. Together with the newcomers, monkey and racer gobies, monogenean *Gyrodactylus proterorhini* was introduced to Europe and Poland (Ondračková *et al.*, 2005, 2009; Francová, 2007; Kvach and Oğuz, 2009; Mierzejewska *et al.*, 2011). Due to the host specificity, the occurrence of the parasite is limited to species of Gobiidae. Although the parasite probably does not threaten the native fish, the fish of Perciformes are closely related to gobies and should be monitored for the presence of Monogenea in the area studied. In addition, the eyefluke *Diplostomum gobiorum*, which had never been reported in Polish fish data, appeared in tubenose goby for the first time in the Włocławek Reservoir. According to Niewiadomska (2003), southern ninespine stickleback, Kessler's gudgeon and the monkey goby from the Gobiidae family are the only confirmed hosts of this parasite. The occurrence of *D. gobiorum* in tubenose goby will be analyzed separately and should be supported by further observation in the studied waters because of possible growth of the parasite population (at least *via* infection of the monkey goby). Larval stages of parasites, mainly the metacercariae of digeneans, formed the most diverse group in the analyzed parasite community (Table I, II, III). In the native range of tested fish hosts, the metacercariae predominated in terms of number, as well as in terms of diversity, in component communities; *Cryptocotyle lingua* and *C. concavum* infect the monkey goby with a prevalence of between 50−100% in the majority of the studied reservoirs, brackish and saltwater, as well as in estuaries. In total, 29 different species of Digenea were noted in *Neogobius fluviatilis* in a natural range including the Black and Azov Seas, Ukrainian rivers in their lower courses and the Danube Delta (Ciurea, 1933; Chernyshenko, 1957; 1960; Kulakovskaya and Koval, 1973; Najdenova, 1974; Kvach, 2002, 2004, 2005); four more digeneans, the new species for this fish host, were detected in a newly-occupied area, including the Danube above the Iron Gates gorge and in the Slovak section of the River (Ondračková *et al.*, 2005; Molnár, 2006). In the Włocławek Reservoir, five further species: *Bucephalus polymorphus*, *Diplostomum paracaudum*, *D. pseudospathaceum*, *Holostephanus luehei* and *Echinochasmus spinosus* joined the list of flukes able to infect *N. fluviatilis*. As far as the racer goby is concerned, *C. concavum*, *C. lingua*, the acanthocephalan *Pseudoechinorhynchus clavula* and the cestode *Proteocephalus* sp. are widely distributed and dominating in this fish in the Ponto-Caspian region. (Najdenova, 1974; Smirnov, 1986; Kvach, 2004). Natively, the *N. fluviatilis* is often infected with microsporidian *Glugea* sp. (Microsporidia) (Najdenova, 1974; Smirnov, 1986; Ovcharenko, 1985), which has not been observed in the Vistula River course. According to Molnár (2006), the specific parasites such as coccidia of three different species (*Eimeria daviesae*, *Goussia kessleri* and *G. szekelyi* n. sp.) seem to be the most characteristic, infecting only the *Neogobius* species and do not seem to infect fish of the genus *Proterorhinus*. The data from Poland support this observation because oospores of *Eimeria* sp. regularly recorded in the monkey goby were not detected in the tubenose goby (of the genus *Proterorhinus*) at all, but only accidentally in the racer goby (a species separated from the genus *Neogobius* and reclassified by Stepien and Tumeo (2006) into the new genus *Babka*). The tubenose goby, usually harboring monogenean *Gyrodactylus proterorhini* (Chiriac and Udrescu, 1957; Ergens, 1967) in its native range, was not infected with this parasite in the Włocławek Reservoir in spite of its occurrence in the other gobies here (Table I and II). Due to the small size and high density of the alien fish tested, their role as transmitters of parasites is crucial, especially for parasites maturing in predatory fish (this only concerns *Bucephalus polymorphus*). As regards the parasites closing their life cycle in fish-eating birds (Digenea, *Eustrongylides* spp.), their transmission *via* the food chain can be limited in the case of gobies, as hunting for small fish living at the bottom (Gobiidae) is difficult, especially in the deeper reservoirs, with littoral zones highly overgrown with macrophytes. The rare and sparse presence of the remains of gobies in cormorant pellets collected around the Włocławski Reservoir confirms the above statement (Wziątek *et al.*, 2010). On the other hand, the growing infection with metacercariae of different species, as well as with pathogenic larvae of nematodes (*Eustrongylides* spp.) possible for the growing populations of Ponto-Caspian gobies, could limit those fish species in the future, at least in the studied reservoir.

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