Non-native gobies share predominantly immature parasites with local fish hosts

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SPECIAL ISSUE: INVASIVE FISHES
RESEARCH PAPER

Non-native gobies share predominantly immature parasites with local fish hosts

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Abstract. Non-native species are known to escape their parasites following introduction into a new range, but they also often acquire local parasites as a function of time since establishment. We compared the parasite faunas of five non-native Ponto-Caspian gobies (Gobiidae) and local fish species (*Perca fluviatilis, Gymnocephalus cernua, Gobio gobio*) in three European river systems; the Rivers Rhine, Vistula and Morava, where Ponto-Caspian gobies were introduced 4-13 years prior to the study. Overall parasite species richness was considerably lower in non-native gobies compared to local fish species, and the same result was found at the component and infra-community levels. Both parasite abundance and diversity greatly varied among the regions, with the highest values found in the River Vistula (Wloclawski Reservoir), compared to a relatively impoverished parasite fauna in the River Morava (Danube basin). While only half of parasite species found in local hosts were acquired by non-native gobies, most of the parasites found in gobies were shared with local fish species related either phylogenetically (percids) or ecologically (benthic gudgeon), including the co-introduced monogenean *Gyrodactylus proterorhini*. As a result, similarity in parasite communities strongly reflected regional affiliation, while phylogenetic distances between fish host species did not play a significant role in parasite community composition. In accordance with other studies, all parasites acquired by gobies in their new range were generalists, all of them infecting fish at the larval/subadult stage, indicating the possible importance of gobies in the life cycle of euryxenous parasites. The absence of adult generalists, particularly ectoparasites with low host specificity, in non-native fish may reflect their generally low abundance in the environment, while an absence of adult endoparasitic generalists was probably related to other factors.

Key words: Ponto-Caspian gobies, parasite acquisition, larva, generalists, species richness

Introduction

Interactions between introduced hosts and parasites have attracted considerable attention in recent decades. After translocation of non-native species into new areas, local species may be at risk of exposure to novel parasites or pathogens. The consequences of introduced parasites for the receiving ecosystem may vary according to a range of factors, including the complexity of the

parasite life cycle, its ability to spill over to native hosts, and the extent of the natural resistance and resilience to infection in these hosts (Dunn 2009). Nevertheless, a number of studies have revealed that introduced species show lower prevalence and parasite species richness compared to their original range (Torchin et al. 2003, Kvach & Stepien 2008, Sellers et al. 2015) as well as to local host species (Gendron et al. 2012). Such escape from natural enemies, articulated in the "enemy-release hypothesis" (Torchin et al. 2003), has been considered as a mechanism by which non-native hosts are advantaged in comparison with native species which may significantly contribute to the success of non-native species in their newly invaded environments (Torchin & Mitchell 2004).

Alongside release from natural parasites, non-native species can be infected with local parasite species over time, potentially altering the host-parasite dynamics in the environment (Tompkins et al. 2015). Depending on the competence of non-native hosts to local parasite species, their exposure may in some cases lead to "spill-back", with increasing parasite numbers in the environment (e.g. Kelly et al. 2009), or to a "dilution effect", whereby parasite infection in a host community is reduced with the addition of new hosts (e.g. Gendron & Marcogliese 2017). Introduced species often acquire a range of local generalist parasites over time, and may even support more diverse parasite communities than their conspecifics in their native range (Poulin & Mouillot 2003, Kołodziej-Sobocińska et al. 2018). However, in some cases the intensity of infection and the number of parasite species remains lower than those in their original range, or than native species in the new range, even in the case of long-established populations. This outcome is particularly associated with species introduced to distant, historically separate regions, such as Ponto-Caspian gobies introduced to North America (e.g. Kvach & Stepien 2008, Gendron et al. 2012), or pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) introduced from North America to Europe (Ondračková et al. 2019a, 2021), but see Poulin & Mouillot (2003) for contrasting results in trans-oceanically introduced brown trout *Salmo trutta* Linnaeus, 1758 and rainbow trout *Oncorhynchus mykiss* Walbaum, 1792.

Range extension of Ponto-Caspian gobies (Actinopterygii: Gobiidae) are some of the best characterised examples of invasive fish species. Over the last three decades these species have proved to be amongst the most successful invasive freshwater fishes (Copp et al. 2005, Roche et al. 2013). Dispersal of aquatic Ponto-Caspian species within Europe has mainly been facilitated by artificial navigation, river interconnection and creation of irrigation canals (Semenchenko et al. 2011, Manné et al. 2013, Roche et al. 2013), while transport via ship ballast water in transoceanic ships most probably contributed to the introduction of two goby species into North America (Charlebois et al. 1997, Corkum et al. 2004). Expansion of particular goby species varies among European river systems, reflecting habitat type, navigation intensity or human-assisted introductions (Semenchenko et al. 2011, Roche et al. 2013, Janáč et al. 2017). As a result, the non-native distribution of Ponto-Caspian gobies is highly variable in European waters. To date, all invaded areas are inhabited by only some of the recently spreading goby species and their relative abundance in fish assemblages is distinctive (Borcherding et al. 2011, Semenchenko et al. 2011, Manné et al. 2013, Roche et al. 2013). Among their non-native ranges, the Lower Rhine as a hot spot of parasite invasion (Sures et al. 2019) has been invaded by the highest number of Ponto-Caspian gobies (five species; Borcherding et al. 2011, 2021).

Parasites of the Ponto-Caspian gobies have been intensively studied in both their native range and area of expansion, resulting in a record of 249 parasite taxa, demonstrating that the number of parasite species reported from non-native populations is rapidly increasing (Kvach & Ondračková 2021). The diversity and abundance of local parasite species acquired in invaded areas reflect, besides duration of the invasion (Gendron et al. 2012, Paterson et al. 2012, Ondračková et al. 2015a), the historical connectivity of river catchments between native and invaded systems. While goby populations introduced to historically connected aquatic habitats were infected with a high diversity of local parasite species (Ondračková et al. 2009, 2012, Francová et al. 2011), local parasite acquisition in populations established in artificially interconnected rivers (e.g. via interconnecting channels) appears lower (Ondračková et al. 2012, 2015b, Kvach et al. 2014). Nevertheless, in the majority of cases the parasite community of gobies consists of species native to the Ponto-Caspian region and only a small proportion is represented by novel local species, a phenomenon observed particularly in gobies translocated into North America (Kvach & Ondračková 2021).

Table 1. Fish species examined for parasites in three European rivers invaded by Ponto-Caspian gobies. n – number of fish dissected, time between sampling and fish introduction, fish standard length (SL, in mm), parasite prevalence (in %), mean abundance and species richness (rarefied for the 20 fish in parentheses).

Site/fish species	Status	Time	n	SL mean (range)	Prevalence (%)	Abundance	Species richness
the River Morava (Danube basin, Black Sea drainage)							
Perca fluviatilis	native		21	92 (73-121)	86	4.0	8 (7.86)
Gobio gobio	native		21	66 (56-78)	100	18.7	9 (8.95)
Neogobius melanostomus	non-native	4	21	65 (56-71)	100	23.7	5 (5.00)
Proterorhinus semilunaris	non-native	12	20	44 (40-56)	100	26.0	6 (6.00)
the River Rhine (North Sea basin)							
Perca fluviatilis	native		25	88 (72-103)	100	19.2	12 (10.90)
Gymnocephalus cernua	native		20	74 (61-100)	100	55.9	12 (12.00)
Neogobius melanostomus	non-native	4	23	70 (51-101)	100	13.4	9 (8.73)
Neogobius fluviatilis	non-native	4	25	71 (55-94)	96	7.6	8 (7.40)
Ponticola kessleri	non-native	6	25	71 (63-78)	100	11.7	9 (8.56)
Proterorhinus semilunaris	non-native	13	21	46 (41-52)	100	42.7	7 (6.86)
the River Vistula (Baltic Sea drainage)							
Perca fluviatilis	native		23	98 (69-137)	100	144.4	21 (20.19)
Neogobius fluviatilis	non-native	6	20	65 (36-113)	100	24.4	6 (6.00)
Babka gymnotrachelus	non-native	8	23	54 (42-83)	100	73.3	12 (11.85)

This study was aimed at investigating the composition of parasite communities in non-native and native fish species, related either phylogenetically (Perciformes) or ecologically (benthic species) in three discrete major river systems in Europe that have recently been invaded by Ponto-Caspian gobies. We first compared the parasite abundance and species richness in native and non-native fish hosts to determine whether the reduced parasite fauna of introduced species is generally applicable. We further analysed the composition of parasite communities, with a focus on the proportion of generalists and specialists and the proportion of adult and immature parasites. Finally, we tested how host phylogenetic distance and sampling site contribute to the similarity in parasite communities in areas affected by the introduction of non-native species.

Material and Methods

Study area

Samples of native and non-native fish were obtained from three major rivers in Europe: 1) In the River Rhine, Germany, near the village of Rees-Grietherbusch (51°48′21″ N, 6°18′20″ E), the fish were collected in the main channel and adjacent side channels in 2012; 2) In the River Vistula, Poland, fish were collected from the Wloclawski Reservoir near the village of Soczewka (52°32′58″ N, 19°34′29″ E) in 2008; and 3) in the River Morava, Czech Republic, the fish were collected in the main river channel near the village of Lanžhot (48°41′28″ N, 16°59′47″ E) in 2012. All samplings were performed during the fish growing season, from May to October. In the Lower Rhine, Ponto-Caspian gobies were first recognised in 1999 (western tubenose goby *Proterorhinus semilunaris* (Heckel, 1837)), followed by bighead goby *Ponticola kessleri* (Günther, 1861) in 2006 and monkey goby *Neogobius fluviatilis* (Pallas, 1814) along with round goby *Neogobius melanostomus* (Pallas, 1811) in 2008 (Borcherding et al. 2011). In the River Vistula, the racer goby *Babka gymnotrachelus* (Kessler, 1857) first appeared in 2000 followed by monkey goby in 2002 (Kostrzewa et al. 2004). The appearance of the tubenose goby in the River Morava dates from 2000, while round gobies were first recorded in 2008 (Roche et al. 2013).

Fish and parasite sampling

Eight fish species were collected; five non-native species of the family Gobiidae, i.e. monkey goby, round goby, bighead goby, racer goby and tubenose goby, and three native species, European perch *Perca fluviatilis* (Linneaus, 1758), ruffe *Gymnocephalus cernua* (Linneaus, 1758) representing phylogenetically related species (Acanthomorpha sensu Bentacur-R et al. 2017) and gudgeon *Gobio gobio* (Linneaus, 1758), representing ecologically related (benthic) species. For species and number of specimens collected at each locality, see Table 1. The fish were collected by electrofishing (a battery-operated SEN backpack unit; Bednář Ltd., Czech Republic) or by using a beach seine (7 m length, 4 mm mesh size) depending on habitat conditions.

Collected fish were transported live in river water to a nearby laboratory, where they were individually sacrificed by cutting the cervical spine and dissected within three days of capture (Kvach et al. 2016). All fish were measured for standard length (SL, to the nearest 1 mm; Table 1) prior to dissection. Fish were examined under a binocular microscope for the presence of metazoan parasites. Collected parasites were preserved in 4% formaldehyde (Acanthocephala, Digenea, Cestoda, Bivalvia), in a mixture of ammonium picrate and glycerine (Monogenea, small Nematoda and Digenea), or in a mixture of glycerine and alcohol (Nematoda). Preserved digeneans and cestodes were stained in ferric acetocarmine, dehydrated in a gradual alcohol series, and mounted in Canada balsam (Georgiev et al. 1986). Parasites were identified using a light microscope equipped with phase-contrast, differential interference contrast and the Lucia 5.0 Image Analysis System to the lowest possible taxonomic level. Larval trematodes were mainly identified to the genus/family level, as metacercariae of many species are difficult to determine by morphology to the species level.

Data analysis

Prevalence and mean parasite abundance were calculated for each fish species and locality sampled. Prevalence was expressed as the percentage of infected fish in a sample and the mean abundance as mean number of parasites per all hosts in a sample. Metazoan parasite community structure was analysed at both the component community (CC; all parasites in a host population, values rarefied to the smallest common sample size within a locality, i.e. 20 fish) and infracommunity (IC; including all parasites on a single host) levels (Bush et al. 1997). Parasites known to infect fish of a single family were denoted as a specialist on the family level (none of the parasites found can be considered as a specialist at the species level), parasites infecting
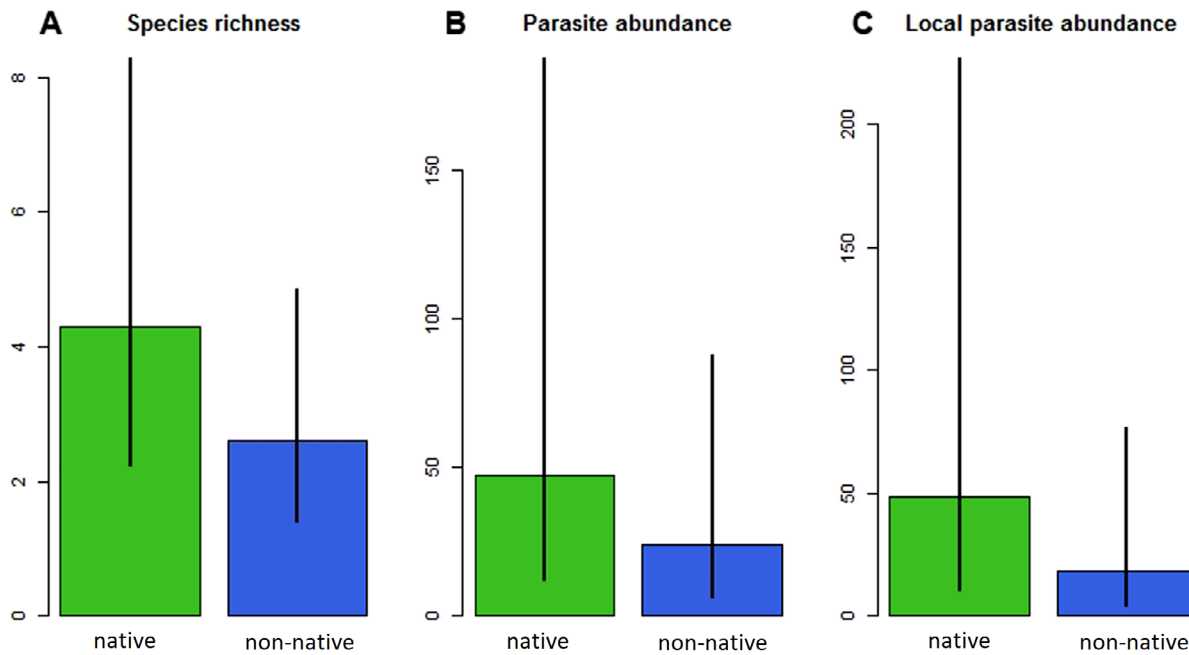
Fig. 1. Estimated overall mean (bars) and 95% CI (vertical lines) of parasite species richness at the infracommunity level (A), mean parasite abundance (B) and abundance of local parasite species (C) in native and non-native fish hosts.

fish hosts of multiple families were denoted as generalists. Similarity in parasite communities among populations was evaluated using the Jaccard index based on presence-absence data (qualitative similarity) and the Bray-Curtis index based on abundance data (quantitative similarity). Rarefaction analysis and similarity between parasite communities were calculated using the PAST software (PAlaeontologicalSTatistics, v.1.77, http://folk.uio.no/ohammer/past/; Hammer et al. 2001).

The differences in parasite assemblage parameters between native and non-native hosts were tested using (i) generalized linear mixed models (GLMM; negative binomial models in abundance, Poisson in species richness, locality and host used as random factors) at the IC level and (ii) linear mixed models at the CC level (LMM; non-integer response variables, log-transformed before analysis, locality used as a random factor).

In order to detect the role of locality on parasite assemblage composition, we calculated CC-level assemblage composition similarities (Bray-Curtis and Jaccard) between all pairs of host-locality combinations. A simple linear model (LM) was used to detect whether the similarities were higher between or within localities, using host genetic distances (calculated as Kimura two-parameter

(K2P) distances in MEGA 10.2.2) as a covariate (Kimura 1980, Kumar et al. 2018). For genetic distance calculations, 6-10 partial sequences of the mitochondrial gene cytochrome *b* (1,037 bp) for each fish species were retrieved from GenBank (see Table S1). The analyses were conducted using R statistical software version 3.5.2 (R Core Team 2018).

## Results

Parasite load in native and non-native host species
Parasite species richness was significantly higher in native compared to non-native hosts at the IC level (GLMM, n = 287, $P = 0.023$; Fig. 1A), the trend also apparent at the CC level (LMM, n = 13, $P = 0.009$). Mean parasite abundance did not significantly differ between native and non-native hosts, at both the IC and CC levels (IC: GLMM, n = 287, $P = 0.167$, Fig. 1B; CC: LMM, n = 13, $P = 0.446$) and the same results were found for the abundance of local parasites, i.e. parasites excluding non-native *Gyrodactylus proterorhini* Ergens, 1967 co-introduced with gobies (IC: GLMM, n = 287, $P = 0.112$, Fig. 1C; CC: LMM, n = 13, $P = 0.290$; Table 1). Nevertheless, the trends were strongly related to the sampling site. Parasite abundance in the River Morava was comparable among host species, except for a generally low infection in perch. On the other hand, there was a significantly lower

Table 2. List of parasite species infecting local fish species, showing their mean abundance and prevalence (in %) in parentheses.

	Stage	Specificity	the River Morava		the River Rhine		the River Vistula
			G. gobio	*P. fluviatilis*	*G. cernua*	*P. fluviatilis*	*P. fluviatilis*
Monogenea							
Gyrodactylus cernuae	A	S			1.2 (5)		
Gyrodactylus gobii	A	S	0.9 (33)				
Gyrodactylus laevis	A	G				0.04 (4)	0.1 (4)
Gyrodactylus luciopercae	A	S			0.8 (5)	0.2 (8)	2.0 (30)
Gyrodactylus markakulensis	A	S	2.6 (48)				
Gyrodactylus proterorhini	A	S					0.2 (9)
Gyrodactylus gasterostei	A	G					27.8 (83)
Paradiplozoon homoion	A	S	0.4 (24)				
Dactylogyrus amphibothrium	A	S			2.5 (65)		
Trematoda							
Bucephalus polymorphus	M	G	5.7 (71)	0.5 (24)			
Rhipidocotyle campanula	A	G					1.9 (22)
Echinostomatidae fam. sp.	M	G	2.9 (52)				
Diplostomum spp.	M	G	4.6 (62)		0.1 (10)	0.04 (4)	14.4 (87)
Ornithodiplostomum scardinii	M	G					0.1 (9)
Tylodelphys clavata	M	G		0.5 (10)	4.3 (70)	12.1 (84)	42.3 (78)
Tylodelphys podicipina	M	G				0.04 (4)	0.3 (13)
Bunodera luciopercae	A	G			0.05 (5)	2.5 (62)	4.2 (52)
Cyathocotylidae fam. sp.	M	G	0.4 (24)				
Apatemon spp.	M	G	0.05 (5)	0.1 (5)			0.04 (4)
Ichthyocotylurus spp.	M	G			44.0 (100)	0.8 (40)	6.7 (91)
Cestoda							
Triaenophorus nodulosus	L	G				0.2 (8)	
Proteocephalus percae	A	S		0.05 (5)	0.4 (25)	0.1 (8)	1.6 (22)
Paradilepis scolecina	L	G				3.0 (28)	
Nematoda							
Anguillicoloides crassus	L	G			1.6 (35)		0.04 (4)
Camallanus lacustris	A	G				0.04 (4)	0.04 (4)
Camallanus truncatus	A	G			0.1 (10)		
Raphidascaris acus	L	G					0.04 (4)
Paracuaria adunca	L	G			0.05 (5)		
Eustrongylides spp.	L	G					1.2 (30)
Acanthocephala							
Pomphorhynchus laevis	SA	G			1.0 (30)		0.1 (4)
Bivaliva							
Anodonta anatina	GL	G	0.4 (19)	2.0 (38)			40.2 (96)
Anodonta cygnea	GL	G					0.9 (52)
Unio tumidus	GL	G		0.8 (19)			
Crustacea							
Ergasilus sieboldi	A	G			0.04 (4)		
Argulus foliaceus	A	G			0.1 (10)	0.04 (4)	0.3 (22)

Stage: A – adult, M – metacercaria, L – larva, SA – sub-adult, GL – glochidium.
Specificity: S – specialist for family-level, G – generalist.
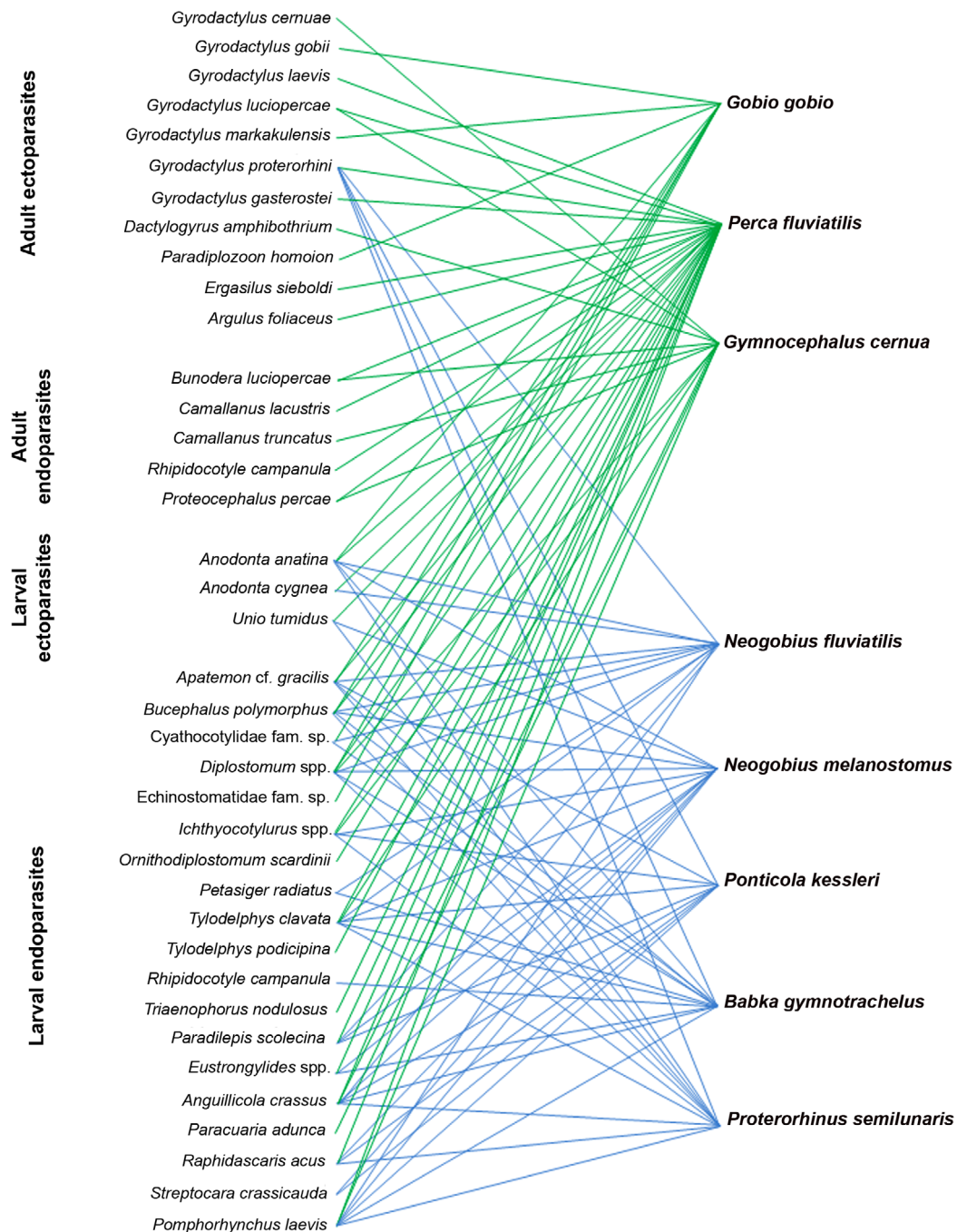
Fig. 2. Host-parasite interactions between native (green lines) and non-native (blue lines) fish hosts and larval/adult ecto- and endo-parasites.

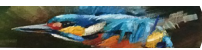abundance in non-native species in the River Vistula (Figs. S1-S3).

Abundance of specialist parasites did not differ between native and non-native fish hosts (GLMM, n = 287, *P* = 0.558; Fig. S4). Local generalists parasitizing fish at the adult stage, including monogeneans, trematodes, cestodes, nematodes and crustaceans, were observed solely in native fish hosts, i.e. all local parasites infecting non-native gobies were immature, parasitizing at larval

or sub-adult stages (Table 2, Fig. S5). However, the abundance and species richness of larval generalists was comparable between native and non-native fish hosts (GLMM, both n = 287, *P* = 0.198 and 0.208), reflecting the high variance in parasite load at individual sampling sites (Fig. S6).

Parasite species composition
A total of 37 metazoan parasite taxa were identified. The majority, i.e. 35 taxa, were identified in native fish hosts, including perch (all three sites),

Table 3. List of parasite species infecting non-native Ponto-Caspian gobies, showing their mean abundance and prevalence in parentheses.

	Stage	Specificity	the River Morava		the River Rhine				the River Vistula	
			N. melanostomus	*P. semilunaris*	*N. fluviatilis*	*P. kessleri*	*N. melanostomus*	*P. semilunaris*	*N. fluviatilis*	*B. gymnotrachelus*
Monogenea										
Gyrodactylus proterorhini	A	S		4.0 (85)	3.2 (56)	0.5 (24)	0.04 (4)	3.9 (86)	4.5 (55)	38.8 (100)
Trematoda										
Bucephalus polymorphus	M	G	14.7 (90)	14.0 (85)	0.04 (4)					0.9 (39)
Rhipidocotyle campanula	M	G								0.9 (26)
Petasiger radiatus	M	G							0.2 (15)	0.5 (13)
Diplostomum spp.	M	G	1.4 (67)	0.4 (40)	0.04 (4)		0.04 (4)		4.1 (70)	6.1 (78)
Tylodelphys clavata	M	G			0.9 (16)	5.8 (48)	0.7 (26)	2.1 (38)		0.3 (9)
Cyathocotylidae fam. spp.	M	G							0.6 (25)	3.0 (74)
Apatemon spp.	M	G		4.6 (55)	0.04 (4)	0.1 (12)		36.4 (95)		2.1 (13)
Ichthyocotylurus spp.	M	G				0.2 (8)	0.7 (22)	0.05 (5)		
Cestoda										
Paradilepis scolecina	L	G			2.2 (28)	0.04 (4)	0.3 (13)			
Nematoda										
Anguillicola crassus	L	G			0.8 (24)	0.4 (16)	1.6 (30)	0.2 (10)		0.04 (4)
Raphidascaris acus	L	G				0.1 (12)	0.9 (26)	0.05 (5)		
Streptocara crassicauda	L	G				0.04 (4)	0.1 (7)			
Eustrongylides spp.	L	G	0.1 (9)							0.1 (9)
Acanthocephala										
Pomphorhynchus laevis	SA	G			0.4 (12)	4.4 (48)	8.9 (48)	0.05 (5)		0.4 (26)
Bivalvia										
Anodonta anatina	GL	G	7.0 (86)	3.1 (50)					15.0 (100)	20.0 (87)
Anodonta cygnea	GL	G							0.05 (5)	
Unio tumidus	GL	G	0.4 (33)	0.1 (5)						

Stage: A – adult, M – metacercaria, L – larva, SA – sub-adult, GL – glochidium.
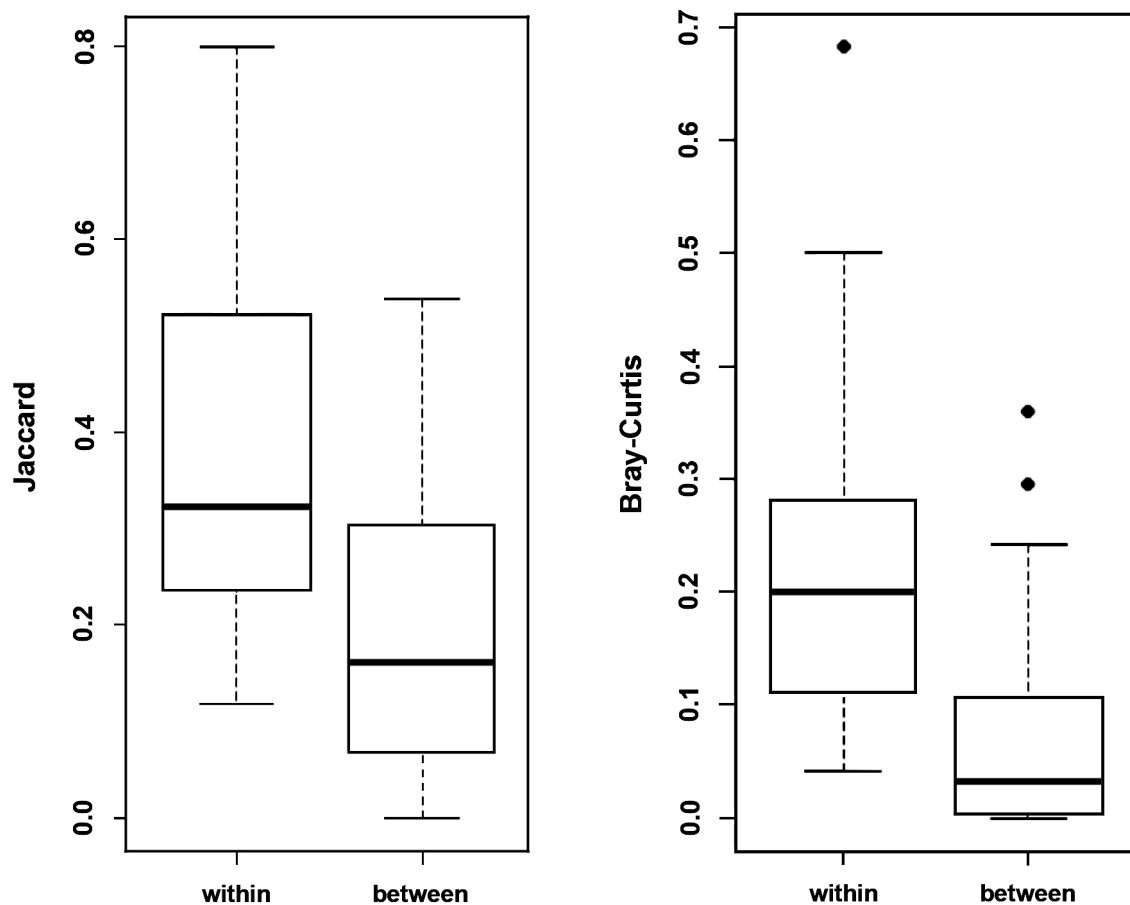Specificity: S – specialist for family-level, G – generalist.

Fig. 3. Median (horizontal lines) similarities in parasite assemblages within the same locality and between different localities. Boxes stand for interquartile range, whiskers for non-outlier (1.5 × interquartile) range, points for outliers.

ruffe (the River Rhine) and gudgeon (the River Morava). Maximum prevalence and abundance were found for metacercariae of *Ichthyocotylurus* spp. infecting ruffe in the River Rhine (100%, 44.0, respectively) and perch in the River Vistula (91%, 6.7), metacercariae of *Tylodelphys clavata* (von Nordmann, 1832) infecting perch in the River Rhine (84%, 12.1) and the River Vistula (78%, 42.3), and metacercariae of *Diplostomum* spp. (87%, 14.4), glochidia of *Anodonta anatina* (Linnaeus, 1758) (96%, 40.2) and *Gyrodactylus gasterostei* (Gläser,

1974) (83%, 27.8) all infecting perch in the River Vistula (Table 2). Parasite infection was relatively low in fish from the River Morava. Perch in the River Vistula were the only native fish species accidentally infected with non-native *G. proterorhini* co-introduced with gobies (Table 2).

Non-native fish hosts, including five Ponto-Caspian goby species, were infected with 18 parasite taxa. Among the most frequent parasite species, *G. proterorhini*, a species co-introduced with goby

Table 4. Results of linear models predicting effects of genetic distance and sympatry (within *vs.* between locality) on qualitative (Jaccard) and quantitative (Bray-Curtis) similarities of parasite assemblages between all pairs of host-site combinations.

Jaccard	df	SS	MS	F	P
genetic distance	1	0.04	0.04	1.97	0.165
sympatry	1	0.68	0.68	33.76	< 0.001
residuals	75	1.50	0.02		
Bray-Curtis	df	SS	MS	F	P
genetic distance	1	0.01	0.01	1.15	0.285
sympatry	1	0.44	0.44	35.43	< 0.001
residuals	75	0.93	0.01		

hosts, showed the highest infection levels with up to 100% prevalence and a mean abundance of 38.8 in racer gobies from the River Vistula. This monogenean parasite was the only specialist (at the host family level) in the parasite community of gobies; all other species were generalists. Of the local parasite species, the highest prevalence and abundance was observed for glochidia of *A. anatina* in the monkey goby (100%, 15.0), racer goby (87%, 20.0) from the River Vistula and round goby (86%, 7.0) from the River Morava, for metacercariae of *Apatemon* cf. *gracilis* (Rudolphi, 1819) in tubenose goby from the River Rhine (95%, 36.4) and *Diplostomum* spp. in racer gobies from the River Vistula (78%, 6.1). The majority of parasites found were shared with native hosts (Fig. 2), and only two parasite species were solely found in non-native gobies; metacercariae of *Petasiger radiatus* (Dujardin, 1845) and larvae of *Streptocara crassicauda* (Creplin, 1829), both species occurring rarely and at low prevalence and abundance (Table 3).

Similarity in parasite communities

Jaccard (qualitative) similarity in parasite community ranged from zero (between round gobies from the River Morava and both bighead and tubenose gobies in the River Rhine) to 0.68 (between tubenose and round gobies in the River Morava). Bray-Curtis (quantitative) similarity ranged from zero (see above) to 0.80 (between round and bighead gobies in the River Rhine). Both qualitative and quantitative similarities were strongly related to the sampling region, irrespective of the genetic distance between hosts (Table 4, Fig. 3).

Discussion

Parasite community structure was compared between native and non-native fish species in three discrete European rivers with the aim of quantifying their parasite load in the invasive environment to characterise the parasite species shared by both host groups. Our results demonstrate: 1) lower parasite species richness in non-native compared to native fish hosts, 2) dominance of immature (mainly larval) parasite species shared between native and non-native hosts, and 3) a strong effect of sampling region on parasite community structure.

Parasite species richness and abundance

The overall number of parasite species infecting native fish hosts (35 spp.) was double that of non-native gobies (18 spp.), despite the lower number of native fish host species (three *vs.* five) and individuals (110 *vs.* 178) in comparison with non-native hosts. Lower parasite species richness in non-native gobies was also apparent at the component community and infracommunity levels, both when compared with local fish hosts examined in this study, and with the native range of the goby species (e.g. Kvach 2005, Francová et al. 2011, Ondračková et al. 2012, Mierzejewska et al. 2014). Our results correspond to those from other studies showing lower species richness in non-native hosts either when compared to their native range (Torchin et al. 2003, Kvach & Stepien 2008, Kvach et al. 2014), or when compared to sympatric local host species (Gendron et al. 2012, Hohenadler et al. 2019), reflecting local host-parasite adaptation and an absence of common history between parasites and novel hosts (Dunn 2009). Using phylogenetically distant native hosts in this study (i.e. Percidae *vs.* Cyprinidae) may have contributed to the overall higher parasite diversity in native hosts, but the parasite species richness still remained high in native (32 spp.) compared to non-native (18 spp.) hosts even after subtracting parasites typical for cyprinids (i.e. specific monogeneans *Gyrodactylus gobii* Schulman, 1953, *Gyrodactylus markakulensis* Gvosdev, 1950 and *Paradiplozoon homoion* Bychowsky & Nagibina, 1959).

Compared to species richness, parasite abundance did not show significant differences between native and non-native fish hosts, possibly due to the high variance among the sampling sites (see Fig. S1-S6). While the trend of lower abundance in non-native hosts was apparent in fish from the River Vistula (Wloclawski Reservoir), higher parasite abundance in non-native gobies was observed in fish from the River Morava. Competence of non-native species to local parasites in new environments varies from avoidance to high susceptibility (e.g. Kennedy 2007, Miller et al. 2008), and a similar picture can be seen in the parasite community in our sampling regions. Some species infected novel hosts (gobies) only rarely, with low prevalence and abundance (e.g. metacercariae of *Rhipidocotyle campanula* Dujardin, 1845, larvae of *S. crassicauda* and *Eustrongylides* sp. or glochidia of *Anodnota cygnea* Linnaeus, 1758). Unfortunately, our data are not sufficient to confirm whether the low infection reflects avoidance of the novel host by parasites, or because parasites are not able to survive on the novel host, potentially leading to

further reduction of parasites in the environment (Gendron & Marcogliese 2017). On the other hand, some parasites showed high infection parameters in gobies, indicating high susceptibility of the host (e.g. metacercariae of *Bucephalus polymorphus* von Baer, 1827 and diplostomid eye flukes, or glochidia of *A. anatina*), with abundances exceeding those in native host species. Integration of non-native hosts in parasite life cycles may increase parasite abundance in the environment, when the novel intermediate host is preyed upon by the definitive host of the parasite (e.g. Ondračková et al. 2015b for *B. polymorphus* case), or the host may contribute significantly to the dispersal of the parasite, as was shown for bivalve glochidia by Šlapanský et al. (2016).

Parasites shared between native and non-native hosts

It has been generally accepted that parasites acquired by non-native species in the new environment are predominantly generalists that infect a wide range of host species (Poulin & Mouillot 2003, Schatz & Park 2021). Accordingly, our results show that in their new range, Ponto-Caspian gobies mainly acquired and shared generalist parasites. Parasites acquired by gobies that were shared with local fish hosts were solely immature parasites infecting fish at their larval or subadult stages and, in these cases, gobies served primarily as their intermediate or paratenic hosts. Contrariwise, co-introduced *G. proterorhini* was the only adult parasite acquired by local fish species. Goby species are known to host a wide range of larval parasites in marine, brackish and freshwater habitats (Zander et al. 1993, Ondračková et al. 2009, Kvach & Winkler 2011, Kvach & Ondračková 2021), highlighting their importance in parasite transmittance (Zander et al. 1993). The high susceptibility of gobies to larval parasites in their non-native range was illustrated for trematodes, in some cases demonstrating opposing effects on parasite dynamics in the environment. While dilution of diplostomid eye-fluke trematodes was observed after the introduction of the round goby in North America (Gendron & Marcogliese 2017), increased abundance indicating parasite spill-back of larval *B. polymorphus* was observed in various intermediate fish species following the introduction of round and tubenose gobies in the River Morava (Ondračková et al. 2015b). In all three rivers sampled in our study, the highest prevalence of local parasite species was found for either metacercariae of trematodes (e.g. *B.*

polymorphus, *Diplostomum* spp., *Apatemon* cf. *gracilis*) or glochidia of bivalves (e.g. *A. anatina*), highlighting the potential importance of non-native gobies in the life cycles of these parasite species.

According to Schatz & Park (2021), the likelihood of parasite acquisition increases with prevalence in the environment. Low prevalence in native hosts of adult generalist crustaceans, such as *Argulus foliaceus* Linnaeus, 1758 and *Ergasilus sieboldi* von Nordmann, 1832, or adult generalist nematodes (*Camallanus lacustris* Zoega, 1776, *Camallanus truncatus* Rudolphi, 1814), reflecting their low prevalence in the environment may, therefore, explain their absence in the parasite communities of non-native gobies, though all four parasite species are known to infect gobies in either their native or non-native range, or both (Kvach & Ondračková 2021). In contrast, the prevalence of, for example, the adult trematode *Bunodera luciopercae* Müller, 1776, a parasite of the intestinal tract of percid, salmonid, esocid and cyprinid fishes (WoRMS 2021), occurred at high prevalence and abundance in perch from the Rivers Rhine and the River Vistula. This parasite species was not found in any goby species, despite some Ponto-Caspian gobies being known hosts of *B. luciopercae* in their non-native range in the Middle Danube and the River Vistula (round goby), or in the River Volga (gorlap goby *Ponticola gorlap* Iljin, 1949) (summarized in Kvach & Ondračková 2021). Fish become infected after ingestion of infected intermediate hosts, which may be various aquatic invertebrates including copepods, cladocerans, amphipods and larval ephemeropterans (summarized in Skorping 1981). Therefore, prey selection is another limiting factor affecting the acquisition of local parasite species infecting fish at the adult stage. Nevertheless, all invertebrate groups serving as intermediate hosts for *B. luciopercae* are preyed upon by gobies (e.g. Grabowska & Grabowski 2005, Borcherding et al. 2013, Brandner et al. 2013, Ondračková et al. 2019b), highlighting that other mechanisms have to be related to the absence of adult endoparasitic species shared between native and non-native species in our study.

Though monogenean parasites are usually host-specific (Whittington et al. 2000), their accidental switch from native hosts to Ponto-Caspian gobies cannot be completely excluded. For example, *G. gobii* was found to infect the monkey goby in Turkey (Ozturk et al. 2002), and the round goby was

accidentally infected with gyrodactylids specific to a benthic fish species, the stone loach *Barbatula barbatula* in its native Danubian range (Francová et al. 2011), indicating that host-specific parasites may occasionally be shared among phylogenetically distant but ecologically related hosts. Similarly, the accidental occurrence of co-introduced *G. proterorhini*, specific to Ponto-Caspian gobies, was found on a local host, the European perch. Nevertheless, its further successful development on a percid host is not expected, as experimentally confirmed by Ondračková (2016).

Regional variance

Parasite communities were significantly affected by sampling region, in contrast to the findings for the phylogenetic distance between host species. Many biological (biotic) and environmental (abiotic) factors are known to affect composition of parasite communities in freshwater ecosystems, including host age, size, diversity and/or density of potential host species (e.g. Poulin 1998, Hechinger & Lafferty 2005) as well as water temperature, salinity, habitat heterogeneity (e.g. Thieltges et al. 2010, Johnson et al. 2016), etc. Possibly, seasonal variance in occurrence of several parasite species (Chubb 1977, 1979, 1982) or environmental conditions (lentic *vs.* lotic waters; Dávidová et al. 2008), for example, may have contributed to higher similarity between the sympatric, though phylogeneticaly less related host species, in contrast to allopatric populations of the same species. Consequently, also acquisition of local parasites following the introduction of non-native species into a new area may reflect a range of biological and environmental factors (Colautti et al. 2004). Time since introduction is an important factor affecting parasite species richness in non-native host populations (e.g. Gendron et al. 2012, Paterson et al. 2012, Ondračková et al. 2015a, Kołodziej-Sobocińska et al. 2018). However, no such association was apparent in the present study (see Table 1), highlighting the importance of regional rather than temporal differences between these non-native goby populations.

Lower competence to local parasites is expected in invasions over geographical barriers (such as transoceanic invasions), where compatible parasite species are less common than, for example, in areas invaded within the same hydrological system. Nevertheless, similarly to Gendron et al. (2012) who observed decreased species richness in gobies introduced to North America in comparison to local fish species, relatively low parasite species richness in non-native hosts seems apparent even in their European range. The reduction of parasite species richness and, in some cases parasite abundance, was more obvious in rivers historically separated from the Ponto-Caspian region, i.e. the Rivers Rhine and Vistula (see Fig. S1-S6). Our results from both river systems correspond to other studies, showing low parasite load in non-native goby species (e.g. Kvach et al. 2014, Ondračková et al. 2015a, Hohenadler et al. 2019). Both these sites showed typical findings, with generally high parasite richness compared to a relatively depauperate parasite community in the River Morava, possibly reflecting real differences in the parasite fauna across the region. Of the 37 parasite taxa found, only seven were common to all three regions, including non-native *G. proterorhini* co-introduced with the goby hosts, with considerable differences in abundance and distribution among host species between sites. Since parasite species richness and prevalence often exhibit strong spatial variation across the range of a natural host (Karvonen et al. 2005), the acquisition of parasites by non-native hosts is also likely to vary across broad geographic scales (Sellers et al. 2015). This conclusion is in agreement with our results, showing that patterns of parasite acquisition by Ponto-Caspian gobies vary regionally, partially reflecting the historical connectivity of the new environment with the native goby range, as well as local parasite diversity.

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Supplementary online material

Fig. S1. Inter-host differences in parasite abundance from three distant river basins. Hosts (green – native, blue – non-native), parasite abundances that do not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points. GG = *Gobio gobio*, PF = *Perca fluviatilis*, PM = *Proterorhinus semilunaris*, NM = *Neogobius melanostomus*, GC = *Gymnocephalus cernua*, NK = *Ponticola kessleri*, NF = *Neogobius fluviatilis*, NG = *Babka gymnotrachelus*.

Fig. S2. Inter-host differences in local parasite abundance from three distant river basins. Hosts (green – native, blue – non-native), parasite abundances that do not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points.

Fig. S3. Inter-host differences in parasite richness from three distant river basins. Hosts (green – native, blue – non-native), parasite richness that does not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points.

Fig. S4. Inter-host differences in abundance of specialist parasites from three distant river basins. Hosts (green – native, blue – non-native), parasite abundances that do not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points.

Fig. S5. Inter-host differences in prevalence of adult generalist parasites from three distant river basins. Hosts (green – native, blue – non-native), parasite prevalances that do not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points.

Fig. S6. Inter-host differences in abundance of larval generalist parasites from three distant river basins. Hosts (green – native, blue – non-native), parasite abundance that does not differ significantly within the same basin share the same lower case letter. Median = horizontal bar, interquartile range = box, non-outlier range (1.5 × interquartile range) = whiskers, outliers = points.

(https://www.ivb.cz/wp-content/uploads/JVB-vol.-70-4-2021-Ondrackova-et-al.-Fig.-S1-S6.pdf)

Table S1. Estimates of evolutionary divergence over sequence pairs between groups. Accession numbers of cytochrome *b* sequences derived from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) for fish hosts used for calculation of phylogenetic distance (https://www.ivb.cz/wp-content/uploads/JVB-vol.-70-4-2021-Ondrackova-et-al.-Tables-S1.xlsx).