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L. Gettova, A. Gilles, A. Simkova

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Metazoan parasite communities: support for the biological invasion of *Barbus barbus* and its hybridization with the endemic *Barbus meridionalis*

L. Gettová 1*, A. Gilles 2 and A. Šimková 1

**Abstract**

**Background:** Recently, human intervention enabled the introduction of *Barbus barbus* from the Rhône River basin into the *Barbus meridionalis* habitats of the Argens River. After an introduction event, parasite loss and lower infection can be expected in non-native hosts in contrast to native species. Still, native species might be endangered by hybridization with the incomer and the introduction of novel parasite species. In our study, we aimed to examine metazoan parasite communities in *Barbus* spp. populations in France, with a special emphasis on the potential threat posed by the introduction of novel parasite species by invasive *B. barbus* to local *B. meridionalis*.

**Methods:** Metazoan parasite communities were examined in *B. barbus*, *B. meridionalis* and their hybrids in three river basins in France. Microsatellites were used for the species identification of individual fish. Parasite abundance, prevalence, and species richness were compared. Effects of different factors on parasite infection levels and species richness were tested using GLM.

**Results:** Metazoan parasites followed the expansion range of *B. barbus* and confirmed its introduction into the Argens River. Here, the significantly lower parasite number and lower levels of infection found in *B. barbus* in contrast to *B. barbus* from the Rhône River supports the enemy release hypothesis. *Barbus barbus × B. meridionalis* hybridization in the Argens River basin was confirmed using both microsatellites and metazoan parasites, as hybrids were infected by parasites of both parental taxa. Trend towards higher parasite diversity in hybrids when compared to parental taxa, and similarity between parasite communities from the *Barbus* hybrid zone suggest that hybrids might represent "bridges" for parasite infection between *B. barbus* and *B. meridionalis*. Risk of parasite transmission from less parasitized *B. barbus* to more parasitized *B. meridionalis* indicated from our study in the Argens River might be enhanced in time as higher infection levels in *B. barbus* from the Rhône River were revealed. Hybrid susceptibility to metazoan parasites varied among the populations and is probably driven by host-parasite interactions and environmental forces.

**Conclusions:** Scientific attention should be paid to the threatened status of the endemic *B. meridionalis*, which is endangered by hybridization with the invasive *B. barbus*, i.e. by genetic introgression and parasite transmission.

**Keywords:** Cyprinid fish, Biological invasion, Hybridization, Metazoan parasite communities

* Correspondence: gettova@mail.muni.cz
1Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

Full list of author information is available at the end of the article

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Background
Concerns over the ecological implications of the introduction of an alien species into new environments are increasing. From the conservation point of view, there is elevated apprehension if the native species represents an endemic or endangered species, which is often characterized by small population sizes, fragmented distribution and low genetic variability (e.g. [1, 2]). A new invader may, therefore, represent a serious problem for the resident if it predates the indigenous species, exploits the same resources, or alters its native habitat [3–6]. Following the introduction event, many native species are endangered through hybridization with the closely related alien species [7–9]. At the same time, the new invader serves as a source of novel parasites to which the local species may display a different degree of susceptibility [10, 11]. Consequently, exposing susceptible local hosts to new parasite species carried by introduced individuals may result in accelerated mortality in native populations. For instance, the parasitic nematode Anguillicoloides crassus was imported to Europe probably as a result of the introduction of the Japanese eel Anguilla japonica and was, subsequently, disseminated in the populations of the European eel Anguilla anguilla (reviewed in [12]). While A. crassus is not highly pathogenic in the Japanese eel probably due to low-intensity infection rates [13], high infection and more serious pathology connected with high mortalities may be detected in wild European eels [14–17].

Populations of invaders established in new habitats typically exhibit fewer parasite species, and a smaller number of host individuals are parasitized (i.e. there is a lower prevalence of infection) when compared to the source populations [18]. This could be the result of the introduction of a restricted number of individuals carrying only a proportion of the original parasite fauna, new and unsuitable environmental conditions for parasites, and the absence or low abundance of suitable hosts required for the parasite life cycles [19, 20]. Such a release from co-evolved parasites may therefore provide an advantage for the performance of a novel host species in new habitats [21]. However, this advantage is often of a temporal nature and parasite species richness and prevalence rates can return to original levels or even be multiplied in a short time by transmission from the local hosts [22]. Co-evolutionary relationships which evolve between hosts and their parasites during their co-existence may, therefore, be steered by ecological forces [23]. Hybridization might even alter the composition of metazoan parasite communities of the two interacting host species, since hybrid individuals are often vulnerable to parasites infecting both parental species [24, 25] and may, therefore, represent “bridges” for parasite infection [26].

In France, two congeneric Barbus species co-exist in several rivers in the Mediterranean basin. The low level of mitochondrial DNA and allozyme variability indicates recent colonization of the French rivers by the common barbel Barbus barbus after the last glaciation [27, 28], where the Mediterranean barbel Barbus meridionalis was already present, probably from the Miocene [27]. Nowadays, the widely distributed European species B. barbus has been found in almost all French river basins and prefers medium-sized to large rivers. By contrast, the occurrence of endemic B. meridionalis is restricted to the Languedoc-Roussillon, Rhône-Alpes, and Provence-Alpes-Côte d’Azur regions in France [27], where it inhabits mainly upper and middle streams of mountain rivers, probably as a result of competition with B. barbus [29]. Nevertheless, hybridization between these two species has previously been reported from the Hérault, Garonne, Orb and Rhône river basins [30].

In our study, we aimed to examine the composition of metazoan parasite communities of B. barbus and B. meridionalis in (i) allopatric areas, (ii) sympatric areas of late origin of the Rhône River basin, and (iii) sympatric areas of recent origin of the Argens River basin. In the Argens River basin, we further intended to confront the metazoan parasite communities of parental taxa with those found in hybrids which resulted from the biological invasion of B. barbus into this watershed. The metazoan parasite abundance, prevalence and species richness in Barbus populations collected from the three areas were analyzed. We focused on the possible threat posed by the introduction of the widely distributed B. barbus to native and endemic B. meridionalis with respect to the transmission of non-native parasite species and the role of hybrids in facilitating parasite transmission between parental taxa.

Methods
Sample collection
From 2007 to 2014, 349 B. barbus (BB), B. meridionalis (BM) and hybrid (H) individuals were collected in France from the allopatric BB and BM populations (site 1 on the Loire River basin and site 14 on the Argens River basin, respectively), the Rhône late sympatric populations (sites 2–3 on the River Ardèche and sites 4–8 on the River Durance of the Rhône River basin), and the Argens recent sympatric populations (sites 9–13 on the Argens River basin); (Fig. 1, Table 1). The full information on the site names and their coordinates are shown in Additional file 1: Table S1. To remove the effect of temporal variation, fish were sampled only in the summer period (i.e. July-August) when the highest parasite diversity and high abundance of many
Metazoan parasite species are expected. The water temperature was measured (in °C) in each locality. Fish were measured (standard body length in mm), transported to the laboratory, and subsequently examined for metazoan parasites.

Microsatellite genotyping of fish individuals

Microsatellite markers developed for the *Barbus* species [34–36] were used to identify the fish species in our study. Genomic DNA was isolated from fin clip samples stored in 96% ethanol using DNeasy Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany). Further diluted DNA (approx. 10 ng/l) served as a template in the following multiplex PCR analysis of microsatellite loci following the protocol described in [34]. Amplicons were analyzed on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) using 500LIZ® Size Standard (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems), and genotypes were finally scored using GeneMapper Software version 4.0 (Applied Biosystems). Due to the tetraploid genome of the investigated *Barbus* spp., only selected microsatellite loci with probable disomic inheritance [34] were applied in our study.

The MICRO-CHECKER program [37] was used to check for microsatellite null alleles in the *Barbus* populations and, subsequently, locus Barb21 was excluded from our study. Overall, 19 loci (Barbus2, Barbus22, Barbus26, Barbus28, Barbus31, Barbus32, Barbus36, Barbus37, Barbus40, Barbus41, Barbus47, Barbus49, Barbus50, Barbus62, Barbus63, Barbus65, Barb59 and Barb79) were used afterwards following a Bayesian clustering approach implemented in the STRUCTURE software [38]. The program was run for five independent runs assuming an admixture model and the model of correlated allele frequencies, using 1,000,000 iterations after a burn-in period of 100,000 iterations for K = 2 clusters. The Introgress package [39] implemented in the R statistical software was used to calculate the hybridization index (h-index) in sympatric populations. Allopatric populations of the Loire River and the Argens River basins (i.e. sites 1 and 14) were set in this software as parental BB and BM populations, respectively. First, the interspecific differentiation index (D) between allelic frequencies in parental populations was computed. Subsequently, 14 microsatellites with D ≥ 0.80 (Barbus22, Barbus26, Barbus31, Barbus32, Barbus37, Barbus47, Barbus49, Barbus50,
Barbus56, Barbus62, Barbus65, Barbus63, Barb59 and Barb79) were selected for estimation of the h-index in sympatric populations, as applied in Andrés et al. [40]. In general, the resultant h-indices of zero and one should be used to determine pure individuals. Since the occurrence of BB in the Argens River basin is a result of introduction from the River Durance, we applied two approaches for BB designation (i) using an h-index of zero and (ii) using an h-index up to 0.11. As similar results were obtained using both approaches (Additional file 2: Table S2; Additional file 3: Table S3; Additional file 4: Table S4), we presented only the results when the individuals with an h-index up to 0.11 were treated as BB and those with an h-index between 0.11 and 1 were treated as H individuals.

Quantitative and qualitative comparisons of metazoan parasite communities

Fish dissection was performed following Ergens & Lom [41]. In our study, fins, gills, eyes, heart, kidney, spleen, hepatopancreas, intestine, gonads, gall-bladder, and swim bladder were examined for all metazoan parasites using a stereo microscope Olympus SZX7. Parasites were fixed as described in Lamková et al. [42] and, subsequently, identified using a light microscope (Olympus BX50) equipped with phase-contrast, differential interference contrast, and Olympus Stream Motion 1.9.2 digital image analysis software. Parasites were identified using the available identification keys and publications providing keys to the identification of metazoan parasites, e.g. [43–46]. Measures of parasite infection, i.e. prevalence and abundance of metazoan parasites were calculated according to Bush et al. [47]. The individual abundance of the myxozoan parasites was not taken into consideration (because these parasites cannot be quantified as in the case of other metazoan parasites) and only the prevalence of this parasitic group was taken into account in the analyses. The effect of sampling effort on parasite species richness was corrected using the Chao1 estimator [48], and was calculated using the EstimateS program [49] on the basis of abundance data excluding data on myxozoan parasites. Similarities between metazoan parasite communities based on presence/absence data (Jaccard index) were computed in PAST [50].

Table 1

| Locality          | Population | n  | T° | Year       | Abundance | Chao1     | Averaged prevalence |
|-------------------|------------|----|----|------------|-----------|-----------|---------------------|
| Loire River       | BB         | 21 | 22.7 | 2008; 2012 | 147.47 ± 151.26 | 12.23 ± 3.79 | 10.77 ± 23.78       |
| Rhône River       | BB         | 13 | 23  | 2012       | 302.92 ± 444.28 | 9.02 ± 1.91 | 11.94 ± 24.70       |
|                   | BB         | 27 | 18  | 2010       | 143.74 ± 254.17 | 13.86 ± 3.01 | 12.57 ± 21.84       |
|                   | BB         | 30 | 20.2 | 2011; 2012 | 184.50 ± 97.24 | 12.58 ± 2.09 | 15.53 ± 28.20       |
|                   | BB         | 27 | 19.9 | 2007; 2010 | 193.44 ± 115.18 | 14.23 ± 2.67 | 16.67 ± 28.05       |
|                   | BB         | 15 | 19.4 | 2010; 2011 | 148.67 ± 113.08 | 10.89 ± 2.81 | 14.39 ± 27.40       |
|                   | BB         | 12 | 21.6 | 2010       | 148.50 ± 190.40 | 12.59 ± 2.42 | 15.35 ± 25.45       |
|                   | BB         | 21 | 17.3 | 2010; 2011 | 164.67 ± 115.30 | 12.11 ± 2.80 | 14.66 ± 29.03       |
| Argens River      | BB         | 14 | 18.8 | 2013; 2014 | 17.86 ± 19.80  | 5.21 ± 1.15  | 8.08 ± 20.81        |
|                   | BM         | 13 |      |            | 20.85 ± 11.90  | 6.72 ± 1.16  | 9.71 ± 21.43        |
|                   | H          | 14 |      |            | 67.21 ± 122.70 | 8.58 ± 2.70  | 9.58 ± 21.29        |
|                   | BB         | 2  | 18.7 | 2013       | 31.00 ± 21.21  | 4.27 ± 1.03  | 10.53 ± 26.40       |
|                   | H          | 21 |      |            | 61.38 ± 125.08 | 8.79 ± 2.03  | 8.39 ± 18.96        |
|                   | BM         | 29 | 17.5 | 2007; 2012; 2014 | 166.45 ± 311.53 | 9.79 ± 2.22 | 9.80 ± 22.41        |
|                   | H          | 17 |      |            | 74.12 ± 117.09 | 10.12 ± 3.17 | 9.90 ± 21.10        |
|                   | BB         | 9  | 17.3 | 2007       | 140.56 ± 119.36 | 8.36 ± 1.50 | 13.16 ± 27.44       |
|                   | H          | 10 |      |            | 89.00 ± 95.82  | 9.39 ± 2.90  | 10.79 ± 21.23       |
|                   | H          | 19 | 16.5 | 2013       | 26.89 ± 31.35  | 6.16 ± 1.40  | 6.65 ± 17.46        |
|                   | BM         | 39 | 15.9 | 2013; 2014 | 356.08 ± 898.61 | 12.63 ± 1.82 | 12.89 ± 21.48       |

*T°: mean water temperature in °C
Statistical analyses
Spearman’s rank correlation was computed between individual admixture q-values and h-index obtained by STRUCTURE software and Introgress package software, respectively. Metazoan parasite abundance and averaged prevalence were log-transformed prior to statistical analyses. Kolmogorov-Smirnov test was used for normality data assessment. Subsequently, Bonferroni post-hoc tests following General Linear Model (GLM) were used to compare the estimated marginal means of total abundance, prevalence and species richness adjusted for fish body length, water temperature, and sampling years between BB, BM and H from different river basins. Since all fish individuals were infected with at least one parasitic species (i.e. the overall prevalence was 100% in each fish population), the average of prevalences for each parasite species across the fish populations was used in our study (further referred to as averaged prevalence).

For the River Argens, GLM analyses were conducted to investigate the potential effects of different factors, i.e. host (BB, BM or H), locality (site), sampling year, water temperature, and host body length on the abundance, averaged prevalence, and species richness of metazoan parasites found in Barbus individuals.

Results
Genetic composition of Barbus spp. populations
Based on microsatellite markers, STRUCTURE analysis confirmed the existence of one allopatric BB population in the Loire River basin (Site 1) and one allopatric BM population in the Argens River basin (Site 14). A low level of admixture between the populations of two Barbus species was revealed in the Rhône River basin (q < 0.12). In contrast, the extent of population admixture in the Argens River basin was high except for the abovementioned the Argens allopatric BM population (Fig. 2). The values of h-index obtained by the Introgress software package resembled the overall picture of the individual admixture obtained by STRUCTURE (Fig. 2) and correlated significantly with the q-values (Spearman’s rank correlation, \( r_{373} = 0.97, P < 0.001 \)). An h-index up to 0.11 was revealed in populations of the Rhône River basin. Using this value as an upper limit for B. barbus individuals, 25 B. barbus, 42 B. meridionalis and 81 hybrids were detected within the Argens River basin (i.e. a system with a very recent introduction of B. barbus), where we considered Barbus populations with the occurrence of hybrids as sympatric populations. In the Argens River basin, the co-existence of both pure species was documented only in Site 9. The Barbus sample originating from Site 13 was composed entirely of hybrid individuals (Table 1, Fig. 2).

Composition of metazoan parasite communities in Barbus spp. populations from different river basins
Examination of the studied Barbus spp. populations for metazoan parasites revealed the presence of parasites belonging to different parasitic groups (Myxozoa, Trematoda, Monogenea, Cestoda, Nematoda, Acanthocephala, Mollusca, Chelicerata and Crustacea). The prevalence and mean abundance of each metazoan parasite species are listed in Table 2. Detailed information on the composition of parasite communities per site is shown in Additional file 5: Table S5. Monogeneans (especially Dactylogyrus spp.) were the most dominant groups of parasites in the Loire allopatric BB and the Rhône late sympatric BB, while they were rare in the Argens recent sympatric BB. Above all, monogeneans and acanthocephalans (specifically Gyrodactylus spp. and Pomphorhynchus tereticollis, respectively)
|                    | Loire allopatric BB | Rhône late sympatric BB | Argens recent sympatric BB | Argens recent sympatric H | Argens recent sympatric BM | Argens allopatric BM |
|--------------------|---------------------|-------------------------|-----------------------------|---------------------------|-----------------------------|---------------------|
| **A ± SD**         |                     |                         |                             |                           |                             |                     |
| **P**              |                     |                         |                             |                           |                             |                     |
| Myxozoa            |                     |                         |                             |                           |                             |                     |
| Myxobolus spp.     | –                   | 38                      | –                           | 60                        | –                           | 28                  |
| Trematoda          |                     |                         |                             |                           |                             |                     |
| Allocotremum isoporum | 0.14 ± 0.36      | 14                      | –                           | –                         | –                           | 2.27 ± 8.25         |
| Apharyngostrigea sp. | –                   | –                       | 0.26 ± 3.07                 | 1                         | 0.28 ± 0.89                 | 0.16 ± 1.44         |
| Clonostomum complanatum | –                   | –                       | 0.39 ± 2.77                 | 12                        | –                           | 0.12 ± 0.53         |
| Diplomastomum spp. | 0.14 ± 0.65        | 5                       | 1.87 ± 6.29                 | 38                        | 0.80 ± 1.53                 | –                   |
| Echinostomatidae gen. sp. | 0.24 ± 1.09   | 5                       | –                           | –                         | –                           | –                   |
| Digenea fam. gen. spp. | 0.05 ± 0.22      | 5                       | 0.19 ± 2.16                 | 1                         | –                           | –                   |
| Holostephanus sp.  | –                   | –                       | 1.68 ± 4.13                 | 24                        | 0.35 ± 1.69                 | 0.02 ± 0.22         |
| Tylodelphys sp.    | –                   | 0.10 ± 0.53             | 5                           | –                         | –                           | 1                   |
| Monogenea          |                     |                         |                             |                           |                             |                     |
| Dactylogyrus sp.   | –                   | –                       | –                           | –                         | –                           | 0.03 ± 0.16         |
| Dactylogyrus extensus | –                   | –                       | –                           | –                         | –                           | 0.07 ± 0.38         |
| Dactylogyrus carpathicus | 3.19 ± 14.39   | 10                      | 57.67 ± 76.65               | 79                        | –                           | 0.05 ± 0.22         |
| Dactylogyrus malleus | 81.95 ± 88.38    | 100                     | 70.14 ± 160.06              | 90                        | 0.60 ± 2.40                 | 0.16 ± 0.70         |
| Gyrodactylus hemibarbi | –                   | –                       | 0.34 ± 0.97                 | 17                        | 0.12 ± 0.43                 | 0.63 ± 1.50         |
| Gyrodactylus katharineri | –                   | –                       | 0.06 ± 0.27                 | 6                         | 0.92 ± 1.63                 | 0.67 ± 1.10         |
| Gyrodactylus markewitschi | 3.48 ± 10.53   | 33                      | 20.59 ± 87.32               | 34                        | 1.12 ± 5.40                 | 17.96 ± 80.32       |
| Gyrodactylus sprostonae | –                   | –                       | –                           | –                         | –                           | 5.91 ± 50.59        |
| Paradiplazoon holoion | –                   | –                       | 0.23 ± 1.02                 | 12                        | 0.04 ± 0.02                 | 0.04 ± 0.19         |
| Cestoda            |                     |                         |                             |                           |                             |                     |
| Bathybothrium rectangulum | 0.90 ± 3.06      | 19                      | 1.08 ± 3.64                 | 16                        | 6.6 ± 15.52                 | 2.02 ± 9.15         |
| Schyzocotyle aehelognathi | –                   | –                       | 0.06 ± 0.37                 | 3                         | –                           | 1.17 ± 2.25         |
| Caryophyllaeus braichycollis | –                   | –                       | 0.01 ± 0.12                 | 1                         | 7.48 ± 22.63                | 2.02 ± 9.15         |
| Proteocephalus torulosus | –                   | –                       | –                           | –                         | –                           | 0.75 ± 3.43         |
| Nematoda           |                     |                         |                             |                           |                             |                     |
| Contracaecum sp.   | –                   | –                       | 0.30 ± 2.15                 | 8                         | –                           | 0.05 ± 0.31         |
| Pseudocapillaria tomentosa | 1.00 ± 4.15      | 10                      | 0.47 ± 2.02                 | 10                        | 0.08 ± 0.28                 | 2.02 ± 2.37         |
| Rhabdolchona hellichi | 53.90 ± 67.03    | 100                     | 14.99 ± 22.15               | 72                        | 2.56 ± 4.55                 | 2.63 ± 9.03         |
| Metazoan parasite                      | Mean abundance (A) ± standard deviation (SD) | Prevalence (P, in %) |
|--------------------------------------|---------------------------------------------|---------------------|
| Nematoda fam. gen. sp. 1             | --                                          | --                  |
| Nematoda fam. gen. sp. 2             | --                                          | 1.01 ± 12.04        |
| Nematoda fam. gen. sp. 3             | --                                          | --                  |
| Acanthocephala                       |                                             |                     |
| Acanthocephalus anguillae            | 0.05 ± 0.22                                  | 0.25 ± 2.04         |
| Acanthocephala fam. gen. spp.       | --                                          | 0.03 ± 0.26         |
| Pomatophyra tereticolis              | 1.67 ± 4.86                                  | 0.79 ± 23.56        |
| Mollusca                             |                                             |                     |
| Anodonta spp.                        | 0.52 ± 0.68                                  | 0.15 ± 0.69         |
| Crustacea                            |                                             |                     |
| Argulus coregoni                     | --                                          | 0.01 ± 0.08         |
| Ergasilus sieboldi                   | 0.43 ± 1.96                                  | 1.32 ± 5.06         |
| Tracheliastes polycolpus             | --                                          | 0.17 ± 0.68         |
| Chelicerata                          |                                             |                     |
| Hydrorhazetes sp.                    | --                                          | 0.01 ± 0.11         |
were dominant in the Argens recent sympatric BM and H. In a single Argens allopatric BM population, nematodes represented the most dominant and abundant parasite group (Table 2, Fig. 3).

**Similarity in metazoan parasite communities between *Barbus* spp. populations**

High Jaccard index value was found between the Argens recent sympatric BB and H individuals (0.71), while lower similarity in parasite communities (0.57) was found between the Argens recent sympatric BM and H. Parasite communities in the Argens sympatric BB were more similar to parasite communities in the Rhône late sympatric BB (0.52) than to those in the Loire allopatric BB (0.43). The parasite communities in the Argens recent sympatric BB and the Argens recent sympatric BM were more similar (0.50) than those in the Argens recent sympatric BB and the Argens allopatric BM (0.43; Table 3).

**Total abundance, averaged prevalence and species richness of metazoan parasites in *Barbus* spp. populations**

Significant differences in abundance, averaged prevalence, and species richness (GLM, abundance: whole model $F_{(4,340)} = 18.05$, $P < 0.001$; averaged prevalence: whole model $F_{(4,1241)} = 1.70$, $P = 0.029$; species richness: whole model $F_{(4,340)} = 23.24$, $P < 0.001$) of metazoan parasites between *Barbus* groups were revealed (see Additional file 3: Table S3 and Additional file 4: Table S4 for detailed statistics). After controlling for the covariates, Bonferroni post-hoc tests revealed no significant differences in metazoan parasite abundance and averaged prevalence between the Loire allopatric BB and the Rhône late sympatric BB ($P > 0.05$). However, significantly lower species richness was revealed in the Rhône late sympatric BB than in the Loire allopatric BB ($P = 0.008$). Significantly lower values of abundance and species richness ($P < 0.001$), and lower but not significantly different averaged prevalence ($P > 0.05$) of metazoan parasites were found in the Argens recent sympatric BB in comparison with the Rhône late sympatric BB. In the Argens River basin, significantly lower metazoan abundance ($P < 0.001$) and species richness ($P = 0.004$), and lower but not significantly different averaged prevalence ($P > 0.05$) were found in BB when compared to BM. Metazoan parasite abundance, averaged prevalence, and species richness in H tended to be intermediate between pure species of the Argens River basin. However, statistically significant difference was only revealed in the case of metazoan parasite abundance between H and BM ($P < 0.001$); (Fig. 4, Additional file 4: Table S4).

**Factors influencing parasitism in the *B. barbus* × *B. meridionalis* recent hybrid zone**

In the Argens River basin, significant effects of locality ($F_{(5,174)} = 11.42$, $P < 0.001$), sampling year ($F_{(3,174)} = 7.99$, $P < 0.001$), water temperature ($F_{(1,174)} = 6.33$, $P = 0.013$), and fish length ($F_{(1,174)} = 5.32$, $P < 0.05$) on metazoan parasite abundance were revealed, while effect of host was not significant in GLM (whole model $R^2 = 0.45$, $F_{(12,174)} = 11.55$, $P < 0.001$). Host ($F_{(2,174)} = 3.85$, $P = 0.012$), locality ($F_{(5,124)} = 20.87$, $P < 0.001$), sampling year ($F_{(3,174)} = 20.22$, $P < 0.001$), water temperature ($F_{(1,174)} = 5.32$, $P = 0.030$), and

![Fig. 3 Metazoan parasites in *Barbus* spp. populations. Proportions of parasite groups in metazoan parasite communities in the populations of *B. barbus* (BB), *B. meridionalis* (BM) and their hybrids (H) from the three river basins](image-url)
fish length ($F_{(1,174)} = 4.16, P = 0.020$) significantly affected the species richness of metazoan parasites (whole model $R^2 = 0.74, F_{(12,174)} = 32.88, P < 0.001$). Neither host, locality, sampling year, water temperature, nor host body length affected significantly averaged prevalence of metazoan parasites (whole model $R^2 = 0.02, F_{(12,709)} = 1.01, P = 0.442$).

**Discussion**

This study explored composition of metazoan parasite communities in *Barbus* spp. populations of three river basins. No significant differences in the intensity of metazoan parasite infection was revealed between the Rhône late sympatric BB and the Loire allopatric BB. At the same

**Table 3** Jaccard similarity indices for metazoan parasite communities in *Barbus* spp. populations

|                | Loire allopatric BB | Rhône late sympatric BB | Argens recent sympatric BB | Argens recent sympatric H | Argens recent sympatric BM | Argens allopatric BM |
|----------------|---------------------|-------------------------|-----------------------------|---------------------------|---------------------------|----------------------|
| Loire allopatric BB | –                   | –                       |                             |                           |                           |                      |
| Rhône late sympatric BB | 0.46                | –                       |                             |                           |                           |                      |
| Argens recent sympatric BB | 0.43                | 0.52                    |                             |                           |                           |                      |
| Argens recent sympatric H  | 0.38                | 0.52                    | 0.71                        | –                         |                           |                      |
| Argens recent sympatric BM | 0.36                | 0.37                    | 0.50                        | 0.57                      | –                         |                      |
| Argens allopatric BM      | 0.30                | 0.28                    | 0.43                        | 0.44                      | 0.58                      | –                    |

*Abbreviations: BB, *B. barbus*; BM, *B. meridionalis*; H, hybrids*
time, lower number of parasitic taxa was revealed in the Rhône late sympatric BB than in the Loire allopatric BB. Low introgression of BM revealed by microsatellite markers in the Rhône late sympatric BB, therefore, does not provide a disadvantage in terms of high levels of parasite infection or high species richness in contrast to the Loire allopatric BB. Yet, a degree to which parasites have a negative impact on host vigour and fitness components is dependent on a particular parasite species, parasite genotype, or co-infection with other parasite species [51, 52]. This is, however, beyond the scope of our study. Several parasite species were shared between the Loire allopatric BB, the Rhône late sympatric BB, and the Argens recent sympatric BB, which indicates that these parasite species followed the expansion of BB. On the other hand, we detected considerably lower diversity and intensity of infection of metazoan parasites in the Argens recent sympatric BB in contrast to the Rhône late sympatric BB. Still, all parasite species except for *Holostephanus* sp. found in our study in the Argens recent sympatric BB were also present in the Rhône late sympatric BB (Table 2). These findings support the view that BB of the Argens River basin originates from the Durance River system. However, parasite species infecting the Argens recent sympatric BB represented only a small proportion of the metazoan parasite fauna found in the Rhône late sympatric BB (Table 2). The enemy release hypothesis suggests that individuals introduced outside their natural ranges may benefit from enemy release, e.g. predators or pathogens [53–55]. Our results concerning the significantly lower levels of parasite infection and lower number of metazoan parasites in the Argens recent sympatric BB when compared to the Rhône late sympatric BB are, therefore, in congruence with the general scenario of parasite loss after host introduction into a new environments [18]. Kennedy & Bush [56] revealed that the parasite communities of native *Onchorhyncus mykiss* populations were dominated by specialist helminth parasites, while the number of specialist helminths declined with the increasing distance of translocated host populations from their original heartland. In fish, monogeneans are considered to be the most host-specific parasites [57]. In our study, five of six monogenean species documented in the Rhône late sympatric BB were found also in the Argens recent sympatric BB. However, the absence of *Dactylogyrus carpathicus*, a highly abundant parasite species of the Rhône late sympatric BB, and the decrease in the abundance and prevalence of *Dactylogyrus malleus* in the Argens sympatric BB resulted in a shift in parasite communities from the dominance of monogenean parasites to a higher proportion of endoparasitic groups in total parasite numbers. Ondráčková et al. [22] showed that the reduction in parasite numbers is dependent on the time after a colonization event and, therefore, a very late arrival of *B. barbus* into the Argens River tributaries can be expected. The first events involving the migration of BB from the River Durance to the waters of the Argens River system probably occurred in the period 1980–1990 [32]. Since an upstream migration range of up to several dozens of kilometres has been documented for BB individuals [58], colonization of the Argens River tributaries might thus have taken place at the turn of this century or even more recently.

Parasites often exhibit a shorter generation time, larger population size, and higher migration and mutation rates than their hosts. Consequently, as a result of co-evolutionary host-parasite interactions, local parasite adaptation, i.e. the better performance of parasites in their local hosts than in foreign ones, is expected [59]. Individuals introduced into novel areas may, therefore, benefit from the higher ability of parasites to adapt to their local hosts. By contrast, lower ability of parasites to infect their local hosts than alien ones or no differences in degrees of host resistance/susceptibility to parasites between local and non-indigenous hosts (i.e. no local adaptation), were previously documented [60, 61]. In our study, with respect to the Argens River basin, the lower parasite diversity and lower parameters of metazoan parasite infection found in introduced BB when compared to the local BM (Fig. 4) indicate that parasites are adapted to their local BM populations. On the other hand, elevated metazoan parasite infection in native BM might be a result of parasite transmission from introduced BB. Out of all the parasite species that infected both the Rhône late sympatric BB and the Argens recent sympatric BB individuals, and might, therefore, have been carried along with host introductions, almost 70% were also found in BM (Table 2). To our knowledge, and with the exception of this study, no investigation of metazoan parasite communities in fish from the Argens River system has so far been undertaken. We, therefore, cannot exclude the possibility that the parasite species that we reported from both BB and BM were already present in the Argens River basin before the introduction of BB. *Myxobolus* spp. parasites as well as endoparasites such as *Pseudopapillaria tomentosa*, *Caryophyllaeus brachycollis* and *Pomphorhynchos tereticollis*, or parasites belonging to the genus *Apharyngostigrea*, infect a wide range of fish hosts beside barbels [62–65]. On the other hand, *Bathybothrium rectangulum* and *Rhabdochona hellichi*, which are commonly found in barbels and rarely found in other fish [57], were already reported from both BB and BM [62, 65]. Similarly, *Gyrodactylus katharineri*, *G. hemibarbi* and *G. markewitchi* found in both *Barbus* species in our study, have already been documented from other cyprinids besides barbels, such as *Cyprinus carpio*, *Alburnus alburnus*, *Gobio gobio*, *Leuciscus cephalus* and *Gymnocephalus cernua* [66–68]; these cyprinids were already reported from the Argens River basin [33]. Finally, in our study, the monogenean parasite *Paradiplazoon*
f  h o s t  n  u m b e ro f  p a r a s i t e  s p e c i e s  w h i c h  species  was  present,  hybrid  individuals  tended  to  be  parasit-
ized  (Fig. 4).  However,  in  localities  where  at least  one  parental  species  was  present,  hybrid  individuals  tended  to  be  parasit-
ized  by  more  parasite  species  (Tables 1 and 2).  Moreover,  the  effect  of  host  on  the  number  of  parasite  species  which
infected  Barbus  populations  was  confirmed  by  the  results  of  GLM  analysis  and  may  be  a  result  of  differences  in  host  ethnology  and  ecological  preference  [69].  Thus,  our  findings  suggest  that  hybrids  represent  “bridges”  for  parasite  infec-
tion  between  invasive  and  endemic  species.  From  this  point  of  view,  it  seems  that  BB  represents  a  potential  threat  to  local  BM  in  terms  of  the  transmission  or  increased  impact  of  metazoan  parasites  on  local  BM  via  hybridization.  In  fact,  all  parasites  that  infected  both  parental  taxa  were  also  detected  in  hybrids  of  the  Argens  River  basin.  Conse-
quently,  we  may  conclude  that  metazoan  parasites  repre-
sent  important  biomarkers  of  BB  and  BM  hybridization  in  the  Argens  River  basin,  as  was  also  reported  for  Dactylo-
gyrus  parasites  in  Alburnus  alburnus  ×  Rutillus  rutulus  hy-
brids  [26].  Šimková  et  al.  [25]  reported  that  Cyprinus  carpio  ×  Carassius  gibelio  hybrids  were  also  parasitized  by  a  greater  variety  of  parasite  species  than  pure  hosts;  however,  they  remained  less  susceptible  to  metazoan  parasites,  which  could  be  a  result  of  so-called  hybrid  vigour  [73].  In  our  study,  hybrids  displayed  higher  fitness  overall  than  BM  in  terms  of  lower  parasite  abundance  and  resembled  BB  more  in  this  respect  (Fig. 4).  Slightly  greater  similarity  in  parasite  communities  was  also  revealed  between  H  and  invasive  BB  than  between  H  and  endemic  BM,  based  on  presence/absence  data.  Phillipart  &  Berrebi  [74]  showed  that  experimental  crossing  between  fe-
male  BB  and  male  BM  resulted  in  F1  hybrids  with  a  similar  size  structure  to  BB  and  favored  female  F1  hybrids.  As  a  result,  the  maternal  effect  may  influence  offspring  (in  our  case,  hybrid)  susceptibility  to  parasites  [75].  Yet,  hybrid  in-
dividuals  displayed  different  levels  of  susceptibility  to  meta-
zoan  parasites  (i.e.  higher  or  lower  abundance),  in  contrast  to  the  parental  taxa  in  localities  where  parental  species  were  also  present  (Table 1).  These  findings  are  consistent  with  the  results  of  GLM,  which  showed  no  effect  of  host  on  metazoan  parasite  abundance,  while  significant  effects  of  host  body  size,  locality,  sampling  year,  and  water  temperature  on  metazoan  parasite  abundance  were  demon-
strated.  Since  parasite  infection  in  hosts  is  unstable  in  the  time  and  space  driven  by  host-parasite  interactions  and  en-
vironmental  forces  [76–78],  our  findings  indicate  that  both  individual  host  characteristics  (i.e.  host  genotype)  and  envir-
onmental  factors  together  significantly  influence  spatio-
temporal  distribution  of  metazoan  parasite  communities  in  the  BB × BM  hybrid  system.  Since  metazoan  parasites  with  the  complex  life-cycle  constitute  a  substantial  fraction  of  metazoan  parasite  communities  in  Barbus  spp.  of  the  Argens  River  basin,  spatial  differences  in  diversity  of  para-
site  communities  found  in  our  study  might  be  also  partially  shaped  by  the  availability  and  abundance  of  inter-
mediate  hosts,  i.e.  the  presence  of  Nematode  sp.  3  in  the  Argens  allopatric  BM.  The  effect  of  intermediate  host  abundance  on  the  parasite  diversity  was  previ-
ously  shown  in  eels  [79].
Conclusions
On the basis of our results, we may conclude that metazoan parasites extend along the expansion range of invasive *B. barbus*. While similar levels of metazoan parasite infection were revealed in *B. barbus* of the Loire River basin (i.e. in the absence of *B. meridionalis*), and *B. barbus* of the Rhône River basin (i.e. where there is a low level of microsatellite introgression from *B. meridionalis*), *B. barbus* recently introduced into the Argens River was shown to profit from enemy release after its arrival from the River Durance by displaying lower susceptibility to metazoan parasites in contrast to the source populations. Concerning the Argens River basin, lower levels of parasite infection in populations of *B. barbus* in comparison to *B. meridionalis* and a similarity in metazoan parasite communities with those found in the Rhône River basin support the idea that the introduction of *B. barbus* into the watersheds of the River Argens from the River Durance is of very recent origin. The infection of hybrids by metazoan parasites found in both parental species in the River Argens supports the existence of hybridization between *B. barbus* and *B. meridionalis* and indicates that parasites along with molecular markers may be used as powerful tools for detecting recent hybridization events. The transmission of parasites via introgressive hybridization and higher parasite infection in *B. barbus* from the Loire River basin and the Rhône River basin indicated in our study may highlight the potential risk of non-native *B. barbus* having an increased disease impact on endangered *B. meridionalis*.

Additional files

- **Additional file 1**: Table S1. Site names and their geographical coordinates. (XLSX 10 kb)
- **Additional file 2**: Table S2. Metazoan parasite abundance, prevalence and species richness in *Barbus* spp. populations. (XLSX 11 kb)
- **Additional file 3**: Table S3. Results of ANCOVA tests. (XLSX 16 kb)
- **Additional file 4**: Table S4. P-values of Bonferroni post-hoc tests. (XLSX 13 kb)
- **Additional file 5**: Table S5. Metazoan parasite communities in *Barbus* spp. populations. (XLSX 21 kb)

Abbreviations
BB: *Barbus barbus*; BM: *Barbus meridionalis*; H: Hybrids; GLM: General linear model

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Availability of data and material
All datasets supporting the main conclusions are included within the manuscript and its additional files. Individual level data are available from the authors on reasonable request.

Authors’ contributions
AS designed and supervised the study; AS, AG and LG conducted the field study; LG performed the data analysis; LG wrote the manuscript; AS critically revised the draft. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
This study was approved by animal care and use committee of Masaryk University in Brno, Czech Republic (approval number CZ01308).

Author details
1Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlárská 2, 61137 Brno, Czech Republic. 2Aix-Marseille Université, IMBE, UMR CNRS 7263, Evolution Génome Environnement, Case 36, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France.

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