# Parasites of the invasive Chinese sleeper *Perccottus glenii* (Actinopterygii: Odontobutidae) in the region of the first introduction of the Carpathian population

by

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## Abstract

Parasites of the invasive Chinese sleeper (*Perccottus glenii*) were studied in five different localities at the site of the first introduction of the Carpathian population of this fish. We recorded eight taxa of parasites in Lviv water bodies. The monogenean *Gyrodactylus perccotti* and the cestode *Nippoteania perccotti* were recorded in all five lakes studied. These parasites represent Far East species introduced together with their host. Also the copepod *Neoergasilus japonicus* is a Far East parasite introduced to Europe with its aquaculture vector. Differences between the surveyed bodies of water were mainly related to their local seasonal conditions and watershed characteristics. The current data confirm the presence of co-introduced populations of *G. perccotti* and *N. perccotti*, which probably affects the future invasiveness of their host. Due to low acquisition of local parasites, we cannot confirm any significant effect of invasive fish on local parasite assemblages in this region. However, the presence of the non-indigenous copepod, *N. japonicus*, requires additional attention in the future.

**Key words:** biological invasions, non-indigenous species, range expansion, host–parasite interaction, fish parasites

# 1. Introduction

The Chinese sleeper, *Perccottus glenii* Dybowski, 1877 (Actinopterygii: Odontobutidae), is a fish species native to China, northwestern Korea, Russian Far East and northern Sakhalin (Mori 1936; Bogutskaya et al. 2008). It is considered invasive in Central and Eastern Europe, where its non-native range extends from the Volga River basin in the east to southern Germany in the west (Reshetnikov 2010, 2013; Reshetnikov, Ficetola 2011; Nehring, Steinhof 2015). A single find was reported from brackish waters of the North-Western Black Sea (Kvach et al. 2021). The main vectors of its spread include aquacultural fish stocks, as well as other anthropogenic introductions such as fish releases by aquarists and anglers using them as live bait, and intentional stocking to control the density of *Carassius* sp. or as prey for the northern pike *Esox lucius* L., 1758 (Reshetnikov 2004; Pupina et al. 2015; Kutsokon 2017; Grabowska et al. 2020).

The fish has negative impact on ecosystems in its non-native range, caused mostly by competition with local fauna and predation (Reshetnikov 2013; Rakauskas et al. 2016; Pupina et al. 2018), which could even lead to the elimination of the local fish fauna and the formation of monospecific communities (Kutsokon et al. 2021). Therefore, this fish has been listed as an Invasive Alien Species (IAS) of Union concern (European Commission 2017).

The Central European population of the Chinese sleeper shares the same haplotypes, originating from one of the few initial introductions from China into the Upper Dniester basin in Ukraine. From there, the species then spread to neighboring riverine basins, such as the Danube, the Dnieper, the Vistula and the Southern Bug basins (Grabowska et al. 2020). The history of this introduced population started in 1972, when the Chinese sleeper was introduced into the Velykyi Lubin fish farm (Dniester River basin) near Lviv, the Carpathian region of Ukraine (Fedoniuk 2005; Reshetnikov 2013; Kutsokon 2017). The population was therefore named the 'Carpathian population', as opposed to two others in Eastern Europe (Kvach et al. 2016a; Grabowska et al. 2020).

Parasites are commonly used as biological tags to distinguish fish populations (Mackenzie 1983; Catalano et al. 2014; Poulin, Kamiya 2015). Four stenoxenous parasite species, specific to shovel-snouts (family Odontobutidae), were co-introduced with the Chinese sleeper from Asia to Europe, e.g. the coccidian *Goussia obstinata* Sokolov & Moshu, 2014, the myxozoan *Henneguya alexeevi* Schulman, 1962, the monogenean *Gyrodactylus perccotti* Ergens & Yukhimenko, 1973 and the cestode *Nippotaenia perccotti* (Akhmerov, 1941)

(Košuthová et al. 2004; Sokolov et al. 2012; Ondračková et al. 2012; Sokolov, Moshu 2014). It was predicted that these parasites can be used as biological markers to analyze vectors and routes of expansion in ecosystems invaded by the Chinese sleeper (Reshetnikov et al. 2011). However, data from the site of the first introduction of the Carpathian population (Lviv region, Ukraine) were very sparse, limited to the gray literature publication of Maslovsky et al. (2010), who reported ciliates *Trichodina* sp. and digeneans *Crepidostomum farionis* (Müller, 1780) from the Vereshchytsia River (Dniester River basin). Thus, the objective of our study was to comprehensively investigate parasite communities of the Chinese sleeper at the site of the first introduction of the Carpathian population.

# 2. Materials and methods

Sampling was conducted in five localities of the Lviv region in Ukraine in two different drainage basins (the Dniester and the Bug) in September–October 2017. A triangular trap (72 x 73 x 80 cm) with a 6 mm net mesh was used for sampling. In the Bug River basin (part of the Vistula drainage basin), fish were sampled in Lake Inzhenerne (50°00′52.7″N; 23°27′55.6″E), Lake on Znesinnia (49°50′54.1″N; 24°03′07.8″E) and Lake on Plastova (49°51′33.6″N; 24°04′47.7″E), while in the Dniester basin these were Lake Maliushevske (50°00′39.7″N; 23°41′00.6″E) and Lake on Stryiska (49°48′02.4″N; 24°01′00.1″E). Only Lake on Stryiska is a natural body of water, while the others are artificial reservoirs. Three of the lakes, Lake on Znesinnia, Lake on Plastova and Lake on Stryiska, are isolated from the mainstream of the river. Two of the reservoirs, Lake Inzhenerne and Lake Maliushevske, are aquaculture ponds built on small rivers. All the studied bodies of water are characterized by lush aquatic vegetation.

The fish were transported alive in aerated cans to the laboratory of Lviv University, where they were kept alive and then dissected two days after sampling, following a known protocol (Kvach et al. 2016b). The standard and total length of each fish was measured. A total of 120 individuals were examined for parasites (Table 1). In each fish, fins, skin, gills, eyes, muscles, viscera and internal organs (gut, liver, gall bladder, spleen, swimming bladder, kidneys, uterine bladder, gonads, mesentery and brain) were removed and examined under a microscope. Ciliates were examined alive under a light microscope. Monogeneans were removed from the host and preserved in GAP (ammonium picrate and glycerin mixture) as semi-permanent slides (Malmberg 1970). Cestodes, trematodes and nematodes were preserved in hot

Abundance and dimensions of the studied fish. n – number of fish, ind.; SL – standard length, mm; TL – total length, mm; m – mean parameters; SD – standard deviation

| Parameters | | Bug basin | | | Dniester basin | |
|---|---|---|---|---|---|---|
| | | Inzhenerne | Znesinnia | Plastova | Maliushevske | Stryiska |
| n | | 18 | 15 | 41 | 26 | 20 |
| SL, mm | m ± SD | 79.7 ± 19.3 | 53.6 ± 11.2 | 53.1 ± 21.4 | 121.9 ± 19.4 | 63.4 ± 4.4 |
| | min. – max. | 54 – 123 | 40 – 82 | 27 – 97 | 100 – 188 | 58 – 75 |
| TL, mm | m ± SD | 93.1 ± 20.9 | 65.1 ± 13.2 | 58.0 ± 28.9 | 142.2 ± 20.1 | 76.1 ± 3.7 |
| | min. – max. | 69 – 140 | 50 – 99 | 31 – 120 | 119 – 214 | 70 – 83 |

4% formalin (Cribb, Bray 2010). Acanthocephalans were pressed between slides and fixed in 70% ethanol. Copepods and glochidia were preserved in 4% formalin. For species identification, cestodes and trematodes were stained in iron acetocarmine, dehydrated in ethanol and mounted in Canada balsam as permanent slides (Georgiev et al. 1986). Acanthocephalans, nematodes and copepods were mounted in glycerol as temporary slides for species identification.

Indices of prevalence (P, %), mean intensity (MI), intensity range (IR) and mean abundance (A) were calculated for each parasite species according to Bush et al. (1997). Differences in parasite abundance and species richness between the sites were tested employing generalized linear models (GLM, Poisson distribution corrected for under-/overdispersion, i.e. quasipoisson), using fish size as a covariate. Differences in parasite assemblage composition were tested using permutational multiple analysis of variance (PERMANOVA, Anderson 2001) and visualized using non-metric multidimensional scaling (NMDS). Fish with no parasites were excluded from both PERMANOVA and NMDS. The response variable (distance matrix of samples, i.e. fish) for each PERMANOVA was calculated using (a) quantitative Bray–Curtis and (b) binary Jaccard dissimilarity as distance measures. PERMANOVA uses a multivariate analogue of Fisher's F ratio to compare variability within groups versus variability between different groups, with P-values obtained using permutations (Anderson 2001). In this study, 999 permutations were conducted for each PERMANOVA. NMDS was conducted using the metaMDS function, which runs NMDS with several random starts and returns the best solution, rotated so that the largest variance of samples is on the first axis (Oksanen et al. 2019).

All analyses were conducted applying R 3.5.2, R Core Team 2018, using the *stats* and *vegan* packages (R Core Team 2018; Oksanen et al. 2019).

# 3. Results

We identified eight taxa of parasites in the Lviv water bodies, including one ciliate, one monogenean, two cestodes, one digenean, one acanthocephalan, one parasitic crustacean and one unionid glochidium (Table 2). Only two parasite species, viz. the monogenean *Gyrodactylus perccotti* and the cestode *Nippoteania perccotti*, were recorded in all five lakes studied. Most of the parasites represent the local European fauna, except for *G. perccotti* and *N. perccotti*, which are Far East species introduced together with their host (Chinese sleeper), and the copepod *Neoergasilus japonicus* (Harada, 1930), an euryxenous Far East parasite.

There was a significant difference between the localities in parasite infracommunity species richness (GLM, df = 4.114, P = 0.002), but not in infracommunity abundance (GLM, df = 4.114, P = 0.315; Fig. 1). Parasite assemblage composition significantly differed between the sites in terms of both Bray–Curtis and Jaccard distances (PERMANOVA, both df = 4,78, both P = 0.001; Fig. 2).

Differences in assemblage species composition were significant in all pairs of localities (PERMANOVA, all P < 0.05), except for Lake on Stryiska and Lake Maliushevske (PERMANOVA, P = 0.498 and 0.676 for Jaccard and Bray–Curtis distances, respectively). Fish from Lake on Stryiska and Lake Maliushevske were typically parasitized by *N. perccotti* (Fig. 2), from Lake on Znesinnia by *G. perccotti* and *Trichodina* sp. (Fig. 2) and from Lake Inzhenerne by *Acanthocephalus lucii* (Müller, 1776) and glochidia (Fig. 2). Fish from Lake on Plastova were parasitized with the widest spectrum of parasites, clearly different from Stryiska, Maliushevske and Inzhenerne lakes (PERMANOVA, all P < 0.005), with a less pronounced but significant difference from Znesinnia (PERMANOVA, P = 0.020 and 0.025 for Jaccard and Bray–Curtis distance, respectively).

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**Table 2**

Infection parameters of the Chinese sleeper (*Perccottus glenii*) in five localities from two different river basins. P – prevalence; MI – mean intensity; IR – intensity range; A – abundance; SD – standard deviation

| Parasite species | Index | Bug basin | | | Dniester basin | |
|---|---|---|---|---|---|---|
| | | Inzhenerne | Znesinnia | Plastova | Maliushevske | Stryiska |
| CILIOPHORA | | | | | | |
| *Trichodina* sp. | P, % | | 13.3 | 22.0 | | 30.0 |
| | MI ± SD | | 5.5 ± 6.4 | 21.1 ± 34.8 | | 4.7 ± 5.3 |
| | IR | | 1–10 | 1–113 | | 1–15 |
| | A | | 0.7 | 4.6 | | 1.4 |
| MONOGENEA | | | | | | |
| *Gyrodactylus perccotti* Ergens & Yukhimenko, 1973 | P, % | 22.2 | 33.3 | 24.4 | 19.2 | 15.0 |
| | MI ± SD | 1.3 ± 0.5 | 1.0 ± 0.0 | 2.7 ± 1.5 | 2.0 ± 1.2 | 7.3 ± 11.0 |
| | IR | 1–2 | 1 | 1–5 | 1–4 | 1–20 |
| | A | 0.3 | 0.3 | 0.7 | 0.4 | 1.1 |
| CESTODA | | | | | | |
| *Nippotaenia perccotti* (Akhmerov, 1941) | P, % | 16.7 | 6.7 | 34.1 | 46.2 | 65.0 |
| | MI ± SD | 3.3 ± 2.3 | 5.0 | 3.4 ± 3.5 | 7.0 ± 4.1 | 3.7 ± 2.1 |
| | IR | 2–6 | 5 | 1–14 | 1–15 | 1–7 |
| | A | 0.6 | 0.3 | 1.2 | 3.2 | 2.4 |
| *Paradilepis scolecina* (Rudolphi, 1819) | P, % | | | 2.4 | | |
| | MI ± SD | | | 2.0 | | |
| | IR | | | 2 | | |
| | A | | | 0.05 | | |
| DIGENEA | | | | | | |
| *Metorchis xanthosomus* (Creplin, 1846) | P, % | | | 19.5 | | |
| | MI ± SD | | | 2.5±1.4 | | |
| | IR | | | 1–5 | | |
| | A | | | 0.5 | | |
| ACANTHOCEPHALA | | | | | | |
| *Acanthocephalus lucii* (Müller, 1776) | P, % | 16.7 | | | | |
| | MI ± SD | 2.3 ± 1.2 | | | | |
| | IR | 1–3 | | | | |
| | A | 0.4 | | | | |
| CRUSTACEA | | | | | | |
| *Neoergasilus japonicus* (Harada, 1930) | P, % | 11.1 | | | | |
| | MI ± SD | 1.5 ± 0.7 | | | | |
| | IR | 1–2 | | | | |
| | A | 0.2 | | | | |
| BIVALVIA | | | | | | |
| Unionidae gen. sp. | P, % | 77.8 | | 14.6 | 7.7 | |
| | MI ± SD | 5.2 ± 4.3 | | 3.3 ± 2.4 | 1.5 ± 0.7 | |
| | IR | 1–17 | | 1–8 | 1–2 | |
| | A | 4.1 | | 0.5 | 0.1 | |
| Species richness | | 5 | 3 | 6 | 3 | 3 |
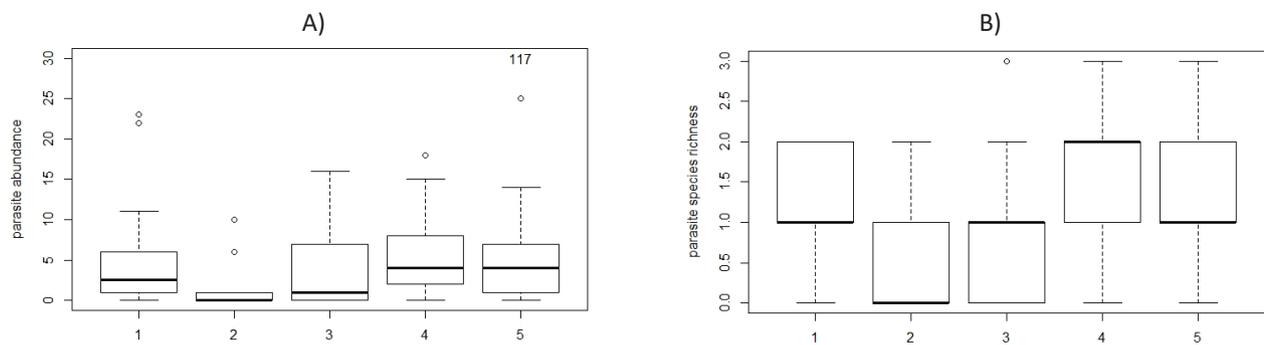
**Figure 1**

Abundance (A) and species richness (B) of fish parasites in five localities. Horizonal bar = median, box = interquartile range, whiskers = non-outlier range (1.5*interquartile range), dots = outliers. Abundance outside the presented y-axis range are written as values. Centroids of the localities are indicated by numbers: 1 = Stryiska, 2 = Znesinnia, 3 = Maliushevske, 4 = Inzhenerne, 5= Plastova.
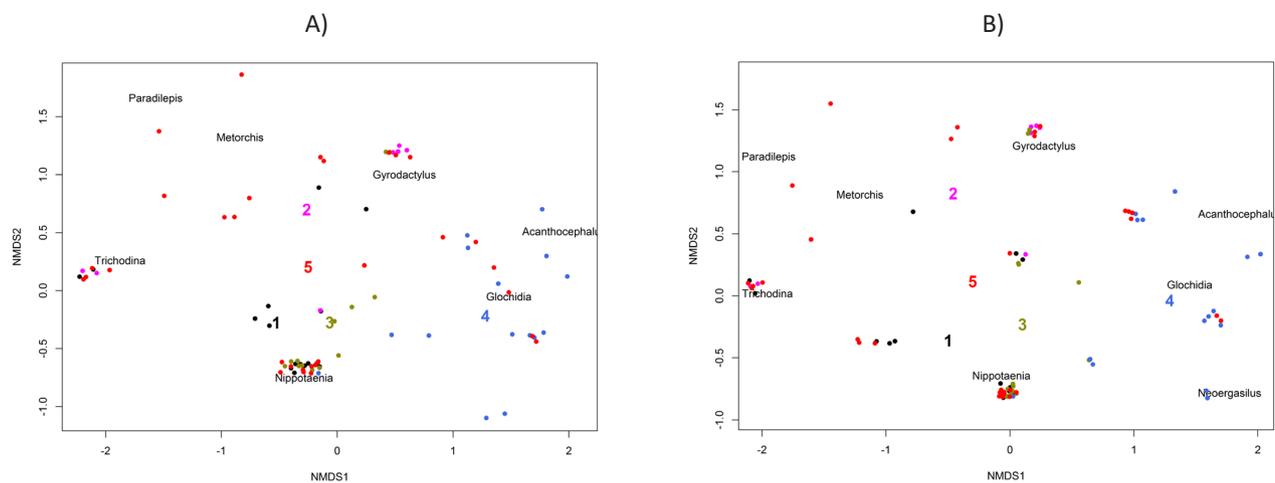


**Figure 2**

Non-metric multidimensional scaling (NMDS) of parasite assemblages in fish from five sites (colors of the dots correspond to site centroids) using Bray–Curtis (A) and Jaccard (B) indices. Centroids of the localities are indicated by numbers: 1 = Stryiska, 2 = Znesinnia, 3 = Maliushevske, 4 = Inzhenerne, 5 = Plastova. NMDS stress = 0.048 (A) and 0.023 (B).

## 4. Discussion

*Gyrodactylus perccotti* and *Nippotaenia perccotti* were the most abundant and widespread parasites of the Chinese sleeper in the Lviv region. Both parasites are specific to the Chinese sleeper, having been introduced with their hosts to Europe from Asia (Košuthová et al. 2004; Ondračková et al. 2012). The monogenean *G. perccotti* was already recorded in the Dnieper, Vistula, Tisza river basins, in the Lower Danube and its deltaic zone, albeit in all cases only in small numbers (Mierzejewska et al. 2012; Ondračková et al. 2012; Antal et al. 2015; Zaichenko 2015; Kvach et

al. 2018, 2020). The cestode *N. perccotti* is known as the most numerous parasite species in the Chinese sleeper parasite community in the Dniester, Vistula and Tisza river basins (Košuthová et al. 2004; Mierzejewska et al. 2010, 2012; Kvach et al. 2013; Antal et al. 2015). The presence of these two parasites at the site of the first introduction of the Carpathian population was assumed earlier (Kvach et al. 2016a) and we have now confirmed their presence in both the Upper Dniester and Upper Bug river basins. Given the high abundance of the parasite in the Lviv region, the fish could be a source for the introduced populations in the nearest water basins.

Both *G. perccotti* and *N. perccotti* are host specific (stenoxenous) parasites that can only infect invasive hosts within the introduced range (parasite co-introduction with a host), therefore potentially giving native species a possible competitive advantage over the invader (Taraschewski 2006). Their presence may also support theories on the introduction pathways, such as in the case of the invasive Chinese sleeper, especially in the Carpathian region (Kvach et al. 2016a).

Another non-native parasite species, the copepod *Neoergasilus japonicus*, is an Asian crustacean introduced to Europe with the fish farming industry, which infects various fish species, mainly cyprinids and percids (Hudson, Bowen 2002). It has also been recorded in the invasive fish species, such as pumpkinseed *Lepomis gibbosus* (L., 1758) from the Czech Republic, and the Chinese sleeper from Latvia (Ondračková et al. 2019; Kvach et al. 2020). The presented finding is the second record of this parasite in the Chinese sleeper in Europe, and the first one in this fish in Ukraine.

Differences between the surveyed bodies of water were mainly due to their local seasonal conditions, and catchment characteristics. The geo-ecological conditions of lakes (both artificial and natural) in the Lviv region change every year depending on both natural and anthropogenic factors, e.g. precipitation, sewage discharge, etc. (Koinova, Chorna 2019). The most different water body (among the studied ones) is Lake on Plastova, which is an artificial pond with a muddy bottom, overgrown with macrophytes. This resulted in high infestation of the Chinese sleeper with trichodinids (Drobiniak et al. 2014) in this lake (see Table 2). The lake is characterized by a high concentration of organic matter in water (Koinova, Chorna 2019), and the presence of a large number of crustaceans, probably due to rich food resources (K. Nazaruk, unpublished data). This may be the source of a wider spectrum of parasites in this water body.

The second lake with good environmental conditions is Inzhenerne, where five parasite species occurred (see Table 2). It is an artificial lake used for fishing purposes. It is the only lake where the acanthocephalan *Acanthocephalus lucii* was found in the Chinese sleeper. The intermediate host of the parasite is the benthic isopod *Asellus aquaticus* (L., 1758), which is known as an indicator of good environmental conditions (Brattey 1986; O'Callaghan et al. 2019).

The similarities in the species composition of parasite assemblages from Lake Maliushevske and Lake on Stryiska (both located in the Dniester basin) were probably not due to their location in the same catchment, but due to the deterioration of environmental conditions in both lakes. Since the first intermediate hosts of *N. perccotti* are planktic crustaceans (Rusinek 1989), their abundant presence in the environment of these lakes (K. Nazaruk, unpublished data) resulted in high levels of fish infestation with the cestode.

Invaders directly or indirectly affect the interactions between invasive and native parasite and host populations and their communities, which is manifested through various mechanisms (Goedknegt et al. 2016). The success of an invasion depends on the ability and chances of a parasite to complete its life cycle in the non-native environment. This is particularly important for parasites with complex life cycles, the invasional success of which depends on the presence of suitable intermediate hosts in the invaded ecosystem (Taraschewski 2006).

Usually, when several non-native species invade the same environment, this mutually increases the invasion success of each of them, which underlies the so-called 'invasional meltdown hypothesis' (Simberloff, von Holle 1999). This may also apply to parasite–host interactions, as invasive hosts may support the spread of non-native parasites introduced by other vectors (Taraschewski 2006; Emde et al. 2014). One piece of evidence for this hypothesis may be the find of the Asian crustacean *N. japonicus*, which is commonly dispersed by cyprinids, with further secondary spread by small-sized invasive fishes such as the pumpkinseed and the Chinese sleeper (Ondračková et al. 2019; Kvach et al. 2020; current data). Its presence in the fishing reservoir, Lake Inzhenerne, is probably strongly associated with the aquaculture vector.

In general, the native parasite acquisition by the Chinese sleeper in the studied region is low. High abundance is mainly exhibited by non-native parasites (see Table 2). The current data confirm the presence of co-introduced populations of the monogenean (*G. perccotti*) and the cestode (*N. perccotti*), which probably affects the future invasiveness of their host. Due to the low acquisition of local parasites, we cannot confirm any significant effect of the invasive fish on local parasite assemblages in the region. However, the presence of the non-indigenous copepod, *N. japonicus*, needs additional attention in the future.

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