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# Metazoan parasites of *Neogobius* fishes in the Slovak section of the River Danube

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**Summary**

Metazoan parasites were investigated in three non-native fishes (monkey goby *Neogobius fluviatilis*, bighead goby *Neogobius kessleri* and round goby *Neogobius melanostomus*) collected from the former and current main channel of the River Danube and from the River Hron, Slovakia, in November 2003. Thirteen parasite species were identified: *Triaenophorus crassus*, *Diplostomum* sp., *Tylodelphys clavata*, *Metorchis xanthosomus*, *Nicolla skrjabini*, *Gyrodactylus proterorhini*, *Pomphorhynchus laevis*, *Contracoecum* sp., *Raphidascaris acus*, *Anguillicola crassus*, *Unio tumidus*, *Anodonta anatina* and *Pseudoanodonta complanata*. The maximum parasite diversity was found in *N. fluviatilis*. Total parasite abundance was significantly higher in *N. kessleri*, but no significant differences among sampling sites were observed. *Pomphorhynchus laevis* and glochidia of *Anodonta anatina* reached 100% prevalence in *N. kessleri* in the new channel of the Danube and, in general, these species were also the most prevalent parasites in all three goby species. For endoparasites, gobies served mostly as intermediate (digenean, cestodes and nematodes) or paratenic (acantocephalan and nematodes) hosts. All parasite species found are common parasites in the Middle Danube basin. No parasites specific to *Neogobius*, known from their native populations, were observed.

## Introduction

The introduction of non-native species into an ecosystem may influence communities by changing species diversity, community structure and the ecological processes, which are dependent on interactions between organisms (Ramakrishnan and Vitousek, 1989). Competition between native and non-native species is central to the study of biological invasions, and parasite release may be important in allowing an introduced species to become invasive (Torchin et al., 2003). Introduction of diseases may also represent serious threats to the health and survival of susceptible native fish populations (Bauer, 1991). Therefore, parasites play three possible roles in animal invasions (Prenter et al., 2004): (i) parasite transmission to native species – parasitized non-native host individuals may be a source of introduction of new parasite species into the native host populations; (ii) parasite loss during translocation – reduced parasite infection of introduced species may have several causes, including reduced probability of introduction of parasites with non-native hosts or early extinction after host establishment, or absence of other required hosts in the new geographical area (Torchin et al., 2003). These hosts may remain free of parasites, or they may be colonized by native parasites; and (iii) parasite transmission to non-native species – depending on the specificity of parasite species, non-native species may be parasitized by native parasites. If introduced species achieve high abundance, then parasites with a wide-host range often use these species as their hosts (Pronin et al., 1997).

Four species of Ponto-Caspian goby have recently invaded the middle and upper sections of the River Danube: monkey goby *Neogobius fluviatilis* (Pallas, 1814), bighead goby *Neogobius kessleri* (Günther, 1861), round goby *Neogobius melanostomus* (Pallas, 1814), racer goby *Neogobius gymnotrachelus* (Kessler, 1857). These four fish species have been introduced to, or expanded into, the Slovak section of the River Danube during the last decade, and all but racer goby have become abundant either locally (Jurajda et al., 2005) or over a wider geographical range (see Copp et al., 2005).

The parasite fauna of *Neogobius* spp. in their introduced (or expanded) geographical range has been studied mainly in round goby in North America (Pronin et al., 1997) and the Baltic Sea (Kvach, 2002a; Rokicki and Rolbiecki, 2002), and no other parasitological studies of invasive gobies in European freshwaters are known. The aim of the present study is to examine the structure of metazoan parasites in bighead, monkey and round gobies and the potential introduction of new parasite species into the native fish host populations.

## Materials and methods

During November 2003, specimens of monkey, bighead and round gobies were collected by electrofishing from the former main channel (downstream of the port, river 1819.5 km) and current main channel (below the Gabčíkovo hydropower station, river 1821 km) of the River Danube and from the River Hron (near its confluence with the Danube), Slovakia. The fish were carried alive back to the laboratory in aerated tanks of water.

In the laboratory, each specimen was individually killed and measured for standard length to the nearest mm (Table 1). Then, all fish were examined under binocular microscope for the presence of metazoan parasites using the methods of Ergens and Lom (1970). Collected parasites were preserved in 4% formaldehyde (Acantocephala, Digenea, Cestoda, Bivalvia), in a mixture of ammonium picrate and glycerine (Monogenea) or in a mixture of glycerine and alcohol (Nematoda). Parasites were identified using a light microscope equipped with phase-contrast, differential interference contrast and Digital Image Analysis (Pro Plus 1.3 for Windows, 1995).

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| Numbers | T | M | F | J | Sampling site | SL (SE) | Min | Max |
|---|---|---|---|---|---|---|---|---|
| Monkey goby | 18 | 7 | 10 | 1 | River Hron | 74.3 (2.6) | 33.5 | 92.4 |
| Bighead goby | 8 | 2 | 6 | 0 | River Hron | 98.8 (2.7) | 88.7 | 107.5 |
| | 14 | 2 | 6 | 6 | Danube former MC | 75.0 (4.6) | 46.4 | 106.7 |
| | 6 | 3 | 3 | 0 | Danube current MC | 99.3 (7.8) | 64.3 | 119.8 |
| Round goby | 7 | 2 | 5 | 0 | Danube current MC | 80.1 (5.9) | 53.8 | 99.2 |

Table 1
Number of monkey, bighead and round goby by sex (T, total; M, male; F, female; J, juvenile) and sampling site in Slovakia (MC, main channel), with mean (in mm), standard error (SE) and minimum–maximum of standard length (SL)

Parasite infection was characterized according to Bush et al. (1997): prevalence was defined as the percentage of fish infected by a given parasite species in a sample, mean abundance as the mean number of parasites per host (infected and non-infected) in a sample, and mean intensity as the mean number of parasites per infected fish, with parasitological range (*sensu* Margolis et al., 1982) indicating the minimum and maximum number of specimens infected. An analysis of variance (ANOVA) among the sampling sites and host species was performed in order to test for differences in parasite abundance between sampling sites.

## Results

With the exception of two specimens of monkey goby, all fish were parasitized. A total of 1018 individuals of 13 metazoan parasite species were recorded (Table 2). The maximum number of parasite species (10) was found in bighead goby. The mean abundance of all parasites was the lowest in monkey goby (5.9), compared with bighead goby (25.9) and to round goby (12.1). Maximum prevalence (100%) was reached by *Pomphorhynchus laevis* (Müller, 1776) and glochidia of *Anodonta anatina* Linnaeus, 1758 found in bighead goby from the new main channel of the Danube. The highest total abundances and intensities of infection were found for *P. laevis* (mean abundance 9.0; mean intensity of infection 17.0), adult digenean *Nicolla skrjabini* (Iwanitzky, 1928) with mean abundance of 3.2 and mean infection intensity of 12.0; and glochidia of *A. anatina* (mean abundance 3.8; mean intensity of infection 5.3).

The proportion of ecto- and endoparasite specimens infecting gobies was similar in monkey and round goby (Fig. 1). In bighead goby, 82% of the parasite community was composed of endoparasites and the ratio of endo- to ectoparasites did not differ markedly among sampling sites. Ectoparasites recorded in gobies were represented by monogeneans infecting fins in bighead goby (*Gyrodactylus proterorhini* Ergens, 1967) and by larval bivalves. Glochidia of three species were found: *A. anatina*, infected the fins, gills and head parts of all three host species; and *Pseudoanodonta complanata* Rossmässler, 1835, infected the gills of monkey goby, and *Unio tumidus* Retzius, 1788, infected the gills of both monkey and bighead goby in the River Hron (Table 2).

*Pomphorhynchus laevis* was the most frequent endoparasite species at all sampling sites, whereby over 76% of worms found (free or encapsulated) were rejected by the fish host. The total prevalence reached 52.5%, when only 9% of examined fish contained live acantocephalans in the intestine. Monkey goby were not found to have worms attached to their intestines (Fig. 2). Rejected acantocephalans occurred mainly on the surface of the intestine and free in abdominal cavity, fewer in the liver and muscle, and occasionally in kidney and spleen. Only one species of adult digenan, *Nicolla skrjabini*, was found in the intestine of all three fish species, compared with three species of larval digenean. All goby species were infected by *Diplostomum* sp. at all sampling sites, whereas *Tylodelphys clavata* (Nordmann, 1832) was found in bighead goby only, and only monkey goby was parasitized by *Metorchis xanthosomus* Creplin, 1846. Plerocercoids of *Triaenophorus crassus* Forel, 1868 were observed in the muscles of bighead goby from the River Hron. A single specimen of larval *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 was found, in the liver of a bighead goby; and one larval nematode *Contracoecum* sp. infected the intestine of a monkey goby. The most common nematode species was larval *Raphidascaris acus* (Bloch, 1779), which was highly prevalent in all three fish species in all sampling sites (Table 2). Considering all fish specimens combined, 96.6% of *R. acus* were located in the wall of intestine (90.7%) or in the liver (9.3%), being surrounded by a thin cyst.

Total parasite infection differed among the fish host species, being significantly higher in bighead goby than in monkey and round goby (ANOVA, $F = 7.42$, d.f. $= 2$, $P = 0.0015$). Concerning particular parasite species, *P. laevis* was significantly more abundant in bighead goby than in monkey and round gobies (ANOVA, $F = 8.60$, d.f. $= 2$, $P = 0.0006$) and *Diplostomum* sp. was significantly more abundant in round goby than in monkey and bighead gobies (ANOVA, $F = 11.07$, d.f. $= 2$, $P = 0.0001$). In bighead goby, no difference was found among sampling sites with respect to total parasite abundance (ANOVA, $F = 2.74$, d.f. $= 2$, $P > 0.05$) or abundance of dominant parasites (*Diplostomum* sp., *P. laevis*, *R. acus*, *A. anatina*).

## Discussion

The introduction of fishes and their parasites into novel environments may represent serious threats to the health and survival of susceptible native fish populations (Bauer, 1991). The parasite fauna of the gobies in our study was similar or less rich than that reported for these fish species in their native waters of the Black and Azov Seas (Najdenova, 1974; Smirnov, 1986). The numbers of parasite species found in monkey and round goby (Table 2) were similar to those in their native range, both in the lower Danube (monkey goby: five species; round goby: nine species) and several estuaries of the Black Sea, Ukraine (monkey goby: three to seven species; round goby: seven to nine species; Kvach, 2001, 2002b,c). However, in bighead goby, the number of parasite species we observed in the Middle Danube (Table 2) was lower than that reported (25 species) for native populations in the lower Danube (Bănărescu, 1964; Kulakovskaya and Koval, 1973; Florescu and Ienistea, 1984). Reduced parasite diversity is often mentioned as a reason for success of introduced species (Shea and Chesson, 2002), and this may explain in part the rapid expansion of bighead goby.

Although the numbers of parasite species in monkey and round goby in their native and expanded ranges were similar,

Table 2
Prevalence (P in %), mean abundance (A) and range of the intensity of infection (minimum and maximum values, i.e. parasitological range, *sensu* Margolis et al., 1982) for metazoan parasites from monkey goby, bighead goby and round goby from the rivers Hron and Danube

| Parasite species | Develomental stage | Location | Hron | | Old Danube channel (bighead goby) | New Danube channel | |
|---|---|---|---|---|---|---|---|
| | | | Monkey goby (n = 18) | Bighead goby (n = 8) | (n = 14) | Bighead goby (n = 6) | Round goby (n = 7) |
| Cestoda | | | | | | | |
| *Triaenophorus crassus* | Plerocercoid | Muscle | | 37.5 (0.75), 2 | | | |
| Trematoda | | | | | | | |
| *Diplostomum* sp. | Metacercaria | Eye lens | 27.8 (0.39), 1–2 | 50.0 (0.63), 1–2 | 28.6 (0.29), 1 | 16.7 (0.17), 1 | 57.1 (3.14), 2–10 |
| *Tylodelphys clavata* | Metacercaria | Eye humour | | | 7.1 (0.07), 1 | | |
| *Metorchis intermedius* | Metacercaria | Muscle | 11.1 (0.11), 1 | | | | |
| *Nicolla skrjabini* | Adult | Intestine | 11.1 (0.17), 1–2 | | 42.9 (0.93), 1–4 | 83.3 (25.17), 7–67 | 14.3 (0.14), 1 |
| Monogenea | | | | | | | |
| *Gyrodactylus proterorhini* | Adult | Fins | | 25.0 (0.25), 1 | | 50.0 (0.50), 1 | |
| Acantocephala | | | | | | | |
| *Pomphorhynchus laevis* | Larva, immature | Intestine*, abdominal cavity, liver, kidney, spleen, muscle | 16.7 (1.33), 3–13 | 50.0 (11.88), 1–50 | 92.9 (18.43), 3–64 | 100 (15.0), 6–36 | 28.6 (1.14), 3–5 |
| Nematoda | | | | | | | |
| *Contracoecum* sp. | Third-stage larva | Intestine | 5.6 (0.06), 1 | | | | |
| *Angiullicola crassus* | Third-stage larva | Liver | | | 7.1 (0.07), 1 | | |
| *Raphidascaris acus* | Third-stage larva | Intestine*, liver | 11.1 (0.67), 1–11 | 62.5 (3.13), 5–6 | 50.0 (0.93), 1–5 | 50.0 (2.67), 2–9 | 57.1 (2.86), 1–9 |
| Bivalvia | | | | | | | |
| *Unio tumidus* | Glochidium | Gills | 22.2 (0.67), 2–4 | 25.0 (1.00), 2–6 | | | |
| *Anodonta anatina* | Glochidium | Gills, fins, head | 72.2 (1.83), 1–8 | 37.5 (1.88), 2–18 | 85.7 (5.29), 1–24 | 100 (7.50), 1–9 | 28.6 (4.86), 2–13 |
| *Pseudoanodonta complanata* | Glochidium | Gills | 11.1 (0.72), 4–9 | | | | |

Values are expressed as P (A), range.
*Located free in the lumen and also within the wall.

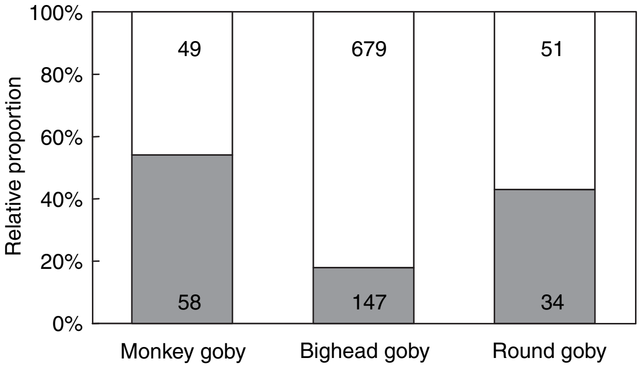


Fig. 1. The relative proportion of ecto- (grey bars) and endoparasites (white bars) for all parasites identified per fish species in monkey, bighead and round gobies from the rivers Danube and Hron, Slovakia. Numbers in each column represent the total number of parasites

species composition of these parasites was quite different. All parasite species found in our study are common and most of them occur in many fish species in the Middle Danube basin (Moravec, 2001). Only three common and widespread species (*Diplostomum* sp., *Tylodelphys clavata* and *P. laevis*) and one species common in the Black Sea drainage area (*Nicolla skrjabini*) occurred both in our samples and in fishes from the lower Danube (Bănărescu, 1964; Kulakovskaya and Koval, 1973; Florescu and Ienistea, 1984). These four parasite species have been found to infect over 35 fish species in the Middle Danube basin in Slovakia (Moravec, 2001).

*Anodonta anatina* was the most prevalent species in our samples and occurred at all sampling sites and in all fish species. Glochidia, the larval stage of bivalves, are frequent parasites of many fish species, including gobiids. Unfortunately, in most studies of fish parasite fauna, glochidia are not determined to species level. In addition to *A. anatina*, monkey goby was parasitized by *P. complanata*, and in the River Hron, both monkey and bighead goby were infected by *U. tumidus*. The presence of these larval stages of unionid mussels in fishes reflects the presence of adult bivalves at that sampling site. Maximum prevalence and abundance values were also recorded for the acantocephalan *P. laevis*. It is a very frequent and widespread intestinal parasite of many fish species in Europe and Palaearctic Asia, and the infection of fish by this parasite is often very heavy. Its development involves an intermediate host, i.e. various species of gammarid crustaceans. It may also include a paratenic host, i.e. many species of small fishes in which the larvae occur in the abdominal cavity on the surface of various internal organs (see Moravec and Scholz, 1991). Elevated *P. laevis* infection in our samples may have been caused by a high proportion of amphipods, the intermediate hosts of this parasite, in fish diet. The stomach contents of fish from the studied area were composed mainly of *Dikerogammarus villosus* (Sovinski, 1894) and *Corophium curvispinum* Sars, 1895 (Z. Adámek, pers. comm.). The majority of *P. laevis* collected from gobies were found on the surface of the intestine and also in internal organs (e.g. liver, kidney and spleen) or in muscles. In this case, gobiids served as paratenic hosts. In addition, four specimens of bighead goby and one round goby probably served as para-definitive hosts (see Odening, 1976) for *P. laevis* (Fig. 2).

Nematode parasites in gobies were represented by three species, all found as larvae; *Anguillicola crassus*, *Contracoecum* sp. and *R. acus* used gobies as intermediate or paratenic host. All these parasites are common in fishes from the Middle Danube basin in Slovakia (Moravec, 2001). *Anguillicola crassus* is a swimbladder parasite of Japanese and European eels, and it uses different fish species as paratenic hosts for the development of its third-stage larvae to be infective to eels (Székely, 1996). In our study, only one specimen of *A. crassus* was found in a liver, surrounded by a thin connective tissue capsule. This encapsulation of nematode larvae has been observed in several cyprinids (Moravec and Konečný, 1994), whereas the occurrence of free larvae in the abdominal cavity is typical for gobiids (Székely, 1996; Székely et al., 1996). Indeed, Székely (1996) concluded that gobies are suitable paratenic hosts for *A. crassus*. The encapsulated larva of *A. crassus* found in our sample may indicate a strong host reaction, which was especially characteristic for the infection of *R. acus* in the material studied. Encapsulated larvae of *R. acus* were collected from all goby species and from all the sites studied. Benthophagous fishes, mainly cyprinids, are typical intermediate or paratenic hosts for *R. acus* (Moravec, 1994). Therefore, gobies with their benthophagous diet are also susceptible to *R. acus* infection.

Monogenean and cestode parasites occurred rarely in our samples. *Gyrodactylus proterorhini* is a common parasite of tubenose goby *Proterorhinus marmoratus* Pallas, 1814 in its native range in the Danube (Ergens, 1967; Ergens and Lom, 1970) and was the only monogenean found in bighead goby. The host switch by *G. proterorhini* between tubenose and bighead goby may be a consequence of their co-occurrence at these particular study sites (Jurajda et al., 2005) and in the

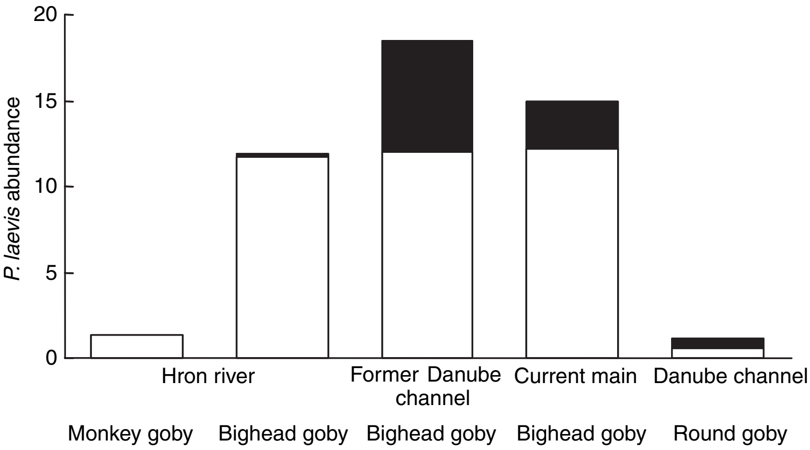


Fig. 2. Proportion of immature *Pomphorhynchus laevis* attached to the intestine (black bars), and larval *P. laevis* free or encysted in the abdominal cavity and other internal organs (white bars) of monkey, bighead and round gobies from the rivers Danube and Hron, Slovakia

Middle Danube in general (Erős et al., 2005; Wiesner, 2005). Plerocercoids of *Triaenophorus crassus* represented the only cestode species found in bighead goby. This parasite has been commonly found in the muscle of tubenose goby, northern pike *Esox lucius* L., Eurasian perch *Perca fluviatilis* L. and European catfish *Silurus glanis* L. from the River Danube (Ergens and Lom, 1970; Moravec, 2001).

In conclusion, none of the parasites collected from bighead, monkey and round goby are novel species to the Middle Danube. Indeed, all of the observed parasite species are commonly found in native fishes from the Slovak section of the Middle Danube. The abundance of parasites varied among the *Neogobius* spp., with bighead goby hosting the most abundant parasite community. But, the total parasite load of bighead goby was relatively low compared with that observed in the species' native populations (Najdenova, 1974). This reduced parasite abundance, combined with strong host reaction to many parasite species, may facilitate the successful establishment and dispersal of non-native goby fishes in novel habitats of the Middle Danube.

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