

## Supplementary material to

The Ponto-Caspian parasite *Plagioporus* cf. *skrjabini* reaches the River Rhine system in Central Europe: higher infestation in the native than in the introduced Danubian form of the gastropod *Theodoxus fluviatilis*

Louisa Marie Rothmeier<sup>1</sup>, René Sahm<sup>2</sup>, Burkard Watermann<sup>3</sup>, Karsten Grabow<sup>1</sup>, Meike Koester<sup>4</sup>, Anna Cichy<sup>5</sup>, Andreas Martens<sup>1</sup>

<sup>1</sup> Institute for Biology, University of Education, Bismarckstraße 10, 76133 Karlsruhe, Germany

<sup>2</sup> German Environment Agency, Schichauweg 58, 12307 Berlin, Germany

<sup>3</sup> LimnoMar Laboratory for Freshwater and Marine Research, Duvenwischen 4, 22359 Hamburg, Germany

<sup>4</sup> Institute for Integrated Natural Sciences, University of Koblenz-Landau, Universitätsstraße 1, 56070 Koblenz, Germany

<sup>5</sup> Department of Invertebrate Zoology and Parasitology, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland

### **Corresponding author**

Louisa Marie Rothmeier

Institute for Biology

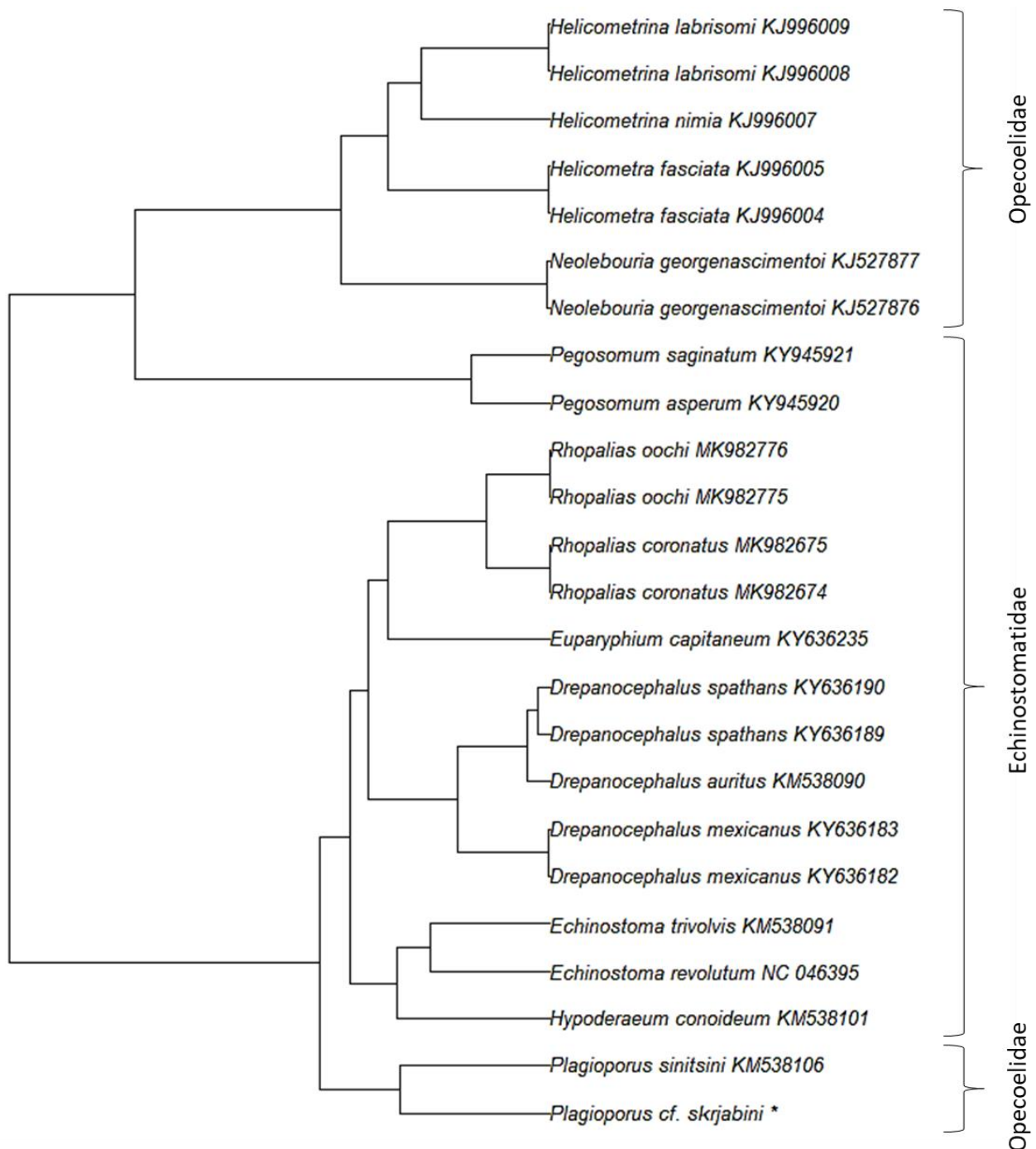
University of Education

Bismarckstraße 10

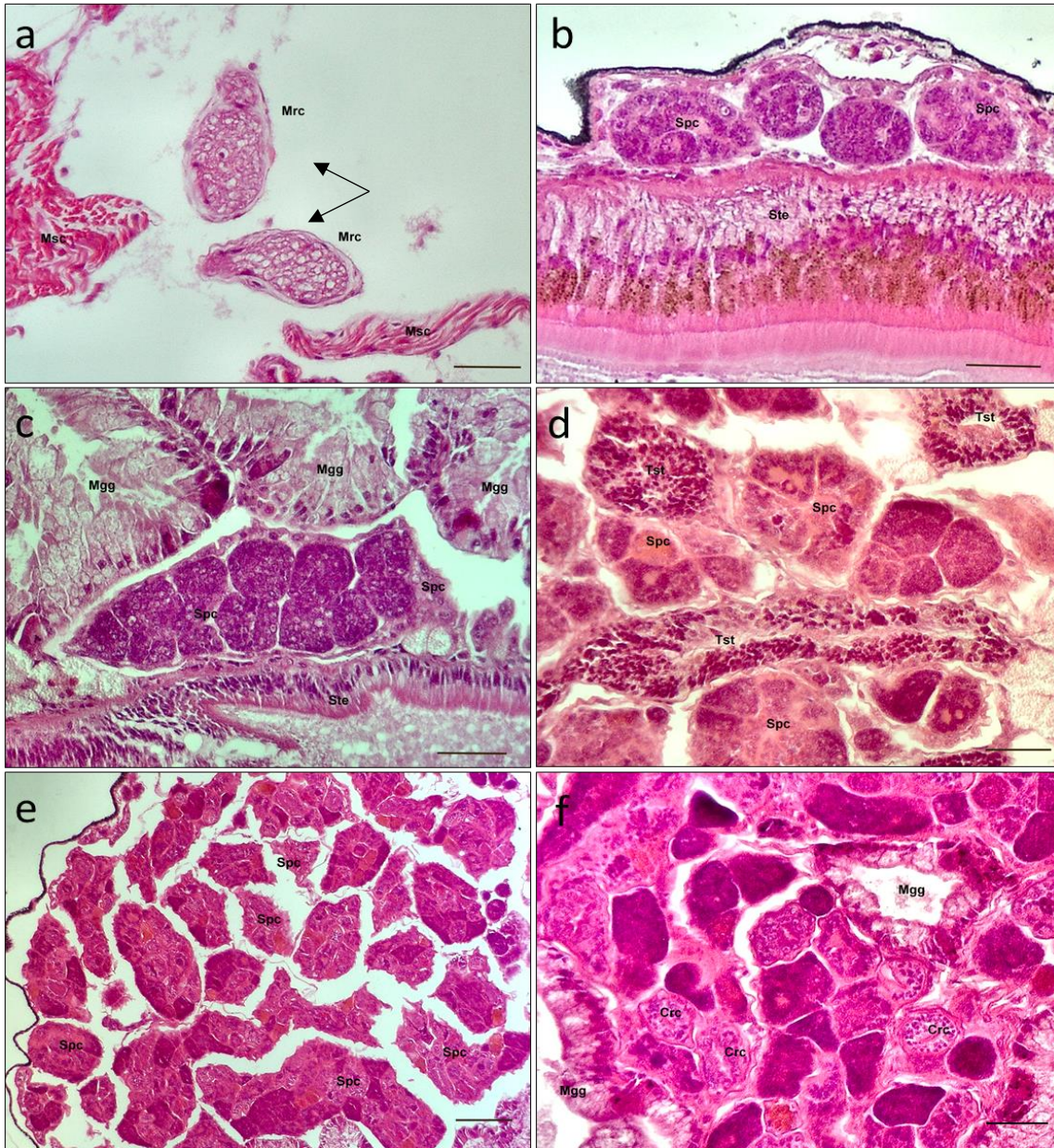
76133 Karlsruhe

Germany

[louisa-rothmeier@gmx.de](mailto:louisa-rothmeier@gmx.de)



**Figure S1** Maximum likelihood tree of *Plagioporus cf. skrjabini* cercariae (marked by an asterisk; GenBank acc. no. MT756015.1, this study) found in the freshwater snail *Theodoxus fluviatilis* and 23 GenBank COI sequences from trematode species belonging to the Echinostomatidae and Opcoelidae families (acc. nos. displayed). Only bootstrap values (n = 1,000) of the main branches are displayed.



**Figure S2** Microscope photographs of histologically prepared sections (2-3  $\mu\text{m}$ ) from tissues of the Northern-European form of *Theodoxus fluviatilis*. Counter-staining with haematoxylin and eosin, bar 50  $\mu\text{m}$ . Crc = cercaria, Mgg = midgut (digestive) gland, Mrc = miracidium, Msc = muscle, Spc = sporocyst, Ste = stomach epithelium, Tst = testis; **a** foot with *Plagioporus* cf. *skrjabini* miracidia between separated muscle bundles (arrows indicating cilia); **b** stomach epithelium with brown pigment deposition and sporocysts of *P. cf. skrjabini*; **c** digestive gland tubuli with sporocysts of *P. cf. skrjabini*; **d** and **e** gonad tissue with intensive *P. cf. skrjabini* infestation (sporocysts) and lacunas; **f** digestive gland tubuli with released cercariae of *P. cf. skrjabini*.

**Table S1** Results of *similarity percentages (simper)* analysis of percentual contributions of *Plagioporus* cf. *skrjabini* prevalence and six histopathological parameters to overall Bray-Curtis dissimilarity of Permutational Multivariate Analysis of Variance (PERMANOVA, 999 permutations) comparing two phylogenetically distinct forms of *Theodoxus fluviatilis* (Danubian and Northern-European).

| Parameter                                             | Contribution to overall<br>Bray-Curtis dissimilarity (%) |
|-------------------------------------------------------|----------------------------------------------------------|
| Prevalence of <i>Plagioporus</i> cf. <i>skrjabini</i> | 28.7                                                     |
| Sexual organs pathological findings                   | 20.4                                                     |
| Stomach pigment deposition                            | 18.7                                                     |
| Digestive gland pigment deposition                    | 16.4                                                     |
| Digestive gland dilatation                            | 8.3                                                      |
| Kidney dilatation                                     | 5.9                                                      |
| Gill dilatation                                       | 1.7                                                      |