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Phylogenetic position of *Ancyrocephalus (sensu lato) curtus* Achmerov, 1952 (Monopisthocotylea, Dactylogyridae), a parasite of fish Perccottus glenii Dybowski, 1877 (Gobiiformes: Odontobutidae)

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Abstract

The genus *Ancyrocephalus sensu lato* is a large assemblage of species of dactylogyrid monopisthocotyleans without clear taxonomic boundaries. Despite an urgent need for revision, only three representatives of this taxon have been molecularly characterised so far. We found specimens of *Ancyrocephalus curtus*, a previously non-genotyped species, in gills of *Perccottus glenii* caught in the River Syumnyur, Amur Basin, Russia. The aim of this study was to assess the phylogenetic position of this parasite using partial sequences of 28S rRNA gene. In the phylogenetic tree, *A. curtus* appeared as a sister taxon to the dactylogyrine genus *Gobioecetes*. The new molecular evidence supports the hypothesis about the non-monophyletic status of *Ancyrocephalus sensu lato*.

Introduction

Ancyrocephalus Creplin, 1839, a large assemblage of dactylogyrid monopisthocotyleans, is a taxonomically problematic genus (Bychowsky and Nagibina 1970; Kritsky and Nitta, 2019; Kmentová *et al.* 2022). The number of species attributed to it has diminished after numerous revisions (e.g., Bychowsky and Nagibina 1970; Beverley-Burton 1984; Dossou and Euzet 1984; Agarwal *et al.* 2001; Kritsky *et al.* 2004; Dmitrieva et al. 2012; Kmentová *et al.* 2018; Kritsky and Nitta 2019). Nevertheless, the concept of *Ancyrocephalus sensu lato* is still applicable to 39 nominal dactylogyrid species with four anchors connected with dorsal and ventral bars and seven pairs of similar or different hooks (WoRMS 2024).

Molecular data are available only on three species of *Ancyrocephalus sensu lato*: *Ancyrocephalus paradoxus* Creplin, 1839, *Ancyrocephalus percae* Ergens, 1966, and *Ancyrocephalus mogurndae* (Yamaguti, 1940). Phylogenetic analyses based on these data have shown that *Ancyrocephalus sensu stricto*, as proposed by Bychowsky and Nagibina (1970) for *A. paradoxus* (type) and *A. percae* from freshwater Palaearctic percid fishes, appears to be a natural taxon (Mathews *et al.* 2021; Kmentová *et al.* 2022; Osaki-Pereira *et al.* 2023). At the same time, *A. mogurndae* clusters with representatives of *Eutrianchoratus* Paperna, 1969, *Gobioecetes* Ogawa & Ito, 2017, *Heteronchocleidus* Bychowsky, 1957, *Pseudodactylogyrus* Gussev, 1965, and *Trianchoratus* Price & Berry, 1966, confirming the polyphyly of *Ancyrocephalus sensu lato* (Wu *et al.* 2006; Mendoza-Palmero *et al.* 2015; Kmentová *et al.* 2022). According to Kmentová *et al.* (2022), *A. mogurndae* diverged from *A. paradoxus* and *A. percae* at the subfamily level: Dactylogyrinae *vs* Ancyrocephalinae.

Ancyrocephalus curtus Achmerov, 1952 is a host-specific gill-associated parasite of the odontobutid fish *Perccottus glenii* Dybowski, 1877. This fish is an invasive species with a rather small heartland (the Middle and the Lower Amur and several neighbouring water systems) and an extensive invader range (water bodies of Europe and Siberia) (Reshetnikov 2010). *Ancyrocephalus curtus* has been recorded only in native populations of *P. glenii* and in some introduced populations confined to water bodies within the Upper Amur Basin (Sokolov and Frolov 2012; Sokolov *et al.* 2013, 2014; Sokolov and Reshetnikov 2020). The type locality of this parasite is Lake Bolon, Amur Basin (Akhmerov 1952). Here we provide the first phylogenetic assessment of *A. curtus* using partial sequences of 28S rRNA gene.

Material and methods

In July 2023, specimens of *A. curtus* (Figure 1) were collected from the gills of *P. glenii* caught in the River Syumnyur, a tributary of Lake Bolon, Amur Basin (49°52’56” N; 136°7’52” E). The parasites were fixed in 96% ethanol and stored at −18ºC. The parasite species was identified according to Akhmerov (1952), Gussev (1955), and Gussev *et al.* (2009).

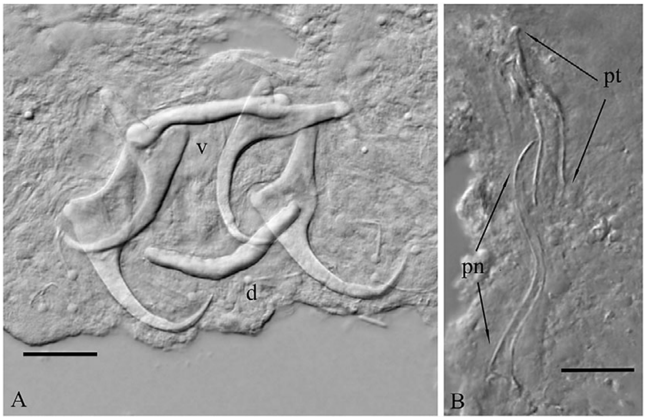

Figure 1. *Ancyrocephalus curtus* from *Perccottus glenii.* **A** – haptoral hard parts. **B** – male copulatory organ. d – dorsal anchors and dorsal haptoral bar; pn – penis; pt –accessory piece; v – ventral anchors and ventral haptoral bar. Scale bar; A, B = 20 μm.

Total DNA was extracted separately from small body fragments (anterior ends cut off at the level of eyespots) of two specimens according to Holterman et al. (2006). The remaining body parts of these specimens were treated with proteinase-K to soften the tissue and were mounted in glycerol-gelatine to study sclerotised structures. The extracted DNA was used as a template in the PCR reaction to amplify the partial D1–D2 domain of the 28S rRNA gene using forward C1 (5'-ACCCGCTGAATTTAAGCAT-3') (Gouÿ de Bellocq *et al.* 2001) and reverse primers D2 (5'-TGGTCCGTGTTTCAAGAC-3') (Lê et al. 1993). Cycling conditions were as follows: 2 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, and a final extension for 10 min at 72° C. PCR products were examined on 1% agarose gels, stained with ethidium bromide, and photographed upon transillumination.

To assess the phylogenetic relationships of *A. curtus*, Bayesian Inference analyses based on partial sequences of 28S rRNA gene were performed. BLAST searches performed on newly obtained sequences demonstrated the highest matching with the sequences of members of the Dactylogyrinae *sensu* Kmentová *et al.* (2022). For the phylogenetic reconstructions, newly obtained sequences were aligned with sequences of dactylogyrinines and some ancyrocephalines (only *A. percae* and *A. paradoxus*) available in the GenBank dataset. Alignments were performed using the Muscle algorithm (Edgar 2004) as implemented in SeaView Version 4.0 (Gouy *et al.* 2010), after which the alignment was adjusted manually. The final length of the alignment was 606 bp. Bayesian algorithm was performed in MrBayes 3.2.7a (Ronquist *et al.* 2012) with the GTR+G+I model. The evolutionary model was estimated with the help of jModeltest 2.1.7 (Darriba *et al.* 2012). In the analysis, 15,000,000 generations of the Markov chain Monte Carlo were simulated, and the selection was performed once every 100 generations. Three species of the Diplectanidae (i.e., *Dolicirroplectanum lacustre* Kmentová, Gelnar & Vanhove, 2021, *Paradiplectanum sillagonum* (Tripathi, 1959), and *Pseudorhabdosynochus grouperi* (Bu, Leong, Wong, Woo & Foo, 1999)) were used as outgroup (Kmentová *et al.* 2022).

Results

The two newly obtained sequences of 28S rRNA gene of *A. curtus* were identical. Bayesian Inference analysis showed that *A. curtus* was a strongly supported sister taxon to *Gobioecetes* (Figure 2). In turn, the *A. curtus* + *Gobioecetes* clade appeared as a strongly supported sister taxon to *A. mogurndae,* and this entire species group was nested in the highly supported clade, which also contained *Eutrianchoratus cleithrium* Lim, 1989*, Heteronchocleidus buschkieli* Bychowsky, 1957*, Pseudodactylogyrus* spp., and *Trianchoratus gussevi* Lim, 1986. The above-mentioned representatives of *Eutrianchoratus*, *Heteronchocleidus*, and *Trianchoratus* formed a clade that had a strongly supported sister relationship to the *A. mogurndae* + (*A. curtus* + *Gobioecetes*) clade. *Pseudodactylogyrus* spp. occupied a basal position to all the species mentioned above.

The clade containing *A. mogurndae*, *A. curtus*, *Gobioecetes* spp., *Pseudodactylogyrus* spp., and representatives of *Eutrianchoratus*, *Heteronchocleidus*, and *Trianchoratus* was resolved within the large, strongly supported Dactylogyrinae clade.

Discussion

In this study, we assessed the phylogenetic position of *A. curtus* from the type-host and the locality very close to the type-locality using molecular data. Our phylogenetic analysis shows that *A. curtus* shares the most recent common ancestor with the clade formed by *Gobioecetes* spp. from Japanese freshwater gobiid fish. The similarities between *A. curtus* and *Gobioecetes* spp. are evident in the morphology of the sclerotised male copulatory organ and the absence of the vaginal armament. These parasite species have a penis shaped as a long, coiled and/or sinuous tube with a conspicuous inflation at the base, and an accessory piece shaped as a separate, distinctly concaved plate, which is not connected to the proximal end of the penis (Gussev 1955; Ogawa and Itoh 2017; Nitta and Nagasawa 2020). The most pronounced differences between *A. curtus* and *Gobioecetes* spp. are associated with the haptoral armatures. The former species has both the dorsal and the ventral haptoral bar, whereas *Gobioecetes* spp. have only the ventral bar (Gussev 1955; Ogawa and Itoh 2017; Nitta and Nagasawa 2020).

Our findings suggest that there is no direct phylogenetic relationship between *A. curtus* and *A. mogurndae.* However, this conclusion is provisional because the identification of *A. mogurndae* specimens for which molecular data are available is not supported by any morphological evidence. *Ancyrocephalus mogurndae* is characterised by the same morphological type of the male copulatory organ as *A. curtus* and *Gobioecetes* spp. (Gussev 1955; Ogawa and Itoh 2017; Nitta and Nagasawa 2020). At the same time, this species differs sharply from *A. curtus* and *Gobioecetes* spp. in the presence of the vaginal armament (Gussev 1955; Ogawa and Itoh 2017; Nitta and Nagasawa 2020). Based on morphological and ecological data, Gerasev (2008) hypothesised that the group within *Ancyrocephalus sensu lato* containing *A. curtus* and *A. mogurndae* might have a monophyletic status. However, our results do not support this hypothesis.

The clade of *A. mogurndae, A. curtus*, and *Gobioecetes* spp. appeared as a member of a monophyletic group within the Dactylogyrinae, which also comprised *Pseudodactylogyrus* spp. and representatives of *Eutrianchoratus*, *Heteronchocleidus*, and *Trianchoratus.* Kmentová *et al.* (2022) proposed to name this group Clade A9. Morphological synapomorphies for Clade A9 are not obvious. The only clearly distinctive feature of its members is that their range (or its native part, in case of invasive *Pseudodactylogyrus* spp.) lies within the Far Eastern and/or South-Eastern regions of Asia (Gussev 1955; Lim 1986, 1989; Buchmann *et al.* 1987).

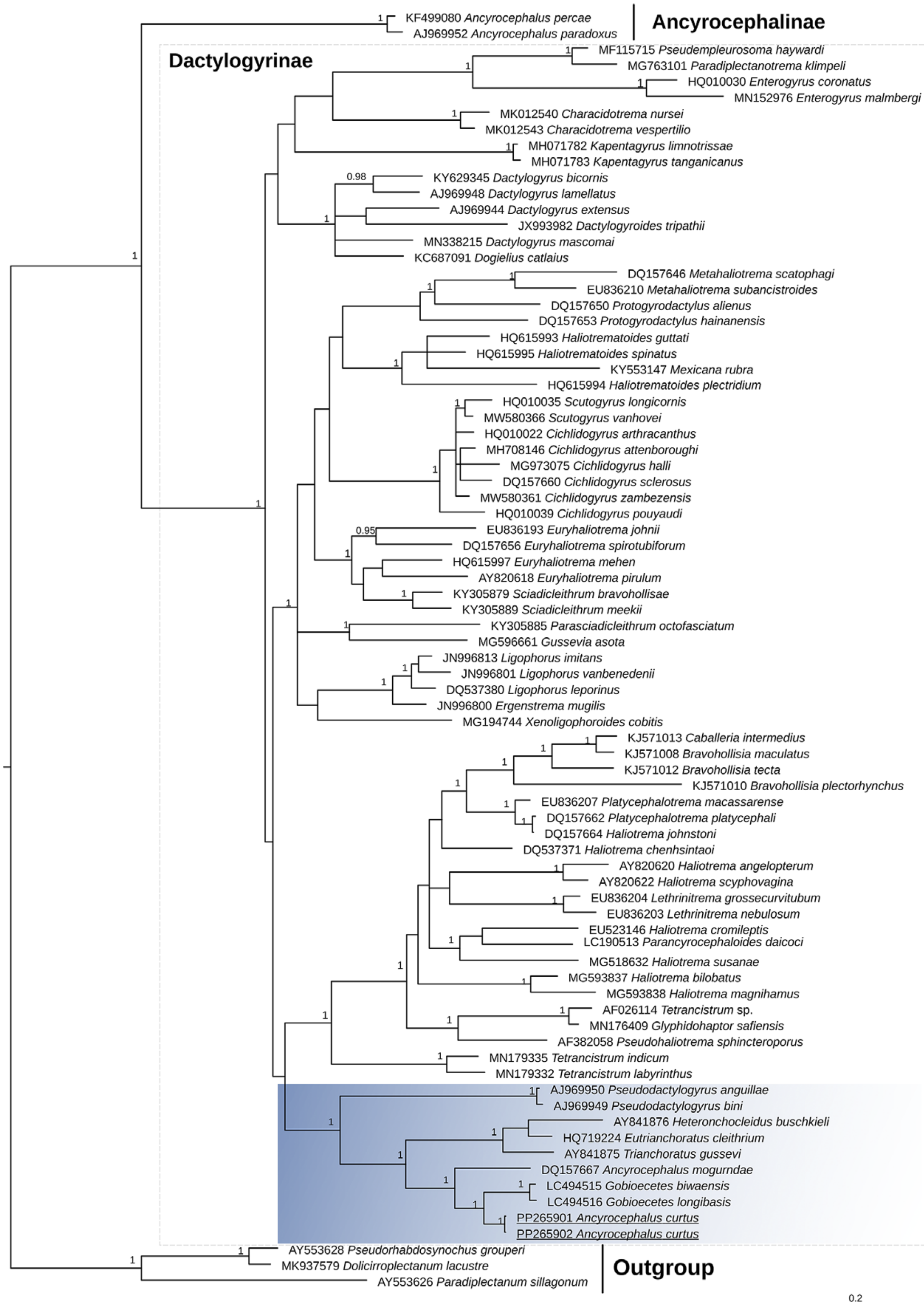

Figure 2. Phylogenetic relationships of *Ancyrocephalus curtus* reconstructed by Bayesian Inference analysis of 28S rRNA gene sequences. Posterior probability values lower than 0.9 are not shown. Newly obtained sequences are underlined.

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The position of *A. mogurndae* and *A. curtus* on our tree combined with morphological differences between them and *Gobioecetes* spp. probably indicates that each of these representatives of *Ancyrocephalus sensu lato* should be assigned to a separate genus. However, we refrain from this taxonomic act until molecular data on other *Ancyrocephalus* spp. from the fishes of the Amur Basin described by Akhmerov (1952) and Gussev (1955) become available.

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

Ethical standard. Not applicable. The host fish is an object of recreational fishing, and therefore, no ethics permit was required under the Russian law.

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