DE GRUYTER

DOI 10.1515/helmin-2016-0006

HELMINTHOLOGIA, 53, 2: 161 – 164, 2016

Research Note

Phylogenetic position of trematode *Amurotrema dombrowskajae* Achmerow, 1959 (Paramphistomoidea: Cladorchiidae) based on partial 28S rDNA nucleotide sequences

S. G. SOKOLOV[1]*, D. I. LEBEDEVA[2], A. P. KALMYKOV[3]

[1]*Centre for Parasitology of the A.N. Severtsov Institute of Ecology and Evolution RAS, 33 Leninskij prosp., Moscow, 119071, Russia, E-mail: *sokolovsg@mail.ru*; [2]Institute of Biology of Karelian Research Centre of RAS, 11 Pushkinskaya st., Petrozavodsk,185910, Russia, E-mail: *daryal78@gmail.com*; [3]Astrakhan Nature Reserve, 119 Naberezhnaya Tsarev, Astrakhan 414021, Russia, E-mail: *kalmykov65@rambler.ru*

Article info

Received September 16, 2015
Accepted November 6, 2015

Summary

The trematode *Amurotrema dombrowskajae* Achmerow, 1959, is the type and the only species of the paramphistomoid genus *Amurotrema* Achmerow, 1959. This intestinal parasite of grass carp (*Ctenopharyngodon idella*) was introduced with host to the Volga River Delta. These naturalized trematodes were found by the authors in 2014 – 2015. In the present study the phylogenetic position of *Amurotrema dombrowskajae* is analysed using partial 28S rDNA nucleotide sequences. Bayesian inference and Maximum Likelihood analyses place this species in a clade with representatives of the family Cladorchiidae - *Megalodiscus temperatus* (Stafford, 1905) and *Indosolenorchis hirudinaceus* Crusz, 1951. Thus, molecular data are consistent with the traditional view on the position of *A. dombrowskajae* in this family.

Keywords: *Amurotrema dombrowskajae; Ctenopharyngodon idella;* Cladorchiidae; 28S rDNA

Introduction

Trematode *Amurotrema dombrowskajae* Achmerow, 1959 is the type species of the monotype genus *Amurotrema* Achmerow, 1959. It is also the one of the few paramphistomoid species (Paramphistomoidea) parasitizing fish in Eurasia. The morphology of this species has been described in sufficient detail by several authors (Akhmerov, 1959; Agapova, 1966; Chen, 1973; Chen, 1985; Sey, 1985).

The native range of *A. dombrowskajae* covers the Amur (Manchurian) Transitional and Sino-Indian zoogeographic regions (Akhmerov, 1959; Strelkov, 1971; Chen, 1973; Chen, 1985). Here the parasite occurs the mostly in grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) (Akhmerov, 1959; Roytman, 1963; Strelkov, 1971; Chen, 1973; Chen, 1985), but it was recorded also from *Spinibarbus denticulatus* (Oshima, 1926) (previously *Spinibarbichthys denticulatus*) (Ha Ky, 1969; Sey, 1985).

In the second half of the 20th century grass carp was introduced to water bodies in various regions of the planet (Shireman and Smith, 1983). Parasite *A. dombrowskajae* with host species was expanded to water bodies of Central Asia (Kazakhstan), East (Bashkiria and Astrakhan Region of the Russian Federation) and Central (Hungary) Europe (Agapova, 1966; Vasil'kov *et al*., 1970; Astakhova & Stepanova, 1972; Molnar, 1984). However, the parasite was usually recorded from introduced grass carp only in the Balkhash-Ili basin (Gvozdev *et al*., 1986) and in the Volga River Delta (Ivanov *et al*., 2008, 2012). That fact designates the naturalization of *A. dombrowskajae* in the aforementioned water bodies.

Here we analyze the phylogenetic position of *A. dombrowskajae* by means of novel ribosomal molecular data using the specimens collected from the Volga River Delta.

Materials and Methods

Trematodes *A. dombrowskajae* were collected from 2 individuals of the grass carp, originating from the wild — Obzhorovo channel in the Buzan River system (the Volga River Delta, Astrakhan Region, Russia); 46°10' N, 49°13' W. In total, three fish (1 in October 2014 and 2 in May 2015) were examined.

Most of the trematodes were fixed in 10 % hot formalin, then stained in acetic carmine, dehydrated by alcohol series, and mounted in Canada balsam. For phylogenetic analyses, we fixed and stored some specimens in 96 % ethanol.

Total genomic DNA for molecular analysis was isolated from two specimens of *A. dombrowskajae* according to the protocol by Miller *et al*. (1988). Specimens for PCR were processed according to the protocols used by Olson *et al*., 2003. Amplification was performed with primers DIGL2 (5' – AAG CAT ATC ACT AAG CGG – 3') and 1500R (5' – GCT ATC CTG AGG GAA ACT TCG – 3'). PCR products were purified and sequenced in both directions at ABI 3130 (PE Applied Biosystems) using PCR primers and internal primers 300F (5' – CAA GTA CCG TGA GGG AAA GTT G – 3') and ECD2 (5' – CTT GGT CCG TGT TTC AAG ACG GG – 3'). Contiguous sequences were processed (assemblage, alignment, tree reconstruction) using Geneouis 8.1.6 (Trial Version). The two alignments (1237 bp) were identical. One of the new sequences was deposited in GenBank with accession number KT321869.

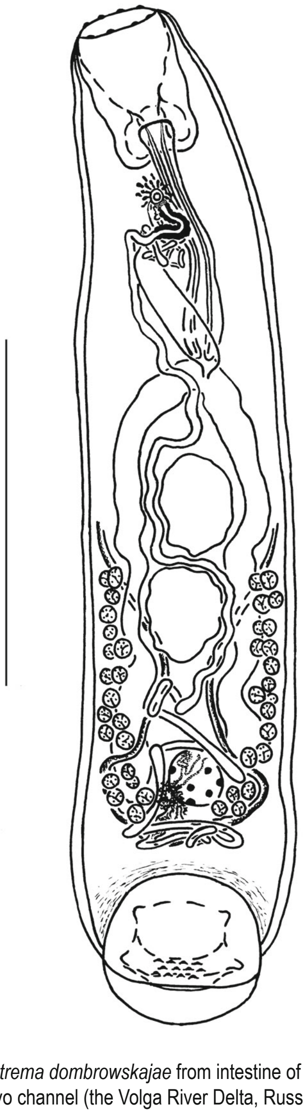

Fig. 1. *Amurotrema dombrowskajae* from intestine of grass carp caught in Obzhorovo channel (the Volga River Delta, Russia), general view Scale bar = 2 mm

The partial 28S rDNA sequences generated in the study were aligned with representatives of different families of the superfamily Paramphistomoidea Fischoeder, 1901 available in GenBank. Bayesian inference and Maximum Likelihood analyses of the 28S rDNA dataset were conducted to explore relationships among these taxa. In addition, jModelTest version 0.1.1 (Posada, 2008) was used to estimate the best nucleotide substitution model for the dataset. Both analyses were conducted using the GTR+G model. Branch support was estimated by bootstrap analyses with 500 replicates. The trematode *Mesometra* sp. of the family Mesometridae Poche, 1926 (superfam. Microscaphidioidea) was designated as an outgroup.

The voucher specimens studied were deposited at the Museum of Helminthological Collections of the Centre for Parasitology of the A.N. Severtsov Institute of Ecology and Evolution RAS, Moscow, Russia (IPEE RAS).

Results and Discussion

Specimens of *A. dombrowskajae* (Fig. 1) were found in the intestines of 2 individuals of the grass carp – 6 specimens in 2014 and 7 specimens in 2015, respectively.

Molecular analyses of partial 28S of *A. dombrowskajae* showed close phylogenetic relationships of the parasite with members of the family Cladorchiidae – *Megalodiscus temperatus* (Stafford, 1905) and *Indosolenorchis hirudinaceus* Crusz, 1951 (Fig. 2). The nearest neighbor species is *M. temperatus*. The distance between the latter and *A. dombrowskajae* is 10.8 %, the distance of *A. dombrowskajae* from *I. hirudinaceus* is 11.9 %.

The taxonomic position of *A. dombrowskajae* has been discussed in many papers. Following Skryabin's (1949) systematics of trematodes, Akhmerov (1959) placed the species into the subfamily Schizamphistomatinae Loos, 1912 of the family Diplodiscidae Cohn, 1904. Yamaguti (1971) transferred the species to the subfamily Dadaytrematinae Yamaguti, 1958, dedicated within Paramphistomidae Fischoeder, 1901. Sey (1988) included *A. dombrowskajae* in the subfamily Helostomatinae Skrjabin, 1949 of the family Cladorchiidae Fischoeder, 1901. Jones (2005) agreed that *A. dombrowskajae* was a member of the family Cladorchiidae, but transferred the species into the subfamily Dadayiinae Fukui, 1929. In contrast to Sey (1988), she considered the subfamily Helostomatinae to be a monotype taxon (Jones, 2005). Morphological markers for this subfamily are distinctive pharyngeal sacs, which consist of long narrow muscular distal part, and extended and non-muscular proximal part. Jones (2005) considered Dadayiinae

Fig. 2. Relationships between *Amurotrema dombrowskajae* and related taxa based on Maximum Likelihood analyses and Bayesian inference of the 28S rDNA dataset. Bootstrap support values are shown above the nodes and posterior probabilities below the nodes. Sequence obtained in the present study is indicated by asterisk (*). *Mesometra* sp. is the outgroup taxon.

and Dadaytrematinae to be synonims. Sey (1988) adhered to a similar opinion, but incorrectly recognized the validity of Dadaytrematinae (Jones, 2005).

The phylogenetic analyses (Fig. 2) showed a clear clustering of *A. dombrowskajae* with other cladorchiid trematodes. This topology is consistent with the view of Sey (1988) and Jones (2005) on the position of the aforementioned species the in family Cladorchiidae. The subfamilial position of *A. dombrowskajae* currently cannot be determined due to the lack of 28S rDNA sequence data for other cladorchiids.

Acknowledgements

The research was partly supported by the RFBR 15 – 29 – 02528 and state order 0221 – 2014 – 0004.

References

Agapova, A.I. (1966): *Parasites of fish from waterbodies of Kazakhstan*. Alma-Ata: Nauka, 342 pp. (In Russian)

Akhmerov, A.Kh. (1959): New genus of fish trematodes. *Trudy gelmintologicheskoy laboratorii AN SSSR*, 9: 45 – 48. (In Russian)

Astachova, T.V., Stepanova, G.A. (1972): On the parasite fauna of the *Ctenopharyngodon idella* from pond and spawning-nursery fisheries in the delta of the Volga River. *Parazitologiia,* 6(4): 364 – 368 (In Russian)

Chen, C.-L. (1973): *An illustrated guide to the fish diseases and causative pathogenic fauna and flora in the Hubei Province*. Beijing: Science Press, 456 pp. (In Chinese)

Chen, X.-T. (1985): *Fauna Sinica: Platyhelminthes: Trematoda: Digenea (1)*. Beijing: Science Press, 697 pp. (In Chinese)

Gvozdev, E.V., Agapova, A.I., Belyakova, J.V. (1986): The developmental cycle of the trematode *Amurotrema dombrowskajae* (Trematoda, Diplodiscidae). *Parazitologiia*, 20 (4): 288 – 293 (In Russian)

Ha, Ky. (1969): *Parasite fauna of some freshwater fishes in North Viet Nam and control measures against their most important diseases*. Avtoreferat Dissertatsii na Soiskaniye Uchenoi Stepeni Kandidata Biologicheskikh Nauk, Zool. Inst. Leningrad: Akad. Nauk SSSR. 18 p. (In Russian)

Ivanov, V.M, Litvinov, K.V., Kalmykov, A.P. (2012): Pathways and the spread of invasive helminths of fish in the Volga River Delta. In: Movsesyan, S.O. (Ed) *Modern problems of general parasitology: The Materials of the International Conference (30 October –1 November, 2012, Moscow).* Moscow. pp. 135 – 138 (In Russian)

Ivanov, V.M, Semenova, N.N., Parshina, O.Yu., Kalmykov, A.P., Fedorovich, V. (2008). New ways of penetrations of new species of trematodes for the Volga River Delta. In: Movsesyan, S.O. (Ed) *Biodiversity and ecology of parasites of terrestrial and water cenoses: The Proceedings of International Symposium dedicated to the 130th Anniversary of acad. K. I. Skryabin (December 9 – 11, 2008, Moscow).* Moscow. pp. 145 – 148 (In Russian)

Jones, A. (2005): Superfamily Paramphistomoidea Fischoeder, 1901. In: Jones, A., Bray, A. Gibson, D.I. (Eds) *Keys to the Trematoda*. Vol. 2. London, UK: CABI Publishing and The Natural History Museum, pp. 221 – 356

Molnar, K. (1984): Parasite range extension by introduction of fish to Hungary. *EIFAC Tech. Pap.*, 42 (suppl. 2): 534 – 540

Miller, S. A., Dykes, D. D., Polesky, H. F. (1988): A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, 16(3): p. 1215

Olson, P.D., Cribb, T.H., Tkach, V.V., Bray, R.A., Littlewood, D.T.J. (2003): Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *Int. J. Parasitol.*, 33(7): 733 – 755. DOI: 10.1016/S0020–7519(03)00049–3

Posada, D. (2008): jModelTest: Phylogenetic model averaging. *Mol. Biol. Evol.*, 25(7): 1253 – 1256. DOI: 10.1093/molbev/msn083

Roytman, V.A. (1963): *Helminthofauna of fishes of the Zeya River basin and its ecological and geographical characteristics*. Avtoreferat Dissertatsii na Soiskaniye Uchenoi Stepeni Kandidata Biologicheskikh Nauk, Inst. Helmonthol. Moscow: 16 p. (In Russian)

Sey, O. (1985). Amphistomes of Vietnamese vertebrates (Trematoda: Amphistomida). *Parasitol. Hung.*, 18: 17 – 24

Sey, O. (1988): Scope of and proposal for systematics of the Amphistomida (Lühe, 1909) Odening, 1974 (Trematoda). *Parasitol. Hung.*, 21: 17 – 30

Shireman, J.V., Smith, C.R. (1983): Synopsis of biological data on the grass carp, *Ctenopharyngodon idella* (Cuvier and Valenciennes, 1884). *FAO Fish. Synop.*, 135: 1 – 86

Skryabin, K.I. (1949): *Trematodes of animals and men. Vol. 3*. Moscow, Publishing House of the USSR Academic Science, 609 pp. (In Russian)

Strelkov, Yu.A. (1971): Trematodes of fish from Amur River basin. *Parasitologichesky sbornik ZIN AN SSSR*, 25: 41 – 76 (In Russian)

Vasiľkov, G.V., Sapozhnikov, G.I., Teteryuk, P.M. (1970): On the study of helminth fauna of pond fishes of the Bashkirskaya ASSR. *Trudy Vsesoyuznogo instituta gelmintologii*, 16: 49 – 52 (In Russian)

Yamaguti, S. (1971): *Synopsis of digenetic trematodes of vertebrates. Vol. 1*. Tokyo, Keigaku. 1074 pp.