Parasitology International 64 (2015) 26–32

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

The life cycle and molecular phylogeny of a gorgoderid trematode recorded from the mussel *Nodularia douglasiae* in the Yodo River, Japan

Misako Urabe [a,*], Ryo Ishibashi [b], Kazuhiko Uehara [b]

[a] Department of Ecosystem Studies, School of Environmental Science, The University of Shiga Prefecture, 2500 Hassaka, Hikone, Shiga 522-8533, Japan
[b] Aquatic Life Conservation Center, Research Institute of Environment, Agriculture, and Fisheries, Koyamotomachi 10-4 Neyagawa, Osaka 572-833, Japan

ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 12 August 2014
Accepted 3 September 2014
Available online 9 September 2014

Keywords:
Subfamily Gorgoderinae
Nodularia douglasiae
Cyprinus carpio
Life cycle
Molecular phylogeny

ABSTRACT

In 2009, a novel larval trematode of the family Gorgoderidae was found in the gonads of *Nodularia douglasiae* (Unionidae) from the lower reaches of the Yodo River, Osaka Prefecture, Japan. This is the first collection of trematodes in a unionid mussel in Japan. We investigated the morphology and life cycle of the trematode, and conducted a molecular phylogenetic analysis with other gorgoderid species, both those collected in the Yodo River water system and those reported in the literature.

Immature adult worms were obtained from the ureters of the common carp *Cyprinus carpio*, the first known instance of a gorgoderid from these fish in Japan. Morphological characteristics and molecular data show that it belongs to the subfamily Gorgoderinae (genus *Phyllodistomum sensu lato*). Regarding the morphology, first intermediate host, and the infection site of adult worms, it resembles *Phyllodistomum elongatum* Nybelin, 1926 from Europe, but no comparable molecular data exist for *Ph. elongatum*. Three cytochrome *c* oxidase subunit I haplotypes were detected in the specimens analyzed, suggesting that the present species is indigenous to the Yodo River water system. The 28S ribosomal DNA data showed that this species is a member of the clade consisting of *Ph.* cf. *symmetorchis*, *Ph. folium*, *Pseudophyllodistomum* and *Xystretrum*. However, its phylogenetic position within the clade differs between the maximum likelihood and maximum parsimony trees, and the sister species of the present species remain unclear.

1. Introduction

The taxonomic situation of many species belonging to the family Gorgoderidae Looss, 1899, is confusing [1,2]. Recently, Cutmore et al. [3] generated a molecular phylogenetic tree of gorgoderids, and identified some new aspects of gorgoderid phylogeny. First, they recognized three subfamilies in the family Gorgoderidae. Second, they showed at least three clades within the subfamily Gorgoderinae Looss, 1899 and suggested that their first intermediate host may be an important key to these clades. Third, the genus *Phyllodistomum* Braun 1899, which is the biggest genus in the family Gorgoderidae and also one of the largest genera in Digenea, was shown to be paraphyletic. Their samples included freshwater gorgoderid species that use cyrenids, dreissenids and sphaeriids as their first intermediate host, but no species were included that use unionids, which are one of the major taxa of intermediate hosts. This left a major gap in their data. Molecular data of species from unionids will add important information to the phylogeny of Gorgoderidae.

In September 2009, one of the authors (R. Ishibashi) found a novel larval trematode in the gonads of *Nodularia douglasiae nipponensis* (syn. *Unio douglasiae nipponensis*) (Martens, 1877) from the lower reaches of the Yodo River, Settsu City, Osaka Prefecture. Its general morphology suggested that it is a gorgoderid cercaria, which had not been known to exist in unionid mussels in Japan. We sampled fishes several times from the same body of water and searched them for the adult form, finding some unknown gorgoderids from the common carp, *Cyprinus carpio* Linnaeus 1758. Unfortunately, despite persistent searching, all of the worms obtained were immature.

In this paper, we report the morphology of the larval gorgoderids from *N. douglasiae* and adults from the common carp, both of which are new in Japan. The DNA sequences of these gorgoderids were compared to those of two other gorgoderids, *Phyllodistomum parasiluri* Yamaguti, 1934 and *Pseudophyllodistomum macrobrachicola* (Yamaguti, 1934), from fishes caught at other localities in the Yodo water system. We also discuss the phylogenetic position and relationship to other known species based on the data currently available.

2. Materials and methods

2.1. Specimens examined

Nine common carp (*C. carpio*) were caught in the main irrigation canal on the left bank of the Yodo River, Koyamotomachi, Neyagawa

* Corresponding author. Tel.: +81 749 28 8308; fax: +81 749 28 8463.
E-mail address: urabe@ses.usp.ac.jp (M. Urabe).

http://dx.doi.org/10.1016/j.parint.2014.09.003
1383-5769/

Table 1
Gorgoderid samples analyzed for DNA sequences.

Accession no. (28S)	Accession no. (COI)	Species	Stage	Collection date	Host	Locality
AB987942	AB987943-AB987944	*Phyllodistomum* sp.	Adult	August 14, 2011	*Cyprinus carpio*	Main canal on the left bank of the Yodo River, Koyamotomachi, Neyagawa City, Osaka, Japan
	AB987945		Sporocyst	October 26, 2010	*Nodularia douglasiae*	Torikai bridge, Yodo River, Settsu City, Osaka, Japan
LC002522	LC002524	*Phyllodistomum parasiluri*	Adult	February 4, 2011	*Silurus asotus*	Chinai, Lake Biwa, Takashima City, Shiga, Japan
LC002521	LC002523	*Pseudophyllodistomum macrobrachicola*	Adult	January 19, 2010	*Anguilla japonica*	Uji River, Uji City, Kyoto, Japan

City, Osaka Prefecture (34°47′17″N, 135°37′01″E), on August 14, 2011, and were examined for parasites. Three crucian carp (*Carassius* sp.), two barbels (*Hemibarbus barbus* Temminck et Schlegel 1846) and two *Pseudogobio esocinus* Temminck et Schlegel 1846 were also collected at the same locality on the same day and examined.

Worms for morphological study were fixed immediately in AFA, stained with Heidenhain's iron hematoxylin, dehydrated, cleared and mounted in Canada balsam following the conventional protocol [4]. Some additional worms were kept in a freezer for DNA analysis (Table 1).

Larval trematodes were collected from the gonads of *N. douglasiae* for both morphological and molecular studies. Samples for morphological studies were obtained from one mussel collected from the left bank of the Yodo River, Shimeno, Neyagawa City, Osaka (34°46′42″N, 135°36′02″E), on January 27, 2010, and two mussels collected from the right bank of the Yodo River, Torikai-Ôhashi, Settsu City, Osaka (34°45′35″N, 135°34′24″E: about 2.5 km downstream of Shimeno) on December 20, 2010. Fresh specimens of cercariae were examined under a Nomarski microscope, with vital staining by neutral red or Nile Blue sulfate. In addition, some sporocysts and cercariae were fixed in AFA, stained in Heidenhain's iron hematoxylin, dehydrated, cleared, and mounted in Canada balsam following the same protocol used for the adult worms.

Samples for molecular study were obtained from one mussel collected from the right bank of the Yodo River, Torikai-Ôhashi, Settsu City, Osaka, on October 26, 2010. Several sporocysts were kept for DNA analysis (Table 1).

Two whole mounts of *Phyllodistomum elongatum* Nybelin, 1926 deposited in Göteborg Natural History Museum, Göteborg, Sweden, were borrowed for morphological comparison. These specimens examined were not designated as type materials by Orvar Nybelin. One whole mount was from *Tinca tinca* (Linnaeus, 1758) (dated October 17, 1923), and the other was from *Abramis brama* (Linnaeus, 1758) (dated June 16, 1923).

Two species collected from the Yodo River system were also added to the phylogenetic analysis: *Ph. parasiluri* from *Silurus asotus* caught in Lake Biwa, and *Ps. macrobrachicola* from *Anguilla japonica* caught in the Uji River, Uji City, Kyoto, Japan. The details of these specimens are found in Table 1.

Table 2
28S ribosomal DNA sequences from DDBJ/GenBank/EMBL for phylogenetic analysis in this study.

Accession no.	Species	Stage	Host	Reference
KF013171	*Phyllodistomum* cf. *symmetrorchis*	Adult	*Clarias gariepinus*	[3]
KF013172	Gorgoderidae sp.	Adult	*Lioconcha castrensis*	[3]
KF013173	*Phyllodistomum vaili*	Adult	*Mulloidichthys flavolineatus*	[3,7]
KF013175	*Phyllodistomum* sp. 5	Adult	*Cephalopholis boenak*	[3]
KF013177	*Pseudophyllodistomum johnstoni*	Metacercaria	*Macrobranchium australiense*	[3]
KF013178	*Xystretrum* sp.	Adult	Rhinecanthus aculeatus	[3]
KF013183	*Phyllodistomum* sp. 4	Adult	*Cephalopholis urodeta*	[3]
KF013184	*Anaporrhutum* sp.	Adult	*Chiloscyllium punctatum*	[3]
KF013188	*Xystretrum solidum*	Adult	*Sphoeroides testudineus*	[3]
KF013189	*Phyllodistomum magnificum*	Adult	*Tandanus tandanus*	[3]
KF013190	*Phyllodistomum* sp. 2	Adult	*Hyporhamphus australis*	[3]
KF013191	*Phyllodistomum hoggettae*	Adult	*Plectropomus leopardus*	[3,7]
KF013192	*Nagmia* sp.	Adult	*Stegostoma fasciatum*	[3]
KF013190	*Plesiochorus* sp.	Adult	*Caretta caretta*	[3]
AY222257	*Degeneria halosauri*	Adult	*Halosauropsis macrochir*	[8]
AY222262	*Nagmia floridensis*	Adult	*Rhinoptera bonasus*	[8]
AY222263	*Xystretrum* sp.	Adult	*Sufflamen chrysopterum*	[8]
AY222264	*Gorgodera cygnoides*	Adult	*Rana ridibunda*	[8]
AY281126	*Phyllodistomum* sp. *Lithania*	Cercaria	*Pisidium amnicum*	[9]
AY281127	*Phyllodistomum folium*	Cercaria	*Dreissima polymorpha*	[10]
EF0325027	*Phyllodistomum staffordi*	Adult	*Ameriurus melas*	[11]
HQ325007	*Gorgoderina* sp.	Adult	*Rana* sp.	[11]
HQ325008	*Phyllodistomum brevicaecum*	Adult	*Umbra limi*	[11]
HQ325016	*Phyllodistomum lacustri*	Adult	*Ictelurus pricei*	[11]
HQ325030	*Xystretrum caballeroi*	Adult	*Balistes polylepis*	[11]
HM486318	*Staphylorchis cymatodes*	Adult	*Chiloscyllium punctatum*	[12]
KC760199	*Phyllodistomum inecoli*	Adult	*Heterandria bimaculata*	[13]
Outgroup				
AY222206	*Prosthenhystera obesa*	Adult	*Hoplias* sp.	[8]