New data on the morphology and phylogenetic connections of *Postlepidapedon opisthobifurcatum* (Trematoda, Lepocreadioidea: Lepidapedidae), a parasite of Antarctic and sub-Antarctic fishes

*S. G. SOKOLOV¹, F. K. KHASANOV¹, I. I. GORDEEV²,³,*

¹Center of Parasitology, A.N. Severtsov Institute of Ecology and Evolution RAS, Leninskij prospect, 33, Moscow, 119071, Russia; ²Lomonosov Moscow State University, Leninskie Gory, 1, Moscow, 119234, Russia, *E-mail: gordeev_ilya@bk.ru; ³Russian Federal Research Institute of Fisheries and Oceanography, V. Krasnoselskaya Str., 17, Moscow, 107140, Russia

**Article info**

Received August 28, 2017  
Accepted January 17, 2018

**Summary**

The trematode *Postlepidapedon opisthobifurcatum* (Zdzitowiecki, 1990) is a common intestinal parasite of the gadiform fishes of the Southern Ocean. In this work, we supplement the description of the species with the anatomy of the terminal part of the reproductive system and with molecular data. The male terminal genitalia are characterised by the presence of the external seminal vesicle and cirrus-sac. The external seminal vesicle is surrounded by aciniform groups of outer prostatic cells. Groups of outer prostatic cells and proximal parts of their ducts are associated with a thin-walled membrane that is connected to the proximal edge of the cirrus-sac. The cirrus-sac is claviform, with a long proximal part accommodating the tubular, thin-walled internal seminal vesicle and ducts of outer prostatic cells. The female terminal genitalia are represented by a thick-walled metraterm, which is surrounded by aciniform groups of glandular cells. Phylogenetic analysis based on 28S rDNA partial sequences data placed *P. opisthobifurcatum* into the monophyletic group Lepidapedidae, including the species *Myzoxenus insolens* (Crowcroft, 1945), *Intusatrium robustum* Durio et Manter, 1968, and *Postlepidapedon uberis* Bray, Cribb et Barker, 1997. However, we were unable to detect direct phylogenetic connections between *P. opisthobifurcatum* and *P. uberis*.  

**Keywords:** Trematoda; *Postlepidapedon opisthobifurcatum*; Lepidapedidae

**Introduction**

The trematode *Postlepidapedon opisthobifurcatum* (Zdzitowiecki, 1990) Zdzitowiecki, 1993 is one of the common intestinal parasites of Antarctic and sub-Antarctic fishes of the families Muraenolepididae and Macrouridae (Zdzitowiecki, 1990; Zdzitowiecki & Cielecka, 1997; Walter et al., 2002; Sokolov & Gordeev, 2013; 2015; Gordeev & Sokolov, 2017). This species was originally included in the genus *Neolepidapedon* Manter, 1954 (see Zdzitowiecki, 1990). Zdzitowiecki (1993) subsequently erected a new genus, *Postlepidapedon*, based on the morphology of the cirrus-sac (presence of elongated, thin-walled internal seminal vesicle and narrow, long ejaculatory duct), the position of the intestinal bifurcation, and a number of other characters to accommodate *Neolepidapedon opisthobifurcatus* Zdzitowiecki, 1990. Other than the type species (*P. opisthobifurcatum*), this genus currently includes five other congeners, described from perciform fishes from the waters off Australia, New Caledonia, and the Philippines (Bray et al., 1997; Bray & Cribb, 2001; Machida, 2004). Bray & Cribb (2012) placed the genus *Postlepidapedon* into the family Lepidapedidae. The aim of the present study is to describe in more detail the morphology of the terminal part of reproductive system of adult *P. opisthobifurcatum* and to define the phylogenetic position of this species based on molecular data.

* – corresponding author
**Materials and Methods**

**Specimen collection and morphological study**

The digeneans were collected during parasitological examination of specimens of *Muraenolepis marmorata* Günther, 1860 (Gadiformes, Muraenolepididae) caught on 18 March 2013 in the central part of the Ross Sea (75°48'S 172°48'W). All hosts (length 49 – 55 cm, weight 0.9 – 1.4 kg) were caught by the fishing vessel *Yantar-35* while it was fishing for the toothfish *Disostichus* spp. at depths ranging from 962 m to 1228 m using bottom longline fishing gear (autoline) “Mustad” (Petrov et al., 2014) inside the Convention for the Conservation of Marine Living Resources (CCAMLR) area. The worms collected for morphological study were fixed in 70 % ethanol under a cover glass without additional pressure and stained with aceticarmine. Trematode specimens of the studied species were deposited in the Museum of Helminthological Collections, Centre for Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, Russia (IPEE RAS).

**DNA extraction, amplification, sequencing, and phylogenetic analysis**

Genomic DNA was extracted from single specimens of adult worms following the protocol used by Tkach et al. (1999). Nuclear 28S rDNA partial fragment, including D1-D3 domains, was amplified using a polymerase chain reaction by the following primers: DIG12 (5'-AAG CAT ATC ACT AAG CGG-3') and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Tkach et al., 2003). The initial polymerase chain reaction was carried out in a total volume of 25 μl containing 0.25 mM of each primer pair, 5 μl DNA in water, 1× Phusion polymerase buffer, 2.5 mM dNTP, and 1 unit of Phusion High-Fidelity DNA Polymerase (New England Biolabs, UK). A negative and positive controls, using both primers, were used. The PCR products were directly sequenced using an ABI Big Dye Terminator v3.1 Cycle Sequencing Kit, as recommended by the manufacturer, with the internal sequencing primers 300F (5'–CAA GTA CCG TGA GGG AAA GTT G-3'), ECD2 (5'–CTT GCT GTG CCG TGT TTC AAG ACG GG–3'), 900F (5'–CCG TCT TGA AAC ACG GACCAA G-3'), and 1200R (Tkach et al., 2003). The sequences obtained have been submitted to NCBI GenBank (Table 1).

**Results**

**Morphology of the terminal part of the reproductive system**

The male terminal genitalia are represented by the external seminal vesicle, cirrus-sac, and complex of outer prostatic cells (Fig. 1). The vas deferens is absent; vas efferentia is joined directly to the external seminal vesicle. The external seminal vesicle is large, 1.5 – 2 times longer than the cirrus-sac, convoluted, and surrounded by aciniform groups of outer prostatic cells radiating into the parenchyma. Groups of outer prostatic cells and proximal parts of their ducts are covered with a thin-walled open-ended membrane. The membrane is divided into two sheets – dorsal and ventral.
Table 1. List of taxa, incorporated into molecular analysis.

| Species                        | Family       | Reference                  | GenBank accession number |
|--------------------------------|--------------|----------------------------|--------------------------|
| Bulbocirrus aulostomi Yamaguti, 1965 | Lepidapedidae | Bray et al. (2009)         | FJ788470                 |
| Intusatrium robustum Durio et Manter, 1968 | Lepidapedidae | Bray et al. (2009)         | FJ788481                 |
| Lepidapedon arlenae Bray et Gibson, 1995 | Lepidapedidae | Bray et al. (1999)         | AJ405262                 |
| Lepidapedon beveridgei Campbell et Bray, 1993 | Lepidapedidae | Bray et al. (1999)         | AJ405263                 |
| Lepidapedon desclersae Bray et Gibson, 1995 | Lepidapedidae | Bray et al. (1999)         | AJ405264                 |
| Lepidapedon discovery Bray et Gibson, 1995 | Lepidapedidae | Bray et al. (1999)         | AJ405265                 |
| Lepidapedon elongatum (Lebour, 1908) | Lepidapedidae | Bray et al. (1999)         | AJ405266                 |
| Lepidapedon gaevskayae Campbell et Bray, 1993 | Lepidapedidae | Bray et al. (1999)         | AJ405267                 |
| Lepidapedon rachion (Cobbold, 1858) | Lepidapedidae | Bray et al. (1999)         | AJ405261                 |
| Muraenolepitrema magnatestis Gaevskaya et Rodjuk, 1988 | Lepidapedidae | This study                 | KY497958                 |
| Myzoxenus insolens (Crowcroft, 1945) | Lepidapedidae | Bray et al. (2009)         | FJ788486                 |
| Neolepidapedon smithi Bray et Gibson, 1989 | Lepidapedidae | Bray et al. (1999)         | AJ405270                 |
| Postlepidapedon opisthobifurcatum (Zdzitowiecki, 1990) | Lepidapedidae | This study                 | KY497957                 |
| Postlepidapedon uberi Bray, Cribb et Barker, 1997 | Lepidapedidae | Bray et al. (2009)         | FJ788492                 |
| Profundivermis intercalarius Bray et Gibson, 1991 | Lepidapedidae | Bray et al. (1999)         | AJ405271                 |
| **Outgroup**                        |              |                            |                          |
| Koseiria xishaense Gu et Shen, 1983 | Enenteridae  | Olson et al. (2003)        | AY222233                 |
| Petalocotyle adenometra Hall et Cribb, 2000 | Gyliauchenidae | Bray et al. (2009)         | FJ788504                 |

Distal ends of the membrane’s sheets are connected to the proximal edge of the cirrus-sac. The cirrus-sac is 0.237 – 0.331 mm long and 0.05 – 0.06 mm maximal wide, and it is composed of internal seminal vesicle, pars prostatica, ejaculatory duct and eversible cirrus. The cirrus-sac is claviform, with a long proximal part accommodatig the proximal part of the internal seminal vesicle and numerous ducts of the outer prostatic cells. The internal seminal vesicle is long, 50 – 70 % the length of the cirrus-sac, tubular, rectilinear or slightly twisted, and thin-walled. The pars prostatica is vesicular. The proximal half of the pars prostatica and distal end of the inner seminal vesicle are surrounded by a field of inner prostatic cells. The cirrus is unarmed and almost cylindrical. The female terminal genitalia are represented by a thick-walled metraterm running over dorsal or dorso-lateral surface of the cirrus-sac, and surrounded by aciniform groups of glandular cells. The length of the metraterm is 0.13 – 0.15 mm, which represents 40 – 60 % of the cirrus-sac length. The male and female canals open into the small genital atrium.

Phylogenetic analysis
Bayesian inference analysis based on sequences containing 900 bp produced topologies in which P. opisthobifurcatum formed a strongly supported clade with Myzoxenus insolens (Crowcroft, 1945), within a polytomic clade also composed of Intusatrium robustum Durio et Manter, 1968 and Postlepidapedon uberi Bray, Cribb et Barker, 1997 (Fig. 2a). In turn, the above-mentioned polytomic clade is weakly supported as a sister group to a large clade of lepidapedids, consisting of M. magnatestis and two monophyletic clades: Lepidapedon spp. and Neolepidapedon smithi Bray et Gibson, 1989 + Profundivermis intercalarius Bray et Gibson, 1991. The trematode Bulbocirrus aulostomi Yamaguti, 1965 is basal taxa to all other Lepidapedidae.
Bayesian inference analysis based on sequences containing 1230 bp has revealed that P. opisthobifurcatum and M. insolens formed a clade that was sister to P. uberi with low support (Fig. 2b) and in turn this clade composed of three species was sister to I. robustum with high support. The species M. magnatestis and B. aulostomi form a strongly supported sister clade to the above-mentioned group of lepidapedids. Postlepidapedon opisthobifurcatum and P. uberi were no more closely related than they were to other lepidapedids.
According to Zdzitowiecki (1990, 1993), the cirrus-sac of *P. opisthobifurcatum* has an elongate-oval or clavate shape and it contains an elongate, but not exceptionally long, thin-walled internal seminal vesicle, a vesicular pars prostatica, a long and narrow ejaculatory duct, and a small cirrus. The external seminal vesicle is long and convoluted, and lies free in the parenchyma.

The outer prostatic cells of *P. opisthobifurcatum* were not found by Zdzitowiecki (1990, 1993). However, the proximal edge of the cirrus-sac and the area in which the external seminal vesicle is located are difficult to observe in whole mounts of this parasite, because they are obscured by the ventral sucker, loops of uterus, and vitelline follicles. The study of the isolated terminal part of the reproductive system revealed outer prostatic cells associated with thin-walled open-ended membrane. The membrane, which covers some clusters of outer prostatic cells, is described for other lepidapedids, in particular *Paralepidapedon sebastisci* (Yamaguti, 1938), *M. magnatestis*, and also for some opecoelids (Shimazu & Shimura, 1984; Sokolov & Gordeev, 2015; Shimazu, 2016). Shimazu & Shimura (1984) consider it as the rudiment of the wall of the membranous sac (=proximal portion of cirrus-sac by Shimazu & Shimura’s terminology), inherent for many lepidapedids and some opecoelids (see Bray, 2005; Cribb, 2005).

The position of *P. uberis* in the obtained phylograms (Fig. 2), separated from *P. opisthobifurcatum*, is consistent with differences in the morphology of the male terminal genitalia. *Postlepidapedon uberis* has a subglobular cirrus-sac with a small proximal part, containing a convoluted internal seminal vesicle. The ejaculatory duct is relatively short and thick-walled. The complex of the outer prostatic cells is absent in *P. uberis* (see Bray & Cribb, 2001). In addition, this species differs from *P. opisthobifurcatum* in the position of vitelline follicles. In *P. uberis* vitelline follicles form two lateral fields that are arranged in hindbody. These fields overlap the intestinal branches ventrally, laterally and dorsally (Bray & Cribb, 2001).

In *P. opisthobifurcatum*, in addition to the lateral fields arranged in hindbody, there are two longitudinal intercaecal rows of the vitelline follicles that lie on the dorsal side of the body. Lateral fields of vitelline follicles in this species overlap the intestinal branches only ventrally and partly laterally (Zdzitowiecki, 1990).

Four other species of the genus *Postlepidapedon*: *Postlepidapedon philippinense* Machida, 2004, *Postlepidapedon secundum* (Durio et Manter, 1968), *Postlepidapedon spissum* Bray, Cribb et Barker, 1997, and *Postlepidapedon quintum* Bray et Cribb, 2001 also differ from *P. opisthobifurcatum* in the shape of the cirrus-sac (oval or elongate-oval without detached proximal part) (Bray et al., 1997; Bray & Cribb, 2001; Machida, 2004). The species *P. philippinense, P. secundum*, and *P. quintum* do not have a complex of the outer prostatic cells. The distal end of the external seminal vesicle of *P. spissum* is encircled by glandular cells, lying unconfined in the parenchyma. (Bray et al., 1997). Moreover, *P. secundum* has a coiled thin-walled internal seminal vesicle, while *P. spissum* and *P. quintum* have a rectilinear, but thick-walled, internal seminal vesicle. *Postlepidapedon philippinense* has rectilinear thin-walled internal seminal vesicle (Machida, 2004). The placement of lateral fields of the vitellarium relative to the intestinal branches in *P. quintum* *P. secundum*, and *P. spissum* is the same as in *P. uberis* (Bray et al., 1997; Bray & Cribb, 2001). Accurate information about mutual location of lateral fields and intestinal branches in *P. philippinense* is absent (see Machida, 2004). We think it is likely that...
Fig. 2. Phylogenetic position of *Postlepidapedon opisthobifurcatum* within the Lepidapedidae based on 28S rDNA sequences containing 900 bp (a) and 1230 bp (b) analysed by Bayesian inference; nodal numbers indicate posterior probabilities. Scale bar shows substitutions per site.
only *P. opisthobifurcatum* among all the species currently recognised as representatives of genus *Postlepidapedon* will ultimately be proven to belong to this genus.

Phylogenetic analysis supports the position of *M. insolens* as the sister taxon of *Phylogenetic analysis supports the position of P. opisthobifurcatum*. This result is unexpected because the indicated species are similar only by the signs common to many lepidapedids (see Bray & Cribb, 1998, 2012; Bray, 2005). Definitive hosts of *P. opisthobifurcatum* are gadiform fishes, and for *M. insolens* – perciform fishes (Bray & Cribb, 1998). Life cycles of these parasites are not known. In turn, *P. opisthobifurcatum* + *M. insolens* clade form a well-supported monophyletic group with the species *I. robustum* and *P. ubiris*. In the phylogenetic model of Lepocreadioidea proposed by Bray et al. (2009) using partial 28S rDNA and nad1 sequences, this species group (without *P. opisthobifurcatum*) was named “clade III.” Bray et al. (2009) noted the absence of a general morphological synapomorphy in representatives of the clade III (at least for adult specimens); therefore, taxonomic reorganisation or revision of this clade is premature.

**Conflict of Interest**

Authors state no conflict of interest.

**Acknowledgements**

The authors are grateful to the crew of f/v Yantar-35 for their help in collecting the specimens. This study was supported by Russian Science Foundation #17-74-10203.

**References**

Bray, R.A. (1998): Lepocreadiidae (Digenea) of 545 – 602 Wallingford, CABI Publishing and the Natural History Museum, pp. 443 – 531

Bray, R.A., Cribb, T.H., Barker, S.C. (1997): *Postlepidapedon* Zditzowiecki, 1993 and *Gibsonivermis* n. g. (Digenea: Lepocreadiidae) from fishes of the southern Great Barrier Reef, Australia, and their relationship to *Intusatrium* Durio & Manter. 1968. Syst. Parasitol., 36: 143 – 155. DOI: 10.1023/A:1005729703302

Bray, R.A., Littlewood, D.T.J., Hendriks, E.A., Williams, B., Henderson, R.E. (1999): Digenean parasites of deep-sea teleosts: a review and case studies of intrageneric phylogenies. Parasitol., 119: 125 – 144. DOI: 10.1017/S0031182000084687

Bray, R.A., Cribb, T.H., Weideli, Cribb, T.H., Weideli, G.D., Dyal, P., Littlewood, D.T.J. (2009): The phylogeny of the Lepocreadioidea (Platyhelminthes, Digenea) inferred from nuclear and mitochondrial genes: Implications for their systematics and evolution. *Acta Parasitol.*, 54: 310 – 329. DOI: 10.2478/s11686-009-0045-z

Cribb, T.H. (2005): Family Opecoelidae Ozaki, 1925. In: Jones, A., Bray, R.A., Gibson D.I. (Eds) Keys to the Trematoda. Vol. 2. Wallingford, CABI Publishing and the Natural History Museum, pp. 443 – 531

Gordeev, I.I., Sokolov, S.G. (2017): Helminths and the feeding habits of the marbled moray cod *Muraenolepis marmorata* Günther, 1880 (Gadiformes, Muraenolepididae) in the Ross Sea (Southern Ocean). *Polar Biol.*, 40: 1311 – 1318. DOI: 10.1007/s00300-016-2055-2

Huls, J.P., Rongquist, F., Nielsen, R., Bollback, J.P. (2001): Bayesian inference of phylogeny and its impact on evolutionary biology. Science, 294: 2310 – 2314. DOI: 10.1126/science.105899

Jeannouin, F., Thompson, J.D., Gout, M., Higgns, D.G., Gibson, T.J. (1998): Multiple sequence alignment with Clustal X. *Trends Biochem. Sci.*, 23: 403 – 405

Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.*, 33: 1870–1874. DOI:10.1093/molbev/msw054

Machida, M. (2004): Four new species of digenean trematodes from wrasses of southern Japan and neighboring waters. *Bul. Nat. Sci. Mus. Ser. A. Zool.*, 30: 105 – 111

Miller, M.A., Peiffer, W., Schiartz, T. (2010): Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE 2010). New Orleans, Louisiana, Institute of Electrical and Electronics Engineers, pp. 1–8.

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

Petrow, A.F., Shust, K.V., Panyova, S.V., Uryupova, E.F., Gordeev, I.I., Sytov, A.M., Demina N.S. (2014): Guidelines for the collection and processing of fishing and biological data on aquatic biotessources of Antarctica for the Russian scientific observers in the CCAMLR convention area. Moscow: VNIRO Publishing, 103 pp.

Shimazu, T. (2016): Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan. IX. Opecoelidae, Opecoelinae. *Bull. Natl. Mus. Nat. Sci.*, 42: 163 – 180

Shimazu, T., Shimura, S. (1984): *Paralepidapedon* g. n. (Trematoda: Lepocreadiidae), with descriptions of metacercariae of *Paralepida* *pedon hoplophathys* (Yamaguti, 1938) comb. n. and of two other
species from sea urchins. *Zool. Sci.*, 1: 809 – 817

*Sokolov, S.G., Gordeev, I.I. (2013): New data on trematodes (Plathelminthes, Trematoda) of fishes in the Ross Sea (Antarctic). Invert. Zool.*, 10: 255 – 267

*Sokolov, S.G., Gordeev, I.I. (2015): New data on trematodes of Antarctic fishes. *Parazitologiya*, 49: 12 – 27 (In Russian)

*Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol.*, 30: 2725 – 2729. DOI: 10.1093/molbev/mst197

*Tkach, V., Grabda-Kazubska, B., Pawlowski, J., Swiderski, Z. (1999): Molecular and morphological evidences for close phylogenetic affinities of the genera *Macrodera*, *Leptophallus*, *Metaleptophallus* and *Paralepoderma* (Digenea, Plagiorchioidea). *Acta Parasitol.*, 44: 170 – 179

*Tkach, V. V., Littlewood, D. T. J., Olson, P. D., Kinsella, J. M., Swiderski, Z. (2003): Molecular phylogenetic analysis of the Microphal-loidea Ward, 1901 (Trematoda: Digenea). *Syst. Parasitol.*, 56: 1 – 15. DOI: 10.1023/A:1025546001611

*Walter, T., Palm, H. W., Pieporka, S., Rückert, S. (2002): Parasites of the Antarctic rattail *Macrourus whitsoni* Regan, 1913 (Macrouridae, Gadiformes). *Polar Biol.*, 25: 633 – 640. DOI: 10.1007/s00300-002-0407-6

*ZdZitowiecki, K. (1990): Little known and new Antarctic Digenea species of the genera *Neolepidapedon* and *Lepidapedon* (Lepocreadiidae). *Acta Parasitol.*, 35: 19 – 30

*ZdZitowiecki, K. (1993): A contribution to the morphology of the Antarctic fish lepocreadiid digeneans, with a description of a new genus. *Acta Parasitol.*, 38: 109 – 112

*ZdZitowiecki, K., Cielecka, D. (1997): Digenea of fishes of the Weddell Sea. I. Parasites of *Macrourus whitsoni* (Gadiformes, Macrouridae). *Acta Parasitol.*, 42: 23 – 30