Syst Parasitol (2023) 100:189–213
https://doi.org/10.1007/s11230-022-10081-1

Integrative approach for discovering of the new species within the genus *Allocreadium* Looss, 1900 (Trematoda: Allocreadiidae) and framing of biogeographical hypotheses for the genus

Konstantin S. Vainutis · Anastasia N. Voronova · Misako Urabe · Vitaly M. Kazarin

Received: 18 March 2022 / Accepted: 4 December 2022 / Published online: 15 December 2022

Abstract In July 2012 new *Allocreadium* species was isolated from *Carassius gibelio* caught in the Arsenyevka River, Primorsky region, Russia. Analyses on the morphometrics and internal organs′ topology revealed that these worms are morphologically closest with *A. isoporum* but both species are independent according to high genetic distances based on the 28S gene fragment (5.434±0.0073%). Unlike *A. isoporum* found earlier in Europe, the new species named *A. pseudoisoporum* **sp. nov.** has a shorter body length and the vitellarium not reaching the posterior end of the body at some distance and its anterior border is on the level of the ventral sucker. *Allocreadium pseudoisoporum* **sp. nov.** differs from seven species previously found in the Russian Far East with the following features: smaller size of the body, suckers′ ratio, range values, and topology of internal structures. Newly localities in the Pavlovka River and the Artyomovka River were discovered for *A. khankaiensis.* Morphological variability of the worms from the Pavlovka River was observed in comparison with *A. khankaiensis* from the Komissarovka River. Using scanning electron microscope, we examined external surfaces of three species (*A. pseudoisoporum* **sp. nov.**, *A. khankaiensis*, *A. hemibarbi*) and observed structures reminiscent sensory receptors. This study was aimed to describe species diversity of allocreadiids inhabiting the south of Primorsky region, and to clarify phylogenetic relationships between the species from the genus *Allocreadium* Looss, 1900 using molecular genetic methods. The phylogenetic Bayesian tree based on the 28S gene showed a clear separation of ten *Allocreadium* species and confirmed the validity of *A. pseudoisoporum* **sp. nov.** *Allocreadium*

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11230-022-10081-1.

K. S. Vainutis (✉) · A. N. Voronova
Pacific Branch of the Federal State Budget Scientific Institution "Russian Federal Research Institute of Fisheries and Oceanography", 4 Alley Shevchenko, Vladivostok, Russian Federation 690091
e-mail: vainutisk@gmail.com

K. S. Vainutis
The Far Eastern State Technical Fisheries University (FESTFU), 52B Lugovaya Street, Vladivostok, Russian Federation 690087

K. S. Vainutis · V. M. Kazarin
Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, pr. 100-letija, 159, Vladivostok, Russian Federation 690022

M. Urabe
Department of Ecosystem Studies, Faculty of Environmental Science, The University of Shiga Prefecture, Shiga 522-8533, Japan

pseudoisoporum **sp. nov.** is most similar to *A. gotoi* (genetic distances – 3.578±0.0051% in 28S, and 18.777±0.0149% in *cox1*), and represented the earliest divergent lineage in *Allocreadium* clade on the phylogenetic tree based on the 28S gene, thereby indicating its proximity to the ancestral node. Also, dichotomous keys for 25 Palearctic species of *Allocreadium* were prepared based on the morphology of the adult worms.

Introduction

The genus *Allocreadium* Looss, 1900 (Trematoda: Allocreadiidae) includes species infecting economically important freshwater fish, is widely distributed throughout the world and possesses a high species diversity compared to other genera within the family Allocreadiidae. Many *Allocreadium* species have been described in Holarctic, Oriental, and Ethiopian regions (e.g., Roitman, 1963; Achmerov, 1960, 1961, 1963; Gao et al., 2008; Shimazu, 2016; Chandra et al., 2019; Vainutis, 2020; Dos Santos et al., 2021). In the most recent phylogenetic works by Vainutis (2020) and Dos Santos et al. (2021), two new species, *A. khankaiensis* Vainutis, 2020 from Primorye, Russia, and *A. apokryfi* Dos Santos, Gilbert, Avenant-Oldewage and Dumbo, 2021 from South Africa were described, the systematic position of *A. hemibarbi* Roitman, 1963 was clarified, and the validity of eight species was confirmed using molecular genetic methods. The type species *Allocreadium isoporum* (Looss, 1894) Looss, 1902 was recorded in different localities of Ukraine, Czech Republic, Lithuania, Poland, Germany, Turkey, European and Asian parts of Russia (Looss, 1894; Linstow, 1896; Shtein, 1957; Koval, 1957; Slusarski, 1958; Wishniewski, 1958; Achmerov, 1960; Roitman, 1963; Strelkov, 1971; Ermolenko, 1992; Moravec, 1992; Aydogdu et al., 2018). However, despite the abundant data on morphology, molecular genetic data (28S rRNA gene) for *A. isoporum* were obtained only from the European part of Russia (Petkevičiūtė et al., 2010, 2018).

The primary aim of the present study was to describe the species diversity of allocreadiids inhabiting the south of Primorsky region, and to clarify the phylogenetic relationships between the species from the genus *Allocreadium* using molecular genetic methods. New dichotomous keys for 25 Palearctic species of *Allocreadium* were prepared based on the morphology of the adult worms.

In addition to classical morphological study, we performed the comparison of external surfaces of 3 *Allocreadium* species using scanning electron microscopy (SEM). To date, images obtained by the SEM were provided for 33 species representing 12 genera of the family Allocreadiidae: *Bunodera* (Lasee et al., 1988; Caira, 1985; Choudhury & León-Règagnon, 2005), *Crepidostomum* (Caira, 1985; Moravec, 2002; Žďárská & Nebesářová, 2003, 2004; Choudhury & León-Règagnon, 2005; Petkevičiūtė et al., 2018; Faltýnková et al., 2020), *Megalogonia* (Caira, 1985), *Allocreadium* (Gao et al., 2008; Aydogdu et al., 2018; Dos Santos et al., 2021), *Margotrema* (Pérez-Ponce de León et al., 2013), *Auriculostoma*, *Paracreptotrema* (Pérez-Ponce de León et al., 2016), *Paracreptotrematoides* (Pérez-Ponce de León et al., 2016), *Pseudoparacreptotrema* (Pérez-Ponce de León et al., 2016; Pérez-Ponce de León et al., 2020), *Auriculostoma* (Razo-Mendivil et al., 2014; Hernández-Mena et al., 2016; Hernández-Mena et al., 2019; Montes et al., 2021), *Wallinia* (Hernández-Mena et al., 2019), and *Creptotrema* (Franceschini et al., 2021). Most of these authors demonstrated SEM of anterior end of the body and total view of the worms without the description of the tegumental surface, except Gao et al. (2008), who described muscular striations on the body surface of *Allocreadium danjiangensis*. Žďárská and Nebesářová (2003, 2004) created the ultrastructure of intrategumental sensory receptors on the forebody of *Crepidostomum metoecus* in detail. We focused on the presence/absence of tegumental sensory organs and their interspecific variation.

Materials and methods

Material collection

Seventeen adult *Allocreadium* samples were isolated from the intestine of one gibel carp *Carassius gibelio* (Bloch, 1782) caught in the Arsenyevka River (the Ussuri River basin; 44°23′29.3″N 133°28′39.2″E), 44 samples of *A. khankaiensis* were isolated from the intestine of 12 individuals of *Rhynchocypris lagowskii* (Dybowski, 1869) from the Pavlovka River (the Ussuri River basin), and two samples of *A. khankaiensis* were

isolated from the intestine of *Barbatula toni* from the Artyomovka River (Fig. 1). Collected samples were defined to the genus level according to morphological characteristics under a light microscope, rinsed in saline, killed in hot distilled water, and stored in 70° ethanol.

Morphological analyses

Seven samples of *A. pseudoisoporum* **sp. nov.** and 11 samples of *A. khankaiensis* for the morphological study were overstained in alum carmine, dehydrated in a graded ethanol series, cleared in clove oil, and mounted in Canada balsam. All measurements are given in millimeters (mm). The holotype and paratypes were deposited to the parasitological collection of the Zoological Museum, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (FSC of Biodiversity FEB RAS), Vladivostok, Russia (e-mail: petrova@ibss.dvo.ru. Deposition date: 10 December 2018).

Dichotomous keys (Table 1) to 25 Palearctic species of *Allocreadium* were prepared in the bracketed variant according to Lobanov et al. (2013).

To explore additional distinguishing features in morphology of *Allocreadium* species, we performed SEM analysis of the tegumental surface of three *Allocreadium* species: 3 samples of *A. pseudoisoporum* **sp. nov.**, 2 samples of *A. khankaiensis*, and 3 samples of *A. hemibarbi*. Worms were dehydrated in a graded ethanol series and subjected to critical-point drying in the air, then mounted on aluminum stubs, carbon coated, and examined with a field-emission electron microscope MERLIN (CARL ZEISS, Germany) at the Center for Collective Use ″Biotechnology and Genetic Engineering″ of the FSC of Biodiversity FEB RAS.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from 12 adults of *Allocreadium* using alkaline lysis method HotShot (Truett, 2006). Fragment of 28S rDNA for 3 specimens of *A. pseudoisoporum* **sp. nov.** was amplified using forward primer Digl2 (5′-AAG CAT ATC ACT AAG CGG-3′) and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Tkach et al., 2003). New 9 sequences of *A. khankaiensis* were obtained using forward primer U178 (5′-GCA CCC GCT GAA YTT AAG-3′) and reverse primer L1642 (5′-CCA GCG CCA TCC ATT TTC A-3′) (Lockyer et al., 2003). Fragment of *cox1* mtDNA for 3 specimens of *A. pseudoisoporum* **sp. nov.** was amplified using forward primer JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) (Morgan & Blair, 1998) and reverse primer CO1-R-trema (5′-CAA CAA ATC ATG ATG CAA AAG G-3′) (Miura et al., 2005). PCR mixture contained 2X DreamTaq Green PCR Master Mix (Thermo Scientific, USA), 0.5 µM forward and reverse primers and 4 µL templates in total volume of 20 µL. The amplification protocol was performed under the following conditions: for 28S rDNA, 3 min denaturation hold at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 54 °C, 2 min at 72 °C, and a 5 min extension hold at 72 °C; for *cox1* mtDNA, 1 min denaturation hold at 94 °C, 30 cycles of 15 s at 94 °C, 30 s at 50 °C, 2 min at 72 °C, and a 7 min extension hold at 72 °C. Each PCR reaction included a negative and positive controls, using both primers to detect possible contamination. PCR products were directly sequenced using the Bright Dye Terminator Cycle Sequencing kit (Nimagen, The Netherlands) as instructed by the manufacturer. Moreover, internal sequencing primers: 300F (5′-AGGGTTC-GATTCCGGAG-3′), 1200R (5′-GGGCATCACA-GACCTG-3′), 900F (5′-CCGTCTTGAAACACGGACCAAG-3′), ECD2 (5′-CCTTGGTCCGTGTTTCAAGACGGG-3′) (Lockyer et al., 2003) have been implemented in case with 28S rDNA. The PCR products were read with an ABI 3500 genetic analyser at the FSC of Biodiversity FEB RAS.

In total, 15 sequences were submitted to the NCBI database: three 28S sequences of *A. pseudoisoporum* **sp. nov.** (accession numbers MK258685–MK258687), nine 28S sequences of *A. khankaiensis* (accession numbers MZ448168-MZ448176) and three *cox1* sequences of *A. pseudoisoporum* **sp. nov.** (accession numbers OM914849–OM914851).

Alignment and phylogenetic analyses

Nine partial 28S rDNA sequences obtained in this study were assembled with SeqScape v.2.6 software and aligned using ClustalW in MEGA X software (Kumar et al., 2018). All analysed sequences of *Allocreadium* spp. were trimmed to the length of the shortest sequence (1239 bp in 28S gene; 687 bp in

Table 1 Dichotomous key to 25 Palearctic species of *Allocreadium* Looss, 1900, based on morphology of the adult worms

1a	Vitelline fields in hindbody.	2
1b	Anterior border of vitelline fields anterior to posterior margin of ventral sucker.	3
2a	Ventral sucker larger than oral sucker.	4
2b	Ventral sucker smaller than oral sucker.	*A. hemibarbi* Roitman, 1963
3a	Anterior border of vitelline fields on the level of ventral sucker.	5
3b	Anterior border of vitelline fields in forebody.	6
4a	Excretory vesicle reaching posterior testis.	7
4b	Excretory vesicle not reaching posterior testis.	8
5a	Excretory vesicle reaching posterior testis.	9
5b	Excretory vesicle not reaching posterior testis.	10
6a	Genital pore anterior to intestinal bifurcation.	11
6b	Genital pore at level of or posterior to intestinal bifurcation.	12
7a	Testes ellipsoid.	13
7b	Testes of irregular shape.	14
8a	Testes rounded or ellipsoid. Seminal vesicle bipartite. Uterine loops reaching posterior testis.	*A. tribolodontis* Shimazu & Hashimoto, 1999
8b	Testes of irregular shape, deeply indented. Seminal vesicle unipartite. Uterine loops pretesticular.	*A. hasu* Ozaki, 1926
9a	Uterus strictly pretesticular or posterior uterine loops covering anterior margin of anterior testis. Proximal part of cirrus pouch reaching posterior margin of ventral sucker.	*A. khankaiensis* Vainutis, 2020
9b	Uterus between ventral sucker and posterior testis. Proximal part of cirrus pouch reaching anterior margin of ventral sucker.	*A. carparum* Odening, 1959 Junior synonym: *A. papilligerum* (Rees, 1968) Moravec, 1984
10a	Body elongate-oval. Testes slightly or deeply indented. Ovary oval or three-lobed.	*A. japonicum* Ozaki, 1926
10b	Body elongate. Testes of irregular shape, slightly indented. Ovary oval, rounded, or irregular shape.	15
11a	Intestinal bifurcation at level of anterior margin of ovary	*A. pseudaspii* (Achmerov, 1960) Bychovskaya-Pavlovskaya, 1962 Synonym: *A. elongatum* (Achmerov, 1960) Bychovskaya-Pavlovskaya, 1962 (preoccupied name)
11b	Intestinal bifurcation at level of or anterior to ventral sucker.	16
12a	Genital pore at level of intestinal bifurcation.	17
12b	Genital pore posterior to intestinal bifurcation.	18
13a	Proximal part of cirrus pouch slightly extending beyond anterior margin of ventral sucker. Vitelline follicles not reaching ventral sucker at short distance	*A. isoporum* (Looss, 1894) Looss, 1902
13b	Proximal part of cirrus pouch reaching the posterior margin of ventral sucker. Vitelline follicles reaching posterior margin of ventral sucker.	*A. pseudoisoporum* **sp. nov.**
14a	Cirrus pouch preacetabular. Anterior border of vitelline follicles at level of posterior margin of ventral sucker.	*A. montanus* Sidorov & Butenko, 1966
14b	Cirrus pouch preacetabular. Anterior border of vitelline follicles at level of ovary.	*A. brevivitellatum* Shimazu, 1992
15a	Cirrus pouch anterior to ventral sucker. Body length 2.56–2.90 mm.	*A. aburahaya* Shimazu, 2003
15b	Cirrus pouch extending to posterior border of ventral sucker. Body length 4.71–4.73 mm.	*A. tamoroko* Shimazu and Urabe, 2013
16a	Intestinal bifurcation at level of ventral sucker.	19

Table 1 continued

16b	Intestinal bifurcation anterior to ventral sucker.	*A. tosai* Shimazu, 1988
17a	Cirrus pouch preacetabular. Anterior border of vitelline fields at level of pharynx.	*A. shinanoense* Shimazu, 2003
17b	Proximal part of cirrus pouch posterior to anterior margin of ventral sucker. Anterior border of vitelline fields posterior to pharynx.	20
18a	Small auricular outgrowths on anterior margin of oral sucker. Anterior border of vitelline follicles at level of intestinal bifurcation.	*A. erythroculteris* (Achmerov, 1960) Bychovskaya-Pavlovskaya, 1962 Junior synonym: *A. maculati* Achmerov, 1963
18b	Perioral outgrowths absent. Anterior border of vitelline follicles at level of anterior margin of ventral sucker.	21
19a	Proximal part of cirrus pouch reaching midlevel of ventral sucker.	*A. gobii* Roitman, 1963
19b	Cirrus pouch preacetabular.	22
20a	Proximal part of cirrus pouch posterior to ventral sucker. Anterior border of vitelline fields between pharynx and intestinal bifurcation.	23
20b	Proximal part of cirrus pouch at midlevel of ventral sucker. Anterior border of vitelline fields at level of anterior margin of ventral sucker.	*A. danjiangensis* Gao, Wang, Xi, Yao, Nie, 2008
21a	Proximal part of cirrus pouch reaching midlevel of ventral sucker.	*A. transversale* (Rudolphi, 1802)
21b	Cirrus pouch preacetabular.	*A. gotoi* (Hasegawa & Ozaki, 1926) Shimazu, 1988
22a	Anterior border of vitelline fields at half distance between suckers.	*A. baueri* Spassky et Roitman, 1960
22b	Anterior border of vitelline follicles at level of posterior margin of oral sucker.	*A. markewitschi* Koval, 1949
23a	Body relatively small (length 2.4-3.3 mm), fusiform.	*A. qianweiensis* Zhang, Yang, 1994
23b	Body large, elongate-oval.	24
24a	Testes large (length 0.4-0.6 mm), irregular shape, entire.	*A. hypophthalmichthydis* (Achmerov, 1960) Bychovskaya-Pavlovskaya, 1962
24b	Testes relatively large (length 0.56-0.64 mm), irregular shape, slightly indented.	*A. conicum* Wang, Jiang, 1985

cox1 gene). Sequences used for the phylogenetic reconstruction were represented in Table 2. Genetic p-distances were estimated by including all substitution types in the MEGA X software. Four species of the family Callodistomatidae (superfamily Gorgoderoidea): *Prosthenhystera obesa* (NCBI Accession number EF032690), *P. oonastica* (KM871182), *P. caballeroi* (KM871186), and *P. gattii* (MF664223) were chosen as outgroup taxa. The undescribed, possibly cryptic, species from Ukraine and China were designated as *Allocreadium* sp. 2 and *Allocreadium* sp. 3, respectively. Phylogenetic analyses were carried out with Bayesian Inference (BI) algorithm (Huelsenbeck et al., 2001) with the GTR+G model (chosen using the Bayesian information criteria (BIC)) + gamma selected in jModeltest v. 2.1.5 software as the best (Darriba et al., 2012). The MCMC algorithm was performed using two independent runs and 400000 generations (the average standard deviation of split frequencies was less than 0.01), 25% of generations were discarded as burn-in in MrBayes v. 3.1.2 software (Huelsenbeck et al., 2001). Additional phylogenetic tree for the same sampling was reconstructed using maximum likelihood (ML) method based on the same GTR+G model in the MEGA X software, with 1000 bootstrap replicates.

Results

Morphological study of *Allocreadium* Looss, 1900

Phylum Platyhelminthes Gegenbaur, 1859
Class Trematoda Rudolphi, 1808
Order Plagiorchiida La Rue, 1957
Suborder Xiphidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003
Superfamily Gorgoderoidea Looss, 1901
Family Allocreadiidae Looss, 1902
Genus *Allocreadium* Looss, 1900

Table 2 Information on the species, host, geographic origin for the partial 28S rDNA sequences used in the phylogenetic reconstructions. Newly obtained sequences are in bold

Parasite species	Host	Locality	Sequence Data Accn.		Reference
			28S	*cox1*	
Family Allocreadiidae					
Allocreadium					
Allocreadium		***pseudoisoporum* sp. nov.**	***Carassius carassius***	**Russia, Primorsky region,**	
Yakovlevsky district, Arsenyevka River (near Yablonovka village)	MK258685-MK258687	OM914849-OM914851	**This study**		
Allocreadium khankaiensis	*Rhynchocypris lagowskii*	Russia, Khankaysky district, Komissarovka River	MK211216, MK211217	MW729428-MW729429	Vainutis, 2020; Vainutis et al., 2021
	Esox reichertii		–	MW729430	Vainutis et al., 2021
	Rhynchocypris lagowskii	**Russia, Chuguevsky district, Pavlovka River (near Pavlovka village)**	**MZ448170-MZ448176**	**-**	**This study**
	Barbatula toni	**Russia, Artyomovsky district, Artyomovka River**	**MZ448168-MZ448169**	**-**	
Allocreadium hemibarbi	*Hemibarbus labeo*	Russia, Khankaisky district, Komissarovka River	MK211220, MK211221	–	Vainutis, 2020
Allocreadium sp. 1	*Phoxinus phoxinus*	Russia, Nadezhdinsky district, tributary of the River Nezhinka (Razdolnaya River basin)	MK211209, MK211210	MK818870-MK818871	Vainutis et al., 2021
Allocreadium apokryfi	*Labeobarbus aeneus*	South Africa, Vaal River	MW907591-MW907595	–	Dos Santos et al., 2021
Allocreadium isoporum	*Alburnus alburnus*	Russia, Karelia, Lake Oster	GU462125, GU462126	–	Petkeviciute et al., 2010
Allocreadium isoporum	*Barbatula barbatula*	Russia, River Il′d, upper Volga River basin	MH143102	–	Petkeviciute et al., 2018
Allocreadium crassum	*Pisidium amnicum*	Finland, Siilaisenpuro River	JF261142-JF261143	–	Petkeviciute et al., 2012
Allocreadium lobatum	*Semotilus corporalis*	Maine, USA	EF032693	–	Curran et al., 2006
	S. atromaculatus	Tobacco Creek, Manitoba, Canada	–	KC899847	Martínez-Aquino et al., 2013
Allocreadium neotenicum	*Hydroporus rufifrons*	United kingdom, Cumbria, Lake District	JX977132	–	Bray et al., 2012
Allocreadium neotenicum	*Pisidium casertanum*	Russia, Crimea, River Burulcha	MH143103	–	Petkeviciute et al., 2018
Allocreadium neotenicum	*Pisidium casertanum*	Norway, Lake Takvatn	MH143104	–	Petkeviciute et al., 2018
Allocreadium gotoi	*Misgurnus anguillicaudatus*	Japan, Nagano, Iiyama, Midori	LC215274	LC215273	Shimazu et al., 2017

Table 2 continued

Parasite species	Host	Locality	Sequence Data Accn.		Reference
			28S	*cox1*	
Allocreadium sp. 2	*Sphaerium corneum*	Ukraine, River Teterev	GU462121	–	Petkeviciute et al., 2010
Allocreadium sp. 3	*Schizothorax parvus*	China	MN969626	–	Unpublished
	Schizothorax yunnanensis		MN969627	–	
Crepidostomum					
Crepidostomum metoecus	*Salvelinus leucomaenis*	Russia, Primorsky territory, Kuznetsovka River	FR821406	–	Atopkin, Shedko, 2014
Crepidostomum oschmarini	*Pisidium casertanum*	Lithuania, River Nedzinge	MH159994	–	Petkeviciute et al., 2018
Crepidostomum affine	*Aplodinotus grunniens*	USA, Mississippi, Pearl River	KF356361	–	Tkach et al., 2013
Crepidostomum illinoiense	*Hiodon alosoides*	USA, Polk Co. MN, Red Lake River	HQ833705	–	Curran et al., 2011
Crepidostomum cornutum	*Lepomis gulosus*	USA	EF032695	–	Curran et al., 2006
Crepidostomum auritum	*Aplodinotus grunniens*	USA, Mississippi, Pearl River	KF250357	–	Tkach et al., 2013
Stephanophiala					
Stephanophiala farionis	*Oncorhynchus masou*	Russia, Primorsky territory, Maksimovka River	FR821399	–	Atopkin, Shedko, 2014
Bunodera					
Bunodera mediovitellata	*Gasterosteus aculeatus*	Russia, Kamchatsky territory, Kamchatka River	MG262549	–	Atopkin et al., 2018
Bunodera vytautasi	*Pungitius pungitius*	Russia, Magadan region, Chernoe Lake	MG262545	–	Atopkin et al., 2018
Bunodera luciopercae	*Thymallus thymallus*	Russia, Karelia, Kamennaya River	MG262544	–	Atopkin et al., 2018
Bunodera acerinae	*Gymnocephalus cernuus*	Russia, Tvertsa River	GU462122	–	Petkeviciute et al., 2010
Acrolichanus					
Acrolichanus auriculatus	*Acipenser schrenkii*	Russia, Amur region, Amur River (near Nikolaevsk-na-Amure city)	FR821371, FR821372	–	Atopkin, Shedko, 2014
	Family Callodistomidae				
	Prosthenhystera				
Prosthenhystera obesa	*Hoplias* sp.	Peru	AY222206	–	Olson et al., 2003
Prosthenhystera oonastica	*Ictalurus furcatus*	USA, Mississippi, Pearl River	KM871180	–	Tkach, Curran, 2015
Prosthenhystera caballeroi	*Astyanax aeneus*	Costa Rica, Guanacaste, Rio Tampisquto	KM871183	–	Tkach, Curran, 2015

Table 2 continued

Parasite species	Host	Locality	Sequence Data Accn.		Reference
			28S	*cox1*	
Prosthenhystera gattii	*Bryconamericus ikaa*	Argentina	MF664223	–	Montes et al., 2020

Generic diagnosis. Perioral papillae absent. Body surface smooth, without spines. Esophagus bifurcates anterior or dorsally to ventral sucker. Ventral sucker can be larger, equal to or smaller than oral sucker, located between first and second thirds of body. Cirrus pouch is sac-shaped, lateral to or anterior to ventral sucker, genital pore anterior to ventral sucker. Testes are tandem, their shape round, oval or irregular. Uterus is pretesticular or reaching posterior testis. Anterior border of vitellarium in anterior part of body or not reaching ventral sucker, posterior border always filling posttesticular space.

Synonyms: Creadium Looss, 1899 (nomen praeoccupatum); *Macrolecithus* Ozaki, 1926; *Neoallocreadium* Achmerov, 1960; *Allocreadioides* Yamaguti, 1971; *Pseudoallocreadium* Yamaguti, 1971.

Type species: Distomum isoporum Looss, 1894 (=*Allocreadium isoporum* (Looss, 1894) Looss 1902).

Type locality: Germany.

Distribution: The range of the genus is the most extensive among other allocreadiid genera and includes the rivers and lakes of Central Asia (Kazakhstan), East (Japan, China, South Korea), South (India) and Western (Turkey) Asia, North (Morocco, Sudan, Egypt), Central, West, and South Africa, European and Asian regions of Russia, Western (Germany, France, Switzerland, Great Britain) and Eastern Europe (Czech Republic, Poland, Ukraine), North (USA, Canada, Mexico) and South America (Argentina).

Allocreadium pseudoisoporum sp. nov.

Taxonomic summary

Type host: *Carassius gibelio* (Bloch, 1782); Cyprininae, Cyprinidae, Cypriniformes.

Localization: intestine.

Locality (type): Arsenyevka River, near the village Yablonovka, Yakovlevsky district, Primorsky region, Russia (44°39′04.2″N 133°32′39.8″E).

Type material: holotype (№ 311-1) and 6 paratypes (№ 311-2-7) were deposited to the parasitological collection of the Zoological Museum, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (e-mail: petrova@ibss.dvo.ru. Deposition date: the 10th of December 2018).

The new species was registered in Zoobank database under the number: urn:lsid:zoobank.org:act:09572997-72F2-4B96-81CF-93FC9C5EC346.

Etymology: The species name *pseudoisoporum* is given due to the high morphological affinity with *Allocreadium isoporum*.

Sample size: 17 samples were found in the one individual of *Carassius gibelio*.

Molecular genetic data: 28S rRNA gene – MK258685–MK258687; *cox1* mtDNA gene – OM914849–OM914851.

Morphological description

Adult (Fig. 2a, b, c; Table 3): Small trematodes, body fusiform, unarmed. Tegument smooth, unarmed. Widest part of body in ovarian region. Forebody short, hindbody 2-3 times longer than forebody. Oral sucker subterminal, round; perioral muscular papillae absent. Eyespots absent. Prepharynx short, opening into subspherical pharynx. Oesophagus long, bifurcating on level of posterior third of ventral sucker. Caeca almost reaching posterior end of body. Ventral sucker on border of anterior and middle thirds of body, round, a little larger than oral sucker. Testes tandem in mid-line of body, in posterior half of body, rounded, almost equal in size. Cirrus pouch club-shaped, thin-walled, posterolateral to ventral sucker, its proximal

Table 3 Measurements (mm) of adult worms for the new species *Allocreadium pseudoisoporum*, *A. isoporum*, and other species previously recorded on the territory of Russian Far East. The newly obtained morphometric values are in bold. References in table: 1 – Koval, 1957; 2 – Slusarski, 1958; 3 – Roitman, 1963; 4 – Skrjabina, 1973; 5 – Moravec, 1984; 6 – Vainutis, 2020

Features	*Allocreadium pseudoisoporum* sp. nov.			*Allocreadium isoporum*[1]	*Allocreadium isoporum*[2]
	Arsenyevka River				
	Holotype	Range	Mean	Range	Range
Body length	**1.625**	**1.10–1.65**	**1.45**	0.817–2.242	2.467–2.62
Body width	**0.35**	**0.35–0.45**	**0.378**	0.304–0.817	0.69 - 0.71
Oral sucker length	**0.20**	**0.17–0.20**	**0.192**	0.114–0.285	0.305–0.308
Oral sucker width	**0.19**	**0.18–0.20**	**0.188**	0.342	0.245–0.246
Forebody*	**0.38 (23.39%)**	**0.34–0.40 (22.42–30.91%)**	**0.37**	–	–
Hindbody	**0.925**	**0.575 - 1.025**	**0.814**	–	–
Ventral sucker length	**0.25**	**0.21–0.25**	**0.234**	0.133–0.342	0.31–0.33
Ventral sucker width	**0.24**	**0.19–0.24**	**0.218**	0.360	0.308–0.38
Pharynx length	**0.09**	**0.085–0.10**	**0.091**	0.124–0.132	–
Pharynx width	**0.095**	**0.085–0.10**	**0.095**	0.114–0.124	–
Oesophagus length	**0.315**	**0.20–0.315**	**0.257**	–	–
Ovary length	**0.17**	**0.12–0.18**	**0.157**	0.114–0.209	–
Ovary width	**0.14**	**0.11–0.16**	**0.135**	0.152–0.228	–
Seminal receptacle length	**0.10**	**0.09–0.12**	**0.106**	–	–
Seminal receptacle width	**0.09**	**0.09–0.11**	**0.098**	–	–
Anterior testis length	**0.21**	**0.15–0.21**	**0.176**	0.114–0.247	–
Anterior testis width	**0.15**	**0.12–0.15**	**0.136**	0.114–0.247	–
Posterior testis length	**0.21**	**0.15–0.21**	**0.188**	0.114–0.247	–
Posterior testis width	**0.14**	**0.13–0.15**	**0.138**	0.114–0.247	–
Cirrus pouch length	**0.265**	**0.205–0.265**	**0.241**	0.152–0.304	–
Cirrus pouch width	**0.085**	**0.07–0.11**	**0.087**	0.057–0.133	–
D_{et}**	**0.33**	**0.195–0.38**	**0.282**	–	–
D_{ov}**	**0.22**	**0.165 - 0.23**	**0.195**		
D_{ev}**	**0.095**	**0.095–0.125**	**0.105**		
VF**	**0.0625**	**0.038–0.063**	**0.049**		
Eggs, length	**0.08**	**0.05–0.085**	**0.074**	0.082–0.090	0.093–0.098
Eggs, width	**0.045**	**0.035–0.05**	**0.047**	0.049–0.057	0.054–0.06
Length/width ratio	**21.53%**	**21.53–31.81%**	**26%**	–	–

Table 3 continued

Features	*Allocreadium pseudoisoporum* sp. nov.			*Allocreadium isoporum*[1]	*Allocreadium isoporum*[2]
	Arsenyevka River				
	Holotype	Range	Mean	Range	Range
Forebody/body length ratio	**23.38%**	**22.42–30.91%**	**25.5%**	–	–
Suckers length, ratio	**1:1.25**	**1:1.15–1.26**	**1:1.21**	–	–
Suckers width, ratio	**1:1.26**	**1: 1.05–1.26**	**1:1.16**	–	–

Features	*Allocreadium isoporum*[3]	*Allocreadium isoporum*[4]	*Allocreadium isoporum* [5]	*Allocreadium khankaiensis*		*Allocreadium hemibarbi* [6]
				This study Pavlovka River (n=11)	Komissarovka River [6]	
	Range	Range	Range	Range		Range
Body length	1.17–1.19	2.0–2.36	3.60–4.08	**1.938-2.871**	1.425–2.75	1.25-1.6
Body width	0.39–0.43	0.68–1.18	0.979–1.115	**0.818-0.859**	0.40–0.86	0.325-0.4
Oral sucker length	0.17–0.18	0.26–0.40	0.380–0.421	**0.265-0.317**	0.175–0.31	0.2-0.26
Oral sucker width	0.17–0.21	0.26–0.40	0.340–0.408	**0.259-0.314**	0.17–0.325	0.19-0.27
Forebody*	–	–	–	**0.488-0.661**	0.335–0.52	0.33-0.44
Hindbody	–	–	–	**1.414–1.86**	0.9–1.925	0.77–1.0
Ventral sucker length	0.17–0.18	0.26–0.40	0.394–0.476	**0.325-0.387**	0.205–0.375	0.14-0.16
Ventral sucker width	0.17–0.21	0.26–0.40	0.353–0.408	**0.32-0.409**	0.215–0.365	0.15-0.18
Pharynx length	0.079–0.084	–	0.136–0.177	**0.134-0.202**	0.09–0.16	0.07-0.10
Pharynx width	0.088–0.098	–	0.190–0.217	**0.118-0.149**	0.10–0.175	0.08-0.10
Oesophagus length	–	–	0.204–0.571		0.165–0.35	0.08
Ovary length	0.11–0.13	0.22–0.32	0.299	**0.189-0.282**	0.135 - 0.29	0.09-0.13
Ovary width	0.11–0.13	0.22–0.32	0.218–0.231	**0.17-0.286**	0.115–0.30	0.08-0.12
Seminal receptacle length	–	–	–	**0.215**	0.035–0.17	0.06
Seminal receptacle width	–	–	–	**0.110**	0.06–0.30	0.06
Anterior testis length	0.14	0.30–0.34	0.312–0.462	**0.295-0.369**	0.175–0.46	0.23-0.285
Anterior testis width	0.11–0.14	0.34–0.42	0.231–0.408	**0.347-0.449**	0.23–0.48	0.23-0.275
Posterior testis length	0.13–0.18	0.30–0.40	0.435–0.530	**0.383-0.456**	0.27–0.46	0.24-0.295
Posterior testis width	0.11–0.14	0.36–0.42	0.258–0.421	**0.328-0.45**	0.27–0.45	0.22-0.28

Table 3 continued

Features	*Allocreadium isoporum*[3]	*Allocreadium isoporum*[4]	*Allocreadium isoporum* [5]	*Allocreadium khankaiensis*		*Allocreadium hemibarbi* [6]
				This study Pavlovka River (n=11)	Komissarovka River [6]	
	Range	Range	Range	Range		Range
Cirrus pouch length	0.13–0.17	0.26–0.30	0.408–0.612	**0.185-0.262**	0.165–0.42	0.17-0.25
Cirrus pouch width	0.079–0.088	–	0.217–0.244	**0.148-0.169**	0.075–0.21	0.08-0.11
D_{et}**	–	–	–	**0.458-0.822**	0.29–0.66	0.205-0.27
D_{ov}**				**0.226–0.335**	–	–
D_{ev}**				**-**	0.075–0.2	–
VF**				**-**	0.04–0.14 × 0.035–0.09	0.045 × 0.035
Eggs, length	0.085–0.09	0.080–0.104	0.090–0.105	**0.055-0.066**	0.07–0.09	0.06-0.075
Eggs, width	0.041–0.05	0.044–0.064	0.057–0.063	**0.037-0.044**	0.05–0.06	0.045-0.05
Length/width ratio	–	–	–	**23.88-36.86%**	28-36.3%	21.6-27.5%
Forebody/body length ratio	–	–	–	**16.98-22.69%**	17.6-27.7%	22-31.5%
Suckers length, ratio	–	–	1:1.03-1.06	**1:1.07-1.45**	1:1.17-1.42	1:1.33–1.62
Suckers width, ratio	–	–	–	**1:1.12-1.61**	1:1.05–1.41	1:1.26-1.5

*Length of the forebody is noted as millimeters followed by percents in parentheses

**D_{et} - distance from posterior end of body to posterior testis; D_{ov} - distance between oral sucker and ventral sucker; D_{ev} - distance between posterior end of body to the posterior border of the vitellarium; VF - vitelline follicles

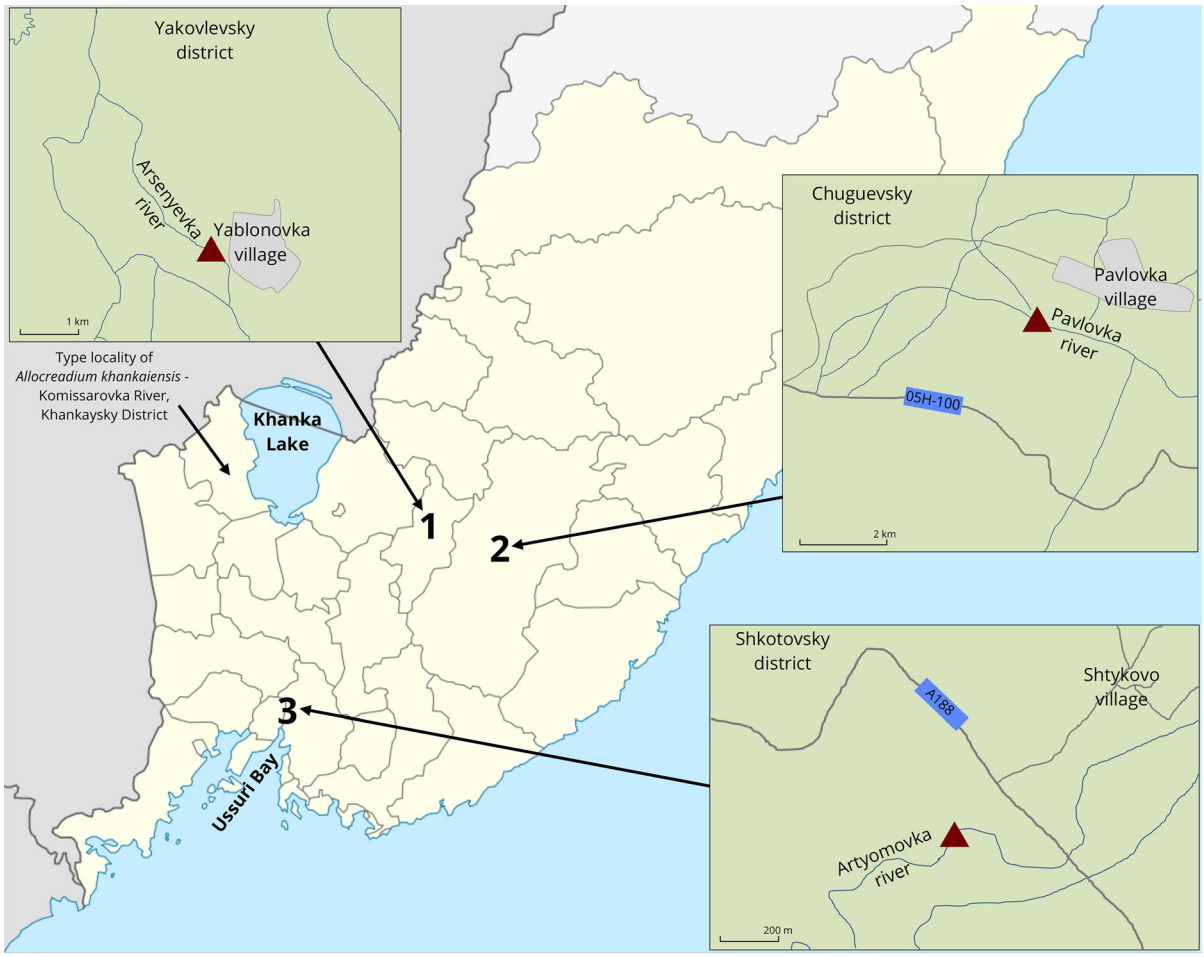

Fig. 1 Map of the material collection localities. (1) Yakovlevsky district: the Arsenyevka River, near the Yablonovka village; (2) Chuguevsky district: the Pavlovka River, near the Pavlovka village; (3) Shkotovsky district: the Artyomovka River, near the Shtykovo village

part reaching posterior margin of ventral sucker; containing seminal vesicle, prostatic part and short cirrus. Seminal vesicle clavate, in proximal part of cirrus pouch, slightly curved forming one loop in the middle of cirrus pouch. External seminal vesicle absent. Genital pore median, anterior to ventral sucker. Ovary pretesticular, subspherical, its anterior margin overlapping posterior margin of ventral sucker. Seminal receptacle in dorsal part of body posterior to ovary, flask-shaped. Laurer′s canal short, posterior to ovary and seminal receptacle. Vitelline follicles relatively large (37.5–62.5 μm), extending in lateral fields of body from posterior margin of ventral sucker to posterior end of body, overlapping testes and caeca in ventral and dorsal sides of body. Follicles not reaching posterior end of body on distance 0.095–0.125 mm. Uterus between ventral sucker and anterior testis, not crossing its anterior margin. Two-nine eggs relatively large. Excretory vesicle Y-shaped, reaching anterior margin of posterior testis. Excretory pore terminal.

Remarks on morphology

Trematodes described in this study belong to the genus *Allocreadium* by following features: the body is fusiform, perioral muscular papillae are absent, vitelline follicles completely fill the posttesticular space, lateral fields of vitellarium extending along the body, without connecting on the dorsal side of the body, to the posterior edge of the ventral sucker, or not reaching it at some distance. Such characters were

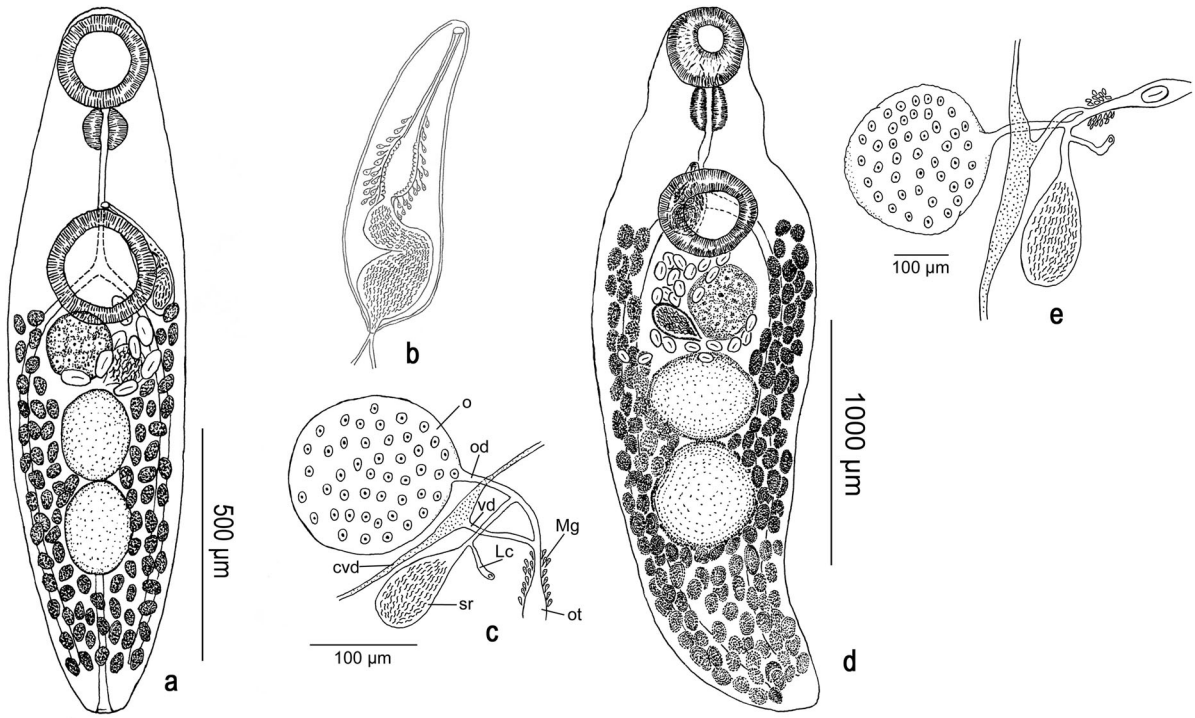

Fig. 2 *Allocreadium pseudoisoporum* **sp. nov.** from the Arsenyevka River (a, b, c) and *A. khankaiensis* from the Pavlovka River (d, e): line drawings. a – Ventral view; b – Cirrus pouch; c – Ovarian complex; d – Ventral view; e – Ovarian complex. Abbreviations: o – ovary; od – oviduct; cvd – common vitelline duct; vd – vitelline duct; Lc – Laurer′s canal; sr – seminal receptacle; Mg – Mehlis glands; ot – ootype

noted for the most *Allocreadium* species, among which, for example: *A. isoporum*, *A. lobatum*, *A. hasu*, *A. japonicum*, *A. tribolodontis*, *A. brevivitellatum*, *A. handiai*, *A. thapari*, *A. gobiosis*, *A hemibarbi*, *A. heteropneustusius* etc.

Allocreadium pseudoisoporum **sp. nov.** is very similar to *A. isoporum* in some diagnostic features: fusiform body, long esophagus bifurcating dorsally to the ventral sucker, ventral sucker larger or equal to oral sucker, vitelline follicles extending to posterior edge of ventral sucker. Previously *A. isoporum* has been noted in various localities including Russian Far East, but the morphological description was given only by a few authors (Looss, 1894; Koval, 1957; Slusarski, 1958; Kozicka, 1956; Roitman, 1963; Skrjabina, 1973; Moravec, 1984). According to the morphometric data, the measurements of *A. pseudoisoporum* **sp. nov.** fall within the ranges of, or smaller than those of *A. isoporum* of Koval (1957); *A. pseudoisoporum* **sp. nov.** has smaller pharynx than *A. isoporum* of Koval (1957) and Moravec (1984); the body length and width of *A. pseudoisoporum* are much smaller than those of *A. isoporum* (Looss, 1894; Lühe, 1909; Layman, 1933; Slusarski, 1958; Skrjabina, 1973; Moravec, 1984); the eggs of *A. pseudoisoporum* are smaller than those of *A. isoporum* (Looss, 1894; Lühe, 1909; Slusarski, 1958; Skrjabina, 1973; Moravec, 1984); and the anterior border of vitelline follicles of *A. pseudoisoporum* are on the level of posterior margin of the ventral sucker, while those of *A. isoporum* generally do not reach the ventral sucker (Looss, 1894; Lühe, 1909; Layman, 1933; Koval, 1957; Slusarski, 1958).

In the aspect of both morphological and geographical criteria, *A. isoporum* from the Zeya River (Amur Oblast) (Roitman, 1963) was the closest one to *A. pseudoisoporum* **sp. nov.** with the extension of the uterus and vitellarium, genital atrium opening, position of the male and female gonads; the size ranges of all body structures overlap in both species, but in *A. isoporum* of Roitman (1963) the length of pharynx, ventral sucker, and cirrus pouch were a little smaller than the minimum values of *A. pseudoisoporum* **sp. nov.** The differences between them are as follows:

Table 4 Genetic p-distances estimated for nine species of the genus *Allocreadium* based on the 28S gene sequences. The newly obtained sequences are in bold

		1	2	3	4	5	6	7	8	9	10	11	12
1	***Allocreadium pseudoisoporum* sp. nov.**		0.0051	0.0053	0.0054	0.0061	0.0061	0.0064	0.0073	0.0067	0.0061	0.0063	0.0069
2	*Allocreadium gotoi*	3.578		0.0026	0.0040	0.0054	0.0054	0.0055	0.0061	0.0063	0.0053	0.0054	0.0062
3	*Allocreadium* sp. 2 Ukraine	3.581	0.815		0.0044	0.0052	0.0052	0.0055	0.0061	0.0062	0.0055	0.0057	0.0065
4	*Allocreadium hemibarbi*	3.581	1.974	2.225		0.0044	0.0047	0.0045	0.0053	0.0053	0.0046	0.0048	0.0058
5	*Allocreadium* sp. 1	4.578	3.446	3.109	2.180		0.0026	0.0044	0.0052	0.0053	0.0053	0.0056	0.0071
6	*Allocreadium khankaiensis*	4.791	3.574	3.237	2.639	0.853		0.0050	0.0056	0.0053	0.0049	0.0058	0.0072
7	*Allocreadium crassum*	4.550	3.329	3.163	2.313	2.434	3.062		0.0030	0.0058	0.0049	0.0049	0.0068
8	*Allocreadium isoporum*	5.434	4.193	4.026	3.163	3.281	3.747	1.143		0.0059	0.0050	0.0050	0.0073
9	*Allocreadium* sp. 3 China	5.770	4.536	4.539	3.412	3.617	3.914	3.671	4.018		0.0049	0.0051	0.0074
10	*Allocreadium neotenicum*	4.627	3.412	3.500	2.646	3.192	3.489	2.905	3.247	3.075		0.0011	0.0064
11	*Allocreadium lobatum*	4.806	3.586	3.675	2.817	3.365	3.663	2.905	3.247	3.248	0.162		0.0066
12	*Allocreadium apokryfi*	6.123	5.310	5.306	4.978	6.250	6.208	5.689	6.228	6.395	5.164	5.345	

*Length of fragment utilized for estimation is 1239 bp. Values of distances are in % (below diagonal), values of standard deviation are in upper diagonal

proximal end of cirrus pouch on level of the posterior edge of the ventral sucker vs. slightly extends beyond the anterior edge of the ventral sucker; ventral sucker noticeably larger than the oral sucker vs. approximately equal to oral sucker (Table 3), which was also noted for *A. isoporum* from other localities (Koval, 1966; Skrjabina, 1973; Moravec, 1984). In *A. isoporum* of Roitman (1963), the most metric values of internal body structures are smaller than those of other isolates of *A. isoporum* (Koval, 1957; Slusarski, 1958; Skrjabina, 1973; Moravec, 1984) and *A. isoporum macrorchis* (Koval, 1957).

In addition, Moravec (1984) reported larger worms of *A. isoporum* from *Squalius cephalus* and *Barbatula barbatula* from the Czech Republic: eggs, body length and width were larger than those of *A. pseudoisoporum* **sp. nov.** and *A. isoporum* of Koval (1957) and Slusarski (1958). The vitellarium of the worms from *S. cephalus* does not reach the ventral sucker, while the vitellarium of the worms from *B. barbatula* reaches the posterior edge of the ventral sucker. The worms from *S. cephalus* have larger metric values than those from *B. barbatula*, but worms from both fish species have uterine loops extending to the posterior testis, that was not observed in *A. pseudoisoporum* **sp. nov.** and *A. isoporum* described by Koval (1957) and Slusarski (1958).

According to Looss (1894), Koval (1957, 1966), Slusarski (1958), Skrjabina (1973), and Moravec (1984) *A. isoporum* is characterized with high morphological variability. Some of its morphoforms may be the independent species. To confirm or refute this assumption, it is necessary to obtain the molecular data for this species from the localities where it was reported by the aforementioned authors. Based on the morphological analysis, *A. isoporum* reported from the Arsenyevka and Amur rivers (Achmerov, 1961; Roitman, 1963; Strelkov, 1971; Ermolenko, 1990, 1992) should be considered *A. pseudoisoporum* **sp. nov.**

The ratio of the suckers′ size of *A. pseudoisoporum* **sp. nov.** is in the lower range values than that of other *Allocreadium* species from the Russian Far East (Table 3). *Allocreadium pseudoisoporum* **sp. nov.** is clearly distinguishable from the *Allocreadium* spp. described in the Amur, Zeya and Komissarovka rivers: *A. elongatum*, *A. erythroculteris*, *A. hypophthalmichthydis*, *A. maculati*, *A. gobii*, *A. hemibarbi*, *A. khankaiensis* (Achmerov, 1960, 1963; Roitman,

1963; Vainutis, 2020) with the features as follows: the size of the body and internal structures is smaller in *A. pseudoisoporum* **sp. nov.** (Table 3); the esophagus of *A. erythroculteris*, *A. hypophthalmichthydis*, *A. maculati*, and *A. hemibarbi* is shorter and bifurcates anterior to the ventral sucker; the uterus of *A. elongatum*, *A. erythroculteris*, *A. hypophthalmichthydis* and *A. maculati* is located between the posterior testis and the ventral sucker, and the vitelline follicles of *A. elongatum*, *A. erythroculteris*, *A. hypophthalmichthydis*, *A. maculati* and *A. gobii* extend to the posterior edge of the pharynx or intestinal bifurcation; in particular, *A. pseudoisoporum* **sp. nov.** differs from *A. hemibarbi* by body fusiform vs. cylindrical; ventral sucker is larger than or equal to oral sucker vs. ventral sucker is smaller than oral sucker.

Most of the maximum values of *A. pseudoisoporum* **sp. nov.** overlap with the minimum values of *A. khankaiensis* recently described from the Komissarovka River (Vainutis, 2020), and much smaller than the ranges of *A. khankaiensis* described from the Pavlovka River (Table 3). *Allocreadium pseudoisoporum* **sp. nov.** differs from the latter as follows: anterior border of vitellarium is on the level of posterior margin of ventral sucker vs. on the level of ventral sucker or anterior to it; ellipsoid testes vs. large testes of irregular shape; proximal part of cirrus pouch reaches back to the posterior margin of ventral sucker vs cirrus pouch reaches to mid-line of ventral sucker.

Allocreadium khankaiensis Vainutis, 2020

Taxonomic summary

Type host: Rhynchocypris oxycephalus Sauvage et Dabry de Thiersant, 1874; Cyprinidae, Cypriniformes.

New host recorded: Rhynchocypris lagowskii (Dybowski, 1869); Cyprinidae, Cypriniformes.

Localization: intestine.

Type locality: Komissarovka River, Khankaysky district, Primorsky region, Russia.

Type specimens: Holotype (No. 309-1) and 9 paratypes (No. 309-2-10).

New locality: Pavlovka River (Ussuri River basin), near the village Uborka, Chuguevsky district, Primorsky region, Russia (44°24′04.4″N 134°13′28.6″E).

New specimens: 11 new voucher specimens (No. 312-1 – 312-11) from the Pavlovka River were deposited to the parasitological collection of the Zoological Museum, Federal Scientific Center of the East Asia Terrestrial

Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (e-mail: petrova@ibss.dvo.ru. Deposition date: the 11th of March 2021).

Prevalence: 71% (12/17).

Mean intensity: 3 (range: 1–6).

Molecular genetic data: 28S rRNA gene – MZ448170-MZ448176; *cox1* mtDNA gene – MW729428-MW729429.

Morphological description (amended)

Adult (Fig. 2 d, e; Table 3): Relatively small trematodes. Body elongate-fusiform, widest at ovarian level. Tegument smooth, unarmed. Forebody short, narrow on anterior end. Hindbody long, evenly tapering to posterior end of body, 62.3–67.3% of body length. Posterior end of body subconical. Eyespots absent. Oral sucker subterminal, rounded. Perioral muscular papillae absent. Ventral sucker rounded on border of first and second fourth of body, larger than oral sucker. Pharynx small, elliptical. Oesophagus short, straight, bifurcating at level of middle or anterior third of ventral sucker. Caeca long, reaching posterior extremity of body. Testes two, large, tandem, in mid-line of body, adjoining each other; anterior testis oval to irregular; posterior testis subspherical. Cirrus pouch small, saccate; its proximal part reaching posterior third of ventral sucker. Cirrus pouch contains internal seminal vesicle, prostatic part, and short unarmed cirrus. External seminal vesicle absent. Internal seminal vesicle large, slightly curved. Genital pore opening anteriorly to ventral sucker, median or dextral. Ovary rounded, approximately half to 3/4 of testes size (42–76.4%), sinistral. Seminal receptacle behind the ovary, dextral, flask-shaped, approximately half to equal size to ovary. Laurer′s canal posterior to ovary and seminal receptacle. Uterus extending between ventral sucker and anterior margin of anterior testis surrounding ovary and seminal receptacle mostly dextrally. Eggs medium-sized, oval, numerous (~200). Vitelline follicles ellipsoid or spherical, size nearly equal to that of eggs, extending from mid-line or posterior third of ventral sucker to posterior extremity of body, closely surrounding testes on both sides, overlapping caeca, and confluent in post-testicular region, ending at some short distance from

posterior end of body. Excretory ducts connecting each other at level of anterior third of posterior testis. Excretory vesicle Y-shaped. Excretory pore terminal.

Remarks on morphology

Size ranges of most organs of *A. khankaiensis* from the Pavlovka River matched the maximum values of *A. khankaiensis* in the original description from the Komissarovka River (Vainutis, 2020), except for the maximum values of body length, forebody length and ventral sucker, which are a little higher in newly observed specimens (Table 3). In contrast, *A. khankaiensis* from the Pavlovka River was a little smaller in egg size than *A. khankaiensis* from the Komissarovka River. Meanwhile, the ratios of the body length to width, forebody to whole body length, and oral to ventral sucker length/width of *A. khankaiensis* from both localities coincided with each other. In total, worms from both rivers had similar morphological characteristics as follows: body widest at ovarian level; intestinal bifurcation dorsal to ventral sucker, on mid-level of latter; cirrus pouch dextro-dorsral to ventral sucker, its proximal part reaching posterior margin of ventral sucker; relatively large tandem testes in relation to body width; relatively large body size. In addition, the distribution of vitelline follicles varied between worms from both rivers: the anterior border of vitellarium at the mid-level of the ventral sucker or anterior to its anterior margin in worms from the Pavlovka River vs. the vitellarium strictly at the level of anterior margin of ventral sucker in worms from the Komissarovka River. The excretory vesicle of *A. khankaiensis* from the Pavlovka River is Y-shaped as well as in *A. khankaiensis* from the Komissarovka River (Vainutis, 2020).

SEM analysis

Scanning electron microscopy of the tegument of *Allocreadium pseudoisoporum* **sp. nov.** (Fig. 3a), *A. khankaiensis* (Fig. 3b), and *A. hemibarbi* (Fig. 3c) revealed that it was covered with the structures reminiscent of small sensory receptors reported from *Crepidostomum metoecus* (Žďárská & Nebesářová, 2003, 2004). The size of the receptors varied in the range of 1.0 – 1.6 μm depending on the species: 1.56 μm in *A. pseudoisoporum* **sp. nov.**; 1.0 – 1.42 μm in *A. khankaiensis*; 1.20 – 1.33 μm in *A. hemibarbi*. Receptors were distributed on the ventral surface of the worms′ body: on forebody, between ventral sucker and oral sucker (Fig. 4a, c, e), mostly on postacetabular region (Fig. 4d, f), less on lateral parts, and absent on the dorsal surface. Unlike *A. pseudoisoporum* **sp. nov.**, the postacetabular uterine region of *A. khankaiensis* and *A. hemibarbi* was densely covered with numerous receptors (Fig. 4d, f). Each species had unique shape of receptors (Fig. 3a-c): *A. pseudoisoporum* **sp. nov.** had elongate receptors, *A. khankaiensis* – elongate-fusiform, and *A. hemibarbi* club-shaped. Small, dome-like papillae, reported for all allocreadiids for which the SEM studies have been done, were observed only on the oral sucker of *A. pseudoisoporum* **sp. nov.**, which has three papillae of 4.21 μm length on each side (Fig. 4b).

Genetic data

Genetic analysis using the 28S rRNA gene

The 28S sequences of three *A. pseudoisoporum* **sp. nov.** individuals were identical. The level of genetic differentiation of *A. pseudoisoporum* **sp. nov.** in relation to *A. isoporum* was rather high (5.434%±0.0073). Genetic distances between closely related *A. pseudoisoporum* and *A. hemibarbi* and between *A. pseudoisoporum* and *A. gotoi* were 3.581%±0.0054 and 3.578%±0.0051, respectively. Genetic distances (Table 4) between all studied species of *Allocreadium* varied in the range of 0.162±0.0011 – 6.395±0.0074%.

Both BI and ML phylogenetic trees maintained the same branch topology (Fig. 5). Species were divided into two clades of which first (basal) was formed by outgroup – the family Callodistomidae and the second consisted of inner group – the family Allocreadiidae. Allocreadiidae split into seven highly supported subclades, representing 5 genera and including 24 species. Genus *Acrolichanus* formed subclade I; subclade II included two species of the genus *Stephanophiala*; single species *Crepidostomum chaenogobii* belonged to the subclade III; subclade IV incorporated four species of the genus *Bunodera*; subclade V – three species of the genus *Crepidostomum* and its sister subclade VI included four species of North American *Crepidostomum* sensu lato. Subclade VII was formed with nine species of the genus

Allocreadium and branched into eight separate groups: *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*. Group *a* belonged to *A. pseudoisoporum* **sp. nov.** Group *b* included *A. gotoi* from Japan and *Allocreadium* sp. 2 from Ukraine. Group *c* was presented with the single species *A. hemibarbi*. Group *d* included *A. khankaiensis* from three localities and *Allocreadium* sp. 1 – both from Primorsky region, Russia. Group *e* included European species *A. isoporum* and *A. crassum*. Group *f* consisted of the single species *Allocreadium* sp. 3 from China, which is the sister group of *A. neotenicum*/*A. lobatum* group (Group *g*) and African species *A. apokryfi* (Group *h*).

Genetic analysis using the *cox1* mtDNA gene

Three identical sequences (843 bp length) were obtained for *Allocreadium pseudoisoporum* **sp. nov.** Genetic distances distinguishing *A. pseudoisoporum* **sp. nov.** from *A. gotoi*, *A. khankaiensis*, *Allocreadium* sp. 1, *A. lobatum* were in the range 18.78±0.015–22.27±0.016% (Supplementary Table 1). Intraspecific genetic distances of the *Allocreadium* species – 0–0.15±0.0014%. In general, interspecific genetic distances varied from 15.72±0.013 to 22.27±0.016%.

Discussion

This study supplemented the cycle of works dedicated to the investigation of the type genus of the family Allocreadiidae. Morphological and molecular studies of the *Allocreadium* species, based both on the original and literature data, allowed us to clarify the taxonomic position of the newly collected *Allocreadium* worms.

Scanning microscopy

To date, images of scanning electron microscopy were provided for 33 species representing 12 genera of the family Allocreadiidae (e.g. Caira, 1985; Lasee et al., 1988; Moravec, 2002; Žďárská & Nebesářová, 2003, 2004; Choudhury & León-Règagnon, 2005; Gao et al., 2008). It was demonstrated that the tegument of allocreadiid worms on the ventral surface of the forebody and on both suckers is smooth with normal tissue striation and only small dome-like papillae (more than 4 μm in diameter). Gao et al. (2008) showed that the surface striations on hindbody of *Allocreadium danjiangensis* are much denser, but shallower than on forebody, and normally the tegument contains round tubercles, which are larger than the papillae. Our SEM sample preparation technique was slightly updated to compress the tegument of worms, leading to release of numerous receptors on the body surface. Thus, only in this study and in studies performed by Moravec (2002) and Žďárská and Nebesářová (2003, 2004) the intra-tegumental sensory receptors were examined in allocreadiids. Rohde and Watson (1989) were first who classified sensory receptors (sensilla) in aspidogastrean trematode *Lobatostoma manteri* and divided them into nine types – ciliate and nonciliate, i.e., bulbs with deep tegument invaginations. Later Žďárská and Nebesářová (2003, 2004) described another five types of sensory receptors in *Crepidostomum metoecus,* but only few of them coincide with sensilla determined by Rohde and Watson (1989). The main problem is inconsistency between existed classifications that should be further resolved; in this study we followed the classification of Žďárská and Nebesářová (2003).

According to latter authors, apical part of type I and type II ciliate receptors is represented by a single cilium partially submerged in the tegument, having the ability to protrude above the tegument surface. Comparing receptors of our samples with those of *Crepidostomum metoecus* from Žďárská and Nebesářová (2003, 2004), the receptors of *A. pseudoisoporum* and *A. khankaiensis* (Fig. 3 a,b) similar in shape with type I – elongate ciliate receptors, while the receptors of *A. hemibarbi* (Fig. 3 c) similar to type II – lobed and broader at the apical part ciliate receptor. Unfortunately, the image and diagrams of lobes of type II sensory receptors provided by Žďárská and Nebesářová (2003) are not visible enough to compare with club-shaped lobed bulbs of *A. hemibarbi*. Structures similar in shape to *A. hemibarbi* receptors were found on the tegument surface of *Stephanostomum egypticum*, but less than 1 μm in size (Abdou & Ashour, 2000). Probably, with the preparation of histological sections and the use of transmission electron microscopy, it will be possible to describe the structures of *A. hemibarbi* as a new receptor type. In aspect of systematics, receptor morphology has a possibility to serve external diagnostic features, for example genus specific, in addition to the extension of

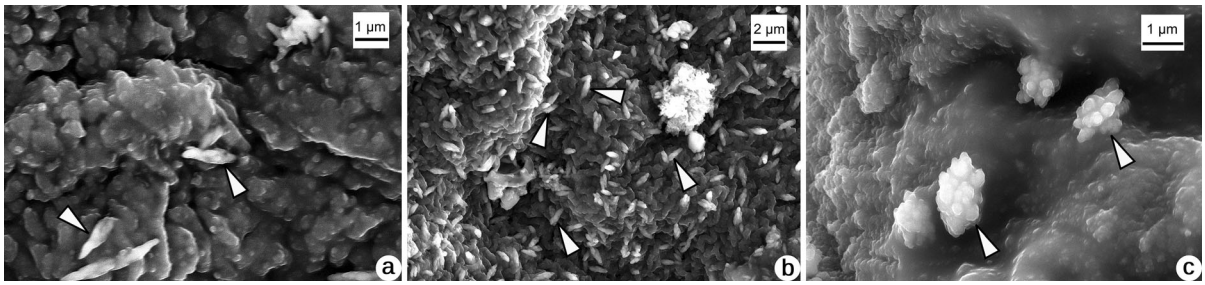

Fig. 3 Scanning Electron Microscopy of the ventral surface of the forebody between oral and ventral suckers in three *Allocreadium* species: *A. pseudoisoporum* **sp. nov.** (a), *A. khankaiensis* (b), *A. hemibarbi* (c). White arrows show some of the presumable sensory receptors represented with the numerous small white spots

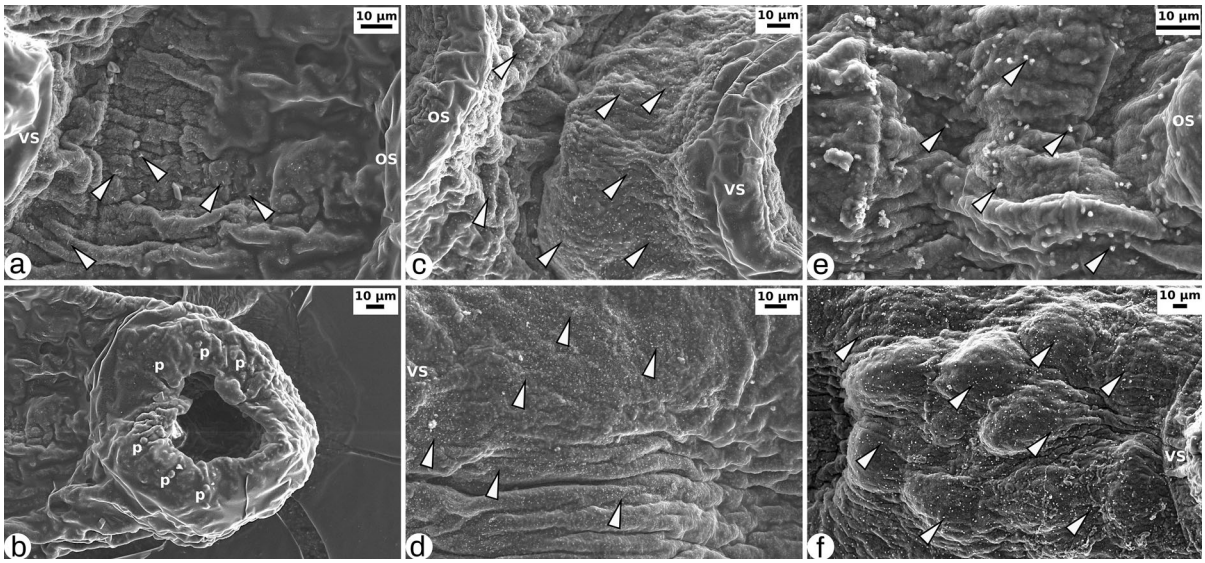

Fig. 4 Scanning Electron Microscopy of the different tegumental regions of three *Allocreadium* species: ventral surface of the forebody between oral and ventral suckers in *A. pseudoisoporum* (a); anterior end of the body in *A. pseudoisoporum* (b); ventral surface of the forebody between the suckers in *A. khankaiensis* (c); ventral side of the postacetabular region in mid-body in *A. khankaiensis* (d); ventral surface of the forebody between the suckers in *A. hemibarbi* (e); ventral side of the postacetabular region (the level of uterine region) in mid-body in *A. hemibarbi* (f). White arrows show the aggregations of the presumable sensory receptors represented with the numerous small white spots. Abbreviation: os – oral sucker; vs – ventral sucker; p – small papillae

small papillae on the ventral surface of the forebody of allocreadiids (family specific feature).

Phylogenetic analysis

Genetic divergence

Genetic variation among *A. pseudoisoporum* **sp. nov.** individuals was 0% according to both 28S and *cox1* genes, confirming the affiliation of all the isolates from the Arsenyevka River to one species. Molecular genetic analysis based on the 28S rRNA gene showed that *A. pseudoisoporum* **sp. nov.** is independent in relation to previously described *A. khankaiensis* and *A. hemibarbi* (Vainutis, 2020), and *A. apokryfi* (Dos Santos et al., 2021). Also, *A. pseudoisoporum* **sp. nov.** is well distinguished genetically (5.43% in 28S) (Fig. 5; Table 4) and geographically isolated from the type European *A. isoporum*. Considering the values noted in the previous studies, the genetic distances (Supplementary Table 1) estimated using *cox1* gene revealed interspecific-level divergence of *A. pseudoisoporum* **sp. nov.** in comparison with *A. gotoi*, *A. lobatum*, *A. khankaiensis*, and *Allocreadium* sp. 1 (Urabe et al., 2020; Vainutis et al., 2021).

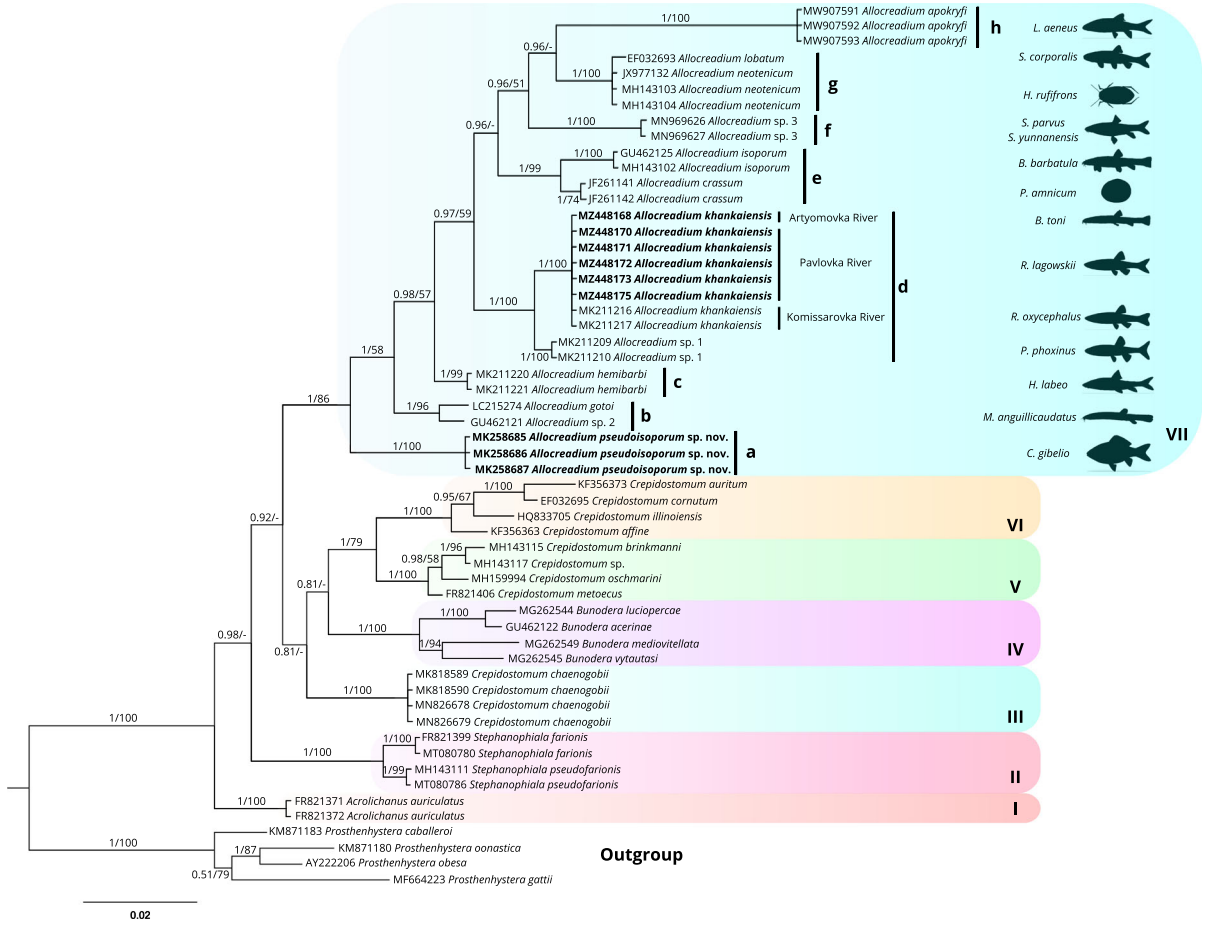

Fig. 5 Generalized phylogenetic tree based on partial 28S rDNA sequences trimmed to the length of 1024 bp for 24 species of the family Allocreadiidae. The newly obtained sequences in this study are in bold. Branch numbers showed as the values of BI (posterior probabilities)/ ML (bootstrap replicates) reconstruction algorithms. Branches with less than 50% support of ML indicated with ″-″

Prior to the results of this study, *A. gotoi* was the earliest divergent species in the genus *Allocreadium* (Petkevičiūtė et al., 2018; Vainutis, 2020), but high nodal support on BI and ML (1/86) reconstructions confirmed the closest position of *A. pseudoisoporum* **sp. nov.** to the ancestral node in relation to the sister groups of *Allocreadium* spp. (Groups *b* – *h*, Fig. 5). Japanese species *A. gotoi* was genetically closest (3.58% in 28S and 18.78% in *cox1*) to East Asian *A. pseudoisoporum* **sp. nov.** These data indirectly confirmed the statements of Lindberg (1955) about the Eurasian and Japanese fauna exchange in Eopleistocene (2.58 – 0.76 million years ago), when the Sea of Japan was a freshwater reservoir. Indeed, nowadays relict fish (*Tribolodon brandtii*, *Parahucho perryi*, *Acipenser mikadoi*) inhabit the Sea of Japan (Zimmerman et al., 2012; Vdovin et al., 2015; Koshelev & Kolpakov, 2020), of them *T. brandtii* and its relatives *T. hakonensis* and *T. sachalinensis* are infected with *Allocreadium tosai*, *A. japonicum*, and *A. tribolodontis* in Japan (Shimazu, 1988, 2016). Probably the ancestors of all modern lineages of *Allocreadium* spp. originated somewhere in East Asia in the interglacial periods of eopleistocene. Then, they could migrate from North East Asia to the North America through the Bering land bridge (Bogatov & Vainutis, 2022). In the Middle Pleistocene, the migration of *Allocreadium* spp. ancestors using the freshwater fish could be interrupted due to the glacial events in the Central Beringia and adjacent mainland areas. These events could probably be the Nome River glaciation in Alaska (Kaufman et al., 1991) and Late

Glacial period in the central Bering Land Bridge (Lozhkin et al., 2011). The following submergence of the most part of the Central Beringia led to the separation of the Eurasian and American parasite fauna. Subsequently, Asian *Allocreadium* spp. diverged and speciated in the inland waters of Northeast Asia, i.e. Russian Far East, Japan, China, and Korea.

According to the values of 28S genetic distances (Table 4), two detected cryptic species, *Allocreadium* sp. 2 and *Allocreadium* sp. 3, could be considered as independent compared to each other and to nine valid species analyzed: 0.815 – 5.306% and 3.075 – 6.395% respectively. Interestingly, in spite of geographic isolation *Allocreadium* sp. 2 showed high degree of gene convergence with *A. gotoi*. It is possible that *Allocreadium* sp. 2 was recently introduced from the fresh waters of Japan or neighboring mainland countries to Europe (particularly Ukrainian fresh waters) through the fish hosts, e.g. *Misgurnus anguillicaudatus*. For example, *A. gotoi* was recorded in China from loaches *M. anguillicaudatus* (Okino et al., 2004) which was introduced into Europe during human activity, that fact has already been confirmed by genetic data (Belle et al., 2017).

The Chinese species *Allocreadium* sp. 3 was sister to the groups *g* and *h* (Fig. 5) and genetically closer to European *A. neotenicum* (3.08%) and American *A. lobatum* (3.25%); however, *Allocreadium* sp. 3 is relatively distant from African *A. apokryfi* (6.395%) (Table 4). Presumably, after the colonization of water bodies in Europe by *Allocreadium* spp., ancestors of *Allocreadium* sp. 3 undergone repatriation to North East Asia with further divergence in China.

The remarks on the origin

Based on the previous phylogeographic studies (Manter, 1963; Vainutis, 2020; Dos Santos et al., 2021; Bogatov & Vainutis, 2022) and including genetic data for nine *Allocreadium* species into phylogenetic analysis, we hypothesize about the origin of the genus *Allocreadium*. Affiliation of five species (*A. pseudoisoporum* **sp. nov.**, *A. gotoi*, *A. hemibarbi*, *A. khankaiensis*, *Allocreadium* sp. 1) to the East Asian region presumes that the ancestral representatives of the genus settled in this region in the past, and then divided into the several phyletic lines (groups a-d, Fig. 5). In the Palearctic region, at least four species are known to have the same morphology as type species *A. isoporum* (vitelline fields in hindbody, not reaching ventral sucker at short distance; ventral sucker larger than oral sucker or the same size; testes ellipsoid): *A. montanus* Sidorov, Butenko, 1966, *A. tribolodontis* Shimazu and Hashimoto, 1999, *A. brevivitellatum* Shimazu, 1992, and *A. pseudoisoporum* **sp. nov.** Recently described *A. apokryfi* (Dos Santos et al., 2021) revealed anatomy similar with *A. isoporum*. Particularly, in the aspect of oldest origin of *A. pseudoisoporum* **sp. nov.**, we suppose that the lack of perioral papillae on the oral sucker, the extension of vitellarium in hindbody, the position of the intestinal bifurcation on the level of the ventral sucker, cirrus pouch anterior to the ventral sucker (exception is *A. pseudoisoporum* **sp. nov.**) could be considered plesiomorphic, characteristic for the common ancestor of *Allocreadium* spp.

Manter (1963) listed 24 valid species, of which 12 are from India, four from Europe, five from North America, two from Africa, and one from Japan. Manter suggested that the genus *Allocreadium* originated in South Asia and, following the cyprinids, spread to Eurasia, North America, partially to Africa, but not to South America. Nowadays, the number of *Allocreadium* species is known to exceed the number indicated by Manter (1963). For instance, species richness in India is about 3.5 times higher than that in the Amur basin (32 and 9 species, respectively). Based on this fact, South Asian origination of the genus *Allocreadium* could be assumed. The taxonomic status of only a small number of *Allocreadium* spp. has been supported with genetic data, the status of the rest needs to be confirmed in future studies. In our study, we suggest the reconsideration of Manter′s hypothesis and supplement it according to the newly accumulated data on biogeography and genetics of the genus *Allocreadium*.

The origination of *Allocreadium* spp. could be associated with their fish hosts (Bogatov & Vainutis, 2022). Stated by Vainutis (2020) the divergence of *Allocreadium* sp. 1 and *A. khankaiensis* occurred between Late Pliocene and Early Pleistocene periods after the separation of the Razdolnaya River and Paleo-Amur. The origination of their hosts (the genus *Rhynchocypris*) is estimated to occur nearly 10 Ma (Cheng et al., 2022). The first cypriniform fish, the definitive hosts of *Allocreadium* spp., appeared about 95 Ma ago in the Late Cretaceous period (Near et al.,

2012). Thus, the key divergence events in the genus *Allocreadium* occurred before the Pleistocene - between 95 and 10 Ma, in the Paleo-Amur region. This is additionally indicated by the origin of another allocreadiid genus *Margotrema* (about 6.53 million years ago), which is younger than the genus *Allocreadium*.

Two parasitophyletic rules can help in explaining the events of anagenesis and cladogenesis between *Allocreadium* spp. and their hosts: Fahrenholz′s and Szidat′s. Fahrenholz′s rule is a direct consequence of Szidat′s rule and concludes that the anagenesis and cladogenesis of hosts are paralleled by the anagenesis and cladogenesis of their parasites (Brooks & McLennan, 1993). Cypriniform fishes, mainly Cyprinidae are infected with numerous *Allocreadium* species worldwide excluding Australia that has been confirmed with multiple studies since Looss (1894). There were single cases of finding *Allocreadium* in other fishes, for example, salmonids (Shimazu, 1988) which could be considered accidental. It is likely that Allocreadium originated in Southeast Asia, because this region possessed the richest diversity of Cyprinidae and was their speciation center (Saitoh et al., 2011). This statement is still difficult to confirm due to the lack of molecular genetic data for the South Asian and Southeast Asian species of *Allocreadium*.

Conclusion

For divergence time estimation, corresponding methodology should be used, considering assumptions relating to clock models, substitution rates and calibrations of node ages. We require the genetic data of larger number of allocreadiid species, and their hosts to analyze the co-phylogenetic relationships and host-parasite associations. The description of the new species *Allocreadium pseudoisoporum* expands our knowledge on the biodiversity of *Allocreadium* spp. in Russian Far East. Based on the comparative morphological analysis, it was confirmed that *A. isoporum* previously found in the rivers of Amur basin belongs to the species *A. pseudoisoporum*. We found out that *A. khankaiensis* is much more widespread in the Primorsky region. Settling of the Artyomovka River by this species can be explained with the natural introduction through minnows extensively inhabiting the entire Amur basin (Berg, 1949; Chereshnev, 1998) including both habitats of *A. khankaiensis* – the Komissarovka and Pavlovka rivers. Shape and size of ciliate tegument receptors provided by SEM can be served as additional diagnostic features, useful in distinguishing *Allocreadium* species.

Acknowledgements The authors are grateful to the head of the Parasitology Department Dr. Vladimir V. Besprozvannykh (Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS) for providing the material collected in the Arsenyevka River in 2012.

Funding The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 121031000154-4).

Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Abdou, N. E., & Ashour, A. A. (2000). Scanning electron microscopy of the tegumental surface of digenetic trematode *Stephanostomum egypticum* from the Red Sea fishes. *Journal of the Egyptian Society of Parasitology*, 30 (1), 341–348.

Achmerov, A. H. (1960). New trematode species from the River Amur. *Helminthologia*, 2 (3-4), 286–394. [In Russian]

Achmerov, A. H. (1961). To the knowledge of the trematode fauna of fishes in the Amur River basin. *Proceedings of GELAN*, 11, 22-31. [In Russian]

Achmerov, A. H. (1963). *Allocreadium maculati* nov. sp. new species of trematode from Amur fish. *Trudy Helmintologicheskoy laboratorii AS USSR*, 13, 250-252. [In Russian]

Atopkin, D. M., & Shedko, M. B. (2014) Genetic characterization of far eastern species of the genus *Crepidostomum* (Trematoda: Allocreadiidae) by means of 28S ribosomal DNA sequences. *Adv. Biosci. Biotechnol.*, 5(3), 209-215. https://doi.org/10.4236/abb.2014.53027

Atopkin, D. M., Sokolov, S. G., Shedko, M. B., Vainutis, K. S., & Orlovskaya, O.M. (2018). Diversity of the genus *Bunodera* Railliet, 1896 (Trematoda: Allocreadiidae) in the northern part of Eastern Europe and North-eastern Asia, estimated from 28S rDNA sequences, with a description of *Bunodera vytautasi* sp. nov. *Parasitology Research*, 117(6), 1765-1772. https://doi.org/10.1007/s00436-018-5858-y

Aydogdu, A., Pérez-Ponce de León, G., Emre, Y., Emre, N., & Yabacı, A. (2018). Prevalence and intensity of

Allocreadium isoporum (Digenea: Allocreadiidae) in three endemic species of cyprinids (*Capoeta* spp.) in Turkey, in relation to season, host size and sex. *Journal of Applied Ichthyology*, 34(5-6). https://doi.org/10.1111/jai.13515

Belle, C. C., Stoeckle, B. C., Cerwenka, A. F., Kuehn, R., Mueller, M., Pander, J., & Geist, J. (2017). Genetic species identification in weatherfish and first molecular confirmation of Oriental Weatherfish *Misgurnus anguillicaudatus* (Cantor, 1842) in Central Europe. *Knowl. Manag. Aquat. Ecosyst.*, 418, 31.

Berg, L. S. (1949). *Freshwater fishes of the U.S.S.R. and adjacent countries*. Acad. Sci. Moscow USSR, Vols. 1, 2, 3 (Edn 4).

Bogatov, V. V., & Vainutis, K. S., (2022). About the origin of the family Allocreadiidae (Trematoda: Plagiorchiida). *Doklady Biological Sciences*, 502(1), 104–108. https://doi.org/10.31857/S268673892201005X

Bray, R. A., Foster, G. N., Waeschenbach, A., & Littlewood, D. T. J. (2012). The discovery of progenetic *Allocreadium neotenicum* Peters, 1957 (Digenea: Allocreadiidae) in water beetles (Coleoptera: Dytiscidae) in Great Britain. *Zootaxa*, Vol. 3577, № 1. P. 58–70. https://doi.org/10.11646/zootaxa.3577.1.3

Brooks, D. R., & McLennan, D. A. (1993). A brief history of parasite evolutionary biology. In: V. A. Funk, & P. F. Cannell (Eds.), Parascript: parasites and the language of evolution (1st ed., pp. 5 - 19). Smithsonian Institution Press, Washington and London.

Caira, J. N. (1985). A revision of the North American papillose Allocreadiidae (Digenea) with independent cladistic analyses of larval and adult forms. Bulletin of the University of Nebraska State Museum. PhD Thesis, 11(3), 1–58.

Chandra, S., Yadav, N., & Saxena, A. (2019). Two new records of digenetic trematode genus *Allocreadium* Looss, 1900 from fresh water fishes of Lucknow, India. *Current Life Sciences*, 5(2), 28-34. https://doi.org/10.5281/zenodo.3262331

Cheng P., Yu D., Tang Q., Yang J., Chen Y., & Liu H. (2022). Macro-evolutionary patterns of East Asian opsariichthyin-xenocyprinin-cultrin fishes related to the formation of river and river-lake environments under monsoon climate. *Water Biology and Security*, 1(2), 100036. https://doi.org/10.1016/j.watbs.2022.100036

Chereshnev, I. A. (1998). *Biogeography of freshwater fish in the Russian Far East*. Vladivostok, Dalnauka. (**In Russian**)

Choudhury, A., León-Règagnon, V. (2005). Molecular phylogenetics and biogeography of *Bunodera* spp. (Trematoda: Allocreadiidae), parasites of percid and gasterosteid fishes. *Canadian Journal of Zoology*, 83(12), 1540–1546. https://doi.org/10.1139/z05-153

Curran, S. S., Tkach, V. V., & Overstreet, R. B. (2006). A review of *Polylekithum* Arnold 1934 and its familial affinities using morphological and molecular data, with description of *Polylekithum catahoulensis* sp. nov. *Acta Parasitologica*, 51(4), 238–248. https://doi.org/10.2478/s11686-006-0037-1

Curran S. S., Tkach V.V., Overstreet R.M. Phylogenetic affinities of Auriculostoma (Digenea: Allocreadiidae), with descriptions of two new species from Peru // Journal of Parasitology. 2011. Vol. 97, № 4. P. 661–670. https://doi.org/10.1645/GE-2641.1

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods*, 9, 772. https://doi.org/10.1038/nmeth.2109

Dos Santos, Q. M., Gilbert, B. M., Avenant-Oldewage, A., & Dumbo, J. C. (2021). Morphological and molecular description of *Allocreadium apokryfi* sp. n. (Digenea: Allocreadiidae) from native *Labeobarbus aeneus* (Cyprinidae) in South Africa, including notes on its biology, evolutionary history and an updated key of African *Allocreadium*. *Folia Parasitologica*, 68: 013. https://doi.org/10.14411/fp.2021.013

Ermolenko, A. V. (1990). *Causative agents of invasive diseases of freshwater fishes from Primorsky Krai*. Vladivostok: FEB AS USSR. [In Russian]

Ermolenko, A. V. (1992). *Parasites of fishes from freshwater ponds of the continental part of Japan sea basin*. Vladivostok: FEB RAS. [In Russian]

Faltýnková, A., Pantoja, C., Skírnisson, K., & Kudlai, O. (2020). Unexpected diversity in northern Europe: trematodes from salmonid fishes in Iceland with two new species of *Crepidostomum* Braun, 1900. *Parasitology Research*, 119, 2439–2462. https://doi.org/10.1007/s00436-020-06724-1

Franceschini, L., Aguiar, A., Zago, A. C., de Oliveira Fadel Yamada, P., Bertholdi Ebert, M., & da Silva, R. J. (2021). Three new species of *Creptotrema* (Trematoda, Allocreadiidae) with an amended diagnosis of the genus and reassignment of *Auriculostoma* (Allocreadiidae), based on morphological and molecular evidence. *Parasite*, 28, 69. https://doi.org/10.1051/parasite/2021065

Gao, D., Wang, G. T., Xi, B. W., Yao, W. J., & Nie, P. (2008). A New Species of *Allocreadium* (Trematoda: Allocreadiidae) from Freshwater Fishes in the Danjiangkou Reservoir in China. *Journal of Parasitology*, 94(1), 176-180. https://doi.org/10.1645/ge-1247.1

Hasegawa, T. & Ozaki, Y. (1926). On a new trematode parasitic in *Misgurnus anguillicaudatus*. *Dobutsugaku Zasshi*, 38, 225–228. [In Japanese]

Hernández-Mena, D. I., Lynggaard, C., Mendoza-Garfias, B., & Pérez-Ponce de León, G. (2016). A new species of *Auriculostoma* (Trematoda: Allocreadiidae) from the intestine of *Brycon guatemalensis* (Characiformes: Bryconidae) from the Usumacinta River Basin, Mexico, based on morphology and 28S rDNA sequences, with a key to species of the genus. *Zootaxa*, 4196(2), 261–277. https://doi.org/10.11646/zootaxa.4196.2.5

Hernández-Mena, D. I., Pinacho-Pinacho, C. D., García-Varela, M., Mendoza-Garfias, B., & de León, G.P. (2019). Description of two new species of allocreadiid trematodes (Digenea: Allocreadiidae) in middle American freshwater fishes using an integrative taxonomy approach. *Parasitology Research*, 118(2), 421–432. https://doi.org/10.1007/s00436-018-6160-8

Huelsenbeck, J. P., Ronquist, F., Nielsen, R., & Bollback, J.P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294, 2310–2314. https://doi.org/10.1126/science.1065889

Kaufman, D., Walter, R., Brigham-Grette, J., & Hopkins, D. (1991). Middle Pleistocene age of the Nome River glaciation, northwestern Alaska. *Quaternary Research*,

36(3), 277-293. https://doi.org/10.1016/0033-5894(91)90003-N

Koshelev, V. N., Kolpakov, N. V. (2020). Sakhalin sturgeon *Acipenser mikadoi* (Acipenseridae): results of study and proposed measures for conservation of the species. *Izvestiya TINRO*, 200(4):791-808. https://doi.org/10.26428/1606-9919-2020-200-791-808 [In Russian]

Koval, V. P. (1957). Trematodes of the genus *Allocreadium* Looss, 1900 in fishes of freshwater ponds of UkSSR. *Naukovi zapiski Kiivs′kogo Universitetu*, v. XVI, XX. *Biologichniy zbirnik*, 14, 201-212. [In Ukrainian]

Koval, V. P. (1966). Family Allocreadiidae Stossich, 1903. In K. I. Skryabin (Ed.), *Trematodes of animals and men* (vol. 22, pp. 185–305). Nauka. (**In Russian**)

Kozicka, I. (1956). Parazyty ryb jeziora Goldapiwo. *Wiadom. parazytol*., 2(5), 207. [In Polish]

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution*, 35(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lasee, B. A., Font, W. F., & Sutherland, D. R. (1988). *Culaeatrema inconstans* gen.n., sp.n. (Digenea: Allocreadiidae) from the brook stickleback (*Culaea inconstans*) in Wisconsin and observations on parthenogenetic populations. *Canadian Journal of Zoology*, 66, 1328–1333. https://doi.org/10.1139/z88-195

Layman, E. M. (1933). Parasitic worms of fishes of Lake Baikal. *Tr. Baikalsk. limnol. stantsii*, 4, 5-98. [In Russian]

Lindberg, G. U. (1955). *Quaternary Period in the Light of Biogeographic Data*. Publishing House of the USSR Academy of Sciences, Leningrad, Monograph. [In Russian]

Linstow, O. F. B. (1896). Helminthologische Mitteilungen. *Arch. Microskop. Anat.*, 48(3), 373-397.

Lobanov, A. L., Kirejtshuk, A. G., Stepanjants, S. D., & Smirnov, I. S. (2013). Diagnostic keys: from the textual dichotomous to computer ones. *Proceedings of the Zoological Institute RAS*, Appendix No. 2, 249–268.

Lockyer, A. E., Olson, P. D., & Littlewood, D. T. J. (2003). Utility of complete large and small subunit rRNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biol. J. Linn. Soc. Lond.*, 78, 155–171. https://doi.org/10.1046/j.1095-8312.2003.00141.x

Looss, A. (1894). *Die Distomen unserer Fische und Frösche Neue Untersuchungen über Bau und Entwickelung des Distomenkörpers*. Bibl. Zool., V. 16, 296 p.

Lozhkin, A. V., Anderson, P., Eisner, W. R., & Solomatkina, T. B. (2011). Late glacial and holocene landscapes of central Beringia. *Quaternary Research*, 76(3), 383–392. https://doi.org/10.1016/j.yqres.2011.08.003

Lühe, M. (1909). Parasitische Plattwurmer. I. Trematodes. In A. Brauer (Ed.), *Die Süsswasserfauna Deutschlands. Eine Exkursionfauna* (vol. 2, is. 17, p. 217). Jena, Gustav Fischer.

Martínez-Aquino, A., Ceccarelli, F. S., & de León, G. P.-P. (2013). Molecular phylogeny of the genus *Margotrema* (Digenea: Allocreadiidae), parasitic flatworms of goodeid freshwater fishes across central Mexico: species boundaries, host-specificity, and geographical congruence. *Zoological Journal of the Linnean Society*, 168(1), 1–16. https://doi.org/10.1111/zoj.12027

MacIntosh, A. J. J., & Frias, L. (2017). Coevolution of hosts and parasites. In A. Fuentes (Ed.), *The international encyclopedia of primatology* (vol. 1, pp. 188–196). Chichester, Wiley/Blackwell.

Manter, H. W. (1963). The Zoogeographical Affinities of Trematodes of South American Freshwater Fishes. *Systematic Biology*, 12(2), 45–70. https://doi.org/10.2307/sysbio/12.2.45

Martínez-Aquino, A., Ceccarelli, F. S., Eguiarte, L. E., Vázquez-Domínguez, E., & de León, G. P.-P. (2014). Do the Historical Biogeography and Evolutionary History of the Digenean *Margotrema* spp. across Central Mexico Mirror Those of Their Freshwater Fish Hosts (Goodeinae)? *PLoS ONE*, 9(7), e101700. https://doi.org/10.1371/journal.pone.0101700

Milton, J., Paray, B. A., & Rather, I. (2018). A review on the biology and physiology of loach *Misgurnus anguillicaudatus* in China. *Indian Journal of Geo-Marine Sciences*, 47(4), 759–765.

Miura, F., Uematsu, C., Sakaki, Y., & Ito, T. (2005). A novel strategy to design highly specific PCR primers based on the stability and uniqueness of 3′-end subsequences. *Bioinformatics*, 21, 4363–4370.

Montes, M. M., Barneche, J., Croci, Y., Balcazar, D., Almirón, A., Martorelli, S., & Pérez-Ponce de León, G. (2021). Description of a new species of *Auriculostoma* (Digenea: Allocreadiidae) from *Characidium heirmostigmata* (Characiformes: Crenuchidae) from Argentina, using morphological and molecular data. *Journal of Helminthology*, 95, e19, 1–8. https://doi.org/10.1017/S0022149X21000109

Montes, M. M., Barneche, J., Croci, Y., Rodriguez Gil, S., Curran, S. S., Ferrari, W., Casciotta, J. R. & Martorelli, S. R. (2020). *Prosthenhystera gattii* n. sp. (Digenea: Callodistomidae), a gallbladder parasite of *Bryconamericus ikaa* from the lower Iguazú River, described based on combined molecular and morphological evidence. *Journal of helminthology*, 94, e151. https://doi.org/10.1017/S0022149X20000322

Moravec, F. (1984). Systematic status of *Allocreadium carparum* Odening, 1959 (Digenea) and three other related species from fishes of Czechoslovakia. *Folia Parasitologica*, 31, 215–224.

Moravec, F. (1992). Observations on the bionomy of *Allocreadium isoporum* (Looss, 1894) (Trematoda: Allocreadiidae). *Folia Parasitologica*, 39(2), 133–144.

Moravec, F. (2002). External morphological differences between *Crepidostomum farionis* and *Crepidostomum metoecus* (Trematoda: Allocreadiidae), parasites of salmonids, as revealed by SEM. *Folia Parasitologica*, 49(3), 211–217. https://doi.org/10.14411/fp.2002.037

Morgan, J. A. T., & Blair, D. (1998). Relative merits of nuclear ribosomal internal transcribed spacers and mitochondrial CO1 and ND1 genes for distinguishing among *Echinostoma* species (Trematoda). *Parasitology*, 116, 289–297.

Near, T. J., Eytan, R. I., Dornburg, A., Kuhn, K. L., Moore, J. A., Davis, M. P., Wainwright, P. C., Friedman, M., & Smith, W. L. (2012). Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National*

Academy of Sciences, 109(34), 13698–13703. https://doi.org/10.1073/pnas.1206625109

Okino, T., Ushirogawa, H., Matoba, K., & Ohyama, F. (2004). Survey of parasitic helminths in loaches imported from China. *Japanese Journal of Zoo and Wildlife Medicine*, 9, 71–78. [In Japanese with English abstract]

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). *International Journal for Parasitology*, 33(7), 733–755.

Ozaki, Y. (1926). On some new species of trematodes of freshwater fishes from Japan (Preliminary report). *Dobutsugaku Zasshi*, 38, 124–130. [In Japanese]

Pérez-Ponce De León, G., Martínez-Aquino, A., & Mendoza-Garfias, B. (2013). A new species of *Margotrema* (Digenea, Allocreadiidae) from the leopard splitfin *Xenotaenia resolanae* (Cyprinodontiformes, Goodeidae) from west-central Mexico. *Zootaxa*, 3670(1), 94–96. https://doi.org/10.11646/zootaxa.3670.1.10

Pérez-Ponce de León, G., Pinacho-Pinacho, C. D., Mendoza-Garfias, B., Choudhury, A., & Garcia-Varela, M. (2016). Phylogenetic analysis using the 28S rRNA gene reveals that the genus *Paracreptotrema* Choudhury, Pérez-Ponce de León, Brooks and Daverdin, 2006 (Digenea: Allocreadiidae) is not monophyletic; description of two new genera and one new species. *Journal of Parasitology*, 102(1), 131–142. https://doi.org/10.1645/15-815

Pérez-Ponce de León, G., Sereno-Uribe, A., García-Varela, M., Mendoza-Garfias, B., Hernández-Mena, D., Pinacho-Pinacho, C., & Choudhury, A. (2020). Disentangling the evolutionary and biogeographical history of the freshwater fish trematode genus *Creptotrema* (Digenea: Allocreadiidae) using an integrative taxonomy approach: The case of Creptotrema agonostomi in Middle American mountain mullets. *Journal of Helminthology*, 94, E171. https://doi.org/10.1017/S0022149X2000053X

Petkevičiūtė, R., Stunzenas, V., Staneviciute, G., & Sokolov, S. G. (2010). Comparison of the developmental stages of some European allocreadiid trematode species and a clarification of their life-cycles based on ITS2 and 28S sequences. *Systematic Parasitology*, 76(3), 169–178.

Petkevičiūtė, R., Stunžėnas, V., & Stanevičiūtė, G. (2012). Clarification of the systematic position of *Cercariaeum crassum* Wesenberg-Lund, 1934 (Digenea), based on karyological analysis and DNA sequences. *Journal of Helminthology*, 86(3), 293–301. https://doi.org/10.1017/S0022149X11000393

Petkevičiūtė, R., Stunžėnas, V., Zhokhov, A. E., Poddubnaya, L. G., & Stanevičiūtė, G. (2018). Diversity and phylogenetic relationships of European species of *Crepidostomum* Braun, 1900 (Trematoda: Allocreadiidae) based on rDNA, with special reference to *Crepidostomum oschmarini* Zhokhov & Pugacheva, 1998. *Parasites & Vectors*, 11, 530.

Razo-Mendivil, U., Mendoza-Garfias, B., Pérez-Ponce de León, G., & Rubio-Godoy, M. (2014). A new species of *Auriculostoma* (Digenea: Allocreadiidae) in the Mexican tetra *Astyanax mexicanus* (Actinopterygii, Characidae) from Central Veracruz, Mexico, described using morphological and molecular data. *Journal of Parasitology*, 100, 331–337. https://doi.org/10.1645/13-352.1

Rohde, K., & Watson, N. (1989). Sense receptors in *Lobatostoma manteri* (Trematoda, Aspidogastrea). *International Journal for Parasitology*, 19(8), 847–858. https://doi.org/10.1016/0020-7519(89)90110-0

Roitman, V. A. (1963). New species of trematodes of fishes of the Amur basin. *Trudy Gel'mintologicheskoi Laboratorii*, 13, 303–312. [In Russian]

Saitoh, K., Sado, T., Doosey, M. H., Bart, Jr, H. L., Inoue, J. G., Nishida, M., Mayden, R. L., & Miya, M. (2011). Evidence from mitochondrial genomics supports the lower Mesozoic of South Asia as the time and place of basal divergence of cypriniform fishes (Actinopterygii: Ostariophysi). *Zoological Journal of the Linnean Society*, 161(3), 633–662. https://doi.org/10.1111/j.1096-3642.2010.00651.x

Shimazu, T. (1988). Trematodes of the genus *Allocreadium* (Allocreadiidae) from a freshwater fish in Nagano, Central Japan. *Bull. Natl. Sci. Mus. Tokyo Ser. A. Zool*., (14) 1–21.

Shimazu, T. (2016). Digeneans Parasitic in Freshwater Fishes (Osteichthyes) of Japan VII. Allocreadiidae: *Allocreadium*, *Bulletin of the National Museum of Nature and Science, Series A (Zoology)*, 42(2), 55–79.

Shimazu, T. (2017). Digeneans parasitic in freshwater fishes (Osteichthyes) of Japan XII. A list of the papers of the series, a key to the families in Japan, a parasite-host list, a host-parasite list, Addenda, and Errata. *Bull. Natl. Mus. Nat. Sci. Ser. A*, 43, 129-143.

Shtein, G. A. (1957). Materials on the parasite fauna of aquatic arthropods from some Karelian lakes. II. Digenetic trematodes (Trematoda), metacercariae. *Uch. Zap. Petrozavodsk. Gos. Univ.*, 8, 120-139. [In Russian]

Slusarski, W. (1958). Formy ostateczne Digenea z ryb lososiowatych (Salmonidae) dorzecza Wisly I Poludniowegu Baltyky. *Acta Parasitologica Polonica*, 6(22), 247-528. [In Polish]

Strelkov, Yu. A. (1971). Digenean flukes of fishes from Amur River basin. In *Parasitological collection of Zoological Institute AS USSR* (Vol. 25, pp. 120-139). Nauka, Leningrad. (**In Russian**)

Tkach, V. V., & Curran, S. S. (2015). *Prosthenystera oonastica* n. sp. (Digenea: Callodistomidae) from ictalurid catfishes in southeastern United States and molecular evidence differentiating species in the genus across Americas. *Systematic Parasitology*, 90(1), 39–51. https://doi.org/10.1007/s11230-014-9531-2

Tkach, V. V., Curran, S. S., Bell, J. A., & Overstreet, R. M. (2013). A new species of *Crepidostomum* (Digenea: Allocreadiidae) from *Hiodon tergisus* in Mississippi and molecular comparison with three congeners. *Journal of Parasitology*, 99, 1114–1121. https://doi.org/10.1645/13-279.1

Tkach, V. V., Littlewood, D. T. J., Olson, P. D., Kinsella, M., & Swiderski, Z. (2003). Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology*, 56(1), 1-15. https://doi.org/10.1023/A:1025546001611

Truett, G. E. (2006). Preparation of genomic DNA from animal tissues. In J. Kieleczawa (Ed.), *The DNA book: protocols and procedures for the modern molecular biology laboratory* (pp. 33–46). Jones and Bartlett Publisher, Sudbury.

Urabe, M., Hashim, N. E. N., Uni, S., Iwaki, T., Halim, M. R. A., Marzuki, M. E., Udin, A. S. M., Zainuri, N. A., Omar, H.,

Agatsuma, T., Uga, S., Takaoka, H., Azirun, M. S., Ramli, R. (2020). Description and molecular characteristics of *Morishitium polonicum malayense* Urabe, Nor Hashim & Uni, n. subsp. (Trematoda: Cyclocoelidae) from the Asian glossy starling, *Aplonis panayensis strigata* (Passeriformes: Sturnidae) in Peninsular Malaysia. *Parasitology International*, 76, 102074. https://doi.org/10.1016/j.parint.2020.102074

Vainutis K. S. (2020). *Allocreadium khankaiensis* sp. nov. and *Allocreadium hemibarbi* Roitman, 1963 (Trematoda: Allocreadiidae) from the Russian Far East: Morphological, molecular, and phylogenetic studies. *Parasitology International*, 76: 102102. https://doi.org/10.1016/j.parint.2020.102102

Vainutis, K. S., Voronova, A. N., & Urabe, M. (2021). Systematics of *Crepidostomum* species from the Russian Far East and northern Japan, with description of a new species and validation of the genus *Stephanophiala* // *Parasitology International*, 84, 102412. https://doi.org/10.1016/j.parint.2021.102412

Vdovin, A. N., Zuenko, Yu. I., Solomatov, S. F., Bolshakov, S. G. (2015). Dynamics of Far-Eastern dace *Tribolodon brandtii* in the Amur bay (Peter the Great bay, Japan sea). Problems of fisheries, 16(1), 107-117. [In Russian]

Wishniewski, L. W. (1958). The development of cycle of *Bunodera luciopercae* (O. F. Muller). *Acta Parasitol. Polon.* 6(11), 290-305.

Žďárská, Z., & Nebesářová, J. (2003). Transmission electron microscopy of intra-tegumental sensory receptors in the forebody of *Crepidostomum metoecus* (Digenea: Allocreadiidae). *Folia Parasitologica*, 50, 215–219.

Žďárská, Z., & Nebesářová, J. (2004). Transmission electron microscopy of presumed sensory receptors in the forebody papillae of *Crepidostomum metoecus* (Digenea: Allocreadiidae). *Folia Parasitologica*, 51(1), 27-32. https://doi.org/10.14411/FP.2004.004

Zimmerman, C. E., Rand, P. S., Fukushima, M., & Zolotukhin, S. F. (2012). Migration of Sakhalin taimen (*Parahucho perryi*): evidence of freshwater resident life history types. *Environmental Biology of Fishes*, 93, 223–232. https://doi.org/10.1007/s10641-011-9908-x