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Description of a new trematode species of *Parasymphylodora* and molecular evidences for asymphylodorine differentiation

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ABSTRACT: Trematodes with diagnostic criteria inherent to Asymphylodorinae were found in the intestine of *Carassius gibelio* from the south of the Russian Far East. Results of 28S rDNA-based phylogenetic analysis showed that the worms belong to *Parasymphylodora* and are closely related to *P. lacustris*. The genetic distance value of 4.52±0.71% between these trematodes corresponds to the interspecific differentiation level for Lissorchiidae. Our discovered trematodes differ from both *P. lacustris* and other *Parasymphylodora* species based on a number of morphometric criteria. Based on these results, they were accepted as a new species of the genus *Parasymphylodora*, named *P. jugiformis* sp.n. The results of the phylogenetic analysis based on 28S rDNA sequence data confirmed the presence of several clades of the generic level within Asymphylodorinae. The molecular characters for the diagnosis of these clades as hypothetical genera are proposed.

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KEY WORDS: trematode, intestine parasites, *Carassius gibelio*, 28S rDNA, taxonomy, nucleotide profiles

Описание нового вида трематод *Parasymphylodora* и молекулярные основания для дифференциации Asymphylodorinae

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РЕЗЮМЕ: На Дальнем Востоке обнаружен и описан новый вид трематод из подсемейства Asymphylodorinae: *Parasymphylodora jugiformis* sp.n. Объект является кишечным паразитом карася *Carassius gibelio*. По результатам филогенетического анализа на основе фрагмента гена 28S рРНК, близкородственным ему оказался ранее описанный вид — *P. lacustris.* Значение генетических дистанций между видами составило 4,52±0,71%, что соответствует уровню межвидовой дифференциации в семействе Lissorchiidae. В ходе сравнительного анализа найдены морфологические признаки, отличающие обнаруженных трематод от других известных представителей

Parasymphylodora, что дает основание ввести в систематику новую таксономическую единицу. Кроме того, в работе сделан акцент на отсутствии четких морфологических различий между представителями *Parasymphylodora* s.lato и *Asymphylodora* s.lato, в связи с чем для дифференциации родов внутри Asymphylodorinae предлагается использовать молекулярные признаки. В данном случае это нуклеотидные фиксированные замены, которые могут характеризоваться в качестве диагностических критериев.

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КЛЮЧЕВЫЕ СЛОВА: трематоды, кишечные паразиты, 28S рДНК, *Carassius gibelio*, систематика, нуклеотидные профили .

Introduction

Trematodes of the family Lissorchiidae Magath, 1917, are cosmopolitans, intestinal parasites of mainly Cypriniformes Bleeker, 1859 fish species. There are two subfamilies within Lissorchiidae: Lissorchiinae Magath, 1917, and Asymphylodorinae Szidat, 1943. Bray (2008) provided morphological diagnoses for four genera of the Asymphylodorinae, including *Asymphylodora* Looss, 1899, *Brahamputotrema* Gupta, 1955, *Prosovitellina* Wang, 1985, and *Wangxiyunia* Bray, 2008. In the latest large-scale revision of the Lissorchiidae trematodes, Bray has synonymized all species belonging to the genus *Parasymphylodora* Szidat, 1943 in *Asymphylodora*. Previously, *Parasymphylodora* was established by Szidat (1943), but the diagnosis for this genus was developed by Kulakova (1972), where the type species was *P. macrostoma* Ozaki, 1925. According to this diagnosis, the representatives of *Asymphylodora* and *Parasymphylodora* differ mainly on the morphology of the excretory vesicle (sac-shaped *vs*. I-shaped). In recent studies, based on molecular data, the genus *Parasymphylodora*, was reinstated within Asymphylodorinae, with molecular characterization of two species, *Parasymphylodora parasquamosa* Kulakova, 1972, and *P. markewitshchi* (Kulakowskaja, 1947), from Lithuanian cyprinid fishes (Petkevičiūtė *et al*., 2022). Later, three new species in *Parasymphylodora* and one new species from the genus *Asymphylodora* were described based on morphological and molecular data (Atopkin *et al*., 2023). Furthermore, Atopkin with coauthors (2023) showed that the shape of the excretory vesicle is not effective in providing differentiation for these genera. According to their results, the morphological characteristics of *Asymphylodora* s. lato and *Parasymphylodora* s. lato agree with the diagnosis for *Asymphylodora*, which corresponds to Bray (2008), and among these trematodes, several highly supported groups of generic level were revealed with a molecular approach.

In the course of studying the fauna of the trematodes of *Carassius gibelio* (Bloch, 1782) in the East Asia region, adult intestinal trematodes were found. Morphologically, these worms corresponded to representatives of the Lissorchiidae. In order to clarify the taxonomic status of these worms, molecular and morphological data were generated for the found specimens.

Material and methods

Adult worms Lissorchiidae were collected from *C. gibelio* caught in the basins of the Ilistaya and Komissarovka Rivers in the south of the Russian Far East (Fig. 1, Table 1). Overall, 15 individuals of *C. gibelio* were investigated. Fish species were identified according to Antonov *et al*. (2019). Of these, 7 individuals were infected with lissorchiid trematodes; the intensity of invasion ranged from 2 to 19 worms. Nine adult trematode specimens that differ morphologically from the lissorchiid species of *Asymphylodora* and *Parasymphylodora*, known for this region, were used for morphological and molecular analyses. The worms are fixed separately in 70% ethanol. Following this fixation, flukes were transferred to 96% ethanol. Five specimens were used for morphological analysis. The samples were soaked in distilled water, stained with alum carmine, dehydrated in an ethanol series, cleared in clove oil, and mounted in a Canada balsam under a coverslip on a slide. All sizes are given in micrometers.

Four samples were used for molecular analysis (Table 1). Total DNA was extracted using DNeasy Blood, Tissue Kits (Qiagen, Hilden, Germany) as

Fig. 1. Map of material collection localities: 1 — Ilistaya River (45°57′34″N, 133°56′07″E); 2 — Komissarovka River (44°59′20″ N, 13°14′65″ E).
Рис. 1. Карта точек сбора материала: 1 — река Илистая (45°57′34″N, 133°56′07″E); 2 — река Комиссаровка (44°59′20″ N, 13°14′65″ E).

consistent with the manufacturer's protocol. Polymerase chain reaction (PCR) amplifications (10 µl) were performed using GoTaq® Green Master Mix. 28S ribosomal DNA (rDNA) was amplified with the primers 28S–A (5′–TCG ATT CGA GCG TGA WTA CCC GC–3′) (Matejusova, Cunningham, 2004) and 1500R (5′–GCT ATC CTG AGG GAA ACT TCG–3′) (Tkach *et al.*, 2003) with an annealing temperature of 55 °C. Negative and positive controls using both primer pairs were included. Polymerase chain reaction (PCR) products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, USA), following the manufacturer's recommendations, with the internal sequencing primers for 28S rDNA. There were used the next internal primers: 300F (5′–CAA GTA CCG TGA GGG AAA GTT G–3′), ECD2 (5′–CTT GGT CCG TGT TTC AAG ACG GG–3′), 1200R (5'–GGG CAT CAC AGA CCT G–3') and 900F (5′–CCG TCT TGA AAC ACG GAC CAA G–3′)

Table 1. List of the taxa incorporated into molecular analysis.
Таблица 1. Список видов, включенных в молекулярный анализ.

Species	n	Host	Location	Author	GenBank number
Lissorchiidae					
Asymphylodorinae					
Parasymphylodora					
Parasymphylodora khankensis	1	*Boreolona* spp.	Komissarovka River Basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914409
Parasymphylodora ussuriensis	1	*Parafossarulus* spp.	Razdolnaya River Basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914394
Parasymphylodora sp. 1	1	*C. gibelio*	Bolshaya Ussurka River basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914415
Parasymphylodora parasquamosa	1	*Bithynia tentaculata*	Ilmėdas Lake, Lithuania	Petkevičiūtė *et al.*, 2022	OP10645
Parasymphylodora markewitschi	1	*S. erythrophthalmus*	Curonian Lagoon, Lithuania	Petkevičiūtė *et al.*, 2022	OP106444
Parasymphylodora markewitschii	1	*B. tentaculata*	Stirniai Lake, Lithuania	Petkevičiūtė *et al.*, 2022	OP106447
Parasymphylodora sp. 2	1	*Leuciscus idus*	Rybinsk Reservoir, Russia	Atopkin *et al.*, 2023	OQ914422
Parasymphylodora lacustris	1	*C. gibelio*	Bolshaya Ussurka River basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914419
***Parasymphylodora jugiformis* sp.n.**	**3**	*C. gibelio*	**Ilistaya River Basin, Primorsky Region, Russia**	**This study**	**PP342581 – PP342583**
***Parasymphylodora jugiformis* sp.n.**	**1**	*C. gibelio*	**Komissarovka River Basin, Primorsky Region, Russia**	**This study**	**PP342584**
Asymphylodora					
Asymphylodora progenetica	1	*Bithynia tentaculata*	Jeruzalė pond, Vilnius, Lithuania	Petkevičiūtė *et al.*, 2020	MT103403
Asymphylodora perccotti	1	*Perccottus glenii*	Bolshaya Ussurka River basin, Primorsky Region, Russia	Besprozvannykh *et al.*, 2012	FR822715
Asymphylodora khasanensis	1	*Stenothyra recognite*	Karasik River Basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914420

Table 1 (continued).
Таблица 1 (продолжение).

Species	n	Host	Location	Author	GenBank number
Lissorchiidae					
Asymphylodorinae					
Asymphylodora s.str.					
Asymphylodora tincae	1	*Anisus vortex*	Verkiai pond, Vilnius, Lithuania	Petkevičiūtė *et al.*, 2022	OP106446
Asymphylodora tincae	1	*Tinca tinca*	Ilmėdas Lake, Lithuania	Petkevičiūtė *et al.*, 2022	OP106440
Asymphylodora sp.	1	*Lithoglyphus naticoides*	Danube River, Hungary	Petkevičiūtė *et al.*, 2020	MT153916
Lissorchiinae					
Lissorchis					
Lissorchis kritskyi	1	*Minytrema melanops*	USA	Curran *et al.*, 2006;	EF032689
L. kritskyi	1	*Carpiodes cyprinus*	USA	Olson *et al.*, 2003	AY222250
L. kritskyi	1	*Carpiodes velifer*	USA	Truong *et al.*, 2021	MT928329
Lissorchis cf. nelsoni	1	*Minytrema melanops*	USA	Truong *et al.*, 2021	MT928354
Asymphylotrema macrocetabulum	1	*Misgurnus anguilicaudatus*	Komissarovka River Basin, Primorsky Region, Russia	Atopkin *et al.*, 2023	OQ914425
Palaeorchis					
Palaeorchis incognitus	1	*Lithoglyphus naticoides*	Elektrėnai water reservoir, Lithuania	Petkevičiūtė *et al.*, 2020	MT103407
P. incognitus	1	*Rutilus rutilus*	Kaunas water reservoir, Lithuania	Petkevičiūtė *et al.*, 2020	MT103408
P. incognitus	1	*L. naticoides*	Kaunas water reservoir, Lithuania	Petkevičiūtė *et al.*, 2020	MT103409
P. incognitus	1	*L. naticoides*	Balaton Lake, Hungary	Petkevičiūtė *et al.*, 2020	MT103410
Posthovitellinum					
Posthovitellinum psilotermim	1	*Cyclocheilos enoplos*	Viet Nam	Truong, *et al.*, 2020	MT928352
Asaccotrema					
Asaccotrema vietnamiense	1	*Rasbora paviana*	Viet Nam	Sokolov *et al.*, 2019	MK863409
Anarchiotrema					
Anarhicotrema ochotense	1	*Anarhichas orientalis*	Sea of Okhotsk, Russia	Sokolov *et al.*, 2022	OM108707
Monorchidae (outgroup)					
Monorchis monorchis	1	*Diplodus vulgaris*	Near Corsica	Tkach *et al.*, 2001	AF184257

(Lockyer *et al.*, 2003; Tkach *et al.,* 2003). PCR product sequences we analyzed using an ABI 3130 genetic analyser at the Federal Scientific Center of East Asia Terrestrial Biodiversity FEB RAS. Sequences were submitted to the GenBank database (will be available after acceptance). Ribosomal DNA sequences were assembled with SeqScapev.2.6 software, provided by Applied Biosystems. Alignment, estimations of the number of variable sites and p-distance calculation were performed with MEGA 7.1 software (Kumar *et al*., 2016). Phylogenetic analysis was performed with Maximum likelihood (ML) and Bayesian Inference (BI) algorithms based on partial sequences of the nuclear large ribosomal subunit rRNA (28S) gene using the newly generated sequences and matching sequences of the all representatives of the family Lissorchiidae available in GenBank (Table 1). The best-fit model of sequence evolution for phylogenetic analysis was GTR+G (Posada, 2003) for both algorithms, estimated with jModeltest version 2.1.5 software (Darriba *et al*., 2012). The ML algorithm was realized in the PhyML 3.1 (Guindon, Gascuel, 2003) software and the BI algorithm in the MrBayes v.3.2.6 software (Ronquist *et al*., 2012). The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al*., 2001). Estimation of ML phylogenetic relationships significance was performed with the approximate likelihood ratio test using Bayesian support (Anisimova, Gascuel, 2006) with 100 repetitions. The Bayesian algorithm was performed throughout 1 000 000 generations during 2 independent runs with estimation of every 1000 generation, final split frequency was not exceeding 0.003. Accession numbers, authority and supporting

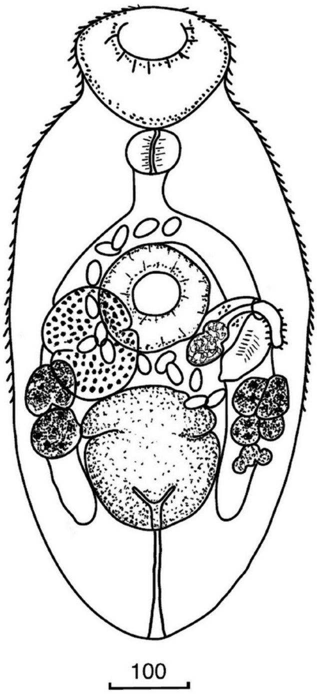


Fig. 2. Holotype of *Parasymphylodora jugiformis* sp.n. ex *Carassius gibelio* from the Ilistaya estuaries, Primorsky Region, Russia.
Рис. 2. Голотип *Parasymphylodora jugiformis* sp.n. от *Carassius gibelio* из эстуария реки Илистая, Приморский край, Россия.

information about rDNA sequences from Gene Bank used for the phylogenetic analyses are provided in Table 1 (Tkach *et al*., 2001; Olson *et al*., 2003; Curran *et al*., 2006; Besprozvannykh *et al*., 2012; Sokolov, Gordeev, 2019; Petkevičiūtė *et al*., 2020; Truong *et al*., 2021; Petkevičiūtė *et al*., 2022). *Monorchis monorchis* (Stossich, 1890), from the family Monorchiidae Odhner, 1911 was used as an outgroup.

Results

Lissorchiidae Magath, 1917
Asymphylodorinae Szidat, 1943
Parasymphylodora jugiformis **sp.n.**
Fig. 2.

TYPE HOST. *Carassius gibelio* (Bloch, 1782).

TYPE LOCALITY. Oxbow of Ilistaya River of south of Russian Far East, 2022.

MATERIAL EXAMINED. Holotype No 238-Tr, paratypes No 239–242-Tr. The samples are stored in the Bioresource Collection of the Federal Scientific Centre of East Asia Terrestrial Biodiversity of the Far East Branch of the Russian Academy of Sciences (reg. number 2797657). E-mail: petrova@dl.ibss.dvo.ru

ETYMOLOGY. The species' name refers based on jug–shaped of body worm.

DESCRIPTION. Adult worm (material examined: 5 specimens) (Fig. 2; Table 2).

Body elongated, jug-shaped. Tegument with small, fine spines. Oral sucker subterminal, transversaleoval. Ventral sucker round, less than oral sucker in anterior part of middle third of body. Prepharynx short. Pharynx round or transversally oval. Oesophagus equal to or slightly shorter than length of pharynx. Oesophageal bifurcation usually anterior to ventral sucker. Intestines extend to level of posterior border of testis. Testis single, elongated oval, from two lobes, usually in middle of hindbody. Cirrus sac saccular, small; sinistrally at level to ventral sucker. Seminal vesicle bipartite. Prostatic cells few. Ejaculatory duct with spines. Genital pore sinistrally lateral, at level middle of ventral sucker. Ovary elongated oval, two-lobed, dextrally of midline, partially covered of ventral sucker, posterior end adjacent to testis, anterior end reaches at level middle of ventral sucker. Laurer's canal and seminal receptacle present. Uterus extending from level of anterior end testis to level of intestinal bifurcating. Metraterm short, elliptical with spines. Eggs oval, yellow, operculated, embryonated. Vitelline follicles large two or four on either side of body. Vitelline fields from level posterior end of ventral sucker to level middle or posterior of half-testes. Excretory vesicle I-shaped, extending to middle of testis.

MOLECULAR DATA. The nucleotide sequences of 28S rRNA gene fragment 1116 bp in length were generated for four specimens of *Parasymphylodora jugiformis* sp.n. All four sequences were identical. GenBank accession numbers are PP342581–PP342584.

REMARKS. Obtained data of 28S rDNA has confirmed the validity of *Parasymphylodora* s.lato and *Asymphylodora* s.lato, as well as the subfamily Asymphylodorinae, which appears as highly supported three clades on both ML and BI phylogenetic reconstructions (Fig. 3). Within this subfamily, groups are segregating: *Parasymphylodora* s.lato (Clade I), two Vietnamese lissorchiid genera (Clade II) and *Asymphylodora* s.lato (Clade III). The level of interspecific differentiation is 1.65±0.38% – 10.42±0.99% within Clade I. The trematodes identified in our study belong to the group *Parasymphylodora* s.lato (Clade I) and are closely related to *Parasymphylodora lacustris* (Atopkin *et al*., 2023) (Fig. 3). The genetic distance between them was 4.52±0.71%, which exceeds the minimum interspecific differentiation value of 1.59 ± 0.41% for Asymphylodorinae, reported in Atopkin *et al*. (2023). At that time, the new species of worm

Table 2. Measurements (micrometer) and other indicators of the two species of adult *Parasymphylodora* worms.
Таблица 2. Измерения (в микрометрах) и другие показатели двух видов взрослых червей *Parasymphylodora*.

Features	*Parasymphylodora jugiformis* sp.n.			*Parasymphylodora lacunae* Atopkin, Ivashko, Besprozvannykh et Zhokhov, 2023 (Atopkin *et al*., 2023)
	Holotype	Range (n=5)	Mean	
Body length	800	616–800	697	708–1,109
Body width	385	246–385	300	277–354
Oral sucker length	162	116–162	137	135–154
Oral sucker width	200	142–200	168	135–166
Forebody	304	216–304	244	250–347
Ventral sucker length	139	116–139	122	135–181
Ventral sucker width	142	116–142	129	154–193
Prepharynx length	0	0–31	13	0–12
Pharynx length	58	46–62	54	50–77
Pharynx width	69	58–77	68	69–85
Oesophagus length	54	19–54	35	65–104
Ovary length	154	108–162	143	77–108
Ovary width	123	85–123	106	100–142
Testis length	185	162–185	175	162–274
Testis width	177	108–177	134	119–188
Cirrus sac length	119	85–119	107	131–193
Cirrus sac width	58	46–58	50	50–77
Metraterm length	96	81–115	97	58–81
Metraterm width	62	39–62	46	31–50
From posterior end of body to testis	131	62–131	97	85–181
Eggs length	31–39	31–39	–	35–39
Eggs width	15–19	15–19	–	19–23
Length/width %	48.1	38–48.1	43	27.3–39.1
Forebody/body length ratio %	38	31.9–42.5	35	26.2–35.3
Suckers length ratio	0.86	0.82–1.0	0.89	1.0–1.18
Suckers width ratio	0.71	0.71–0.82	0.77	1.1–1.33
Vitilarium follicles number	–	4–8	–	6–8

differs from the other species of the genus in the range 6.47±0.8% – 8.82±0.97%. Regarding morphological features, adult worms detected in the intestines of *C. gibelio* correspond to the diagnosis of the genus *Asymphylodora* by Bray (2008). The significant feature distinguishing these trematodes from other species of *Parasymphylodora* s.lato and *Asymphylodora* s.lato is proportion of size of oral and ventral suckers: oral sucker well over ventral sucker *vs* oral sucker equal or less ventral sucker (Kulakowskaja, 1947; Lambert, 1976; Kulakova, 1972, 1982; Našincová, Scholz, 1994; Besprozvannykh *et al*., 2012; Atopkin *et al*., 2023). The morphological and metric data also indicate that the trematodes in our material differ from those of a genetically close species, *P. lacustris*. Namely, they differ from *P. lacustris* in the smaller size of the ventral sucker and cirrus sac, smaller values of the proportions of length and width of suckers, and the length and width of the body (Table 2). In addition to the differences above: ovary elongated oval *vs* transverse oval, anterior end ovary at the level of middle ventral sucker *vs* ovary posterior to ventral sucker; cirrus sac and metraterm left to ventral sucker *vs* cirrus sac and metraterm mostly covered ventral sucker, accordingly. Thus, based on the results of morphological and molecular analyses, we conclude that the trematodes ex *C. gibelio* belong to the new species of the genus *Parasymphylodora*, *P. jugiformis* sp.n.

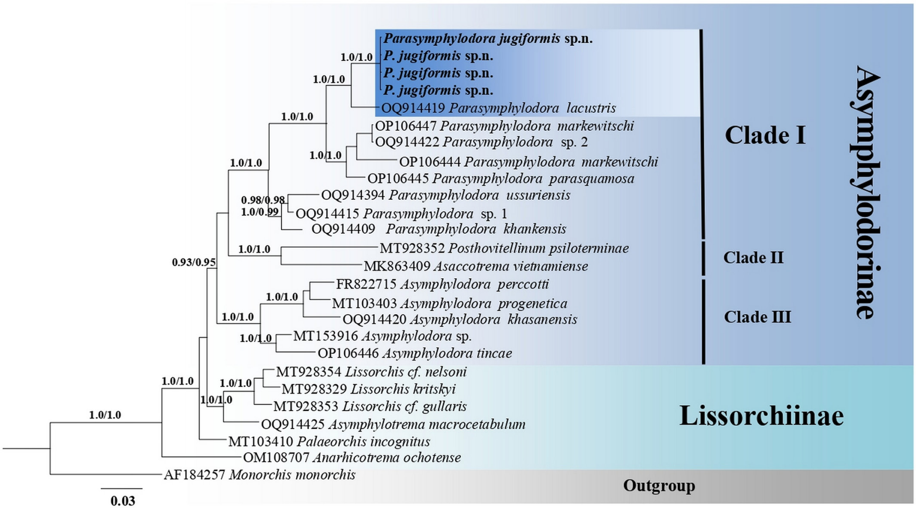


Fig. 3. Reconstruction of phylogenetic relationships of the family Lissorchiidae obtained with Bayesian and Maximum likelihood algorithms based on partial 28S rRNA gene (alignment length 1116 bp). Nodal numbers — posterior probabilities for BI and ML that indicate statistical support of the phylogenetic relationships, only significant values (0.9–1.0) are showed.

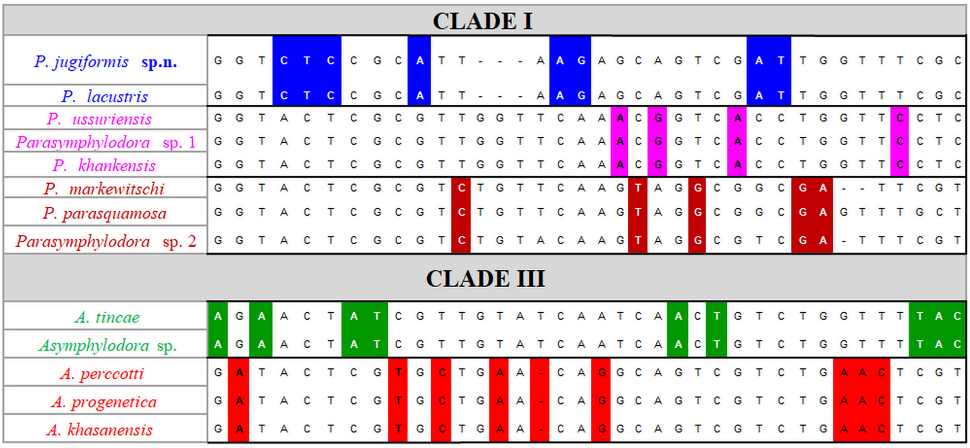
Рис. 3. Реконструкция филогенетических отношений в семействе Lissorchiidae, полученная с использованием алгоритмов Байеса и максимального правдоподобия, по данным участка гена 28S рРНК (длина выравнивания 1116 п.н.). Указанные цифры над ветвями — апостериорные вероятности, которые обозначают статистическую поддержку филогенетических отношений. Показаны значения в пределах 0.9–1.0.

Discussion

The results of the phylogenetic analysis of Lissorchiidae are consistent with the findings of previous studies on this family (Petkevičiūtė *et al*., 2022; Atopkin *et al*., 2023), confirming that Asymphylodorinae is a monophyletic and Lissorchiinae is a paraphyletic (Fig. 3). The *p*-distance values within and between clades, representing *Parasymphylodora* s.lato (Clade I) and *Asymphylodora* s.lato (Clade III), agree with the intergeneric divergence level of Asymphylodorinae, reported in Atopkin *et al*. (2023). Thus, the level of differentiation is 5.84±0.78% to 10.42±0.99% within Clade I and 6.44±0.80% to 8.15±0.89% within Clade III. It shows intergeneric relations within clades. This is supported by the value of the pairwise comparisons of the distances between the three clades, which are in the range of 8.36±0.62% to 16.00±1.17%.

At the same time, there are no morphologically significant differences between the species of Clade I and Clade III suitable for intergeneric differentiation (Atopkin *et al*., 2023). For this reason, we believe that the molecular characteristics revealed for can be used as diagnostic indicators of genera within this subfamily. Namely, nucleotide profiles of sequences of 28S rRNA gene are showing fixed substitutions between species of trematodes *Parasymphylodora* s.lato and *Asymphylodora* s.lato that can be used as diagnostic criteria both from generic level and higher ranks of taxa (Fig. 4). These characteristics look most efficient for generic differentiation of trematodes within Asymphylodorinae, but we do not exclude the possibility that these profiles will undergo some corrections after new molecular data on Asymphylodorinae becomes available.

The practice of using molecular characteristics for the delimitation of morphologically similar taxa was applied at the species level for the representatives of the genus *Gyrocotyle* Design, 1850 (Cestoda) from chimaeriform holocephalans (Bray *et al*., 2020). These authors provide unique nucleotide characters of 18S rDNA and 28S rDNA as molecular diagnoses for two new *Gyrocotyle* species, with remarks that “there are no reliable characters to differentiate this species or indeed any of the gyrocotyloidean species circumstanced by molecular means.” Ad-



CLADE I																																					
P. jugiformis **sp.n.**	G	G	T	C	T	C	C	G	C	A	T	T	-	-	-	A	A	G	A	G	C	A	G	T	C	G	A	T	T	G	G	T	T	T	C	G	C
P. lacustris	G	G	T	C	T	C	C	G	C	A	T	T	-	-	-	A	A	G	A	G	C	A	G	T	C	G	A	T	T	G	G	T	T	T	C	G	C
P. ussuriensis	G	G	T	A	C	T	C	G	C	G	T	T	G	G	T	T	C	A	A	A	C	G	G	T	C	A	C	C	T	G	G	T	T	C	C	T	C
Parasymphylodora sp. 1	G	G	T	A	C	T	C	G	C	G	T	T	G	G	T	T	C	A	A	A	C	G	G	T	C	A	C	C	T	G	G	T	T	C	C	T	C
P. khankensis	G	G	T	A	C	T	C	G	C	G	T	T	G	G	T	T	C	A	A	A	C	G	G	T	C	A	C	C	T	G	G	T	T	C	C	T	C
P. markewitschi	G	G	T	A	C	T	C	G	C	G	T	C	T	G	T	T	C	A	A	G	T	A	G	G	C	G	G	C	G	A	-	-	T	T	C	G	T
P. parasquamosa	G	G	T	A	C	T	C	G	C	G	T	C	T	G	T	T	C	A	A	G	T	A	G	G	C	G	G	C	G	A	G	T	T	T	G	C	T
Parasymphylodora sp. 2	G	G	T	A	C	T	C	G	C	G	T	C	T	G	T	A	C	A	A	G	T	A	G	G	C	G	T	C	G	A	-	T	T	T	C	G	T
CLADE III																																					
A. tincae	A	G	A	A	C	T	A	T	C	G	T	T	G	T	A	T	C	A	A	T	C	A	A	C	T	G	T	C	T	G	G	T	T	T	T	A	C
Asymphylodora sp.	A	G	A	A	C	T	A	T	C	G	T	T	G	T	A	T	C	A	A	T	C	A	A	C	T	G	T	C	T	G	G	T	T	T	T	A	C
A. perccotti	G	A	T	A	C	T	C	G	T	G	C	T	G	A	A	-	C	A	G	G	C	A	G	T	C	G	T	C	T	G	A	A	C	T	C	G	T
A. progenetica	G	A	T	A	C	T	C	G	T	G	C	T	G	A	A	-	C	A	G	G	C	A	G	T	C	G	T	C	T	G	A	A	C	T	C	G	T
A. khasanensis	G	A	T	A	C	T	C	G	T	G	C	T	G	A	A	-	C	A	G	G	C	A	G	T	C	G	T	C	T	G	A	A	C	T	C	G	T

Fig. 4. Nucleotide profiles of sequences of 28S rRNA gene showing differences among species that can be used as diagnostic criteria for the genetic level of subfamily Asymphylodorinae. Fixed substitutions of the sequences fragment: 37 bp from 1116 bp.
Рис. 4. Нуклеотидные профили последовательностей гена 28S рРНК демонстрируют различия между видами, которые могут быть использованы в качестве диагностических критериев на генетическом уровне для подсемейства Asymphylodorinae. Фиксированные замены на выбранном участке последовательностей: 37 п.н. из 1116 п.н.

ditionally, the 18S rDNA sequence dataset was used for differential diagnoses for the Myxozoa species (Molnár *et al*., 2010). It should be noted that the differentiation of taxa based on molecular data is not in conflict with the International Code of Zoological Nomenclature (ICZN). The statement does not impose any restrictions on the types of characters that can be utilized for taxon delimitation (Article 13, Point 13.1.1) (Ride *et al*., 1999). Alongside this, there are data indicating the absence of a correlation between morphological and molecular characters in certain groups of Digenea (Izrailskaia *et al*., 2019; Gonchar *et al*., 2019; Atopkin *et al*., 2020, 2023; Izrailskaia *et al*., 2024). However, for the present, we leave unchanged the taxonomy of the Asymphylodorinae until the receipt of new morphological and molecular data simultaneously for other members of the subfamily.

Conclusion

The results of morphometric and molecular investigations have confirmed the classification of a new species of trematodes, *Parasymphylodora jugiformis* sp.n., found in the *Carassius gibelio* host within the East Asian region. This discovery enhances our understanding of lissorchiid species diversity. Moreover, the fixed nucleotide substitutions between *Parasymphylodora* s. lato and *Asymphylodora* s. lato can be used as diagnostic criteria for identification at the generic level and higher taxa with morphological similarities within the subfamily Asymphylodorinae.

Compliance with ethical standards

CONFLICTS OF INTEREST: No potential conflict of interest was reported by the authors.

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