

Sindiplozoon coreius n. sp. (Monogenea: Diplozoidae) from the gills of *Coreius guichenoti* (Cyprinidae) in China

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ABSTRACT

Sindiplozoon coreius n. sp. is described from the gills of *Coreius guichenoti* in Sichuan province, China. There is a smooth tegument and a cup-like widened area in the posterior part of the worm body, which are particular features of the genus *Sindiplozoon*. There are no branched intestinal caeca before the widened area, but some branches reach the fourth clamp in the hind body; there was no cross striation on the anterior arch of the anterior clamp jaw and medial part of the posterior jaw, which are distinguished from the other species in *Sindiplozoon*. In addition, *S. coreius* n. sp. shared the highest ITS2 sequence identity (96.0%) with *S. ctenopharyngodoni*. The established phylogenetic tree showed that the two species of *Sindiplozoon* formed a sister group. The k2p genetic distance between the new species and other diplozoids was higher than 3.4%, which suggested interspecific differentiation.

1. Introduction

Monogeneans of the Diplozoidae Palombi, 1949 are blood-feeding ectoparasite on the gills of freshwater cyprinid fishes in Europe, Asia, and Africa [1]. Two juvenile individuals fuse to form the typical X-shaped adult worm in a permanent copula. The Diplozoidae is represented by two subfamilies: Diplozoinae Palombi, 1949 and Neodiplozoinae Khotenovsky, 1980. Five genera are included in the Diplozoinae [1]. Of the recorded species in Diplozoidae in the world, almost half are found in China: 1 in *Diplozoon*, 25 in *Paradiplozoon*, 2 in *Inustiatus*, 1 in *Eudiplozoon*, and 6 in *Sindiplozoon* [2–7].

The genus *Sindiplozoon* was erected by the “cup-like” widened area in the posterior region and smooth tegument of the worm body [8]. But *S. ctenopharyngodoni* was first described by Ling in 1973 from *Ctenopharyngodon idellus* [9]. Then the other 5 *Sindiplozoon* species were recorded, *S. strelkowi* from *Ctenopharyngodon idella* and *Hemibarbus labeo* in Heilongjiang River, China and the Amur River, Russia [8], *S. fujianensis* from *Leptobotia pellegrini* [5], *S. diplozoon* from *Erythroculter mongolicus* and *Elopichthys bambusa* [8], *S. hunanensis* from

Parabotia fasciata [10], *S. xenocypris* from *Xenocypris argentea* and *X. davidi* [11].

C. guichenoti (Cyprinidae) is endemic fish in the upper reaches of the Yangtze River. Due to environmental pollution and the construction of hydropower stations, the population of *C. guichenoti* declines sharply, and now it becomes a rare protected fish in the Yangtze River [12]. During the artificial breeding, a large amount of *C. guichenoti* was caused death by diplozoids, which would affect the breeding and release of *C. guichenoti*. Attachment of the clamps and central hooks resulted in inflammation, hyperaemia and haemorrhages in the gills and hyperplasia [13].

We collected some specimens of diplozoid from *C. guichenoti* in the Yangtze River. Its morphological characteristics differed from all other *Sindiplozoon* species, and the internal transcribed spacer of the ribosomal gene (ITS2) supported the establishment of a new species. The present study aims to provide both a morphological and molecular characterization of this new *Sindiplozoon* species.

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2. Materials and methods

2.1. Sample collection

The fish *C. guichenoti* infected diplozooids was obtained from the upper reaches of the Yangtze River, Sichuan Province in December 2020. Gills were removed and examined under a dissecting microscope. Some diplozooids were collected for morphological identification, and some worms were preserved in 90% ethanol for molecular analysis.

2.2. Morphological identification

Fresh diplozooid specimens were stained with Magenta Ferric Chloride, decolorized with concentrated hydrochloric acid and alcohol mixture, dehydrated with gradual ethanol series, cleared in xylene, and mounted in Canada balsam. Some haptors of specimens were fixed in a picric acid poly-ethanol mixture to examine sclerites [14]. The body and some external and internal organs were measured on whole-mounted specimens. The terminology of the sclerites of the clamps and central hooks followed that of Shimazu et al. [15]. Measurements are given in micrometers unless otherwise stated. Drawings were made with the aid of Photoshop software. The type specimens were deposited in the Museum of the Institute of Hydrobiology, Chinese Academy of Sciences (collection name code IHB-P).

2.3. Molecular analysis

Genomic DNA of a single ethanol-fixed diplozooid was extracted using TIANamp Genomic DNA Kit according to standard protocols suggested by the manufacturer. Amplification of ITS2 region was done using universal primers D (5'-GGCTYRYGGNGTCGATGAAGAACGCAG-3') and B1 (5'-GCCGGATCCGAATCCTGGTTAGTTTCTTTCC-3') [16]. All the polymerase chain reaction (PCR) were performed using 1.0 µl of DNA under the following cycling parameters: 10 min at 90 °C, 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 75 s at 72 °C; and a final extension of 10 min at 72 °C. PCR products were detected on ethidium bromide-stained 1% agarose gels. Purified DNA fragments were sequenced. The sequences obtained were submitted to the NCBI database for BLAST searches. Phylogenetic analyses (Bayesian inference) were performed with 9 previous sequenced European and Chinese diplozooid species submitted to nucleotide databases (see Table 1) using Phylosuite [17] with optimized model and the monogenean *Neoheterobothrium hirame* was used as outgroup, a tree was visualized in iTOL. Pairwise distances (kimura 2-parameter) between species was constructed in MEGA X.

3. Results

3.1. A new species

Family Diplozooidae Palombi, 1949.

Subfamily Diplozoinae Palombi, 1949.
Genus *Sindiplozoon* Khotenovsky, 1981.
Species *Sindiplozoon coreius* n. sp., 2020.

3.1.1. Morphological description and characterization

3.1.1.1. *Sindiplozoon coreius* n. sp.. Type host: *C. guichenoti* (Sauvage Dabry de Thiersant, 1874) (Cyprinidae).

Type locality: The fish host population is reserved in the upper reaches of the Yangtze River, such as the Jinsha River, the Minjiang, Jialing, and Wujiang rivers.

Site of infection: Gills.

Type material: Type specimens are deposited in the Institution of Hydrobiology, Chinese Academic of Sciences, Wuhan, China.

Etymology: The specific name “*coreius*” means the genus name of the host.

Description: Two adult individuals partially fused in an X-shape, divided into anterior, fusion area and posterior parts (Fig. 1A). Tegument smooth in the whole body. Mean total body length 7.25 (1.80–11.36, $n = 10$) mm.

Anterior part of body dorsoventrally flat, elongate, 4.22 (1.02–6.81, $n = 10$) mm long and 1.04 (0.31–2.03, $n = 10$) mm wide. Crescent-shaped mouth sub-terminal on anteroventral surface. Mouth open into the buccal cavity with two large, muscular buccal suckers, joined proximally. The buccal sucker 117 (79–160, $n = 9$) × 102 (73–130, $n = 9$). Pharynx oval, muscular 101 (66–127, $n = 4$) × 67 (52–75, $n = 4$). Pharynx open into intestine. Intestine median, extending to the fusion area with lateral diverticula on either side of the anterior part of the body. Vitelline follicles numerous, well developed in the anterior part of the body.

Reproductive organs are located in the fusion area. Genital pore open on the fusion area. Ovary single, oval-shaped, in anterior to the fusion area. Testis single, composed of 5 small oval balls, posterior to the ovary. Eggs elliptical with long, curly filament attached to operculum, 289 (253–316, $n = 5$) × 102 (96–122, $n = 5$) (Fig. 1B).

Posterior part of body 2.56 (0.67–4.73, $n = 10$) mm long, no ridges. “Cup-like” widened areas 1.10 (0.35–1.37, $n = 8$) × 0.87 (0.47–1.28, $n = 8$) mm, in both ventral and dorsal sides, between fusion area and opisthaptor, muscular, nearly round to elliptical. Intestine lacks branches in the fusion area, long branches extend from the widened area to the fourth clamp. Opisthaptors 590 (340–960, $n = 10$) × 780 (390–1040, $n = 10$) with four pairs of clamps and one pair of central hooks. Clamp I, smallest, 125 (110–144, $n = 6$) × 68 (61–106, $n = 6$). Clamp II 151 (124–187, $n = 6$) × 76 (68–117, $n = 6$). Clamp III, largest, 158 (141–199, $n = 6$) × 85 (72–126, $n = 6$). Clamp IV 145 (121–168, $n = 6$) × 80 (60–109, $n = 6$) (Fig. 2A). Clamps consist of sclerotized structures: median sclerite u-shaped, anterior end of median plate thickened with a trapezoid outgrowth. Anterior clamp jaw consists of two curved sclerites. The posterior clamp jaw comprised of medial and lateral parts. Anterior arch of anterior clamp jaw and medial part of the

Table 1

List of diplozooid species used for genetic comparison and phylogenetic analysis with *Sindiplozoon coreius* n. sp., including their host species, locality, GenBank ID and sequence length.

Parasite species	Host species	Locality	GenBank ID	Length (bp)
<i>Diplozoon paradoxum</i>	<i>Abramis brama</i>	Kyjovka River, Czech Republic	AJ563372	769
<i>D. paradoxum</i>	<i>A. brama</i>	Scamandre pond, Camargue, France	AF369759	1002
<i>Eudiplozoon nipponicum</i>	<i>Cyprinus carpio</i>	Moroava River, Czech Republic	AJ300710	755
<i>Eudiplozoon nipponicum</i>	<i>C. carpio</i>	Camargue, France	AF369758	977
<i>Inustiatius inustiatius</i>	<i>Hypophthalmichthys molitrix</i>	Tangxun Lake, China	DQ098893	771
<i>Inustiatius aristichthysi</i>	<i>Hypophthalmichthys nobilis</i>	Tangxun Lake, China	DQ098894	771
<i>Paradiplozoon bliccaae</i>	<i>Blicca yoercna</i>	Moroava River, Czech Republic	AJ300712	736
<i>Paradiplozoon homoion</i>	<i>Rutilus rutilus lacustris</i>	Irtys River (Chinese section), China	KP340972	764
<i>Sindiplozoon ctenopharyngodoni</i>	<i>Ctenopharyngodon idella</i>	Tangxun Lake, China	DQ098898	834
<i>Sindiplozoon coreius</i> n. sp.	<i>Coreius guichenoti</i>	Sichuan, China	MW992745*	722

* New sequence obtained in the present study.

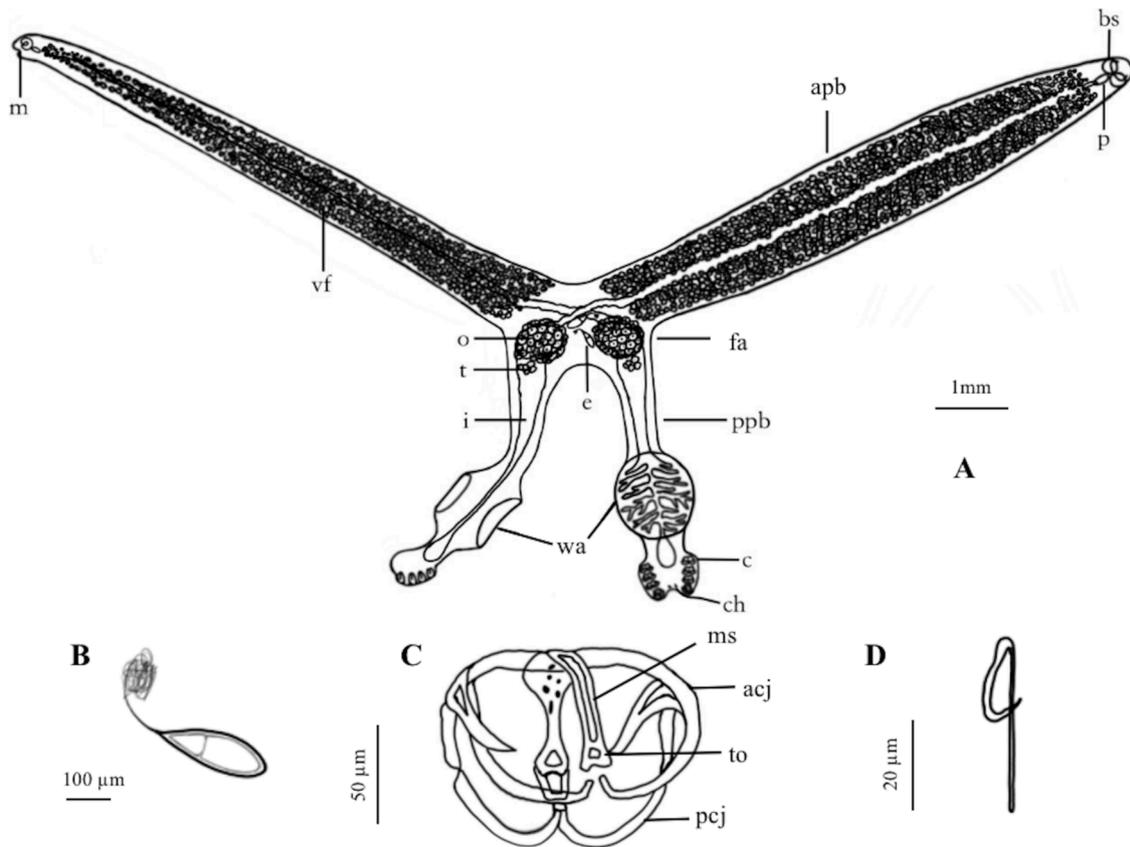


Fig. 1. *Sindiplozoon coreius* n. sp. (A) adult pair, the left half is a lateral view, and the right half is a ventral view. Apb: anterior part of body; bs: buccal suckers; c: clamps; ch: central hooks; e: eggs; fa: fusion area; i: intestine; m: mouth; o: ovary; p: pharynx; ppb: posterior part of body; t: testis; vf: vitelline follicles; wa: widened area. (B) egg, with long, curly filament. (C) clamp. Acj: anterior clamp jaw; ms: median sclerite; pcj: posterior clamp jaw; to: trapezoid outgrowth. (D) central hooks.

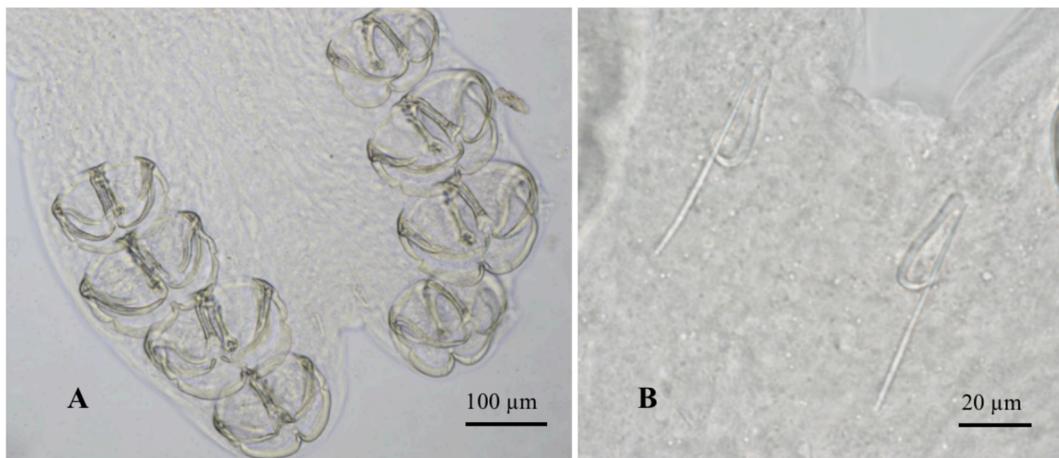


Fig. 2. Opisthaptors of *Sindiplozoon coreius* n. sp. with four pairs of clamps (A) and a pair of central hooks (B).

posterior jaw lacks cross striation (Fig. 1C). Central hooks are located between the terminal protrusion of the haptor and the first pair of clamps, formed by a handle and a sickle through a connection. The sickle bends in the direction of handle, and has wing in the end which bend in the direction of the connection. Central hook sickle 29 (28–30, $n = 3$), handle 36 (34–38, $n = 3$) (Fig. 1D, Fig. 2B).

3.2. Molecular analysis

The ITS sequences obtained from three specimens were identical with 1656 bp long and deposited in the GenBank database with

accession number MW992745. The total length of the full ITS2 rDNA sequence was 722 bp.

S. coreius n. sp. had the highest sequence similarity (96.0%) with *S. ctenopharyngodoni*, and 85.0% with *Paradiplozoon sapae*. The genetic distances (Kimura 2-parameter) between *S. coreius* n. sp. and other diplozooids were higher than 3.4% (Table 2).

According to the BI analysis method (Fig. 3), the new species merged with *S. ctenopharyngodoni* (DQ098898) into a clade, and then formed a sister group with *Paradiplozoon* species in Europe and *Diplozoon* species.

Table 2

Pairwise distance (kimura 2-parameter in %) for diplozoids taxa based on the complete ITS2 sequences available in NCBI.

	1	2	3	4	5	6	7	8	9
1 <i>Diplozoon paradoxum</i>									
2 <i>D. paradoxum</i>	0.13								
3 <i>Eudiplozoon nipponicum</i>	22.17	21.95							
4 <i>Eudiplozoon nipponicum</i>	22.50	21.58	0.14						
5 <i>Inustiatus inustiatus</i>	30.58	31.19	27.72	27.83					
6 <i>Inustiatus aristichthysi</i>	30.39	31.00	27.72	27.83	0.40				
7 <i>Paradiplozoon bliccaae</i>	4.36	4.50	22.00	22.24	31.27	31.31			
8 <i>Paradiplozoon homoion</i>	6.76	6.81	23.29	22.35	30.39	30.21	7.34		
9 <i>Sindiplozoon ctenopharyngodoni</i>	17.40	18.03	24.26	24.36	28.30	28.30	19.06	17.10	
10 <i>Sindiplozoon coreius</i> n. sp.	19.61	19.57	26.94	25.95	32.86	32.86	21.60	18.49	3.40

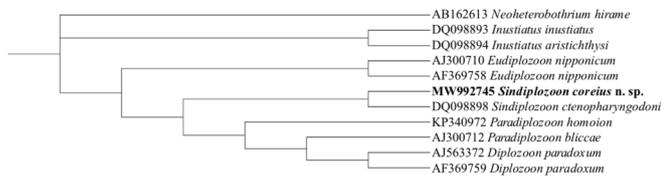


Fig. 3. Bayesian tree resulting from analysis of the ITS2 sequences of diplozoid species using GTR + G model. The monogenean *Neoheterobothrium hirame* was used as outgroup.

4. Discussion

Species in the two genera, *Sindiplozoon* and *Inustiatus* have only been recorded from China in the Yangtze River, the Pearl River and the Heilongjiang River (Amur River) [7]. The “cup-like” widened area and smooth tegument in the posterior part of the body are the typical features of *Sindiplozoon* and *Inustiatus* in Diplozoinea [5,7,9]. The position of the genital pore is the key feature to differentiate the two genera with the genital pore in the fusion area for the *Sindiplozoon* species, but in the anterior part of the body for the *Inustiatus* species [5]. The “cup-like” widened area and smooth tegument in the posterior part of the body are the obvious characteristics of the diplozoid on the gills of *C. guichenoti*, and the genital pore opens to the fusion area. So this diplozoid belongs to the genus *Sindiplozoon*.

So far six species in the genus *Sindiplozoon* have been recorded (Table 3). There are obvious differences between *S. coreius* n. sp. and the other six species of *Sindiplozoon* in the testis shape, presence/absence of branched intestine in the fusion area and widened area, presence/absence of striation on posterior and anterior clamp jaw. *S. coreius* n. sp. resembles *S. diplodiscus* the closest in morphology; a single testis with

Table 3

Comparison of main characters between species of *Sindiplozoon*.

<i>Sindiplozoon</i> species	Host fish	Testis	Intestine	Clamps	Refs.
<i>S. diplodiscus</i>	<i>Mylopharyngodon piceus</i> , <i>Erythroculter mongolicus</i> , <i>Elopichthys bambusa</i>	1 piece, composed of a few small balls	multiple lateral branches in fusion area and widened area	anterior clamp jaw with striation	[8]
<i>S. fujianensis</i>	<i>Leptobotia pellegrini</i>	1 piece with branches, lump-shape	well developed in fusion area, then branches are thinner, long but not dense in widened area	anterior and posterior clamp jaw with striation, anterior end of median plate thickened	[5]
<i>S. hunanensis</i>	<i>Parabotia fasciata</i>	2 pieces, one behind the other, lump-shape, the last one branched	well developed in fusion area, long but not dense in widened area	anterior and posterior clamp jaw with striation, anterior end of median plate thickened	[10]
<i>S. xenocypris</i>	<i>Xenocypris argentea</i> , <i>Xenocypris davidi</i>	1 piece, lump-shape	no branch in fusion area, a few in widened area	two rows of particles can be seen in the front of median plate	[11]
<i>S. ctenopharyngodoni</i>	<i>Ctenopharyngodon idellus</i>	1 piece, oval, or lump-shape	no branch in fusion area, then many long lateral branches in posterior body	posterior clamp jaw with striation, anterior end of median plate thickened	[9]
<i>S. strelkowi</i>	<i>Ctenopharyngodon idella</i> , <i>Hemibarbus labeo</i>	1 piece, composed of three small balls	no branch in fusion area, long in widened area	anterior clamp jaw with striation, multiple regular strips on the edge of the front half of anterior end of median plate	[8]
<i>S. coreius</i> n. sp.	<i>Coreius guichenoti</i>	1 piece, composed of five small balls	no branch in fusion area, long and many branches in widened area	no striation, anterior end of median plate thickened and trapezoid outgrowth	

several balls, multiple intestine branches in the widened area [8]. But the latter have intestine branches in the fusion area and cross striations on the anterior arch of the anterior clamp jaw [8], while these characteristics are absent in the new species.

Although six *Sindiplozoon* species have been described, the ITS2 sequence of only *S. ctenopharyngodoni* is available in GenBank. The 96.0% ITS2 sequence identity and 3.4% genetic divergence to *S. ctenopharyngodoni* suggested interspecific divergence between *S. coreius* n. sp. and *S. ctenopharyngodoni*. Higher than 1.0% genetic distances were detected among other diplozoid species [3,4]. Lower than 1.0% and higher than 20% sequence divergence was suggested the intraspecific and intergeneric distance, respectively [18]. The phylogenetic tree based on BI method suggested that *S. coreius* n. sp. firstly clustered together with *S. ctenopharyngodoni* and then formed a sister group with *Paradiplozoon* species from Europe and *Diplozoon* species on the derived clade, which is consistent with previous results [3,4,15,19,20].

Except for some *Paradiplozoon* species, such as *P. homoion*, *P. gracile* and *Perlohmanna skrjabini*, diplozoids are characterized by high host specificity [4,18,21]. For the six reported *Sindiplozoon* species, only *S. diplodiscus* was recorded on three host species [8]. *S. coreius* n. sp. was firstly recorded on *C. guichenoti*. *S. coreius* n. sp. should be examined on more fish species to determined its host specificity.

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Availability of data and material

Data is available from the authors upon reasonable request.

Declaration of Competing Interest

The authors declare no conflict of interest.

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