



Gill parasites of *Schizocypris altidorsalis* (Pisces: Cyprinidae), a threatened freshwater fish in Iran

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ABSTRACT

Context. *Schizocypris altidorsalis* is a fish of very restricted distribution, with its populations being known only from few water reservoirs on the border of Iran and Afghanistan. **Aims.** The aim was to investigate the occurrence and diversity of host-specific Monogenea on this fish. **Methods.** In total, 400 fish were purchased from the market and examined for the presence of Monogenea. Specimens were characterised morphologically and genetically. **Key results.** In total, 26.5% of fish were found to be infected with Monogenea. Further morphological and molecular studies based on the sequences of the 18S, 28S and ITS2 regions suggested that they all belong to a new species, herein named as *Paradiplozoon jalalii*. Our results suggest that this monogenean is a highly specific parasite of *Schizocypris altidorsalis*. **Conclusions.** Monogeneans are highly specialised host-specific parasites infecting fish gill. They can adversely affect respiration and excretory functions in fish, making them more vulnerable to illness, leading to population declines. **Implications.** Determining effective strategies for safeguarding endangered fish can be challenging in conflict zones, such as the area studied here. Additionally, the parasite identified in our study, with its blood-feeding habits, could contribute to the extinction of its fish host, subsequently leading to its own extinction. An unanswered question lingers regarding the potential extinction of other symbionts of *Schizocypris altidorsalis* if the fish faces extinction.

Keywords: conservation, Diplozoidae, fish, freshwater fish, Iran, Monogenea, new species, threatened species.

Introduction

Reports of fish mortality attributed to gill parasites are abundant (Johnsen and Jensen 1986; Obiakezie and Taege 1991; Leis *et al.* 2023). Gill parasites hold particular importance because they can lead to significant health issues in the infected fish. The gills play several vital roles in fish, including respiratory and excretory functions. Infections by parasites can disrupt these crucial systems, resulting in malfunctions and, consequently, significant health problems for the fish.

One of such parasite groups that poses threats to aquatic life includes Monogenea, a group comprising 6000–7000 species (Khotenovsky 1985), predominantly acting as ectoparasites on the gills and skin of fish (Sayyaf Dezfuli *et al.* 2021). These parasites are known to contribute to fish mortality or a decline in health, particularly when fish is under stress such as in farms with high-density stock (Sayyaf Dezfuli *et al.* 2021). The attachment and feeding activities of a substantial number of monogeneans on fish gills can result in injuries to epithelial cells, hyperplasia, and oedema, leading to the atrophy of capillaries and lamellae. This, in turn, adversely affects the respiratory function of the host, ultimately leading to death (Whittington 2012).

Currently, the cyprinid *Schizocypris altidorsalis* Bianco & Bağnağrescu, 1982 is known from a very limited distribution within the Chah Nimeh water reservoirs of Zabol, situated in the Sistan and Baluchistan Province, Iran (see Brian W. Coad's *Freshwater fishes of Iran* at www.briancoad.com). This species belongs to the family Cyprinidae, which includes a diverse array of carp and minnow species. The Chah Nimeh reservoirs consist of three interconnected lakes nestled along the border between Iran and Afghanistan. These

lakes are characterised by their natural and artificial origins, i.e. they are large natural holes in Sistan and Baluchistan Province into which Hirmand River water is directed, turning it to an artificial lake. The Chah Nimeh reservoirs provide the only habitat for *S. altidorsalis*. Known locally as ‘anjak’ (see www.briancoad.com), *S. altidorsalis* has become an integral part of the cultural and culinary heritage of the surrounding communities. Its delicate flavour and succulent flesh, along with limited options for other food resources, have made it a sought-after edible fish among the locals, a highly disadvantaged population (Moudi *et al.* 2022).

Schizocypris altidorsalis faces a multitude of threats that further compound its conservation status. Pollution, a persistent issue in many aquatic ecosystems, has been identified as one of the significant challenges for this fish. Mirnia *et al.* (2019) showed that industrial waste and agricultural runoff can disrupt the delicate balance of the reservoirs, affecting water quality and diminishing the fish’s ability to thrive. In addition to pollution, climate change poses a growing threat to *S. altidorsalis* and its habitat. Bazzi *et al.* (2021) shed light on the potential impacts of changing environmental conditions on the fish’s distribution, reproductive patterns and overall survival. Rising temperatures, altered rainfall patterns and shifts in water availability could disrupt the delicate ecological equilibrium that sustains this unique species.

Regrettably, the outlook for the survival of *S. altidorsalis*, a freshwater fish with a restricted distribution in an extremely unsafe area to conduct research, remains grim. This species has received scant international attention and is not listed as vulnerable or endangered. Inevitably, highly host-specific symbionts, including parasites of this fish, will also be subject to extinction, if their host becomes extinct. For long, ecologists have advocated for investigations aimed at comprehending the significant ecological role played by parasites, and protecting them from extinction as much as possible. Carlson *et al.* (2020) urged documentation of parasite declines and extinctions, as part of a more wholistic conservation practice.

In the case of our study, this circumstance extends beyond the fish itself as well. The limited distribution of *S. altidorsalis* poses a significant threat to the existence of unique symbionts that exhibit a high level of host specificity, residing on or within the fish. If the fish species were to disappear, these symbionts, too, face the dire prospect of extinction. Species dependent on threatened hosts may become extinct through either direct or indirect human action (Moir *et al.* 2012). One group of these symbionts is Monogenea, a class of ecologically and economically significant platyhelminth parasites of freshwater and marine animals (Gilbert and Avenant-Oldewage 2021), which can cause alterations at the population and organismal levels. Species of Monogenea are generally highly host-specific parasites that may face extinction when their host goes extinct, causing an ‘unintentional extinction’ (Jørgensen 2016).

Hence, the primary objective of this study was to examine the presence of monogenean parasites on the gills of *S. altidorsalis* and characterise them.

Materials and methods

Fish collection

The origin of the studied fish were Chah Nimeh water reservoirs, Zabol, Iran, as depicted in Fig. 1. The Chah Nimeh reservoirs consist of three interconnected lakes nestled along the border between Iran and Afghanistan. These lakes provide a vital habitat for *Schizocypris altidorsalis*. In total, 400 fish (Fig. 2a) were procured from a local fish market. The sampling was conducted during two seasons, namely, autumn 2020 (October and November) and winter 2021 (December and January). Two hundred fish were examined during each season to assess any potential variations.

On acquisition, the fish were promptly transported to the laboratory on ice. Once in the laboratory, the fish were measured for their length and weight as part of the morphological analysis. Additionally, the specimens were thoroughly examined for any signs of infection or infestation with monogenean parasites (Fernando *et al.* 1972) on the same day as the fish’s arrival in the laboratory.

Parasite collection and identification

Within 6–12 h after fish were caught, they were examined for parasites. The excised gills were placed in separate Petri dishes filled with water. With a stereomicroscope, the surfaces of all the gills were inspected. On detection (Fig. 2b), the Monogenea parasites were extracted from the gills with fine dissection needles. Once extracted, the parasites were carefully washed, followed by counting the total number of individuals found on each gill. To enable further analysis, the collected Monogenea parasites were preserved in 70% ethanol for subsequent morphological and molecular investigations. The preserved parasites were then sent to Shamsi’s Parasitology Laboratory at Charles Sturt University, Australia, where the specimens underwent morphological examination and molecular analyses of their taxonomic classification and genetic characteristics. Parasites were morphologically examined as previously described by Gussev (1983) and Jalali *et al.* (2005), and identified in accordance with previous publications (Galli *et al.* 2010; Huang *et al.* 2023; Nejat *et al.* 2023). The terminology to describe various body-part structures is mainly in accordance with Galli *et al.* (2010). All measurements are given in millimetres unless otherwise stated.

A small piece of seven parasites was transferred into separate 1.5-mL autoclaved Eppendorf tubes for molecular study. Voucher material (specimens) has been deposited in the Queensland Museum under the accession numbers G241122 (holotype) and G241123–241127 (paratypes).

DNA was extracted using DNeasy Blood and Tissue kits (Qiagen, Hilden, Germany), as per the manufacturer’s instructions, and modified (Shamsi *et al.* 2018) to be eluted in 40 μ L of elution buffer. Polymerase chain-reaction (PCR) amplification of the fragments of the *ITS2*, *18S* and *28S* of



Fig. 1. Map of the study area. The black box on the inset shows the study region. Map has been produced using ArcGIS Pro (ver. 3.1, ESRI, Redlands, CA, USA).

the rDNA region was performed as previously described (Roohi *et al.* 2019; Shamsi *et al.* 2021; Nejat *et al.* 2023). Representative samples were sent to the Australian Genome Research Facility (AGRF), Brisbane, Qld, Australia, and were subjected to Sanger sequencing by using the same primer sets as for PCR. Sequence data including chromatograms were observed initially through Sequence Scanner Software (Applied Biosystems Genetic Analysers). The evolutionary (pairwise) genetic distance was calculated using MEGA 10 (ver. 10.1, see <https://www.megasoftware.net>; Kumar *et al.* 2016). The phylogenetic relationships among the species were inferred using the maximum-likelihood analysis, Tamura-Nei model in MEGA 10. Closely related species of Monogenea were used as outgroup. For ITS-2 tree, analysing of phylogenetic trees built in previous works (Huang *et al.* 2023; Nejat *et al.* 2023) for *Paradiplozoon* spp. was useful as well. *Gyrodactylus salmonis*, *Hexabothrium appendiculatum*, and *Octomacrum europaeum* were used as the outgroups for 18S, 28S and ITS2 phylogenetic trees respectively. The reliability of the phylogenetic tree was assessed by the bootstrap method with 1000 replications. The pairwise comparison using the p-distance model in MEGA

10 was performed to evaluate the genetic distance between the sequences. After analysing phylogenetic trees built in previous works (Huang *et al.* 2023; Nejat *et al.* 2023) for *Paradiplozoon* spp., a closely related species of Monogenea, *Octomacrum europaeum*, was selected as an outgroup. Details of the sequences used to build phylogenetic trees in the present study can be found in Table 1.

Parasites prevalence, abundance and intensity

The prevalence (p), and mean intensity (MI) of monogeneans were calculated as follows (Bush *et al.* 1997):

$$p = (\text{number of infected fish} \div \text{total number of examined fish}) \times 100$$

$$\text{MI} = (\text{number of parasites} \div \text{number of infected hosts})$$

The data were entered into an Excel spreadsheet and transferred into Stata (ver. 11, StataCorp., College Station, TX, USA). Fisher's exact test was used to compare parasite

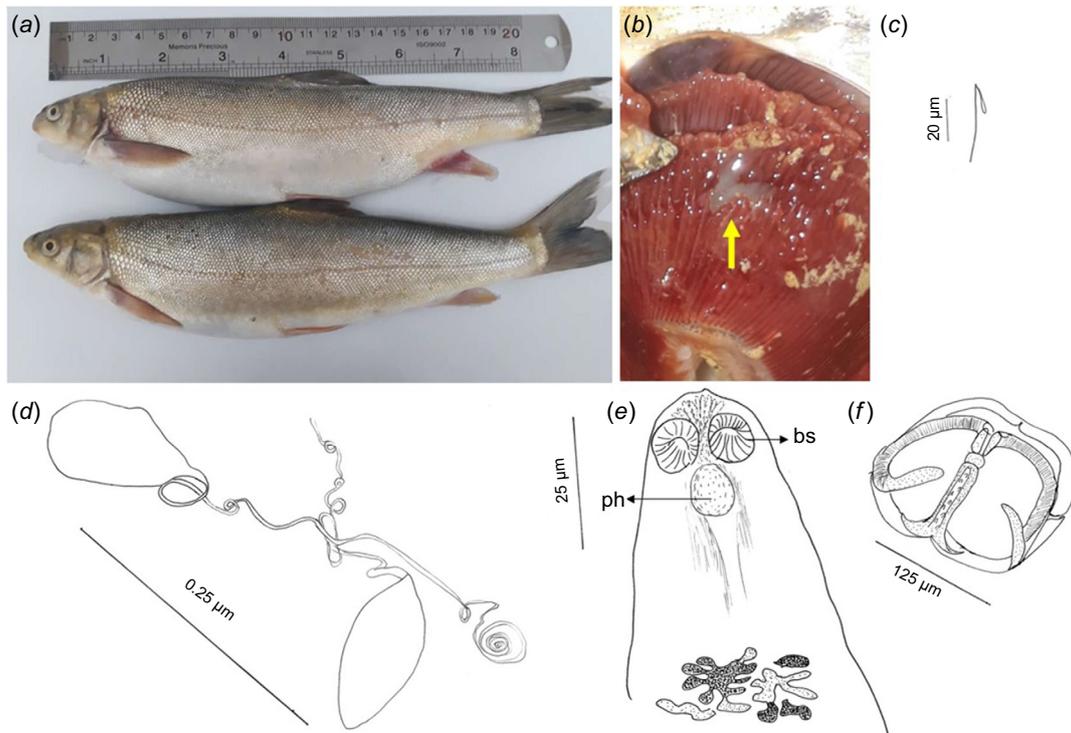


Fig. 2. Fish and parasites that were collected in the present study, including (a) *Schizothorax altidorsalis*, (b) gills infected with monogenean parasite, (c) illustration of hook, (d) illustration of the eggs, (e) illustration of the taxonomically important features of the parasite in the anterior end, and (f) clamp. Abbreviations denote pharynx (ph) and buccal sucker (bs).

prevalence, and mean intensity in different seasons and years. *P*-values of <0.05 were considered as significant.

Results

The parasite specimens found in this study were subjected to morphological examination, which placed them in the family Diplozoidae and the genus *Paradiplozoon*. This classification was based on several distinguishing features, including the absence of dilatations of the middle part of the posterior end of the body, absence of musculo-glandular organs anterior to the buccal suckers, and absence of folds on the posterior part of the body, and the attachment of an egg filament on the opposite end of the uterus opening. However, there were morphological, morphometric (Table 2) and molecular differences between specimens in the present study and the previously described *Paradiplozoon* spp., as presented below. A new species was recognised, and is described below.

Paradiplozoon jalalii, sp. nov.

(Fig. 2–4.)

Type host: *Schizocypris altidorsalis* Bianco & Bănărescu, 1982.

Site of infection

Gills.

Type locality

Chah Nimeh water reservoirs (Fig. 1), Zabol, Province of Sistan and Baluchistan, Iran.

Type material

Holotype (G241122), 5 paratypes (G241123 to G241127), deposited in Queensland Museum, Australia.

Etymology

The new species is named after the late Professor Behiar Jalali in recognition of his dedicated research on monogenean parasites in Iran.

Description

Adults forming typical couples, two adults with X-shape body (Fig. 2b, 3a, b), split into anterior and posterior sections, and smooth tegument throughout body; total body length 4.189 (2.275–5.200, *n* = 7); anterior part 3.030 (1.575–4.500, *n* = 11) long and posterior part 0.900 (0.500–1.200, *n* = 11) long from the fusion area to haptor end; buccal suckers

Table 1. Details of the sequences used to build phylogenetic trees in the present study.

Taxon	Host scientific name (common name)	Locality	GenBank accession number	DNA region	References
<i>Paradiplozoon jalalii</i>	<i>Schizocypris altidorsalis</i> (Anjak)	Iran	PZ203003-6	18S	Present study
<i>Eudiplozoon nipponicum</i>	<i>Cyprinus carpio</i> (common carp)	Czechia	AJ287510	18S	Olson and Littlewood (2002)
<i>Discocotyle sagittata</i>	<i>Salmo trutta</i> (sea trout)	Isle of Man, UK	AJ287504	18S	Olson and Littlewood (2002)
<i>Microcotyle sebastis</i>	<i>Sebastes</i> sp.	North Sea, UK	AJ287540	18S	Olson and Littlewood (2002)
<i>Neomicrocotyle pacifica</i>	<i>Caranx hippos</i> (black jack)	Chamela Bay, Mexico	AJ228787	18S	Olson and Littlewood (2002)
<i>Monaxine formionis</i>	–	Mumbai, India	KT267181	18S	Verma and Verma (2022)
Mexicotyle sp.	<i>Scomberomorus</i> sp. (mackerel)	Paraná, Brazil	AJ287539	18S	Olson and Littlewood (2002)
<i>Gotocotyla secunda</i>	<i>Scomberomorus commerson</i> (Spanish mackerel)	Heron Island, Australia	AJ276425	18S	Olson and Littlewood (2002)
<i>Microcotyle erythrinii</i>	<i>Pagellus erythrinus</i>	France	AM157195	18S	Badets <i>et al.</i> (2011)
<i>Paradawesia</i> sp.	<i>Scomberomorus</i> sp. (mackerel)	Paraná, Brazil	AJ287555	18S	Olson and Littlewood (2002)
<i>Choricotyle australiensis</i>	<i>Rhabdosargus sarba</i> (goldlined seabream)	Coffs Harbour, NSW, Australia	AF382069	18S	Olson and Littlewood (2002)
<i>Probursata brasiliensis</i>	<i>Oligoplites</i> sp. (leatherjack)	Paraná, Brazil	AJ276426	18S	Olson and Littlewood (2002)
<i>Narcinecotyle longifilamentus</i>	<i>Narcine entemedor</i>	Mexico	MN447332	18S	Torres-Carrera <i>et al.</i> (2020)
<i>Pellonicola elongatus</i>	–	Lucknow, India	KX094553	18S	Unpublished
<i>Diclybothrium armatum</i>	<i>Acipenser schrenckii</i> (Amur sturgeon)	Amur River, Russia	KP96254	18S	Rozhkovan and Shedko (2015)
<i>Gyrodactylus salmonis</i>	<i>Oncorhynchus mykiss</i> (rainbow trout)	Veracruz, Mexico	JN230350	18S	Rubio-Godoy <i>et al.</i> (2012)
<i>Paradiplozoon jalalii</i>	<i>Schizocypris altidorsalis</i> (Anjak)	Iran	PZ203006-II	28S	Present study
<i>Eudiplozoon nipponicum</i>	<i>Cyprinus carpio</i> (common carp)	Czechia	AF382037	28S	Olson and Littlewood (2002)
<i>Microcotyle erythrini</i>	<i>Pagrus pagrus</i> (red porgy)	Guardamar del Segura, Spain	MN814848	28S	Villora-Montero <i>et al.</i> (2020)
<i>Microcotyle erythrini</i>	<i>Pagellus erythrinus</i> (common pandora)	France	AM157221	28S	Badets <i>et al.</i> (2011)
<i>Microcotyle whittingtoni</i>	<i>Dentex dentex</i> (common dentex)	Guardamar del Segura, Spain	MN814847	28S	Villora-Montero <i>et al.</i> (2020)
Mexicotyle sp.	<i>Scomberomorus</i> sp. (mackerel)	Brazil	AF382041	28S	Olson and Littlewood (2002)
<i>Paradawesia</i> sp.	<i>Scomberomorus</i> sp. (mackerel)	Brazil	AF382042	28S	Olson and Littlewood (2002)
<i>Microcotyle isyebi</i>	<i>Boops boops</i> (bogue)	Guardamar del Segura, Spain	MN814850	28S	Villora-Montero <i>et al.</i> (2020)
<i>Diclidophora denticulata</i>	<i>Pollachius virens</i> (saithe)	North Sea, northern Europe	AY157169	28S	Lockyer <i>et al.</i> (2003)
<i>Paraheterobothrium chilense</i>	<i>Hippoglossina macrops</i>	Japan	LC658939	28S	Ogawa and Itoh (2022)
<i>Probursata brasiliensis</i>	<i>Oligoplites</i> sp. (leatherjack)	Brazil	AF382049	28S	Olson and Littlewood (2002)
<i>Heterobothrium praeorchis</i>	<i>Takifugu flavipaterus</i> Matsuura (Japanese name: komon-fugu)	Japan	LC658931	28S	Ogawa and Itoh (2022)
<i>Heterobothrium matsubarai</i>	<i>Takifugu stictonotus</i> (Japanese name: goma-fugu)	Japan	LC658934	28S	Ogawa and Itoh (2022)
<i>Neoheterobothrium</i> sp.	<i>Syacium papillosum</i> (flounder)	Yucatan Shelf (Gulf of Mexico)	MT429192	28S	Soler-Jiménez <i>et al.</i> (2021)
<i>Heterobothrium victorwepeneri</i>	<i>Amblyrhynchotes honckenii</i> (evileye blaasop)	South Africa	MW115857	28S	Acosta and Smit (2021)

(Continued on next page)

Table 1. (Continued).

Taxon	Host scientific name (common name)	Locality	GenBank accession number	DNA region	References
<i>Choricotyle australiensis</i>	<i>Rhabdosargus sarba</i> (goldlined seabream)	Australia	AF382046	28S	Olson and Littlewood (2002)
<i>Paradiplozoon jalalii</i>	<i>Schizocypris altidorsalis</i> (Anjak)	Iran	PZ2030012-18	ITS2	Present study
<i>Paradiplozoon yarkandense</i>	<i>Schizothorax</i> fish (Cyprinidae: Schizothoracinae)	China	MN892638	ITS2	Arken et al. (2022)
<i>Paradiplozoon yarkandense</i>	<i>Schizothorax</i> fish (Cyprinidae: Schizothoracinae)	China	MN892637	ITS2	Arken et al. (2022)
<i>Paradiplozoon bingolensis</i>	<i>Cyprinion macrostomum</i>	Iraq	OP588754	ITS2	Nejat et al. (2023)
<i>Paradiplozoon bingolensis</i>	<i>Cyprinion kais</i>	Turkey	OP588752	ITS2	Nejat et al. (2023)
<i>Paradiplozoon krugerense</i>	<i>Labeo rosae</i>	South Africa	LT574865	ITS2	Dos Santos and Avenant-Oldewage (2016)
<i>Paradiplozoon moroccoensis</i>	<i>Luciobarbus lepineyi</i>	Morocco	MT417735	ITS2	Benovics et al. (2021)
<i>Paradiplozoon moroccoensis</i>	<i>Luciobarbus lepineyi</i>	Morocco	MT417734	ITS2	Benovics et al. (2021)
<i>Afrodiplozoon polycotyleus</i>	<i>Labeobarbus marquensis</i>	South Africa	LT719088	ITS2	Prikrylová et al. (2018)
<i>Paradiplozoon bliccae</i>	<i>Ladigesocypris ghigii</i>	Turkey	OP588760	ITS2	Nejat et al. (2023)
<i>Paradiplozoon bliccae</i>	<i>Petroleuciscus ninae</i>	Turkey	OP588758	ITS2	Nejat et al. (2023)
<i>Paradiplozoon homoion</i>	<i>Squalius cii</i>	Turkey	OP588791	ITS2	Nejat et al. (2023)
<i>Paradiplozoon homoion</i>	<i>Garra rufa</i>	Iraq	OP588784	ITS2	Nejat et al. (2023)
<i>Paradiplozoon homoion</i>	<i>Rhodeus amarus</i>	Turkey	MT028131	ITS2	Aydođdu et al. (2020)
<i>Paradiplozoon homoion</i>	<i>Squalius cii</i>	Turkey	OP588793	ITS2	Nejat et al. (2023)
<i>Paradiplozoon skrjabini</i>	<i>Gnathopogon elongatus elongatus</i>	Japan	LC731724	ITS2	Nitta and Nagasawa (2023)
<i>Diplozoon paradoxum</i>	<i>Abramis brama</i>	Czechia	AJ563372	ITS2	Matejusová et al. (2004)
<i>Paradiplozoon hemiculteri</i>	<i>Hemiculter leucisculus</i>	China	KY290761	ITS2	Jirsová et al. (2018)
<i>Sindiplozoon ctenopharyngodoni</i>	<i>Ctenopharyngodon idella</i>	China	DQ098898	ITS2	Gao et al. (2006)
<i>Paradiplozoon gracile</i>	–	–	KP340973	ITS2	Unpublished
<i>Paradiplozoon sapae</i>	<i>Abramis sapa</i>	Czechia	AJ300713	ITS2	Matejusová et al. (2001)
<i>Paradiplozoon barbi</i>	–	–	MN688771	ITS2	Unpublished
<i>Sindiplozoon</i> sp.	<i>Coreius guichenoti</i>	China	MW992745	ITS2	Cao et al. (2022)
<i>Eudiplozoon nipponicum</i>	<i>Cyprinus carpio</i>	China	DQ098897	ITS2	Gao et al. (2006)
<i>Eudiplozoon nipponicum</i>	–	–	OP376058	ITS2	Unpublished
<i>Octomacrum europaeum</i>	<i>Alburnoides bipunctatatus</i>	Poland	MT441500	ITS2	Benovics et al. (2021)

(Fig. 2c, 3g, h) one pair, larger than pharynx, circular, opening subterminal, 0.097 (0.080–0.120, $n = 12$) × 0.095

(0.080–0.115, $n = 12$), glandular structures absent; pharynx (Fig. 2c, 3g, h) circular, below buccal suckers, 0.080

Table 2. Comparison of the measurements of the taxonomically important features of the specimens of *Paradiplozoon jalalii* in the present study with those of closely related taxa.

Item	<i>Paradiplozoon jalalii</i> sp. nov. (present study)				<i>Paradiplozoon schizothorazi</i> (Galli et al. 2010)				<i>P. yarkandense</i> (Arken et al. 2022)			
	Number of specimens measured	Minimum	Maximum	Mean	Number of specimens measured	Minimum	Maximum	Mean	Number of specimens measured	Minimum	Maximum	Mean
Body length	7	2.275	5.200	4.189	Not stated	4.1	6.1	Not stated	58	1.10	3.05	2.13
Length of the anterior part	11	1.575	4.500	3.030	Not stated	1.0	1.8	Not stated	52	0.57	2.07	1.39
Length of the posterior part	11	0.500	1.200	0.900	Not stated	2.1	3.8	Not stated	52	0.35	0.93	0.55
Total anterior and posterior parts	11	2.075	5.675	3.930	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated	Not stated
Length of suckers	12	0.080	0.120	0.097	Not stated	0.11	0.16	Not stated	52	0.05	0.08	0.06
Width of suckers	12	0.080	0.115	0.095	Not stated	Not stated	Not stated	Not stated	52	0.04	0.07	0.05
Pharynx (length)	9	0.060	0.100	0.080	Not stated	0.06	0.09	Not stated	41	0.046	0.049	0.047
Pharynx (width)	9	0.050	0.075	0.066	Not stated	Not stated	Not stated	Not stated	41	0.041	0.043	0.042
Clamps 1 (length)	6	0.083	0.103	0.093	Not stated	0.12	0.17	Not stated	54	0.05	0.08	0.07
Clamps 1 (width)	6	0.100	0.145	0.122	Not stated	0.17	0.20	Not stated	54	0.09	0.12	0.11
Clamps 2 (length)	6	0.080	0.123	0.094	Not stated	0.12	0.16	Not stated	53	0.08	0.10	0.09
Clamps 2 (width)	6	0.120	0.158	0.139	Not stated	0.21	0.24	Not stated	53	0.14	0.18	0.15
Clamps 3 (length)	6	0.085	0.115	0.096	Not stated	0.13	0.16	Not stated	52	0.09	0.11	0.10
Clamps 3 (width)	6	0.120	0.155	0.135	Not stated	0.21	0.26	Not stated	52	0.14	0.19	0.16
Clamps 4 (length)	6	0.080	0.120	0.096	Not stated	0.13	0.18	Not stated	53	0.07,	0.09	0.08
Clamps 4 (width)	6	0.120	0.138	0.126	Not stated	0.22	0.26	Not stated	53	0.12	0.17	0.14
Central hooks length	3	0.013	0.015	0.014	Not stated	0.019	0.022	Not stated	33	0.039	0.045	0.042
Shaft length	2	0.038	0.038	0.038	Not stated	0.042	0.049	Not stated	28	0.019	0.026	0.023
Eggs dimensions	2	0.233 × 0.153	0.267 × 0.113	–	Not stated	0.13–0.33 × 0.15–0.24	Not stated	Not stated	29	0.18 × 0.06	0.22 × 0.08	0.20 × 0.07

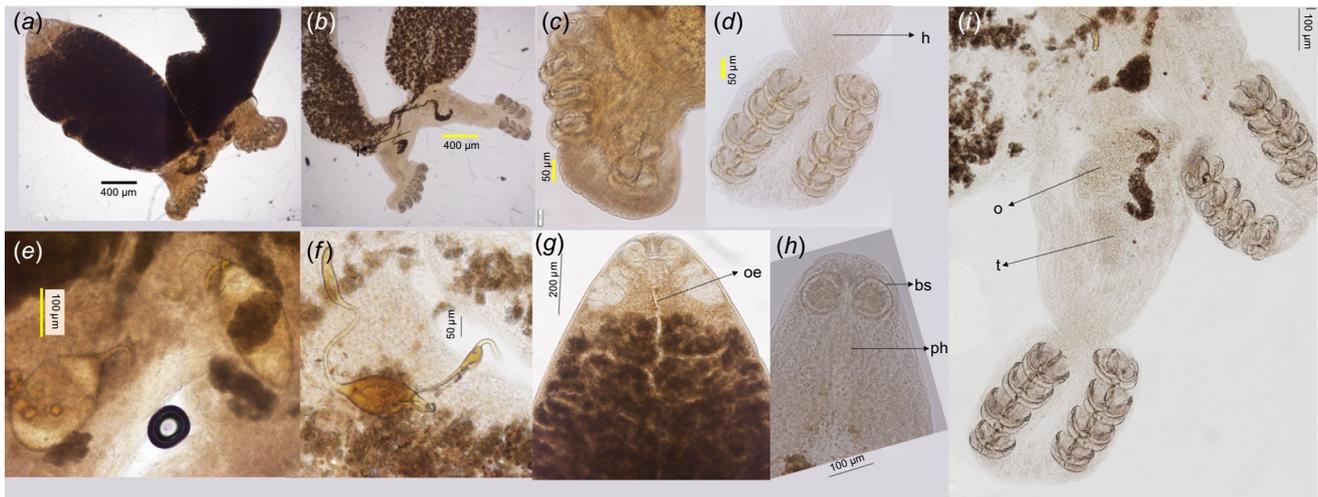


Fig. 3. Light microscopy images of the *P. jalalii* found in the present study, showing variations in the morphological characteristics such as (a, b) overall anatomy of the body, (c, d) clamps, (e) mature and (f) immature eggs, and (g, h) anterior ends among different individuals with identical *ITS2*, *18S* and *28S* sequences. Image (i) shows ovary (o) and testis (t). Other abbreviations include bs, buccal sucker; h, haptor; oe, oesophagus; ph, pharynx.

(0.060–0.100, $n = 9$) \times 0.066 (0.050–0.075, $n = 9$), opening into branched intestine. Reproductive organs located anteriorly in the posterior part of the body; testis (Fig. 3i) circular; ovary (Fig. 3i) larger and anterior to testis; eggs (Fig. 2e, 4d) 0.325 (0.316–0.333) \times 0.233 (0.233–0.233, $n = 2$) in size, with filament in the pointed end and rounded in the other end; up to three eggs were found in one individual; haptor with four pairs of clamps (Fig. 3b, d, i) and one pair of central hooks (Fig. 4a, c) in each haptor; posterior arch of the clamps with cross-striation; first clamp (the most posterior) 0.093 (0.083–0.103, $n = 6$) \times 0.122 (0.100–0.145, $n = 6$), second clamp 0.094 (0.080–0.123, $n = 6$) \times 0.139 (0.120–0.158, $n = 6$), third clamp 0.096 (0.085–0.115, $n = 6$) \times 0.135 (0.120–0.155, $n = 6$), the fourth clamp 0.096 (0.080–0.120, $n = 6$) \times 0.126 (0.120–0.137, $n = 6$); central hook (Fig. 4a, c) sickles 0.014 (0.013–0.015, $n = 3$) long, hook handles 0.038 (0.038–0.038, $n = 2$) long; each clamp (Fig. 4a–c) consists of sclerotised structures, including median sclerite, trapezoid outgrowth on median sclerites and jaws. The anterior end of the median sclerite has numerous perforations that extend almost the entire length of the median region.

Differential morphology

Paradiplozoon stands out as the most diverse genus within the Diplozoinea subfamily, and its distinguishing feature for species identification is primarily associated with its clamp structure. Specifically, the key morphological components include the median sclerite and its associated structures, such as the trapeze spur, as well as the anterior and posterior joining sclerites (Matejusová et al. 2004; Dos Santos et al. 2015; Huang et al. 2023). There are two distinct species of *Paradiplozoon* that parasitise *Schizothorax* spp., namely, *Paradiplozoon schizothorazi* (Iksanov, 1965) and *P. yarkandense*

(Galli et al. 2010; Arken et al. 2022). As a result, a comparative analysis of the morphological traits of *P. jalalii* sp. nov. was conducted, showing notable distinctions when compared with both *P. schizothorazi* (Iksanov, 1965) and *P. yarkandense* Arken et al. (2022).

There were differences in the size of the various body parts between *P. jalalii* and *P. yarkandense* (Table 2). For example, body length of the new species was larger than that of *P. yarkandense*, and hooks in the new species were smaller than hooks in both *P. schizothorazi* and *P. yarkandense*. They were also different in the anatomy, with the oral sucker being oval, and testis being of irregular shape in the latter, whereas sucker and testis were circular in the new species. *P. jalalii* and *P. schizothorazi* were very similar in the size of their body; however, the hook length (both the shaft and the hook itself) and the clamps were much smaller in the new species. The anatomy and measurements of the central hook and clamps are normally considered to be taxonomically significant, which were also different between *P. jalalii* and *P. schizothorazi*. In addition, the anterior end of the median sclerite in the new species has distinct Y-shaped projection absent in *P. schizothorazi* and *P. yarkandense*.

Molecular results

We generated the *18S*, *28S* and *ITS2* sequences of the specimens investigated in our study (GenBank Accession numbers PZ203003–PZ203018). Among the six specimens, we obtained identical *18S* sequences, with a length of 1033 bp. Notably, there were no *18S* sequences belonging to *Paradiplozoon* spp. in GenBank at the time of our analysis. The BLAST analysis showed less than 95% similarity with *Eudiplozoon nipponicum* (AJ287510) or pairwise genetic distances ranging from 5.23 to 5.66%.

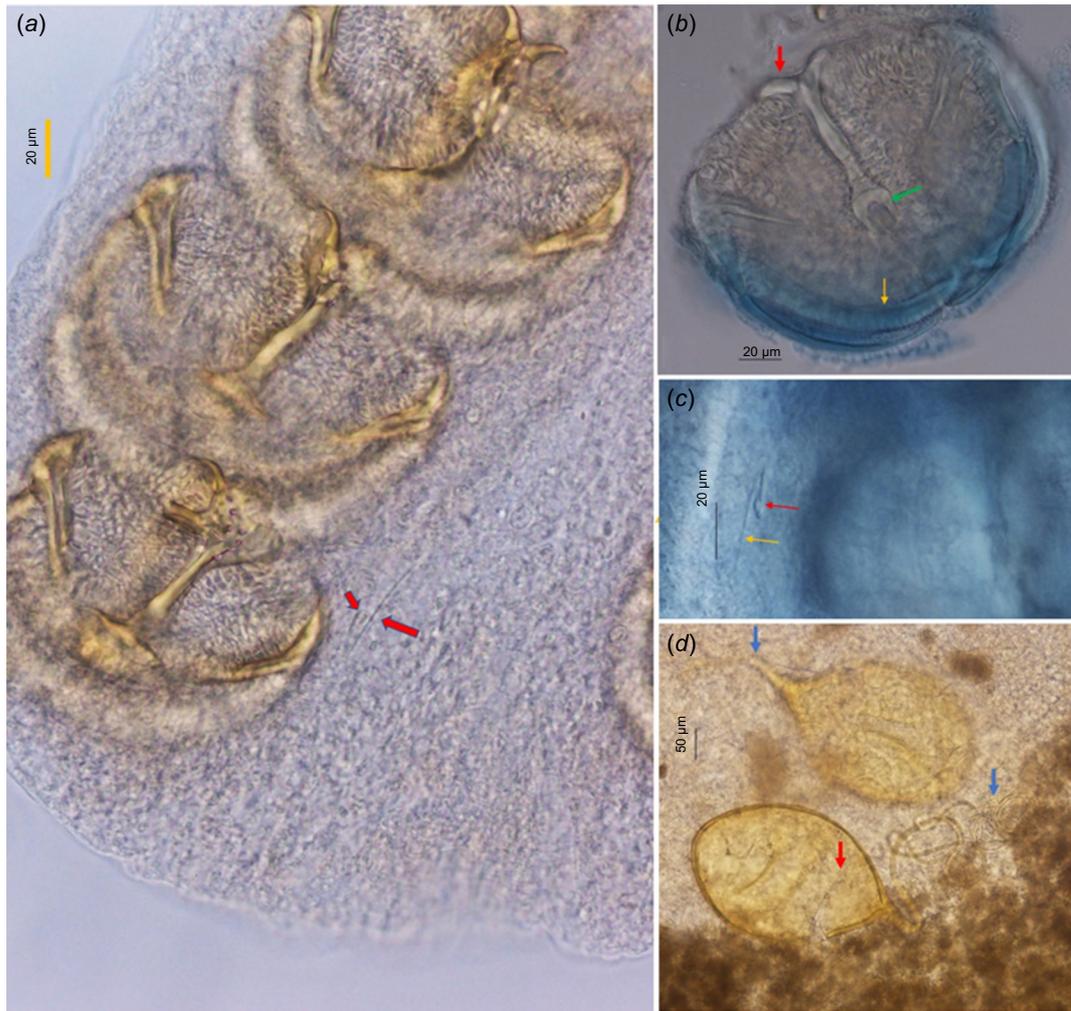


Fig. 4. Light microscopy images of the clamps in *P. jalalii* found in the present study, showing details of the clamps and eggs: (a) showing the position and size of hook (red arrows) in the opisthohaptor, (b) clamp in posterior view, showing the median plate (red and green arrows) and medial sclerite of posterior jaw (yellow arrow), (c) handle (yellow arrow) and body of the central hook, and (d) egg filament (blue arrow) and operculum (red arrow).

For the 28S region, sequences obtained from four specimens were also identical and spanned 1328 bp. In the BLAST results, the two most closely related diplozoids were *E. nipponicum* (AF382037) and *Paradiplozoon* sp. (KU519493), with estimated pairwise genetic distances of 13.70 and 15.87% respectively.

Additionally, we obtained ITS-2 sequences from six specimens, all of which were identical and 774 bp in length. For ITS2 comparisons, we selected 31 diplozoid sequences from GenBank. After aligning the data, the final dataset contained 882 positions, with 314-bp conserved sites, 547-bp variable sites, 424 parsimony-informative sites and 122-bp singleton sites. Similarly, for the 18S dataset, of the total 2040-bp sites, 1515 bp were conserved and 450 bp were variable. In the ITS2tree, the sequences of the newly described species formed a well-supported monophyletic group with *P. yarkandense*, another Monogenea species also found on

Schizothorax fish, and then clustered with other *Paradiplozoon* spp. These findings strongly suggest that all the specimens in our study belong to a single and distinct species.

Moreover, our phylogenetic analyses (Fig. 5) provided further support for the genetic distinctiveness of the examined specimens compared with previously characterised species. This reaffirmed their classification as a distinct species within the genus *Paradiplozoon*, especially evident in the topography of the tree based on ITS2 sequences. Notably, only one to two diplozoid sequences from published works were available in GenBank for 18S and 28S comparisons.

Table 3 shows the details of the infection rate of fish with *Paradiplozoon jalalii*. The maximum number of *Paradiplozoon jalalii* parasites found per fish in this study was two, with the majority of fish being infected by only one parasite (Table 3). Mean intensity was 1.0–1.3. This study showed no significant

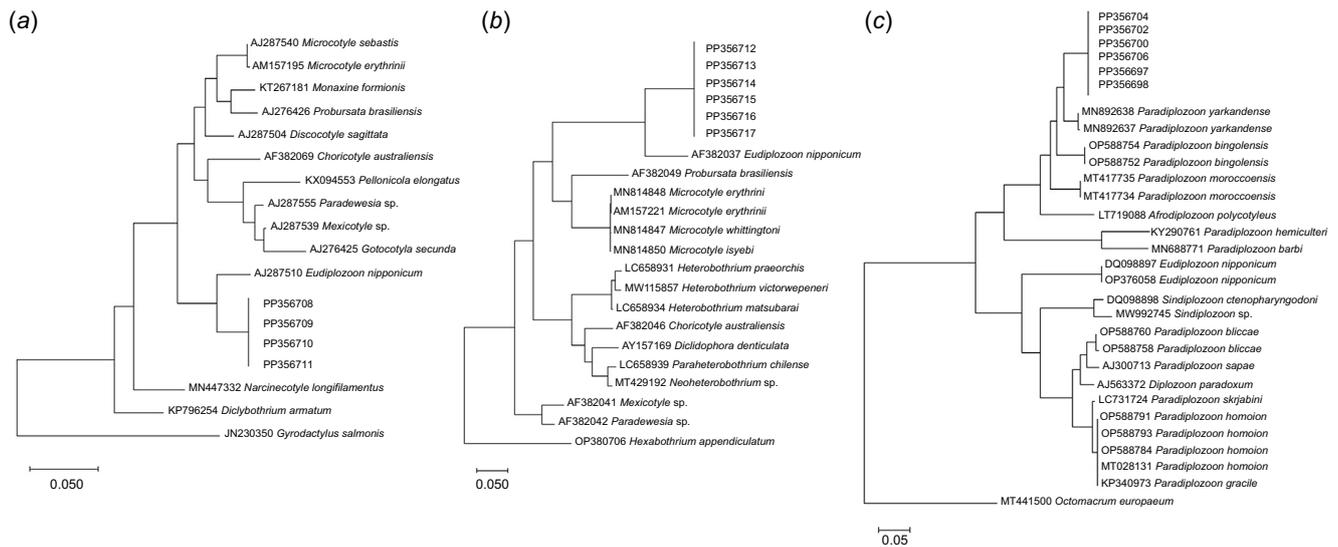


Fig. 5. Phylogenetic trees based on the (a) 18S, (b) 28S and (c) ITS2 sequences.

Table 3. Summary of number of *P. jalalii* sp. nov. found in the fish in the present study.

Season and year	Number of fish examined	Number of fish infected	Prevalence (%)	Total number of parasites	Number of fish with one parasite	Number of fish with two parasites
Autumn 2020 (November)	200	52	26	66	38	14
Winter 2021 (February)	200	66	33	66	42	12
Total	400	106	26.5	132	80	26

difference in the prevalence, mean intensity and mean abundance of *Paradiplozoon jalalii* between different years (Table 3).

Discussion

This is the first study reporting monogenean parasites of *Schizocypris altidorsalis*. There was no opportunity in the study region where fish was collected to conduct a histopathology study. Therefore, the details of the damage caused by this parasite on fish gill remains unknown. Because, generally, the number of parasites is a crucial contributing factor to the occurrence of disease by Monogenea (Thoney and Hargis 1991), we speculate that it is unlikely that *P. jalalii* causes serious harm to the fish, owing to low number of the parasites found in the examined fish individuals. Although it is possible that the actual number of parasites was higher as we only collected and examined already dead fish.

In regard to the number of the parasites found in the fish host in the present study, the absence of the difference in prevalence and abundance of parasites in different seasons and years could be attributed to the narrow range of temperature fluctuations observed in the study area. Over the course of a 30-year period, the average temperatures in the area have

been reported as being 7–15°C during autumn, and 1–12°C during winter (Zare Abianeh et al. 2015). Population of diplozoid Monogenea, including *Paradiplozoon* spp. on their fish hosts, exhibits seasonal variations, which are more pronounced during the warmer months (Gilbert and Avenant-Oldewage 2016).

There is limited knowledge about monogenean fauna of eastern regions of Iran that are influenced by Indian faunal region (Jalali et al. 2000). The present study also shows identification of a new species of diplozoid Monogenea in this region. Accurate identification and delimitation of diplozoid monogeneans have been always challenging. Morphologically they lack sclerotised genitalia and possess only small sclerites in their haptors, which are often difficult to accurately visualise owing to their orientation and position within tissue (Gläser and Gläser 1964; Khotenovsky 1985). Dos Santos and Avenant-Oldewage (2020) reviewed currently available genetic data, to uncover insights from the current sequence data, suggested improvements for future studies, and highlighted potential pitfalls to be avoided. Like several other studies about other parasitic taxa (Barton et al. 2022; Shamsi et al. 2024), Dos Santos and Avenant-Oldewage (2020) pleaded for a more integrated taxonomic approach and the inclusion of voucher material alongside the sequence data obtained from several additional markers, and concluded that

analyses of a substantial amount of further morphological and genetic data are needed before an accurate study of the taxonomy and evolutionary history of diplozoid species can be achieved. These challenges over specific identification of the existing taxa makes introducing a new species challenging. With these in mind, we believe our study provides evidence for a new species of Monogenea, herein named *Paradiplozoon jalalii*, on a fish species, *Schizocypris altidorsalis*. Both fish and the parasite have been found only in a small area in the Province of Sistan and Baluchistan in Iran (R. Froese and D. Pauly, FishBase, ver. 10/2023, see www.fishbase.org). The reference materials are available in Queensland Museum for morphological examination by interested parties. The gDNA of this valuable species is also available in Shamsi's Parasitology Laboratory at Charles Sturt University and can be handed over to interested researchers in the future.

The new species was different from previously known species on the basis of its size and also morphology of the clamps and hooks. Clamps and hooks serve as apparatus for maintaining the attachment of adult diplozoids to their hosts. The morphological characteristics and size of the clamps and hook have always been the primary basis for distinguishing among diplozoid Monogenea. However, it is important to note that factors such as host size, developmental stage, and environmental pollution can influence individual size and the shape and size of the clamp, resulting in intraspecific morphological variations within the same species of diplozoid (Pecínková *et al.* 2005; Arken *et al.* 2022). These characteristics can also be subject to different fixation and examination methods and varying observation angles by different researchers, affecting the morphological results to some extent.

Noting that not all diplozoid monogeneans have been genetically characterised yet, phylogenetic analyses using sequences of *18S*, *28S* and *ITS2* regions also supported the distinction of our specimens from previously described species. The phylogenetic position of *Paradiplozoon jalalii* in the *ITS2* tree (Fig. 5c) suggests that this species belongs to the genus *Paradiplozoon*, a paraphyletic genus (Huang *et al.* 2023). The present species is a sister of *Eudiplozoon* in the *28S* and *18S* trees (Fig. 5a, b); however, these trees do not include any or many other *Paradiplozoon* species in the analysis because of the lack of comparable data available in the GenBank. Future studies providing comparable sequences will further elucidate the taxonomic status of the specimens in the present study.

Although the phylogenetic trees constructed in the current study provide support for distinguishing the new species, they also prompt questions regarding the validity of certain previously described species. Notably, the unexpected position of *Afrodiplozoon polycotyleus* in Fig. 5c warrants attention. However, because of the lack of comparable sequence data for multiple regions across all diplozoids, drawing definitive conclusions about the phylogenetic relationships of these

taxa has been shown to be challenging and falls outside the scope of the present study.

Finding of a highly host-specific parasite in the present study is also of significance because this parasite itself may be at risk of extinction, potentially owing to environmental factors or changes in host populations.

Although no pathological impact by the new species on its fish host can be concluded at this stage, this very parasite itself may have the potential to drive the extinction of its cyprinid fish hosts, if it was found to be pathogenic for its host. It is known that some *Paradiplozoon* spp. have blood-feeding habits (Smyth and Halton 1983; Rohlenová *et al.* 2011). The call for the conservation of parasite species began nearly three decades ago (Windsor 1995; Durden and Keirans 1996) and has persisted, although not consistently, over time. Preserving parasite species is crucial (LyMBERG and Smit 2023) because they are integral components of natural ecosystems, just as important as the charismatic vertebrates that typically receive the majority of conservation attention and funding. Parasites play vital roles in maintaining the structure and functioning of ecosystems and also serve as valuable indicators of ecosystem health. Lists of threatened species, such as the International Union for Conservation of Nature's (IUCN) Red List, continue to be widely used tools for biodiversity conservation (Moir and Brennan 2020). However, many unique species in conflict zones, such as the area studied here, often do not appear on these lists and it becomes challenging to determine effective strategies for safeguarding endangered populations. Parasite species dependent on threatened hosts may become extinct through either direct or indirect human action (Moir *et al.* 2012).

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