

## Research Article

**First molecular identification of a non-indigenous parasite, *Sciadicleithrum iphthimum* Kritsky et al., 1989 (Monogenea: Dactylogyridae) in India**Kalpana Km<sup>1</sup>, Haren Ram Chiary<sup>2</sup>, Km Gazala<sup>1,3</sup>, Anshu Chaudhary<sup>1</sup>, Bindu Sharma<sup>3</sup> and Hridaya Shanker Singh<sup>1,4</sup><sup>1</sup>Molecular Taxonomy Laboratory, Department of Zoology, University Road, Chaudhary Charan Singh University, Meerut, Uttar Pradesh 250004, India<sup>2</sup>Department of Zoology, Kirori Mal College, University of Delhi, Delhi 110007, India<sup>3</sup>Laboratory of Molecular Parasitology, Department of Zoology, University Road, Chaudhary Charan Singh University, Meerut, Uttar Pradesh 250004, India<sup>4</sup>Maa Shakumbhari University, Punwarka, Saharanpur, Uttar Pradesh 247120, IndiaCorresponding author: Anshu Chaudhary ([anshuchaudhary81@gmail.com](mailto:anshuchaudhary81@gmail.com))

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**Abstract**

Fish translocation for the ornamental fish sector has resulted in the co-introduction of their parasites in a new region. In this study, we are characterized a non-indigenous monogenean, *Sciadicleithrum iphthimum*, which infected an introduced cichlid, *Pterophyllum scalare* (Angelfish) imported into India for ornamental purposes. The present species was identified using morphological and molecular data using 18S and 28S rDNA sequences, and they were generated for the first time. We are providing here a morphological description and phylogenetic analysis of *S. iphthimum*, which appeared genetically close to *Sciadicleithrum* spp. and nested within dactylogyrid parasites.

**Key words:** cichlid fish, trematoda, molecular, *Sciadicleithrum* sp., Meerut, ribosomal RNA**Introduction**

Imports of freshwater fish species are widespread across the globe and are identified as one of the leading causes worldwide of changes in aquatic ecosystems (Sala et al. 2000; Leprieur et al. 2008; Butchart et al. 2010; Brosse et al. 2021). Cichlid fishes are commonly parasitized by dactylogyrid monogeneans that are host-specific. Neotropical cichlids have been parasitized by five different genera of gill monogeneans: *Gussevia* Kohn & Paperna, 1964; *Sciadicleithrum* Kritsky et al., 1989; *Trinidactylus* Hanek et al., 1974; *Tucunarella* Mendoza-Franco et al., 2010; and *Parasciadicleithrum* Mendoza-Palmero et al., 2017. However, *Sciadicleithrum* (26 spp.) is the most diversified genus, followed by *Gussevia* (17 spp.) of neotropical monogeneans (Cohen et al. 2013). Until recently, the monogenean parasites of non-native fish in India have not been studied much (see Tripathi et al. 2010; Chaudhary et al. 2014; Chiary et al. 2014; Tripathi 2015; Tripathi et al. 2022; Tripathi and Matey 2023 for exceptions). Despite

these few recent studies, the complete picture about the presence of monogeneans in native fish in India is far from known.

Dactylogyridae Bychowsky, 1933, is one of the most species rich families within the Monogenea and comprises more than 1000 species and 166 genera to date (Cribb et al. 2002; Pugachev et al. 2010; Kmentová et al. 2022). Neotropical cichlid, the angelfish *Pterophyllum scalare* (Schultze, 1823), is popular worldwide as an ornamental freshwater fish species due to its body coloration, which contributes to its high economic value (Patil et al. 2015; Jayalekshmi et al. 2017). *Pterophyllum scalare* is frequently parasitized by species of genera *Gussevia* and *Sciadicleithrum*. To the best of our knowledge, only two species of the genus *Gussevia* and *Sciadicleithrum* parasitize *P. scalare*, i.e., *Gussevia spiralocirra* Kohn & Paperna, 1964, and *Sciadicleithrum iphthimum* Kritsky et al., 1989. Kritsky et al. (1989) proposed the *Sciadicleithrum* to comprise the dactylogyrids of Neotropical cichlid fishes. The genus is characterized by overlapping gonads, a coiled male copulatory organ with clockwise rings, unmodified anchors, a ventral bar with two umbelliform membranes or cavities on the anterior bar margin, and similar marginal hooks with undilated shanks and erect thumbs. Bellay et al. (2009) revised the generic diagnosis of *Sciadicleithrum* spp. and mentioned that they have overlapping gonads, a male copulatory organ with less than two rings, and the shaft and points of the ventral and dorsal anchors are in the presence of longitudinal lateral grooves that were also supported by previous studies (Kritsky et al. 1989, 1994; Mendoza-Franco et al. 1997, 2007; Mendoza-Franco and Vidal-Martínez 2005; Vidal-Martínez et al. 2001a, b; Bellay et al. 2008; Carvalho et al. 2008; Yamada et al. 2009).

Morphological methods were mainly used for the identification of monogenean species, but recently, the use of molecular markers has indicated their utilization for the accurate identification of species. On the other hand, molecular data on monogenean species from India is still limited, and most of the identified species were described only under the traditional morphospecies notion. In the case of *Sciadicleithrum*, including the present species, molecular data is available for 13 species on the GenBank database, along with 22 sequences from unknown species. To the best of our knowledge, there are no sequences available on the GenBank database for any species of *Sciadicleithrum* to date from India.

In this study, *S. iphthimum* infecting the gills of imported Amazon cichlids (*P. scalare*) is reported using morphological and molecular data from the 18S and 28S rDNA gene sequences.

## Materials and methods

During a survey of exotic fish monogenean parasites, 47 freshwater angelfish *P. scalare* of size 8 to 12 cm were collected in 2020–2022 from the sellers at aquarium markets of Meerut (29°01'N; 77°45'E), Uttar Pradesh,

India. Specimens of fish were brought to the laboratory of the Department of Zoology, Chaudhary Charan Singh University, Meerut, on ice in an insulated box. The gills of these fish were examined on the day of arrival and dissected under a stereomicroscope (Motic SMZ-168 series, Xiamen, People's Republic of China) for the presence of monogenean parasites. The monogeneans were recovered from the gills with the help of fine dissection needles. Monogeneans were washed repeatedly in saline water and preserved in ethanol at 70% and 90% (molecular grade) for further morphological and molecular analyses, respectively. For morphological examination, parasites were heat-killed by using freshwater and, after that, preserved in 70% ethanol. Furthermore, they were stained with acetocarmine, dehydrated through ascending grades of ethanol (70, 90, and 100%), cleared in xylene, and finally mounted in Canada balsam. For the study of hard structures, they were placed with a drop of water on a slide, slightly flattened under a coverslip, fixed, and mounted in glycerin for examination. The overall prevalence and intensity of infection were calculated further in the taxonomic summary section and determined according to Bush et al. (1997).

For morphological examination, monogeneans were selected and processed according to Gussev (1973). Morphometric analyses were conducted using a compound microscope, the Olympus CH30 (Tokyo, Japan), equipped with AxioCam Erc5s (Olympus, Tokyo, Japan). Morphological identification was based on their morphological traits, such as body and organ shape and measurements of the hard parts of the haptor and reproductive organs, according to Kritsky et al. (1989). All measurements are in micrometers and given in the range, followed by the mean in parentheses with the standard deviation. A dash (-) indicated that measurements could not be made or were not available. The voucher specimens of monogenean parasites were deposited in the Museums, Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India (HS/mon/2022/10) and the Museum d'Histoire naturelle, Geneva, Switzerland (MHNG-PLAT-0144327).

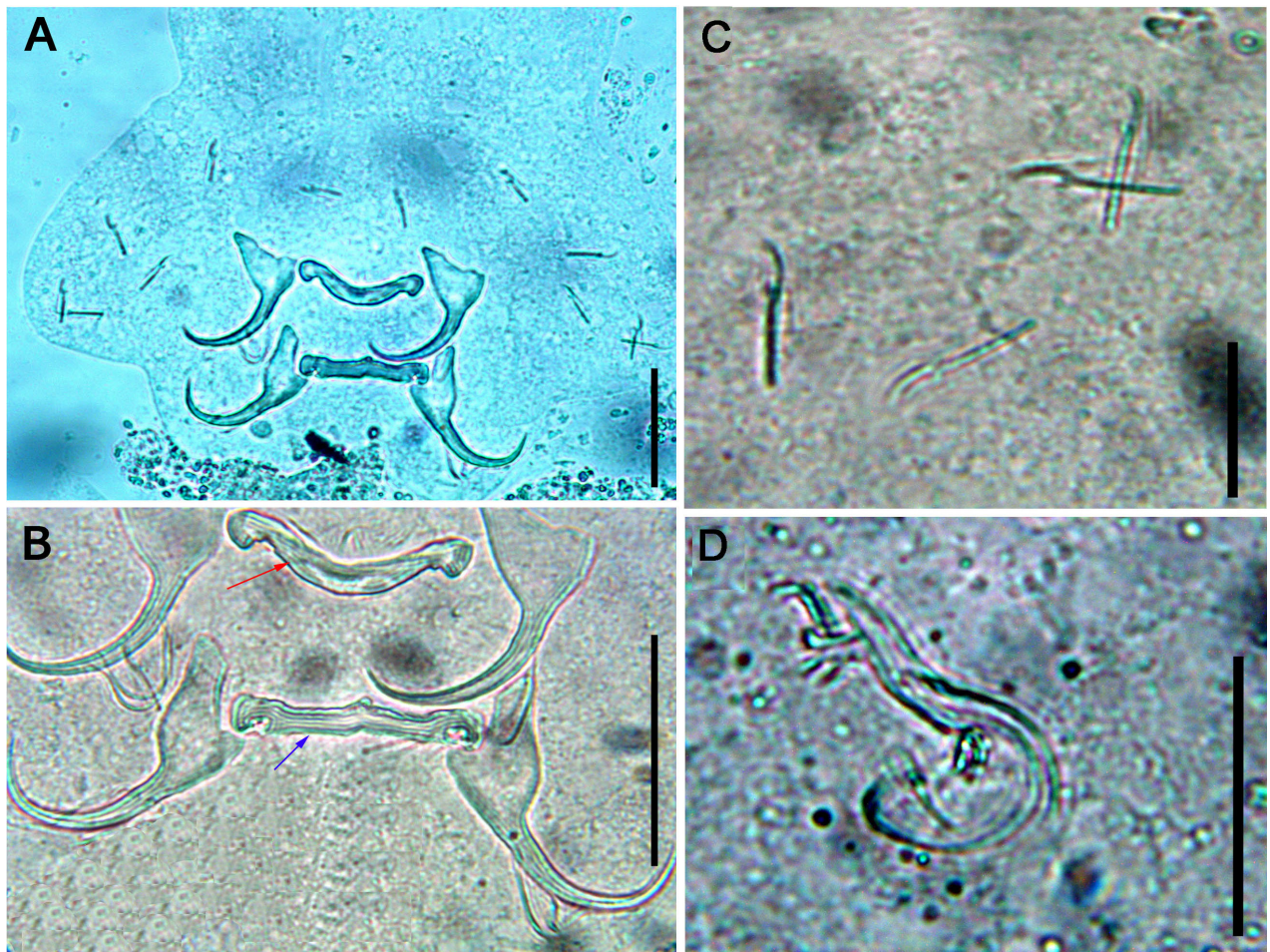
Genomic DNA of monogenean parasites ( $n = 2$ ) was extracted using the QIAGEN DNeasy™ Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer instructions. Sequences of the 18S rRNA gene of ribosomal RNA were amplified using the primers WormA+1270R and 930F+WormB (Littlewood and Olson 2001), while for the amplification of the 28S gene sequence, the primers Ancy55F+Ancy1200R (Plaisance et al. 2005) were used. Polymerase chain reactions (PCR) were performed according to Shinad et al. (2021) in 25  $\mu$ l reactions. The following thermocycling profile was applied: DNA denaturation (94 °C for 3 min); 35 cycles of amplification at 94 °C for 40 s, 55 °C for 45 s for all the mentioned primer sets; and 72 °C for 1 min extension with a final extension of 7 min at 72 °C and hold at 4 °C. Afterwards, PCR products were purified using a Purelink™ Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen,

**Table 1.** *Sciadicleithrum* species included in the phylogenetic analysis of 28S gene of rDNA with information on the host, locality and GenBank accession number. HNA = host name not available and asterisk shows species sequenced during the present study.

Species name	Host	Location	Accession no.	References
<i>Sciadicleithrum variabilum</i>	<i>Cichlasoma amazonarum</i>	Peru	ON261194	Seidlová et al. 2022
<i>Sciadicleithrum variabilum</i>	<i>Symphysodon aequifasciatus</i>	Brazil	ON261219	Seidlová et al. 2022
<i>Sciadicleithrum variabilum</i>	<i>Heros</i> sp.	Venezuela	ON261222	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 1	<i>Heros severus</i>	Venezuela	ON261221	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 2	HNA	Brazil	ON261206	Seidlová et al. 2022
<i>Sciadicleithrum iphthimum</i> *	<i>Pterophyllum scalare</i>	India	OQ800931, OQ822829	Present study
<i>Sciadicleithrum</i> sp. 3	<i>Kronoheros umbriferus</i>	Colombia	ON261220	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 4	<i>Crenicichla lenticulata</i>	Brazil	ON261207	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 5	<i>Crenicichla lugubris</i>	Brazil	ON261208	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 6	<i>Geophagus surinamensis</i>	Colombia	ON261210	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 7	<i>Geophagus surinamensis</i>	Colombia	ON261211	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 8	<i>Satanoperca daemon</i>	Brazil	ON261212	Seidlová et al. 2022
<i>Sciadicleithrum</i> sp. 9	<i>Satanoperca daemon</i>	Brazil	ON261213	Seidlová et al. 2022
<i>Sciadicleithrum ergensi</i>	<i>Cichla monoculus</i>	Peru	ON261216	Seidlová et al. 2022
<i>Sciadicleithrum ergensi</i>	<i>Cichla monoculus</i>	Peru	ON261219	Seidlová et al. 2022
<i>Sciadicleithrum umbilicum</i>	<i>Cichla monoculus</i>	Peru	ON261217	Seidlová et al. 2022
<i>Sciadicleithrum splendidae</i>	<i>Parachromis friedrichsthalii</i>	Mexico	KY305891	Mendoza-Palmero et al. 2017
<i>Sciadicleithrum splendidae</i>	<i>Parachromis friedrichsthalii</i>	Mexico	KY305890	Mendoza-Palmero et al. 2017
<i>Sciadicleithrum bravohollisae</i>	<i>Paraneetroplus fenestratus</i>	Mexico	KY305879	Mendoza-Palmero et al. 2017
<i>Sciadicleithrum bravohollisae</i>	<i>Paraneetroplus fenestratus</i>	Mexico	KY305878	Mendoza-Palmero et al. 2017
<i>Sciadicleithrum mexicanum</i>	<i>Amphilophus amarillo</i>	Nicaragua	OL960045	Santacruz et al. 2022
<i>Sciadicleithrum mexicanum</i>	<i>Rocio octofasciata</i>	Mexico	KY305887	Mendoza-Palmero et al. 2017
<i>Sciadicleithrum mexicanum</i>	<i>Rocio octofasciata</i>	Mexico	KY305886	Mendoza-Palmero et al. 2017
<i>Sciadicleithrumni caraguense</i>	<i>Cribroheros longimanus</i>	Nicaragua	OL960044	Santacruz et al. 2022
<i>Sciadicleithrumni caraguense</i>	<i>Cribroheros longimanus</i>	Nicaragua	OL960043	Santacruz et al. 2022
<i>Sciadicleithrum</i> sp. 10	<i>Amatitlania nigrofasciata</i>	Nicaragua	OL960046	Santacruz et al. 2022
<i>Sciadicleithrum meekii</i>	<i>Thorichthys meeki</i>	Mexico	KY305889	Mendoza-Palmero et al. 2017

Löhne, Germany). Amplicons were sequenced from both strands with the above-mentioned PCR primers using an ABI Big Dye Sequencing Kit (Applied Biosystems, Foster City, California, USA). For the consensus sequences of monogenean specimens of *S. iphthimum*, chromatograms of forward and reverse sequences were assembled and edited to remove poor-quality sequences on both ends using the Geneious Pro 5.4 platform (Kearse et al. 2012).

Newly generated sequences of the partial 18S rRNA gene were submitted to GenBank under the accession numbers (OQ800933, OQ800932, OQ822829, OQ800931). Two newly generated partial sequences of the 18S and 28S genes of *Sciadicleithrum* specimens were aligned using the default parameters of the Muscle algorithm implemented in Molecular Evolutionary Genetics Analysis (MEGA) version 11 (Tamura et al. 2021) along with other closely related sequences retrieved from the GenBank (see Table 1). To select the best model of evolution for the 18S and 28S datasets, the software jModelTest version 2.1.10 (Darriba et al. 2012) was used. Based on the Akaike Information Criteria (AIC), the software recovered GTR+I+G as the best-fitting evolutionary model. Phylogenetic analyses were run under Maximum Likelihood (ML) with 1000 bootstraps and Bayesian Inference (BI) criteria using MEGA version 11 and TOPALi version 2.5 software (Milne et al. 2009), respectively. Bayesian Inference (BI) trees were



**Figure 1.** Micrographs of *Sciadicleithrum ipthimum* from *Pterophyllum scalarae*. (A) Haptoral hard parts (B) An enlarged view of the dorsal (DB) and ventral bars (VB), VB indicated by red arrow while DB indicated by blue arrow (C) Marginal hooks (D) Male copulatory organ. Scale bars: 35  $\mu\text{m}$  (A, B), 15  $\mu\text{m}$  (C), 20  $\mu\text{m}$  (D). Photograph by Kalpana Km.

used based on running two independent MCMC runs of four chains for 10 million generations, then sampling tree topologies every 1000 generations, and the first 25% of the sampled trees were discarded as burn-in for each data set. The genetic divergence among monogenean spp. was estimated using the uncorrected “p-distances” (pairwise distances) method with the program MEGA version 11. Species of *Protygyrodactylus amacleithrium* (Price & McClellan, 1969) Gusev, 1973 and *Protygyrodactylus hainanensis* Pan, Ding & Zhang, 1995, were used as outgroups for the 18S and 28S rDNA genes, respectively.

## Results

Family Dactylogyridae Bychowski, 1933

Genus *Sciadicleithrum* Kritsky, Thatcher & Boeger, 1989

### *Sciadicleithrum ipthimum* Kritsky et al., 1989

Description (n = 9) (Figure 1: *Sciadicleithrum ipthimum* microphotograph). The monogeneans from 29 infected hosts were found with a range of 2–5 parasites per fish. Morphological measurements of the present specimens are

**Table 2.** A comparison of the morphometrics (measurements given in  $\mu\text{m}$ ) of *S. iphthimum* infected the freshwater exotic cichlid *Pterophyllum scalare* from India; ranges given in parentheses.

Body parts	<i>S. iphthimum</i>	<i>S. iphthimum</i>	<i>S. iphthimum</i>
Reference source	Kritsky et al. 1989 (N = 19)	Tripathi et al. 2010	Present study (N = 09)
Locality	Brazil	India	India
<b>Body:</b>			
Body length	329 (270–364)	252 (225–313)	363.98 (314.28–398.57)
Body width	92 (71–104)	113 (77–123)	75.36 (65.75–82.85)
Pharynx diameter	24 (20–27)	24 (18–26)	27.71 (20.01–36.67)
Haptor length	59 (52–65)	41 (36–46)	55.14 (48.57–62.85)
Haptor width	79 (57–88)	88 (86–100)	72.86 (65.71–81.42)
<b>Dorsal anchor:</b>			
Length	35 (33–37)	34 (31–37)	34.58 (31.67–36.67)
Width	17 (15–19)	–	15.31 (13.33–17.08)
<b>Dorsal bar:</b>			
Length	31(28–33)	31(23–29)	32.70 (30.83–34.16)
Width	–	–	4.30 (3.21–5)
<b>Ventral anchor:</b>			
Length	32 (27–35)	35 (32–38)	32.16 (30–34.16)
Width	19 (16–21)	34 (31–37)	17.91 (16.67–19.16)
<b>Ventral bar:</b>			
Length	29 (27–32)	26 (29–33)	31.60 (28.75–33.50)
Width	–	–	4.91 (4.16–5.41)
<b>Female reproductive organs:</b>			
Ovary length	55 (42–64)	50 (37–38)	62.76 (58.57–66.70)
Ovary width	17 (12–24)	26 (22–29)	24.99 (22.85–27.14)
Egg length	–	50	54.13 (50.85–60)
Egg width	–	35	43.39 (40–46.45)
<b>Male reproductive organs:</b>			
Testis length	–	30 (22–36)	27.85 (24.28–31.42)
Testis width	–	18 (13–21)	17.09 (14.29–20)
Copulatory tube length	24(23–25)	25 (21–27)	29.59 (26.10–32.76)
Accessory piece length	–	17 (14–19)	18.29 (16.33–20.10)
Hooks length:	12 (11–14)	12 (11–14)	12.58 (11.25–14.17)

given in the Table 2. Body fusiform comprises a maximum width at mid-length. Cephalic region broad, cephalic lobes moderately developed. Eyes divided into two pairs, with posterior pair being larger than anterior pair and located closer to spherical pharynx. Peduncle narrows, moderately long, with a sub hexagonal haptor. Anchor dissimilar, each with well-developed elongate superficial root, short deep root, knob like, shaft slightly curved, elongate point with well-developed sleeve sclerites. Dorsal anchor with broad base, elongated inner root, curved shaft and recurved point. Ventral anchor broader than dorsal anchor with poorly differentiated roots evenly curved shaft and recurved point. Straight dorsal bar present, broad ends with medial projection. Yoke-shaped ventral bar with bulbous end and cavities present on anterior bar margins. Hooks similar in length, shank with dilated end and filament hook loop present. Copulatory tube double-walled throughout its length with a maximum width at distal end, a poorly defined ring that frequently appears U-shaped present, and an accessory piece with a variable sheath. Vagina not observed.

#### *Taxonomic summary*

Host: Angelfish, *Pterophyllum scalare* (Schultze, 1823), (Perciformes: Cichlidae).

Previous record: *P. scalare* at Rio Solimoes near Marchantaria Island, Amazon, Brazil by Kritsky et al. (1989).

Locality: Meerut (29°01'N; 77°45'E), Uttar Pradesh, India.

Site of infection: Gill filaments.

Prevalence, and intensity of infection: 61.7% and 2.5.

Material deposited: Voucher specimens were submitted to the collection of the Museum, Department of Zoology, Chaudhary Charan Singh University, Meerut (U.P.), India (HS/mon/2022/10) and to the Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG-PLAT-0144327).

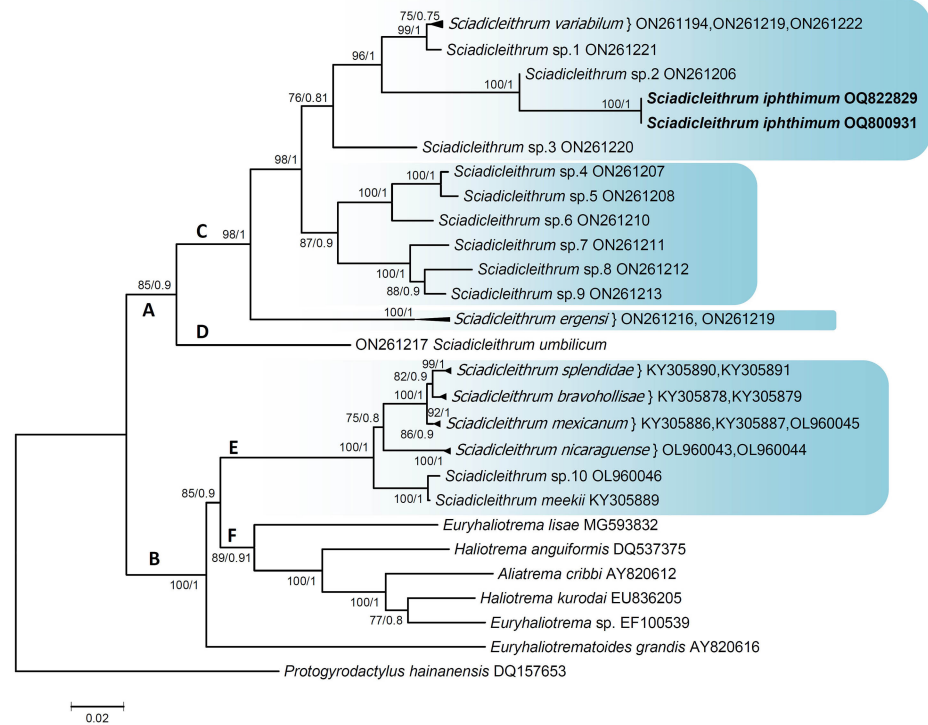
Molecular sequence data: For each gene, we have generated sequences from two isolates of *Sciadicleithrum iphthimum*: 18S gene OQ800933 (1700 bp), OQ800932 (1580 bp) and 28S gene, OQ822829 (786 bp), OQ800931 (875 bp).

### Remarks

Tripathi et al. (2010) redescribed the same species from *P. scalare* (Cichlidae), collected from aquarium markets in Uttar Pradesh, India, and provided detailed morphological structures like a non-sclerotized funnel-shaped vagina and an oval egg with a very short knob-like filament at the posterior end. Besides, Tripathi et al. (2010) mentioned the presence of two semi-distinct forms of *S. iphthimum*, some forms having hooks with slightly inflated shanks but some others having hooks with slender shanks that were deficient in the original description by Kritsky et al. (1989). After that, Bittencourt et al. (2014) also reported *S. iphthimum* from the same fish, *P. scalare*, from the Igarapé Fortaleza basin, eastern Amazon, Brazil. The specimens of *S. iphthimum* collected in the present study are similar to those of the original description by Kritsky et al. (1989), although in our specimens we also identified an anterior medial projection on the dorsal bar and well-developed sleeve sclerites present on the dorsal as well as on ventral anchors. In our study, we identified hooks of one type as similar to those described by Kritsky et al. (1989) as having slender shanks, although Tripathi et al. (2010) describe two types of hooks. A comparative analysis of measurements of body parts of the *S. iphthimum* isolates from different studies is shown in Table 2. The present specimens are longer in comparison to the specimens of Kritsky et al. (1989) and Tripathi et al. (2010) (Table 2). The length of the dorsal anchor and bar is more or less similar to our specimens. The ventral anchor and bar length are also almost the same as in the present study specimens (Table 2). In regard to the male copulatory organ, the copulatory tube of the present isolates is much closer to the specimens of Tripathi et al. (2010). The lengths of the hooks are more or less similar (Table 2).

### Molecular analyses

In the phylogeny of 18S and 28S rRNA sequences amplified; only the 28S gene was used for analysis because the 18S tree did not provide much information



**Figure 2.** Tree topology based on partial 28S ribosomal DNA sequences of *Sciadicleithrum* species. GenBank accession numbers precede species names. Species sequenced in the present study is shown in bold. The alphabets represent the well-supported clades. The support values are composed above the nodes as follows: ML/BI. Bootstrap score and posterior probabilities values below 75 or 0.75 are not considered well-supported. The branch length scale bar indicates the number of substitutions per site.

in comparison to the 28S, which gives a better picture than the other genes (Figure 2) available for *Sciadicleithrum* spp. to compare. The alignment of *S. iphthimum*, using the 28S rRNA gene, was used for phylogenetic analysis. The tree topologies based on the ML and BI methods were identical; therefore, the ML tree is represented with ML bootstrap (BS) and BI probability (PP) values at the nodes of phylogenetic trees (Figure 2). The phylogenetic reconstruction of 28S gene sequences results in a well-supported tree for *Sciadicleithrum* species (Figure 2). The details about the host species and locality are shown in Table 1. The tree topology based on the alignment of the 28S rRNA gene produced two main clades, A and B (Figure 2). The main clade A was well supported by bootstrap support and Bayesian inference posterior probabilities (BS = 85; PP = 0.9) (Figure 2). Clade A comprises *Sciadicleithrum* spp. from Peru, Brazil, Venezuela, and Colombia (Seidlová et al. 2022), including specimens of *S. iphthimum* from India, all parasites of cichlid hosts (Table 1, Figure 2). Main clade B was also well supported by bootstrap values and Bayesian posterior probabilities (BS = 100; PP = 1) and was divided into two subclades E and F. Clade E included the parasites of cichlid hosts from Mexico and Nicaragua (Mendoza-Palmero et al. 2017; Santacruz et al. 2022) (Table 1, Figure 2). Clade F is related and comprises a sister group to the *Sciadicleithrum* spp. (Figure 2). *Sciadicleithrum* spp. (ON261221 and ON261206), *Sciadicleithrum*



*variabilum* (Mizelle and Kritsky, 1969), Kritsky et al. (1989) from Brazil, Peru, and Venezuela (ON261194, ON261219, and ON261222), all generated by Seidlová et al. (2022), along with Indian isolates of *S. iphthimum*, show genetic divergence in a range of 0.10 to 0.13 (Figure 2).

## Discussion

Biological invasions are now attracting more attention due to their impact on indigenous fauna and global environmental changes (Simberloff 2013; Shvydka et al. 2020). In the Indian region, less research effort has been put into studies pertaining to invasive parasites. Therefore, it is no surprise that the co-introduced parasites commonly travel along with their invasive host in the Indian region. We need a more complete approach to resolving the origin and identification of invasive species that should be supplemented with molecular data from co-introduced parasites that can make it easier to trace and investigate invasions. One of the most species-rich fish families is the Cichlidae (Heckel, 1840), with 202 genera and more than 1,700 spp. (Nelson et al. 2016; Fricke et al. 2019). The main geographical areas of cichlid distribution are mainly Africa and Neotropical America, and the family Cichlidae represents a very diverse group in terms of ecology, morphology, and behavior. Their development has been marked by rapid speciation and adaptive radiation (Kullander 2003; Kocher 2004).

To the best of our knowledge, to date, 26 species of the genus *Sciadicleithrum* have been reported worldwide (Paschoal et al. 2016). From *P. scalare*, *S. iphthimum* was described by Kritsky et al. (1989) at Rio Solimoes, near Marchantaria Island, Amazon, Brazil. The species was then reported from India by Tripathi et al. (2010) and by Bittencourt et al. (2014) in Brazil. Later, this species was also reported by Mendoza-Franco et al. (2018) from Mexico. In previous studies, Seidlová et al. (2022) examined the phylogeny of *Sciadicleithrum* spp. and concluded that *Sciadicleithrum* is a polyphyletic genus. This is in agreement with our study showing that *Sciadicleithrum* form divergent phylogenetic clades (Figure 2). Furthermore, it is also consistently suggested that the addition of more molecular data from different geographical regions for *Sciadicleithrum* spp. into phylogenetic analyses will shed more light. The molecular divergence of *Sciadicleithrum* spp. between them and their establishment in the tree could be due either to the host closeness or their geographical regions. It is hard to infer at this level of data scarcity, but it goes more to the side of geographical locality. In clade A, all the *Sciadicleithrum* spp. belongs to the South American countries, i.e., Brazil, Colombia, Peru, and Venezuela, including our monogenean specimens that were also co-introduced from some of these South American countries to India. Despite this, in clade E, *Sciadicleithrum* spp. belong to all the hosts that were distributed in Mexico and Nicaragua on the basis of the phylogenetic reconstruction in the present study, which suggests their closeness according to the closeness of the geographical locality.

However, our results showed that *S. iphthimum* 28S analyses represent distinct lineages and predict its validation with good BI and PP values. We believe that the present study is the first to deduce the phylogenetic relationship of *S. iphthimum* using the 28S gene, which was used to complement the morphological characterization. The results showed that the various *Sciadicleithrum* spp. from diverse cichlid hosts are distributed in different geographical regions and are genetically closely related, while *S. iphthimum* forms a distinct clade from other congeneric species. We also mentioned here that the present data belongs to *S. iphthimum* from India and were found close to *Sciadicleithrum* sp. 2 from Brazil, collectively situated in clade A that comprises countries i.e., Brazil, Colombia, Peru, and Venezuela of the Amazon River basin. In this study, we predicted that this parasite is imported in India from Brazil, and even though minor morphological variations were found, specimens from India and Brazil showed clear similarities. Considering previous records of *S. iphthimum*, it is suggested that further studies should be corroborated with molecular data.

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### Author's contribution

KK was responsible for sample collection, morphological investigation, and analysis. KG and HRC are helping with the interpretation of the data. KK and AC drafted the initial manuscript. AC, BS, and HSS are responsible for critically reviewing the manuscript. The authors declare that there is no conflict of interest.

### Ethics and permits

All national and institutional guidelines for the care and use of animals were followed.

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