

Supplement

Supplement S1. Polymerase chain reaction (PCR) conditions and primers used to amplify the domains D2 and D3 of the nuclear 28S rRNA gene in the present study.

Primers (also used for sequencing): D2A (5'-ACAAGT ACC GTG AGG GAA AGT-3') forward + D3B (5'-TGC GAA GGA ACC AGC TAC TA-3') reverse

PCR reactions (25 µl total volume) consisted of: 2.5 µl of 10× PCR buffer minus MgCl₂, 1.5 µl of MgCl₂ (50mM), 2 µl of dNTPs (2.5mM), 0.25 µl of each primer (10mM), 0.2 µl of Platinum Taq DNA polymerase (5 U/µl) (Invitrogen), 0.25 µl of BSA (10 mg / mL), 16.0 µl of water and 2.0 µl of genomic DNA

PCR cycling conditions: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, annealing at 50°C for 1min and elongation at 72°C for 1 min, followed by a post-amplification extension step at 72°C for 5 min

Note: Attempts to amplify the 18S rRNA failed probably due to DNA fragmentation, since samples were fixed in formalin and genetic markers were to amplify a long fragment (\approx 1,800bp).

Supplement S2. Details on the definition of morphological / life history characters and states used in the present study.

Morphological and life history traits matrix of all representatives used in the present study (see as follows the details on characters and their respective states).

Species	Cuticle surface	Cephalic outgrowth	Number of outer circle cephalic papillae	Number of inner circle cephalic papillae	Peribuccal ring of teeth	Esophageal teeth	Esophagus structure	Esophageal gland	Ventriculus	Vulva	Protusions on female tail	Structure of gubernaculum distal end	Relative size of spicules	Pair of far anterior precloacal papillae	Structure of male tail	Site of infection of gravid females	Habitat	Host Order	Geographic Origin	References
<i>Afrophilometra hydrocyoni</i>	1	0	0	2	0	0	1	1	1	0	1	?	?	?	?	0	1	3	2	[1]
<i>Alinema amazonicum</i>	0	0	0	2	1	0	1	2	1	1	2	2	1	1	0	1	1	1	0	[2, 3]
<i>Caranginema americanum</i>	1	0	0	1	0	1	2	1	1	0	1	1	0	0	1	2	0	H	8	[4, 5]
<i>Digitiphilometroides marinus</i>	1	0	0	2	0	0	1	1	1	0	1	?	?	?	?	1	0	H	4	[6, 7]
<i>Nilonema senticosum</i>	1	0	0	0	0	0	0	1	0	0	0	?	?	?	?	1&4	1	5	0	[3, 8]
<i>Philometra arafurensis</i>	0	0	0	2	0	0	1	1	1	0	1	4&6	0	0	2	3	0	B	4	[7]
<i>Philometra bagri</i>	0	0	1	2	0	0	1	1	1	0	1	?	?	?	?	2&5	1	1	2	[1, 9]
<i>Philometra brevispicula</i>	0	0	0	2	0	0	1	1	1	?	1	1	0	0	1	5	0	B	5	[10]
<i>Philometra cyprinirutili</i>	0	0	0	2	0	0	1	1	?	0	1	2	1	0	1	1	1	0	1	[11–14]
<i>Philometra diplectri</i>	0	0	0	2	0	0	1	1	1	0	0	1	0	0	1	5	0	E	8	[15]
<i>Philometra floridensis</i>	0	0	0	2	0	0	1	1	1	0	0	2	1	0	2	3	0	B	8	[16]
<i>Philometra globiceps</i>	0	0	1	2	0	0	1	1	1	0	0	4&6	0	0	2	3	0	E	5	[17]
<i>Philometra gracilis</i>	0	0	0	2	0	0	1	1	1	0	1	?	?	?	?	5	0	B	4	[18]
<i>Philometra gymnosardae</i>	0	0	0	1	0	0	1	1	1	0	1	?	1	0	3	1	0	A	4	[19]
<i>Philometra iraqiensis</i>	0	0	0	2	0	0	1	1	1	0	1	?	?	?	?	1&3	0	7	4	[20]
<i>Philometra kotlani</i>	0	0	0	2	0	0	1	1	?	?	1	2	0	0	2	1	1	0	1	[21–27]
<i>Philometra lagocephali</i>	0	0	0	2	0	0	1	1	1	0	0	?	?	?	?	1	0	4	4	[28, 29]
<i>Philometra lateolabracis</i>	0	0	0	2	0	0	1	1	1	0	1	4&6	0	0	3	3	0	C	6	[1,30]
<i>Philometra latii</i>	0	0	0	2	0	1	1	1	1	0	0	?	?	?	?	1	1	D	2	[1]
<i>Philometra longa</i>	0	0	0	2	0	0	1	1	1	0	0	3&4	0	0	2	1	0	8	7	[31]
<i>Philometra madai</i>	0	0	0	1	0	0	1	1	1	0	0	4	1	0	3	3	0	B	6	[30, 32]
<i>Philometra morii</i>	1	0	0	2	0	1	1	1	1	0	1	2	1	0	1	2&5	0	E	5	[33]
<i>Philometra nattereri</i>	0	0	0	2	0	0	2	1	1	0	1	?	?	?	?	5	1	3	0	[34]
<i>Philometra nemipteri</i>	0	0	0	1	0	0	1	1	1	0	0	4	1	0	3	3	0	B	6	[35]
<i>Philometra obturans</i>	1	0	1	1	0	0	1	1	?	0	1	2	1	0	1	5&6	1	6	1	[36–38]
<i>Philometra ocularis</i>	0	0	1	1	0	0	1	1	1	0	1	?	?	?	?	5	0	E	4	[39–44]
<i>Philometra ovata</i>	0	0	1	2	0	0	1	1	1	0	1	2	1	0	2	1	1	0&F&G	1	[45–47]
<i>Philometra pellucida</i>	0	0	0	2	0	0	1	1	1	0	0	?	?	?	?	1	0	4	4	[48–50]

<i>Philometra rara</i>	0	0	1	2	0	0	1	1	1	0	1	4&6	0	0	2	3	0	E	4	[51]
<i>Philometra rischta</i>	0	0	0	2	0	0	1	1	1	0	1	3	0	0	1	2&5	1	0	1	[12, 23, 52]
<i>Philometra saltatrix</i>	0	0	0	2	0	0	1	1	1	0	1	5	0	0	2	3	0	A	5	[51, 53–55]
<i>Philometra sciaenae</i>	0	0	0	2	0	0	1	1	1	0	0	4	0	0	3	3	0	B	6	[32, 35]
<i>Philometra spiriformis</i>	0	0	0	2	0	0	1	1	1	0	1	?	?	?	?	5	1	D	2	[1]
<i>Philometroides grandipapillatus</i>	1	0	2	2	0	0	1	1	1	0	0	?	?	?	?	0&2	0	H	6	[10]
<i>Philometroides moravecí</i>	1	0	0	2	0	1	2	1	1	0	1	2	0	0	2	2&5	1	G	1	[56, 57]
<i>Philometroides sanguineus</i>	1	0	0	?	0	1	1	1	?	0	0	2	0	?	2	0	1	0	1	[58, 59]
<i>Philometroides seriolae</i>	1	0	0	1	0	0	1	1	1	0	0	?	?	?	?	7	0	H	6	[60, 61]
<i>Philometroides stomachicus</i>	1	0	0	2	0	0	1	1	1	?	0	?	?	?	?	8	0	B	4	[18]
<i>Rumai rumai</i>	0	1	0	2	0	0	0	0	0	0	0	?	?	?	?	5	1	5	0	[8]
<i>Philonema oncorhynchi</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	9	1	[62]

Description of characters and their respective states that were used for phylogenetic analyses

CHARACTER: Cuticle surface

Refers to the presence of ornamentations in the cuticle.

States:

0 = Smooth at least in one sex

1 = With cuticular ornamentations at least in one sex

CHARACTER: Cephalic outgrowth

Refers to the presence of apical outgrowth at cephalic end.

States:

0 = Present

1 = Absent

CHARACTER: Number of outer circle cephalic papillae

Refers to the number of papillae of outer circle, at cephalic end.

States:

0 = 8 papillae in double pairs not fused at least in one sex

1 = 8 papillae in double pairs fused at least in one sex

2 = 8 papillae (still 4) close one another forming dorsal and ventral rows

CHARACTER: Number of inner circle cephalic papillae

Refers to the number of papillae of inner circle, surrounding the oral opening at cephalic end, excluding the amphids.

States:

0 = 2 papillae single

1 = 4 papillae single

2 = > 4 papillae single (6 papillae)

CHARACTER: Peribuccal ring of teeth

Refers to the presence of ring of teeth around the oral opening.

States:

0 = Absent

1 = Present

CHARACTER: Esophageal teeth

Refers to the presence of teeth in the lobes of esophagus.

States:

0 = Absent

1 = Present

CHARACTER: Esophagus structure

Refers to the morphology of esophagus, regarding presence or absence of anterior bulbous inflation.

States:

0 = Not swollen near oral opening in female

1 = With anterior bulbous inflation not separated from remaining part of oesophagus in female

2 = With anterior bulbous inflation well separated from remaining part of oesophagus in female, or with any specialization

CHARACTER: Esophageal gland

Refers to the presence or absence of esophageal gland, including the morphology of this gland.

States:

0 = Absent

1 = Unicellular esophageal gland

2 = Multicellular esophageal gland (more than one nucleus)

CHARACTER: Ventriculus

Refers to the presence or absence of a well-developed ventriculus, between esophagus and intestine.

States:

0 = Absent

1 = Present

CHARACTER: Vulva

Refers to the presence or absence of functional vulva.

States:

0 = Absent

1 = Present

CHARACTER: Protusions on female tail

Refers to the presence of caudal projection on female tail, including the number of projections.

States:

0 = Absent

1 = 2 papilla-like protusions

2 = 4 papilla-like protusions

CHARACTER: Structure of gubernaculum distal end

Refers to the shape of the gubernaculum distal end, including the presence of ornamentations.

States:

0 = Absent

1 = Smooth (in a shape of spear lacking ornamentation and dorsal barbs)

2 = 1 dorsal barb on distal end

3 = Two dorsal barbs on distal end

4 = Distal end with lamellate-like structures (ornamentations)

5 = Transverse annulation on dorsal side present

6 = With dorsal protuberance

CHARACTER: Relative size of spicules

Refers to the relative size of spicules in male.

States:

0 = Equal

1 = Unequal

CHARACTER: Pair of far anterior precloacal papillae

Refers to the presence or absence of anterior precloacal papillae.

States:

0 = Absent

1 = Present

CHARACTER: Structure of male tail

Refers to the shape of male tail, regarding the presence and the morphology of caudal mound.

States:

0 = Absent

1 = Caudal mound unseparated in lobes

2 = Caudal mound separated in two lobes

3 = Caudal mound separated in more than two lobes

CHARACTER: Site of infection of gravid females

Refers to the tissue/organ, where the gravid females were found.

States:

0 = Fins

1 = Body cavity including connective tissues (e.g. mesentery)

2 = Subcutaneous tissue including superficial muscles

3 = Gonads

4 = Swimbladder

5 = Head tissues

6 = Circulatory system

7 = Musculature of body

8 = Stomach wall

CHARACTER: Habitat

Refers to the type of environment that in which parasite host inhabits.

States:

0 = Marine

1 = Freshwater

CHARACTER: Host Order

Refers to the order or (*misc.*) of fish host.

States:

0 = Cypriniformes

1 = Siluriformes

3 = Characiformes

4 = Tetraodontiformes

5 = Osteoglossiformes

6 = Esociformes

7 = Mugiliformes

8 = Belontiiformes

9 = Salmoniformes

A = Scombriformes

B = Eupercaria

C = Acropomatiformes

D = Carangaria

E = Perciformes

F = Cupleiformes

G = Gobiiformes

H = Carangiformes

CHARACTER: Geographic Origin

Refers to biogeographic occurrence of species.

States:

0 = Neotropical

1 = Palaearctic

2 = Afro-Tropical

4 = Tropical Indo-Pacific

5 = Temperate Northern Atlantic

6 = Temperate Northern Pacific

7 = Temperate Australasia

8 = Tropical Atlantic

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Table S1. Species of fish hosts used in the cophylogenetic analysis, associated with their order (or *misc*), family and GenBank accession numbers.

Host species	Order (or <i>misc</i>)	Family	<i>cox1</i> mtDNA
<i>Arapaima gigas</i>	Osteoglossiformes	Osteoglossidae	NC010570*
<i>Arothron stellatus</i>	Tetraodontiformes	Tetraodontidae	NC056962*
<i>Bagrus bajad</i>	Siluriformes	Bagridae	LC487110
<i>Calophrysus macropterus</i>	Siluriformes	Pimelodidae	KT952410
<i>Carassius gibelio</i>	Cypriniformes	Cyprinidae	NC014177*
<i>Caranx hippos</i>	Carangiformes	Carangidae	FJ347905
<i>Diplectrum formosum</i>	Perciformes	Serranidae	JN312338
<i>Epinephelus morio</i>	Perciformes	Serranidae	KF836470
<i>Epinephelus septemfasciatus</i>	Perciformes	Serranidae	HM593054
<i>Esox lucius</i>	Esocidae	Esociformes	NC004593*
<i>Gobio gobio</i>	Cypriniformes	Gobionidae	NC008662*
<i>Gymnosarda unicolor</i>	Scombriformes	Scombridae	AP012510*
<i>Hydrocynus forskahlii</i>	Characiformes	Alestidae	JF800983
<i>Hyporhamphus australis</i>	Beloniformes	Hemiramphidae	KX781932
<i>Hyporthodus haifensis</i>	Perciformes	Epinephelidae	KT023567
<i>Lagocephalus sceleratus</i>	Tetraodontiformes	Tetraodontidae	JQ681800
<i>Lateolabrax japonicus</i>	Acropomatiformes	Lateolabracidae	HM180646
<i>Lates niloticus</i>	Carangaria	Latidae	DQ108015
<i>Leuciscus aspius</i>	Cypriniformes	Leuciscidae	HM392013
<i>Lutjanus johnii</i>	Eupercaria	Lutjanidae	EU148537
<i>Lutjanus griseus</i>	Eupercaria	Lutjanidae	GU224916
<i>Lutjanus sebae</i>	Eupercaria	Lutjanidae	EU600104
<i>Nemipetrus virgatus</i>	Eupercaria	Nemipteridae	FJ237833
<i>Oncorhynchus nerkae</i>	Salmoniformes	Salmonidae	MK216599
<i>Pagrus major</i>	Eupercaria	Sparidae	FJ237863
<i>Pennahia argentina</i>	Eupercaria	Sciaenidae	AB547245
<i>Percottus glenii</i>	Gobiiformes	Odontobutidae	KX224108
<i>Planiliza klunzingeri</i>	Mugiliformes	Mugilidae	MT943741
<i>Pomatomus saltatrix</i>	Scombriformes	Pomatomidae	AB355904*
<i>Protonibea diacanthus</i>	Eupercaria	Sciaenidae	DQ107820
<i>Pseudaspius leptocephalus</i>	Cypriniformes	Leuciscidae	LC277264
<i>Pygocentrus nattereri</i>	Characiformes	Serrasalminidae	KU288904
<i>Rachycentron canadum</i>	Carangiformes	Rachycentridae	EF609583
<i>Rutilus rutilus</i>	Cypriniformes	Leuciscidae	ON097817
<i>Sciaenops ocellatus</i>	Eupercaria	Sciaenidae	EU752180
<i>Seriola quinqueradiata</i>	Carangiformes	Carangidae	MK560632
<i>Uranoscopus scaber</i>	Perciformes	Uranoscopidae	KM538637

*Sequences retrieved from whole mitochondrial genome.

Table S2. Correspondence between abbreviations and species names from fish hosts and philometrid parasites, used in the cophylogenetic analysis (see Figure 3A of the manuscript).

Abbreviation	Host species	Abbreviation	Parasite species
Arag	<i>Arapaima gigas</i>	Afrh	<i>Afrotylometra hydrocyoni</i>
Aros	<i>Arothron stellatus</i>	Alina	<i>Alinema amazonicum</i>
Bagb	<i>Bagrus bajad</i>	Cara	<i>Caranginema americanum</i>
Calom	<i>Calophrys macropterus</i>	Digm	<i>Digitipilometroides marinus</i>
Carag	<i>Carassius gibelio</i>	Nils	<i>Nilonema senticosum</i>
Carh	<i>Caranx hippos</i>	Phidg	<i>Philometroides grandipapillatus</i>
Dipf	<i>Diplectrum formosum</i>	Phidm	<i>Philometroides moravecii</i>
Epim	<i>Epinephelus morio</i>	Phids	<i>Philometroides sanguineus</i>
Epis	<i>Epinephelus septemfasciatus</i>	Phidse	<i>Philometroides seriola</i>
Esol	<i>Esox lucius</i>	Phidst	<i>Philometroides stomachicus</i>
Gobg	<i>Gobio gobio</i>	Phila	<i>Philometra arafurensis</i>
Gymu	<i>Gymnosarda unicolor</i>	Philba	<i>Philometra bagri</i>
Hydf	<i>Hydrocynus forskahlii</i>	Philbr	<i>Philometra brevispicula</i>
Hypa	<i>Hyporhamphus australis</i>	Phile	<i>Philometra cyprinirutili</i>
Hypoh	<i>Hyporthodus haifensis</i>	Phild	<i>Philometra diplectri</i>
Lags	<i>Lagocephalus sceleratus</i>	Philf	<i>Philometra floridensis</i>
Latj	<i>Lateolabrax japonicus</i>	Philgl	<i>Philometra globiceps</i>
Latn	<i>Lates niloticus</i>	Philgr	<i>Philometra gracilis</i>
Leua	<i>Leuciscus aspius</i>	Philgy	<i>Philometra gymnosardae</i>
Lutj	<i>Lutjanus johnii</i>	Phili	<i>Philometra iraqiensis</i>
Lutjg	<i>Lutjanus griseus</i>	Philk	<i>Philometra kotlani</i>
Luts	<i>Lutjanus sebae</i>	Phillate	<i>Philometra lateolabracis</i>
Nemv	<i>Nemipetrus virgatus</i>	Phillati	<i>Philometra lati</i>
Oncn	<i>Oncorhynchus nerkae</i>	Phillg	<i>Philometra lagocephali</i>
Pagm	<i>Pagrus major</i>	Phillo	<i>Philometra longa</i>
Pena	<i>Pennahia argentina</i>	Philma	<i>Philometra madai</i>
Perg	<i>Percottus glenii</i>	Philmo	<i>Philometra morri</i>
Plak	<i>Planiliza klunzingeri</i>	Philna	<i>Philometra nattereri</i>
Poms	<i>Pomatomus saltatrix</i>	Philne	<i>Philometra nemipteri</i>
Prod	<i>Protonibea diacanthus</i>	Philob	<i>Philometra obturans</i>
Psel	<i>Pseudaspius leptocephalus</i>	Philoc	<i>Philometra oculares</i>
Pygn	<i>Pygocentrus nattereri</i>	Philoon	<i>Philomena oncorhynchi</i>
Racc	<i>Rachycentron canadum</i>	Philov	<i>Philometra ovata</i>
Rutr	<i>Rutilus rutilus</i>	Philp	<i>Philometra pellucida</i>
Scio	<i>Sciaenops ocellatus</i>	Philra	<i>Philometra rara</i>
Serq	<i>Seriola quinqueradiata</i>	Philri	<i>Philometra rischta</i>
Uras	<i>Uranoscopus scaber</i>	Philsa	<i>Philometra saltatrix</i>
		Philsc	<i>Philometra sciaenae</i>
		Philsp	<i>Philometra spiriformis</i>
		Rumar	<i>Rumai rumai</i>