The following supplement accompanies the article

Podocotyle atomon (Trematoda: Digenea) impacts reproductive behaviour, survival and physiology in *Gammarus zaddachi* (Amphipoda)

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Supplementary data

Table S1. Primers used for the molecular identification of trematode specimens

Primer	Sequence (5'–3')	PCR settings		Gene	Reference
		Temp. (°C)	Cycles		
Worm-A Worm-B	RCGAATGGCTCATTAAATCAG ACGGAAACCTTGTTACGACT	56.5	40	ssrDNA (18S)	Olson et al. (2003)
LSU-5 1500R	TAGGTCGACCCGCTGAAYTTAAGCA GCTATCCTGAGGGAAACTTCG	56.5	40	lsrDNA (28S)	Olson et al. (2003)
GA1 L5	AGAACATCGACATCTTGAAC TTCACTCGCCATTACT	45	35	5.8S and ITS2	Petkevičiūtė et al. (2004)

Table S2. GenBank accession numbers for the sequence data used in the phylogenetic analysis

Species	GenBank ID		Reference(s)
	18S	28S	
Opecoelidae			
Bathypodocotyle margolisi	KU320583	KU320596	Bray et al. (2016), Martin et al. (2018a)
Buticulotrema thermichthysi	KF733987	KF733984	Bray et al. (2012)
Halosaurotrema halosauropsi	AJ287514	AY222207	Martin et al. (2018a)
Scorpidotrema longistipes	MK052939	MK052936	Martin et al. (2018a)
Holsworthotrema enboubalichthys	MK052940	MK052938	Martin et al. (2018a)
Holsworthotrema choaderma	MK052941	MK052937	Martin et al. (2018a)
Anomalotrema koiae	KU320582	KU320595	Bray et al. (2016)
Pseudopecoeloides tenuis	KU320592	KU320605	Bray et al. (2016)
Allopodocotyle sp. A	KU320586	KU320599	Bray et al. (2016)
Maculifer sp.	AY222109	AY222211	Olson et al. (2003)
Magnaosimum brooksae	MG813906	MG813907	Martin et al. (2018b)
Peracreadium idoneum	AJ287558	AY222209	Olson et al. (2003), Cribb et al. (2001)
Propycnadenoides philippinensis	KU320591	KU320604	Bray et al. (2016)
Pseudoheterolebes stellaglobulus	MH933882	MH933877	Martin et al. (2018c)
Allopodocotyle epinepheli	KU320585	KU320598	Bray et al. (2016)
Bentholebouria blatta	KU320593	KU320606	Bray et al. (2016)
Hamacreadium mutabile	KU320588	KJ001209	Bray et al. (2016)
Macvicaria macassarensis	AJ287533	AY222208	Olson et al. (2003), Cribb et al. (2001)
Pacificreadium serrani	KU320589	KU320602	Bray et al. (2016)
Pedunculacetabulum inopinipugnus	MF805699	MF805700	Martin et al. (2018d)
Podocotyloides gracilis	MF805692	MF805693	Martin et al. (2018d)
Choerodonicola arothokoros	MG844417	MG844418	Martin et al. (2018e)
Podocotyloides parupenei	MF926408	MF926409	Martin et al. (2018d)
Trilobovarium parvvatis	KY551561	KY551562	Martin et al. (2017)
Polypipapiliotrema citerovarium	MH823962	MH823957	Martin et al. (2018f)
Polypipapiliotrema stenometra	MH823959	MH823954	Martin et al. (2018f)
Polypipapiliotrema ovatheculum	MH823961	MH823956	Martin et al. (2018f)
Polypipapiliotrema hadrometra	MH823960	MH823955	Martin et al. (2018f)
Helicometra boseli	KU320587	KU320600	Bray et al. (2016)
Outgroups			
Zalophotrema hepaticum (Brachycladiidae)	AJ224884	AY222255	Olson et al. (2003), Cribb et al. (2001)
Stephanostomum pristis (Acanthocolpidae)	DQ248209	DQ248222	Bray et al. (2005)

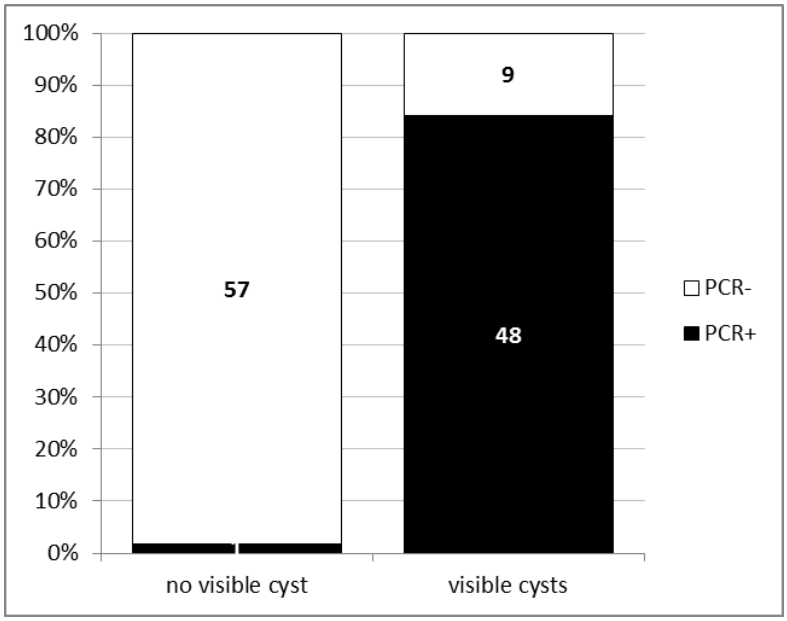

Fig. S1. Evaluation of trematode prevalence: Cyst observation versus PCR. Two methods were compared to evaluate the prevalence of the trematode in *G. zaddachi*. At first, PCRs were performed using primers specific to Digenea (GA1/L5, Petkevičiūtė et al. 2004; Table S1), on DNA extracted from the full bodies of 115 animals in order to detect the presence of such parasite in each animal. PCR-negative samples were screened on a minimum of three occasions. Presence of DNA in these extracts was checked using the primers Ef/Er, which are widely used to amplify amphipods 18S rDNA (Englisch et al. 2003). In the absence of amplification using these primers, we considered that DNA quality and/or DNA concentration was not good enough to to look for the presence of trematodes by PCR. PCR conditions for GA1/L5 and Ef/Er primers are those described in Petkeviciute et al. (2004) and Slothouber Galbreath et al. (2010) respectively. No amplification was obtained from negative controls (deionized water). Additionally, visible presence of cysts was also recorded for each animal. Concordance between the presence of cysts and PCR results was then evaluated. Generally, the two methods agreed, with 84.2% of the animals that had one or more visible cysts (n = 57) being PCR-positive and only one animal with no visible cysts (n = 58) being PCR-positive. All the animals which were PCR-negative, but visibly infected, had only one cyst. It was concluded that in these cases, either the parasite had died, or the parasite/host DNA ratio was too weak to detect presence of the trematode by PCR. Presence of visible cysts was deemed the more reliable criteria to evaluate trematode infections in *G. zaddachi* and was used for subsequent experiments.

Table S3. Results of BLAST analyses performed on 18S, 28S and 5.8S/ITS2 sequences obtained from the *G. zaddachi* trematode. Best hits (with highest similarity scores) are shown for each sequence.

Species (GenBank accession number)	Subfamily	Reference(s)	Coverage (%)	Similarity (%)	e-value
18S					
Opecoelidae sp. (AY218105)	Unknown	Giribet et al (2004)	100	99	0.0
Halosautrema halosauropsi (AJ287514)	Podocotylinae	Martin et al. (2018a)	100	99	0.0
Buticuloterma thermichthysi (KF733987)	Podocotylinae	Bray et al. (2012), Martin et al. (2018a)	100	99	0.0
Bathypodocotyle margolisi (KU320583)	Podocotylinae	Martin et al. (2018a)	100	99	0.0
28S					
Podocotyle atomon (MH161437)	Podocotylinae	Sokolov et al. (2018), Martin et al. (2018a)	99	98	0.0
5.8S/ITS2					
Opecoelidae gen. sp. (AJ241809)	Unknown	Jousson et al. (1999)	61	94	0.0
Podocotyle scorpaenae (AJ241794)	Unknown	Jousson et al. (1999)	62	94	0.0
Podocotyloides brevivesiculatus (MF805697)	Unknown	Martin et al. (2018d)	62	94	0.0

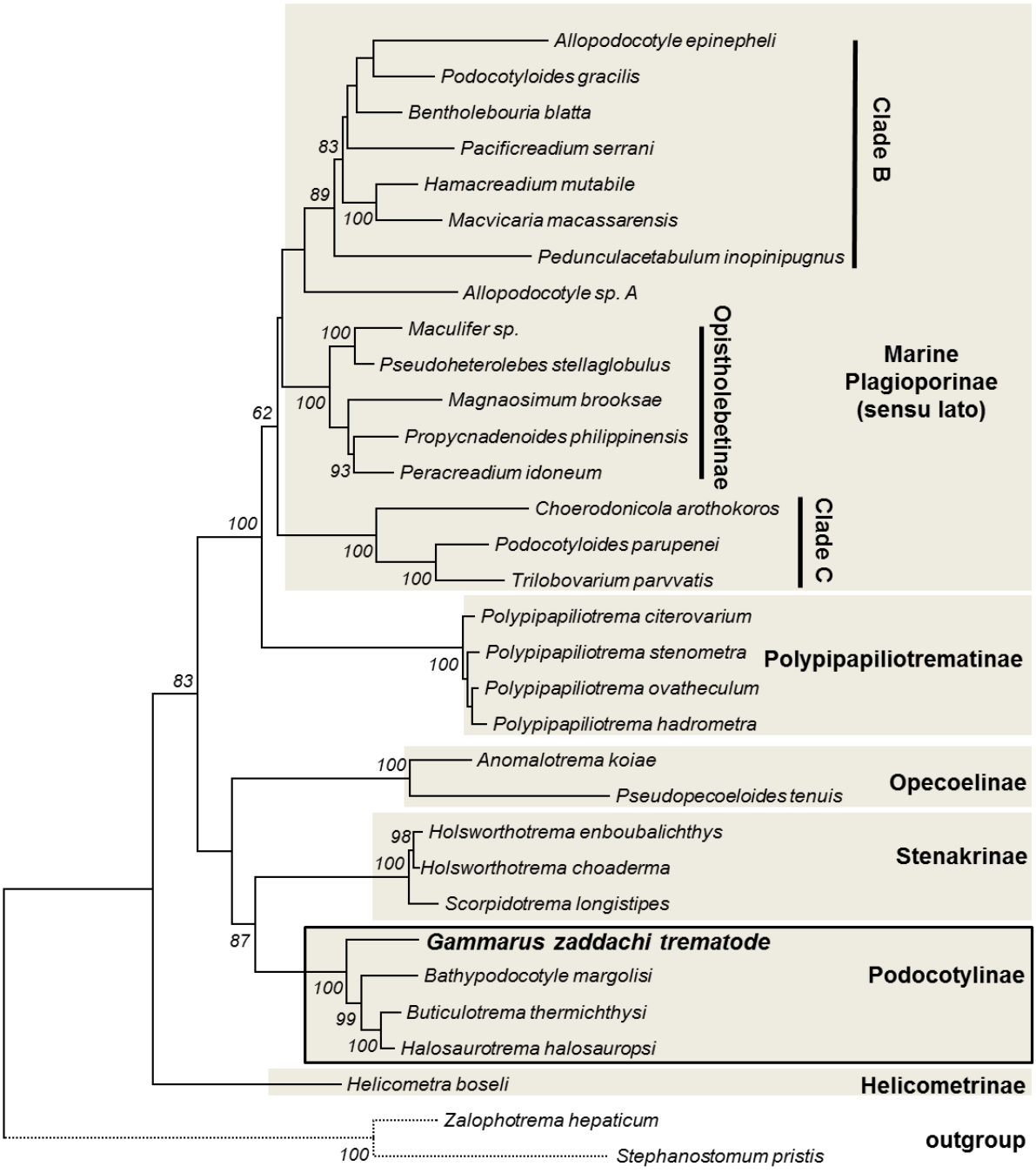

Fig. S2. Maximum Likelihood analysis of concatenated 18S and 28S rDNA alignment of Opecoelidae. Bootstrap values above 80% are indicated. The trematode species analyzed in this study is indicated in bold.

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