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Phylogeny Based Taxonomy Validation and *In Silico* Primer Designing of Piscine Tapeworm, *Senga lucknowensis* using Mitochondrial (COX-1) Gene

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Abstract: In the present study emphasis on the phylogenetic analysis and *in silico* primer designing of 10 primer set of tapeworm, *Senga lucknowensis* parasitizing the most commonly edible fish *Channa striata* that has been since long known for its healing properties upon consumption. Cestode, *Senga* attaches itself to the host intestine through its scolex and is known to cause severe damage to the submucosal layer. Phylogenetic analysis confirmed the taxonomic validation of the worm and designed Primer set will be of use in wet lab in PCR for desired gene amplification.

Keywords: *Senga lucknowensis*, Cestode, Tapeworm, Piscine, COX1, Genome

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Introduction

Aquaculture has since ages served as an excellent way for income and has prominently improved the economy of US\$ 145.4 billion (Aohagi *et al.*, 1992; Sosa *et al.*, 2016). With the increased cases of parasitic diseases in fishes the aquaculture productivity is decreasing. Upon entry into the host body the parasite starts feeding upon the host and reduces host reproductive capacity thus leading to overall reduced growth and performance of the fishes.

Parasitic infection caused due to cestode genus, *Senga* in *Channa striata* and *Channa punctata* are common in India (Tandon *et al.* 2005; Bhure *et al.*, 2010; Sawarkar 2012; Solunke *et al.*, 2012). *Channa striata* has been since long consumed in its raw form due to antiseptic properties found in the muscles of this fish. Cestode, *Senga* attaches itself with its scolex to the submucosal layer of intestine of the host that leads to ruptured serosa layer, vacuolization and

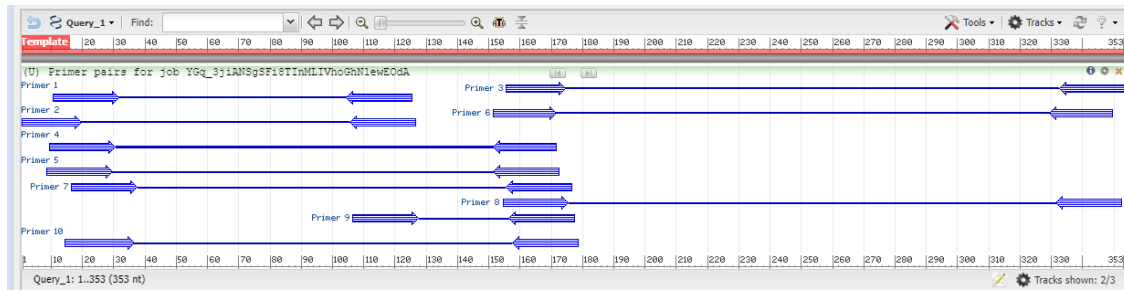


Fig. 1: Ten set of primers designed using computational tools.

irregular villi processes (Kaur *et al.*, 2014). Hence, identification of organism at molecular level and studying its taxonomic status is important.

Cytochrome c oxidase (COX1) gene is extensively used both as molecular marker for confirming the taxonomic validity as it is an evolutionary conserved segment and as well as for gene expression studies to further provide insights into the biology of host parasite interaction of these parasites for disease control (Chauhan *et al.*, 2022). The objective of this study was to ascertain the identity of *Senga lucknowensis* using COX-1 gene and designing primer sets for PCR based studies.

Materials and Methods

Retrieval of the sequence:

Mitochondrial cytochrome c oxidase subunit 1 (COX 1) gene of cestode, *Senga lucknowensis* genome was used in the present study. Sequence retrieval was done from NCBI (National Centre of Biotechnology Information) database (<http://www.ncbi.nlm.nih.gov>) under accession number ON803646.

Primer Designing:

In silico primer designing was performed using tools of NCBI (Figs. 1, 2).

Phylogenetic Analysis and Multiple Sequence Alignment:

Upon sequence retrieval of parasite under accession number ON803646, it was aligned with other *Senga* sp. for phylogenetic analyses. The sequences were aligned using the ClustalW program in MEGA6 (Hall, 1999). Multiple Sequence Alignment and phylogenetic tree was

constructed using Neighbour Joining Method (Figs. 3-5).

Results

Primer Set: The partial COX 1 gene of cestode, *Senga lucknowensis* was analyzed and 10 set of primers were designed which can be in future used for gene amplification of this piscine cestode (Figs. 1, 2).

Phylogenetic Analysis and Multiple Sequence Alignment:

Alignment description with top 10 matches:

Alignment of partial cox-1 gene was performed and it was analyzed that present cox-1 partial gene of cestode, *Senga lucknowensis* showed similarity with 10 gene sequences of *Senga lucknowensis* and *Senga* sp.

Phylogenetic Studies:

For confirming the taxonomic validation of the parasite phylogenetic analysis was performed and tree was constructed through Neighbour –Joining method. The highlighted sequence in yellow is sequence of interest investigated during the present study.

Multiple Sequence Alignment (MSA):

Multiple Sequence Alignment of the gene of interest was also performed and it was analyzed that it showed similarity with multiple genome sequences of *Senga* sp.

Discussion

The genus *Senga* was established by Dollfus (1934). Parasitic infections reduces the overall fitness of the host. *Senga lucknowensis* attaches

Primer pair 1										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	CACCAGACGCTTTTGGGTTTT	Plus	21	11	31	59.86	47.62	6.00	0.00	
Reverse primer	GCAGTCTTTACATCCAGCCCT	Minus	21	125	105	60.07	52.38	3.00	2.00	
Product length	115									
Primer pair 2										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TTGGGTGTTCACACAGACG	Plus	19	1	19	59.85	57.89	6.00	2.00	
Reverse primer	AGCAGTCTTTACATCCAGCCC	Minus	21	126	106	60.07	52.38	3.00	0.00	
Product length	126									
Primer pair 3										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TGGTGTCCCTACGGGGATT	Plus	19	156	174	59.92	57.89	4.00	1.00	
Reverse primer	AAGTAGGCCACCAAAACCAA	Minus	21	353	333	60.62	47.62	4.00	0.00	
Product length	198									
Primer pair 4										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TCACCAGACGCTTTTGGGTTT	Plus	21	10	30	60.68	47.62	6.00	1.00	
Reverse primer	CCCCGTAGGACACCAATTA	Minus	20	171	152	58.79	55.00	4.00	2.00	
Product length	162									
Primer pair 5										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TTCACCAAGCGCTTTTGGGTT	Plus	21	9	29	60.68	47.62	6.00	2.00	
Reverse primer	TCCCCGTAGGACACCAATTA	Minus	21	172	152	60.27	52.38	4.00	2.00	
Product length	164									
Primer pair 6										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TAATTGGTGCCCTACGGGG	Plus	20	152	171	58.79	55.00	4.00	2.00	
Reverse primer	GAGCCACCAACCAACCAAGTA	Minus	20	349	330	58.02	50.00	2.00	2.00	
Product length	198									
Primer pair 7										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	ACGCTTTTGGGTTTATGGCT	Plus	21	17	37	59.03	42.86	2.00	0.00	
Reverse primer	TTAATCCCCGTAGGGACACCA	Minus	21	176	156	60.27	52.38	4.00	1.00	
Product length	160									
Primer pair 8										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	TTGGTGTCCCTACGGGGATTA	Plus	21	155	175	60.27	52.38	4.00	2.00	
Reverse primer	AGTGAGCCACCAACCAACGA	Minus	21	352	332	61.02	52.38	4.00	0.00	
Product length	198									
Primer pair 9										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	GGCTGGATGTAAAGACTGCTG	Plus	21	107	127	58.99	52.38	3.00	2.00	
Reverse primer	CTTAATCCCCGTAGGGACACC	Minus	21	177	157	59.58	57.14	4.00	1.00	
Product length	71									
Primer pair 10										
	Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity	
Forward primer	AGACGCTTTTGGGTTTATGGC	Plus	22	15	36	60.03	45.45	2.00	2.00	
Reverse primer	CCTTAATCCCCGTAGGGACAC	Minus	21	178	158	59.58	57.14	4.00	1.00	
Product length	164									

Fig. 2: Primer set designed highlighting length, Tm, self compatibility , Template stand and GC content.

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
✓	Senga lucknowensis isolate PBI_029 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Senga lucknowe...	652	652	100%	0.0	100.00%	600	KR780798.1
✓	Senga visakhapatnamensis isolate PBI_028 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Senga visakhap...	652	652	100%	0.0	100.00%	600	KR780797.1
✓	Senga lucknowensis isolate 1 cytochrome c oxidase subunit I (COX1) gene .partial cds .mitochondrial	Senga lucknowe...	652	652	100%	0.0	100.00%	353	ON803646.1
✓	Polyonchobothrium sp. PCLS/05/08 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Polyonchobothi...	403	403	96%	1e-107	87.98%	394	FJ172983.1
✓	Senga sp. M250B cytochrome c oxidase subunit I (COX-1) gene .partial cds .mitochondrial	Senga sp. M250B	403	403	99%	1e-107	87.43%	432	EU912552.1
✓	Senga magna isolate PBI_537 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Senga magna	398	398	100%	4e-106	86.97%	600	KR780821.1
✓	Senga sp. PCLS/09/08 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Senga sp. 9 AM-...	398	398	96%	4e-106	87.68%	394	FJ172987.1
✓	Senga sp. PCLS/04/08 cytochrome c oxidase subunit I (cox1) gene .partial cds .mitochondrial	Senga sp. 4 AM-...	398	398	96%	4e-106	87.68%	394	FJ172982.1
✓	Senga sp. M258A cytochrome c oxidase subunit I (COX-1) gene .partial cds .mitochondrial	Senga sp. M258A	398	398	99%	4e-106	87.14%	432	EU912553.1
✓	Senga ophiocephalina mitochondrion .complete genome	Senga ophiocep...	392	392	100%	2e-104	86.69%	13816	KX434430.1

Fig. 3: Top 10 sequences showing 96-100% similarity with *Senga lucknowensis*.

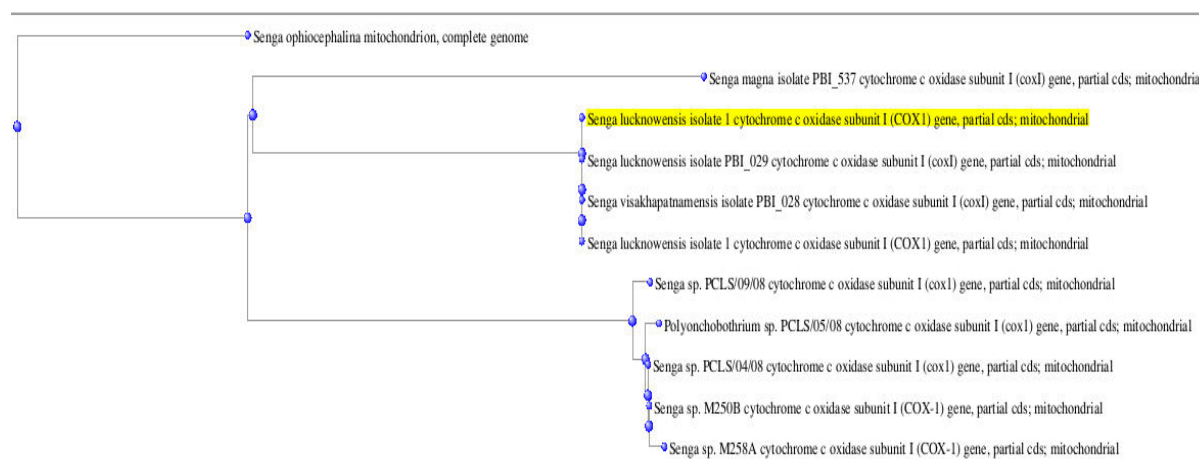


Fig. 4: The taxonomic position of *Senga lucknowensis* in yellow showing close resemblance with other *Senga* sp.



Fig. 5: Multiple Sequence Alignment results with top 10 cestode matches. Red region depicts the regions of similarity within the genome of the parasites.

itself to the host body by adhering to the GI tract mucosal layer thus causing severe damage to the fish intestine. The present study confirms the presence of *Senga lucknowensis* in *Channa striata* intestine. Also the phylogenetic studies confirms the taxonomic validity of the tapeworm. Upon analysis it showed 96-100% similarity with *Senga lucknowensis* and *Senga* sp. 10 pair of primers were also designed that had GC content ranging between 42-55%. A high GC content requires a higher temperature to dissociate thus, the chances of amplification of gene is high (Sosa *et al.*, 2016).

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