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Hypothesis

Morphological and molecular identification of the metacestode parasitizing the liver of rodent hosts in bamboo growing areas of mizoram, northeast India

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Abstract:

In Mizoram (Northeast India), rodent outbreaks are known to occur periodically with the onset of bamboo flowering causing a tremendous destruction to food grains and as per the folk belief, often resulting in famine. In an exploratory survey of rodent pests in bamboo growing atreas for their helminth parasite spectrum, metacestodes of tapeworms were frequently encountered infecting the liver lobes and body cavity of the host. The morphological criteria were found to be closely consistent with the metacestode of Taenia species. In molecular characterization of the parasite, the ribosomal DNA (ITS1, ITS2) and mitochondrial COI were amplified and sequenced. Based upon both morphological data and molecular analysis using bioinformatic tools, the metacestode is identified as confirmed to be representing Cysticercus fasciolaris. The adult form of which (Taenia taeniaeformis) commonly occurs in felid and canid mammalian hosts.

Keywords: metacestode; rodent; internal transcribed spacer; ribosomal DNA; polymerase chain reaction

Background:

In the state of Mizoram (Northeast India) the rodent outbreaks are intertwined with the onset of bamboo flowering, causing a tremendous destruction to food crops. This gregarious bamboo flowering, which is known locally as 'Mautam', occurs periodically after every 48±1 years and since the last confrontation with the flowering was in 1959, it was again expected during the period 2007-2009. About 31% of the total forest area of Mizoram is covered by bamboo forests, of which about 90% is covered by M. baccifera alone, which has been flowering since the year 2003. Although various measures have been taken to control outbreak of rodents in Mizoram, exhaustive studies on their parasites have never been ISSN 0973-2063 (online) 0973-8894 (print)

undertaken. In view of their underlying threat as serious pests of crop plants and also as reservoir of zoonoses, a study on the parasite of rodent hosts in Mizoram was undertaken. Studies on the phylogeny of tapeworms (Eucestoda) have achieved considerable progress and the ribosomal DNA (rDNA) clusters have been used for genetic studies [1]. PCR techniques that utilize the second internal transcribed spacer (ITS2) sequences and mitochondrial coding region COI, have been used to be a reliable tool in identifying the platyhelminth parasite species and their phylogenetic relationships [2-11]. Mitochondrial DNA has minimal non-coding DNA and no introns and it has been used in taxonomic studies as it is a rapidly evolving genome as compared to nuclear DNA [12]. For the design of

oligonucleotide primers which are used for amplification of variable region of the genome conserved coding regions are ideal and the cytochrome oxidase subunit1 (COI) gene quiet useful for studying closely related species.

Cestodes of the family Taeniidae are parasites of carnivore animal and human hosts which use mammals as their intermediate hosts where the larval stage develops in the tissues causing significant harm to the host. As they are of great medical and veterinary significance several studies have been focused at the species level [13-15]. Approximately 40 species of the genus Taenia have been recognized based on morphological studies of the adult specimen [16]. Phylogenetic relationships of taeniid cestodes have been constructed using morphology, host specificity and biological traits. However it is still difficult to come up with a reliable conclusion on the phylogenetic relationship between the members of Taeniidae [17]. However, recent studies have shown high level of interspecific variations for molecular characters, which are useful for characterization of species of Taenia and many new taeniid species have also been reported using more recent molecular biological techniques [18-20]. Taenia taeniaeformis is a taeniid cestode parasite, the adult form of which is found in cats and other carnivores and uses rodents as the intermediate hosts where the larval form or the cysticercus develops as a fluid filled larva in different organs [21-23]. Taxonomic revisions of Taenia spp have been done based on morphological criteria [24]. During an exploratory survey of rodent pests (burgeoning coincident with bamboo-flowering times in forests of Mizoram Northeast India) for their helminth parasite spectrum, metacestodes of cyclophyllidean tapeworms were frequently encountered infecting the liver lobes and body cavity of the host. The present study aimed at identifying this larval form based on morphological studies supplemented with molecular characterization.

Methodology:

Rattus rattus the commonly prevalent rodent species in the region were collected and examined. The parasites were found encysted in the liver tissues. The capsules were opened by

making a small slit to release the parasite. The recovered parasites were processed for morphological studies following standard procedures of fixation and stained whole mount preparation. Measurements of the specimens were taken using stage and ocular micrometers and/or morphometric software in the image analyser (Leica DM 1000).

Note: The Nucleotide sequence data reported in this paper have been submitted to the GenBank with the accession numbers FJ939133-FJ939135.

Scanning electron microscopy (SEM)

The specimens were fixed in 10% neutral buffered formalin (NBF) and processed as per the protocol described elsewhere **[25].** The gold coated specimens were observed using LEO 435 VP scanning electron microscope at electron accelerating voltages between 10 and 20 kV.

Molecular characterization

The genomic DNA of the parasite was isolated and amplified following standard procedure as described earlier [26] using the universal trematode primers of Schistosoma species [27]. These include ITS1: BD1 (forward) and 4S (reverse); ITS2: 3S (forward) and A28 (reverse); CO1: JB3 (forward) and JB4 (reverse). The PCR amplification was performed following the standard protocol with minor modifications as described elsewhere [28-29]. The PCR product was purified and sequenced in both directions on an automated sequencer. The sequences were submitted to GenBank for obtaining their accession number. The Sequences obtained were analysed using bioinformatic tools such as Basic Local Alignment Search Tools (BLAST), ClustalW and the extent of variation was compared by doing pairwise alignment of the nucleotides. Initially the sequencesc were aligned using ClustalW multiple alignment with the default gap and extention penalties used by this program. The phylogenetic trees of the metcestodes were constructed using distance and character based method in MEGA 4.0 [30]. Branch support was given using 1000 bootstrap replicates.

1	aaatttttgg	tttttgtatt	gtgtggatgc	ctgggtgtgt	gtgagtcaga	gggcagcaaa			
61	ggagtgaatt	aatttaatac	agattgattt	gtacattacc	ctcttgactt	ccattgcgtc			
121	cataggeteg	cgtctggcca	tgtctgtgct	agtgtcgtat	aataaaatat	cacttagcgt			
181	aacgtgcagt	ggetegggeg	attcccttcc	ctgcccgcct	ctggtcgtgt	geggtgetgt			
241	cctgtgcggt	gcgtttcagt	gcatgtcatg	tagtgtaagg	ttgacggtgc	tgccgaggac			
301	agggcagtac	aggtcagctg	tgctgtcctg	tgctgtgctg	tgcagtgctg	tgctgcgttg			
361	cgttgcactc	ctggtcgtcc	tatccacaag	acggtggata	ggggctgtat	ggagtggcgc			
421	attgtgtgtc	gegettgtge	ggtagtggtg	cttgcagttc	acggatcgtg	gcccagtgag			
481	tggtggtgtg	gtgctggcgc	gattggcttt	gtcagttatt	cgcgtctgta	gtgcacgcgc			
541	acgtgaagtg	gtctgcgcgg	gtgcgcccac	ctaggcggtt	gggcagcggc	agcggcagcg			
601	gcattaatag	cagcggcgga	ggcgcaccgt	gtttgggtta	gggtgtgctg	atgcagcatc			
661	tacaacagca	acagcaacaa	acagcagtaa	caactgacct	cggattaggc	gagagtaccc			
721	gctgaacata	agcatatcaa	taat						
			TTS	20					
			116	52					
1	agagatatgt	ttttttta	aataaaaaaa	aaaaaatata	tgaggaggg	aaaaatattt			
	tgtcttcctt								
121					gtttatacac				
181					agtgatggat				
241	tgcagttgcg	tccgtccgcg	ccccatcatg	tgtcatgtgt	ttggtgtagt	ggtgatggtt			
301	gtgcgtgagt	gtaaggcgat	gtgaagtgga	gaagtggaat	ggcatggcaa	taactgtgcg			
361	cggtggatca	ctcggctcgt	gtgtcgatga	agggtgcagc	caactgtgag	aattagtgtg			
421	tagtagccag	aggtgetete	gta						
			TTS	21					
			111	,1					
1	gtatcatgta	acactttatc	taaaacacaa	gccgataata	ctataccagt	aaccccacca			
61	aacgtaaaca	gaataataaa	agaaacaatt	catcataaaa	caggatcact	ctttttgact			
	cgagcattca								
	atcatagtta								
	tgatgacete								
	taaaaaccaa		agacatacta	attcttaaac	atatatgtct	aataatacca			
361	aatccaggaa	gaat							
COL									

Figure 1: ITS1, ITS2 and COI sequence of Taenia taeniaeformis ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 7(8):393-399 (2011)

Metacestode_Mizoram	GGTGCGTTTCAGTGCATGTCATGTAGTGTAAGGTTGACGGTGCTGCCGAG	Metacestode_Mizoram	AGAGATATGTTTTTTTTGGGTGGGGGG		
T_taeniaeformis_Hyderabad	GGTGTGGGTGCAGTGGCTTCCCTCTTTCGCAAGGCTGTTGCTGGTGCCCTG	T_taeniaeformis_Hyderabad	TCTTGTGCGTGTAGGCGTGGAAGAGATGTGTGTGTGTGTG		
Metacestode Mizoram	GACAGGGCAGTACAGGTCAGCTGTGCTGTC	Metacestode Mizoram	GGGGGGGTGTGTGAGCAGGGGGGGGGGTGTTTTGTCTTCCTTC		
T_taeniaeformis_Hyderabad	GCTGCGGCACTACGGTGCAAGGCTTGATCCCTGTGCTCCGCTGCGCCTGTG * **** *** * **	T_taeniaeformis_Hyderabad	GTGGTGGTGAGGGGTTCAGTGGTGTTTGGTCTTCCCTTCCCTTGCGCG * ** **** * * * ** * ** * * * * * ** **		
Metacestode_Mizoram	CTGTGCTGTGCTGTGCAGTGCTGTGCGTGCGTTGCGT	Metacestode_Mizoram	CGTGTCCGTCTCTTCTCTTTCTGTGTGTGTGTGTGTGT		
T_taeniaeformis_Hyderabad	CTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTG	T_taeniaeformis_Hyderabad	TGTGCGCGTATCTCGGTCTCGTGGGGTGTGTGTGTGTGCGTGGGAGCAGGGG *** *** **** **** * **************		
Metacestode_Mizoram	TGCACTCCTGGTCGTCCTATCCACAAGACGGTGGATAGGGGGCTGTATGGA	Metacestode_Mizoram	AGTGGGAGCCCGGTCCATCCCGGGGGGGGGGGGGGTTTATACACATGCGCG		
T_taeniaeformis_Hyderabad	TGCACTCCTGGCCGTCCTATCCACAAGACGGTGGATAGGGGCTGTATGGA	T_taeniaeformis_Hyderabad	ACCGCTCCACACGTCCATGCCGGAGGGGGCAGAGACATGTGCACACGCGCG * * * * ****** **** *** * * * * * * *		
Metacestode_Mizoram	GTGGCGCATTGTGTGTCGCGCTTGTGCGGTAGTGGTGCTTGCAGTTCACG	Metacestode_Mizoram	CGCGATTTTGTGTGTGTGTGTGCGTGTGTGCGAGGCGTAAGATAGTGATG		
T_taeniaeformis_Hyderabad	GTGGCGCATTGTGTGTGCGCGCTGTGCGCGGTAGTGGTGCTTGCAGTTCCCG	T_taeniaeformis_Hyderabad	CACGTGTTTGTGTGTGTGCGGTGTGTGCGAGGCGTAAGATAGTGATG * * *****		
Metacestode_Mizoram	GATCGTGGCCCAGTGAGTGGTGGTGGTGGTGGCGGGGCGGGATTGGCTTTGTC	Metacestode_Mizoram	GATGCGACGGTTGTGCAGTTGCGTCCGTCCGCGCCCCATCATGTGTCATG		
T_taeniaeformis_Hyderabad	GATCGTGGCCCAGTGAGTGGTGGTGGTGGTGCTGGGCGCGATTGGCTTTGTC	T_taeniaeformis_Hyderabad	GATGCGACGGTTGTGCAGTTGCGTCCGTCCGCCCCCATCATGTGTCATG		
Metacestode_Mizoram	AGTTATTCGCGTCTGTAGTGCACGCGCACGTGAAGTGGTCTGCGCGGGGTG	Metacestode_Mizoram	TGTTTGGTGTAGTGGTGATGGTTGTGCGTGAGTGTAAGGCGATGTGAAGT		
T_taeniaeformis_Hyderabad	AGTTATTCGCCTCTGTAGTGCACGCGCACGTGAAGTGGTCTCCCGCGGGTG	T_taeniaeformis_Hyderabad	TGGTTGGTGTAGTGGTGATGGTTGTGCGTGAGTGTAAGGCGATGTGAAGT		
Metacestode_Mizoram	CGCCCACCTAGGCGGTTGGGCAGCGGCAGCGGCAGCGGCATTAATAGCAG	Metacestode_Mizoram	GGAGAAGTGGAATGGCATGGCAATAACTGTGCGCGGTGGATCACTCGGCT		
T_taeniaeformis_Hyderabad	CGCCCACCTA6GCGGTTGGGCAGCCGCATTATACCAG	T_taeniaeformis_Hyderabad	GGAGAAGTGGAATGGCATGGCAATAACTGTGCACTTAATCACTCGGCT		
Metacestode_Mizoram	CGGCGGAGGCGCACCGTGTTTGGGTTAGGGTGTGCTGATGCAGCATCTAC	Metacestode_Nizoram	CGTGTGTCGATGAAGGGTGCAGCCAACTGTGAGAATTAGTGTGTAGTAGC		
T_taeniaeformis_Hyderabad	CGGCGGAGGCCCACCGTGTTTGGGTTAGGGTGTGCTGTGCGATGCAGCATCTAC	T_taeniaeformis_Hyderabad	CGTGTGTCGATGAAGGGTGCAGCCAACTGTGTGATAAAAGC		
Metacestode_Mizoram	AACAGCAACAACAACAGCAGTAACAACTGACCTCGGATTAGGCGAG	Metacestode_Mizoram	CAGAGGTGCTCTCGTA		
T_taeniaeformis_Hyderabad	AACAGCAACAGCAACAAACAGCAGTAACAACTGAC	T_taeniaeformis_Hyderabad	CGGCCTACTTGT- * ** ** **		
	ITS2	ITS1			
		1101			

Figure 2: Pairwise Alignment of ITS2 and ITS1 sequences of Taenia taeniaeformis

T taeniaeformis Japan	ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT
T taeniaeformis Kazakhstan	ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT
Metacestode Mizoram	ATTCTTCCTGGATTTGGTATTATTAGACATATATGTTTAAGAATTAGTAT
T taeniaeformis Kolkata	ATTCTTCCTGGATTTGGTATTATTAGACATATATGCTTAAGAATTAGTAT

T taeniaeformis Japan	GTCTTCGGATGTGTTTGGTTTTTTGGTTTATGTTTGCTATGTTTTCTA
	GTCTTCGGATGTGTTTGGTTTTTTGGTTTATTGTTTGCTATGTTTTCTA
Metacestode Mizoram	GTCTTCGGATGTGTTTGGTTTTGTTTGGTTTGTTTGCTATGTTTTCTA
T taeniaeformis Kolkata	GTCTTCGGATGTGTTTGGTTTTTTGGTTTGGTTTGCTATGTTTTCTA

T taeniaeformis Japan	TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
T taeniaeformis Kazakhstan	TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
Metacestode Mizoram	TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG
T taeniaeformis Kolkata	TAGTTTGTTTAGGAAGAAGGGTGTGAGGTCATCATATGTTTACTGTTGGG

T taeniaeformis Japan	TTAGATGTAAAGACGGCTGTGTTTTTTAGTTCTATAACTATGATTATTGG
T taeniaeformis Kazakhstan	
Metacestode Mizoram	TTAGATGTAAAGACGGCTGTGTTTTTTAGCTCTATAACTATGATTATTGG
T taeniaeformis Kolkata	TTAGATGTAAAGACGGCTGTGTTTTTTAGTTCTATAACTATGATTATTGG
, <u> </u>	*****
T taeniaeformis Japan	AGTTCCTACGGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
T taeniaeformis Kazakhstan	AGTTCCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
Metacestode Mizoram	AGTACCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG
T taeniaeformis Kolkata	AGTCCCTACAGGTATAAAGGTTTTTACATGATTGTATATGTTGTTGAATG

T taeniaeformis Japan	CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
T taeniaeformis Kazakhstan	CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
Metacestode Mizoram	CTCGAGTCAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT
T taeniaeformis Kolkata	CTCGAGTTAAAAAGAGTGATCCTGTTTTATGATGAATTGTTTCTTTTATT

T_taeniaeformis_Japan	ATTCTGTTTACGTTTGGTGGGGGTTACTGGTATAGTATTATCAGCTTGTGT
	ATTCTGTTTACGTTTGGTGGGGGTTACTGGTATAGTATTGTCAGCTTGTGT
Metacestode_Mizoram	ATTCTGTTTACGTTTGGTGGGGGTTACTGGTATAGTATTATCGGCTTGTGT
T_taeniaeformis_Kolkata	ATTCTGTTTACGTTTGGTGGGGGTTACTGGTATAGTATTATCGGCTTGTGT
T taeniaeformis Japan	TTTAGATAAAGTGTTACATGATAC
T taeniaeformis Kazakhstan	
Metacestode Mizoram	TTTAGATAAAGIGITACAIGATAC
T taeniaeformis Kolkata	TTTAGATAAAGTGTTACATGATAC
KOIKata	111AGA1AAGIGI1ACAIGAIAC

Figure 3: Multiple alignment of CO1 sequences of Taenia taeniaeformis geographical isolates

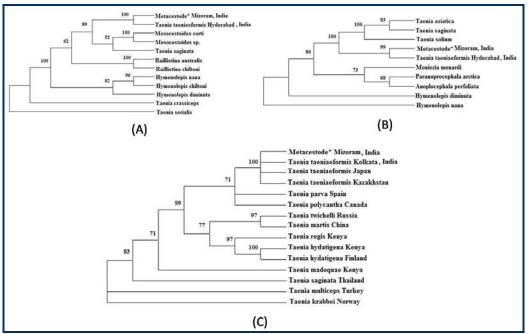


Figure 4: MP phylogenetic trees of the metacestode for- (A) ITS2 (B) ITS1 (C) COI. The numbers on the branches refer to bootstrap values.

Results:

Morphological Analysis

The morphological examination of the parasite revealed typical taeniid features: body measuring 0.9-9.6 cm in length; large scolex 0.8-1.6 mm long and 1.1-2.09 mm wide, with four prominent lateral suckers; rostellum armed with double rows of 34 - 42 hooks, the outer larger hooks 0.36-0.42 mm in length, the smaller inner hooks 0.23-0.27 mm, all hooks typically taenoid type having long blunt handle with sharp pointed blade; and short or elongated and segmented strobila terminating with a bladder, thus resembling a small tapeworm but without reproductive organs (Figure. 5). All these morphological characters are in consistence with those of *Cysticercus fasciolaris*, the adult form of which is *Taenia taeniaeformis*.

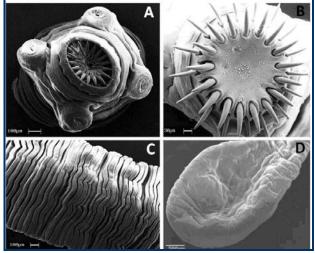


Figure 5: SEM **(A-D)** view of the metacestode. **(A)** Scolex with hooks and four lateral suckers; **(B)** Double rows of hooks on the ISSN 0973-2063 (online) 0973-8894 (print) Bioinformation 7(8):393-399 (2011)

scolex; **(C)** A portion of the Strobila showing segmentation, suggestive of proglottidization; **(D)** The terminal bladder like end

Molecular Analysis

The rDNA ITS and mtCOI regions of the metacestode were successfully amplified using primers as mentioned above (Figure. 1). PCR amplification of ITS regions and the mtCOI showed a single band of size 744bp, 443bp and 374bp, respectively (Figure. 6). The nucleotide sequences obtained from the PCR products were put to BLAST and compared with other available cyclophyllidean cestode sequences from GenBank (Table. 1 see supplementary material). The BLAST hits result shows that the sequences of the metacestode are closer to those of species of Taenia, with maximum similarity to Taenia taeniaeformis. In pairwise alignment of the ITS2 and flanking regions of the query sequences with the sequences of Taenia taeniaeformis from Hyderabad India isolate shows the presence of 6.4% mismatches. Similarly, with regard to ITS1 pairwise alignment of query sequences with the sequences of T. taeniaeformis from Hyderabad India shows 14.3% mismatches (Figure. 2), multiple alignment of COI of query sequences with three different isolates shows the presence of 2.1% mismatches with no gap (Figure. 3). Phylogenetic trees were obtained by comparing the ITS and mtCOI sequences of the metacestode with other cyclophyllidean cestode species using the NJ and MP methods (Figure. 4). The topology of the trees obtained through both the methods emerged to be quiet similar placing both the Taenia taeniaeformis and the query sequences in the same clade; giving high bootstraps values of 90% and above. Bootstsrap value of 99% in the tree constructed for ITS1 and 100% for ITS2 with T. taeniaeformis obtained from Wistar rats indicates perfect phylogenetic accuracy and reliable grouping. In the trees constructed for COI the query sequences are placed in the same clade with T. taeniaeformis isolates from Hokkaido (Japan),

Kazakhstan and Kolkata (India) showing high bootstrap values of 100%.

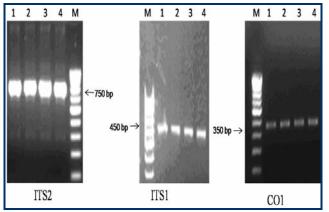


Figure 6: PCR products of metacestode DNA using different primer sets (M) = 100bp DNA ladder

Discussion:

The LM and SEM observations clearly shows the presence of double rows of hooks and four lateral suckers which are the distinguishing characters for T. taeniaeformis. The size and the number of hooks are important features for identification of Taeniidae species. The numbers of hooks reported in the metacestode under study were also found to be in confirmity with those of T. taeniaeformis [31]. Ribosomal DNA or the rDNA clusters has also been widely used for taxonomic studies. Mitochondrial genes have also been used to study phylogenetic relationships as they have fast evolutionary rate [32-33]. In analysis of the sequences of the rDNA ITS2 and ITS1 and the mtCO1 and comparing with the so far known sequences of other cyclophyllidean cestodes, the sequence of ITS and CO1 showed close similarity with the sequence of T. taeniaeformis showing high bootstrap value of 90% and above. If the bootstrap value is 70% or higher than the topology at that branch is considered reliable or correct [34]. Intraspecific variations of four different isolates of T. taeniaeformis by using several criteria such as morphology of the parasite, infectivity, protein component of the parasites and restriction fragment length polymorphism of the parasite DNA have also been reported earlier by [35]. In the present study when pairwise and multiple alignment of the query sequences was done with different isolate only a few mismatches and gaps were seen. On the basis of the morphological similarities with earlier studies supplemented by close matching of the ITS and the mitochondrial CO1 sequences of the metacestode under study with T. taeniaeformis it can be concluded that the parasite found in the liver cysts of rodents in the study area is indeed the metacestode of T. taeniaeformis the adult of which occurs in the various carnivorous animals.

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Supplementary materials:

Table 1: Taennid species and their various geographical isolates used in the study with respective GenBank accession numbers.

Name of parasites	Host	Geographical	Accession No.			
		Isolate	ITS2	ITS1	CO1	
Metacestode*	Rattus rattus	Mizoram, India	FJ 939133	FJ939134	FJ939135	
Taenia taeniaeformis	Wistar rat , Apodemus sylvaticus , Rattus	India ,	EU 051352	EU051351	EU544597,	
	norvegicus , Rattus rattus	Kazakhstan, Japai	n		AB221484, EF090612	
Taenia asiatica	PMD-3 Clone	Taiwan		AY606272	-	
Taenia saginata	Bos indicus	China , Thailand	AY825542	AY392045	AB465239	
Taenia solium	Sus scrofa domestica	Kolkata, India		EF090614		
Taenia crassiceps	Mus sp.	USA	DQ099564	-	-	
Taenia serialis	Canis latrans	USA	DQ099574		-	
Taenia hydatigena	Canis familiaris , Rangifer tarandus	Kenya, Finland			AM503316,	
		2	-	-	EU544552	
T. Regis	Panthera leo	Kenya	-	-	AM503330	
T. Twichelli	Gulo gulo	Russia	-	-	EU544598	
T. martis	Myodes rufocanus	China	-		EU544558	
T. madoquae	Canis mesomelas	Kenya	-	-	AM503325	
T. multiceps	Ovis aries	Turkey	-	-	EF393620	
T. krabbei	Vulpes lagopus	Norway	-	-	EU544579	
T. polyacantha	Lemmus trimucronatus	Canada	-	-	EU544595	
T. parva	Apodemus sylvaticus	Spain	-	-	EU544580	
Hymenolepis diminuta	Rodents, R. rattus	Australia , India	FJ939132	AF461125	-	
Hymenolepis nana	Rodents, Mesocricetus auratus	Australia ,	AB494477	AF461124		
• •		Uruguay			-	
Hymenolepis microstoma	Laboratory rodents	Japan	AB494478	-	-	
Raillietina australis	Dromaius novahollandiae	Australia	AY382317	-	-	
Raillietina chiltoni	Dromaius novahollandiae	Australia	AY382319	-	-	
Mesocestoides corti	Canis familaris	USA	AF119696	-	-	
Mesocestoides sp.	Canis familaris	USA	AF119697	-	-	
Moniezia monardi	Capricornis crispus	Japan	-	AB367791	-	
Paranoplocephala arctica	Dicrostomyx spp.	Finland	-	AY299558	-	
Anoplocephala perfoliata	Equus ferus caballus	Germany	-	AJ578151	-	