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Morphology, Molecular and Chromosomal Identification of *Adenoscolex oreini* Fotedar, 1958 (Cestoda: Caryophyllidea) from Jammu & Kashmir, India

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Research Article

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Abstract

Introduction: Caryophyllids are unique among Eucestoda in having a monopleuroid body plan *i.e.*, they have neither internal proglottidization nor external segmentation and have a single set of reproductive organs. They are intestinal parasites of cypriniform and siluriform freshwater fishes; intermediate hosts are aquatic annelids. Knowledge of chromosome sets of caryophyllidean tapeworms has increased within the past 40 years. To date, 23 species of all four existing families have been studied cytogenetically and karyotypes of 14 species have been completed. The diploid chromosome number of all but one species ranges from 14 to 20; *Caryoaustralus sprenti*, represents the exception, having only six chromosomes. *Adenoscolex oreini* recovered during the present investigation belongs to this order.

Material and methods: The genomic DNA was isolated from the parasite of *Adenoscolex oreini* collected from the fish hosts of *Carassius carassius*; *Schizothorax niger; Schizothorax curvifrons; Schizothorax labiatus; Schizothorax esocinus* and *Schizothorax plagiostomus* from River Jhelum, River Sindh, Dal, Manasbal and Wular lakes of Kashmir valley by using standard ethanol precipation technique. The resultant DNA was separated by electrophoresis through 1.5% (w/v) agarose gel in TAE buffer stained with ethidium bromide, transilluminated under ultraviolet light and then photographed. The known size fragments of 100 bp ladder in agarose gel were used as marker. The rDNA regions spaning ITS regions were amplified by PCR.

Results: During the helminthological survey of fishes in Kashmir, India, specimens of genus *Adenoscolex* were collected from *Carassius carassius* and *Schizothorax* spp fishes. The morphological and molecular study inferred with partial sequence of 28S rDNA and chromosome analysis confirmed the specimens as *Adenoscolex oreini*. Phylogenetic analysis further confirmed its taxonomic status, as it comes under the same clade formed by other same family members reported from other geographical regions. This study first time describes the molecular identification of *Adenoscolex oreini* from Kashmir.

Conclusion: It is concluded that *Adenoscolex oreini* can be differentiated on the basis of morphology, molecular and karyological features. By using molecular approach, there is marked difference between the nucleotide sequences of this tapeworm. The sequences of ITS region of 28S rDNA gene have been proved a valuable tool for taxonomic studies of closely related taxa including cestodes from fresh water bodies of fishes. The variation in size of smallest bivalent and other bivalents indicates that the somatic chromosomes will show a marked variation in the length of longest and shortest chromosome in *Adenoscolex oreini.* It is apparent that the systematics of the *Adenoscolex* and its position among the basal tapeworms (Eucestoda) is still unresolved, because of the low number of molecular characters used and very low consistency index of the trees; it is premature to speculate on some of the molecular and evolutionary implications of the present data. New material of most genera is needed for analyses of molecular data.

Keywords: Cestode; Adenoscolex oreini; Kashmir; Carassius carassius and Schizothorax; 28S rDNA.

Abbreviations: PCR: Polymerase Chain Reaction; CI: Centromeric Indices; SM: Sub Metacentric; ST: Sub Telocentric; SD: Standard Deviation; NJ: Neighbour Joining Method MP: Maximum Parsimony; TCGA: The Centre for Genomic Applications

Introduction

The valley of Kashmir is gifted with a large number of Lakes, Rivers, streams, and ponds, which harbor variety of fishes and is famous for its natural fresh water Lakes, distributed in its length and breadth at varying altitudes. Important lakes and rivers of Kashmir valley surveyed are Wular Lake, Dal Lake, Manasbal Lake and Anchar Lake while among rivers; the River Jhelum and the River Sindh are the most important [1-6].

Cestodes have received considerable attention of systematists, not only because they are ubiquitously distributed, having radiated with their hosts into all habitats, but because of their importance as pathogens of humans and fishery (*Cyprinus carpio, Carassius carassius, Schizothorax* species) [7]. They exhibit a range of morphological, physiological, biochemical, and ecological adaptations, which make them suitable models for studies of various biological phenomena [8,9]. A high diversity of scolex morphology also makes cestodes a suitable model for studies on morphological adaptations [10,11]. Earlier approaches were mainly based on observed morphological characters without considering interspecific differences and without any knowledge on population variability and genetic characteristic, which resulted in inflation of descriptions of conspecific taxa. Thus, several approaches have recently been taken to more rigorously circumscribe species for producing accurate inventories.

Several tools for studying cestode micromorphology such as scanning and transmission electron microscopes have been used to provide accurate and, most importantly, more stable morphological characters [12,13]. Correctly stated –"it is much more 'scientific' to identify specimens with machines than doing it by simply looking at them", molecular approaches are now integrated with morphological ones to provide much reliable results [14-17].

Caryophyllids are unique among Eucestoda in having a monopleuroid body plan *i.e.*, they have neither internal proglottidization nor external segmentation and have a single set of reproductive organs. They are intestinal parasites of cypriniform and siluriform freshwater fishes; intermediate hosts are aquatic annelids Knowledge of chromosome sets of caryophyllidean tapeworms has increased within the past 40 years [1]. To date, 23 species of all four existing families have been studied cytogenetically and karyotypes of 14 species have been completed. The diploid chromosome number of all but one species ranges from 14 to 20; *Caryoaustralus sprenti*, represents the exception, having only six chromosomes [2]. *Adenoscolex oreini* recovered during the present investigation belongs to this order.

The present aim was to carry out modern systematic studies to carry out Molecular taxonomy of *Adenoscolex* spp. from fishes of Kashmir valley by isolating the genomic DNA of the parasites using a standard SDS-protinase K procedure, Polymerase chain reaction (PCR); DNA sequencing and chromosomal analysis of these parasites, so as to define or redefine the new genera and species of the helminth parasites of fishes in Kashmir valley. This study would lay down the foundation for the establishment of database of parasitofauna of fishes in water bodies of Kashmir Valley.

Materials and Methods

Collection of the Hosts: The fish hosts examined during the present study includes *Schizothorax esocinus* Heckel, 1838; *Schizothorax plagiostomus* Heckel, 1838; *Schizothorax curvifrons* Heckel, 1838; *Schizothorax niger* Heckel, 1838; *Schizothorax labiatus* McClelland, 1842; and *Carassius carassius*. Ten to fifteen fishes per survey were collected using gill nets or bought directly from fisherman. The live fishes were killed by a blow on the top of the cranium and were subsequently dissected by making an insertion from the anus towards the head.

Collection of Parasites: Fishes collected from water bodies were taken to laboratory, Department of Zoology, University of Kashmir and were immediately dissected. An incision was made in the mid-ventral body, cutting around the anus and urogenital opening. The entire alimentary canal and other visceral organs were detached and kept in separate Petri dishes containing normal saline (0.65% NaCl). With the aid of magnifying glasses, these detached organs were searched for parasites. The parasites recovered were kept in normal saline (0.65 NaCl) for some time.

Identification of Parasites: Parasitic specimens were identified using reference keys [18-21].

Molecular Characterization

DNA Extraction: Identified specimens of Cestodes were fixed in 96-100% ethanol. The genomic DNA was isolated from the parasite of *Adenoscolex oreini* collected from the fish hosts of *Carassius carassius*; *Schizothorax niger; Schizothorax curvifrons; Schizothorax labiatus; Schizothorax esocinus* and *Schizothorax plagiostomus* from River Jhelum, River Sindh, Dal, Manasbal and Wular lakes of Kashmir valley by using standard ethanol precipation technique [3]. The resultant DNA was separated by electrophoresis through 1.5% (w/v) agarose gel in TAE buffer stained with ethidium bromide, transilluminated under ultraviolet light and then photographed. The known size fragments of 100 bp ladder in agarose gel were used as marker. The rDNA regions spaning ITS regions were amplified by PCR (Table 1).

***Adenoscolex oreini* Fotedar, 1958**	**Primer Designed (5.8S- ITS-2)**		**GenBank Accession Number**	**Author and Year**
	Forward	5/- GTCGATGAAGAGCGCAGC-3/	EMBLZ269; AF229028 & AF229029	[22,23]
	Reverse	5/-AGGAGGCGAATCACTAT-3/		
Sequences submitted to GenBank	SUB2816972 *Adenoscolex oreini* complete sequence.			

Table 1: Primers used.

Polymerase Chain Reaction (PCR): PCR was performed using the primer pairs for (ITS1 and ITS2), which were selected based on conserved sequences of *Adenoscolex* spp. PCR was carried out in a 25 µl reaction volume, containing 50-100 ng of genomic DNA with 20pmols of the primer (both forward and reverse). The PCR amplification of ITS1 and ITS2 regions was performed following the standard protocol, with minor modifications. The PCR products were run in 1.5% agarose gel to determine their size, with a 100 bp ladder and viewed in Kodak Gel logic 100 imaging system and purified by using spin columns [24]. The thermal gradient of these marker regions started with an initial denaturation at 94 ºC (5 minutes), denaturation for 35 cycles (94ºC for 30 seconds), annealing, 28S at 55 ºC (2 minutes), extension 72ºC (2 minutes) and final extension at 72ºC (7 minutes).

Sequencing of the Amplified Product

The purified products were bidirectionally sequenced using the forward and reverse primers using automated sequencers- ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, California, USA) with Big Dye (3.1) terminator protocol at UPE Central Instrumentation Facility of School of Life Sciences, North-Eastern Hill University, facility at The Centre for Genomic Applications (TCGA), New Delhi was used. Sequencing was performed five times each to avoid any ambiguity. All sequences obtained were deposited in GenBank using the submission tool Bankit and their accession numbers acquired.

Karyology

Whole living specimens of *Adenoscolex* spp. were placed in physiological saline (0.65% NaCl) containing colchicine (0.05%) for 3–4 hours at room temperature and transferred into distilled water for about one hour for hypotony and fixed in ethanol-glacial acetic acid (3:1), with two changes, 15 minutes each. Spread preparation of mitotic and meiotic chromosomes was made as described by Petkeviciute [25]. Small posterior mature portions of fixed worms were transferred into drop of 60 % acetic acid on a slide and torn into fine pieces with the help of tungsten needles. The slides were then placed on a heating plate at 45ºC and the drop of cell suspension was slowly drawn along the slide until it evaporated. Slides were dehydrated in an

ethanol series (30%, 50%, 70%, 90% and 100%, 5 minutes each) and stored at –20^{0}C. Slides were stained with 4% Giesma solution (pH. 6.8) in phosphate buffer for 30 minutes, rinsed in tap water and allowed to dry. The best chromosome plates were photographed and used for morphological studies.

Photomicrography

Leica DM LS2 trinocular photomicroscope with 100X x 10X magnification lens was used for taking the photographs and analyzes the chromosomes. The photographs were then developed on Kodak high quality photo paper. For karyotyping, chromosomes were cut out of the photomicrographs and paired on the basis of size and centromere position. The homologues were cut and arranged in metacentric, submetacentrics, meta-submetacentric, telocentric, subtelocentrics and acrocentric pairs of chromosomes arbitrarily. Relative lengths of chromosomes were calculated by the division of the individual chromosome length by the total haploid length and centromeric indices (ci) were determined by division of the length. Measurements are based on all chromosomes from 10 best metaphase spreads of parasites. The terminology relating to centromere position follows that of Levan *et al.* 1964. A chromosome is metacentric (m) if the ci falls in the range of 37.5–50.0, submetacentric (sm) if 25.0–37.5, subtelocentric (st) if 12.5–25.0; acrocentric (a) if < 12.5 and telocentric if 0. When the centromere position was on the borderline between two categories, both are listed.

Statistical Analysis

Parametric as well as the non-parametric tests were used for analyzing relative length of chromosomes. A computer program (Minitab for windows) was used for data analysis. The descriptive data was given as a mean ± standard deviation (SD). Chi-Square analysis was used to see the statistical significance in chromosome lengths. Pearson's correlation was used to find correlation between different species of helminths. Correlation analysis of data was carried out by using SPSS 16.5 package programme. Student's t-test was used to test the differences which were considered to be significant when the p-value obtained was less than 0.05.

Results

Morphology

Generic Diagnosis*:* Smooth scales with well marked gland cells which are continued in body region, cirrus sac and utero-vaginal canal open separately at the beginning of posterior seventh of the body; lower horns of ovary bent inwards giving the appearance of inverted 'A', uterine coils extended beyond anterior horns of ovary but never anterior to cirrus sac; well developed receptaculum seminis; vitelline fields partly cortical and partly medullary being mostly at the level of inner longitudinal muscle layer; post-ovarian vitelline were present.

Species Identification*:* Comparatively smaller than *Adenoscolex fotedari* (about 38mm); No. of excretory vesicles 14-18; Shape of ovary like inverted 'A' lower horns of which are strongly bent inwards. Whilest in *Adenoscolex fotedari* ovary is H-shaped and the two horns of 'H' are not bent on either side; number of excretory vesicles 18-30 (Table 2).

Particulars	Fayaz, 1993	Ashiq, 2008	Fayaz *et al* 2009	Present study
Total body length	49.5	30.5	51	32-37
Scolex	0.87-1.64	1.38 x 0.9	0.84-1.06	0.90-1.40
Neck	1.0-1.1	---------	1.0-1.1	0.9-1.1
Testes	0.144-0.224 x 0.048-0.192	0.18-0.21 x 0 .09-0.16	0.15-0.24 x 0.050-0.19	---------
Vas deference	---------	0.5 x 0.45	---------	---------
Ovary length	---------	2.15	---------	---------
Ovary width	---------	0.5	---------	---------
Wings of ovary	1.4-2.7 x 0.17-0.3	---------	1.46-2.5 x 0.17-0.5	---------
Ovary isthmus	0.31-0.71 x 0.07-0.16	---------	0.35-0.73 x 0.07-0.18	0.33-0.71 x 0.06-0.15
Egg	0.052-0.076 x 0.028-0.056	0.52-0.06 x 0.23-0.26	0.059-0.074 x 0.028-0.059	0.050-0.071 x 0.027-0.055
Host	*Schizothorax* spp.	*Schizothorax* spp.	*Schizothorax* spp.	*Carassius carassius* & *Schizothorax* spp.
Site	Intestine	Intestine	Intestine	Intestine

Table 2: Comparative characteristics (measurements in mm) of *Adenoscolex oreini* Fotedar, 1958.

Description: Body elongated with unarmed scolex. Mature worm broader and thicker in posterior part of the body. Gland cells developed extensively in scolex region being visible to naked eye, extended posteriorly in 3 well-developed columns for more than 3 quarter of anterior body length. A single set of male and female genital organs open separately to posterior end through male and female genital openings (Figures 1 & 2). Testis and vitellaria scattered while other genital organs restricted to posterior 1/7th of body. Testis rounded, extended from scolex to cirrus pouch, bounded by vitelline follicles, vas deferens loosely convoluted tube, median anterior to cirrus sac. Cirrus sac muscular and pear shaped. Vitellaria numerous. Ovary single at 1/7th posterior end of the body more or less H shaped. Two long limbs connected by a transverse ovarian isthmus Uterus coiled structure containing ova at different stages of development unite with vagina forming an utero-vaginal chamber to open exterior by female genital aperture. Capsules operculate ovoid, boat shaped with a small protuberance near basal region.

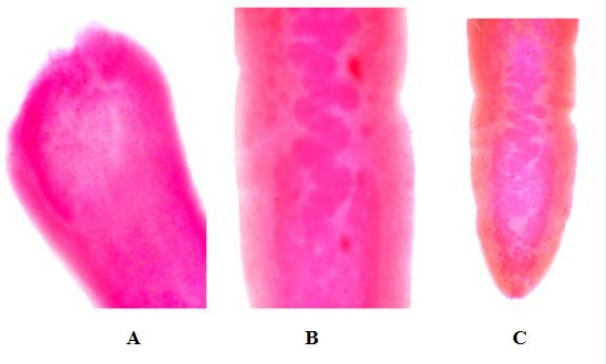

Figure 1: Photograph of *Adenoscolex oreini* Fotedar, 1958. A-Anterior end; B-Middle region; C-Posterior end.

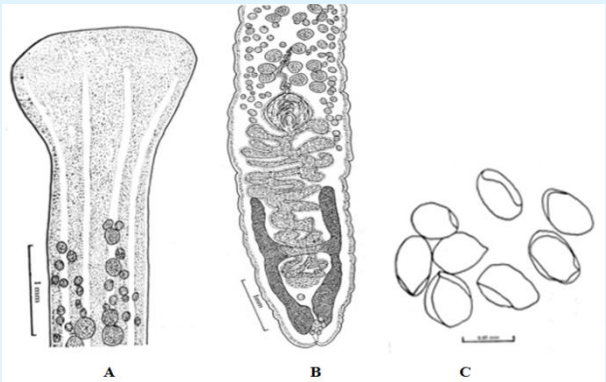

Figure 2: Camera lucida drawing of *Adenoscolex oreini* Fotedar, 1958. A: Anterior end; B: Posterior region; C: Eggs.

Remarks: From the above Table with regard to morphological characteristics/measurements of the parasite, it shows close resemble with *Adenoscolex oreini*. The identifying characters include; scolex and neck, Body length 30-51mm, ovary isthmus and egg size. The studies are in close confirmity with that of Fayaz; Ashiq and Fayaz, *et al.* [26-28].

Molecular characterization

PCR amplification was carried out to amplify ITS region by using Automated sequencers-ABI prisim 3130XL Genetic Analyzer (New Delhi-India). The size of the amplified product was found to be 954 bp long (Figure 3). In BLAST search of the sequence, it showed similarity with other Cestodes. The sequence obtained was submitted to GenBank SUB2816972 (Table 3). Sequences were compared with other sequences of Cestodes available in GenBank. When the BLAST search was performed, the query sequence showed maximum similarity with 28S sequence of *Breviscolex* spp. which belongs to the same family Capingentidae. The BLAST hits show that the sequences of the *Adenoscolex oreini* are close to those of *Breviscolex orientalis,* since there is no information available in GeneBank for ITS sequences pertaining to *Adenoscolex* species. The pairwise alignment of the ITS sequences and the flanking regions of the query sequences with the sequences of *Breviscolex orientalis* showed the presence of 9.22% mismatches with 0.94% gaps. All positions containing gaps and missing data were eliminated. The results obtained through Neighbour Joining method (NJ) and Maximum Parsimony (MP) are constructed (Figure 4).

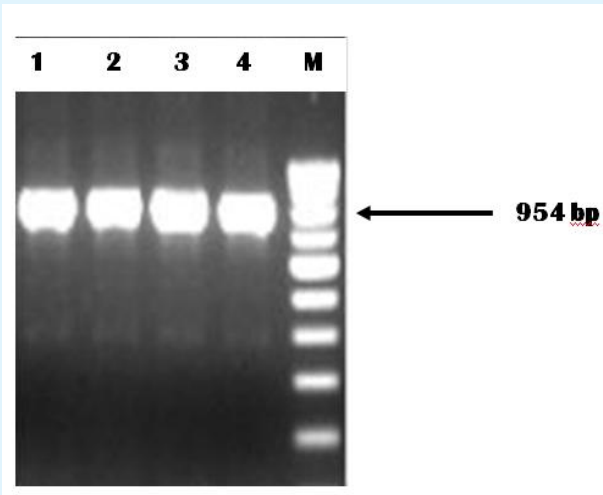

Figure 3: PCR product of *Adenoscolex oreini* Fotedar, 1958 [M = marker; bp = base pairs (100 bp ladder); 1, 2, 3, 4=DNA of *Adenoscolex* spp.].

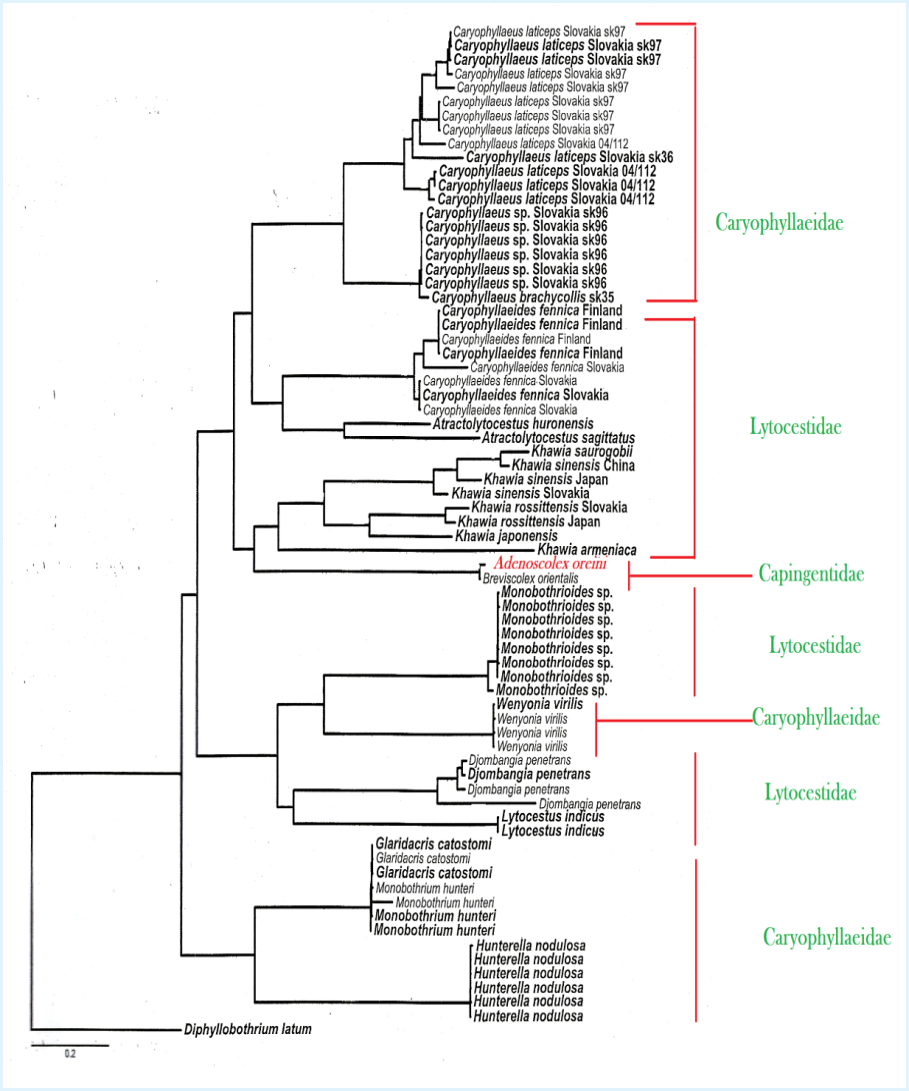

Figure 4: Phylogenetic Neighbour Joining (NJ) tree of 28S rDNA ITS sequences of Family Caryophyllaeidae; Lytocestidae and Capingentidae of Class Cestoda which shows that *Adenoscolex oreini* is very close to *Breviscolex oreintalis* belonging to same Family.

S. No.	Cestode Species	Host	GenBank Accession No.	Family	Base pairs	Authors	Country
1	*Adenoscolex oreini* Fotedar, 1958*	*Carassius carassius; Schizothorax niger; Schizothorax curvifrons; Schizothorax labiatus; Schizothorax plagiostomus*	SUB2816972	Capingentidae	954 bp	Present study	India
2	*Breviscolex orientalis*	*Hemibarbus barbus*	AF286978	Capingentidae	2075 bp	Olson *et al.*,[2001]	Japan
3	*Breviscolex orientalis*	*Hemibarbus barbus*	JQ034117	Capingentidae	1605 bp	Brabec *et al.* [2012]	Japan

Table 3: Cestode species used for molecular comparison of ITS rDNA sequences along with their hosts, country and GenBank accession numbers for corresponding sequences (*Query sequence).

It is observed that *Adenoscolex oreini* having GenBank accession number SUB2816972 shows 90.78% similarity with that of *Breviscolex orientalis* having JQ034117.1 GenBank accession number. Out of 954 base pairs of *Adenoscolex oreini* 866 bp are identical with that of *Breviscolex orientalis* with 9 gaps.

Karyology analysis

Analysis of mitotic metaphase spreads from seven specimens showed that the modal diploid complement of *Adenoscolex oreini* contains 20 chromosomes (2n=20). The karyotype (Figure 5a & 5b) included eight metacentric; one submetacentric and one acrocentric chromosome pair. First four pairs of metacentric elements are distinctly larger than the remaining chromosomes and contributed 48.62% to the total chromosome length. The chromosomes are middle sized; the largest measured 8.56 µm and the smallest were 1.94 µm long (Table 4). The total chromosome length of the haploid complement was 58.29 µm. Fundamental arm number is 38. The homologues of pairs 6 and 7 could not be distinguished clearly and are statically less significant (Students T-test; $P<0.05$; $P=0.003$). First three pairs of chromosomes are all metcentric and there is less difference of their relative lengths and are statically less significant $P<0.05$; $P=0.008$; Students T-test). Last three pairs of chromosomes are less significant statically ($P>0.05$; $P=0.045$; Students T-test) and there is difference in their relative lengths. In order to better visualize the existing differences in chromosome morphology, ideograms were constructed using the centromere indexes and relative length values (Figure 6).

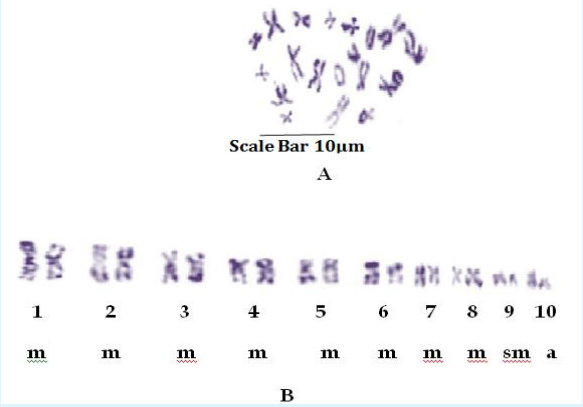

Figure 5: (a) Chromosome preparation of *Adenoscolex oreini*, (b) Karyotype constructed from mitotic cells of *Adenoscolex oreini* (m= Metacentric; sm= submetacentric).

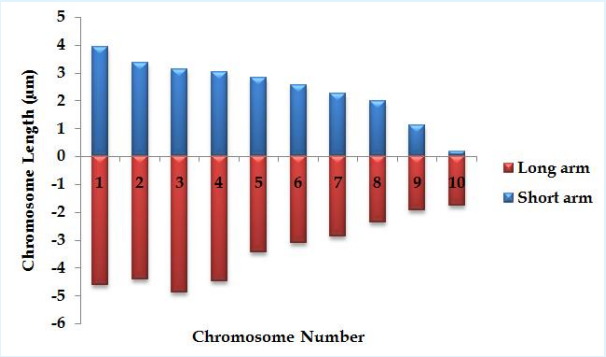

Figure 6: Haploid idiogram of *Adenoscolex oreini* Fotedar, 1958.

Chromosome pair number	Length of short arm (µm) 'S'	Length of long arm (µm) 'L'	Total Length/Absolute Length (µm) L+S	Arm Ratio (L/S)	Relative Length (%)	Centromeric Index (ci)	Classification	
1	3.93	4.63	8.56	1.18	14.69	45.91	Metacentric	
2	3.37	4.42	7.79	1.31	13.36	43.26	Metacentric	T-Value = -80.57 P-Value = 0.008
3	3.11	4.87	7.98	1.57	13.69	38.97	Metacentric	
4	3.03	4.49	7.52	1.48	12.9	40.29	Metacentric	
5	2.83	3.45	6.28	1.22	10.77	45.06	Metacentric	
6	2.57	3.11	5.68	1.21	9.74	45.25	Metacentric	T-Value = -17.65 P-Value = 0.003
7	2.27	2.88	5.15	1.27	8.84	44.08	Metacentric	
8	1.97	2.36	4.33	1.2	7.41	45.5	Metacentric	T-Value = -4.55 P-Value = 0.045
9	1.13	1.93	3.06	1.71	5.25	36.93	Submetacentric	
10	0.17	1.77	1.94	10.41	3.33	8.76	Acrocentric	

Table 4: Measurements and classification of chromosomes of *Adenoscolex oreini* Fotedar, 1958.

Discussion

Molecular Characterization: The present study used the molecular characterization of *Adenoscolex oreini* from different water bodies of the Kashmir valley is the first of its kind to determine the complete ITS sequence of 28S rDNA in combination with phylogenetic tree to show the interrelationships of this species with other cestode orders. During present observation, the ITS sequence of 28S rDNA showed 954 bp in which 90.78% sequences are similar to that of *Breviscolex orientalis*, which belongs to the same family of Capingentidae. Brabec *et al* and Olson *et al* showed that *Breviscolex orientalis* from the fish host of *Hemibarbus barbus* contains 707 partial sequence and 2075 complete sequence base pairs respectively out of which nearly 90% sequences matched in our study hence our results confirmed the position of *Adenoscolex oreini* in the family Capingentidae [16,29]. In relation to phylogenetics the present result also showed that, the *Adenoscolex oreini* is very close to that of family Capingentidae and Caryophyllaidae but with different host species in which the present species were collected from 6 species of fishes. Showed the phylogenetic relationship of the monozoic tapeworms from the morphological characters in which they concluded that the non-monotypic families namely the Capingentidae; Caryophyllaeidae and Lytocestidae, appeared to be paraphyletic, which are in close agreement with the present observations of *Adenoscolex oreini* studied at molecular level [30].

Only a few caryophyllidean cestodes have been sequenced till date [31-35]. Therefore, it is impossible to assess relationships of individual genera based on current molecular data. In the recent study by Olson *et al*, relationships of ten species of nine caryophyllidean genera of all families (Balanotaeniidae – 1 species, Capingentidae – 1, Caryophyllaeidae – 3, Lytocestidae – 5 species of 4 genera) have been analysed. Their study has shown, as in the case of the present data, incongruency with the current classification, because none of the non-monotypic families formed monophyletic clades. *Monobothrioides* Fuhrmann & Baer, 1925 (Lytocestidae) and *Balanotaenia* Johnston, 1924 (Balanotaeniidae) were most basal groups in a combined analysis of 28S and 18S rDNA based on Bayesian inference, whereas other two lytocestid genera viz., *Atractolytocestus* Anthony, 1958 and *Khawia* Hsu, 1935 were most derived [35]. Similarly, as in the present study, *Adenoscolex*, which is the only genus with species found in the intestine of the host, was the most basal Capingentidae in the tree inferred from sequences of the 28S rDNA gene.

Various authors who worked on molecular characterization of family Caryophllidae include Anthony; Jones and Mackiewicz; Chubb, *et al.*; Buckler, *et al.*; Scholz, *et al.*; Oros, *et al.*; Bouzid, *et al.*; Oros, *et al.*; Kralova-Hromadova, *et al.*; Orosova, *et al.*; Ash, *et al.*; Bazsalovicsova, *et al.*; Scholz, *et al.*; Bazsalovicsova, *et al.* who noted different sequences and relationship within the family but none showed the present form [37-48].

It is apparent that the systematics of the Caryophyllidea and its position among the basal tapeworms (Eucestoda) is still unresolved [32,49,50]. Because of the low number of molecular characters used and very low consistency index of the trees, it is premature to speculate on some of the molecular and evolutionary implications of the present data. New material of most genera is needed for analyses of molecular data. These data should help us understand not only the evolutionary relationships of this unique cestode but also the evolution of the life cycles.

Karyology

The diploid chromosome number of *Adenoscolex oreini* is 20 and it is the first report of chromosome number of a genus from family Capingentidae of order Caryophyllidea from the Kashmir valley. However, in the family Caryophyllidae the chromosome number has been reported by Mackiewicz and Jones; Grey and Mackiewicz; Vijayaraghavan and Subramanyam; Grey; Grey and Mackiewicz; Petkeviciute and Kuperman; Petkeviciute; Bombarova, *et al.;* Orosova, *et al.* for *Archigetes appendiculatus* (2n=18); *Hunterella nodulosa* (2n=14); *Glaridacis laruei* (2n=16); *Lytocestus indicus* (2n=16); *Capingens singularis* (2n=14); *Glaridacris catostomi* (2n=20); *Caryophyllaeus laticeps* (2n=20); *Khawia sinensis* (2n=16); *Caryophyllaeides fennica* (2n=20) and *Khawia saurogobii* (2n=16), respectively [2,51-57]. It seems that the chromosome number of *Adenoscolex oreini,* a member of family Capingentidae is higher as compared to that of the members of family Caryophyllaeidae. The variation in size of smallest bivalent and other bivalents indicates that the somatic chromosome shows a marked variation in the length of longest and shortest chromosome in *Adenoscolex oreini.* The same has been reported by Mackiewicz and Jones in *Hunterella nodulosa.* Therefore, this type of chromosome complement may be characteristic of Caryophyllidea in general [50].

In individual species, chromosome morphology differs significantly; a predominance of bi-armed elements (symmetrical karyotypes) was detected in four species of the family Caryophyllaeidae and one lytocestid species, one-armed acrocentric chromosomes

(asymmetrical karyotypes) prevailed in four species of Caryophyllaeidae and Lytocestidae and the rest of five karyotypes with rather balanced chromosome morphology occurs in both families. Because of this variation, no relevant hypothesis on karyotype evolution can be made on basis of traditional karyological analyses among the four families of the Caryophyllidea. It is noteworthy that recent cladistic analysis by Oros, *et al.*, based on unweighted morphological characters, are only partly congruent with the existing classification into four families that is based on the placement of the internal, longitudinal musculature. It is apparent that classical karyological data are hardly sufficient to resolve phylogenetic and systematic relationships within insufficiently investigated animal groups such as the Caryophyllidea. However, the recent inputs of molecular approaches into the cytogenetics of Caryophyllidea raises hopes that new data will substantially help in elucidating the phylogenetic relationships within this unique group of monozoic tapeworms [43,44,58-60].

Conflict of Interest

Authors declare that there is no conflict of interest regarding the present research work.

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