

Redescription on *Raillietina echinobothrida* (Pasquale, 1890) (Cestoda: Davaineidae) and Study of Conserved Domain across Divergent Phylogenetic Lineages of Class Cestoda

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Abstract

Raillietina (Fuhrmannetaa) *echinobothrida*, (Magnin, 1881) cestode parasite of *Gallus gallus domesticus* is redescribed on the basis of type material from Aurangabad, Marathwada, Maharashtra, India. The present worms resemble with *R. echinobothrida*, (Magnin, 1881) in having all essential morphological characters. having scolex oval, rostellum elongated/rounded, presence of four suckers, short neck, mature proglottids are broader than long, testes rounded and excretory canal long tube. But the same differ due to number of testes. Hence it is redescribed. We carried out the phylogenetic analyses to observe molecular resemble of related proteins.

Keywords: *R. (Fuhrmannetaa) echinobothrida*; Magnin, 1881; *Gallus gallus domesticus*; MSA; Conserve domain; Phylogenetic analysis

Introduction

Raillietina echinobothrida is an endoparasite, and this tapeworm belonging to the class Cestoda. This is avian gastrointestinal parasite of family *Davaineidae* (Cestoda: Cyclophyllidea), and is the most pathogenic and prevalent species infecting *Gallus domesticus* Linnaeus, 1758. *R. echinobothrida* requires two hosts, birds and ants, for completion of its life cycle. *R. echinobothrida* is a hermaphrodite worm having both the male and female reproductive organs in its body. The parasite is responsible for 'nodular tapeworm disease' in poultry [1,2]. This tapeworm is responsible for stunted growth of young chicken, also emaciation of adult and decreased egg production of hen. *R. echinobothrida* does not cause gross pathological damages, but in severe lesions on the intestinal walls and diarrhoea could arise. It ostensibly resulted in ill health [3]. Phylogenetic analyses have become essential to research on *R. echinobothrida* for the evolutionary tree of life. It shows taxonomical classification, identification, and naming of organisms, which is usually richly informed by phylogenetics. Multiple sequence alignments are the resource for the annotation of functional units in proteins called as conserved domain. This Conserved domains can be thought of as distinct functional, structural units of a protein from *R. echinobothrida*. In molecular evolution of *R. echinobothrida* such domains may have been utilized as building blocks, and may have been recombined in different arrangements to modulate protein function, which can be determined by sequence and structure analysis [4,5].

Material and Methods

Study cestode

Sixty five cestode were collected from the intestine of *Gallus gallus domesticus* from Aurangabad district Maharashtra state, India, during the period Dec-2006 to Dec-2009. Thirty cestode were preserved in hot 4% formalin and specimen were stained with Haris Haematoxyline and Borax carmine stain and passed through various alcoholic grades. Cleared in xylene, mounted in DPX and drawing are made with aid of camera lucida. All measurements are given in the millimetre [6,7].

Sources and sequence information of Cestoda class

We have taken seven species of Cestoda class, in which targeted is nucleotide-binding domain of the sugar kinase/HSP70/actin protein data were used to observe molecular resemble of related protein by phylogenetic analysis [8,9].

Multiple sequence alignment of Cestoda class

Multiple sequence alignment [MSA] is conducted by COBALT that aligns protein sequences of similar Cestoda class using a combination of distance matrix and approximate parsimony methods. Numerical setting method is used to study the relative entropy threshold, in bits, that must be met for an alignment column to be displayed in red. A larger number indicates higher degree of conservation. The relative entropy is computed as:

$$\sum_i f_i \log_2 (f_i / p_i),$$

where i is residue type, f_i is residue frequency observed in the multiple alignment column, and p_i is the background residue frequency.

Identity setting used for only columns with one residue type will be colored in red [10,11].

Construction of a phylogenetic tree for actin protein

Phylogenetic analyses were performed by Fast minimum evolution algorithm and Neighbor Joining algorithms to allow the reconstruction phylogenetic tree of the molecular evolutionary history of various

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aligned sequences that are useful to align highly evolved gene families clearing evolutionary relationships such as multiple actin proteins [12,13]. Trees were obtained by the methods Fast minimum evolution algorithm and Neighbor Joining algorithms. Evolutionary distance is studied by Grishin (protein) model [14,15] and distance between two sequences modelled as expected fraction of amino acid substitutions per site given the fraction of mismatched amino acids in the aligned region and can be computed for fraction of mismatched amino acids larger than 0.75 [5,3].

Results and Description

Observation of *R. echinobothrida*

In *R. echinobothrida*, the scolex is having four rounded sucker which overlaps each other and measures 0.242 (0.1619-0.3155) in length and 0.1262 (0.0970-0.1553) in width. The mature proglottids are 2-3 times broader than long and measures 0.4563 (0.4271-0.4854) in length and 0.9805 (0.9028-1.0581) in width. Testes are oval to rounded, 20-25 in number and measures 0.0291 in diameter. Cirrus pouch is elongated in shape, preovarian, situated in centre of proglottids and measures 0.1262 (0.1067-0.1456) in length and 0.0436 (0.0339-0.0533) in width. The vas deference is long, curved tube and measures 0.2621 (0.2524-0.2766) in length and 0.0097 (0.0048-0.0145) in width. The gravid segments are broader than long and contain numerous egg capsules. Each egg capsule contains 4-5 eggs (Figures 1 and 2).

Systematic position

Raillietina (Fuhrmannetta) echinobothrida

Class: Eucestoda

Order: Davaineidea

Family: Davaineidae

Genus: *Raillietina*

Species: *Raillietina echinobothrida*

Taxonomy summary

Type species: *Raillietina (Fuhrmannetta) echinobothrida*

Host: *Gallus gallus domesticus*

Habitat: Intestine

Locality: Aurangabad M.S., India

Period of collection: Dec. 2006-Dec.2009

Deposition: Helminthology Research Lab, Dept. Of Zoology, Dr. Babasaheb AmbedkarMarathawada University, Aurangabad.

Evolutionary distance

This study, 7 β -actin from Cestoda class is summarized to study

the evolutionary distance. The identification of the origin of β -actin protein, multiple sequences analysis, observing the conserved amino acid residues and reconstruct the phylogenetic tree specify the evolutionary history, relationship of Cestoda with different species

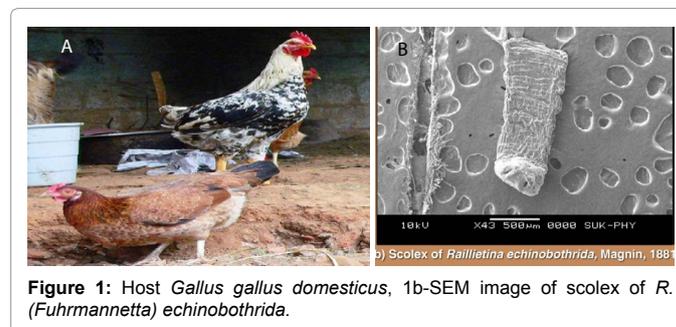


Figure 1: Host *Gallus gallus domesticus*, 1b-SEM image of scolex of *R. (Fuhrmannetta) echinobothrida*.

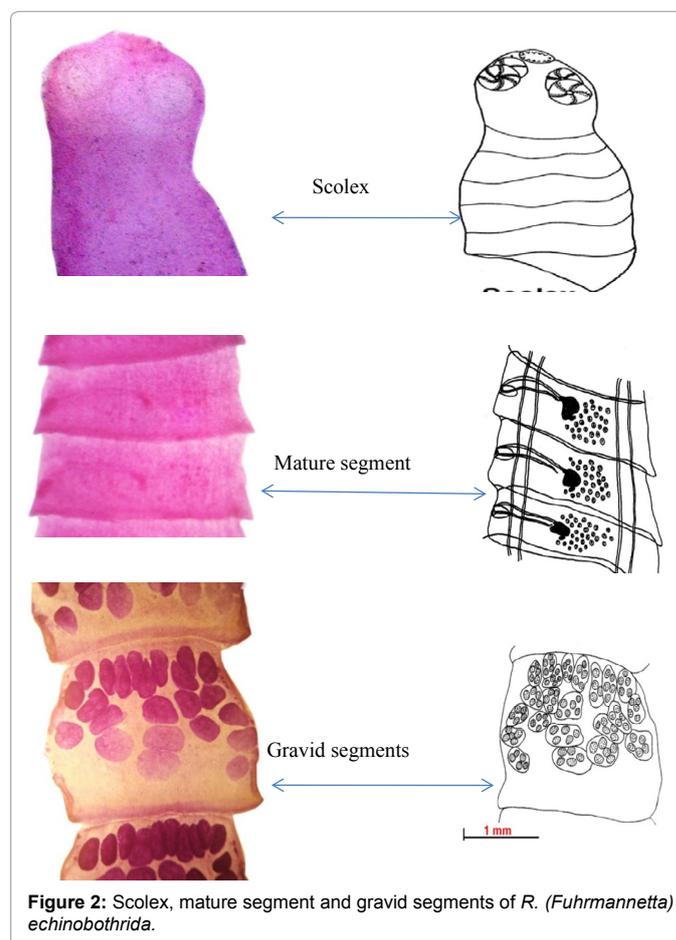


Figure 2: Scolex, mature segment and gravid segments of *R. (Fuhrmannetta) echinobothrida*.

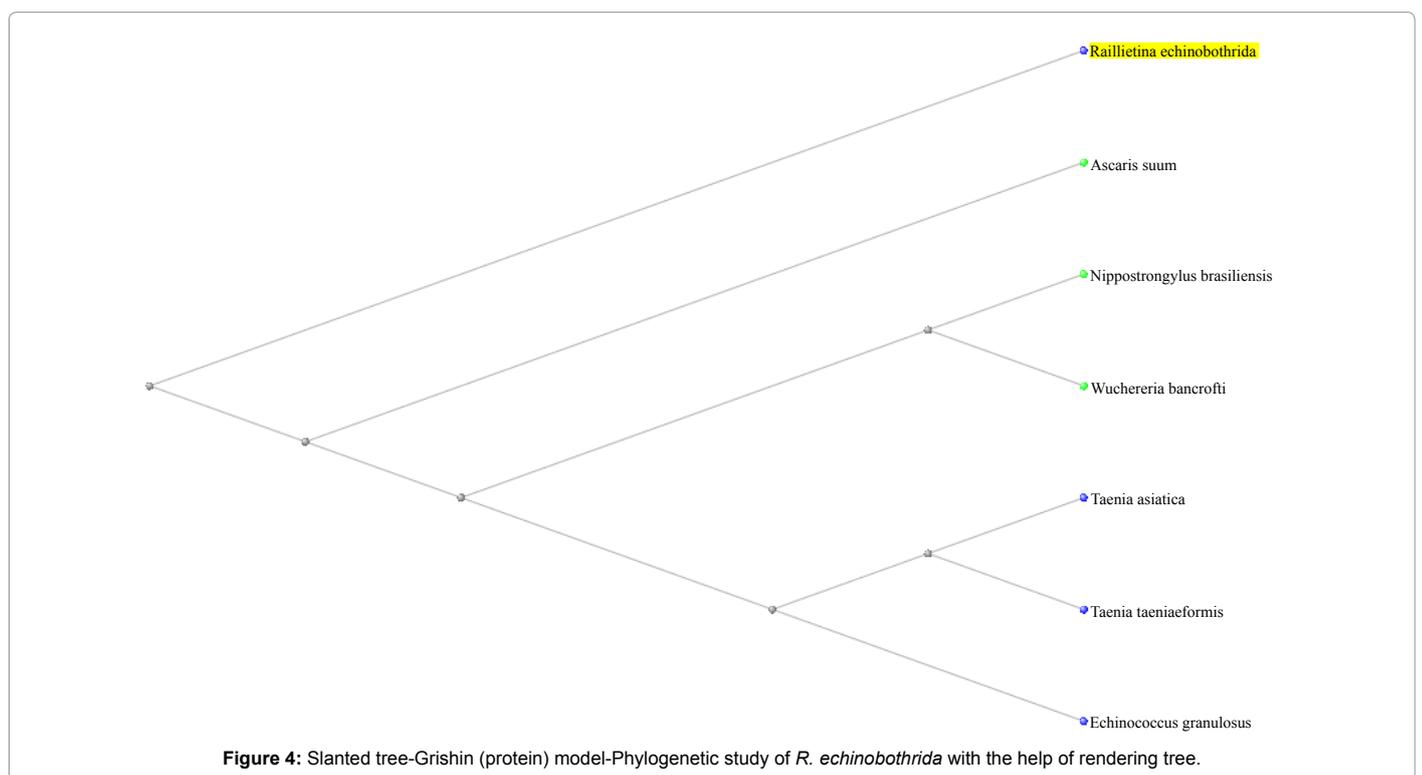
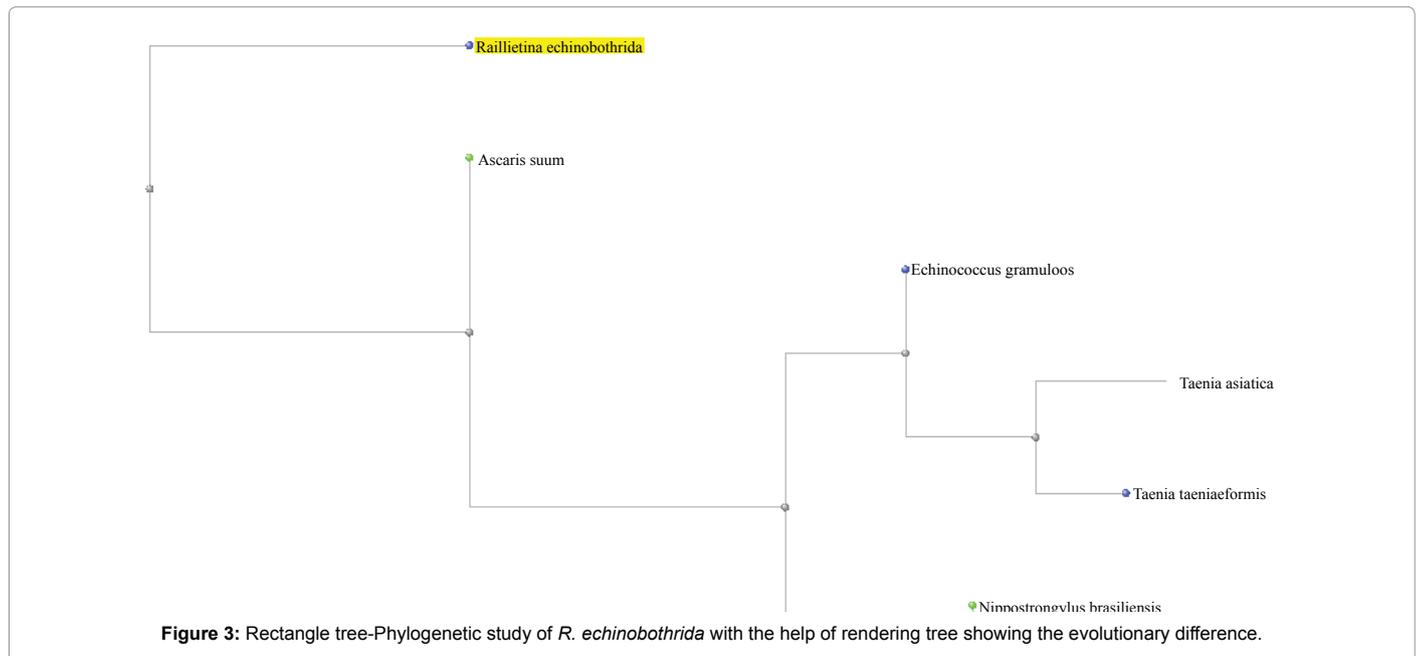
Accession	Species Name	Max score	Total score	Query cover	E value	Ident
AGA19608.1	<i>β-actin [Raillietina echinobothrida]</i>	235	235	100%	2.00E-77	100%
ERG81274.1	<i>actin-2 [Ascaris suum]</i>	176	176	78%	3.00E-53	93%
BAI81968.1	<i>actin [Nippostrongylus brasiliensis]</i>	174	174	75%	3.00E-53	96%
ACP21243.1	<i>actin II [Echinococcus granulosus]</i>	172	172	75%	4.00E-53	96%
EJW72694.1	<i>actin-2 [Wuchereria bancrofti]</i>	174	174	75%	5.00E-53	96%
ABN14926.1	<i>cytoskeletal actin 2 [Taenia asiatica]</i>	174	174	74%	6.00E-53	98%
BAJ07368.1	<i>actin [Taenia taeniaeformis]</i>	174	174	75%	8.00E-53	96%

Table 1: Sequences producing significant alignments.

(Table 1). Rectangle tree shows rectangular shaped rooted tree, where root is places in the longest edge. Fast minimum evolution algorithm produce un-rooted tree such as ones shown as radial or force in the tabs below. The rooted trees are created by placing a root in the middle of the longest edge (Figure 3). Slanted tree shows similar to rectangle, but with triangular tree shape. Neighbor Joining algorithms produce un-rooted tree such as ones shown as radial or force in the tabs below. The rooted trees are created by placing a root in the middle of the longest edge (Figure 4).

Multiple sequence alignment

Multiple sequence alignment analysis shows columns with no gaps are colour in blue or red. The red colour indicates highly conserved regions and blue indicates less conserved ones. The Conservation analysis can be used to select a threshold for determining which columns is colour in red (Figure 5). Multiple sequence alignment identify conserved motifs and to predict functional role in the variable sites as well as conserved sites show the sequence divergence profile



AGA19608	1	-----NKERMR[7]GV[13]MVGMGNKDSYVGDEAQSKRGILSLKYPI	56
ERG81274	1	MCD-EEVAALVVDNGSGMCKAGFAGDDAPRAVFPFI-----GV MVGMGQKDSYVGDEAQSKRGILTLKYPI	65
BAI81968	1	-----APRAVFPISVGRPRHQGV MVGMGQKDSYVGDEAQSKRGILTLKYPI	46
ACP21243	1	-----GV MVGMGQKDSYVGDEAQSKRGILTLKYPI	30
EJW72694	1	MCD-EEVAALVVDNGSGMCKAGFAGDDAPRAVFPISVGRPRHQGV MVGMGQKDSYVGDEAQSKRGILTLKYPI	72
ABN14926	1	MSEgDEIAALVVDNGSGMCKAGFAGDDAPRAVFPISVGRPRHQGV MVGMGQKDSYVGDEAQSKRGILTLKYPI	73
BAJ07368	1	MSEgEEIAALVVDNGSGMCKAGFAGDDAPRAVFPISVGRPRHQGV MVGMGQKDSYVGDEAQSKRGILTLKYPI	73
AGA19608	57	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFE-----	110
ERG81274	66	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRIT [40]	183
BAI81968	47	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRIT [20]	144
ACP21243	31	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNCPAMYVAIQ-----	97
EJW72694	73	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRIT	150
ABN14926	74	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNCPAMYVAIQAVLSLYASG---	148
BAJ07368	74	EHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNCPAMYVAIQAVLSLYASGRIT [17]	168

Figure 5: Multiple sequence alignment by COBALT of various Cestoda class species. An alignment will display the following symbols denoting the degree of conservation observed in each column. '*' indicate that, the residues in that column are identical in all sequences in the alignment 12 is 12.50%. ':' indicate that strongly similar, conserved substitutions have been observed, 4 is 4.17%. '.' indicate that weakly similar, semi-conserved substitutions are observed 1 is 1.04%.

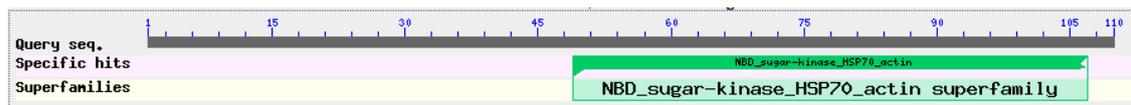


Figure 6: Conserved domain analysis shows nucleotide-binding domain of the sugar kinase/HSP70/actin superfamily.

of these actin proteins, which demonstrate the sequence enrichment strategy of these sequences for adaptation to different physiological systems. Here we observed that from all sequences of actin proteins that Cys, Lys, Asp, (Hydrophilic amino acid) Pro, Gly, (hydrophobic amino acid) which is conserved in all peptides having a common ancestor. That all of these peptides share eight highly conserved cysteine, which were involved in the formation of β -strands are almost conserved. Cysteine (C) is conserved in all sequences at 8 sites.

Conserved domain analysis

Molecular study of Cestoda class species shows one conserved domain is nucleotide-binding domain of the sugar kinase/HSP70/actin superfamily. The nucleotide-binding region residues are conserved and the nucleotide sits in a deep cleft formed between the two lobes of the nucleotide-binding domain (NBD). Substrate binding to superfamily members is associated with closure of this catalytic site cleft. Functional activities of nucleotide-binding domain, including hexokinases, actin, and HSP70s, are modulated by allosteric effectors, which may act on the cleft closure (Figure 6).

Conclusion

The presences of various species of the Cestoda were noted on the basis of actual sighting. Above survey with the primary objective of collecting and identifying the species, Sampling for estimation population of available species and understanding the community structure of amphibians in different habitat types. Phylogenetic analysis of Cestoda class signifies that nucleotide-binding domain of the sugar kinase protein is important components of Cestoda species, are originated from proteins enriched with different sequence specific substitution strategy for biological needs. Comparative analyses specify that the nucleotide-binding domain of the sugar kinase protein

demonstrates how proteins are generated within the nature's testing ground for tailor-made biologic needs. Tracing the natural protein engineering scheme of sodium/calcium exchanger protein enrich our knowledge which in turn helps to molecular phylogeny.

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