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Description on *Tylocephalum salunkhi* N. Sp. (Cestoda: Lecanicephalidea) and Study of Conserved Domain across Divergent Phylogenetic Lineages of Class Cestoda

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Abstract

Tylocephalum salunkhi n. sp. cestode parasite of *Trygon sephen* is described on the basis of type material from Ratnagiri (West Coast of Maharashtra, India). The present worms resemble with *Tylocephalum marsupium* in having all essential morphological characters. Species having scolex oval, rostellum elongated/rounded, presence of four suckers, 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 new species described.

Keywords: *Tylocephalum salunkhi n.* sp.; *Trygon sephen*; MSA; Conserve domain; Phylogenetic analysis

Introduction

Tylocephalum salunkhi n. sp. is an endoparasite, and this tapeworm belonging to the class Cestoda. This is pisian gastrointestinal parasite of family *Tetragonocephalidae* (Cestoda: Lecanicephalidea), and is the most pathogenic and prevalent species infecting *Trygon sephen* [1-3].

The genus *Tylocephalum* was erected by Linton, with its type species *T. pingue* from *Rhinoptera quadriloba* at Woods Hole, Shipley et Hornell (1906). Total 19 species of this genus recorded till now. Linton reported *T. marsupium* from *Aetobatis narinari*, (Euphrasen, 1790) at Tortugas Chincholikar (1976) added one new species to this genus i.e. *T. madhukari* from Trygon sp. at Ratnagiri. Jadhav and Shinde described *T. singhii* from *Trygon zugei* Muller and Henle, at Bombay, India Wankhede and Jadhav (2003) added new species *T. gajanane* from *Trygon sephen* at Bombay (West Coast of India). Later on Pawar et al. added two new species of this genus *T. babulae* from *Trygon zugei* and *T. shindei* from *Trygon sephen* at Ratnagiri (West Coast of Maharashtra, India).

Phylogenetic analyses have become essential to research on Cestoda class 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 Cestoda class. In molecular evolution of Cestoda class species 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 Methods

Study of Cestode

Sixty nine cestodes were collected from the intestine of *Trygon sephe* from Ratnagiri (West Coast of Maharashtra, India), during the period Dec-2006 to Dec-2009. Thirty five cestodes were preserved in hot 4% formalin and specimen were stained with Haris Haematoxyline and Borax carmine stain and passed through various alchoholic grades. Cleared in xylene, mounted in DPX and drawing are made with aid of camera lucida. All measurements are given in the milimeter [6,7].

Sources and sequence information of Cestoda class

We have taken sixty three (63) species of Cestoda class, in which targeted is NADH dehydrogenase subunit 3 protein data were used to observe molecular resemble of related protein by phylogenic 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: $\sum i\ fi \log2\ (fi/pi)$, where i is residue type, fi is residue frequency observed in the multiple alignment column, and pi 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 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 modeled 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 [16,17].

Results and Description

Observation of Tylocephalum salunkhi n. sp. (2009)

A new species of the cestode genus *Tylocephalum salunkhi* n. sp. (2009) obtained from the host *Trygon sephen* is described. A detailed examination of specimens has allowed us to erect a new species *Tylocephalum* to accommodate the worm. Microscopic observation shows remarkable differences from other known species of *Tylocephalum*. The new species is designated as *Tylocephalum salunkhi n.* sp. (2009) (see keys).

Neck absent	1
Neck present	2
1} Vitellaria granular	3
Vitellaria follicular	4
2} Scolex cushion shaped	*T. yorkei*, Southwell, 1925.
Scolex globular	5
Scolex sub-globular	6
Scolex variable in shape	*T. dierma*, Shipley et Hornell, 1906.
Scolex quadrangular in shape	7
Scolex rounded in shape	*T. bombayensis* Jadhav, 1983.
3} Ovary " H" shaped	*T. hanumanthraoae* Shinde, et.al., 1989.
Ovary compact	*T. madhukarae* Chincholikar and Shinde, 1980
Ovary "U" shaped	*T. alibagensis* Bhagwan and Mohekar,2003
Ovary lobate	*T. marsupium* Linton, 1916.
Ovary bilobed	*T. gajananae* Wankhede and Jadhav, 2003.
4} Scolex circular at the anterior part	*T. aetiobatidis* Shipley et Hornell, 1906.
Scolex globose	*T. pingue* Linton, 1890.
Anterior region of scolex smaller than posterior region	*T . minimum* Subhapradha, 1955.
Anterior region of scolex larger than posterior region	*T. elongatum* Subhapradha, 1955.
5} Vagina anterior to cirrus pouch	*T. mehdii* Bhagwan et.al., 2002.
Vagina posterior to cirrus pouch	8
Vagina posterioventral to cirrus pouch	*T. shindei* Pawar and Jadhav, 2005.
6} Testes below 20 in number	*T. salunkhi* n.sp.
Testes above 20 in number	*T. squatinae* Yamaguti, 1934
7} Vagina dorsolateral to cirrus pouch	*T. bonasum* Ronald A. Campbell et.al., 1984.
Vagina posterior to cirrus pouch	*T. aurangabadensis* Jadhav et.al., 1987.
8} Genital pore lies at marginal	*T. babulae Pawar* and Jadhav, 2005.

Genital pore Sub-marginal	*T. shindei* Pawar and Jadhav 2005.

Tylocephalum salunkhi n.sp. - Comparison keys

The scolex is divided into two regions, anterior and posterior. Presences of four suckers which are oval in shape two are placed towards the anterior side and two are placed towards the posterior side of the scolex. Mature segments are longer than broad. The cirrus pouch is oval, elongated, large, Testes 12-13 in number and pre-ovarian. Ovary bi-lobed, 'V' shaped. The vitellaria are follicular in shape (Figures 1 and 2).

Systematic Position

Tylocephalum salunkhi n. sp. (2009)

Class: *Eucestoda*

Order: *Lecanicephalidea*

Family: *Tetragonocephalidae*

Genus: *Tylocephalum*

Species: *Tylocephalum salunkhi n.* sp. (2009)

Figure 1: Host Trygon sephen.

Taxonomy summary

Type species: *Tylocephalum salunkhi n.* sp. (2009)

Host: *Trygon sephen*

Habitat: Intestine

Locality : Aurangabad M.S., India

Period of collection: Dec. 2006- Dec.2009

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

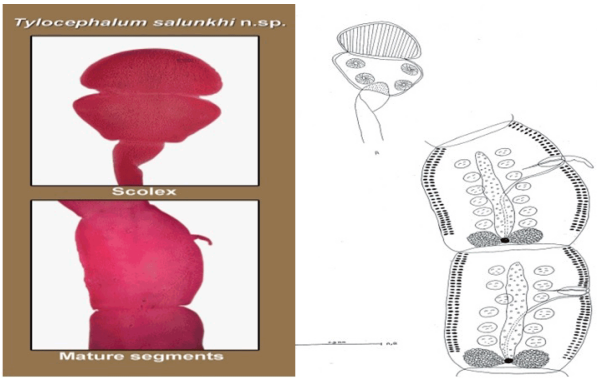

Figure 2: Scolex, mature segment segments of *Tylocephalum salunkhi n.* sp. (2009).

Evolutionary distance

This study, sixty three NADH dehydrogenase subunit 3 protein from Cestoda class is summarized to study the evolutionary distance. The identification of the origin of NADH dehydrogenase subunit 3 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 (Table 1).

Description	Accession No of NADH dehydrogenase subunit 3 protein	Identity %	E value	Total Score
Tylocephalum sp. DTJL-2012	gi|374094534|gb|AEY84602.1|	100	3.00E-71	219
Kotorella pronosoma	gi|374094534|gb|AEY84602.1|	74.14	1.00E-47	159
Pachybothrium hutsoni	gi|374094534|gb|AEY84602.1|	74.14	1.00E-46	157

Clistobothrium montaukensis	gi|374094534|gb|AEY84602.1|	71.55	3.00E-46	156
Diphyllobothrium dendriticum	gi|374094534|gb|AEY84602.1|	70.69	2.00E-43	149
Acanthobothrium sp. DTJL-2012	gi|374094534|gb|AEY84602.1|	71.55	5.00E-43	147
Diplogonoporus balaenopterae	gi|374094534|gb|AEY84602.1|	68.97	6.00E-43	147
Diphyllobothrium klebanovskii	gi|374094534|gb|AEY84602.1|	68.97	2.00E-42	146
Diphyllobothrium nihonkaiense	gi|374094534|gb|AEY84602.1|	68.1	2.00E-41	144
Hymenolepis diminuta	gi|374094534|gb|AEY84602.1|	69.23	3.00E-41	143
Sparganum proliferum	gi|374094534|gb|AEY84602.1|	68.97	1.00E-40	141
Anchistrocephalus microcephalus	gi|374094534|gb|AEY84602.1|	70.69	2.00E-40	141
Haplobothrium globuliforme	gi|374094534|gb|AEY84602.1|	66.38	2.00E-40	141
Proteocephalus macrocephalus	gi|374094534|gb|AEY84602.1|	81.48	2.00E-38	136
Rhodobothrium sp. DTJL-2012	gi|374094534|gb|AEY84602.1|	72.41	2.00E-38	136
Diphyllobothrium stemmacephalum	gi|374094534|gb|AEY84602.1|	65.52	3.00E-38	135
Grillotia pristiophori	gi|374094534|gb|AEY84602.1|	68.97	5.00E-38	135
Litobothrium nickoli	gi|374094534|gb|AEY84602.1|	71.55	2.00E-37	133
Spirometra erinaceieuropaei	gi|374094534|gb|AEY84602.1|	68.97	1.00E-36	131
Abothrium gadi	gi|374094534|gb|AEY84602.1|	70	2.00E-36	131
Diphyllobothrium klebanovskii	gi|374094534|gb|AEY84602.1|	73.68	2.00E-36	130
Dipylidium caninum	gi|374094534|gb|AEY84602.1|	66.09	5.00E-36	129
Didymobothrium rudolphii	gi|374094534|gb|AEY84602.1|	64.6	9.00E-36	129
Tetrabothrius erostris	gi|374094534|gb|AEY84602.1|	61.21	1.00E-34	126
Nippotaenia chaenogobii	gi|374094534|gb|AEY84602.1|	68.1	2.00E-31	117
Taenia madoquae	gi|374094534|gb|AEY84602.1|	57.98	2.00E-30	115
Taenia ovis	gi|374094534|gb|AEY84602.1|	57.76	5.00E-30	114
Versteria mustelae	gi|374094534|gb|AEY84602.1|	61.26	7.00E-30	114
Khawia baltica	gi|374094534|gb|AEY84602.1|	65.85	1.00E-29	113
Taenia solium	gi|374094534|gb|AEY84602.1|	56.48	3.00E-29	112
Taenia saginata	gi|374094534|gb|AEY84602.1|	57.14	3.00E-29	112
Caryophyllaeus brachycollis	gi|374094534|gb|AEY84602.1|	65.85	5.00E-29	112
Monobothrioides sp. JB-2012	gi|374094534|gb|AEY84602.1|	63.04	1.00E-28	110
Taenia crassiceps	gi|374094534|gb|AEY84602.1|	63.92	2.00E-28	110
Echinobothrium harfordi	gi|374094534|gb|AEY84602.1|	70.53	5.00E-28	109
Khawia parva	gi|374094534|gb|AEY84602.1|	51.67	3.00E-27	107
Taenia taeniaeformis	gi|374094534|gb|AEY84602.1|	53.51	3.00E-27	107
Taenia asiatica	gi|374094534|gb|AEY84602.1|	54.31	5.00E-27	106
Taenia pisiformis	gi|374094534|gb|AEY84602.1|	58.62	7.00E-27	106

Wenyonia virilis	gi|374094534|gb|AEY84602.1|	46.55	1.00E-26	105
Khawia armeniaca	gi|374094534|gb|AEY84602.1|	59.04	1.00E-26	105
Breviscolex orientalis	gi|374094534|gb|AEY84602.1|	65.85	2.00E-26	105
Taenia twitchelli	gi|374094534|gb|AEY84602.1|	63.79	2.00E-26	105
Echinococcus equinus	gi|374094534|gb|AEY84602.1|	65.43	3.00E-25	102
Echinococcus vogeli	gi|374094534|gb|AEY84602.1|	64.04	5.00E-25	101
Caryophyllaeides fennica	gi|374094534|gb|AEY84602.1|	61.32	6.00E-25	101
Echinococcus canadensis	gi|374094534|gb|AEY84602.1|	64.04	8.00E-25	101
Khawia sinensis	gi|374094534|gb|AEY84602.1|	67.06	8.00E-25	100
Caryophyllaeides fennica	gi|374094534|gb|AEY84602.1|	60.75	9.00E-25	100
Echinococcus ortleppi	gi|374094534|gb|AEY84602.1|	62.92	2.00E-24	100
Fasciola hepatica	gi|374094534|gb|AEY84602.1|	64.63	2.00E-24	100
Fasciola gigantica	gi|374094534|gb|AEY84602.1|	65.85	3.00E-24	99.8
Glaridacris catostomi	gi|374094534|gb|AEY84602.1|	69.41	7.00E-24	98.6
Monobothrium hunteri	gi|374094534|gb|AEY84602.1|	69.41	9.00E-24	98.2
Khawia saurogobii	gi|374094534|gb|AEY84602.1|	61.54	3.00E-23	97.1
Glaridacris catostomi	gi|374094534|gb|AEY84602.1|	68.24	5.00E-23	96.7
Caryophyllaeus laticeps	gi|374094534|gb|AEY84602.1|	64.13	7.00E-23	96.3
Hydatigera parva	gi|374094534|gb|AEY84602.1|	55.45	7.00E-23	95.9
Taenia martis	gi|374094534|gb|AEY84602.1|	60.34	2.00E-22	94.7
Echinococcus oligarthrus	gi|374094534|gb|AEY84602.1|	66.67	1.00E-21	92.8
Opisthorchis viverrini	gi|374094534|gb|AEY84602.1|	51.69	1.00E-21	92.8
Taenia multiceps	gi|374094534|gb|AEY84602.1|	62.62	2.00E-21	92.4
Taenia hydatigena	gi|374094534|gb|AEY84602.1|	58.62	2.00E-21	92.4

Table 1: Sequences producing significant alignments.

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 (Figures 3 and 4).

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 5).

MSA

Multiple sequence alignment analysis shows columns with no gaps are colored in blue or red. The red color indicates highly conserved regions and blue indicates less conserved ones. The Conservation analysis can be used to select a threshold for determining which columns are colored 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 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 cysteines which were involved in the formation of β-strands are almost conserved. Cysteine (C) is conserved in all sequences at 8 sites. Multiple sequence alignment by COBALT of various Cestoda class species.

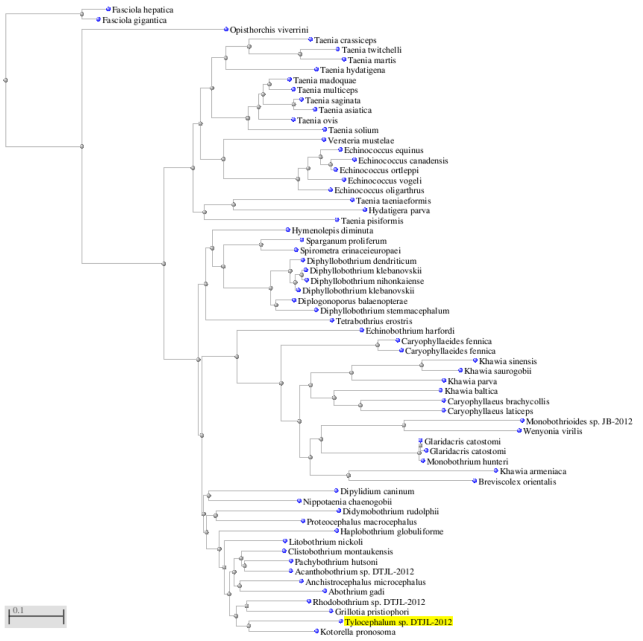

Figure 3: Rectangle tree - Fast minimum evolution algorithm model- Phylogenetic study of *Raillietina echinobothrida* with the help of rendering tree showing the evolutionary difference.

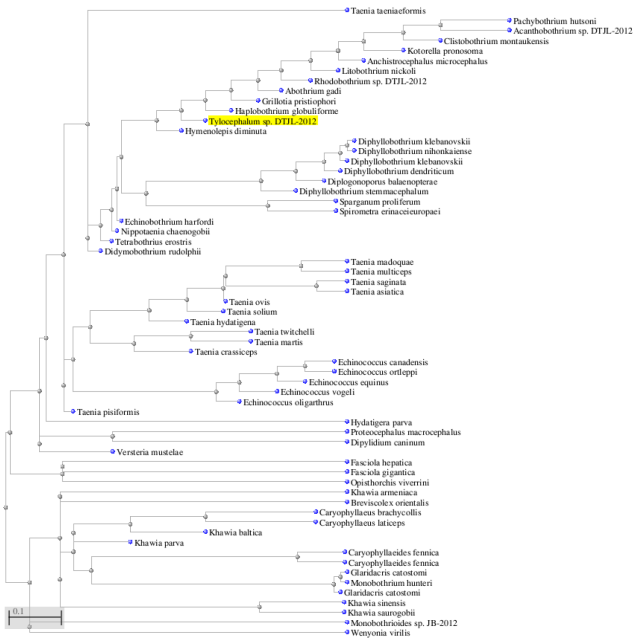

Figure 4: Rectangle tree –Cobalt Model- Phylogenetic study of *Raillietina echinobothrida* with the help of rendering tree showing the evolutionary difference.

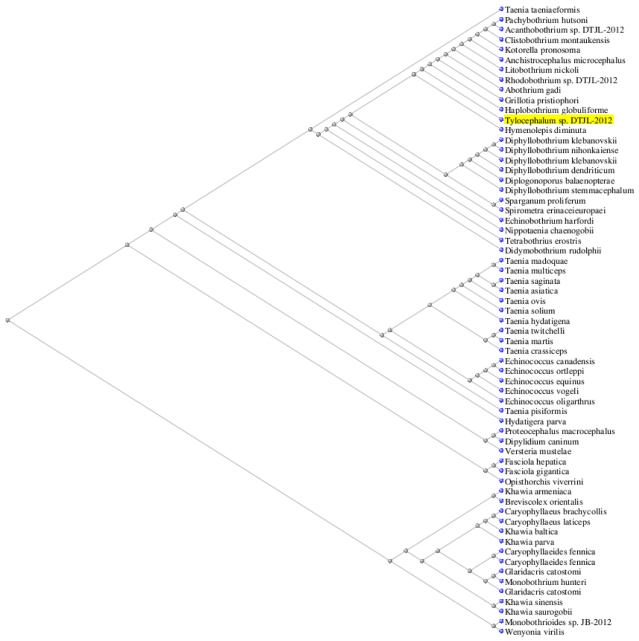

Figure 5: Slanted tree - Grishin (protein) model- Phylogenetic study of *Raillietina echinobothrida* with the help of rendering tree.

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 %.

Conserved domain analysis

Molecular study of Cestoda class species shows one conserved domain is NADH-ubiquinone/plastoquinone oxidoreductase, chain 3 (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 Cestodes in different habitat types. Phylogenetic analysis of Cestoda class signifies that NADH-ubiquinone/plastoquinone oxidoreductase, chain 3 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 NADH-ubiquinone/plastoquinone oxidoreductase, chain 3 protein demonstrates how proteins are generated within the nature's testing ground for tailor-made biologic needs. Tracing the natural protein engineering scheme of three NADH dehydrogenase subunit 3 protein enrich our knowledge which in turn helps to molecular phylogeny.

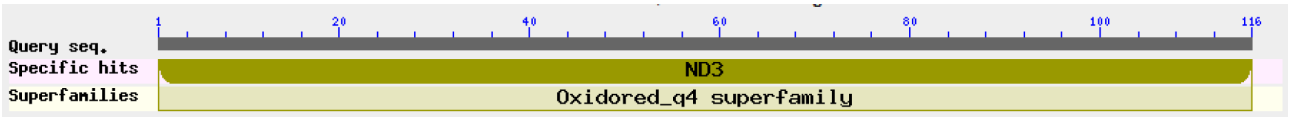

Figure 6: Conserved domain analysis shows NADH-ubiquinone/plastoquinone oxidoreductase, chain 3.

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