



# REVIEW OF RESEARCH

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## MORPHOLOGICAL AND MOLECULAR STUDY OF *STILESIA* SP. (CESTODA: ANAPLOCEPHALIDEA) A CESTODE PARASITE OF THE DOMESTIC GOAT *CAPRA HIRCUS* (L.) IN JALNA DISTRICT (M.S.), INDIA

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### ABSTRACT

The genus *Stilesia* was erected by Railliet, 1893. *Stilesia hircusae* Sp. Nov. is collected in the intestine of *Capra hircus*, Linnaeus, 1758 (Family: Bovidae) from Jalna district (M.S.), India. The present Cestode i.e. *Stilesia hircusae* Sp. Nov. differs from other all known species is having scolex large, squarish, mature proglottids almost 25 to 28 times broader than long, testes small in size, oval to rounded, two lateral fields, 10-12 in each segments, cirrus pouch medium, oval, ovary small in size, compact, nut shaped, vitelline gland is absent. In molecular characterization of the parasites, the genomic DNA were amplified and sequenced. Based upon both morphological data and molecular analysis using bioinformatics tools, the Cestode is identified as confirmed to be representing *Stilesia* Sp. in mammalian host i.e. Goat.

**KEYWORDS:** - Anoplocephalidea, Cestode, *Capra hircus* and Jalna.

### INTRODUCTION

The genus *Stilesia* was established by Railliet in 1893 from *Ovis aries* in Europe, Asia and Africa as *Stilesia globipunctata*. In 1896, Railliet also added *Stilesia vittata* and Wolffhugel, 1903 described *Stilesia hepatica*. Later on Leiper, 1936 added *Stilesia okapi*. In India Kadam et al, 1980 described *Stilesia leperi*. In 1981 Kalyankar, added *Stilesia caballeroi*. Shinde, et al, 1982 described *Stilesia southwellii*. Jadhav, et al., 1982 added *Stilesia aurangabadensis*. Malhotra and Capoor, 1983 added two species to this genus i.e. *Stilesia garhwalensis* and *S. kotwarensis*. *Stilesia marathwadensis* is added by Shinde, et al., 1985. In 1999 two species are added to this genus i.e. *Stilesia jadhavae* by Jadhav and *Stilesia yawalensis* by Kalse, et al. In 2001, Deshmukh and Shinde added *Stilesia dhondgae*. In 2004 four new species were added to this genus i.e. *S. pandeyi*, by Nanware and Jadhav, *Stilesia ambajogaiensis* by Pawar, et al. *Stilesia indapurensis* by Khadap, et al. and *Stilesia daulatabadensis* by Shelke and Shinde. In 2005, Nanware et al. added *Stilesia jadhavi* and Padwal and Jadhav described *Stilesia govindaein* 2006 and later on *Stilesia shrigondaensis*, added by Pokale, et al. 2008. Later on Humbe, et al. added *Stilesia bordeae*, 2013 and Shaikh Kalim, 2014 added *Stilesia kalamae*. In recent year Ravi Solunke, 2015 erected new species i.e. *Stilesia alii* from *Capra hircus* (L.), Nikam Priyanka 2015 added *Stilesia shindei* from *Capra hircus* (L.). Lastly Sanap N.P. 2016 reported new species *Stilesia gangakhedensis* from *Capra hircus* (L.) and *Stilesia Indiana* from *Capra hircus* (L.) by Pawar R.G. 2016.

Molecular phylogenetics is the analysis of hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationship. The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree. Molecular phylogenetic is one aspect of

molecular systematics, a broader term that also includes the use of molecular genetics markers have been developed into powerful tools to analyze genetic relationships and genetic diversity. Recent efforts based on comparative analysis of morphology and on molecule have advanced our understanding of tapeworm systematics considerably (Hoberg *et al.*, 1997 and Hoberg *et al.* 1999) and more generally of their position within the phylum platyhelminthes (Littlewood, D. T. J. *et al.*, 1999 and 2001).

The present communication, deals with the morphological and molecular identification of genus *Stilesia hircusae*, Sp. Nov. collected from *Capra hircus* (L).

#### **MATERIAL AND METHODS:**

Cestode parasites were collected from the intestine of *Capra hircus* at Jalna district (M.S.) India. These Cestodes preserved in hot 4% formalin and stained with Aceto-caramine or Harris haematoxylin, passed through various alcoholic grades, cleared in xylene, mounted in D.P.X. and drawings are made with the aid of camera lucida. All measurements are given in millimeters, otherwise mentioned. The identification is made with the help of Systema Helminthum.

Cestodes intended for molecular analysis were fixed with 95% ethyl alcohol. DNA Extraction was carried out using Genelute Mammalian Genomic DNA extraction kit (Sigma, G1N70-1KT). Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The Extracted DNA was stored at -20°C for further use. The DNA isolated was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). Gel electrophoresis was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The band size obtained for amplification of Partial 18S rRNA region is ~1095bp and ~1084bp. The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). For sequencing of 18S rRNA PCR product 18s 5F- 5' (CTGGTTGATYCTGCCAGT 3') sequencing primer was used. The DNA sequences were analyzed using online BLASTn (nucleotide Basic Local Alignment Search Tool) facility of National Centre for Biotechnology Information (NCBI). The BLAST results were used to find out evolutionary relationship of Worms. Altogether ten sequences, including sample were used to generate phylogenetic tree. The tree was constructed by using Maximum Likelihood method MEGA 7 software.

#### **RESULTS:**

**Morphological description:-** Twenty specimens of Cestode parasites were collected from the intestine of *Capra hircus* (L.) at Jalna district (M.S.) India during the month of August 2015.

These cestodes preserved in 4% formalin and stained with Acetocarmine or Harris haematoxylin or Borax carmine, passed through various alcoholic grades, cleared in xylene, mounted in D.P.X. and drawings are made with the aid of camera Lucida. All measurements are given in millimeters, otherwise mentioned.

All the cestodes are long consisting scolex, neck and proglottids. Proglottids are immature and mature.

The scolex is large in size, muscular, Squarish in shape and measures, 3.719 (3.624-3.815) in length and 2.899 (2.670-3.128) in width.

Suckers are large in size, bulgy appearance of sucker, oval to rounded in shape, four in numbers, arranged in two pairs, one pair placed in each half region of the scolex and measures, 1.144 (0.953-1.335) in diameter.

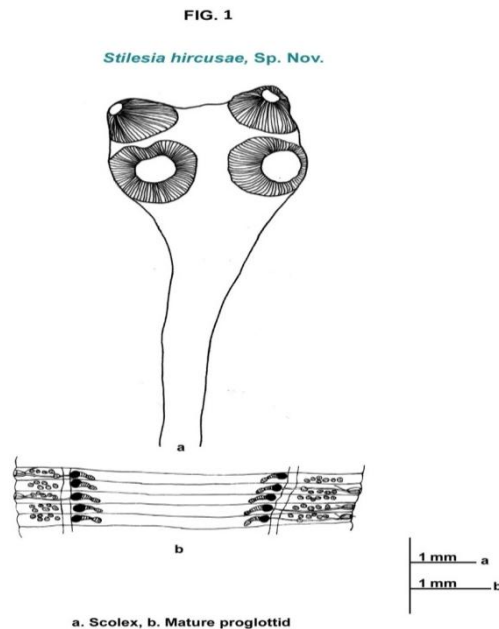
The neck is very long and measures, 3.515 (3.230-3.800) in length and 0.760 (0.570-0.950) in width.

The mature proglottids are broader than long, almost twenty five to twenty eight times broader than long, proglottids with a double set of reproductive organs and measures, 4.62 (4.455-4.785) in length and 0.165 (0.132-0.198) in width.

The testes are small in size, oval to rounded in shape, in two groups, 5-7 in each group, 10-12 in each segment and measures, 0.082 (0.066-0.099) in diameter.

The cirrus pouch is medium, elongated, oval in shape, irregular, marginal, pre-ovarian and measures, 0.231 (0.198-0.264) in length and 0.099 in width.

Cirrus is short, thin, slightly curved, contained within the cirrus pouch and measures, 0.297 (0.264-0.33) in length and 0.049 (0.033-0.066) in width.



The vas deferens is short, thin, straight or slightly curved, short as compare to cirrus and measures, 0.082 (0.066-0.099) in length and 0.049 (0.033- 0.066) in width.

The Ovary is small in size, compact, rounded, nut shaped and measures, 0.181 (0.165-0.198) in diameter.

The vagina is thin tube, arises from posterior to cirrus pouch, runs a long distance and open into the ootype and measures, 1.237 (1.15-1.320) in length and 0.033 (0.033-0.033) in width.

The uterine cap is large in size, elongated, narrow proximally, broad distally, situated and attached to one side of the ovary, towards aporal side and measures, 0.132 in length and 0.099 in width.

Ootype is small, rounded in shape and measures, 0.082 in diameter.

The par-uterian organ are elongated, cylindrical in shape, present two in numbers and measures, 0.363 (0.330-0.396) in length and 0.099 (0.066-0.132) in width.

The genital pores large in size, oval in shape, irregularly alternate, marginal, situated 1/3<sup>rd</sup> from anterior margin in the aporal side of the segments and measures, 0.0825 (0.066-0.099) in length and 0.082 (0.066-0.099) in width.

The longitudinal excretory canals are large in size, present on both the lateral sides of the segments along the body length and measures, 0.132 (0.099-0.165) in width.

## DISCUSSION

The genus *Stilesia* was erected by Railliet, 1893. The worm under discussion is having scolex large, squarish, mature proglottids almost 25 to 28 times broader than long, testes small in size, oval to rounded, two lateral fields, 10-12 in each segments, cirrus pouch medium, oval, ovary small in size, compact, nut shaped, vitelline gland is absent.

1. The present parasite differs from *Stilesia globipunctata* (Rivolta, 1874) Railliet, 1893 in having scolex small in size, rounded, testes 4-7, cirrus pouch small, pyriform, ventral to vagina, ovary spherical, vagina dorsal to cirrus pouch and reported from *Ovis aries* in Europe .

2. The worm under discussion differs from *Stilesia vittata*, Railliet, 1896, in having testes 5-9 each in two lateral groups, vas deferens closely coiled, cirrus pouch elongated, cylindrical, ovary rounded compact, genital pore in anterior half of the segment and reported from *Camelus dromedarius* in Africa.
3. The present tapeworm differs from *Stilesia hepatica*, Wolfhugel, 1903, in having testes 6-7, in each side, pre ovarian, vas deferens not closely coiled, but extending up to excretory canal, ovary small, compact, oval, vagina anterior to cirrus pouch, genital pore in the middle of the segment and reported from the liver of *Ovis aries*.
4. The worm under discussion differs from *Stilesia okapi*, Leiper, 1936 in having testes 2-3 testes in each lateral side and reported from *Okapi* in Africa.
5. The present parasite differs from *Stilesia leiperi*, Kadam, *et al.* 1980, which is having testes 5-6, on each side, cirrus pouch cylindrical, vas deferens not closely coiled but extend beyond long excretory canals, ovary medium, almost circular, compact with small acini and genital pore in anterior half of the segment and reported from *Ovis bharal* (*L.*)
6. The present worm differs from *Stilesia caballeroi*, Kalyankar, *et al.*, 1981 in having the scolex very small, testes 1 to 11 in number testes on each side, disposed in 2 to 3 rows.
7. The present form differs from *Stilesia southwelli*, Shinde, *et al.* 1982, which is having quadrangular scolex, mature proglottids 5 times broader than long, testes 4 in each two lateral groups, vas deferens very much coiled, reaching upto ovary, ovary small, round without acini, genital pore at 1/3<sup>rd</sup> from anterior margin of the proglottid, par uterine organs two in each proglottid, transversely elongated, containing eggs (10-15) in numbers.
8. The present worm differs from *Stilesia aurangabadensis*, Jadhav, *et al.*, 1982, in having the scolex spherical, testes rounded, in two lateral groups, 5 on each lateral side of the proglottid and lateral to ovary, vas deferens not coiled, reaches up to longitudinal excretory canal, cirrus pouch cylindrical, ovary medium in size, compact, circular in poral half and reported from *Ovis bharal* (*L.*) in India.
9. The present worm differs from *Stilesia garhwalensis*, Malhotra and Capor, 1983, in having the size of scolex 0.510-0.840 in diameter, testes 0-9 in numbers, size of cirrus pouch 0.011-0.101 in diameter, size of ovary 0.009-0.097 in diameter, genital pore situated at anterior 1/3<sup>rd</sup> level of lateral margin of proglottid.
10. The present cestode differs from *Stilesia kotwarensis*, Malhotra and Capoor, 1983 in having testes 1 to 12 in numbers, ovary small, spherical, situated inner to the longitudinal excretory canal on poral side, genital pore at anterior 1/3<sup>rd</sup> level of lateral margin of proglottid and reported from *Ovis bharal* (*L.*) in India.
11. The present form differs from *Stilesia marathwadensis*, Shinde, *et al.*, 1985, is having proglottids broader than long, testes 5-7 in number, rounded, in two groups and cirrus pouch cylindrical.
12. The present worm differs from *Stilesia jadhavae*, Jadhav, 1999 in having the mature proglottids 8 times broader than long, testes 5-7 in numbers, Ootype 0.045 in diameter, ovary 0.197 in diameter, vagina anterior to cirrus pouch and reported from *Ovis bharal* (*L.*) in India.
13. The present cestode differs from *Stilesia yawalensis*, Kalse, *et al.* 1999, in having scolex quadrangular, mature proglottids broader than long, testes rounded, in 2 groups (5-6 in number), vas-deference thin, curved, cirrus pouch oval, ovary medium, globular, a single mass, vagina thin and genital pore marginal.
14. The present parasite differs from *Stilesia dhondgae*, Deshmukh, *et al.*, 2001 in having scolex quadrangular, broad anteriorly, testes oval, 8-10 in numbers, arranged in 2 rows, vas deferens short, cirrus pouch small, oval, situated middle to posterior side, ovary bilobed and par uterine organ simple.
15. The present worm differs from *Stilesia capari*, Patil, *et al.*, 2002 in having scolex globular, testes 8-9 in numbers, vas deferens short, cirrus pouch small, ovary sac like and vagina anterior to cirrus pouch.

16. The present cestode differs from *Stilesia ambajogaensis*, Pawar, *et al.*, 2004 in having scolex quadrangular, testes 25-30 in numbers, cirrus pouch cylindrical, elongated, ovary small, oval, vagina anterior to cirrus pouch and reported from *Bos indicus*, in India.
17. The present tapeworm differs from *Stilesia. pandeyi*, Nanware and Jadhav, 2004, in having scolex large, with four suckers, neck short and wide almost four times broader than long, mature proglottids almost 17 times broader than long, with convex lateral margins and slight projections at posterior corners of proglottid, testes are on two lateral fields, twenty in number, cirrus pouch small, elongated, at one third of anterior margins of proglottid, obliquely placed, cirrus thin, slightly curved, vas deferens short, medium, obliquely placed, ovary small, oval, with 3-4 short, blunt acini, vagina thin tube, runs obliquely, Ootype small and rounded, genital pores medium, oval.
18. The present cestode differs from *Stilesia indapurensis*, Khadap, *et al.*, 2004 in having scolex medium, quadrangular, broad anteriorly, mature proglottids squarish, testes oval, 8 to 9 in numbers, vas deferens curved, cirrus pouch large elongated, vagina thin, par uterine organs two in each mature segment.
19. The present parasite differs from *Stilesia daulatabadensis*, Shelke and Shinde, 2004 in having scolex globular, medium, squarish, testes acraspedote, eleven in number, 7 on poral and 4 on aporal side, vas deferens medium, slightly curved, cirrus pouch medium, oval, ovary medium, oval, single mass, vagina thin, long, anterior to cirrus pouch, slightly curved and par uterine organ 2 in number.
20. The present worm differs from *Stilesia jadhavii*, Nanware and Jadhav, 2005 in having scolex globular, testes 14 in numbers, ovary lobulated and vagina anterior to cirrus pouch.
21. The present cestode differs from *Stilesia govindae*, Padwal and Jadhav, 2006 in having scolex medium, elongated, mature proglottids 15 times broader than long, testes 12-14 in numbers, cirrus pouch cylindrical, sac like, vas deferens long, ovary lobulated and vagina postero-ventral to cirrus pouch.
22. The present cestode differs from *Stilesia shrigondaensis*, Pokale, *et al.*, 2008 in having scolex medium, rounded, mature proglottids broader than long, testes 10-12 in numbers, cirrus pouch oval, vas deferens short, ovary Medium in size, oval and vagina thin tube.
23. The present cestode differs from *Stilesia bordeae*, Humbe, *et al.*, 2013 in having scolex medium, globular, mature proglottids almost 6-7 times broader than long, testes 6 - 11 in each segment, cirrus pouch oval, sac like, vas deferens long coiled and ovary compact.
24. The present cestode differs from *Stilesia kalamae*, Shaikh Kalim, 2014 in having scolex globular, mature proglottids 7-8 times broader than long, testes 7-8 in each group, cirrus pouch oval, vas deferens short, thin tube, ovary compact, oval.
25. The present cestode differs from *Stilesia alii*, Ravi Solunke, 2015 in having scolex large, nearly quadrangular, mature proglottids 12-13 times broader than long, testes 9-10 in numbers, vas deferens short, thin, slightly curved, cirrus pouch medium, oval, ovary medium, oval, par uterine organ two in numbers and genital pore marginal.
26. The present cestode differs from *Stilesia shindei*, Nikam Priyanka, 2015 in having scolex squarish, mature proglottids 22-24 times broader than long, testes 9-12 in numbers, ovary medium, rounded and genital pore unilateral, irregularly alternate.
27. The present cestode differs from *Stilesia gangakhedensis*, Sanap N. P., 2016 in having scolex large, quadrangular, small, squarish, mature proglottids broader than long, acraspedote, testes 9 in numbers, vas deferens medium, thin, slightly curved, cirrus pouch small, cylindrical, ovary medium, oval, single mass, with uterine cap, vagina thin, posterior to cirrus pouch, Par utrine organ two in numbers and genital pore small, oval, anterior half of segment.
28. The present cestode differs from *Stilesia indiana*, Pawar R. G., 2016 in having scolex medium globular, quadrangular, mature proglottids broader than long, testes two lateral fields, oval, 4 to 5 in numbers, vas deferens short, medium in size, obliquely placed, curved, cirrus pouch small,

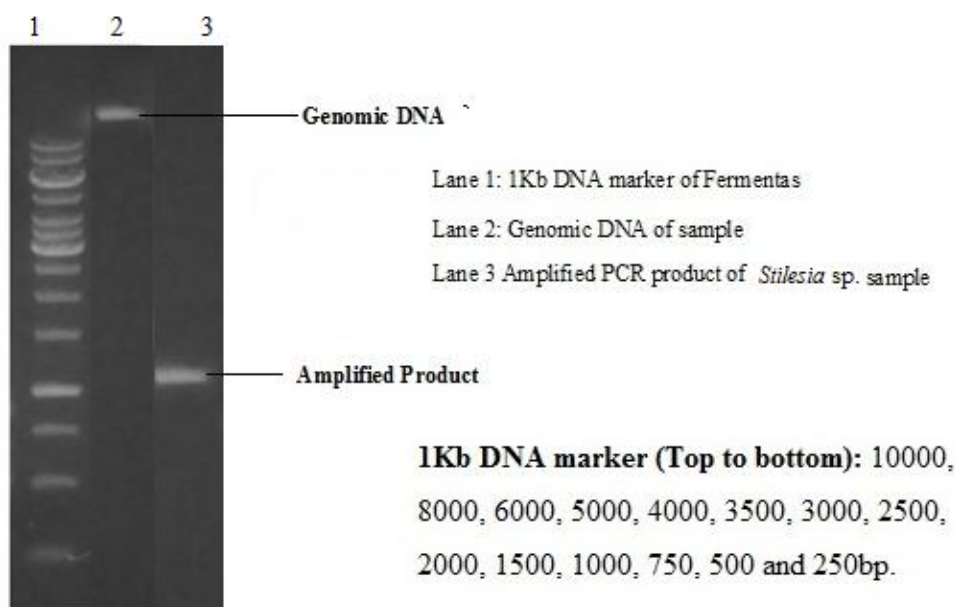
elongated, ovary small compact, 'U' shaped, vagina thin tube, posterior to cirrus pouch and genital pore small, oval, marginally placed, regularly alternate.

The above noted characters are valid enough to erect a new species hence the name *Stilesia hircusae*, Sp. Nov. is proposed, after the species name of host, *Capra hircus* (L).

**MOLECULAR DATA:-** A comparison of the partial sequences of the 18s rRNA gene of the present cestodes with those of other cestodes, in a phylogenetic context, provided further support for placing this species as a new one within *Stilesia* Sp. thus confirming taxonomic conclusion based on morphological data.

In the phylogenetic trees (Fig. 3) obtained by maximum parsimony analysis of the 18s rRNA sequence data set, a close to the species *Stilesia sp.* KD-2015 is clear with a maximum identity 100% (Table no.1). After partial 18s rRNA gene Sequence of *Stilesia* sample DNA sequences length is 767bp (Fig. 2)

**Fig. 2: Genomic DNA and Amplified partial 18S rRNA gene for worm sample**



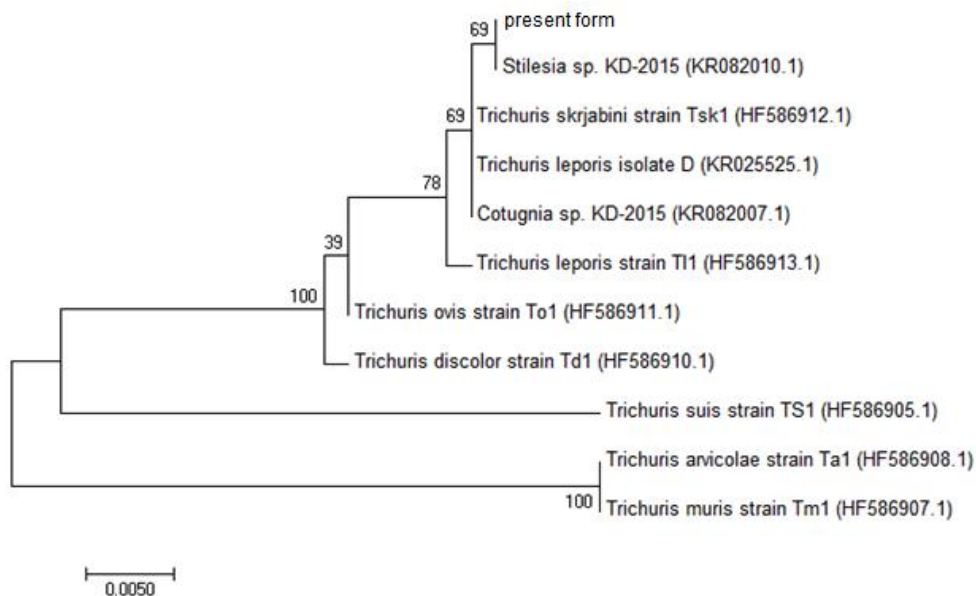
### Partial 18s rRNA gene Sequence (767bp)

GCAAGAACTGATGTCCACTTGGATAACTGTGGAAATTCTAGAGCTAATACATGCCTCGAAGCTTCGGCGCTAA  
TCGCGTCGGAGCGCATTATTAGTACAAAACCAATCGGGCGTTGGCTCTTAGCCTTCGTCCGCCAAAGGTTGGT  
GAATCGGAATAACTATGCTGATCGCACGGTCCAGCACCGGGGACGAATCTTTGAAATGACTTGCTCATCAACTT  
TCGATGGTACGCTACGTGCTTACCATGGTGACAACGGTTAACGGAGAATCAGGGTTGACTCCGGAGAGGGAG  
CCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCACGAAATTACCCACTCCCGGATCGGGGAGGTAGT  
GACGAAAAATAACGGAACGTATCTCCATGAGACGCGTTACCGAACGACCGAGCCGTACATAAGCTCGGCTAA  
ATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGTAATTCAGCTCCAATAGCGTATATTAAGTTGCTGC  
GGTTAAACCGCTCGTAGTTGGATTGCGGTGACGACGACGGTCGTCCTAAGCAGGAGTCGTTCCGTCGCTCGTC  
ACCTGTTTGATCAAGATCGTCCTGGATGCTCTTCGGTGAGTGTCTTGGCGACTTGAACGTTTACTTTGAGAAA  
ATGGGAGCGCTCAAGGCAGGGCCGTAGAGCTTGAACAGTGGTGCATGAAATAATGAAAGATGGCCTCGGTGCT  
ATTTTGTTGGTTT ACGGGCAATGGAGGCAATGG

**Table 1: Phylogenetic neighbors of *Stilesia* sp.sample based on partial 18s rRNA gene sequence**

Description	Max score	Total score	Query cover	E value	Ident	Accession
<a href="#">Stilesia sp. KD-2015 18S ribosomal RNA gene, partial sequence</a>	1423	1423	100%	0.0	100%	<a href="#">KR082010.1</a>
<a href="#">Trichuris skrjabini 18S rRNA gene, strain Tsk1</a>	1389	1389	99%	0.0	99%	<a href="#">HF586912.1</a>
<a href="#">Trichuris leporis 18S rRNA gene, strain Tl1</a>	1373	1373	99%	0.0	99%	<a href="#">HF586913.1</a>
<a href="#">Cotugnia sp. KD-2015 18S ribosomal RNA gene, partial sequence</a>	1360	1360	96%	0.0	99%	<a href="#">KR082007.1</a>
<a href="#">Trichuris leporis isolate D 18S ribosomal RNA gene, partial sequence</a>	1358	1358	96%	0.0	99%	<a href="#">KR025525.1</a>
<a href="#">Trichuris ovis 18S rRNA gene, strain To1</a>	1352	1352	99%	0.0	98%	<a href="#">HF586911.1</a>
<a href="#">Trichuris discolor 18S rRNA gene, strain Td1</a>	1347	1347	99%	0.0	98%	<a href="#">HF586910.1</a>
<a href="#">Trichuris arvicolae 18S rRNA gene, strain Ta1</a>	1192	1192	97%	0.0	95%	<a href="#">HF586908.1</a>
<a href="#">Trichuris muris 18S rRNA gene, strain Tm1</a>	1192	1192	97%	0.0	95%	<a href="#">HF586907.1</a>
<a href="#">Trichuris suis 18S rRNA gene, strain TS1</a>	1190	1190	99%	0.0	95%	<a href="#">HF586905.1</a>

**Fig. 3: Phylogenetic tree for *Stilesia* sp. sample using partial 18s rRNA gene sequence.**



**CONCLUSION:**

After discussion we conclude that the morphological and molecular observation (sequence of its 18S rRNA gene) is different in the present investigation. The present study clearly demonstrates that species should be considered to be a member of genus *Stilesia* (Cestoda: Anaplocephalidea).

In molecular analysis the phylogenetic neighbors of *Stilesia* Sp. based on partial 18S rRNA gene are shown in table no. 1 and fig. 3. On the basis of position of sequences of given *Stilesia* sample in

phylogenetic tree, the sample showed 100 % similarity with the *Stilesia* sp. i.e. KD-2015 having accession no. [KR082010.1](#).

After above discussion in both morphological and molecular observations the present worm is a member of genus *Stilesia* (Cestoda: Anoplocephalidea). In the molecular observation the sample in phylogenetic tree showed 100% similarity with the *Stilesia* Sp. i.e. KD-2015 having accession no. KR082010.1 studied by Thosar *et al.* 2015, but in this study the identification only generic level there is no species identification. On the morphological observation the cestode parasites differs from other *Stilesia* Sp. with several characters and these characters are valid enough to erect a new species for the cestode hence the name *Stilesia hircusae*, Sp. Nov. is proposed after the species name of host.

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