

Molecular and Morphological Identification of Two Cestode Species Parasitizing Sheep and Goats in Aurangabad District (m.s.) India

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Abstract

Cestodes species of sheep and goat are collected from intestines in Aurangabad district (M.S.) India, were subjected to a complete parasitological examination. Cestode parasites were processed for morphological and morphometric analysis aimed at identifying them to the species level. Cestode parasites identity was confirmed through amplification and sequencing of COI-5P gene marker. Phylogenetic analysis of COI-5P gene marker of two *Moniezia* Sp. showed that it claded with *Moniezia expansa* from isolated with a sequence similarity index of 99%. Thus, the molecular and morphological identification revealed that the two species of cestode parasites i.e. *Moniezia babaii*, Sp. Nov. and *Moniezia yamagutii*, Sp. Nov.

Key words:- Aurangabad, COI-5P, Molecular identification, *Moniezia* Sp.

Introduction

Sheep and goats rearing contribute significantly to the agrarian economy especially in areas where crop and dairy farming are not economical and it plays an important role in the livelihood of a large production of small and marginal farmers and landless labourers (Acharya, 1982).

Goats and sheep have numerous gastrointestinal parasites, many of which are shared by both species. The most important include cestodes (tapeworms), nematodes (round worm) and trematodes (flukes). Helminth parasitism especially, gastrointestinal parasitism, is one of the major health problems severely limiting the animal production in dairy animals. In spite of significant production losses, which may run into millions of rupees (Shan and Choudhry, 1995).

Cestode parasites of Sheep and goats shows to be useful as model parasites for the study of animal invasions and environmental global change. Introduction of *Moniezia* Sp., and *Stilesia* Sp. have been associated with imports of grazing animals. In various target areas, susceptible mites serving as intermediate hosts were either naturally present or were introduced from the donor continent of the parasite or from progressive stages of a global biota changes. Over the helminths extended range, in addition to domestic stock animals or naturalized mammals can also serve final hosts.

Parasitic infection is cestode parasites belonging to genus *Moniezia* and *Stilesia*. The genus *Moniezia* was established by Blanchard, 1891. Recently Sunita borde et al., 2017 erected *Moniezia* (*B*) *bordae*, Jadhav V.M. et. al. 2018 erected *Moniezia* (*B.*) *madhavae* and lastly Amol Thosar, et al., 2020 erected *Moniezia* (*B.*) *shilae*.

The phylogeny of the order Anaplocephalidea has been studied at generic and sub familiar level or as a part of studies on the phylogeny of the Eucestoda in general, mainly in comparative morphology and relationships among individual orders. In addition to morphological characters that are often variable, difficult to homologies, molecular data have been widely used in phylogenetic studies of Cestodes generally and these Cestodes particularly using many genes and developed techniques as attempts in solving many taxonomic problem. Aim of these study was to evaluate the phylogenetic position of the Cestode genera. Two *Moniezia* Sp. of the sheep and Goat i.e. *Ovis bharal* (L.) and *Capra hircus* (L.) respectively from Aurangabad district (M.S.), India on the basis of morphological and molecular data (using molecular markers) among Anaplocephalidea.

The present communication deals with molecular and morphological identification of two cestodes species i.e. *Moniezia babaii*, Sp. Nov. and *Moniezia yamagutii*, Sp. Nov. from sheep and goats in Aurangabad district (M.S.) India.

Material and Methods

Morphological identification: - Cestode parasites were collected from the intestine of sheep and goat in Aurangabad district (M.S.) India. These Cestodes preserved in 4% formalin and stained with Aceto-carmine 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 millimetres, otherwise mentioned. The identification is made with the help of Systema Helminthum.

Molecular identification:- Cestodes intended for molecular analysis were fixed with 95% ethyl alcohol and transported to wet laboratory facility of Paul Hebert Centre for DNA Barcoding.

DNA isolated from tissue of cestode parasites using Promega wizard genomic Kit, Quantified using Nanodrop spectrophotometer (ND 1000, Thermo Corporation, USA) and quality checked on 1% Agarose gel electrophoresis. Gel was visualized using gel documentation system (Biorad Inc., USA). DNA diluted to have 100 ng/μl concentration and stored at -200C for further use. These DNA samples were processed further for molecular studies where we employed mitochondrial markers and microsatellite marker studies.

From mitochondrial genome we have selected two markers for amplifying Cytochrome oxidase-1 gene. CO-I gene amplification was performed in 25 μl PCR reaction using Kapabiosystems PCR kit. The reaction constitute 12.5 μl of 10% trehalose, 5.5 μl H₂O, 2.5 μl 10X PCR buffer, 0.4 μl MgCl₂, 2.0 μl 2.5mM dNTPs, 0.5 μl 10mM primers (TGTAACGACGGCCAGTATTCAACCAATCATAAAGATATTGG (Hebert et al., 2004)) and 0.1 μl taq polymerase (5 units/μl) and 1 μl of 100 ng/ μl of template DNA.

The PCR thermal cycle conditions includes an initial denaturation step of 2 min at 95°C, 35 cycles of 30 sec at 94°C, 40 sec at 52°C, and 1 min at 72°C, with final extension at 72°C for 10min. Amplified PCR products were visualized on 1.5% agarose gel as shown in figure 3.

PCR products were processed for cleanup to remove unincorporated nucleotide and residual primers using 0.25 μl Exonuclease-I (20 units/μl) and 0.5μl of Shrimp Alkaline Phosphatase enzyme (1

unit/ μ l) followed by cycle sequencing reaction using BigDye[®] Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) with 16thfold dilution. During COI gene amplicon sequencing M13 primers were used whereas, for D-loop and Cyt b amplicon sequencing same PCR primers were used. The thermal cycler conditions were an initial denaturation of 2 min at 96°C and 35 cycles of 30 sec at 96°C, 15sec at 55°C, and 4 min at 60°C. The Cycle sequencing is followed by sequencing clean-up by ethanol precipitation followed by dissolving template in HiDi formamide and bidirectional sequenced in ABI 3130 Genetic analyzer.

Sequences obtained for CO-I, region were aligned and assembled in Codon Code aligner v4.0.3 (CodonCode, Dedham, MA, USA) using Muscle algorithm and Mega 5 (Tamura et al., 2011) using Clustal-W algorithm.

Sequences have been deposited in NCBI GenBank under the following inclusive accession numbers for CO-I (JX024320 - JX423844).

Genetic divergence values for CO-I region were calculated using K2P parameters (Kimura, 1980). Publicly available cestode parasites sequences obtained from BOLD and GenBank (NCBI) were also included in a separate analysis. Neighbour-joining trees (Saitou and Nei, 1987) of K2P distances were generated using Mega 5 (Tamura et al., 2011).

Results

Morphological Description

***Moniezia* (B.) *babaii*, Sp.Nov. (Fig.1)**

Eight specimens of the Cestode parasites were collected from the intestine of *Capra hircus* (L.) at Aurangabad (M.S.) India. These cestodes were preserved in 4% formalin then prepared the permanent slides (mentioned in material and methods) and identified.

The Cestodes are long consisting scolex, neck and proglottids. Proglottids are immature, mature and gravid. The scolex is large in size, globular in shape and measures, 4.484(4.750-4.218) in length and 2.850(3.610-2.090) in width. The Suckers are large, round in shape, four in numbers, arranged in two groups, one pair is overlapping and measures, 0.874(0.988-0.760) in length and 0.893(1.026-0.760) in width. The neck is long and measures, 12.749(12.730-12.768) in length and 0.671(1.786-1.330) in width.

Mature proglottids are nearly three time broader than long, each proglottids with a double set of reproductive organs and measures, 1.848(1.881-1.815) in length and 10.148(10.065-10.230) in width. The testes are small, oval to rounded in shape, 150-170 in numbers, distributed in posterior half of the segment and measures, 0.083 (0.099-0.066) in diameter. The vas-deference is thin, long tube, coiled up to anterior side of the ovary, measures, 0.743(0.759-0.726) in length and 0.050(0.066-0.033) in width. The Cirrus pouch is small, oval in shape, situated in middle margin of the segments and measures, 0.429 (0.660-0.198) in length and 0.264(0.363-0.165) in width. The Cirrus is thin, long tube, curved, inside the cirrus pouch and measures, 1.815 (1.980-1.650) in length and 0.049(0.066-0.033) in width.

The Ovary large in size, compact, butterfly shaped, two in numbers in each side of the segment and measures, 0.957 (1.188-0.726) in length and 0.924 (1.188-0.660) in width. The Vagina posterior to cirrus pouch, thin, long tube reaches to the Ootype and measures 1.403 (1.485-1.320) in length and 0.082(0.099-0.066) in width. The Ootype is small, rounded in shape, anterior to the ovary and measures, 0.182 (0.198-0.165) in diameter. The Genital pores are small, oval, marginal, middle in position and measures, 0.347 (0.627-0.066) in length and 0.099(0.132-0.066) in width. The Vitelline gland are small, oval to rounded in shape, compact, post-ovarian and measures, 0.446(0.594-0.297) in length and 0.380(0.429-0.330) in width. The Inter-proglottidal glands large, oval to rounded in shape, arranged in double row, present in between longitudinal excretory canals of the segment, 20-25 in numbers and measures, 0.248 (0.396-0.099) in diameter.

The longitudinal excretory canals are thin, present on both the sides of segments along the body lengths and measures, 0.049(0.066-0.033) in width.

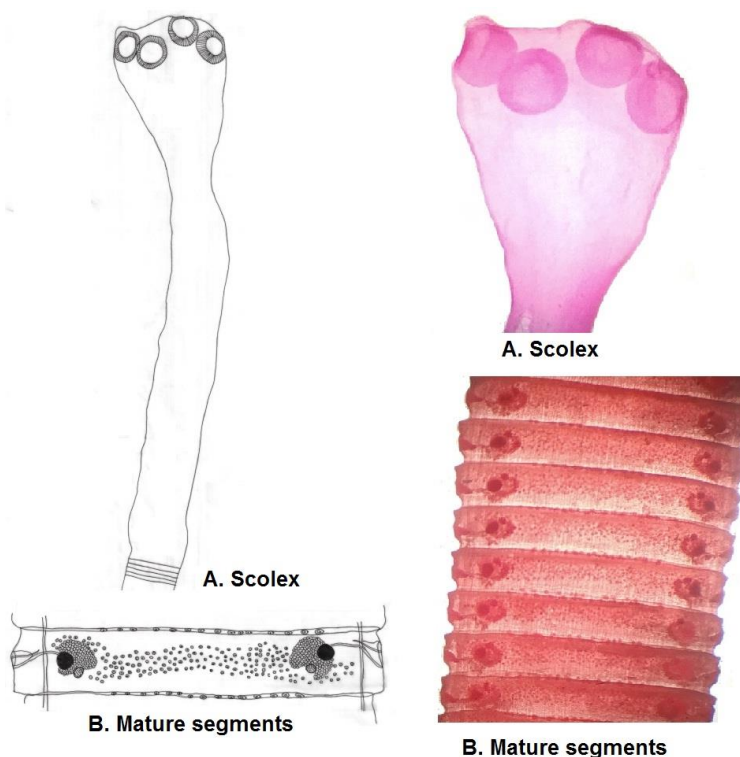


Fig.1 *Moniezia babaii*, Sp. Nov.

***Moniezia* (B.) *yamagutii*, Sp. Nov. (Fig. 2)**

Four specimens of the Cestode parasites were collected from the intestine of *Ovis bharal* (L.) at Aurangabad (M.S.) India. These cestodes were preserved in 4% formaline then prepared the permanent slides (mentioned in material and methods) and identified.

The Cestodes are long consisting scolex, neck and proglottids. Proglottids are immature, mature and gravid. The scolex is large in size, globular in shape and measures, 5.624(5.7-5.548) in length and

4.066(4.978-3.154) in width. The Suckers are large, ablique, circular in shape, four in numbers, arranged in two groups, overlapping and measures, 1.349(1.406-1.292) in length and 1.311(1.330-1.292) in width. The neck is long and measures, 8.550(8.74-8.36) in length and 3.059(3.230-2.888) in width.

Mature proglottid are almost three time broader than long, each proglottid with a double set of reproductive organs and measures, 2.343(2.541-2.145) in length and 10.263(10.296-10.230) in width. The testes are small, oval to rounded in shape, 65-75 in numbers, distributed in posterior half of the segment and measures, 0.116 (0.165-0.066) in diameter. The vas-deference is long, thin tube, measures, 1.436(1.452-1.419) in length and 0.050(0.066-0.033) in width. The Cirrus pouch is small, elongated, oval in shape and measures, 0.677 (0.693-0.660) in length and 0.545(0.561-0.528) in width. The Cirrus is thin, long tube, curved, inside the cirrus pouch and measures, 0.050 (0.066-0.033) in length and 0.058(0.082-0.033) in width.

The Ovary large in size, compact, rounded, two in numbers in each side of the segment and measures, 1.271(1.287-1.254) in length and 1.040 (1.320-0.759) in width. The Vagina posterior to cirrus pouch, thin, long tube reaches to the Ootype and measures 1.997 (2.079-1.914) in length and 0.025(0.033-0.016) in width. The Ootype is small, rounded in shape, anterior to the ovary and measures, 0.182 (0.264-0.099) in diameter. The Genital pores are medium in size, oval, marginal, bilateral, middle in position and measures, 0.083(0.099-0.066) in length and 0.050(0.066-0.033) in width. The Vitelline gland are large, rounded, compact, post-ovarian and measures, 0.347(0.363-0.330) in diameter. The Inter-proglottidal glands large, oval to rounded in shape, arranged in single row, present in between longitudinal excretory canals of the segment, 28-33 in numbers and measures, 0.166 (0.182-0.149) in diameter.

The longitudinal excretory canals are thin, present on both the sides of segments along the body lengths and measures, 0.029(0.033-0.024) in width.

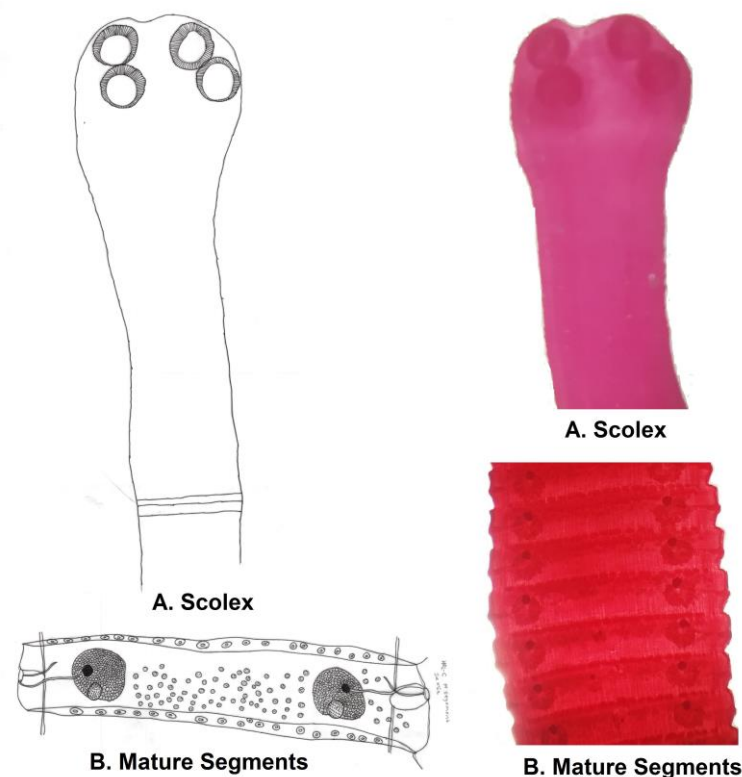


Fig. 2 . *Moniezia yamagutii*, Sp. Nov.

Molecular Data

A comparison of the partial sequences of the COI-5P 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 *Moniezia babaii* Sp. Nov. and *Moniezia yamagutii*, Sp. Nov. thus confirming taxonomic conclusion based on morphological data.

In the phylogenetic trees (Fig. 4 and Fig 5) obtained by maximum parsimony analysis of the COI-5P sequence data set, two species close to the species *Moniezia expansa* is clear with a maximum identity 99% (Table no.1 and 2). After partial COI-5P gene Sequence of *Moniezia babaii* Sp. Nov., and *Moniezia yamagutii*, Sp. Nov. Sample DNA sequences length is 368 bp. (Fig.3).

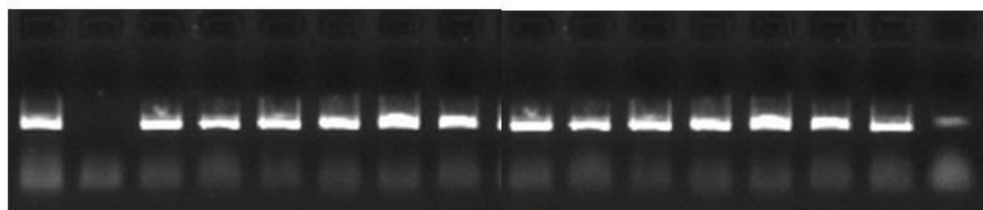


Fig. 3. Amplification of COI-5P gene from sample

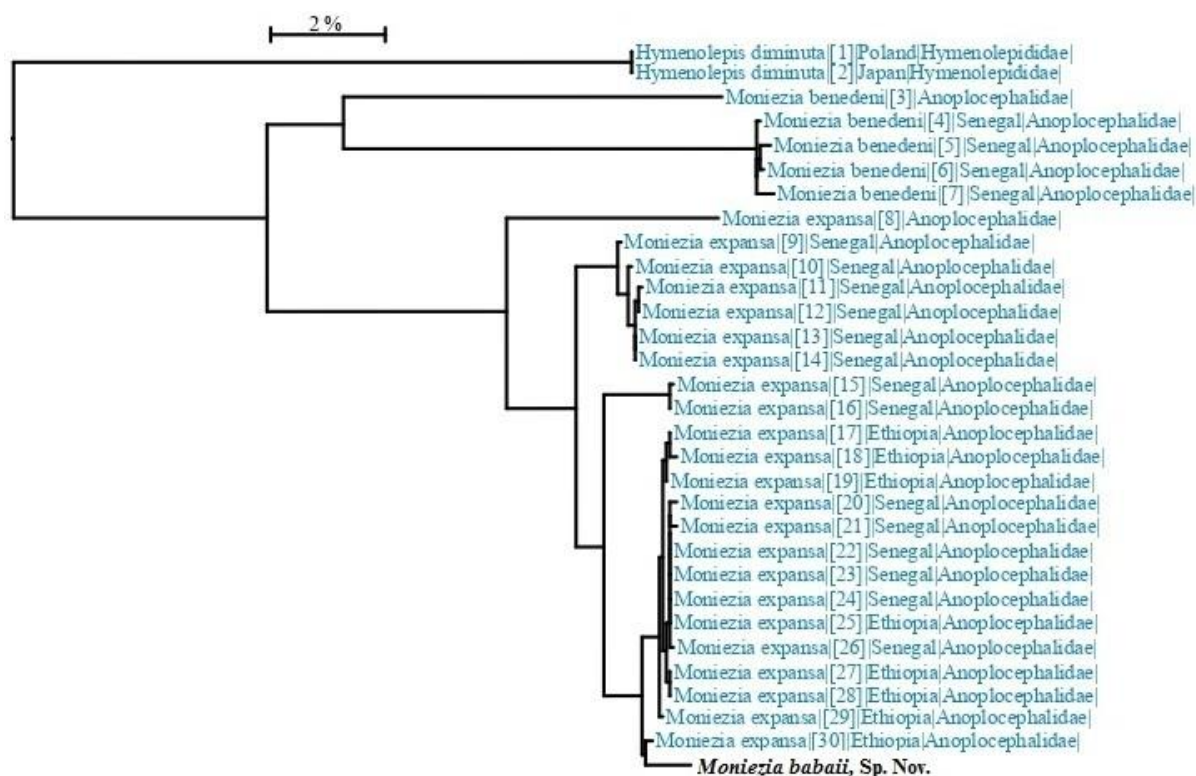


Fig. 4. Phylogenetic tree for *Moniezia babaii*, Sp. Nov. using COI-5P gene sequence

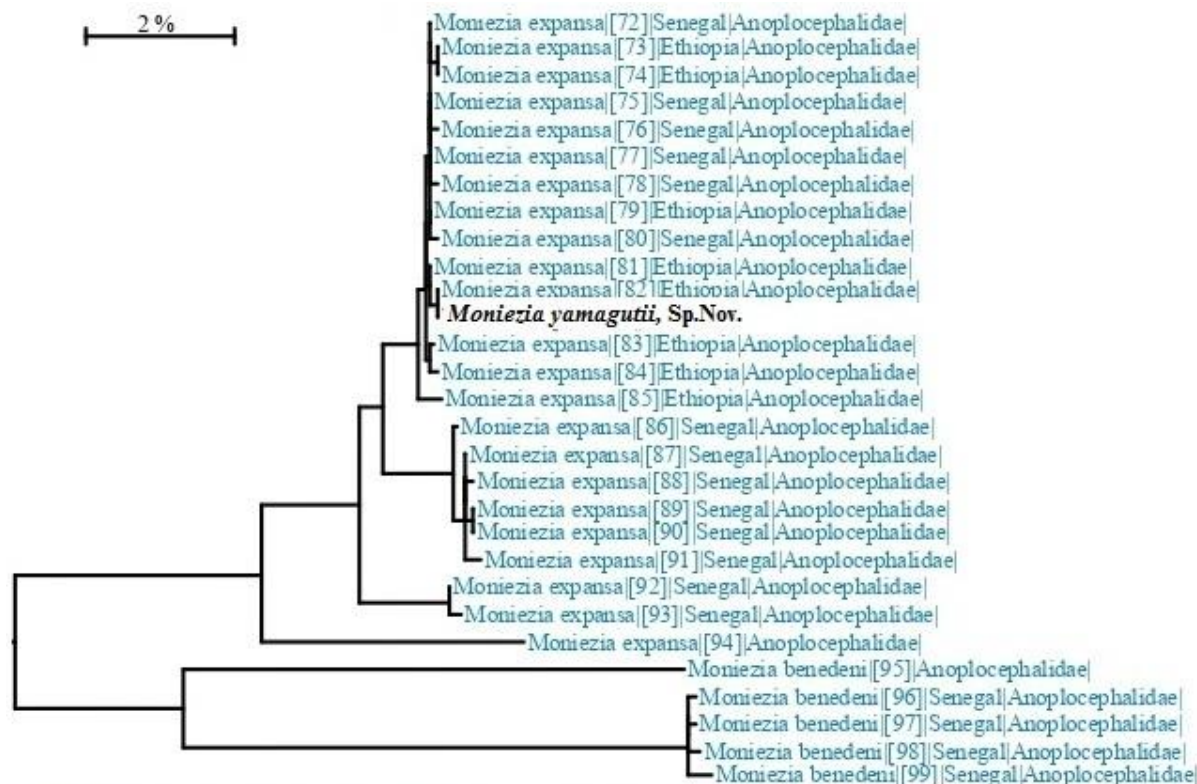


Fig. 5. Phylogenetic tree for *Moniezia yamagutii*, Sp. Nov. using COI-5P gene sequence

Table 1: Phylogenetic neighbors of *Moniezia babaii*, Sp. Nov. based on COI-5P gene sequence

Scientific Name	Max Score	Total score	Query cover	E value	Percentage identity	Acc. Len	Accession
<i>Moniezia expansa</i>	671	671	1	0	99.46%	1590	AB821382.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821385.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821381.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821380.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821379.1
<i>Moniezia</i>	665	665	1	0	99.19%	1590	AB821378.1

<i>expansa</i>							
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821376.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821375.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	1590	AB821374.1
<i>Moniezia expansa</i>	665	665	1	0	99.19%	14104	LC102496.1

Table 2: Phylogenetic neighbors of *Moniezia yamagutii*, Sp. Nov. based on COI-5P gene sequence

Scientific Name	Max Score	Total score	Query cover	E value	Percentage identity	Acc. Len	Accession
<i>Moniezia expansa</i>	1134	1134	1	0	99.21%	1590	AB821382.1
<i>Moniezia expansa</i>	1129	1129	1	0	99.05%	1590	AB821381.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821383.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821380.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821379.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821378.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821376.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821375.1
<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	1590	AB821374.1

<i>Moniezia expansa</i>	1123	1123	1	0	98.89%	14104	LC102496.1
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Discussion

The genus *Moniezia* was erected by Blanchard in 1891. The worm *Moniezia babaii* Sp. Nov., under discussion is having the scolex large, globular, mature proglottids nearly three times broader than long, testes small in size, round to oval, 150-170 in numbers, cirrus pouch oval, ovary butterfly shaped, vitelline gland post ovarian, inter proglottidal glands 20-25 in numbers.

The worm *Moniezia yamagutii* Sp. Nov., under discussion is having the scolex large, globular, mature proglottids nearly three times broader than long, testes small in size, round to oval, 65-70 in numbers, cirrus pouch oval, ovary compact, rounded, vitelline gland post ovarian, inter proglottidal glands 28-33 in numbers.

- The present worm differs from *Moniezia (B) benedeni*, Moniez, 1879, Skrjabin and Schulz, 1937, which is having numerous proglottids broader than long, posterior proglottids fleshy, testes 500 in numbers, arranged in two groups, cirrus pouch short and wide, vas deferens with 2-3 coils, ovary compact, in the center of the segments, eggs well developed, inter proglottidal glands liner and close to the posterior margin of the segments, arranged transversely and reported from the Calves and Lambs.
- The present cestode differs from *Moniezia (B) pallida*, Monnig, 1926, which is having the uterus external, dorsal and ventrally over excretory canals, the inter-proglottidal glands varying in size and reported from the host horse in South Africa.
- The present parasite differs from *Moniezia (B) aurangabadensis*, Shinde, *et al.* 1985, which is having the scolex quadrangular, testes small, 1100-1200 in numbers, vas deferens coiled, cirrus pouch cylindrical, oval with some rounded acini, gravid proglottids broader than long, uterus reticulate, inter proglottidal glands 12-15 in numbers and reported from *Ovis bharal* (L.).
- The present tapeworm differs from *Moniezia (B) bharalae*, Shinde, *et al.*, 1985, which is having testes rounded, 190-200 in numbers, vas deferens short, elongated, fusiform, genital pores bilateral, sub marginal, ovary compact, inter proglottidal glands arranged in two rows, small in size, 38-44 in numbers and reported from *Ovisbharal* (L.).
- The present form differs from *Moniezia (B) warananagarensis*, Patil, *et al.* 1997, which is having scolex large, globular, testes 300-320 in numbers, distributed throughout the proglottids, in single field, ovary indistinctly lobed with 13-15 short, blunt acini, transversely elongated, inter proglottidal glands, 56 in numbers, oval, medium in size, cirrus pouch medium, oval, transversely elongated, slightly obliquely placed and extend beyond longitudinal excretory canal.
- The present cestode differs from *Moniezia (B) kalawati*, Nanware, *et al.* 1999. Which is having squarish scolex, oval shaped cirrus pouch, testes small, oval, distributed throughout the segment, 172 in numbers, ovary medium, short, blunt acini, and 54 inter proglottidal glands in the inter segmental region, medium, oval either single or paired, irregularly arranged in the central width of the segments and leaving space on each lateral side.
- The present tapeworm differs from *Moniezia (B) murhari*, Kalse, *et al.*, 1999, in having the scolex

squarish, testes 405-415 in numbers, cirrus pouch elongated in the anterior region of the segments, ovary inverted horse shoe shaped, indistinctly bilobed each with numerous short, blunt, round, acini and inter proglottidal glands 63 in numbers.

- The present parasites differs from *Moniezia (B) caprai*, Pokale, et al, 2004, which is having the scolex is medium, squarish, with large four suckers, without rostellum, testes oval in shape, 255-260 in numbers, cirrus pouch is medium in size and ovary medium in size, kidney shaped.
- The present worm differs from *Moniezia (B) shindei*, Pawar, et al., 2004 in having scolex large, mature segments craspedote, testes 190-200 (195) in numbers, scattered all over segment and ovary a single mass, large, oval, cirrus pouch oval, elongated, in center of the segment and vitelline gland large, oval, internal to ovary.
- The present cestode differs from *Moniezia (B) hircusae*, Tat and Jadhav B. V., 2004 which is having scolex large, globular, mature segments big, craspedote, testes 168 in numbers, small, scattered in a single field, ovary large, oval, a single mass, in anterior half of the segment, inter proglottidal glands 14-15 in numbers, large, oval and cirrus pouch in anterior 1/3rd region of the segment.
- The present cestode differs from *Moniezia (B) rajalaensis*, Borde, et al. 2007, in having scolex large, globular, mature proglottids Squarish, Broader than long, testes 250-260 in numbers, medium, scattered throughout proglottids, ovary large, horse shoe shaped, inter proglottidal glands 31-32 in numbers, large, oval and cirrus pouch oval.
- The present cestode differs from *Moniezia (B) caprae*, Nanware S.S., 2010 in having scolex large, mature segment big, almost three and a half times broader than long, testes 84-85 in numbers, medium in size, oval in shape, ovary large, bilobed, inter proglottidal glands 40 in numbers, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) govindae*, Padwal, et al., 2011 in having scolex large, globular, mature proglottids big, broader than long, testes 100-140 in numbers, medium, scattered throughout proglottids, ovary large, compact, nut shaped, inter proglottidal glands 40-42 in numbers, large, oval and cirrus pouch elongated.
- The present cestode differs from *Moniezia (B) babai*, Humbe, et al., 2011 in having scolex globular, mature segment four times broader than long, testes 190-220 in numbers, small in size, rounded in shape, ovary large, rounded, inter proglottidal glands 18-20 in numbers, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) ovisae*, Humbe, et al., 2011 in having scolex broad anteriorly and narrow towards neck, mature segment two times broader than long, testes 155-165 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 32-35 in numbers, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) osmanabadensis*, Humbe, et al., 2012 in having scolex globular, mature segment five times broader than long, craspedote, testes 170-200 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 38-40 in numbers, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) devraoi*, Humbe, et al., 2013 in having scolex quadrangular, mature segment four times broader than long, testes 160-180 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 40-45 in numbers, oval, rounded and

cirrus pouch on each side.

- The present cestode differs from *Moniezia (B) shegaonensis*, Barote, *et al.*, 2013 in having scolex globular, mature segment four to five times broader than long, testes 190-220 in numbers, small in size, rounded in shape, ovary compact, inter proglottidal glands 20-25 in number, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) shivajiraovae*, Barote, *et al.*, 2014 in having scolex squarish, large in size, mature segment six to eight times broader than long, testes 84-95 in numbers, small in size, rounded in shape, ovary horse-shoe shaped, inter proglottidal glands 40-42 in numbers, oval, rounded and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B)sureshi*, Ravi Solunke, 2015 in having scolex oval, quadrangular, mature segment four to five times broader than long, testes 180-185 in numbers, single field, unevenly distributed, ovary medium, horse-shoe shaped, in appearance having numerous short, blunt acini, inter proglottidal glands 18-19 in numbers, oval and cirrus pouch on each side.
- The present cestode differs from *Moniezia (B) jadhavii*, Amol Thosar, *et. al.*, 2015 in having scolex squarish, mature segment craspedote, five times broader than long, testes 210-220 in numbers, small, oval to round, ovary horse-shoe shaped, compact, inter proglottidal glands 46-52 in numbers, arranged lineally in one or two rows, cirrus pouch small oval.
- The present cestode differs from *Moniezia (B) marathwadensis*, Shaikh Kalim, 2015 in having scolex quadrangular, mature segment five times broader than long, testes 125-130 in numbers, small, oval in shape, ovary compact with numerous blunt acini, inter proglottidal glands 50-52 in numbers, arranged lineally in one or two rows, cirrus pouch large, elongated, oval.
- The present cestode differs from *Moniezia (B) bhalchandrai*, Kalse A.T. *et. al.*, 2016 in having scolex quadrangular, mature segment rectangular in shape, almost four and half times broader than long, testes 196-200 in numbers, oval in shape, ovary medium in size, inverted cup shaped, inter proglottidal glands 13-14 in numbers, oval in shape, highly muscular, single regularly and lineally arranged, cirrus pouch large, oval in shape.
- The present worm differs from *Moniezia (B.) bordeae*, Sunita Borde, *et al.*, 2017 in having scolex quadrangular, mature segment nearly four to five times broader than long, testes 130-170 in numbers, spread in the medulla in between the longitudinal excretory canals, ovary bean shaped, small, forms concavity posteriorly, inter proglottidal glands 5-9 in numbers, arranged single row, cirrus pouch on each side and reported from *Ovis bharal* (L.).
- The present cestode differs from *Moniezia (B) madhavae*, Jadhav V.M. *et. al.*, 2018 in having scolex quadrangular, mature segment near five times broader than long, testes 45-60 in numbers, medium in size, oval in shape, ovary distinctly bilobed, inter proglottidal glands 40-42 in numbers, oval in shape, cirrus pouch small in shape, curved.
- The present worm differs from *Moniezia (B.) shilae*, Amol Thosar, *et al.*, 2020 in having the scolex quadrangular, mature proglottids nearly four times broader than long, craspedote in shape, testes small in size, oval to rounded, 180-210 in numbers, cirrus pouch oval, ovary large, compact, horse-shoe shaped, vitelline gland post ovarian, inter proglottidal glands 26-30 in numbers and reported from *Capra hircus* (L.).

We conclude that the morphological observation, clearly demonstrate that these two species should be considered to be a member of genus *Moniezia* (Cestoda: Anaplocephalidea).

In molecular analysis the phylogenetic neighbors of two *Moniezia* Sp. based on COI-5P gene are shown in table. On the basis of position of sequence of the given two *Moniezia* species sample in the phylogenetic tree, the sample showed closest similarity with the *Moniezia expansa*.

Key to the Species of the genus *Moniezia*, Blanchard, 1891.

- Mature segments broader than long - 1
- Mature segments Squarish - *M. (B.) pallida*, Monnig, 1926.
- Mature segments Craspedote - 2
- Testes below 60 in numbers - *M. (B.) Madhavae*Jadhav V.M. *et. al.*, 2018.
- Testes between 65 -75 in number - ***Moniezia (B.) babaii*, Sp. Nov.**
- Testes between 80-100 in numbers - *M.(B.) caparae*, Nanware, *et.al.*, 2010.
- Testes in between 100-130 in number - 3
- Testes in between 130-150 in numbers - *M. (B.) kalawati*, Nanware, *et al.*,1999.
- Testes in between 150- 175 in numbers - 4
- Testes in between 175-200 in numbers - 5
- Testes in between 200-300 in numbers - 6
- Testes in between 300 -400 in numbers - *M. (B.) warnanagarensis*, Patil, *et al.*, 1997.
- Testes in between 400 -450 in numbers -*M. (B.) murhari*, Kalse, *et al.*1999.
- Testes in between 450 -500 in numbers -*M.(B.) benedeni*, Moneiz, 1879,Skrjabinet *al.*, chulz, 1937.
- Testes above 500 in numbers - *M. (B.) aurangabadensis*, Shinde, *et al.*, 1985.
- Inter proglottidal glands between10-15 in numbers - *M. (B.)hircusae*, Tat and Jadhav, 2004.
- Inter proglottidal glands between 16-20 in numbers - *M. (B.) babai*, Humbe, *et al.*, 2011.
- Inter proglottidal glands between 20-25 in numbers - *M. (B.) shegaonesis*, Barote, *et al.*, 2013.
- Inter proglottidal glands between 25-30 in numbers - *M.(B.) shilae*,AmolThosar, *et al.*, 2020

Inter proglottidal glands between 30-35 in numbers	-M. (B.) <i>ovisae</i> Humbe, et al., 2011.
Inter proglottidal glands between 35-40 in numbers	- M. (B.) <i>osmabadensis</i> , Humbe, et al., 2012.
Inter proglottidal glands between 40-45 in numbers	- M. (B.) <i>shivajiraovae</i> , Barote, et al., 2014.
Inter proglottidal glands above 50 in numbers	-M. (B.) <i>shindei</i> , Pawar, et al. 2004.
Inter proglottidal glands above 25	- M.(B.) <i>marathwadensis</i> , Shaikh Kalim 2015.
Inter proglottidal glands between 40-50 in number	- M. (B.) <i>govindae</i> , Padwal, et al. 2011.
Scolex quadrangular	- M.bordae, Sunita Borde, et al. 2017
Scolex quadrangular	- M. (B.) devraoi, Humbe, et al., 2013.
Scolex globular	- <i>Moniezia (B.) yamagutii</i>, Sp. Nov.
Inter proglottidal glands below 15	-M. (B.) bhalchandrai, Kalseet. al., 2016.
Inter proglottidal glands between 15-20 in numbers in two rows-	M. (B.) sureshi, Ravi Solunke, 2015.
Inter proglottidal glands between 20-45 in numbers	- M. (B.) bharalae, Shinde, et al., 1985.
Inter proglottidal glands between 45-55 in numbers	- M.(B.) <i>jadhavii</i> , Amol Thosar, et al, 2015.
Scolex squarish	- M. (B.) <i>caparai</i> , Pokale, et al., 2004.
Scolex globular	-M.(B.) <i>rajalaensis</i> , Borde, et.al., 2007.

After above discussion in both morphological and molecular observations the Cestode is same at generic level but the species are different and these differentiating characters are valid enough to erect a new species for the Cestode and hence the name *Moniezia (B) babaii*, Sp. Nov. is proposed after Late Professor Dr. Baba Jadhav, well known scientist in Helminthology and Ex-head and professor, Department of Zoology, Dr. Babasaheb Ambedkar University, Aurangabad-431004 and *Moniezia (B) yamagutii*, Sp. Nov. is proposed after Late Professor Sattu Yamagutti, well known scientist in Helminthology.

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