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Morphological and Molecular Studies of *Moniezia* Sp. (Cestoda: Anaplocephalidea) A Parasite of the Domestic Goat *Capra Hircus* (L.) in Aurangabad District (M.S.), India.

KEYWORDS: Anaplocephalidea, Aurangabad, *Capra hircus*, India, *Moniezia.*

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ABSTRACT *Moniezia Sp.Nov. (Cestoda: Anaplocephalidea) is collected in the intestine of Capra hircus, Linnaeus, 1758 (Family: Bovidae) from Aurangabad district (M.S.), India. The present Cestode i.e. Moniezia Sp. Nov. differs other all known species is having the scolex almost squarish, mature proglottids nearly five times broader than long, Craspedote in shape, testes small in size, round to oval, 210-220 in numbers, cirrus pouch oval, ovary horse-shoe shaped, vitelline gland post ovarian.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 Moniezia Sp. in mammalian host i.e. Goat.*

INTRODUCTION

The genus *Moniezia* was established by Blanchard, 1891. Skrjabin and Schulz (1937) divided this genus in to three subgenera as follows:

1) Inter proglottidal glands grouped in rosettes----------*Moniezia*.
2) Inter proglottidal glands arranged linearly----------*Blancharieziа*.
   (Some time absent)

3) Inter proglottidal glands absent----------*Baeriezia*.

The present worm agrees in all characters with subgenus *Blancharizia*. Skrjabin and Schulz (1937) having two species as *M. (B.) benedeni* (Moniez, 1879), Skrjabin et al Schulz 1937 and *M. (B.) pallida*, Monnig, 1926. Later on two more species were added by Shinde et. al (1985) from the host *Ovis bharal* as *M.(B.)aurangabadensis* and *M. (B.) bharalae* at Aurangabad, M.S. India. Later on Patil et. al added *M. (B.) warananagarensis* from *Capra hircus*(L.) in 1997. In 1999 Nanware et.al.erected *M. (B.) kalawati* from *Capra hircus* (L.).Kalse et.al.(1999) added *M.* (*B.*) *murhari* from the same host, Pokale, *et.al.* (2004) added *M. (B.) caprai* from *Capra hircus* (L.). Pawar et.al (2004) added *M (B) shindei* from *Capra hircus (L.).* Tat and Jadhav B. V.(2004) added *M.* (B.) *hircusae*, from *Capra hircus (L.).* Borde et.al.(2007) added *Moniezia (B) rajalaensis* from *Capra hircus (L.),* Padwal et. al,(2008) added *M (B) govindae* from *Capra hircus (L.), M. (B) Caprae* is added by Nanware S. S. (2010). Later on Humbe et. al added four more species i.e. *M. (B.)babai*, 2011, *M. (B.) ovisae*,2011, *M. (B.) osmanabadensis*, 2012 and *M. (B.) devraoi*, 2013. Lastly Barote et. al added two more species i.e. *M. (B.) shegaonesis* and *M. (B.) shivajiraovae,* 2014.

The phylogeny of the order Anaplocephalidea has been studied at generic and subfamiliar 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 *Moniezia* Sp. parasite of the Goat i.e. *Capra hircus* (L.) from Aurangabad district (M.S.), India on the basis of morphological and molecular and molecular data (using molecular markers) among Anaplocephalideans.

The present communication, deals with the description of a new species, *Moniezia (B) jadhavii* Sp. Nov. Collected from the *Capra hircus* (L.) at Aurangabad City (M.S.) India, in the month of Feb. 2015.

MATERIAL AND METHODS

Cestode parasites were collected from the intestine of *Capra hircus* at. Aurangabad 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 xyline, 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). 25mg of tissue was minced and transferred to 1.5ml microcentrifuge tube. 180µl of Lysis solution T and 20 µl of proteinase K were added. The samples were mixed and incubated at 55^{0}C to digest the tissue completely. 20 µl of RNase A solution was added and incubated at room temperature for 2min. Then 200µl of lysis solution C was added and incubated at 70^{0}C for 10 min. The column was prepared for binding by adding 500µl of Column preparation solution to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000 rpm

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for 1 min. 200µl of ethanol was added to the lysate and mixed by vortexing. The entire lysate was transferred into the treated binding column and centrifuge at 10,000rpm for 1 min. The binding column was then placed in fresh 2ml collection tube. 500µl of Wash solution was added to the binding column and centrifuge at 10,000 rpm for 3min. This step was repeated twice. The column was again transferred to a new tube. 200µl of elution buffer was added directly into the centre of the binding column and centrifuge at 10,000rpm for 1min. Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -20^{0}C for further use.

The DNA isolated was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler (T-Personal 48). The PCR reaction mix contained 2.5µl of 10X buffer, 1µl of each primer (Table 6), 2.5µl of 2.5mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1µl Template DNA and 8.5µl nuclease free water. The PCR amplification cycle consist of, a cycle of 5 min at 94°C; 35 cycles of 1min at 94°C, 1 min at 56°C, 2 min at 72°C; and additionally 1 cycle of 7 min at 72°C. The reagents used are procured from GeNei. 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.

The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, AP-PCR-50). 100µl of PCR-A buffer was added to the 25µl of reaction. The sample was mixed and transferred to column placed in 2ml collection tube and centrifuge at 10,000 rpm for 1min. the filtrate was discarded. 700µl of W2 buffer was added to the column and centrifuge at 10,000rpm for 2min. This step was repeated twice. The column was transferred to a new tube. 25µl of Eluent was added into the column and incubated at room temperature for 2min. Then centrifuge at 10,000rpm for 5min. It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of 18S rRNA PCR product 18s 5F-5' (CTGGTTGATYCTGCCAGT 3') sequencing primer was used and for sequencing 28S rRNA PCR product LSU5F 5' (TAGGTCGACCCGCTGAAYTTAAGCA) 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 twenty sequences, including sample were used to generate phylogenetic tree (Figure 1-4). The tree was constructed by using MEGA 5 software (Saitou N. and Nei M.,1987; Felsenstein J.1985 and Tamura K. et al 2011).

RESULTS

Morphological description: - Twelve specimens of the Cestode parasites were collected from the intestine of *Capra hircus (L.)* from Aurangabad District (M.S.), India, during the period of Feb.-2015. These Cestodes were preserved in 4% formalin and stained and then mounted, slide were observed for identification.

The Cestodes are long consisting scolex, neck and proglottids. Proglottids are immature, mature and gravid. The scolex is large in size, almost squarish in shape and measures 2.475 (2.178-2.772) in length and 1.898 (132-2.475) in width. The Suckers are large, oval to round in shape, four in numbers, arranged in two pairs and measures 0.636 (0.627-0.644) in diameter. The neck is long and measures 14.8 in length and 1.056 (0.858-1.254) in width. Mature proglottids are broader than long, nearly five time broader than long, Craspedote in shape, each proglottids with a double set of reproductive organs and measures 2.673 (2.211-3.135) in length and 10.23 (9.90-10.56) in width. The testes are small, round to oval in shape, 210-220 in numbers, scattered all over the segment in between longitudinal excretory canal and measures 0.083 (0.033-0.132) in diameter. The Cirrus pouch is small, oval in shape, situated in middle margin of the segments and measures 0.578 (0.495-0.660) in length and 0.182 (0.099-0.264) in width. The Cirrus is thin, large, inside the cirrus pouch, slightly curved and measures 0.513 in length and 0.05 in width. The vas-deference is long, thin tube, measures 0.989 in length and 0.066 in width. The Ovary large, compact, horse-shoe shaped, two in numbers, on each lateral side of the segments with big acini, and measures 0.875 (0.759-0.990) in length and 0.809 (0.495-1.122) in width. The Vagina posterior to cirrus pouch, long tube reaches to the Ootype and measures 0.991 in length and 0.05 in width. The Ootype is small, oval to rounded, anterior to the ovary and measures 0.149 (0.132-0.165) in length and 0.132 (0.132-0.132) in width. The Genital pores large, oval, marginal, bilateral, protruded outside, middle in position and measures 0.231 in length and 0.05 in width. The Vitelline gland large, rounded, post-ovarian, near the con-cavity of the ovary and measures0.248 in diameter. The Interproglottidal glands present in between two proglottids, they are large, oval to round, arranged lineally in one or two rows in between longitudinal excretory canals, 46-52 in numbers and measures 0.182 in diameter. The longitudinal excretory canals are thin, present on both the sides of segments along the body lengths and measures 0.215 (0.132-0.297) in width.

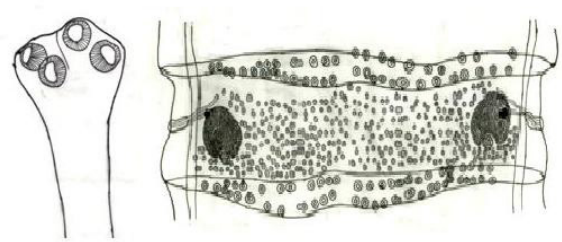

Fig. 1. *Moniezia jadhavii* Sp. Nov.

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 *Moniezia jadhavii* Sp. Nov. thus confirming taxonomic conclusion based on morphological data.

In the phylogenetic trees (Fig. 2) obtained by maximum parsimony analysis of the 18s rRNA sequence data set, a close to the species *Moniezia expansa* is clear with a maximum identity 97% (Table no.1). After partial 18s rRNA gene Sequence of Moniezia sample DNA sequences length is 1040bp (Fig. 3)

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Table 1: Phylogenetic neighbors of Moniezia Sp. based on partial 18s rRNA gene sequence

Description	Max score	Query cover	E value	Ident	Accession
Moniezia expansa gene for small subunit ribosomal RNA, partial sequence, isolate: MOE03	2152	92%	0.0	97%	AB862303.1
Moniezia expansa gene for small subunit ribosomal RNA, partial sequence, isolate: MOS05	2152	92%	0.0	97%	AB862301.1
Moniezia expansa isolate 2 18S ribosomal RNA gene, partial sequence	2152	92%	0.0	97%	GU817405.1
Moniezia expansa gene for small subunit ribosomal RNA, partial sequence, isolate: MOS01	2146	92%	0.0	97%	AB862300.1
Moniezia expansa gene for small subunit ribosomal RNA, partial sequence, isolate: MOS15	2134	92%	0.0	97%	AB862302.1
Moniezia benedeni gene for small subunit ribosomal RNA, partial sequence, isolate: MB03	1860	92%	0.0	93%	AB862304.1
Moniezia benedeni isolate 2 clone 1 18S ribosomal RNA gene, partial sequence	1772	92%	0.0	92%	GU817403.1
Moniezia benedeni isolate 2 clone 2 18S ribosomal RNA gene, partial sequence	1764	92%	0.0	92%	GU817404.1
Moniezia benedeni isolate 1 clone 2 18S ribosomal RNA gene, partial sequence	1760	92%	0.0	92%	GU817402.1
Moniezia benedeni isolate 1 clone 1 18S ribosomal RNA gene, partial sequence	1760	92%	0.0	92%	GU817401.1
Bertiella studeri isolate M01 18S ribosomal RNA gene, partial sequence	1011	77%	0.0	95%	GU323707.1
Mosgovoyia ctenoides partial 18S rRNA gene, fragment 1	1003	50%	0.0	92%	AJ555164.1
Hymenolepis nana genome assembly H_nana_Japan, scaffold HNAJ_contig0006266	996	76%	0.0	93%	LM407632.1
Hymenolepis nana 18S ribosomal RNA gene, complete sequence	992	76%	0.0	93%	AY193875.1
Andrya cuniculi partial 18S rRNA gene, fragment 1	989	63%	0.0	93%	AJ555162.1
Hymenolepis microstoma 18S rRNA gene	981	74%	0.0	93%	AJ287525.1
Hymenolepis diminuta strain WMS-il1 18S ribosomal RNA gene, partial sequence	979	79%	0.0	93%	JX310720.1
Hymenolepis diminuta 18S ribosomal RNA gene, complete sequence	979	79%	0.0	93%	AF286983.1
Fimbriaria sp. OLBM1 18S ribosomal RNA gene, complete sequence	974	76%	0.0	93%	AF286982.1
Wardoides nyrocae 18S rRNA gene	972	79%	0.0	93%	AJ287587.1

Figure 2: Phylogenetic tree for *Moniezia* Sp. using partial 18s rRNA gene sequence

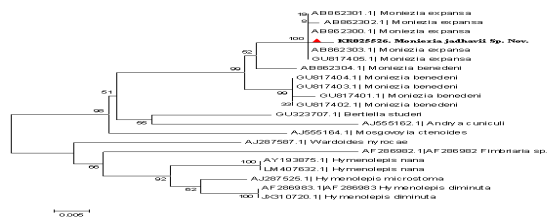
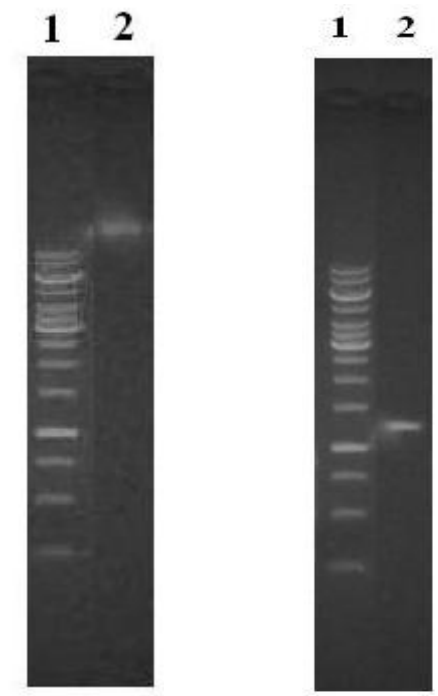


Fig.3. a) Genomic DNA extracted from worms

b) Amplification of partial 18S and 28S rRNA gene for worm samples



a)
Lane 1: 1Kb DNA Ladder of Fermentas

Lane 2: Genomic DNA of sample

b)
Lane 1: 1Kb DNA Ladder of Fermentas

Lane 2: Amplified PCR product of Moniezia Sp.

1Kb DNA marker (Top to bottom): 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250bp.

DISCUSSION

The genus *Moniezia* was erected by Blanchard in 1891. The worm under discussion is having the scolex almost squarish, mature proglottids nearly five times broader than long, Craspedote in shape, testes small in size, round to oval, 210-220 in numbers, cirrus pouch oval, ovary horse-shoe shaped, vitelline gland post ovarian.

1. The present worm differs from *Moniezia (B) benedeni,*

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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.

2. The present cestode differs from *Moniezia (B) pallid, Monnig*, 1926, which is having the uterus external, dorsal and ventrally over excretory canals, the interproglottidal glands varying in size and reported from the host horse in South Africa.
3. The present parasite differs from *Moniezia (B) aurangabadensis*, Jadhav B. V. *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.).*
4. The present tapeworm differs from *Moniezia (B) bharalae* Jadhav B.V. *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 number and reported from *Ovisbharal (L.).*
5. The present form differs from *Moniezia (B) warnanagarensis*, Patil *et.al*. 1997, which is having scolex large, testes 300-320 in number, 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.
6. 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 number, 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.
7. The present tapeworm differs from *Moniezia (B) murhari* Kalse*et.al,* 1999, in having the scolex squarish, testes 405-415 in number, 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.
8. 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.
9. The present worm differs from *Moniezia (B) shindei,* Pawar*et. al.,* 2004 in having scolex large, mature segments craspedote, testes 190-200 (195) in number, scattered all over segment and ovary a single mass, large, oval, cirrus pouch oval, elongated, in centre of the segment and vitelline gland large, oval, internal to ovary.
10. The present cestode differs from *Moniezia (B) hircusae* Tat and Jadhav B. V., 2004 which is having scolex large, mature segments big, craspedote, testes 168 in number, medium, small, scattered in a single field, ovary large, oval, a single mass, in anterior half of the segment, inter proglottidal glands 14-15 in number, large, oval and cirrus pouch in anterior 1/3rd region of the segment.
11. The present cestode differs from earlier described *Moniezia (B) rajalaensis,* Borde *et.al*. 2007in 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 number, large, oval and cirrus pouch oval.
12. The present cestode differs from earlier described *Moniezia (B) caprae,* Nanware S.S., 2010 in having scolex large, mature segment big, almost three and a half times border than long, testes 170 in numbers, medium in size, oval in shape, ovary large, bilobed, inter proglottidal glands 40 in number, oval, rounded and cirrus pouch on each side.
13. The present cestode differs from earlier described *Moniezia (B) govindae,* Padwal *et. al.,* 2011 in having scolex large, globular, mature proglottids big, craspedote, testes 100-140 in numbers, medium, scattered throughout proglottids, ovary large, compact, nut shaped, inter proglottidal glands 40-42 in number, large, oval and cirrus pouch elongated.
14. The present cestode differs from earlier described *Moniezia (B) babai,* Humbe et al.,2011 in having scolex globular, mature segment four times border than long, testes 190-220 in numbers, small in size, rounded in shape, ovary large, rounded, inter proglottidal glands 18-20 in number, oval, rounded and cirrus pouch on each side.
15. The present cestode differs from earlier described *Moniezia (B) ovisae,* Humbe et al.,2011 in having scolex broad anteriorly and narrow towards neck, mature segment two times border than long, testes 155-165 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 32-35 in number, oval, rounded and cirrus pouch on each side.
16. The present cestode differs from earlier described *Moniezia (B) osmanabadensis,* Humbe et al.,2012 in having scolex globular, mature segment five times border than long, craspedote, testes 170-200 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 38-40 in number, oval, rounded and cirrus pouch on each side.
17. The present cestode differs from earlier described *Moniezia (B) devraoi,* Humbe et al.,2013 in having scolex quadrangular, mature segment four times border than long, testes 160-180 in numbers, small in size, rounded in shape, ovary large, bilobed, inter proglottidal glands 40-45 in number, oval, rounded and cirrus pouch on each side.
18. The present cestode differs from earlier described *Moniezia (B) shegaonesis,* Barote et al,.2013 in having scolexglobular, mature segment four to five times border 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.
19. The present cestode differs from earlier described *Moniezia (B) shivajiraovae,* Barote et al,.2014 in having scolexsquarish, large in size, mature segment six-toeighttimes border than long, testes 84-95 in numbers, small in size, rounded in shape, ovary horse-shoe shaped, inter proglottidal glands 40-42 in number, oval, rounded and cirrus pouch on each side.

We conclude that the morphological observation as well as

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the sequence of its 18S rRNA gene obtained in this study, clearly demonstrate that this species should be considered to be a member of genus *Moniezia* (Cestoda: Anaplocephalidea) but species name is differ in both the observation. Several characteristics are differs from other *Moniezia* Sp.

In molecular analysis the phylogenetic neighbors of *Moniezia* Sp. based on partial 18S rRNA gene are shown in table no. 1. On the basis of position of sequence of the given *Moniezia* sample in the phylogenetic tree, the sample showed closest similarity with the *Moniezia expansa*.

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) jadhavii,* 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.

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

Mature segments broader than long - 1
Mature segments Squarish - *M. (B.) pallid,* Monnig, 1926

Mature segments Craspedote - *2*
1) Testes below 100 in numbers-*M.(B.) Caparae,* Nanwaree*t.al.*,2010
Testes in between 100-150 in number- *M.(B.) govindae,* Padwal *et.al.* 2011

Testes in between 150-200 in numbers- 3
Testes in between 200-300 in numbers- 4

Testes in between 300-400 in numbers- M. *(B.) warnanagarenisis*,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 ,Skrjabin et. al.Schulz, 1937

Testes in between 1100-1200 in numbers- *M. (B.) aurangabadensis*, Shinde*et.al.*, 1985

2) Inter proglottidal glands between10-15 in number- *M. (B.) hircusae*,Tat and Jadhav, 2004

Inter proglottidal glands between 16-20 in number- *M. (B.) babai, humbe et al., 2011*

Inter proglottidal glands between 20-25 in number- *M. (B.) shegaonesis*,Barote et al., 2013

Inter proglottidal glands between 30-35 in number- 5

Inter proglottidal glands between 36-40 in number- *M. (B.) osmabadensishumbe et al., 2012*

Inter proglottidal glands between 46-52 in numbers- ***M. (B.) jadhavii* Sp. Nov.**

Inter proglottidal glands 76in number- *M. (B.) shindei,* Pawar*et.al.* 2004

3) Scolex globular - M. *(B.) bharalae,* Shinde*et.al.*, 1985

Scolexsquarish -M. *(B.)kalawati* ,Nanware*et.al.* ,1999

Scolex quadrangular -
6

4) Scolexsquarish - *M. (B.) caparai*,Pokale et al. , 2004
Scolexglobular - M. *(B.) rajalaensis,* Borde*et.al.*, 2007

5) Testes in between 50-100 in numbers - *M. shivajiraovae*,Barote et al., 2014

Testes in between 150- 200 in numbers- *M. (B.) ovisae, humbe et al., 2011*

6) Ovary oval in shape - *M. (B.) devraoi, humbe et al., 2013*

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