Research Paper Zoology

Morphological and Molecular Characterization of *Senga*, Dollfus, 1934 (Cestoda: Ptychobothridae) From a Fresh Water Fish in Aurangabad District (M.S.), India.

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ABSTRACT

The present communication deals with the description of new species of genus *Senga* from fresh water fish *Mastacembelus armatus*. The differentiating character of new form are scolex triangular, neck absent, testes 285 to 295 in number, ovary bilobed. In molecular analysis of present parasites, the genomic DNA were amplified and sequenced. Morphologically the genus of this parasite is *Senga* but molecularly they come closer to other *Bothriocephalus*.

KEYWORDS Aurangabad, *Bothriocephalus*, Molecular analysis, Ptychobothridae, *Senga*.

INTRODUCTION

The genus *Senga* was established by Dollfus, 1934 with its type species *S. besnardi* from *Betta splendens* at Vinecunes, France. *S. ophiocephalina* Tseng, 1933 as *Anchistrocephalus ophiocephalina* from *Ophiocephalus argus* at Taimen, China and identified with a form previously recorded by Southwell, 1913 as *Anchitrocephalus polyptera* (*Anchitrocephalus*) Monticelli, 1890 Syn. *Anchistrocephalus* Luhe, 1899 from *Ophiocephalus striatus* in Bengal, India *S. pcynomera* Woodland, 1924 as *Bothriocephalus pcynomera* from *Ophiocephalus marulius* at Allahabad, India. *S. lucknowensis*. Johri, 1956 from *Mastacembelus armatus* in India.

Fernando and Furtado, 1963 recorded *S. malayana* from *Channa striata*, *S. parva* and *S. filiformis* from *Channa micropeltes* at Malacca. Ramadevi and Hanumantha Rao, 1966 reported the plerocercoid of *Senga* sp. from *Panchax panchax*. Tadros, 1968 synomised the genus *Senga* with the genus *Polyonchobothrium* and proposed new combinations for the species. Furtado and Chauhan, 1971 reported *S. pahangensis* from *Channa micropeltes* at Tesak Bera. Shinde, 1972 redescribed *S. besnardi* from *Ophiocephalus gachua* in India. Ramadevi and Rao, 1973 reported another species of *S. visakhapatanamensis* India.

Ramadevi (1976) described the life cycle of *S. visakhapatnamensis* from *Ophiocephalus punctatus* in lake at Kondakaria, Andhra Pradesh, India. But they do not agree with Tadors statements. Wardle, McLeod and Radinovsky, 1974 put *Senga* as a distinct genus in the family Ptychobothridae. Deshmukh, 1980 reported *S. khami* from *Ophicephalus marulius*, a fresh water fish from Kham river at Aurangabad. Jadhav and Shinde, 1980 reported *S. godavarii* from *M. armatus* at Nanded, M.S. India. One more species *S. aurangabadensis* was added by Jadhav and Shinde, 1980 from *M. armatus* at Aurangabad M.S. India. A new addition made by Kadam et.al. 1981 as *S. paithaenansis* from host *M. armatus.* Majid et. al., 1984 added *S. raoi* and *S. jagannathae* from *Channa punctatus.* Two more new species erected by Jadhav et.al. 1991 as *S. maharashtrii* and *S.gachuae* from the intestine of *M. armatus.*

Monzer Hasnain, 1992 added *S. chauhani* from *Channa punctatus*. Tat and Jadhav, 1997 added *S. mohekarae* from the intestine of the *M. armatus*, at Parli, Dist. Beed, M.S. India. Patil and Jadhav added *S. tappi* from *M. armatus* in 2003. Jadhav, 2005 made the review article of the genus *Senga* from freshwater fishes from Maharashtra state, India. Pande et.al, 2006 added two new species i.e. *S. ayodhensis* from *Amphinuous cuchia* and *S. baughi* from *Rita rita.* Kalse A. T, 2009 added one new species *senga panzarensis* from *Mastacembelus armatus*. Bhure et.al, 2010 added one new species *S.madhavii* from *Mastacembelus armatus.*Pardeshi P.R, 2011 added one new species *Senga rupchandensis* from *Channa striatus*. . Lastly Fartade et.al. added two new species i.e. *S. nandedensis*, 2014 and *S. madhukarii*, 2015.

The phylogeny of the order Ptychobothridea 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 *Senga* Sp. parasite of the *Mastacembelus armatus* from Aurangabad district (M.S.), India on the basis of morphological and molecular and molecular data (using molecular markers) among Ptychobothrideans.

The present communication deals with the description of new species, *Senga govindii* Sp. Nov. collected from intestine of fresh water fish *Mastacembelus armatus,* Lecepede, 1800 from river Kham at Aurangabad district (M.S.) India, in the month of Feb.2015.

MATERIAL AND METHODS

Cestode parasites were collected from the intestine of *Mastacembelus armatus.* 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. The identification is made with the help of Systema Helminthum.

For the molecular analysis Cestode 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 micro centrifuge 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 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, 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 ~1000bp.

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).

RESULTES

Morphological description: - Nine parasites were collected from *Mastacembelus armatus* were flattened, preserved in 4% formalin whole amount of slides were prepared for anatomical studies. Drawing was made with the help of camera lucida. All measurement given in millimeters.

The worms are considerably long, thin milky white in colour with scolex, immature, mature and gravid segment. The scolex is well developed, triangular in shape and it measures about 2.689(1.106-4.272) in length and 2.251(0.763-3.739) in width. The anterior part of scolex is a prominent rostellum armed with 52 hooks. Hooks are arranged in semicircle, measures 0.0795 (0.063-0.096) in length and 0.0105 (0.006-0.015) in width. The scolex bears bothria extended up to the posterior end measures 2.041(0.763-3.319) in length and 0.629 (0.114-1.144) in width.The neck is absent. Mature segment rectangular in shape, broader than long and measures 2.861(2.785-2.937) in length and 3.624 (3.395-3.853) in width. Testes are 285 to 295 in number. Testes are oval, rounded small in size measures 0.133(0.076-0.19) in length and 0.1045 (0.019-0.19) in width. The cirrus pouch is oval in shape. Cirrus is thin tube. Ovary is bilobed each lobe is different with long isthmus measures 0.686(0.495-0.877)in length and 0.381 (0.228-0.534) in width, situated in the middle of segment. The vagina is thin tube. Genital pore is small rounded. Uterus is filled with numerous eggs. The vitellaria are follicular and arranged in two lateral margin of the segment which having one to two rows.

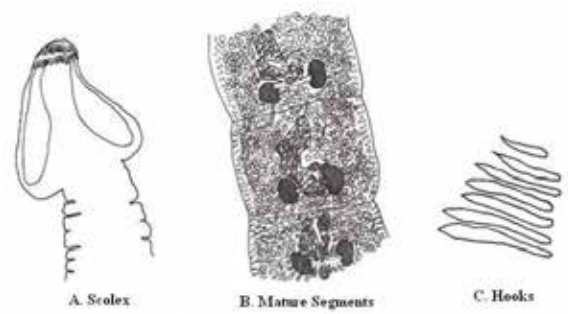


Fig. 1. *Senga govindii*, Sp. Nov.

Molecular description:-

Partial 18s rRNA gene Sequence (1065bp)

Table 1: Phylogenetic neighbors of Sample E based on partial 18s rRNA gene sequence

Description	Max score	Query cover	E value	Ident	Accession
Bothriocephalus acheilognathi 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1495	99%	0.0	93%	HM367066.1
Bothriocephalus sp. KBD1 18S ribosomal RNA gene, complete sequence	1489	98%	0.0	93%	AF267289.1
Echinophallus wageneri 18S ribosomal RNA gene, complete sequence	1463	99%	0.0	92%	DQ925311.1
Bothriocephalus scorpii 18S rRNA gene	1452	99%	0.0	92%	AJ228776.1
Bothriocephalus claviceps 18S ribosomal RNA gene, complete sequence	1450	97%	0.0	92%	AF267288.1
Paraechinophallus japonicus gene for 18S ribosomal RNA, partial sequence	1424	99%	0.0	92%	AB559561.1

Paraechinophallus japonicus 18S ribosomal RNA gene, complete sequence	1424	99%	0.0	92%	DQ925315.1
Anchistrocephalus microcephalus 18S rRNA gene	1413	99%	0.0	91%	AJ287473.2
Parabothriocephalus segmentatus gene for 18S ribosomal RNA, partial sequence	1402	99%	0.0	91%	AB559563.1
Parabothriocephalus segmentatus 18S ribosomal RNA gene, complete sequence	1402	99%	0.0	91%	DQ925314.1
Amphicotyle heteropleura 18S ribosomal RNA gene, complete sequence	1402	99%	0.0	91%	DQ925303.1
Bathycestus brayi 18S ribosomal RNA gene, complete sequence	1391	99%	0.0	91%	DQ925306.1
Anonchocephalus chilensis 18S ribosomal RNA gene, complete sequence	1386	99%	0.0	91%	DQ925304.1
Probothriocephalus sp. KBD1 18S ribosomal RNA gene, complete sequence	1386	99%	0.0	91%	AF267298.1
Ptychobothrium belones 18S ribosomal RNA gene, complete sequence	1338	99%	0.0	90%	DQ925317.1
Abothrium gadi 18S rRNA gene	1319	99%	0.0	90%	AJ228773.2
Marsipometra hastata 18S ribosomal RNA gene, complete sequence	1314	99%	0.0	90%	DQ925313.1
Anantrum tortum 18S ribosomal RNA gene, complete sequence	1310	96%	0.0	90%	AF286992.1
Bathybothrium rectangulum 18S ribosomal RNA gene, complete sequence	1288	99%	0.0	89%	DQ925305.1
Triaenophorus crassus 18S ribosomal RNA gene, complete sequence	1286	99%	0.0	89%	DQ925318.1

Figure 2: Phylogenetic tree for Sample E using partial 18s rRNA gene sequence

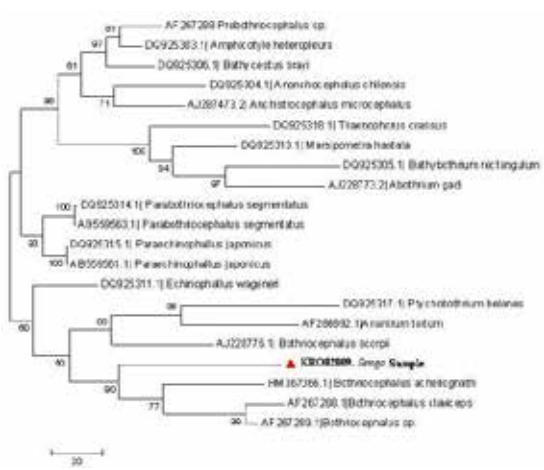
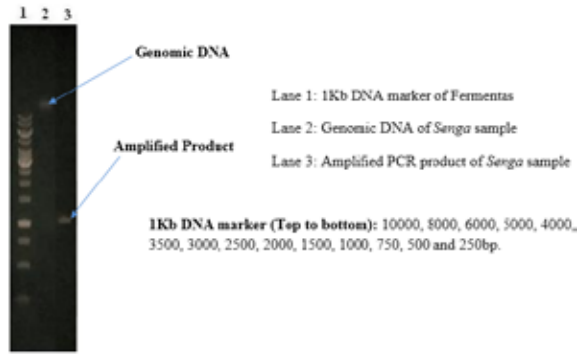


Figure 3: Genomic DNA and Amplified partial 18S rRNA gene of *Senga* sample.



Maximum Parsimony analysis of taxa:- The evolutionary history was inferred using the Maximum Parsimony method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree is drawn to scale with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. The analysis involved 21 nucleotide sequences. There were a total of 3665 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

DISCUSSION

The genus *Senga* was established by Dollfus, 1934 with the type species *Senga besnardi* from *Betta splendens*. The present worm comes closer to all the known species of the genus *Senga* Dollfus, 1934 in general topography of organs. But differs due to some characters from following species.

1. The present worm differs from *S. besnardi* Dollfus, 1934 in the shape of scolex which is triangular, hooks 50 in numbers, testes 160-175 in numbers, ovary compact and reported from *Betta splendens* in France.
2. The present cestode differs from *S. ophiocephalina* Teseng,1933 in having hooks 47-50 in numbers, testes 50-55 in numbers, ovary bilobed but equatorial in position, vitellaria lobate and reported from *Philocephalus argua argua* in China.
3. The present tapeworm differs from *S. pcynomera* Woodland, 1924 in having scolex elongated, hooks 68 in numbers, mature segments are indistinct, ovary discontinuous into two groups and reported from *Philocephalus marulius* in India.
4. The present parasites differs from *S. lucknowensis* Johri,1956 in having hooks 36-48 in numbers, ovary post equatorial, vitellaria lobulate and discontinuous in two groups.
5. The present cestode differs from *S.malayana* Furnando and Furtado, 1964 in having scolex circular, hooks 60 in numbers, ovary slightely bilobed, post equatorial, vitellaria lobate, discontinuous in two groups and reported from *Channa striata,* in Malacca
6. The present tapeworm differs from *S.parva* Furnando and Furtado, 1964 in having hooks 38-40 in numbers, testes 150-180 in numbers and reported from *Channa micropeltis*, in Malacca.
7. The present cestode differs from *S. pahangensis* Furtado et. al., 1971 in having triangular scolex, hooks 52 in numbers, neck short, segmentation clear, testes laterally situated in the proglottids, vitellaria lobulated and reported from *Channa micropeltis*, in Tasek, Bera.
8. The present tapeworm differs from *S. visakhapatanamensis*

Ramadevi et. al., 1973 in having circular scolex, hooks 46-52 in numbers, testes 50-55 in number, vitellaria lobulated and reported from *Ophiocephalus punctatus,* in India.

9. The present worm differs from *S. khami* Deshmukh and Shinde,1980 having scolex rectangular, oval, shallow bothria, hooks 55-57 in numbers, short neck, testes rounded, 155 in numbers and arranged in two fields, cirrus pouch is elongated, vitellaria follicular and reported from *Ophiocephalus marulius,* in India.
10. The present cestode differs from *S. aurangabadensis* Jadhav et. al., 1980 in having oval scolex, hooks 50-52 in numbers; in two half rows, overlapping on each other, mature segment broader than long, testes 240-260 in numbers and vitellaria follicular.
11. The present tapeworm differs from *S. godavarii* Shinde et. al., 1980 in having hooks 40-42 in numbers, arranged in two half rows, testes rounded, 220-230 in numbers, cirrus pouch is oval, situated in anterior half of the segment and vitellaria follicular.
12. The present worm differs from *S. paithanensis* Kadam et. al., 1981 which shows prominent, large, triangular scolex, hooks 54 in numbers, neck present, testes oval to rounded, 130-135 in numbers, arranged in two lateral groups, vagina posterior to cirrus pouch and vitellaria follicular.
13. The present cestode differs from *S. raoi* Majid and Shinde,1984 in having hooks 46 in numbers, testes 65-170 in numbers, vagina posterior to cirrus pouch and reported from *Channa punctatus,* in India.
14. The present cestode differs from *S.jagannathae* Majid and Shinde,1984 in having hooks 44 in numbers, testes 240 - 250 in numbers, ovary compact, vagina anterior to cirrus pouch and reported from *Channa punctatus,* in India.
15. The present parasite differs from *S. gachuae* Jadhav et. al.,1991in having hooks 22-25 in numbers, neck present, testes 60-70 in numbers, vitellaria follicular and reported from *Channa gachua,* in India.
16. The present cestode differs from *S. maharashtrii* Jadhav et. al., 1991 which shows muscular scolex, hooks 45-46 in numbers, large, arranged in two half crowns, testes oval 80-90 in numbers and vitellaria follicular.
17. The present worm differs from *S.chauhani* Monzer Hasnain,1992 in having scolex oval, hooks 40-44 in numbers and testes 200-210 in numbers, vitellaria non lobate and reported from *Channa punctatus,* in India.
18. The present cestode differs from *S. mohekarae*, Tat and Jadhav,1997 which shows elongated scolex, hooks 151 in numbers, neck short and broad, testes 300-310 in numbers and vitellaria follicular.
19. The present parasite differs fom *S. armatusae* Hiware,1999 in having scolex triangular, hooks 32-40 in numbers, vagina anterior to cirrus pouch and vitellaria follicular.
20. The present cestode differs from *S. tappi* Patil et. al., 2003 which is having triangular scolex, hooks 42-44 in numbers, neck is very short and squarish, testes 285-295 in numbers, small, rounded, distributed in 2 fields, vagina anterior to cirrus pouch and vitellaria follicular.
21. The present parasite differs from *S.ayodhensis* Pande et. al., 2006 in having conical scolex, hooks 29 in numbers, testes numerous, vitellaria follicular and reported from *Amphinuous cuchia,* in India.
22. The present cestode differs from *S.baughi* Pande et. al., 2006 in having hooks 28 in numbers, neck present, testes 40-50 in numbers, ovary compact, vitellaria follicular and reported from *Rita rita,* in India.
23. The present worm differs from *S.panzarensis* et.al. 2008, having scolex triangular, no.of hooks 58, neck absent, testes 40-45, ovary compact, vitellaria 4-5 lateral side reported from *Mastacembelus armatus* in India.
24. The present worm differs from *S.madhavii* Bhure et.al. 2010 having scolex triangular, hooks 40-42 in numbers, testes 200-225 in numbers, vitellaria granular reported from *Mastacembelus armatus* in India.
25. The present worm differs from *S.rupchandensis* Pardeshi et.al. 2011, having scolex tubular, hooks 42-45 in numbers, testes 350-370 in numbers. Reported from *Channa striatus*.
26. The present worm differs from *S. nandedensis* Asawari M. fartade, et.al 2014 having solex triangular, hooks 60-62. Mature segment rectangular, genital pore small and rounded, reported from *Mastacembelus armatus* in India.
27. The present worm differs from *S.madhukarii,* Asawari Fartyade,et.al 2015 having scolex cylindrical in shape, hooks 45, Mature segment rectangular, testes 130 in numbers reported from *Mastacembelus armatus* in India.

The above noted characters are valid enough to erect a new species hence the name *S. govindii* Sp.Nov.is proposed in honour of Professor Dr. G. B. Shinde, well known scientist in Helminthology and Ex-Registrar and Ex-Professor, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004.

In molecular analysis the phylogenetic neighbors of the present worm based on partial 18S rRNA gene are shown in table no. 1. On the basis of position of sequence of the given the present sample in the phylogenetic tree, the sample showed closest similarity with the *Bothriocephalus acheilognathi* (93%) where as the morphological characteristics between the genus *Bothriocephalus* and the present worm differentiating in having scolex is compressed and unarmed, while the present worm scolex is armed with hooks (Yamaguti,1956). But the present worm with same character that is the Scolex similar with *Senga*, Dollfus, 1934 along with other characters hence the present worm erect as the genus *Senga* with new species *Senga govindii.*

A Key to the species of the genus *Senga* Dollfus, 1934

Neck present	-	1
Neck absent	-	2
1) Scolex circular	-	*S. malayana*, Furnando and Furtado, 1964.
Scolex rectangular	-	S. khami, Deshmukh and Shinde, 1980.
Scolex triangular	-	3
Scolex pear shaped	-	4
Scolex oval	-	5
2) Scolex circular	-	*S.visakhapatnamensis*, Ramadevi et.al.1973.
Scolex conical	-	S. ayodhensis, Pande et.al. 2006.
Scolex cylindrical	-	S. madhukarii, Fartade et. al. 2015
Scolex tubular	-	S. rupchandensis Pardeshi 2011.
Scolex elongated	-	S. pcynomera, Woodland 1924.
Scolex oval	-	6
Scolex pear shaped	-	7
Scolex triangular	-	8
3) Vitellaria follicular	-	9
Vitellaria lobulate	-	S.pahangensis, Furtado et.al. 1971.
4) Testes below 50	-	*S.baughi*, Pande et.al. 2006
Testes above 50	-	S. gachuae, Jadhav et.al 1999
Testes in bet^{n} 100-200	-	S.parva, Furnando and Furtado, 1964
Testes in bet^{n} 200-300	-	S.jagannathae, M. A. Majid and G. B. Shinde, 1984.
5) Hooks below 100	-	*S. chauhani*, Monzer Hasnain, 1992
Hooks above 100	-	S.mohekarae, Tat and Jadhav, 1997
6) Testes below 100	-	*S. maharashtrii*, Jadhav and Tat 1991
Testes above 100	-	S. aurangabadensis, Jadhav et.a1980
7) Vitellaria lobulate	-	10
Vitellaria follicular	-	S.godavarii, Shinde

et.al. 1980.
Vitellaria granular - S.raoi, M. A. Majid and Shinde1984

8) Testes below 100 - *S. panzarensis*, Kalse. A. T 2009.
Testes in bet^{n}100-150 - S. nandedensis Fartade et al. 2014
Tetses in bet^{n} 150-200 - S. besnardi, Dollfus, 1934
Testes in bet^{n} 200-250 - 11
Testes in bet^{n} 250- 300 - ***Senga* govindii, Sp. Nov.**

9) Hooks below 50 - *S. tappi*, D. N. Patil 2003.
Hooks above 50 - S. paithanensis, Kadam et.al. 1981.
10) Hooks below 50 - *S. luknowensis*, Johri, 1956
Hooks above 50 - S. ophiocephalina, T seng, 1933
11) Vitellaria follicular - *S. armatusae*, C. J. Hiware, 1991
Vitellaria granular - S. madhavii, Bhure et.al. 2010

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